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Bacteriological quality of ice creams in Baghdad

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Abstract

In order to assessment of distribution pattern and bacteriological quality of ice creams (locally produced and imported) sold in Baghdad, a total of thirty pooled samples (three replicates from each type, n=90) were randomly collected from different markets and homemade regions in Baghdad during May till July (2015), in which they processed and analyzed for prevalence of *Escherichia coli* and *E. coli O157* by different dairy microbiological procedures. The results showed isolation and identification of 14 (46.7%) isolates out of 30 pooled samples as 8 (53.4%) isolates from 15 locally produced and homemade ice creams, in which the serotype *O157* was detected in 5 (33.4%) samples (4 isolates in homemade types and one isolate in machine types) and 6 (40%) isolates from 15 imported ice creams, in which 2 (13.4%) isolate was identified as a serotype *O157*. The mean log count of *E. coli* to *E. coli O157* in locally produced ice creams range from 2 to 7 $\log_{10}$ cfu g^{-1}, while in imported ones range from 1.7 to 3 $\log_{10}$ cfu g^{-1}. Results profile provide useful information on biosafety and hazard analyses critical control points of hygienic environmental measurements of *E. coli* and *E. coli O157* in ice creams marketed in Baghdad.

الجودة البكتريولوجية للمثلجات اللبنية في بغداد

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الخلاصة

من أجل تقييم نمط الانتشار والجودة البكتريولوجية للمثلجات اللبنية (المنتجة محليا والمستوردة) المباعة في بغداد، جمعت ثلاثين انموذج جمعي (ثلاثة مكررات من كل نوع، ما مجموعه تسعين مكرر) بصورة عشوائيا من مختلف الأسواق والمنتجة محليا داخل البيوت في بغداد خلال ايار حتى تموز (2015)، حيث عوملت النماذج وتم تحليلها وفق طرائق مكروبيولوجيا الألبان لغرض التحري عن تلوث تلك المنتجات ببكتريا القولون ونمطها المصلي الممرض *O157*. أظهرت النتائج عزل وتشخيص 14 (46.7%) عزلة من 30 انموذج جمعي: 8 (53.4%) عزلات من 15 أنموذج محلية الصنع، حيث شخص النمط المصلي *O157* في 5 (33.4%) نماذج (4 عزلات من المثلجات اللبنية المنتجة داخل البيوت وعزلة واحدة من تلك المنتجة بوساطة ماكينة المثلجات) و6 (40%) عزلات من 15 انموذج مستورد، كان منها 2 (13.4%) عزلة النمط المصلي *O157*. تراوحت معدلات العد اللوغاريتمي لبكتريا القولون الى نمطها المصلي *O157* في المثلجات اللبنية المحلية من 2 – 7 مستعمرة / غرام (مل) منه، في حين كانت القيم للمستورد منها 1.7 – 3 مستعمرة / غرام (مل). نوعية النتائج وفرت معلومات مفيدة في مجال السلامة الأحيائية والمخاطر الناجمة من نقاط المراقبة الحرجة للتلوث الميكروبي ضمن القياسات البيئية الصحية لبكتريا القولون ونمطها المصلي *O157* في المثلجات اللبنية المباعة في بغداد.

Introduction

Food-borne diseases are important public health and economic burden; and result in considerable morbidity and mortality rate (1-5). Exposure of processed food to contaminated raw material increases the chance of cross-contamination. Other factors associated with cross-contamination and food-borne illnesses are improper food processing, cooking, storage and handling (1,6). Problems associated with food-borne disease are vomiting, diarrhea, liver and kidney failure, neural and brain disorders and in the most severe cases, death. Long term problems related to food-borne disease are paralysis and reactive arthritis (1,7).

Detection of an indicator *E. coli* in foods means presence of pathogenic and toxigenic microbes in it. Due to the low infective dose, severity of the disease symptoms and the case fatality rate, *E. coli O157:H7* was considered a harmful threat in food safety (8,9). *E. coli O157* contaminates water and food products. Milk was considered a high risk food as it's highly nutritious and serves as an ideal medium for bacterial growth (10). Dairy farms act as reservoirs for several food-borne pathogens such as *Escherichia coli*, *Listeria*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter* and *Salmonella* (11-13). Dairy cattle are the major source of *E. coli* strains in milk that contaminate milk and meat through direct and indirect contact with the cattle and the dairy farm environments. Shiga-toxin producing and Enterohemorrhagic strains are highly pathogenic to human with low infectious dose, causing food-borne disease through consumption of contaminated water or food (14,15).

Ice cream, a milk based product, was a major product of the dairy industry and has becomes a dominant consumer product for large segments of the population. Ice cream was sold both in a package form (cups, cones and cartons) and in 'open' containers at retail outlets or ice cream parlours, which was distributed manually in scoops, cones or sundaes. Ice cream was a good medium for a microbial growth due to its nutrient content, almost neutral pH (6–7) and long storage duration. Ice cream was rich in fat, sugar, emulsifier and stabilizer. Flavor enrichment of ice cream was an optional addition of fruits nuts, candies, syrups and other flavoring ingredients. Ice cream should contain extremely low bacterial load, but higher bacterial counts have been reported by many researchers. This could be due to insufficient hygienic measurements, environments, handlers, improper pasteurization process or post-process contamination. Bacterial contamination was the main danger posed by ice cream. Freezing ice-cream and other frozen dairy products slow bacterial growth but doesn't kill them, which begin to grow again as food thaws. The risk of food-borne illness increases after ice cream has been opened and used. Relatively low storage temperature and pasteurization steps during its processing were considered to eliminate most of the hazard microorganisms. However, there was still a remained concern over the microbial safety of ice creams. During the processing after a pasteurization step, there was a potential hazard by an addition of contaminated ingredients or an improper handling of the final products including an abuse of the storage temperature. This was especially important in the

preparation of soft ice cream as its final stage of production was carried out at the point of sale (16-23).

The objective of this study was to evaluate the microbial load in ice creams sold in the Baghdad markets and to determine the degree of contamination and distribution pattern with *E. coli* and *E. coli O157* as measurements of bacteriological quality, due to limited information of these indicator microorganisms in locally produced and imported ice creams in Baghdad.

Materials and Methods

Collection and Processing of Samples: Thirty pooled samples of ice creams were collected randomly from different markets and regions in Baghdad during May till July (2015): fifteen locally produced from ice cream machines (10 pooled samples: 3 replicates from each) and from some homemade regions (5 pooled samples: 3 replicates from each); and fifteen imported pooled samples (3 replicates from each). All samples were collected aseptically in sterile bags and ice container, then transported as soon as possible to zoonotic diseases laboratory, in which they processed and analyzed by different dairy microbiological procedures with some modifications (23-26).

Modified isolation procedures were used, in which each pooled and well mixed replicates were divided in to two separate unit (direct and indirect processing steps): directly well mixed replicates units were cultured on freshly prepared McConkey, Sorbitol-McConkey and Eosin Methylene Blue agars (one g\ml well mixed thawed sample part was streaked by sterile loops and swabs by dilution procedure on three replicates agars for each pooled unit) at 37 ©C for 18-48 hours; and indirectly replicates units were enriched by tryptone soya yeast extract broth for 24 hour at 37 ©C, then cultured on same agars and temperature above. In this pathway samples units were cultured by dilution formula: one g\ml pooled part of sample unit to nine parts of broth diluent (23-26).

Pure isolated colonies were counted by Miles-Misra technique in accordance to McFarland's opacity tubes (27), then picked up and recultured on tryptone soya yeast extract broth at 37 ©C for 24 hours, then cultured on tryptone soya yeast extract agar at 37 ©C for 24 hours for further identification procedures. Electronic rapid code compendium computerized designed biochemical panel test system for *Enterobacteriaceae* with serotyping latex test kit for serotype *O157* were used for confirmation procedure of isolates (28). Data were statistically analyzed by Chi-square test in accordance with SPSS (29).

Results and Discussion

Results profile revealed contamination with *E. coli* in Table (1) and *E. coli O157* in Table (2) especially from homemade ice creams, which was an indicator of fecal contamination, pre and post contamination sources and a public health hazard. The results showed isolation and identification of 14 (46.7%) isolates out of 30 pooled samples as 8 (53.4%) isolates from 15 locally produced and homemade ice creams, in which the serotype *O157* was detected in 5 (33.4%) samples (4 isolates in homemade types and one isolate in machine types) and 6 (40%) isolates from 15 imported ice

creams, in which 2 (13.4%) isolate was identified as a serotype *O157*. The mean log count of *E. coli* to *E. coli O157* in locally produced ice creams range from 2 to 7 log_{10} cfu g^{-1}, while in imported ones range from 1.7 to 3 log_{10} cfu g^{-1}. According to Jay (26) increase or decrease 0.5 microbial load log_{10} cfu g^{-1} in food indicate significant differences. This explains the need of strict monitoring and surveillance for effective measures of hygiene and sanitary practice during production and importation of ice creams and various milk products.

Table (1) Isolation percentages and mean log count of *E. coli* from ice creams in Baghdad.

Type of Pooled Samples		Number	Isolation %	From 15	From 30	Mean log count cfu\g or ml
Locally produced ice creams	Machine type	10	1 (10%)	6.7%	3.4%	2.698 [a]
	Homemade type	5	2 (40%)	13.4%	6.7%	7 [b]
Imported ice creams		15	4 (26.7%)		13.4%	3 [a]

a,b: Indicate significant differences among isolates for mean log count vertically at level (P≤0.05).

Table (2) Isolation percentages and mean log count of *E. coli O157* from ice creams in Baghdad.

Type of Pooled Samples		Number	Isolation %	From 15	From 30	Mean log count cfu\g or ml
Locally produced ice creams	Machine type	10	1 (10%)	6.7%	3.4%	2 [a]
	Homemade type	5	4 (80%)	26.7%	13.4%	5 [b]
Imported ice creams		15	2 (13.4%)		6.7%	1.7 [a]

a,b: Indicate significant differences among isolates for mean log count vertically at level (P≤0.05).

The possible sources of these indicator coliforms in ice creams could be contaminated or low quality raw milk and milk powders or post processing contamination from nose, hands, skin and clothing of handlers. Coughing, talking and sneezing produce droplets which could settle on ice creams during transportation, storage and retailing. This was an indication of poor sanitation practices on the food commodity with unrestricted importation rules (16-23).

The high incidence of indicator organisms in ice creams has been highly associated statistically with high counts in the same product. The accepted standard for coliforms in ice creams was 10 colonies of bacteria/ml or g (19,30). Contamination load levels of *E.*

coli and *E. coli O157* in ice creams in Baghdad markets, 46.7% exceeded the accepted standard by the Food and Drug Administration (FDA) and the U. S. Department of Agriculture (USDA) for manufactured milk products (19).

The coliforms especially *E. coli* and *E. coli O157* in packed ice creams might have been mainly due to post pasteurization contamination due to prolonged storage under inadequately clean freezers with or without other foods (mixed contamination) as well as freeing-thawing cycles due to electricity shuts, which indicate poor sanitation and hygienic practices by personnel or management (16-23). A proper application of Good Manufacturing Practice and Hazard Analysis Critical Control Points System will minimize the possibility of the contamination of ice creams with pathogens. However, the present study indicates that low hygienic measurements can affect the microbial quality and increase the risk further from the food-borne pathogens of ice creams, especially with dangerous serotypes like *E. coli O157*.

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Solar radiation maps in Iraq using geographic information systems

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Abstract

The design and operation of any solar energy system requires a good knowledge of the solar radiation data in a location. This data finds application in agriculture, climatology, meteorology, etc. This radiant energy is necessary for the metabolism of the environment and its inhabitants. Since the solar radiation reaching the earth's surface varies with climatic conditions of a place, a study of solar radiation under local climatic condition is essential. Global solar radiation is of economic importance as renewable energy alternatives. In this paper we determine the global solar radiation incident on horizontal surface for Baghdad city using computer program CROPWAT 8.0 on monthly average daily climate date for 22 Iraqi stations. The period (2004-2014) were taken to determine through statistical analysis the regression coefficient (a and b) from Angstrom's formula which have values of 0.267 and 0.474 respectively. These values were used to estimate global solar radiation for other cities in Iraq (Basrah province as a case study) because of the lack of recording instrument for solar radiation in climate stations in Iraq. High correlation (0.99) between Rs / Ra vs. n/N about 0.99 was found. Also in this paper we derived solar radiation maps using data from 22 Iraqi stations for the period (2004-2014).

خرائط الإشعاع الشمسي بالعراق باستخدام نظم المعلومات الجغرافية

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الخلاصة

إن تصميم وعمل أي نظام للطاقة الشمسية يتطلب معرفة جيدة لبيانات الإشعاع الشمسي في الموقع. إن هذه البيانات لها تطبيقات بالزراعة، علم المناخ، الأنواء الجوية...الخ. إن الطاقة الإشعاعية تكون ضرورية للكائن و الساكنين بالبيئة. بما إن الإشعاع الشمسي الواصل لسطح الأرض يتغير مع الظروف المناخية للمكان، فان دراسة الإشعاع الشمسي تحت الظروف المناخية المكانية يكون ضروري .إن الإشعاع الشمسي الكلي يعتبر ذو أهمية اقتصادية كبدائل للطاقة المتجددة. في هذا البحث تم حساب الإشعاع الشمسي الكلي الساقط على سطح أفقي لمدينة بغداد باستخدام برنامج CROPWAT النسخة 8.0 وباستخدام البيانات المناخية للمعدلات الشهرية في 22 محطة

عراقية. تم اعتماد الفترة (2004–2014) و استخدام التحليل الإحصائي لحسب معامل الارتباط (a و b) من صيغة انكسترون ، حيث كانت القيم 0.267 و 0.474 على التعاقب. تستخدم هذه القيم لتقدير الإشعاع الشمسي الكلي للمدن الأخرى في العراق (محافظة البصرة كدراسة حالة) بسبب النقص في أجهزة تسجيل الإشعاع الشمسي في المحطات المناخية بالعراق .تم الحصول على معامل ارتباط عالي (0.99) بين Rs/Ra و n/N. كذلك تم في هذا البحث اشتقاق خرائط الإشعاع الشمسي باستخدام البيانات من 22 محطة عراقية للفترة (2004 – 2014)،

Introduction

The growing populations of the world, the fast depleting reserves of fossil fuels, and the awareness of environmental impact have led the researchers to think of alternate sources of energy for a safer life on this earth. Therefore, the whole world is looking for non-exhaustible and renewable energy sources for their future. Among the all renewable energies, solar energy is the best option if it can be used in a cost effective manner; because the technology is also environmentally sound. Solar energy is a renewable resource, and is environmentally friendly. Unlike fossil fuels that are only found in selected regions of the world, solar energy is available just about everywhere on earth. The added advantage of solar energy is that it is provided for free and is not susceptible to price fluctuations associated with fossil fuels. Solar radiation may be harnessed for use either as solar thermal or photovoltaic. An accurate knowledge of solar radiation distribution at a particular geographical location is of vital importance for the development of many solar energy devices. All human cultures require the production and use of energy. Energy is used for transportation, heating, cooling, cooking, lightening, and industrial production (1, 2). In fact, energy is the life blood of economies around the world and global economic growth depends on adequate reliable and affordable supplies of energy. Incident shortwave solar radiation at the Earth's surface of fundamental important to the Earth's biosphere and climate. Shortwave radiation is the prime energy source for terrestrial and marine photosynthesis and is a major term in the global surface heat budget (3, 4, 5). Iraq suffers from a severe shortage of solar radiation data to be measured by institutions interested in measuring the different kinds of radiation. The researchers resorted to extract the estimated values by using various mathematical formulas that rely on measurements of various climate stations or satellites. In Iraq, solar radiation measurements are available only for very limited stations (6). The Middle East is a great source of solar energy that has not yet been fully exploited, due to the lack of proper meteorological stations specialized for solar radiation has made it hard to study this great amount of energy source in such region (7). Iraq is one of the areas suitable for the investment of solar energy as the amount of solar energy falling on square kilometer of earth surface about 200 million kilowatts annually (8). Global Solar Radiation (Rs) has a fundamental importance for life on earth. Rs is used in various type of calculation, e.g. in estimating reference evaporation. However, its measurement is costly and time consuming. Efforts have therefore been made in order to find away to estimate (Rs) (9). Kimball found a correlation that had an approximately linear shape between Rs and sunshine duration(10), various climate model have been developed for use in predictive the

monthly average global solar radiation, the popular one being the Angstrom-type regression equation developed by Angstrom in 1924, it is given by the following expression[1]:

$$\frac{H_o}{H_a} = a + b(n/N)$$

For many developing countries, solar radiation measurements are not easily available because of the incapability to afford the measuring equipments and techniques involved as given by. Therefore, it is necessary to develop methods to predict solar radiation from the available meteorological data.

Materials and Methods

Baghdad is the capital of Iraq, in the mid of its area about 204.2 km², having one observation meteorological station on Baghdad international airport (33. 18°N 44. 24°E), 31.7 m above sea level. This paper interested in calculating monthly average daily global solar radiation , computed in CROPWAT V 8.0 calculations, which represents the amount of extraterrestrial radiation reaching a horizontal plane on soil surface, that is computing the fraction of extraterrestrial radiation scattered, reflected or absorbed by the atmospheric gases, clouds and dust (11). Radiation is expressed in MJ /m² /day. But it's multiplied by the factor 11.575 to transfer to the units of watt /m², also calculating through statistical analysis the, b regression coefficients from Angstrom formula to estimate monthly average daily global radiation (Rs) on horizontal surface which relates solar radiation to extraterrestrial radiation and relative sunshine duration (12, 13, 14). The extraterrestrial radiation (Ra) represents the radiation received at the top of the earth's atmosphere on a horizontal surface, depending on latitude, date and time of the day. The coefficients computed from the mean monthly data did not practically differ from those computed using the mean daily data. The monthly average of clearness index (Rs/Ra) should usually lie between about 0.25 and 0.75 at any place. Data of actual mean monthly of total daily sunshine duration measured in Baghdad air port, al Basrah hai al Hussain in Iraq meteorological organization and seismology for the period (2004-2014) (15). We compare our calculated monthly average daily solar radiation falling on horizontal surface by CROPWAT V. 8.0 for Baghdad, Basrah hai al Hussain observation stations by Angstrom formula with monthly average daily data for solar radiation (1983 – 2005) for NASA surface meteorology and solar energy (16). If the solar radiation, R_s, for any area in Iraq is not measured, it can be calculated with the Angstrom formula which relates solar radiation to extraterrestrial radiation and relative sunshine duration:

$$R_{s=} \left(a_{s+} b_s \frac{n}{N}\right) \mathrm{R_a} \qquad (1)$$

Where

R_s: solar or shortwave radiation [MJ m^{-2} day^{-1}],

n: actual duration of sunshine [hour],

N: maximum possible duration of sunshine or daylight hours [hour],

n/N: relative sunshine duration,

R_a extraterrestrial radiation [MJ m^{-2} day^{-1}], a_s regression constant, expressing the fraction of extraterrestrial radiation reaching the earth on overcast days (n = 0), a_s+b_s fraction of extraterrestrial radiation reaching the earth on clear days (n=N). R_s is expressed in the above equation in MJ m^{-2} day^{-1}. Depending on atmospheric conditions (humidity, dust) and solar declination (latitude and month), the Angstrom values a_s and b_s will vary. Where no actual solar radiation data are available and no calibration has been carried out for improved a_s and b_s parameters, the values a_s = 0.25 and b_s = 0.50 are recommended. The extraterrestrial radiation, R_a, and the daylight hours or maximum possible duration of sunshine, N, are given by Equations 2 and 9. Values for R_a and N for different latitudes. The actual duration of sunshine, n, is recorded with a Campbell Stokes sunshine recorder.

Extraterrestrial radiation for daily periods (R_a)

The extraterrestrial radiation, R_a, for each day of the year and for different latitudes can be estimated from the solar constant, the solar declination and the time of the year by:

$$R_a = \frac{24(60)}{\pi} G_{sc}\, d_r\, [w_s \sin(\varphi) \sin(\delta) + \cos(\varphi)\cos(\delta)\sin(\omega_s)] \quad (2)$$

Where

R_a extraterrestrial radiation [MJ m^{-2} day^{-1}],

G_{sc} solar constant = 0.0820 MJ m^{-2} min^{-1},

d_r inverse relative distance Earth-Sun (Equation 3),

ω_s sunset hour angle (Equation 5 or 6) [rad],

ϕ latitude [rad] ,

δ solar decimation (Equation 4) [rad].

The inverse relative distance Earth-Sun, d_r, and the solar declination, δ, is given by:

$$d_r = 1 + 0.033\cos\left(\frac{2\pi}{365} J\right) \quad (3).$$

$$\delta = 0.409 \sin\left(\frac{2\pi}{365} J - 1.39\right) \quad (4).$$

Where J is the number of the day in the year between 1 (1 January) and 365 or 366 (31 December).

The sunset hour angle, ω_s, is given by:

$$\omega_s = \arccos\left[-\tan(\varphi)\tan(\delta)\right] \quad (5).$$

As the arccos function is not available in all computer languages, the sunset hour angle can also be computed using the arctan function:

$$\omega_s = \frac{\pi}{2} - arc\ \tan\left[\frac{-\tan(\varphi)\tan(\delta)}{X^{0.5}}\right] \quad (6).$$

Where

$$X = 1 - [\tan(\varphi)]^2[\tan(\delta)]^2 \quad (7).$$

The conversion from decimal degrees to radians is given by:

$$[Radians] = \frac{\pi}{180}\,[decimaldegrees] \quad (8).$$

The daylight hours, N, are given by:

$$N = \frac{24}{\pi}\omega_s \quad (9).$$

Where ω_s is the sunset hour angle in radians given by Equation 5 or 6.

Result and Discussion

We used the result of calculated solar radiation Rs as input for Angstrom formula to calculate the coefficients a and b. For Baghdad city Angstrom equation is:

Rs/Ra = 0.26633 + 0.47397 n/N

Where Ra is calculated from equation (2), N from equation (9)

The value a, b equal to 0.26633, 0.47397 respectively was very close to the value of FAO publications which equal to 0.25, 0.5 that will be used as a default value in calculating monthly average solar radiation by Angstrom formula. Figure (5) represent scatter plot of Rs/Ra and n/N for period (2004 – 2014) in Baghdad city. The values of r =0.98667 and R^2 = 0.973 represent high relation between Rs/Ra and n/N, also scattered data in diagram is very close to regression line, which means a high correlation between them.

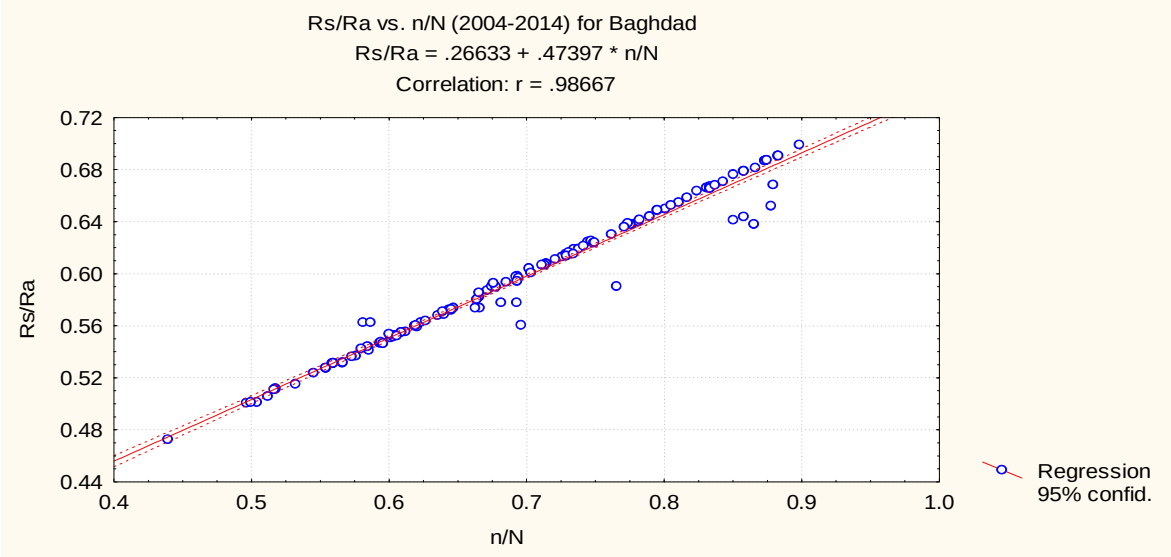

Fig (5) Correlation between Rs/Ra and n/N (2004-2014) in Baghdad city.

Figure (6) represent a comparison between a mean monthly solar radiation calculated by CROPWAT version 8.0 for the period (2004 – 2014) with monthly average daily data for solar radiation for the period (1983 – 2005) for NASA surface meteorology and solar energy for Baghdad city. We used monthly data for different period because we try to make a comparison between our calculated solar radiation with measured by surfaces station or by satellite, and with that published by NASA surface meteorology and solar energy, the two line overlapped reflect first that our calculation depending on five climate elements, second overlap between the two lines appear in April, July, and August, where the weather in April was affected by unstable weather system while in July, August were affected by dust phenomenon.

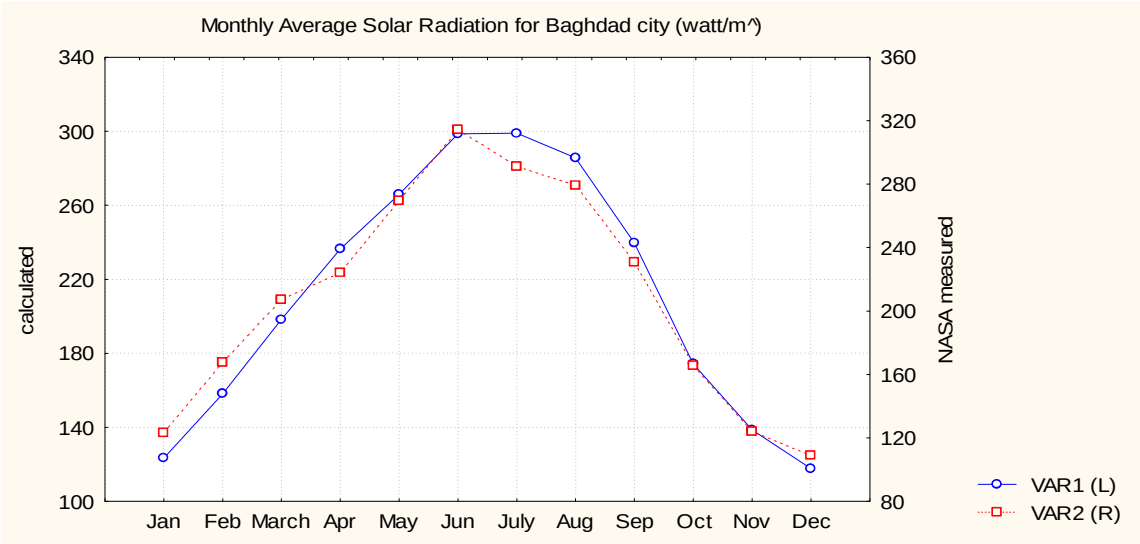

Fig (6) Comparison between calculate by CROPWAT and measured by NASA (monthly average daily of solar radiation) for Baghdad city.

For Basrah city we use calculated constant coefficient for Baghdad city to calculate monthly average daily solar radiation incident on horizontal surface Rs by Angstrom formula, Figure (7) represent the relation between Rs and Rm monthly average daily solar radiation measured by NASA for the period (1983 – 2005) with r =0.98554 and R^2 = 0.971 represent high relation between Rs and Rm. Data was very close to regression line which represent an evident of high correlation between them.

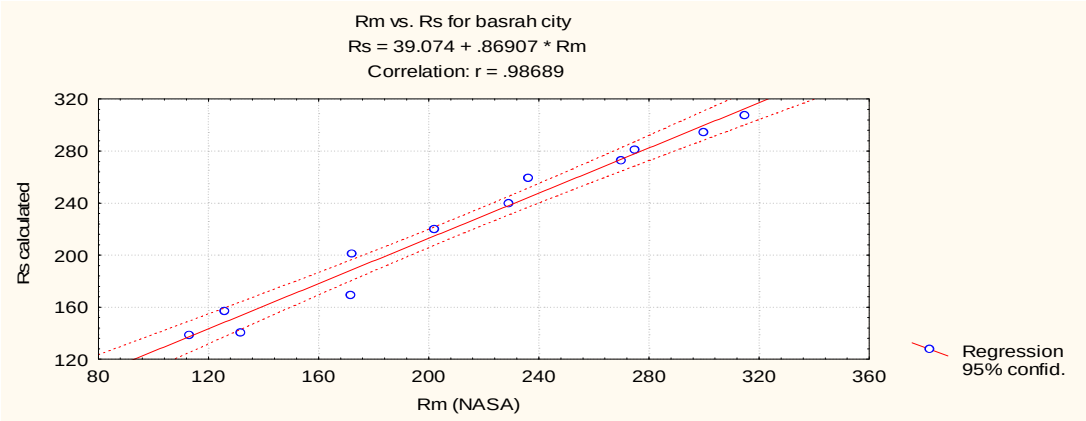

Fig (7) Correlation between global solar radiation calculated by Angstrom formula and global solar radiation measured by NASA.

Figure (8) represent a comparison between a mean monthly solar radiation calculated by Angstrom formula (2004 – 2014) with monthly average daily data for solar radiation (1983 – 2005) for NASA surface meteorology and solar energy, the two line overlapped in June reflecting that our calculation depend on five climate elements were correct. The two lines seems similar in the general shape, but theirs values interfere in January, February, March due to unstable weather.

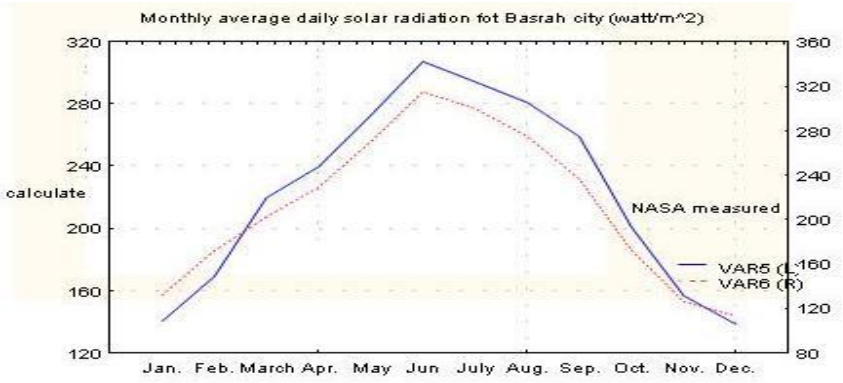

Fig (8) Comparison between calculated and measured monthly average daily of solar radiation for Basrah city.

In order to have a monthly distribution maps of solar radiation, the climate data are used in calculating solar radiation By CROPWAT version 8.0 for (2004 – 2014) for 22 climate stations in Iraq, these elements data are Maximum temperature, Minimum temperature, RH%, sunshine duration, wind speed.

Figures (9) to (12) represent samples of solar radiation maps for the years 2004 and 2013 for January and July only.

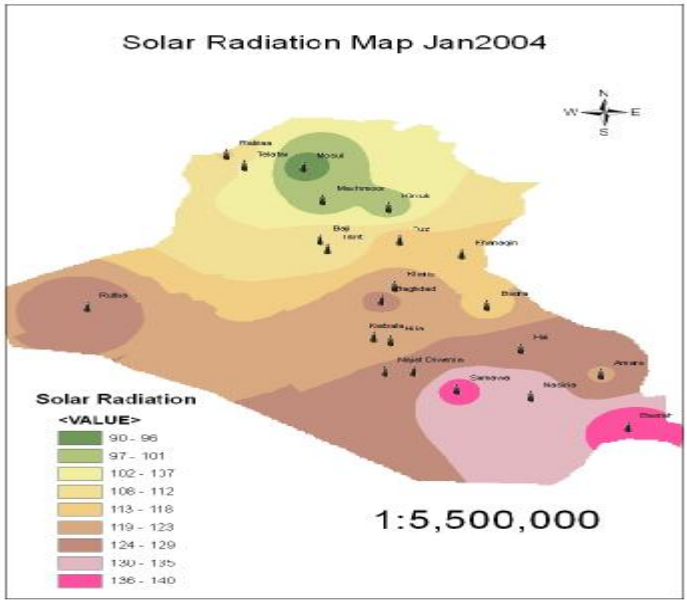

Figure (9): Solar Radiation map for January 2004.

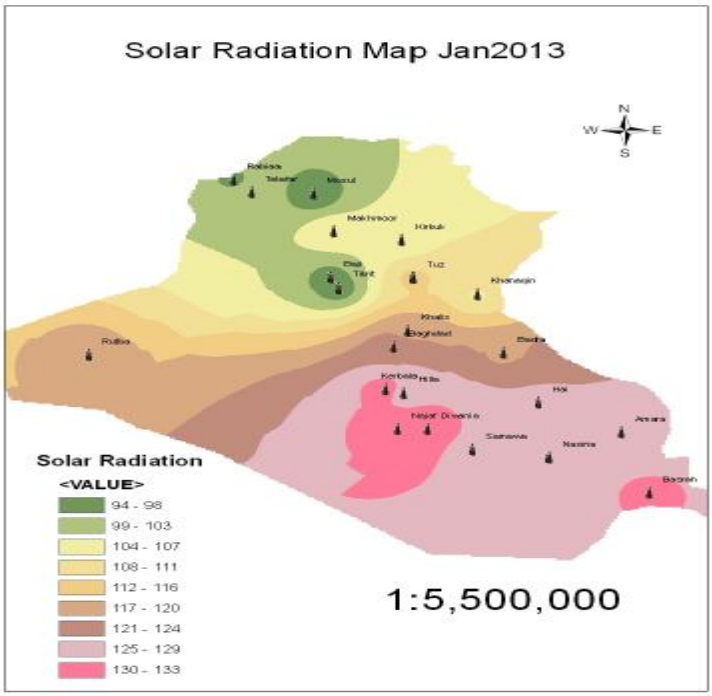

Figure (10): Solar Radiation map for January 2013.

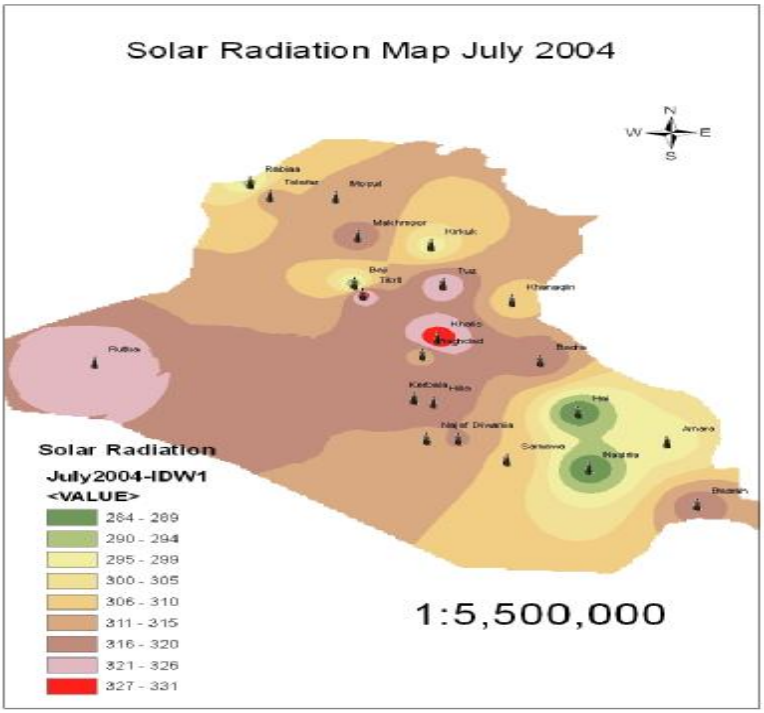

Figure (11): Solar Radiation map for July 2004.

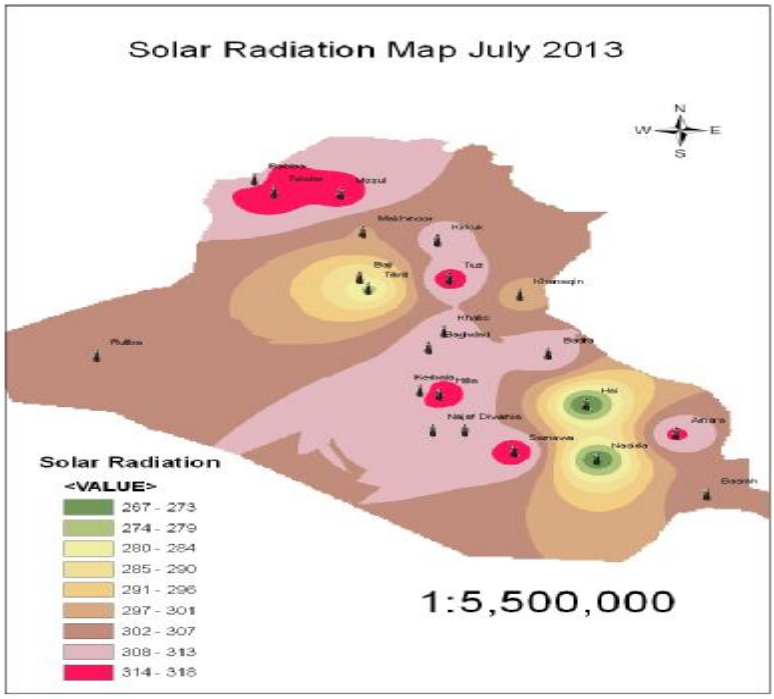

Figure (12): Solar Radiation map for July 2013.

The determination of global solar radiation at any site is vital for many scientific, engineering and environmental applications. In order to estimate daily solar radiation, a method of calculation based on daily sunshine duration was proposed to be applicable in Baghdad region, Iraq. In developing countries such as Iraq, due to absence or malfunction of measuring instruments, reliable solar radiation data is not available. In Iraq, Global Solar Radiation data on horizontal surface is recorded at only few stations. In the absence and scarcity of trustworthy solar radiation data, the need for an empirical model to predict and estimate global solar radiation seems inevitable. These models use climatological parameters of the location under study. Among all such parameters, sunshine hours are the most widely and commonly used. The models employing this common and important parameter are called sunshine-based models. Sunshine-based models use only bright sunshine hours as input parameter while others use additional climatological data together with bright sunshine hours. In some of the models geographical and seasonal parameters are also taken into account to reflect the latitudinal and seasonal variation of the air mass.

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Immunohistochemical study of TGF-β and TNF-α in treated mice infected with hydatid cysts

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Abstract

The present study was designed to estimate the chemical and immunological therapy of infected mice strain Balb /c with secondary hydatid cysts. Sixty white mice were immunized by Hydatid cyst fluid antigen (HCF Ag) and antioxidant and use of three chemical drugs in an attempt to treat secondary Hydatid cysts in mice which are Oxfendazole (OFZ) a concentration of 30mg/kg, (PZQ) Praziquantel a concentration of 40 mg/kg and albendazole (ABZ) Albendazole a concentration of 10 mg /kg of body weight, the drugs were given weekly for four months as a single dose or mixed. The results showed that the highest efficiency relative to the treatment of mice was in treatment with OFZ+PZQ (96.70%), while the treatment with a drug, OFZ, OFZ + ABZ and ABZ + PZQ therapeutic efficiency (93.75%, 82.50%) and (77.50%), respectively. There was variable values of expression of Transforming growth factor-β (TGF-β) and Tumor necrosis factor Alpha (TNF-α) by immunohistochemistry examination in the spleen of treated mice and control groups. In conclusion, the efficacy of OFZ + PZQ promising drugs in the immunochemical treatment by detection TGF-β and TNF-α in infected mice.

دراسة مناعية كيمونسيجية TGF-β و TNF- α في علاج الفئران البيضاء المخمجة بالاكياس العدرية

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الخلاصة

تم تصميم الدراسة الحالية لتقييم كفاءة معالجة داء الأكياس العدرية الثانوية في الفئران البيضاء Balb/c مناعيا وكيميائيا. استخدم لهذا الغرض ستون فارا تم تمنيعها بمستضد سائل الكيس العدري Hydatid Cyst Fluid Antigen (HCFAg) مع تجريع الفئران بمضادات الاكسدة، واستعمال ثلاثة عقاقير كيميائية في محاولة لعلاج داء الاكياس العدرية الثانوية في الفئران وهي الاوكسفيندازولOxfendazole (OFZ)، البرازكوينتل (PZQ) Praziquantel والألبيندازول (Albendazole(ABZ بتركيز30 ،40 ,10ملغم/كغم من وزن الجسم،على التوالي، والعقار يعطى اسبوعيا لمدة اربعة اشهر بشكل عقار مفرد او ممتحد وبنفس التراكيز اعلاه. اظهرت النتائج ان اعلى كفاءة نسبية للعلاج كانت في الفئران المعاملةOFZ+PZQ وبنسبة 96.70 %، بينما اظهرت المجاميع المعاملة بعقار OFZ, OFZ+ABZ و ABZ+PZQ كفاءة علاجية بنسبة 93.75 %، 82.50% و77.50% على التوالي. واظهرت نتائج المناعة الكيمونسجية Immunohistochemistry (I.H.C) ، وجود بروتين عامل تحويل النمو Transforming growth factor-β (TGF-β) و Tumor Necrosis Factor Alpha عامل التنخر الورمي

ألفا(TNF-α) في طحال الفئران المعالجة. نستنتج من الدراسة الحالية كفاءة عقار OFZ+PZQ كنوع من العلاجات المناعية الكيميائية الواعدة الاستخدام في علاج الاكياس العدرية.

Introduction

Cystic Echinococcosis or hydatidosis is one major a zoonotic parasitic infection of many mammalian species and public health problems in many countries like Iraq, it's caused by the larval stages of the tapeworm *Echinococcus granulosus* (1). Unlike the surgical removing of the cysts the researchers went to find many therapeutic modalities by stimulating the immune system using different antigens of hydatid cyst (2 , 3).

Resistance of the host to the parasite by cellular immune T cell (Th1) while susceptibility to infection depends on the secretions (cytokines) of Th2 (4), play an important role in regulating the response to cellular or humoral immunity during all the stages of infection. Transforming growth factor (TGF-β) is one of the dynamics to cellular regulatory released by lymphocytes, phagocytic cells and Dendritic cell and works on the growth, proliferation, cell differentiation, programmed cell death (Apoptosis), and in control the immune system, and reduces the toxic factors in the immune response and chronic inflammatory diseases (5) and urges the cystic fibrosis in many cases worms. The proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) in parasitic diseases, infectious diseases and acute infections, and there is a relationship between TNF-α and the number and extent of the damage in the affected tissue. (6).

The current study was an attempt to treated the hydatidosis in mice by immunization with antigens of Hydatid Cyst Fluid antigens (HCFAgs), with antioxidants to reduce the oxygenic free radicals and strengthen the immune system, while the drug was used from Benzimidazole derivatives is Oxfendazole individually or mixed with another drug such as Albendazole and Praziquntel as a chemical against infection. Mice experiment in this study using Immunohistochemical technique (IHC) staining to determine the expression of TGF- β and TNF-α in the spleen of mice.

Materials and Methods

1-Lab animals: sixty white males' mice strain Balb / c, aged 4-5 weeks and weighing 20 ± 5 gr, obtained from the Drug Control in Baghdad. Adapted and managed in optimal environmental conditions.

2-Preparations of antigen: Hydatid cysts of infected sheep were collected to prepare HCFAg according to (7), and select concentration (3.36 mg/ml) according to the method of (8), and isolated protoscolices (PSCs) estimated viability and their number by (9), a single dose challenge (2000PSCs±5).

3-preparation of drugs: Three drugs prepared according to (10).used in an attempt to treat hydatid cysts in mice are- :

-Oxfendazole (OFZ) a concentration of 30 mg/kg of body weight, equivalent to 0.04 mg / ml (11) were obtained on the property locally (Synanthic ®, Fort, Dodge, Mexico.

-Praziquantel (PZQ) a concentration of 40 mg/kg of body weight, equivalent to 0.06 mg / ml 1.

-Albendazole (ABZ) a concentration of 10 mg/kg of body weight, equivalent to 0.01 mg / ml.

Drugs given to mice groups in single dose or mixed, as follows- :

1-OFZ. 2- OFZ + ABZ. 3- OFZ + PZQ. 4-ABZ + PZQ.

4-Experimental designs: (n=60) mice were immunized at day 0 with 0.2 ml of HCFAg S/C after mixed with an equal volume of incomplete Freund adjuvant, after 21 days given booster dose consists of hydatid fluid 0.2 ml with an equal volume of complete Freund adjuvant, and with immunization the mice injected with 0.2 ml antioxidants (Pharmaton R, Switzerland) daily/orally for month.The challenge dose (2000 PSCs ± 5) I / P in day 30 of the first day of immunization and at the same time injected a group of positive control (15) mice the same initial dose of primates, and the negative control group injected (15) mice 0.2 ml sterile buffer.

The immunized group's mice administered 0.2 ml of the above drugs orally, one dose a week for four months. Four months post challenge dose sacrificed all the animals and examined their internal organs; form the number and sizes of hydatid cysts developed in mice after immunization, treatment and used the equation (12) to determine the relative efficiency of the treatment and the percentage of reduction the number and size of hydatid cysts.

$$\text{Efficacy of treatment \%} = \frac{\text{NO.of H.cyst in positive control group - NO.of H.cyst in treated group}}{\text{NO.of H.cyst in positive control group}} \times 100$$

4-Immunohistochemical (IHC) examination: to detect tumor necrosis factor (TNF-α) and transforming growth factor (TGF-β), according to the manufacturer's instructions (Envision Detection System, Rabbit, Mouse, DakoRral TM).

5-Statical analysis: Data were expressed as mean ± standard deviation (SD) and Analysis of Variance (ANOVA) test was used for differences between groups. Values $p< 0.05$ was regarded as statistically significant.

Results and Discussion

The relative efficiency of the treatment post autopsy showed the mice in control positive presence secondary hydatid cysts distributed irregularly, single or as transparent clustered and forms spherical, oval and irregular and the percentage of infection in which 100%, (Figure- 1) and the highest therapeutic efficiency in mice treated with OFZ + PZQ was (96.70%), while the treatment groups with OFZ, OFZ+ABZ and ABZ + PZQ therapeutic efficiency were (93.75%, 82.50% and 77.50%), respectively.

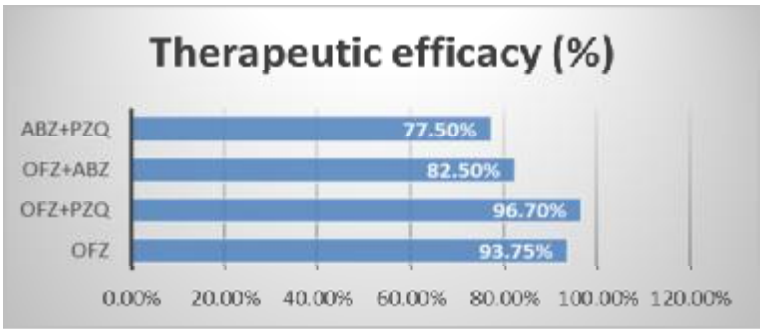


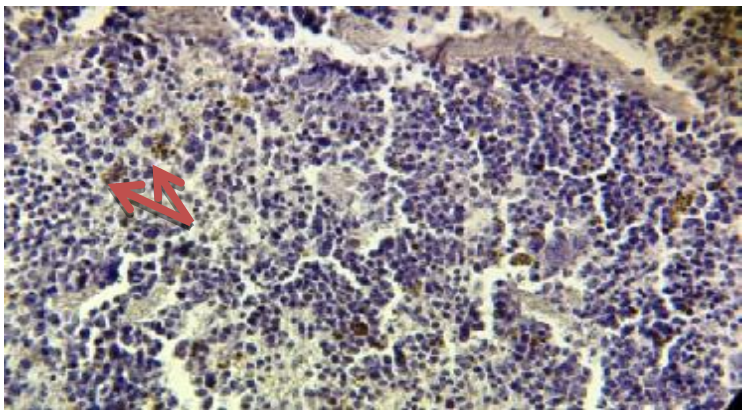
Figure (1): The percentage of therapeutic efficacy in treated mice groups for four months.

Table (1) showed significant differences between the animal groups post treatment with OFZ, OFZ + PZQ, OFZ + ABZ and ABZ + PZQ; the level of expression of TGF-β in a manner IHC in spleen (Figures-1,2,3,4) were (17.5 ± 8.3,12.3 ±3.22,8.8 ± 4.3and 10.1 ± 6.0), respectively, as compared with positive and negative control.

Table (1): expression of TGF-**β** in treated mice and control groups

Groups	Spleen	
OFZ	8.3±17.5 a**,b**	
OFZ+PZQ	3.22±12.3 a**,b**	
OFZ+ABZ	4.3±8.8 a*,b**	
ABZ+PZQ	6.0±10.1 a**,b**	
Negative control	2.1±3.2	
Positive control	35.6±15.2	

a; comparison with negative group. b; comparison with positive group.
* Significant differences on (p<0.05). **Significant differences on (p<0.001).



Figure(1): Histologic section of spleen in mouse treated with OFZ; showed the expression of TGF-**β** in lymphocytes (arrow) stained with (DAB stain, 200 X)

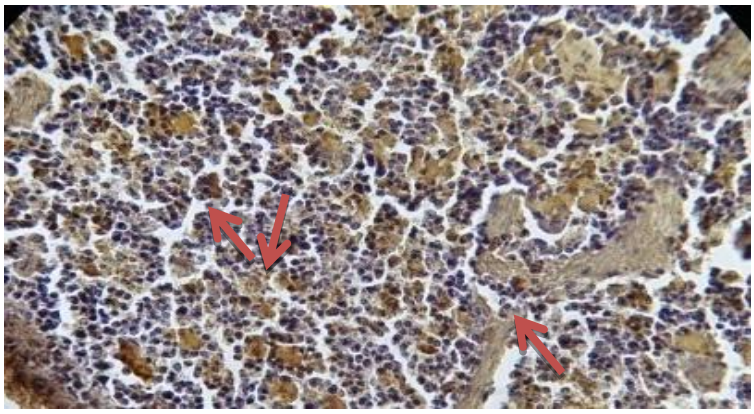


Figure (2): TGF-**β** expression in lymphocytes of spleen in mouse treated with OFZ+PZQ (arrow), stained with (DAB stain, 400 X**)**

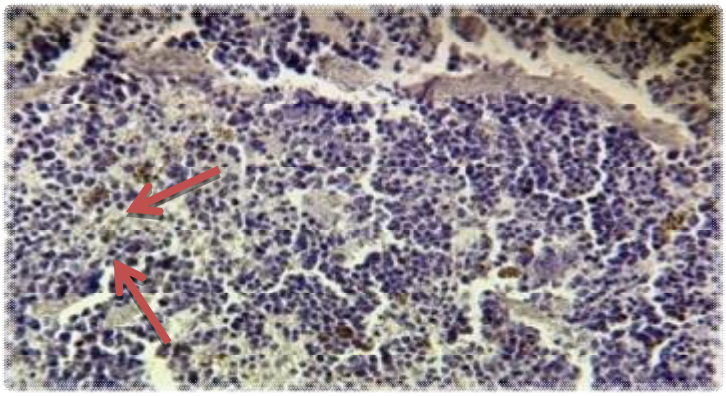


Figure (3): Histologic section of spleen mouse treated with OFZ+ABZ; showed the expression of TGF-**β** in lymphocytes (arrow) stained with (DAB stain, 400X**)**

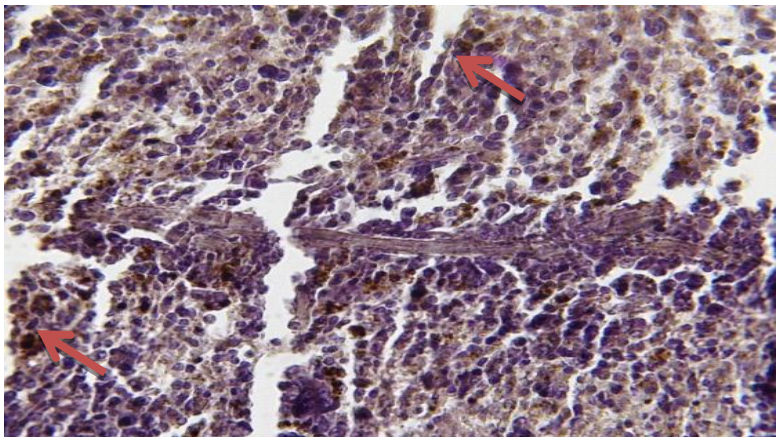


Figure (4): Histologic section of spleen in mouse treated with ABZ+PZQ; showed the expression of TGF-**β** in lymphocytes (arrow) stained with (DAB stain, 200X**)**

The effective immunization should stimulate the cellular and humoral immunity, also natural killer cells and other lymphocytes which are contributed to the reduction of injury (12), the current study involved the using of antioxidants and HCFAg in the immunization of mice to increase the response to cellular immunity. The OFZ effective medicine in the treatment of tapeworms, including Echinococcosis cystic disease in dogs, cats, and ruminants; sheep, goats, pigs and poultry, as well is absorbed by the intestines for about 50% of the administered dose and ultimately into the bloodstream and stays in plasma for more than 144 hours after taking a drug dose compared with ABZ, OFZ has a half-life longer than ABZ who stays for 60 hours after treatment (13). OFZ had ability to penetrated all three layers of the hydatid cyst; kill living tissue of the parasite, the destruction of internal Endocyst, calcification of the cyst, killed initial primates by 93.3% and decrease the number of hydatid cyst fertile (14 and 15).

OFZ+PZQ have influence on decrease the size of cyst and their numbers as explained from table-1 that showed high values as compared with OFZ and OFZ+ABZ by damaged the germinal membrane with integral tissue adventitia, and peel off the laminate layer of the cyst that was compatible with expression of TGF-β in red pulp of spleen and moderate expression in the white pulp, were phagocytic cells and lymphocytes (16).

TGF- β and IL-10 response to Echinococcosis, by cellular immune modulation, which induces particles and antigens parasite, the survival of the parasite inside the body and stability depends on the Shuffle efficient mechanisms that begin while the parasite developed toward the hydatid cyst, where attracts the host immune response towards the cytokines such as IL-4, IL-5, IL-10, inhibitory of the immune modified for immunity by TGF- β and IL-10, which stops the reaction, where the parasite in hydatid cyst to stimulate the immunity of fluid and antigens primates, which plays an important role in the developing of chronic disease (Ghasemi and Ghavami, 2008 (17).

Table (2) there were highly significant differences on ($p < 0.001$) of expression TNF-α in spleen (Figures- 5, 6, 7, 8) of treated mice (24.21, 32.11, 16.2 and 52.2), respectively, as compared with the control negative and positive groups. (7.4 and4.2).

Table-2: Expression of TNF-**α** in treated mice groups

Groups	Spleen	
OFZ	24.21±5.3a**,b**	
OFZ+PZQ	32.11±12.8a**,b**	
OFZ+ABZ	16.2±6.2a**,b**	
ABZ+PZQ	52.2±7.9a**,b**	
Negative control	7.4±4.1	
Positive control	4.2±2.3	

a; comparison with negative group. b; comparison with positive group.
*Significant differences on ($p<0.05$). ** Significant differences on ($p<0.001$).

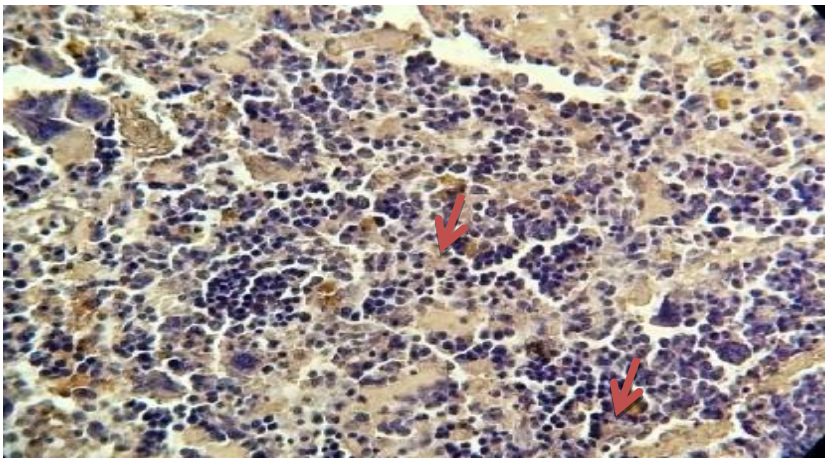


Figure (5): Histologic section of spleen in mouse treated with OFZ; showed the expression of TNF-**α** in lymphocytes (arrow) stained with (DAB stain, 400X**)**

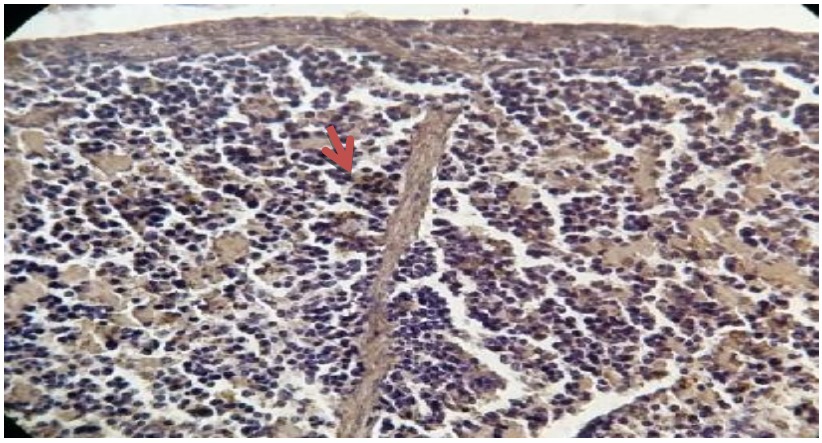


Figure (6): Histologic section of spleen in mouse treated with OFZ+PZQ; showed the expression of TNF-**α** in lymphocytes (arrow) stained with (DAB stain, 200X**)**

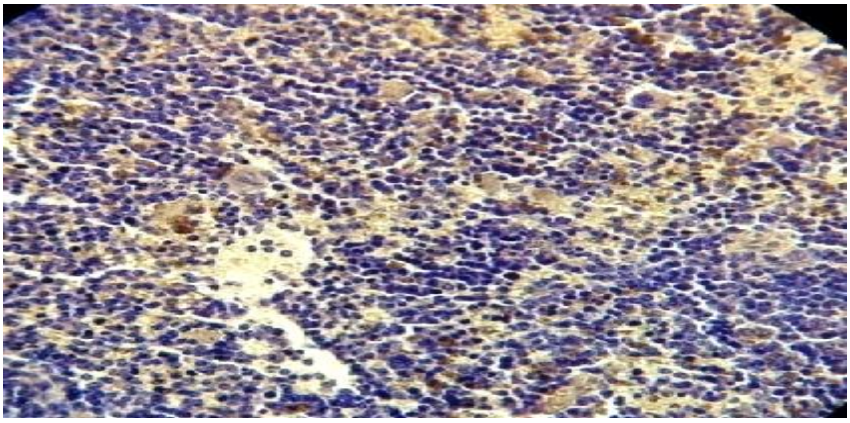


Figure (7): histologic section of spleen in mouse treated with OFZ+ABZ; showed the expression of TNF-**α** in lymphocytes stained with (DAB stain, 200X)

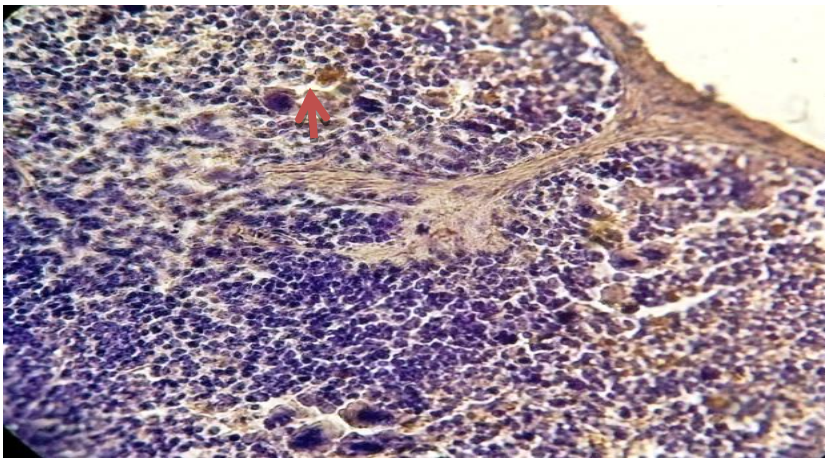


Figure (8): Histologic section of spleen in mouse treated with ABZ+PZQ; showed the expression of TNF-**α** in lymphocytes (arrow) stained with (DAB stain, 100X**)**

Tumor necrosis alpha TNF-α mainly produced by the active T cells and plays an important role in immune regulation, inhibited the growth of hydatid cysts in mice (18). The present study appeared there was a significant difference in expressions of TNF- α between the treated groups there was high in spleen of animals treated with ABZ+PZQ than in liver tissue as compared with other drugs used in present study. It seems that there was correlation between MHC in different treated groups of mice (19). TNF- α a cytokine of cellular-mediated infections occurring in the body by stimulating T-lymphocytes and the production of IL-2, the latter raises the efficiency of immune system and increases the viability of macrophages to attack the foreign bodies. TNF-α play a role in early stage of the larval growth that necessary for protection of the host, the highly values of TNF-α in the treated animal groups in current study were had influences; on killing and prevent the growth of parasite, increase the level of IL-2, to response Th1 that play a role against *E. granulosus*. Tissue sections of the spleen in the current study, showed high levels of TNF-α in mononuclear cells in red pulp while negative reactions in the white pulp that agreed with the study (20), and the role of TNF-α to eliminate parasitic infections. According to our information a available. The present results is the first in Iraq of detection TGF-β and TNF-α in infected mice ,OFZ+PZQ drug appeared the effective therapeutic role of immunogenic therapy in reduction the growth of hydatid cysts.

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The impact of overlapping addition of peppermint and fennel oil to broiler diet on some productive traits

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Abstract

The aim of this study was to investigate the effect of overlapping addition of peppermint and fennel oil at different rates on some productive qualities and performance of broiler chickens. Three hundred lohman broiler males were purchased from a local hatchery and reared for 42 days. Chicks were randomly divided into four treatments, each treatment assigned to 15 experimental units and reared in 15 identical pens (15 birds / $1m^2$). After a week from purchasing, birds were weighed and randomly assigned to five different diets contains different peppermint and fennel oil concentrations. Feed intake and body weight gain were recorded weekly, while feed conversion ratio was calculated. It was noted that body weight gain was improved at week four of the experimental period for birds fed rations supplemented with 0.2% peppermint and 0.1% fennel oil compared with birds fed the other diets. A significant difference ($p < 0.05$) was shown in feed conversion ratio for the birds fed T4 (0.1% of each kind of oils) was found in the fourth week of the experiment (1.44 gm), compared with birds fed control diet. Therefore, feeding broilers rations supplemented with different concentrations of peppermint and fennel oil significantly ($p<0.05$) increased weight gain and feed conversion ratio compared to birds fed the control diet, which indicate that the overlapping addition of peppermint and fennel oil to birds rations might be effective and improved their productive traits.

تأثير إضافة مزيج من زيت النعناع والشمر على بعض الصفات الإنتاجية في علائق فروج اللحم

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الخلاصة

استهدف البحث دراسة تأثير إضافة مزيج من زيت النعناع و الشمر بتراكيز مختلفة على الأداء الإنتاجي لفروج اللحم . واستخدم في الدراسة 300 طير لومان (ذكور) خلال فترة 42 يوم ، حيث قسمت الطيور الى اربع معاملات ، كل معاملة مكونه من 15 طير و تمت تربيتهم بحظائر مخصصة (15 طائر /متر مربع) . ووزعت بشكل عشوائي على خمس علائق غذائية تحتوي على نسب مختلفة من زيوت النعناع و الشمر . ولغايات حساب الزيادة الوزنية و كمية تناول الغذاء تم أخذ القياسات كل اسبوع ، كما تم حساب كفاءة التحويل الغذائي في نهاية التجربة . أظهرت نتائج الدراسة ان الزيادة الوزنية قد تحسنت في الاسبوع الرابع من التجربة للطيور التي استهلكت العليقة المحتوية على 0.2

% نعناع و 0.1 % شمر مقارنة مع المعاملات الأخرى . وبينت الدراسة وجود فروق معنوية في الاسبوع الرابع من التجربة ($p < 0.05$) في مـ±شر التحويل الغذائي للطيور التي غذيت على العليقة المحتوية على تركيز 0.1 % من نوعي الزيوت المستخدمة . وتوصلت الدراسة الى أن الطيور المغذاه على عليقة ذات تراكيز مختلفة من المزيج المستخدم في الدراسة قد عمل على تحسين الصفات الانتاجية لها .

Introduction

The steady growth in population in the recent years was one of the main causes of increased demand for food products in general and the increasing demand for white meat in particular. White meat considered affordable, available in the market, and accessible to different strata of society. This prompted the researchers to looking for ways to increase the efficiency of production and improving the quality of the product by using different feed additives. The use of feed additives as growth enhancers in animals rations go back to the forties of the last century, as the continues use and addition of industrial antibiotics to feed has led to the emergence of bacterial resistance against antibiotics in humans and this is why the EU countries stopped their use as feed additives in 2006 (1). Different types of additives are important to be used in broiler feed in order to produce high quality meat, reduce many diseases, and provide high feed conversion ratio. In general, feed additives increases growth rates in poultry through improving digestion processes, increasing absorption of nutrients from feed, and reduce the energy consumed by animal during the digestion processes (2).

Researchers started to choose several natural alternatives to feed additives such as herbal and medicinal plants. Most of these materials are from natural sources and it works toward improving the gut environment and strengthen the immune system through its anti-bacterial and fungi action (3). Some common plants used for medicinal purposes are mint, and fennel. The main aim of adding these plants oil to birds is to increased energy in the ration, and to maintain volatile compounds from being lost through feed processing (4, 5).

The study of Qusibati et al., (2) emphasized on controlling the production efficiency in broilers fed rations with different ratios (0.1% and 0.2%) of dried and grounded mint leaves and grounded red chili fruits. It has been found that the birds, which fed with chili (0.1%), showed an improvement in weight by 1.03% without significant differences, and by adding 0.2% it was increased live weight by 1.08% significantly, and increased feed conversion ratio of birds fed on red chili. As well as, the results showed that adding mint by 0.1% increased live weight by 1.02%.

Moreover, other studies using herbal plants showed how adding these plants might affect bird's performance. A study performed by Abbas (6) showed that the use of 3 g / kg feed from fenugreek seeds, parsley, and basil in the diets of broiler chickens during a period of forty two days improved final body weight and feed conversion ratio in favor of basil seeds compared to other plants. On the other hand, when comparing the use of essential oils of basil and thyme plants (600 mg oil / kg feed) with adding anti-bacterial compounds (500 mg / kg) feed conversion efficiency of diet was found to be greater when adding the herbal oils, and that referred to the catalytic effect of basil which improved broiler growth and performance (7).

Therefore, the goal of this study was to find the effect of overlapping addition of peppermint and fennel oil at different rates on some productive qualities and performance of broiler chickens of Lohman strain.

Materials and Methods

Experimental birds: One day old of three hundred lohman broiler males were obtained from a local hatchery and reared for 45 days during the period from 1 April to 15 June, 2015 in a controlled environmental poultry house at the research station of the Faculty of Agriculture at Jerash University. Chicks were divided into four groups, each group assigned to 15 experimental units and reared in 15 identical pens (15 birds / 1m^2). Each pen was provided with an adequate number of thermostatically controlled heaters and an electric fan for air circulation and distribution. A thermometer was used to monitor the temperature.

Management practices: Birds were offered *ad libitum* feed and water. Temperature was decreased gradually during the experimental period from 32 ºC at one day old to reach 22 ºC at the end of the experiment. After a week from purchasing the birds, they were weighed and randomly assigned to the different treatments.

Experimental rations: Five different experimental diets were used to investigate the effect of peppermint and fennel oil addition to broiler diet on some productive traits. At the first week of bird age, a commercial broiler starter diet was used for the five groups containing 3026 Kcal ME/kg and a 22.1% crude protein, after this week; one group were kept on the same diet for another 14 days and then fed a grower diet to from day 15 until day 28 of age, then a finisher diet from day 29 until the end of the experiment period which contains 3048 Kcal ME/kg and 18.2 % crude protein (Table 1). The other groups of birds were offered the same diets but with the addition of different amounts of peppermint and fennel oil.

Experimental setting and measurements: The aim of this project was to evaluate the impact of overlapping addition of peppermint and fennel oil to broiler diet on some productive traits. The experimental diets used in this experiment are shown in Table 1. The addition of peppermint and fennel oil was divided to different ratios and was added to the rations (each kilogram of oil was added to 1000 kg of the diet), the experimental diets used in this experiment are:

Treatment 1 (T1): Control diet, without any addition of oil.
Treatment 2 (T2): Adding 0.1% of peppermint oil with 0.1% of fennel oil.
Treatment 3 (T3): Adding 0.1% of peppermint oil with 0.2% of fennel oil.
Treatment 4 (T4): Adding 0.2% of peppermint oil with 0.1% of fennel oil.
Treatment 5 (T5): Adding 0.2% of peppermint oil with 0.2% of fennel oil.

Peppermint and fennel oil were supplemented to the different groups of birds as a percentage from the total feed offered (1 kg or 2 kg of the different oils on each 1000 kg of the diet). Birds were weighed from second week and repeated every week at the same time until the end of the experimental period. Feed intake and body weight gain were recorded weekly, while feed conversion ratio was calculated.

Table (1): Ingredients and calculated chemical composition for broiler diets.

Diet	0-14 days	15-28 days	29-42 days
Ingredients and composition			
Yellow corn	61.9	68.5	73.3
Soy-bean meal (44% CP)	35.5	28.8	24
Dicalcium-phosphate	2.0	2.0	2.0
Premix*	0.12	0.2	0.2
DL-methionine	0.1	0.1	0.1
Choline	0.1	0.1	0.1
Salt	0.3	0.3	0.3
Nutrient chemical composition **			
ME (kcal kg^{-1})	3014	3058	3115
Crude Protein	22.2	19.9	18.1
ME/CP	135.7	153.6	172
Calcium (%)	1.07	0.95	0.94
Phosphorus (%)	0.76	0.74	0.73
Lysine (%)	1.26	1.09	1.02
Methionin (%)	0.49	0.48	0.46
Methionin and cystine (%)	0.89	0.75	0.69
Sustain (%)	0.40	0.39	0.34

*: 1 kg of premix contains: 12000000 IU vitamin A, 2500000 IU vitamin D3, 10000 mg vitamin E, 2000 mg vitamin K3, 1000 mg Vitamin B1, 5000 mg vitamin B2, 10 mg vitamin B12, 30000 mg Nicotinic acid, 3000 mg Ca-pantothenate, 1000 mg folic acid, 50 mg biotin, 40000 mg Fe, 5000 mg CU, 60000 mg Mn, 100 mg I, 60000 mg Zn, 150 mg Co, 10000 mg B.H.T

**: The chemical composition of nutrients for each feed ingredient was calculated using NRC tables (8).

Chemical composition of oils: Fennel oil contains many volatile oils such as Anethol and Fenchonp. It also contains Esthagole as well as vitamins A, C, and some menials such as phosphorus, calcium, iron, and potassium. Peppermint oil consists of menthol, methyl acetate, methofuran, and limonene.

Statistical analysis: Data were analyzed using PROC MIXED of SAS (9) with bird considered the experimental unit for the treatment × week effects. The mean separation was performed using an F-protected t-test. Treatment means are reported as least square means by using Duncan test with a probability of $P \leq 0.05$.

Results and Discussion

Bird body weight: Table (2) shows the effect of adding peppermint and fennel oil to broiler diets on body weight through the experimental period. Body weight for birds consumed T4 diet (Adding 0.2% of peppermint oil with 0.1% of fennel oil) was significant ($p < 0.05$) when compared to birds consumed other treatments in week 2, 4 and 6 of the experimental period.

Table (2): Effect of adding peppermint and fennel oil to broiler diets on body weight (gram) during the experimental period.

Treatments	Body weight means (gm/bird/week)		
	Week 2	Week 4	Week 6
T1	358.24ab ±27.17	1250.25b ±25.21	2102.15c ±18.13
T2	351.03ab ±36.82	1304.47ab ±18.20	2228.27ab ±31.57
T3	361.31ab ±27.38	1331.18ab ±23.11	2254.28ab ±45.34
T4	396.29a ±14.21	1392.79a ±14.78	2309.20a ±29.43
T5	342.55b ±18.24	1301.89ab ±36.38	2173.57bc ±34.32
Significances	*	*	*

Means with different superscripts in the same column are significantly different $p<0.05$

Weekly body weight gain: Table (3) shows the effect of adding peppermint and fennel oil to broiler diets on body weight gain. There was a significant differences ($p< 0.05$) between treatments during the fourth week of the experiment in weight gain for the benefit of T4 (1007.04 gm) compared to other treatment T1 (901.01 gm). There were no significant differences in the second and sixth week in the rate of weight gain despite the existence of morphological differences, but this was not significant at $p<0.05$. When the cumulative gain of body weight increase was calculated during the experimental period, results showed there significant differences ($p<0.05$) where treatment T4 birds had greater weight (2293.40 gm) than birds consumed the control diet (2085.35 gm) and T5 (2155.18 gm) but not significantly differ ($p<0.05$) with birds consumed diets T2 and T3.

Average weekly feed intake: Table (4) indicates the presence of significant differences between treatments at the age of six weeks in broilers feed intake, where the superiority at the 5% level ($p<0.05$) for the benefit of T2 and T3 (1896.03 and 1879.86 gm respectively), compared to the control T1 (1662.17 gm). There were no significant differences ($p> 0.05$) in feed intake for birds consumed diets with T4 and T5, and when the cumulative feed intake was calculated, a significant differences between treatments was found and the cumulative feed intake was greater for birds consumed diets treated with T3 and T4.

Table (3): Effect of adding peppermint and fennel oil to broiler diets on weekly body weight gain (gram) during the experimental period.

Treatments	Body weight gain (gm/bird/week)			
	Week 2	Week 4	Week 6	Cumulative body weight gain
T1	321.32 ±25.22	901.01b ±13.46	859.82 ±24.04	2084.35c ±21.29
T2	316.56 ±14.20	980.22a ±12.1	931.49 ±25.45	2228.22ab ±38.27
T3	326.09 ±10.34	979.60a ±17.43	931.84 ±23.12	2237.54ab ±45.01
T4	362.22 ±5.13	1007.04a ±17.01	924.67 ±21.35	2293.40a ±28.21
T5	306.45 ±15.26	969.39a ±23.90	879.34 ±12.44	2155.18bc ±45.37
Significances	N.S	*	N.S	*

Means with different superscripts in the same column are significantly different p<0.05
N.S there is no significant differences between treatments.

Table (4): Effect of adding peppermint and fennel oil to broiler diets on feed intake (gram) during the experimental period.

Treatments	Feed intake (gm/bird/week)			
	Week 2	Week 4	Week 6	Cumulative feed intake
T1	321.34 ±12.48	1405.53 ±35.46	1662.17b ±39.30	3389.04b ±28.37
T2	333.74 ±21.51	1412.83 ±26.32	1896.03a ±18.49	3642.60ab ±35.11
T3	348.60 ±24.01	1467.03 ±16.21	1879.86a ±13.37	3695.49a ±42.17
T4	381.27 ±26.76	1511.93 ±12.34	1839.76ab ±35.21	3732.96a ±32.43
T5	329.80 ±26.40	1472.06 ±21.23	1788.13ab ±39.44	3589.99ab ±37.54
Significances	N.S	N.S	*	*

Means with different superscripts in the same column are significantly different p<0.05
N.S there is no significant differences between treatments.

Feed conversion ratio: Table (5) shows that best feed conversion ratio was found in birds consumed diet treated with T2 in the fourth week of the experiment(1.44 gm) when compared to T1 (1.56 gm) accompanied by the presence of significant differences (p< 0.05). There were no significant differences (p<0.05) in the second and sixth week

feed conversion ratio despite the existence of morphological differences. On the other hand, the cumulative feed conversion ratio during the period of the experiment showed no significant differences ($p<0.05$) between the different treatments. However, it was the best conversion ratio for birds at T4 (1.62 gm) and least with birds at T5 (1.66 gm). Growth rate: Table (6) shows the absence of significant differences ($p> 0.05$) between treatments for birds growth rate during the second and the sixth week, while the results showed a significant difference between treatments during the fourth where the superiority at the 5% level for the benefit of T2 (119.66 gm) compared to the T1 (113.33 gm) which recorded the lowest growth rate.

Through the results obtained from tables (2 and 3), which showed that the best treatment was T4 for adding 0.2% of peppermint oil and 0.1% of the fennel oil, it has been proven to improve the growth and increased live weight, and increase the use of feed contains such oils. The results showed a significant superiority for the treatment T4 when compared to other treatments. A study conducted by Ankaril et al., (10) found an improvement in live weight when mint was added to the ration. Moreover, results were also consistent with Galib and al-Kassie study (11), where they noticed an improvement in growth when using mint. Our result differs with Ashayerizadh et al., (12) who did not find any difference in growth or live weight when mint was added to the rations in their experiment. The effect of mint on increasing animal growth rate could be explained by its properties; mint found to act as a resistant to both E. coli and Anoukadat and improve animal appetite and feed intake (13, 14).

Table (5): Effect of adding peppermint and fennel oil to broiler diets on feed conversion ratio (gram) during the experimental period.

Treatments	Feed conversion ratio (gm/bird/week)			
	Week 2	Week 4	Week 6	Cumulative feed conversion
T1	1.00 ±0.01	1.56a ±0.03	1.93 ±0.01	1.62 ±0.02
T2	1.05 ±0.08	1.44b ±0.02	2.03 ±0.08	1.63 ±0.04
T3	1.06 ±0.02	1.49ab ±0.03	2.02 ±0.02	1.65 ±0.05
T4	1.05 ±0.09	1.50ab ±0.05	1.99 0.07	1.62 ±0.04
T5	1.07 ±0.03	1.52ab ±0.04	2.03 ±0.09	1.66 ±0.03
Significances	N.S	*	N.S	N.S

Means with different superscripts in the same column are significantly different $p<0.05$
N.S there is no significant differences between treatments.

Table (6): Effect of adding peppermint and fennel oil to broiler diets on growth rate (gram) during the experimental period.

Treatments	Growth rate (gm/bird/week)		
	Week 2	Week 4	Week 6
T1	157.28 ±5.22	113.33b ±3.21	51.94 ±4.28
T2	163.47 ±3.20	119.66a ±6.10	53.98 ±5.68
T3	164.93 ±2.84	116.44ab ±9.34	52.84 ±4.04
T4	168.71 ±1.25	113.69ab ±2.01	50.42 ±1.34
T5	161.99 ±2.96	114.25ab ±4.04	51.18 ±1.44
Significances	N.S	*	N.S

Means with different superscripts in the same column are significantly different $p<0.05$
N.S there is no significant differences between treatments.

Fennel oil also found to increase appetite because it contains Fenchone oil and other compounds that increases feed intake. Peppermint have several active substances such as Trepenoids which had a positive role in reducing the microbiology harmful and affected the intestinal flora, which improved the physical conditions microbial community on one hand, and on the other hand, these active substances worked to increase appetite in birds (15).

Tables (4 and 5) showed that when using the mixture of mint oil, fennel with various proportions, it worked on increasing concentrations of Flavenoids and Terpenoids and Fenchone compounds in the mixture as well as the presence of active substances individually in the two plants oil which may have increased appetite and palatability of feed by the birds, and reflected positively on the improvement of the rate of live body weight and increase the weight. The fact that the cumulative feed conversion ratio measures the relationship between feed consumption and increase the weight, it was noticed an improvement for some experimental treatments despite not arriving at the significant level. For the combination treatment T5 in broiler ration has become a negative overlap and inversely the bird to accept feed and the level of his appetite, which has reduced feed consumption when compared to other treatments and the impact of recent treatment in the cumulative feed conversion ratio.

Oil mixtures that were added to the different treatments have shown an improvement in the value of the feed conversion ratio in the fourth week, and this is consistent with a study that was performed by Kussaibati et al. (16), which showed improvement in feed conversion ratio when using mint as additive to broilers diet. Another studies supported our findings, it was found that the use of mint improves feed intake efficiency and feed conversion ratio (11, 12). Feed conversion ratio improvement

may be attributed to the increase in metabolic activation process and improve the level of absorption of intestinal villi in birds consumed these oils leading to lift take advantage of the food rate.

Conclusion

Experimental birds fed diets supplemented with 0.2% of peppermint oil with 0.1% of fennel oil 0.5% gained significantly ($p<0.05$) higher weight and had an increased feed conversion ratio compared to birds fed other treatments which indicate that a mixture of peppermint and fennel oil might be effective and improved growth performance.

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Evaluation of the immunogenicity of biofilm produced by *Escherichia coli* isolated from animals infected with diarrhea

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Abstract

This study was conducted to detect the ability of *E. coli* isolated from diarrhea to produce biofilm and protection against diseases cause by these bacteria compared with whole cell sonicated antigen. One hundred two fecal samples (52 fecal samples from cows, calves and 50 fecal samples from sheep,goat) were collected from College of veterinary medicine-university of Baghdad, College of Agriculture-university of Baghdad, Dora zone and Abu-Ghraib zone. Samples were cultured on MacConkey and Eosin Methylene Blue agar and after purification of cultured bacteria, biochemical tests, API 20 E System and RapID ™ ONE System kit were done. Results showed that 91 out of 102 fecal samples have the characteristics belong to *E. coli,* the ability of these isolates to produce biofilm were detected and the results showed that 38 out of 49 *E. coli* isolates from fecal samples of cow produce biofilm (77.55%) and 39 out of 42 *E. coli* isolates from fecal samples of sheep produce biofilm (92.85%) with different thickness ranged between (0.2-2)mm, while 11 isolates from 49 fecal samples of cow and calves and 39 isolates from 42 fecal samples of sheep had not produce biofilm. The results showed that protein concentration of biofilm was 92 mg/ml for one isolate and 70 mg/ ml for the whole sonicated antigen of the bacteria that produce biofilm. Three types of bacterial antigens were prepared as follow; Whole cell sonicated antigen (WCA) of biofilm producer *E. coli,* biofilm extract antigen (biA) with protein concentration 3.5 mg/ml and biofilm extract antigen (biA) with protein concentration 14 mg/ml, then these antigens were injected in 50 healthy White BALB mice. Results showed that survival time of animals in the immunized group with biofilm antigen of high protein concentration (14 mg/ml) was longer (652.8 hrs) than animals immunized with whole sonicated antigen of protein concentration 3.5mg/ml antigen (378hrs) and then the group with biofilm antigen of protein concentration 3.5mg/ml (513.6 hrs), heavy bacterial isolation were recorded in the internal organs of the immunized infected animals at 12-48hrs post infection, while moderate bacterial isolation at day 30 post infection. Histopathological examination showed thrombus formation in the lung and kidney, congestion of the blood vessels in the heart and lung, death in animals during 12, 2hrs .Immunized animals showed variable degrees of mononuclear cell infiltration, the results showed that all animals of the control positive group, five animals of group injected with(WCA) produce biofilm ,three animals of the group injected with

(biA) 3.5mg/ml and one animal of group injected with (biA) 14mg/ml was died during 12-48 hours post infection, vacuolar degeneration in the epithelial cell and inflammatory cells infiltration in the lung and kidney of animals died at 12-24-48hrs post infection, granulomatous lesions were seen in most internal organs of animals in groups 2, 3 and 4 at day 30 post challenge, while mild inflammatory reaction was recorded in most internal organs of group 4 at day 30 post challenge.

تقييم القابلية المناعية على انتاج الغشاء الحيوي المتكون من بكتريا الاشيريكيا القولونية المعزولة من الحيوانات المصابة بالإسهال

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الخُلاصة

تم جمع 102 عينة براز (52 عينة براز من الابقار والعجول و 50 عينة براز من الاغنام والماعز) من عدة مواقع شملت كلية الطب البيطري- جامعة بغداد، كلية الزراعة- جامعة بغداد ، الدورة وابو غريب زرعت جميع هذه العينات على وسط الماكونكي ووسط صبغة المثيل الزرقاء وبعد تنقية البكتريا اجريت الاختبارات الزرعية والمجهرية والكيموحيوية و اختبار ال (Api 20 E system) اضافة لاختبار (RapID ™ ONE System) . أظهرت النتائج ان91 عزلة من مجموع 102 التي نمت على وسط صبغة المثيل الزرقاء تحمل صفات البكتريا *E. coli* . أظهرت النتائج أن 91 عزلة من مجموع 102 من عينات البراز كانت تحمل صفات جرثومة ال *E.coli* ، تم الكشف عن قابلية هذه العزلات لانتاج الغشاء الحيوي اذ اظهرت النتائج ان 38 عزلة من اصل 49 عزلة من عينات البراز للابقار كانت منتجة للغشاء الحيوي و بنسبة (77.55%) و39 عزلة من مجموع 42 عزلة من عينات البراز للاغنام كانت منتجة للغشاء الحيوي و بنسبة (92.85%) وقد تراوح سمك الغشاء الحيوي المنتج لجميع هذه العزلات بين(2 -0.2) ملم بينما كانت11 عزلة من عينات البراز للابقار من مجموع 49 و3 عزلات من عينات البراز للاغنام من مجموع 42 غير منتجة للغشاء الحيوي. قُدر تركيز البروتين لمستخلص الغشاء الحيوي بواسطة اختبار البايوريت وقد بلغ 92 ملغم/مل لاحدى العزلات المختبرة و 70 ملغم/مل لمستخلص البكتريا المتكسرة المنتجة للغشاء الحيوي . شملت الدراسة تقييم دور الغشاء الحيوي كمستضد ، لذلك حضرت ثلاثة أنواع من المستضدات :المستضد المتكسر الكلي للجرثومة التي تنتج غشاء حيوي ،مستضد مستخلص الغشاء الحيوي ذو تركيز البروتين 3.5 ملغم/مل و مستضد مستخلص الغشاء الحيوي ذو تركيز البروتين 14 ملغم /مل ثم حقنت هذه المستضدات كل على انفراد في الفئران المختبرية البيضاء وبواقع (50) فأره. أظهرت النتائج أن مدة بقاء الحيوانات في المجموعة الممنعة بمستضد مستخلص الغشاء الحيوي بتركيز (14 ملغم/مل) كانت الأطول اذ بلغت 652.8 ساعة مقارنة بالحيوانات الممنعة بالمستضد المتكسر الكلي للجرثومة اذ بلغت 378ساعة بينما بلغت مدة بقاء الحيوانات في المجموعة الممنعة بمستضد مستخلص الغشاء الحيوي بتركيز 3.5 ملغم/مل 513.6ساعة كما سجل عزل بكتيري كثيف للاعضاء الداخلية في المدة (12-48) ساعة بعد الاصابة للحيوانات الممنعة بينما ظهر عزل متوسط في اليوم الثلاثين بعد الاصابة

Introduction

E .coli is a normal inhabitant of the intestines of most animals and humans. Some *E. coli* strains can cause a wide variety of intestinal and extra –intestinal diseases , such as diarrhea ,urinary tract infections , septicemia , mastitis and neonatal meningitis (1). The formation of bacterial biofilms of *E.coli* in a host in general seems to be based on

current evidence to a large extent an intra-cellular event (2). The diseases caused by a particular strain of *E. coli* depend on distribution and expression of many virulence determinants such as biofilm formation, adhesion ,production of haemolysin, enterotoxin, shiga toxin ,endotoxin and capsules formation (3).

Microbial biofilms were extremely complex microbial ecosystems consisting of microorganisms attached to a surface and embedded in an organic polymer matrix of microbial origin , As well as microbial components , non – cellular materials such as mineral crystals , corrosion particles, clay or silt particles , or blood components , may also be found in the biofilm matrix , so a biofilm has to be kept general and thus may be redefined as " microbial cells immobilized in a matrix of extra cellular polymers acting as an independent functioning ecosystem, homeostatically regulated" (4).

Diarrheal diseases were major problem in third world countries which are responsible for death of millions of people and animals each year, diarrhea is an alteration in normal bowel movement and it is characterized by an increase in the water content, volume, or frequency and decrease of dry matter of feces (5, 6, 7). It can be either acute or chronic (8).

Diarrhea accounts of 46% of calves and lambs mortality (9).the most common causes of acute diarrhea are bacterial and viral infections (10, 11).Infections with *E. coli* being one of the major causative agents (12).

Materials and Methods

MacConkey and Eosin Methylene Blue agar were used for growth and isolation of *E.coli* from fecal samples were collected from (sheep , goats , cows and calves) suffering from diarrhea .Morphological , cultural and biochemical tests in addition to API20 E system and RapID ™ ONE System were used for the diagnosis of isolates .

Bacterial Virulence test: After all confirmatory tests were done to identify the bacteria, suspension of bacterial isolate that produced biofilm with the highest thickness was used in preparation of challenge dose which was made by harvesting a cultured plate and 0.3 ml was injected in 5 mice to examine its virulence.

Preparation of challenge dose: The preparation of the bacterial suspension and the counting were made using McFarland's tubes according to procedure described by (13). This bacterial suspension containing 1.5 $\times 10^8$ cfu/ml of *E.coli* was injected intra peritoneal (I/P) by insulin syringe.

Experimental design: Fifty mice were divided equally into five groups, ten mice in each :

Group1:10 mice as positive control.

Group2:10 mice immunized with 0.25 ml (WCA) from *E.coli* produce biofilm S/C with protein concentration (3.5 mg\ml) at day zero as primary dose followed by booster dose after 15 days.

Group3:10 mice immunized with 0.25 ml (BiA) S/C with protein concentration (3.5 mg /ml) as a primary dose followed by booster dose after 15 days.

Group 4:10 mice immunize with0.25 ml (Bi A) S/C with protein concentration (14 mg/ ml) as a primary dose followed by booster dose after 15 days.

Group 5: 10 mice (negative control) were injected with 0.25 ml of PBS S/C and repeated after 15 days.

After 30 days the first four groups were challenged with 0.5 ml(1.5×10^8 CFU/ml) of *E.coli* I/P and group five were injected with PBS I/P, animals which were died after 12 , 24, 48 and 72 hours were examined for bacterial isolation. After 30 days the remained animals were sacrificed for its bacterial count and histological study of kidney, heart and lung.

Results and Discussion

This study showed that all non-immunized infected animals were died during 12,24 hours particularly at 24 hours post infection (6 animal died) with mean survival time 19.2 hours , also heavy bacterial isolation was obtained from examined organs depending on number of colony grown on EMB agar was done as showed in Table(1).

One animal of group immunized with whole cell sonicated antigen were died at 12hrs post infection ,one animal died at 24hrs and three died at 48hrs post infection with survival time 378hrs. While immunized animal with biofilm antigen with protein concentration 3.5mg/ml, two animal died at 24hrs and one animal died at 48hrs post infection with survival time 513.6hrs and heavy bacterial isolation from internal organ of animal of these groups that died during 24-48hrs with moderate bacterial isolation at day 30 post infection Table (2), however one animal of immunized group with biofilm of high protein concentration (14 mg/ml) were died at 48hrs with moderate bacterial isolation, the survival time of this group is 652.8hrs.

These results indicated that *E. coli* isolates in this study induced septic shock in non-immunized infected animal and most immunized animal with sonicated antigen of biofilm producing strain, while immunized animal with biofilm with high concentration provide immune response that activate the peritoneal macrophage and destroyed most of inoculated bacteria at site of injection in lag phase of growth.

Table (1): Bacterial isolation (on EMB) from the internal organs of the control positive and immunized mice infected with virulent *E. coli* at 12-48hrs post challenge

	Hours		
Groups	12hrs	24hrs	48hrs
Group 1	+++	+++	0
Group2	+++	+++	++
Group 3	0	+++	++
Group 4	0	0	++

+++ heavy (over than 11 colonies), ++ moderate (6-10) colony

Table (2) :Bacterial isolation (on EMB agar) from the internal organs of the immunized mice infected with virulent *E. coli* at 30 days post challenge

At 30 days post challenge				
Group 2	8×10	6×10^2	2×10^3	1×10^4
Group 3	5×10	3×10^2	1×10^3	0
Group4	3×10	1×10^2	0	0

Table (3):Survival time of mice

Hours / Groups	12hrs	24hrs	48hrs	72hrs	720hrs		Total hrs
Group 1 (positive control) 10 mice	4	6	0	0	0	192/10	19.2
	48	144	0	0	0		
Group 2 (WCA) produce biofilm 3.5 mg/ml 10 mice	1	1	3	0	5	3780/10	378
	12	24	144	0	3600		
Group 3 (bi A) 3.5mg/ml 10 mice	0	2	1	0	7	5136/10	513.6
	0	48	48	0	5040		
Group 4 (bi A) 14mg/ml 10 mice	0	0	1	0	9	6528/10	652.8
	0	0	48	0	6480		

Results of Histopathological examination:

At 12hours post-infection:

Control positive group (Group 1):

Lung: The histopathological section in the lung showed neutrophils infiltration in the pleura with fibrin networks and inflammatory cells in alveolar spaces, thrombus formation in the blood vessels and increased thickness of interalveolar septa were the main lesions in the lung (Fig:1)

Kidney: The microscopic section of the kidney Showed congested blood vessels with neutrophils in their lumen (Fig: 2).

Heart: The lesions in the heart expressed congested blood vessels between cardiac muscle fiber (Fig: 3) .

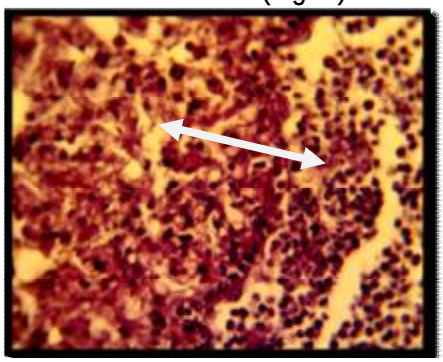


Figure (1): Histopathological section in the lung of animal at 12 hrs. post-infection shows neutrophils infiltration in the pleura with fibrin networks and inflammatory cells in alveolar spaces (H&E stain 40X)

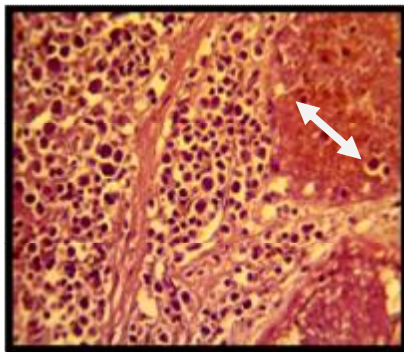


Figure (2): Histopathological section in the kidney of animal at 12 hrs. post- infection shows congested blood vessels with neutrophils in their lumen (H&E stain 40X)

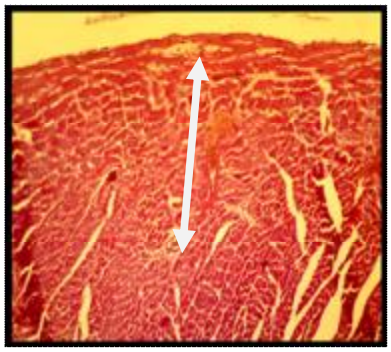


Figure (3): Histopathological section in the kidney of animal at 12 hrs. post-infection shows moderate congested blood vessels between mascular layer (H&Estain40X)

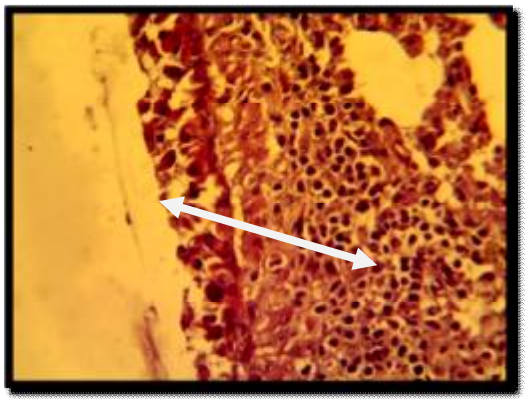


Figure (4): Histopathological section in the lung of immunized animal with whole sonicated Ags of biofilm producing strain with protein concentration 3.5mg\ml at 12 hrs post-infection shows vacuolar degeneration in the epithelial cells with mononuclear cells infiltration in the wall of bronchi (H&E stain 40X) .

24 hours post – infection:

Control positive group (group 1):

Lung: The histopathological section in the lung expressed thrombus formation with neutrophils infiltration in the alveolar spaces (Figure 5), in other section. Neutrophils in dilated congested blood vessels were seen in addition in the wall of alveoli (Figure 6).

Kidney: The main lesions in the kidney consisting from neutrophils in dilated blood vessels and vacuolar degeneration and sloughing of epithelial cells of renal tubules (Figure 7) in addition to thrombus in the blood vessels (Figure 8) .

Heart: The histopathological section in the heart showed congested blood vessels with proteinous material in dilated blood vessels (Figure 9).

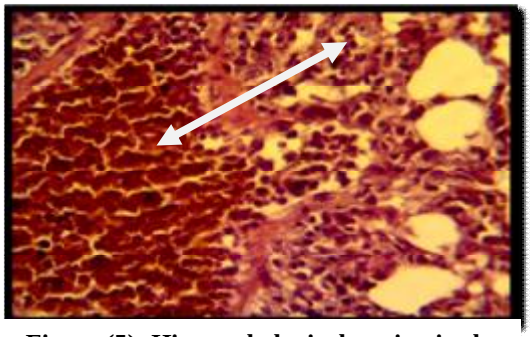


Figure (5): Histopathological section in the lung of control positive group at 24 hrs. post- infection shows thrombus formation with neutrophils infiltration in the aleveolar spaces (H&Estain40X)

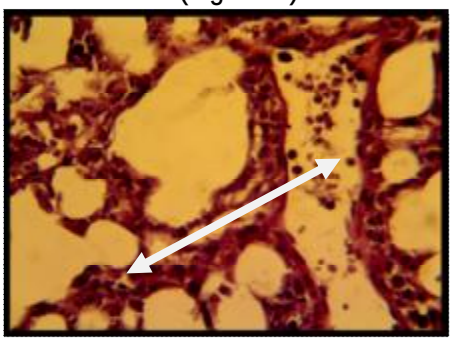
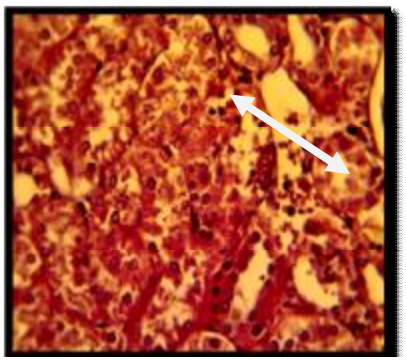
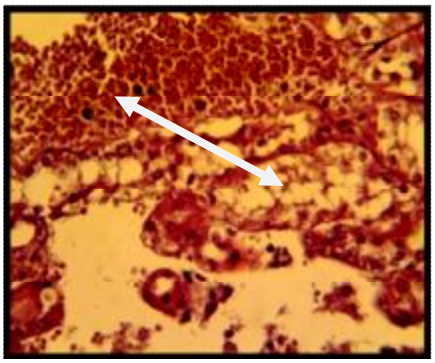


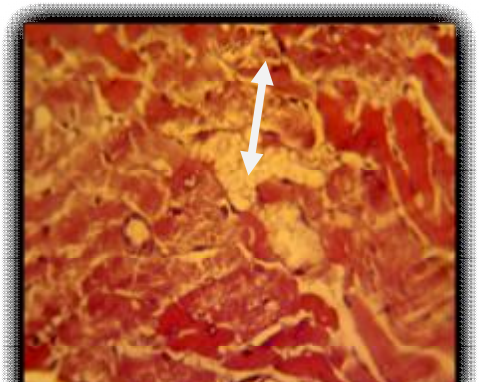
Figure (6): Histopathological section in the lung of control positive group at 24 hrs. post-infection shows inflammatory cells particularly neutrophilsin dilated congested blood vessels and in the wall of alveoli (H&Estain40X)



Figure(7): Histopathological section in the kidney of control positive group at 24 hrs. post- infection shows neutrophils in dilated blood vessels and vacuolar degeneration &sloughingof epithelial cells of renal tubules (H&Estain40X)



Figure(8): Histopathological section in the kidney of control positive group at 24 hrs. post- infection shows thrombus with sever vacuolar degeneration &sloughingof epithelial cells of renal tubules (H&Estain40X)



Figure(9): Histopathological section in the heart of control positive group at 24 hrs. post-infection shows congested blood vessels with proteinous material in dilated blood vessels (H&Estain40X)

E. coli isolate (group 2): Histopathological examination showed aggregation of mononuclear cells around congested blood vessels of the lung (Figure 10) also the kidney expressed, aggregation of mononuclear cells between degenerative renal tubules(Fig:11). in addition edema between cardiac muscle fiber were seen (Fig: 12).

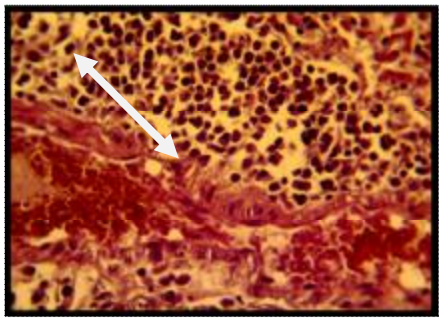


Figure (10):Histopathological section in the lung of immunized animal with whole cell sonicated Ag at 24 hrs. post- infection shows aggragation of mononuclear cells around congested blood vessels (H&Estain40X)

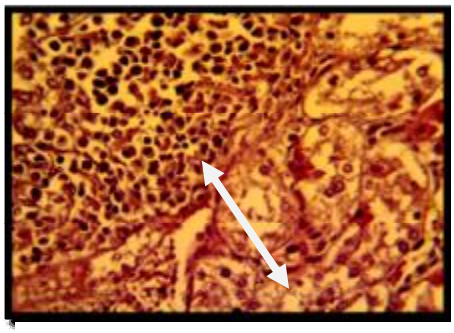


Figure (11):Histopathological section in the lung of immunized animal with whole cell sonicated Ag at 24 hrs. post- infection shows aggragation of mononuclear cells between degenerative renal tubules (H&Estain40X)

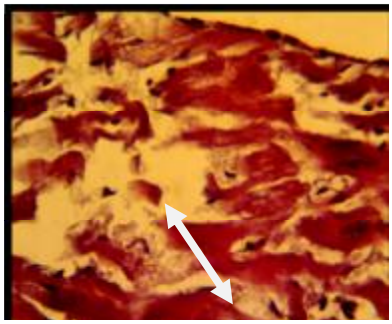


Figure (12):Histopathological section in the heart of immunized animal with whole cell sonicated Ag at 24 hrs. post- infection shows odema between cardiac muscle fiber (H&Estain40X)

Lung: The histopathological section in the lung showed mononuclear cells aggregation around blood vessels and bronchiole (Figure 13).

Kidney: The microscopic examination revealed that kidney showed macrophages, lymphocytes and neutrophils aggregation around congested blood vessels between renal tubules and around glomeruli were the main lesions in kidney (Figure14).

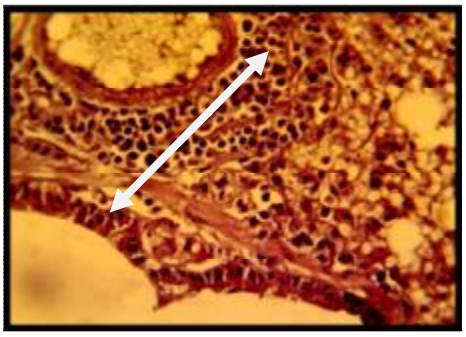


Figure (13):Histopathological section in the lung of immunized animal with biofilm producing strain with protein concentration 3.5 mg/ml at 24 hrs. post-infection shows mononuclear cell aggragation around blood vessels and bronchiole (H&Estain40X)

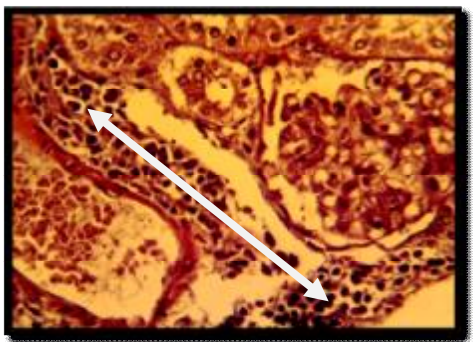


Figure (14):Histopathological section in the kidney of immunized animal with biofilm producing strain with protein concentration 3.5 mg/ml at 24 hrs. post- infection shows macrophages, lymphocytes and neutrophils aggragation around congested blood vessels between renal tubules and around glomeruli (H&Estain40X)

E. coli isolate (group 2): Histopathological section in the the kidney expressed cellular debris in the lumen of renal tubules (Figure 15) in the Lung except proliferation of lymphoid tissues.

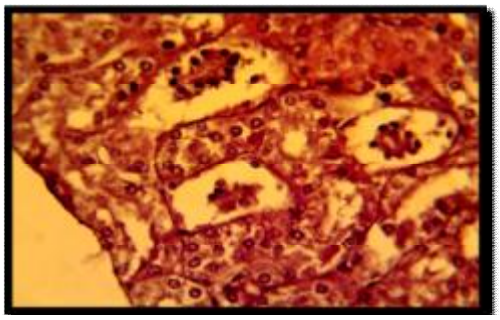


Figure (15): Histopathological section in the kidney of immunized animal with biofilm producing strain with protein concentration 3.5 mg/ml at 48 hrs. post- infection shows celluler debris in the lumen of renal tubules ↔ (H&Estain40X)

Animals immunized with biofilm antigen (bi A) with protein concentration 3.5 mg/ml (group 3): Lung: the present study explained that the lesion in the lung revealed mononuclear cells infiltration around bronchi (Figure 16).

Kidney: The kidney showed acute cellular degeneration in the epithelial cells of renal tubules with atrophy of glomerular tufts were seen (Figure 17).

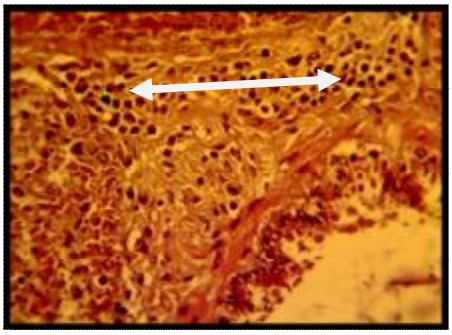
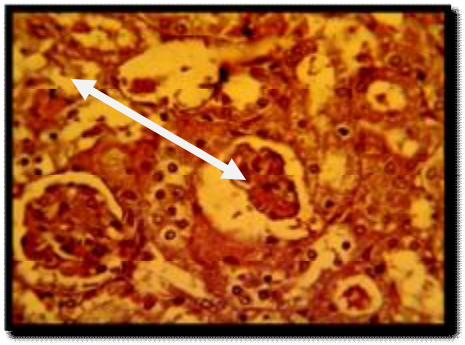


Figure (16): Histopathological section in the lung of immunized animal with biofilm Ags of biofilm producing strain with protein concentration 3.5mg\ml at 48hrs. post-infection shows mononuclear cells infiltration around bronchi (H&E stain 40X).



Figure(17): Histopathological section in the kidney of immunized animal with biofilm Ags of biofilm producing strain with protein concentration 3.5mg\ml at 48hrs. post-infection shows acute cellular degeneration in the epithelial cells of renal tubules with atrophy of glomerular tufts (H&E stain 40X).

Lung: The lesion in the examined organ showed neutrophils, alveolar macrophages, fibrin and edema in the alveolar spaces (Figure 18).

Kidney: There were moderate degeneration of epithelial cells of renal tubules (Figure 19)

Heart: The heart expressed few mononuclear cells infiltration in the epicardium (Figure 20).

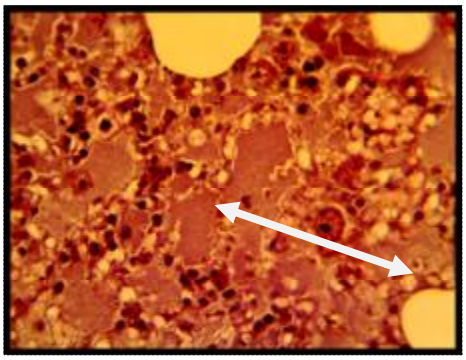
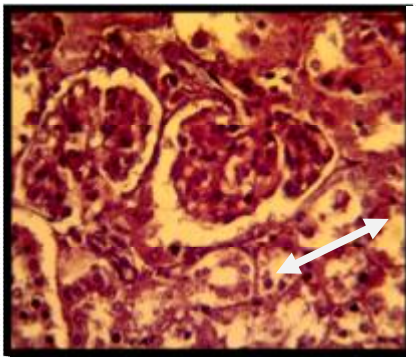
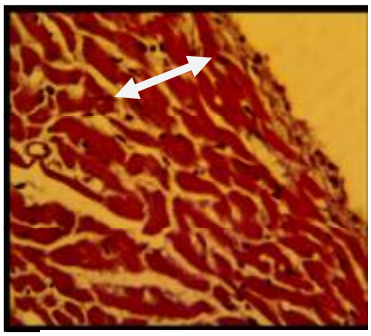


Figure:18.Histopathological section in the lung of immunized animal with biofilm producing strain with protein concentrat 14mg/ml at 48hr post-infection shows neutrophils, alveolarmacrophages, fibrin and edema in the alveolar spaces (H&E stain 40X).



Figure(19):Histopathological section in the kidney of immunized animal with biofilm Ags of biofilm producing strain with protein concentration 14 mg\ml at 48hrs. post-infection shows moderate degeneration of epithelial cells of renal tubules (H&E stain 40X).

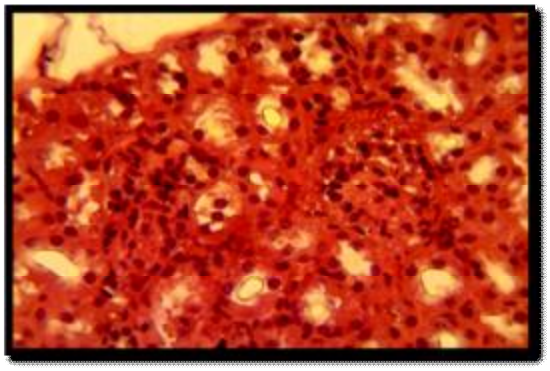


Figure(20): Histopathologic section in the heart of immunize animal with biofilm Ags of biofil producing strain with prote concentration 14 mg\ml at 48h post-infection shows mononucle cells infiltration in the epicardiu (H&E stain 40X).

Animal sacrificed at day 30 post – infection:

Animals immunized with whole sonicated antigen (WCA) of biofilm producing *E. coli* isolate (group 2): Kidney : the microscopic section in the kidney showed no clear lesion (Fig :21).

Heart: The histopathological section of the heart showed no clear lesion (Fig: 22).



Figure(21):Histopathological section in the kidney of immunized animal with sonicated Ags of biofilm production strain with protein concentration 3.5 mg/ml at 30 day post- infection shows no clear lesion (H&E 40X).

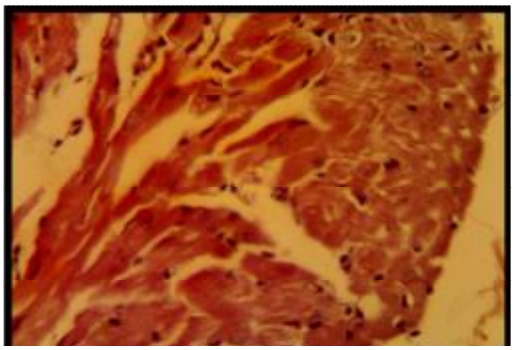
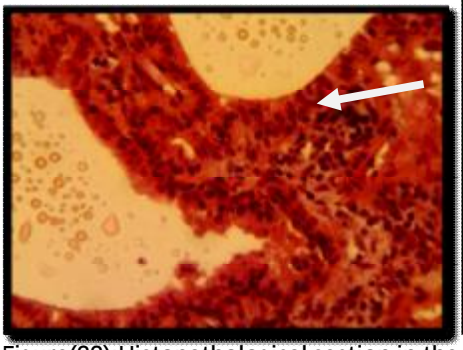


Figure (22). Histopathological section in the heart of immunized animal with sonicated Ags of biofilm production strain with protein concentration 3.5 mg/ml at 30 day post- infection shows no clear lesion (H&E 40X).

Animals immunized with biofilm antigen (bi A) with protein concentration 3.5 mg/ml (group 3):

Lung: The microscopic examination revealed that lung expressed hyperplasia of escheated lymphoid tissue with granulomatous lesion. (Fig: 23), (Fig: 24).

Heart: The heart showed aggregation of mononuclear cell mainly neutrophils between cardiac inflammatory muscle fibers (Figure 25).



Figure(23):Histopathological section in the lung of immunized animal with biofilm Ags of biofilm production strain with protein concentration 3.5 mg/ml at 30 day post-infection shows hyperplasia of escheated lymphoid tissue (H&E 40X).

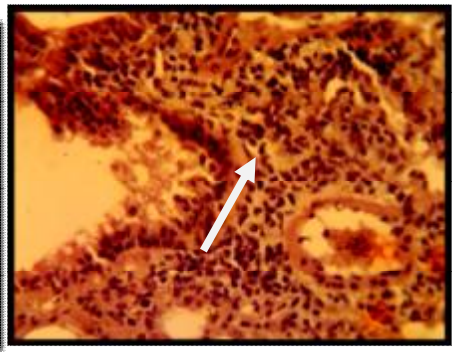


Figure (24): Histopathological section in the lung of immunized animal with biofilm Ags of biofilm production strain with protein concentration 3.5 mg/ml at 30 day post- infection shows hyperplasia of escheated lymphoid tissue (H&E

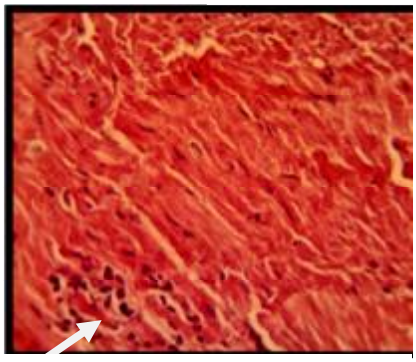


Figure (25):Histopathological section in th heart of immunized animal with biofilm A of biofilm production strain with protein concentration 3.5 mg/ml at 30 day post-infection shows aggregation of mononucl cell mainly neutrophils between cardiac inflammatory muscle fibers (H&E 40X)

Animals immunized with biofilm antigen (bi A) with protein concentration 14 mg/ml (group 4):

Kidney: the histopathological section in the kidney expressed no clear lesion (Fig: 26).

Heart: the microscopic examination of the heart expressed no clear lesion (Fig: 27).

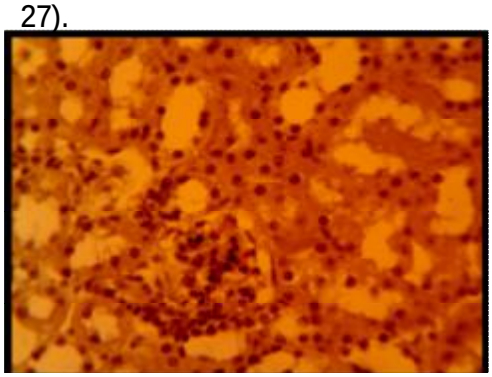


Fig: 26. Histopathological section in the kidney of immunized animal with biofilm Ags of biofilm production strain with protein concentration 14 mg/ml at 30 day post- infection shows no clear lesion (H&E 40X).

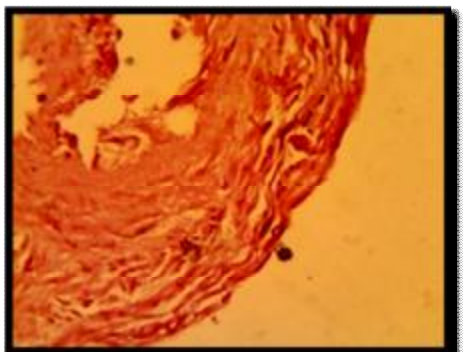


Fig: 27. Histopathological section in the heart of immunized animal with biofilm Ags of biofilm production strain with protein concentration 14 mg/ml at 30 day post-infection shows no clear lesion (H&E 40X).

Control negative group (group 5):
There is no lesion in lung, heart, (Figures 28, 29).

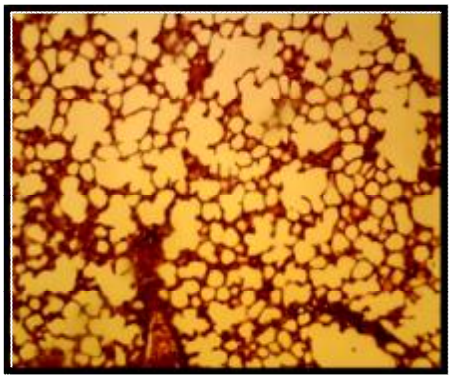


Fig: 33.Histopathological section in the Lung of the control negative group showed the normal structure of the lung (H&E 40 X).

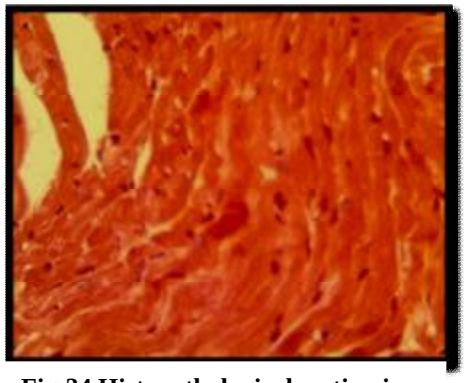


Fig:34.Histopathological section in the heart of the control negative group showed the normal structure of the heart (H&E 40 X).

Histopathological examination showed thrombus formation in the lung and kidney, congestion of the blood vessels and vacuolar degeneration in the heart and lung, death in animals during 12, 24hrs these results are consonant with those previously reported by several investigators (7). reported that typical pathological finding when induce chronic infection.

The infection characterized by a rapid and massive multiplication of bacteria and a general necrosis of the infected quarter. The ability of *E. coli* to cause disease has been attributed to its ability to produce virulence factors which allow them to colonize and subsequently produce disease these results came in agreement with what was reported by (14).

Immunized animals showed variable degrees of mononuclear cell infiltration between degenerative renal tubules with edema between cardiac muscle fiber, The results showed the development of the bacterial pathogenesis which attributed to severity and ability of *E. coli* to grow and multiply and induce inflammation in Kidney, heart and lung , these results came in agreement with (15) who explained that aggregated mononuclear cell indicated immune response (16). Showed that the animals immunized with WCA *E. coli* Ags strengthen the immune responses against virulent *E. coli* (17) expressed that the antigen stimulated immune response with variable degrees according to type of antigen and the animals that immunized with biofilm with high protein concentration express low mortality rate and all the above results came in agreement with the present results.

At 30 days post infection, showed no clear lesion in most of the internal organs, the main lesion in the immunized animal is granulomatous lesions (18) also showed granulomatous lesion is considered as the strongest immune defense against pathogen

which present till destruction of the pathogen this result in agreement with the current study. There is also variable degree of inflammatory cells infiltration mainly neutrophils.

On the basis of above information, it will be suggested that biofilm antigen stimulated both cell mediated immune response and humeral immune response which synergistic to destroyed this pathogen.

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Trace elements and microbial contamination of groundwater and its evaluation in the Rahaliya–Ekhedhur area, west Razzaza lake-central Iraq

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Abstract

This study aims to investigate the diversity, and distribution of some harmful pollutants in groundwater in the Rahaliya–Ekhedhur Area, west Razzaza Lake- central Iraq. The recognizing and studying trace elements and microbes along with major constituents in groundwater is necessary for its evaluation for drinking and irrigation purposes. Fifteen groundwater samples were collected during April 2013 (wet period) from Rahaliya, Shithatha and Ekhedhur groundwater wells. All samples were analyzed for trace elements (Fe, Co, Cu, Zn, Cd, Pb and Mn) and for Microbial parameters (total microbial count, Coliforms, Pseudomonas, Alcaligenes, Escherichia, Citrobacter, Salmonella, Shigella, Klebsiella, Proteus and Enterobacter). The results indicated that the groundwater is of mixed origin, very hard (TH ranging from 227.5 to 1032.8 ppm), having high pH values, and total dissolved solids (TDS) are ranging between 1700-2750 mg/l, so that the water is of brackish type. On the basis of the major cation and anion concentration the groundwater is of (mixed Mg and Na -SO_4) group having two families which are (Na_2SO_4) family with $Na^+ > Ca^{2+} > Mg^{2+}$-$SO_4^{2-} > Cl^- > HCO_3^-$ and ($MgSO_4$) water family with $Mg^{2+} > Ca^{2+} > Na^+$-$SO_4^{2-} > Cl^- > HCO_3^-$. Generally the groundwater in the area were not recommended for drinking purposes, due to high salinity and it is Doubtful to unsuitable-Unsuitable for irrigation, whereas it can be used to cultivate sensitive crops in areas of medium to coarse grained soil. The results indicated that about 50 % of the groundwater samples are contaminated according to the total microbial count. No harmful bacteria were detected in the investigated samples.

تقييم تلوث المياه الجوفية بالعناصر النزرة والبكتيريا في منطقة الرحالية – الاخيضر ، غرب بحيرة الرزازة – وسط العراق

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الخلاصة

كان توزيع وتنوع الملوثات الضارة في المياه الجوفية في منطقة الرحالية – الاخيضر ،غرب بحيرة الرزازة – وسط العراق هو من اهداف هذة الدراسة . ان دراسة وتحديد العناصر النزرة والبكتيريا اضافة الى المكونات الرئيسية للمياه الجوفية لها ضرورة في تقييم تلك المياه لاغراض الشرب والسقي . جمعت خمسة عشر عينة من المياه الجوفية خلال شهر نيسان 2013 (كفترة رطبة) من الابار في مناطق الرحالية – شثاثة - الاخيضر. تم تحليل كافة العينات

للعناصر النزرة (Fe, Co, Cu, Zn, Cd, Pb and Mn) والمكونات البكتيرية (total microbial count, Coliforms, Pseudomonas, Alcaligenes, Escherichia, Citrobacter, Salmonella, Shigella, Klebsiella, Proteus and Enterobacter) اظهرت النتائج بان المياة الجوفية كانت ذات اصول مختلطة وذات عسرة كلية عالية جدا (تراوحت قيم العسرة الكلية بين 227.5 و 1032.8 جزء من المليون) قيم عالية للحامضية (pH) و للاملاح الذائبة الكلية (تراوحت قيمها بين 1700 و 2750 مليغرام / لتر) وبذلك تعتبر من النوع العكر . صنفت المياة الجوفية استنادا الى تراكيز الايونات الرئيسية الموجبة والسالبة الى انها مختلطة من مجموعتين كبريتات المغنيسوم والصوديوم وتتكون من عائلتين كبريتات الصوديوم على شكل $Na^{+}>Ca^{2+}>Mg^{2+}$-$SO_4^{2-}>Cl^{-}$ والاخرى كبريتات المغنيسيوم على شكل $Mg^{2+}>Ca^{2+}>Na^{+}$-$SO_4^{2-}>Cl^{-}>HCO_3^{-}$. بصورة عامة فان $HCO_3^{-}>$ المياة الجوفية في المنطقة ليست صالحة لشرب الانسان ولاغراض الري او انها مصنفة كمشكوك بها لارتفاع الملوحة العالي بينما يمكن استخدامها لزراعة بعض المحاصيل الحساسة في المناطق ذات نسجة التربة المتوسطة والخشنة . كما اظهرت النتائج بان 50 % منها تقريبا ملوثة بالعدد الكلي للبكتريا ولم تحدد النتائج وجود بكتريا ضارة بالعينات المدروسة.

Introduction

The quality of groundwater is of nearly equal importance to quantity. The quality required of groundwater supply depends upon its purpose, thus, the needs for drinking water, industrial water, and irrigation water vary widely (1). To maintain the sustainability of the renaissance agricultural development in the Rahaliya –Ekhedhur Area, pollution indicators must follow-up to the groundwater especially after increasing the sources of pollution (agricultural fertilizers,…….etc.). In the absence of infrastructure (healthy drinking water and sewage) which makes people's in this area using shallow groundwater in the area, so it must be study the sources of pollution, especially trace and microbial pollutants which are detrimental to human health which that is the ultimate objective of this research .Studying the trace elements concentrations in water is of great importance due to their direct effects on human and animal's health in addition to plants growing. The source of these elements is due to rocks weathering beside human activities. Some trace elements, such as Cu and Zn, are necessary in low concentration for all livings, while most of them present toxicity hazards at high concentrations (2). Several studies were achieved that concerning the hydraulic characteristics and the hydrochemical analysis properties of the groundwater of Rahhalia, Shithatha or Ekhedhur (3 , 4), but no one investigate the trace and microbial pollutants in groundwater. Therefore, the area from Rahhalia to Ekhedhur is chosen for further deals of the hydrochemical analyses for future utilization of the groundwater (Figure 1).

The studied area is located in the middle part of Iraq in Anbar and Karbala governorates, to the west of the Razzaza Lake. The area is extending from Rahhalia to the Ekhedhur passing through Shithatha city between latitudes (32º 25` to 32º 50`) North and longitudes (43º15` to 43º 40`) East (Figure 1). The area is characterized by arid climate of hot dry summer ,cold dry winter with annual rainfall (90 mm) mainly during January to April and annual evaporation (2954 mm) (4).

The geological formations in the study area consist of Dammam, Euphrates, Nfayil and Injana, Formations and Quaternary deposits (5).Quaternary sediments cover vast area near Razzaza Lake which represented by gypcrete, inland sabkha, depression fill,

flood plain and Aeolian sediments (Figure 1) (6). The studied area has two fault systems, Abu Jir and Imam Ahmad bin Hashim which are deepening more than 150 m for the period before Miocene and its main trend is to the northwest - southeast (3). The hydrogeology situation depends on the nature of the structural, geological, type of formations, nature of the water bearing rocks and cavities. The nature of rocks consists of carbonate rocks with large thickness. The ground water aquifers are represented by three aquifers, (Tayarat – UmmRadhuma), (Dammam – Euphrates) and Nifayil formations (7). The study area is considered to be one of the important areas due to the existence of several economic projects (industrial and agricultural products) such as the production of washed sand factory and fractionalizing gravels factory (4).

The unconfined aquifer in the studied area is represented by the Euphrates - Nifayil Formations (5 and 7). The confined aquifers represented by the Dammam Formation which is one of the most important aquifers in south western Iraq. It is composed of variable carbonate rocks mainly limestone, dolomatic limestone and dolomite, with secondary marl and chert. The Dammam aquifer is considered to be the main source of water in the area, (6). The aims of this study are to investigate the hydrochemical analyses, Microbial parameters and to evaluate the quality of ground water suitability for different purposes at Rahhalia - Ekhedhur area.

Materials and Methods

Fifteen groundwater samples were collected during wet period (April 2013) from Rahaliya, Shithatha and Ekhedhur groundwater wells of different depths 80-120 m (Figure 1). The GPS (Global Position System) was used to determine the locations (Longitude, Latitude and Elevation) for each well.

A-The analysis of the concentration of cations (K^+, Na^+, Mg^{2+}, Ca^{2+}) and anions (Cl^-, HCO_3^-, SO_4^{2-}) in addition to (Electric conductivity EC, Total dissolved salts TDS and pH) as well as trace elements determination (Fe, Co, Cu, Zn, Cd, Pb and Mn). They have been done in the chemical laboratory of the General Commission for Groundwater according to the international standards (8). The results of major ions, trace elements, TDS, EC and pH were shown in Tables 1 and 2.

B- Microbial investigation : Bacteriology analyses are included most probable number (MPN) is a qualitative method for coliform counts were made in the Central Environmental Laboratory, the College of Science, University of Baghdad.

Bacterial isolates were preserved by lyophilization and were routinely cultured at 37^oC on Luria-Bertani agar plates. The analyses were carried out according to standard methods (8 and 9).

Total coliform viable or colony forming units (CFU)were enumerated using the multiple-tube fermentation (MTF) is recommended by APHA(8) and Nogueria and Nakamura (9), incubation at 35 ± 0.5 ^oC for 48± 3h., if the water sample yielded presumptive positive result simultaneously inoculation in to Brilliant- green lactose bile broth fermentation tube at any time within incubation 35 ± 0.5^oC for 48± 3h., was used to confirm the presence of the total coliform group, standard MPN technique was used for quantitative enumeration of fecal coliform group of bacteria as described by

APHA(8), standard MPN technique was applied using glucose azide broth cultivating medium, for 24-72 hrs at 37°C. Results were expressed as MPN of fecal streptococci 100 ml-1 of sample. As described by Harly and Prescott (10), the heterotrophic plate count was estimated in milliliter sample by pour plate method using Nutrient agar medium.

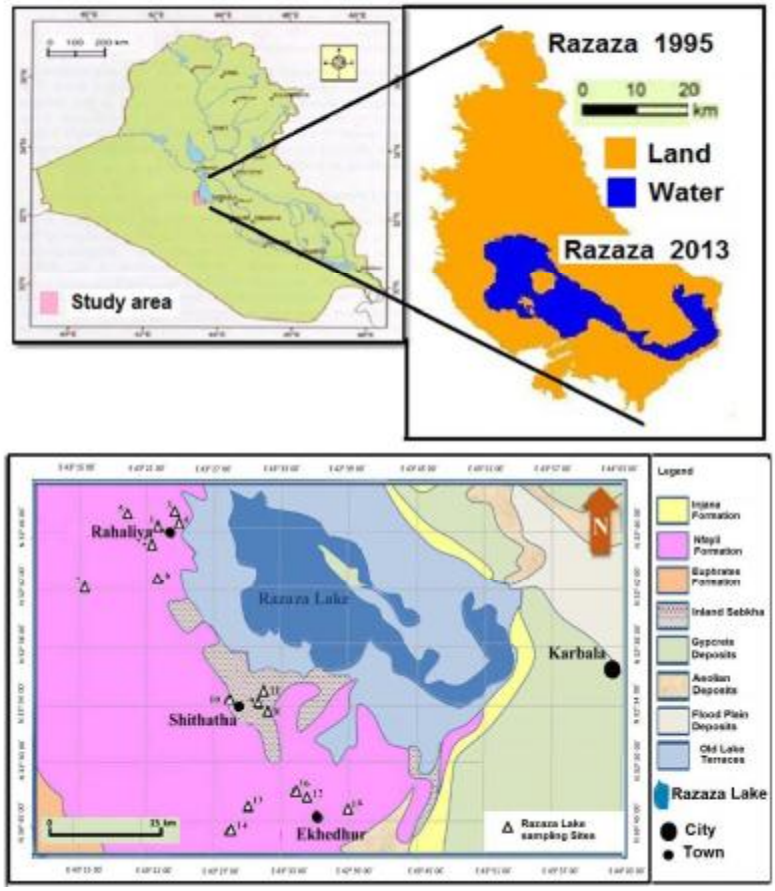

Figure 1: Location and geological map of the study area (4 and 6).

Results and Discussion

A- Physical and chemical analysis: The groundwater of Rahaliya, Shithatha and Ekhedhur area is characterized by wide variation in TDS (ranging from 1700 to 2420 ppm (Table 1).The EC (ranging from 2520 to 3710 μs/cm with pH values ranging from 7.08 to 7.61.

Comparison of TDS values with classifications of Todd (1), Altoviski (11) and Drever (12), showed that the groundwater in the studied area is classified as slightly-brackish water and all the water samples are not useful for drinking. The total hardness (TH) ranging from 228 to 773 ppm that classified as very hard water due to wide exposures of limestone in the recharge area according to Todd (1) classification. It can be concluded that the type of groundwater and Razzaza lake water indicate excessively mineralized water according to Detay (13) classification. Appling Sulin's (14) classification, the

ground water samples fall in the zone of A< 1 that represent marine water in semi confined aquifer and in the zone of B<1 that represent meteoric origin of Na+K - Sulphate .

According to Schoeller (15) classification the groundwater samples show that the group are SO_4 and Cl and the families are Na-SO_4, Mg-SO_4 , Ca-SO_4, Na -Cl . While according to Piper's (16) classification the groundwater samples fall in (e –class) which represents earth alkaline water with increase portion of alkali with prevailing sulphate and chloride and some of these samples fall in (g – class) which represents alkaline water with prevailing sulphate and chloride. The application of Piper's diagram (16) shows that all water samples have non carbonate hardness (secondary salinity)>50%. Applying Hassan, et al (17), method to classify the ground water of Rahaliya – Ekhedhur area and identifying their water types are shown in Table 2. The Rahaliya – Shithatha and Ekhedhur groundwater have clearly indicated that the sulphate and chloride groups are dominant. The sulphate group with two families (sulphate -sodium and sulphate-magnesium) the first family with one major water type rNa>.rCa>rMg; SO_4> rCl, while the second family with two water types rMg> rCa> rNa; rSO_4> rCl and rMg> rNa >rCa; rSO_4> rCl water type. The chloride group contains one major family (chloride -sodium family) and one water type which is rNa >rMg> rCa; rCl>rSO_4 water type.

This variation can be attributed to the lithological and mineralogical contents of the geological formation or to the spatial variation in controlling factors that are responsible for sedimentation and dissolution of different minerals. Hassan, et al (3) and (17) concluded that sulphate and chloride ions are the dominant ions in the study area.

Table (1) Physical and Chemical properties of groundwater samples.

Well No	Na+K epm (%)	Mg epm (%)	Ca epm (%)	Cl epm (%)	SO_4 epm (%)	HCO_3 epm (%)	TDS ppm	EC µs/cm	pH	T.H (ppm)
1	61.57	19.96	18.45	54.67	32.24	13.08	2137	3710	7.31	649
2	69.27	14.13	16.58	32.31	61.09	6.59	1740	2570	7.12	277
3	72.09	12.50	15.38	36.31	47.86	15.82	1848	2760	7.08	321
4	78.90	8.38	12.7	36.85	42.35	20.78	1860	2560	7.15	228
5	45.97	18.37	35.64	38.04	48.23	13.71	1755	2520	7.30	604
6	32.03	35.99	32.58	35.42	58.96	5.61	1932	2650	7.40	655
7	32.7	43.97	32.32	35.27	59.09	5.61	1820	2600	7.50	649
8	31.41	36.5	32.08	35.33	59.49	5.17	1932	2550	7.61	687
9	31.26	36.59	32.16	35.36	59.4	5.23	1700	2560	7.16	687
10	34.22	34.37	31.39	35.59	59.14	5.55	1919	2650	7.31	653
11	42.2	27.93	29.79	41.67	45.25	13.07	1872	2540	7.15	773
12	47.51	17.84	34.63	37.43	47.58	14.98	1723	2560	7.34	604
13	74.40	11.57	14.01	40.25	42.47	17.27	2000	2670	7.34	321
14	55.47	24.29	20.24	49.38	39.13	11.47	2420	3000	7.31	686
15	42.00	18.61	35.38	37.5	47.52	14.96	1812	3020	7.21	620

Table (2) Rahaliya– Shithatha groundwater classification according to Hassan, *et al* (3).

	Family	Group	Index	Water type	Spring No.	%
1	Sulphate- Sodium	Sulphate	23; 32	rNa>.rCa>rMg;rSO_4>rCl	2,3,4,5,11,12 13,15	53.3%
2 3	Sulphate- Magnesium		53; 32	rMg>rCa>rNa; rSO_4>Cl	6,8,9	20.0%
			33; 32	rMg>rNa>rCa;rSO_4>Cl	7,10	13.3 %
4	Chloride-Sodium	Chloride	13;12	rNa>rMg>rCa;rCl>SO_4	1,14	13.3 %

B- Trace elements: Seven trace elements are analyzed for all water samples. These are Fe, Co, Cu, Zn, Cd, Pb and Mn. The water samples are compared with the WHO (18) and IQS (19) standards specifications for trace elements in drinking water. The results of some trace elements concentrations (Table 3) discussed as follows:

(1) Zinc (Zn): The Zinc ions is essential element in plant and animal but excessive amounts will be harmful to human life (18,20 and 21).The Zn concentration in water samples of the study area in wet period ranges between (0.011- 0.051) ppm with average (0.025) ppm (Table 3).

(2) Manganese (Mn): Manganese is one of the essential toxic trace elements, which means that it is not necessary for humans to survive, but can be toxic when too high concentrations are present in a human body (18 and 21). The Mn concentration in water samples of the study area in wet period ranges between (0.025 -0.089) ppm with average (0.052) ppm, (Table 3).

(3) Lead (Pb): The Lead ions quantity in surface and groundwater is little and also its natural mobility is low (12). The Pb concentration in water samples of the study area in wet period ranges between (0.042– 0.050) ppm with average (0.046) ppm, (Table 3).

(4) Cadmium (Cd): The geochemical characters of cadmium are similar to that of zinc but (Cd) is much less abundant in earth's crust (20 and 21).The Cd concentration in water samples of the study area in wet period ranges between (0.002- 0.005) ppm with average (0.004) ppm, (Table 3).

(5) Copper (Cu): The copper is considered toxic for human life if its concentration exceeds the permissive limit in drinking water (18 and 21). The Cu concentration in water samples of the study area in wet period ranges between (0.023- 0.042) ppm with average (0.034) ppm, (Table 3).

(6) Cobalt (Co): Fertilizers and waste disposal are considered sources for cobalt (20 and 21). The Co concentration in water samples of the study area in wet period ranges between (0.015- 0.091) ppm with average (0.038) ppm, (Table 3).

(7) Iron (Fe): Iron is essential for human, but it becomes toxic when the concentration increases (18 and 20). The Fe concentration in water samples of the study area in wet period ranges between (0.23 -0.30) ppm with average (0.28) ppm, (Table 3).

Table (3) Trace elements concentrations of water samples (ppm).

Water samples	Zn	Mn	Pb	Cd	Cu	Co	Fe
W1	0.012	0.030	0.050	0.005	0.035	0.04	0.30
W2	0.011	0.029	0.050	0.005	0.039	0.047	0.29
W3	0.013	0.025	0.045	0.005	0.028	0.046	0.28
W4	0.013	0.051	0.042	0.004	0.04	0.025	0.25
W5	0.025	0.044	0.046	0.004	0.039	0.091	0.30
W6	0.021	0.048	0.043	0.005	0.035	0.015	0.23
W7	0.035	0.047	0.050	0.004	0.036	0.016	0.30
W8	0.023	0.055	0.045	0.005	0.033	0.04	0.27
W9	0.041	0.06	0.044	0.004	0.030	0.041	0.26
W10	0.051	0.061	0.046	0.002	0.031	0.05	0.23
W11	0.032	0.075	0.043	0.005	0.03	0.03	0.29
W12	0.041	0.077	0.042	0.003	0.041	0.04	0.30
W13	0.021	0.072	0.050	0.002	0.042	0.016	0.25
W14	0.018	0.068	0.047	0.003	0.027	0.027	0.29
W15	0.019	0.089	0.046	0.002	0.023	0.055	0.30
Min.	0.011	0.025	0.042	0.002	0.023	0.015	0.23
Max.	0.051	0.089	0.050	0.005	0.042	0.091	0.30
Mean	0.025	0.052	0.046	0.004	0.034	0.038	0.28

C –Total Bacterial Indicator: The total coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae ; they are a group of bacteria found in the intestine of human and other animals; also occur naturally in the soil, vegetation and surface water (22). Most members of the coliform group when found in drinking water, they are indicating that bacterial contamination of the water has occurred (8). Drinking water contaminated with these organisms can cause stomach and intestinal illness including diarrhea, nausea, and even lead to death. Use of normal intestinal organisms as indicator for fecal pollution rather than the pathogens themselves is a universally accepted principle for monitoring and assessing the microbiological safety of water supplies (18), which presence of small numbers of coliform organisms (1-10 organisms per 100 ml) particularly in untreated groundwater may be limited sanitary significant provided fecal coliform organisms are absent. But when the number of coliform bacteria exceeds the usual levels, it becomes very serious and needs immediate attention to protect inhabitants life from outbreaks of pathogenic disease (18).

The results of the total microbial count, Coliforms, Pseudomonas, Alcaligenes, Escherichia, Citrobacter, salmonella, Shigella, Klebsiella, Proteus and Enterobacter are

tabulated in Table 4. About 50 % of the groundwater samples (Nos. 2, 4, 5,6, 7, 9, 11, & 14) are contaminated according to the total microbial count, while coliforms were not detected in the groundwater sites, with the appearance of some types of organisms as Pseudomonas, Escherichia, Shigella and Entrobacter (Table 4).

At some wells were found high amount of Heterotrophic plate count and total coliform compared to other locations because of these locations more affected by human activity and domestic sewage effluent to the water sources, therefore unsatisfactory for drinking, and may be due to the level of organic matter in the water (23). Possible sources of contamination include leaking septic systems, runoff from agricultural lots and organically enriched water such as industrial effluents or from decaying plant materials and soils (18).

Table (4) Results of bacterial analyses of groundwater samples represented in the (MPN) and the type of bacteria in the study area.

S. No.	Total microbial count	Pseudo monas	Alcali genes	Esche richia	Citrob acter	Salmo nella	Shig ella,	Klebs iella	Pro teu s	Entero bacter
1	35	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	194	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
3	43	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	70	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
5	93	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
6	80	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	240	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
8	20	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9	185	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
10	23	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	135	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
12	40	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
13	30	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
14	58	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
15	43	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Evaluation of groundwater quality: The groundwater chemical analyses indicated that they are unsuitable for human drinking purposes according to Iraqi standard (19) and World Health Organization standard (18) (Table 5). While, they are ranging from acceptable for all types of animals and poultry according to Crist and Lowery (24) classification to very good for animal drinking according to Altoviski (11) and Ayers and Westcot (25) classifications . It is clear that all water samples are not suitable for industrial purposes for their high values of hardness and high salts concentrations according to Hem (21), while, they are suitable for building purposes according to Altoviski (11). It is located within the permissible limits and the doubtful limits according

to Ayers and Westcot (25) classifications for irrigation purpose. Microbial analysis results indicated that about 50% of groundwater samples are contaminated according to their total microbial count. Therefore, the microbial investigation showed that all of the groundwater samples are unsuitable for drinking purposes.

Table (5) Water Samples with the standards of Drinking Water (18 and 19).

Parameter	IQS (19)	WHO (18)	Water samples in wet period		Exceeding limits
			Range	Average	
EC(μs/cm)	1500	1530	2000-3850	3042.3	Exceed
TDS(ppm)	1000	1000	1514-2750	2176.2	Exceed
pH (ppm)	6.5-8.5	6.5-8.5	7.1-7.8	7.29	Not Exceed
T.H(mg/l)	500	_	285.4-1032.8	700.3	Exceed
Ca(ppm)	150	75	65-241	151.8	Exceed
Mg(ppm)	100	125	30-132	78.23	Exceed
Na (ppm)	200	200	141-490	335.8	Exceed
K (ppm)	_	12	2-15	11.63	Exceed
Cl (ppm)	350	250	240-740	418.7	Exceed
SO_4(ppm)	400	250	490-907	621.9	Exceed
NO_3(ppm)	50	50	1.4-9	4.15	Not Exceed
Zn (ppm)	3	3	0.011-0.051	0.025	Not Exceed
Mn (ppm)	0.1	0.1	0.025-0.089	0.052	Not Exceed
Pb (ppm)	0.05	0.05	0.042-0.05	0.046	Not Exceed
Cd (ppm)	0.005	0.005	0.002-0.005	0.004	Not Exceed
Cu (ppm)	1	1	0.023-0.042	0.034	Not Exceed
Co (ppm)	_	0.5	0.015-0.091	0.038	Not Exceed
Fe (ppm)	0.3	0.3	0.23-0.30	0.28	Not Exceed

Conclusion

1- The groundwater is classified as slightly-brackish, very hard water and is of excessively mineralized water.

2- The groundwater is of mixed Mg and Na - SO_4 group having two families which are (Na_2SO_4) and ($MgSO_4$).

3- The ground water in the area were not recommended for drinking purposes because of high salinity and it is Doubtful to unsuitable-Unsuitable for irrigation; whereas it can be used to cultivate sensitive crops in areas of medium to coarse grained soil . It is clear that all water samples are not suitable for industrial, while, they are suitable for building purposes.

4- The microbial investigation showed that all of the groundwater samples are unsuitable for drinking purposes.

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Anti-mutagenic effect of marigold (*Calendulla officianalis*) extract against mitoxantrone in albino male mice

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Abstract

This study focused on the effect of methanolic extract of marigold (*Calendula officinalis*) on some cytogenetic parameters in albino male mice. The results showed that marigold has the ability to increase the mitotic index and decreased in micronucleus formation in mice, comparing with the negative control. In contrast, mitoxantrone caused a reduction in mitotic index and an increase in micronucleus formation and sperm head abnormalities. While the interaction between plant extract and mitoxantrone drug showed the ability of marigold to minimize the cytotoxic effect of mitoxantrone. It was concluded from this study that marigold extract has anti-mutagenic properties against a mutagenic drug.

التأثير المضاد للطفرة لمستخلص الاقحوان (*Calendula officinalis*) ضد المايتوزانترون في ذكور الفئران البيض

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الخلاصة

ركزت هذه الدراسة على تاثير المستخلص الميثانولي لنبات الاقحوان على بعض قياسات الوراثة الخلوية في ذكور الفئران البيض. وقد اظهرت النتائج ان الاقحوان لديه القدرة على زيادة معامل الانقسام الخلوي مع انخفاض معنوي في تشكيل النوى الصغيرة في الفئران، مقارنة مع السيطرة السالبة. على العكس من المايتوزانترون الذي سبب انخفاض في معامل الانقسام وزيادة في تكوين النوى الصغيرة مع ازدياد في تشوه رؤوس النطف. في حين ان التداخل بين المستخلص النباتي والمايتوزانترون أظهر قدرة الاقحوان على خفض التاثير السام للمايتوزانترون. يستنتج من هذه الدراسة ان مستخلص الاقحوان لديه خصائص مضادة للطفرات ضد الادوية المطفرة.

Introduction

Throughout the ages, humans have relied on nature for their basic needs for the production of food-stuffs, shelters, clothing, means of transportation, fertilizers, flavors and fragrances, and, not the least, medicines (1). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies (2). One of these plants is *Calendula officinalis* L., is cultivated worldwide, and its uses as a medicinal plant dates back to the 12th Century. Calendula is widely used in folkloric medicine, and more than

35 medicinal properties have been attributed to the plant flowers, for instance, anti-inflammatory, analgesic, anti-ulcer, bactericidal and diuretic (3). Moreover, many investigations have revealed its anti-mutagenic and anti-carcinogenic properties (4, 5). Calendula can be taken internally to treat fevers and also to promote menstruation (6). The flowers are made into extract tinctures, balms and salves and applied directly to the skin to help wound healing and to soothe in flamed and damaged skin (7). As well as, its aqueous extract is used as eye washes, gargles or compresses to treat conjunctivitis, pharyngitis and other inflammatory conditions of the skin and mucous membranes (8, 9). Jimenez-Medina *and colleagues* (2006) demonstrated that the principal chemical components of aqueous extract of calendula are: polysaccharides, proteins, fatty acids, carotenoids, flavonoids, triterpenoids and saponins, and accordingly several biological and pharmaceutical potentials have been suggested. Phyto-pharmacological studies of different calendula extracts are in agreement of such scope, and have shown anti-viral activity, anti-HIV properties of therapeutic interest (10), and anti-genotoxic properties (11), as well as anti-cancer effects (12).

This study was designed to evaluate the cytogenetic anti mutation effects of methanol extract of *C. officinalis* on mitotic index, micronucleus formation and sperm head abnormalities of albino male mice.

Material and Methods

1- Calendula plant (*Calendula officinalis* L.): The late herbalist Dr. Raad Al-Mawlla (Deparment of Biology, College of Science, University of Baghdad) supplied and identified the flowers of calendula (*Calendula officinalis* L.), which collected in June 2006. The plant was grown in a local garden in Baghdad. The flowers were left at room temperature to dry, and after dryness, they were powdered with a coffee grinder.

2- Plant extract: The flower powder was extracted with methanol; 10 grams of the powder were extracted with 150 ml of solvent at 60°C for three hours using the Soxhlet apparatus. Then, the resulted extract solution was evaporated by a rotary evaporation. The collected crude deposit extract was frozen at -20°C until use to prepare the required doses (13). Three doses (37.5, 75.0 and 112.5 mg/kg), based respectively on 10, 20 and 30% of the LD_{50} dose (300-375 mg/kg) in mice (14), were prepared.

3- Mitoxantrone drug: The solution was diluted with sterile distilled water to prepare a subcutaneous dose of 0.33 mg/kg, which is the recommended dose in the treatment of leukemia in humans (Company leaflet, Ebewe Pharma Company, Austria). Therefore, the mouse dose was 0.008 mg/mouse.

4- Laboratory animals: Albino male mice (*Mus musculus*) were the laboratory animals, which were used to carry out the investigations of the present study. They were obtained from Biotechnology Research Centre (Al-Nahrain University). Their age range was 8-9 weeks, and their weight was 23-27 grams at the beginning of experiments. They were caged in the animal house of the supplier, in which the temperature was 23-26°C, and a light:dark periods of 10:14 hours/day. The animals

had free excess to food (standard pellets) and drinking water (*Ad libitium*) during all experiments.

5- Experimental design: In all cases, a single dose/day (0.1 ml) of the tested material was injected subcutaneously for seven days, and on day 8, the animals were killed to carry out laboratory assessments.

1. Dose determination: groups of 8 mice were given: distilled water (negative control), mitoxantrone at 0.33 mg /kg (positive control) or one of three doses (37.5, 75.0 and 112.5 mg/kg) of calendula methanol extract.
2. Interaction study: Groups of 8 mice were given: a single dose (determined in experiment 1) of the plant extract for six days and on day 7, they were injected with mitoxantrone (pre-treatment); mitoxantrone on day 1, while on days 2-7 they were injected with the plant extract (post-treatment); or distilled water (negative control).

6- Metaphase index: The metaphase index was assessed on somatic cells obtained from the bone marrow and spleen of investigated mice, according to a pre-established method (15), the animal was injected intraperitoneally with 0.25 ml of colchicin solution, and the animal was sacrificed by cervical-dislocation. Femur bone and spleen were removed and transferred to two Petri dishes containing 5 ml of PBS. The femur bone was cleaned from muscles and other tissues, and both ends were cut. Then, the bone marrow was obtained with PBS (5 ml) and collected in a test tube. While the spleen was punctured with the needle of insulin syringe, and its cellular content was obtained by repeated injections of PBS. The cells were collected in a test tube. Then the cell suspension of both tubes was gently pipetted, and centrifuged (2000 rpm) for 5 minutes. The cell deposit was suspended in 10 ml of a warm (37°C) hypotonic KCl (0.075M), for 30 minutes the tubes were centrifuged (2000 rpm) for 5 minutes, and the cell deposit was slowly suspended in 5 ml of cooled fixative (4ºC), and incubated at 4°C. After 30 minutes repeated fixative, to prepare a single cell suspension. Few drops of the fixed cell suspension were dropped vertically from a height of about 3 feet on a cleaned slide to give chance for nuclei and chromosomes to spread well. The slides were air-dried, stained with Giemsa stain. The slides were examined under oil immersion lens (100X), and at least 1000 cells (divided and non-divided cells) were scored. Then, the percentage of metaphase cells (metaphase index) was calculated according to the following equation:

$$\text{Metaphase index (\%)} = \left(\frac{\text{Number of Metaphase Cells}}{\text{Total Count}} \right) \text{x } 100$$

7- Micronucleus test: The procedure of 16 was followed with some modification to assess the micronucleus formation in the bone marrow of mice. Two ml of AB human plasma (heat inactivated) were injected in the bone cavity to wash out the bone marrow cells, using insulin syringe. The test tube was centrifuged at (2000 rpm) for 5 min, and after discarding the supernatant, the cell deposit was smeared on a clean slide, which was air-dried. The smear was stained with Giemsa stain for 15 minutes, then washed with distilled water, and air-dried.

The slide was examined under oil immersion lens (100X), and polychromatic erythrocytes (PCE) were inspected for the formation of micronucleus. A total of 1000 cells were randomly examined, and the micronucleus index was calculated using the following equation:

$$\text{Micronucleus index (micronucleus/cell)} = \frac{\text{Number of Micronuclei}}{\text{Total Count of PCE}}$$

8- Sperm-head abnormality assay: The procedure 17 was followed with some modification, to assess the sperm-head abnormalities (SHA) in mice. The animal was sacrificed by cervical dislocation and dissected to remove the epididymis, which was transferred to a Petri dish containing 5 ml of normal saline. The epididymis was cut into small pieces and dispersed with Pasture pipette. The spermatozoa containing saline was transferred to a test tube, to a centrifugation (1000 rpm) for 5 minutes, and drops from spermatozoa deposit was smeared on a clean slide and air-dried. The smear was stained with eosin for 15 minutes.

At least 1000 spermatozoa were examined randomly under oil immersion lens (100X), and the percentage of spermatozoa with abnormal head was scored using the following equation:

$$\text{SHA index (\%)} = \left(\frac{\text{Number of Spermatozoa with Abnormal Head}}{\text{Total Count}} \right) \text{x } 100$$

Statistical analyses: The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using the computer programme SPSS version 7.5.The difference was considered significant when the probability value was equal or less than 0.05.

Treatment efficiency was calculated according to (Perez-Serrano *et al*., 1997), as following equation:

$$\text{Treatment efficiency} = \left(\frac{\text{Treatment Group - Negative Group}}{\text{Negative Group}} \right) \times 100$$

Results and Discussion

To explore the anti-mutagenic effects of *C. officinalis* methanolic extract on the induced genetic damage in mice, three assessments were carried out; they were MN formation in polychromtic cells of bone marrow and metaphase index of both bone marrow and spleen cells, which both are good parameters of mutagenic evaluations (18). Three doses (37.5, 75.0 and 112.5 mg/kg) of *C. officinalis* methanol extract were evaluated for their cytogenetic effects in albino male mice, together with mitoxantrone (positive control), distilled water (negative control). In the present study three parameters were performed:

1- Metaphase Index as Figure 1, were scored in samples of bone narrow and spleen, and therefore the metaphase index was based on the percentage of these cells.

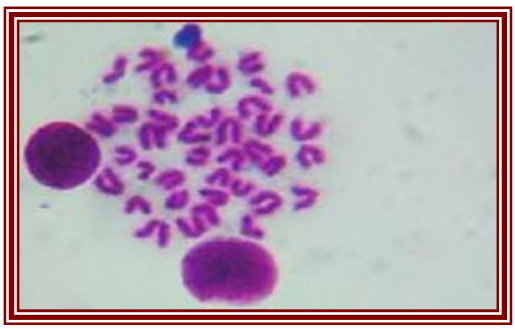

Figure (1): A metaphase preparation from bone marrow of mouse treated with the first dose (37.5mg/kg) of calendula methanol extract.

A: Bone marrow: A treatment with mitoxantrone caused a significant reduction in the metaphase index (0.45%) as compared to negative control (2.96%). In contrast, the three doses of methanol extract were associated with an increased index (4.63, 3.78 and 3.23%, respectively), but the difference was significant in the first two doses as compared to the distilled water negative control (2.96%), (Table 1).

Table (1): Metaphase index of bone marrow cells (mean ± standard error) in albino male mice treated with methanol extract of *Calendula officinalis*.

Groups	Dose (mg/kg)	Mean ± Standard Error (%)	Treatment Efficiency (%)
Positive Control (mitoxantrone Drug)	0.33	0.45±0.03	-87.78
Negative Control (Distilled Water)	0.00	2.96±0.14	
Methanol Extract	37.5	4.63±0.21	+56.51
Methanol Extract	75.0	3.78±0.08	+27.75
Methanol Extract	112.5	3.23±0.10	+9.14

B: Spleen: The negative control showed a significant increased metaphase index (1.33%) as compared to positive control (0.48%). Such increase was apparent in the three doses of methanol extract (2.50, 2.15 and 1.50%, respectively), but a significant difference was reached in the first two doses as compared to the corresponding negative control (1.33%), (Table 2).

Table (2): Metaphase index of spleen cells (mean ± standard error) in albino male mice treated with methanol extract of *Calendula officinalis*.

Groups	Dose (mg/kg)	Mean ± Standard Error (%)	Treatment Efficiency (%)
Positive Control (mitoxantrone Drug)	0.33	0.48±0.15	-64.15
Negative Control (Distilled Water)	0.00	1.33±0.09	
Methanol Extract	37.5	2.50±0.15	+88.68
Methanol Extract	75.0	2.15±0.13	+62.26
Methanol Extract	112.5	1.50±0.04	+13.21

C: Calendula Extract-Mitoxantrone Interactions (Metaphase Index of Bone Marrow cells): Methanol extract (37.6 mg/Kg) was significantly effective in enhancing the metaphase index of bone marrow cells in the pre-treatment interaction as compared to the corresponding control. In post-treatment, the methanol extract showed no effect (3.57 *vs*. 3.53%), as compared to the corresponding control (Table 3).

Table (3): Metaphase index of bone marrow cells (mean ± standard error) in albino male mice after interactions (pre and post-treatments) between methanol extract of *Calendula officinalis* and mitoxantrone drug.

Groups	Mean ± Standard Error(%)		Treatment Efficiency (%)	
	Pre-	Post-	Pre-	Post-
Control I (Distilled Water)	0.60±0.04	3.53±0.23		
Methanol Plant Extract (Ideal Dose)	2.29±0.12	3.57±0.20	+280.00	+1.41

D: Extracts-Mitoxantrone Interactions (Metaphase Index of Spleen cells): For methanol extract (37.6 mg/Kg) was effective in a significant enhancement of metaphase index in pre- and post- treatment as compared to the corresponding control, (Table 4).

Table (4): Metaphase index of spleen cells (mean ± standard error) in albino male mice after interactions (pre- and post-treatments) between methanol extract of *Calendula officinalis* and mitoxantrone drug.

Groups	Mean ± Standard Error (%)		Treatment Efficiency (%)	
	Pre-	Post-	Pre-	Post-
Control I (Distilled Water)	0.30±0.04	0.73±0.05		
Methanol Plant Extract (Ideal Dose)	0.93±0.11	2.58±0.09	+16.25	-253.42

Several studies showed the calendula contain the flavonoids as active compounds, which may be responsible of the biological activities (bactericidal, anti-inflammatory, antiviral, anti-tumor, anti-mutagen and anti-genotoxic) (19, 20, 21, 22).

The flavonoid compounds, in addition to antioxidant activity, serve other bio-functions like anti-mutagenic and anti-tumor activities (23, 24). Antioxidant systems are frequently inadequate, and damage from reactive oxygen species is proposed to be involved in carcinogenesis (25). Reactive oxygen species can damage DNA and division of cells with unrepaired or misrepaired damage can lead to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression may result. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species increases the risk of DNA damage and this will lead to mutations, and can increase the exposure of DNA to mutagens (26, 27).

2- Micronucleus index: The micronucleus formation was assessed in polychromatic erythrocytes of bone marrow, in which the mitoxantrone caused a significant increase of micronucleus frequency (0.0160 micronucleus/ cell) as compared to distilled water negative controls (0.0070 micronucleus/cell). The first dose of methanol extract (37.5 mg/kg) had no effect on the frequency of micronuclei, while the next two doses (75.0 and 112.5 mg/kg) showed a significant increase of such frequency as compared to the corresponding negative control, (Table 5).

Table (5): Micronucleus formation in bone marrow cells (mean ± standard error) of albino male mice treated with methanol of *Calendula officinalis,* distilled water (negative controls) and mitoxantrone drug (positive control).

Groups		Dose (mg/kg)	Mean ± Standard Error (%)	Treatment Effeciency
Positive Control(mitoxantrone Drug)		0.33	0.0160±0.0007	+128.5710
Negative Control (Distilled Water)		0.00	0.0070±0.0004	
	Methanol Extract	37.5	0.0075±0.0006	+7.1428
	Methanol Extract	75.0	0.0115±0.0006	+67.8570
	Methanol Extract	112.5	0.0117±0.0006	+67.8570

- Extracts-mitoxantrone interactions (micronucleus Index): For methanol extract, neither pre nor pos-treatment were able to show a significant reduction of micronucleus formation as compared to the corresponding controls, (Table 6).

Table (6): Micronucleus formation in bone marrow cells (mean ± standard error) of albino male mice after interactions (pre- and post-treatments) between the ideal dose (37.6 mg/kg) of methanol extract of *Calendula officinalis* and mitoxantrone drug.

Groups	Mean ± Standard Error (micronucleus/cell)		Treatment Efficiency (%)	
	Pre-	Post-	Pre-	Post-
Control I (Distilled Water)	0.0117±0.0006	0.0070±0.0004		
Methanol Plant Extract (Ideal Dose)	0.0102±0.0004	0.0072±0.0005	-12.71	+4.28

Mitoxantrone drug had cytotoxic effect causing decreased in mitotic index with increase in micronucleus formation, because of this drug generates DNA double strand breaks through its effect on the formed topoisomerase II cleavable complex, and such effect mimics the action of ionizing radiation. A radiation-induced DNA damage has been ascribed to the production of cytokines, in particular, IL-6 and its induction of release has been correlated with the formation of cyclobutane pyrimidine dimers (28, 29).

In pre- and post treatment of calendula methanol extract showed ability to minimized cytotoxic effect of mitoxantrone drug, that may result from electrophile scavengers and antitoxidation properties of the plant, therefore several biological functions can be achieved; a stimulation of the immune system, inhibition of DNA adducts with carcinogens, inhibition of hormonal actions and metabolic pathway associated with the development of cancer, and inducing phase I or II detoxification enzymes (30, 31).

3- Sperm head abnormality index: Different sperm-head abnormalities and frequencies were encountered (Figure 2) as a result of treatment with mitoxantrone (7.15%), distilled water (6.80%) and the three doses (37.5, 75.0 and 112.5 mg/kg) of methanol extract (6.35, 7.20 and 8.95%, respectively). However, these differences did not maintain any significant level (P > 0.05), (Table 7).

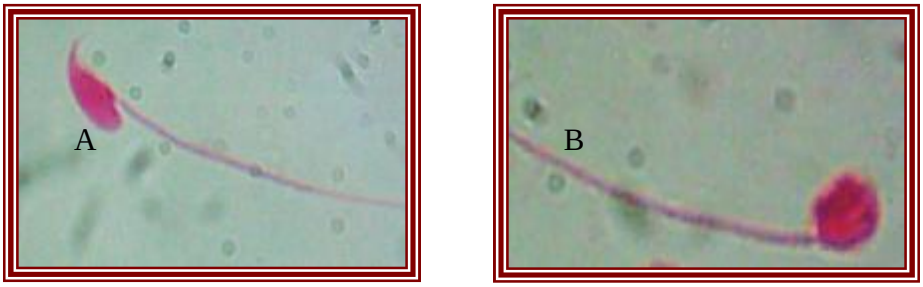

Figure (2): Normal (A) and abnormal (B) Sperm-heads in mice treated with mitoxantrone.

Table (7): Sperm-head abnormalities (mean ± standard error) in albino male mice treated with methanol extract of *Calendula officinalis,* distilled water (negative controls) and mitoxantrone drug (positive control).

Groups		Dose (mg/kg)	Mean ± Standard Error (%)	Treatment Effeciency
Positive Control (mitoxantrone Drug)		0.33	7.15±0.74	+5.15
Negative Control (Distilled Water)		0.00	6.80±0.82	
	Methanol Extract	37.5	6.53±0.63	-4.11
	Methanol Extract	75.0	7.20±0.47	+5.88
	Methanol Extract	112.5	8.95±0.24	+31.62

- Calendula Extract-Mitoxantrone Interactions (sperm-head abnormalities): Interaction treatment of methanol extract failed show a significant difference in the frequency of sperm head abnormalities between the treated animals and the corresponding controls, (Table 8).

Table (8): Sperm-head abnormalities (mean ± standard error) of albino male mice after interactions (pre- and post-treatments) between the ideal dose (37.6 mg/kg) of methanol extract of *Calendula officinalis* and mitoxantrone drug.

Groups	Mean ± Standard Error (%)		Treatment Efficiency (%)	
	Pre-	Post-	Pre-	Post-
Control I (Distilled Water)	4.9±0.2	5.2±0.1		
Methanol Plant Extract (Ideal Dose)	4.7±0.1	4.7±0.2	-4.04	-10.57

The effects of flavonoid on reactive oxygen species can be achieved through several metabolic pathways. Flavonoids may have an additive effect to the endogenous scavenging compounds by increasing the activity of detoxifying enzymes such as glutathione transferase (GST) and superoxide dismutase (SOD). Flavonoids have also shown to inhibit nitric oxide in a dose dependently manner and also it has been reported that flavonoids have the most potent inhibitor of xanthineoxidase. Another possible mechanism by which flavonoids act on reactive oxygen species is through interaction with various enzyme systems, and some effects may be a result of a combination of radical scavenging and an interaction with enzyme functions (32). When reactive oxygen species are associated with the presence of iron, lipid peroxidation results; however, specific flavonoids are known to chelate iron, thereby removing a causal factor for the development of free radicals, and it has been stated that

flavonoids, as antioxidants, can inhibit carcinogenesis in this manner (33). The flavonoids content which possess anti-mutagenic and anti-tumor activities, play important role in the detoxification of mutagenic compounds (34, 35).

Summary of this study, *calendula officinalis* plant extract play important role in reduce mutagenic effect of mitoxantrone drug without any side effect as a result from pharmaceutical properties of this plant.

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Bioinformatics of 14-3-3γ in Bovine

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Abstract

The 14-3-3 proteins are from families maintains regulatory molecules expressed in all eukaryotic cells. It was discovered before thirty years, it is attributes of 14-3-3 proteins are able to connect a large number of signaling proteins are functionally diverse, including kinases, phosphatases and transmembrane receptors. 14-3-3 proteins play an important role in a variety of vital regulatory processes, such as protein regulation, apoptotic cell death and cell cycle control. The aim of this study, the bioinformatics of structure protein 14-3-3γ, in addition to that it provides insights into the mechanisms of their functions. We use some web and programs to verify from protein of 14-3-3γ, domains, post-translational modifications, Secondary structure and Subcellular local prediction 14-3-3γ. Our result show positive areas in helices structures of 14-3-3γ, prediction the subcellular localization of 14-3-3γ is in the nucleus and cytoplasm and Modification phospho site. Therefor the biological activities of 14-3-3 proteins involve protein motifs outside of the phosphoamino acid binding domain.

Introduction

The 14-3-3 proteins are a family of regulatory proteins, found in the all eukaryotes. 14-3-3 proteins serve as molecular scaffolds by modification the conformation of its binding partners (1). The 14-3-3 proteins are highly conserved over a wide range of eukaryotic species and many organisms express multiple isoforms. While lower eukaryotes, for example, yeast, containing only two genes 14-3-3, either eukaryote Supreme possess up to 15 genes from 14-3-3 genes. For instance, in mammals, seven isoforms (β, ε, η, γ, τ, ζ and σ) have been identified to date. With the exception of mammalian sigma isoform, all 14-3-3 proteins can form both homo- and heterodimers (sigma isoform preferentially forms homodimers).During the formation of a functional from a wide variety of binding partners, 14-3-3 proteins are participating, in many biologically important processes, including regulation of the cell cycle, control of metabolism, apoptosis, control of gene transcription and the subcellular localization of their substrates to enhance a particular signal or sequester and inhibit a particular pathway (2).

In silico prediction and analysis of 14-3-3γ characteristics involved a variety of web tools and software. BLAST searches were carried out with the 14-3-3γ DNA, mRNA or protein sequences against GenBank databases. None of the tools predict the structure of genes different from those already known of the 14-3-3γ. In short, bioinformatics is a management information system for molecular biology and has many practical applications.The aims of bioinformatics are organised data in a way that allows researchers to access existing information and to submit new entries as they are produced, such as, the 3D macromolecular structures for Protein Data Bank. The second aim is to develop tools and resources that aid in the analysis of data. For instance, having sequenced a particular protein, it is of benefit to compare it with previously characterised sequences. This needs more than just a simple text-based search and programs such as FASTA and PSI-BLAST (3) must consider what comprises a biologically significant. The third aim is to use tools to analyse the data and interpret the results in a biologically meaningful manner. Usually, biological studies examined individual systems in detail and often compared those with just a few of which are relevant. In bioinformatics, recently conduct global analyses of all the available data with the aim of uncovering common principles that apply across many systems and highlight novel features.

Protein sequence databases: Classified databases, protein sequence to the primary, secondary or compound. Preliminary databases containing more than 300,000 proteins sequence and function as a repository of raw data. Some stores are more common, such as SWISS-PROT and PIR International (4)McGarvey et al, annotate the sequences as well as a description of the functions of proteins and the building domain, and post-translational modifications. Composite databases such as OWL (5) Bleasby et al and the NRDB (6)Bleasby and Woottontranslate the data sequence and the candidate of the primary databases to produce different groups of combined non-redundant, which is more complete than the individual databases include the data sequence of the protein coding regions are translated in the databases of DNA sequences. While, the secondary databases contain information derived from the sequence of the protein and help the user determine whether a new sequence belongs to the family of a protein known. One of the most popular is PROSITE (7), a database of short sequence patterns and profiles that characterize biologically significant sites in proteins. PRINTS (8) Offered in this concept, a compendium of protein fingerprints conservation groups of motifs that characterize a protein family. Usually are separated motifs along the protein sequence, but may be contiguous in 3D space when it is folded protein. Using several motifs can be encoded fingerprints of protein folds and functions more flexible than PROSITE.Finally, Pfam(9) contains a large collection of multiple sequence alignments and profile Hidden Markov Models covering many common protein domains. PFAM-A includes precise alignments collected manually while, PFAM-B is a compilation of automated full database SWISS-PROT. Low complexity and disordered regions were predicted by SEG (10). And the ability to form disulphide bonds was predicted by Predict Protein Disulfide(11). To predict the ability to direct a particular organelle SignalP 3.0 prediction

was carried out (12). Identified the structural properties of proteins by synchrotron small angle X-ray scattering (SAXS) and analytical ultracentrifugation (13).

Structural databases: The Protein Data Bank, PDB, provides a primary archive of all 3D structures of macromolecules such as proteins, RNA, DNA and various complexes. Most of the ~13,000 structures are solved by x-ray crystallography and nuclear magnetic resonance (NMR), but also included some theoretical models. As the information contained in individual PDB entries can be difficult to extract, PDB sum (14) provides a separate Web page for every structure in the PDB displaying detailed structural analyses, diagrams and data on interactions between different molecules in a given entry. Three major databases rating of the structure of proteins in order to determine the structural and evolutionary relationships: CATH (15), SCOP (16), and FSSP databases (17). Include all structural hierarchical classification in terms of increasing groups of proteins in the similarity in the lower levels of the classificationtree. In addition, numerous databases focus on particular types of macromolecules. The database includes these nucleic acids NDB for structures related to nucleic acids, the HIV protease database (18) for HIV-1, HIV-2 and SIV protease structures and their complexes, and ReLiBase(19) for receptor-ligand complexes.

Protein-protein interactions: Generally, proteins must interact with each other to carry out the biochemical. Thus, mapping out protein-protein interactions are another important aspect of proteomics. Protein interactions are considered among the strong interactions that allow the formation of stable complexes and weaker ones that exist transiently. Proteins involved in the formation of complexes are usually more tightly Co regulated in expression of those involved in transient interactions. Proteins can react with other proteins to form pathways and these interactions are stored in specific databases. APID2NET (20) and CytoScape(21) integrate several of these databases. All the protein-protein interaction information for 14-3-3γ was combined and visualized using CytoScape. BINGO CytoScape plugin (22) was used to retrieve. The Gene Ontology classifications of all the interactors and compared them to the gene classification frequencies in the human genome (23).

Materials and Methods

In silico prediction and analysis of 14-3-3γcharacteristics involved a variety of webtools and software

Protein structure: There are four main levels of protein structure. The amino acid sequence is generally referred to as the primary structure. The secondary structure occurs when the sequence of amino acids is linked by hydrogen bonds, forming mainly alpha helices, beta strands and loops or coils. The secondary helical, strand and loop structures interact with each other to assemble into a compact globular structure called the tertiary structure.

Secondary structure: There are several methods available for predicting the ability of a sequence to form alpha-helices and beta-strands. Jpred(24). PHD Predict Protein (25). PSIPRED (26) and Network protein sequence analysis (http://npsa-pbil.ibcp.fr/cgi-bin/npsaautomat.pl?page= npsa_gor4.html/). Were used to predict the 14-3-3γ

secondary structures by using the default values. Jpred receives the amino acid sequence as an input, and predicts the secondary structure based on two neural networks systems, a form of learning method, and filtered by PSI-BLAST. PHD Predict Protein uses three neural networks also filtered by PSI-BLAST whilst Network protein sequence analysis was used to predict coiled regions by comparing the input sequence with a database of known parallel two-stranded coiled-coils. Transmembrane helices in integral membrane proteins are composed of stretches of predominantly hydrophobic residues separated by polar connecting loops and were predicted by PHD htm Predict Protein (25) and by TM Pred(27).

Domains: Assigning sequence to protein family is a very valuable way of predicting protein function. Database searches can also be used to find specific protein families or domains through the use of profiles. Protein profiles are built from common patterns observed in multiple sequence alignments of related sequences. Analysis of the domain search using the SWISS-MODEL program http://swissmodel.expasy.org/ also compared to a protein family database, Pfam database (28), and to an integrated resource of protein family and domains, InterPro(29).

Post-translational modifications: To identify phosphorylation sites were uploaded to the phosphorylation site database PHOSIDA (www.phosida.com), and the evolutionary analysis was performed as previously described (30). And the ability to form disulphide bonds was predicted by Predict Protein DiSulFind(11) and Scratch protein predictor program (http://scratch.proteomics.ics.uci.edu/).

Sub-cellular localization: To determine the sub-cellular localization in 14-3-3γ as (Mer& Andrade-Navarro, 2013) (31) and by using WoLF PSORT (Protein Subcellular Localization Prediction) (http://www.wolfpsort.org/).

Phylogeny of the 14-3-3**γ**: 14-3-3γ protein is ubiquitous and detected in practically in different eukaryotes .The tree was built by phylogenetic analysis of the primary structure using the phylogenetic analysis. (http://www.phylogeny.fr/version2_cgi/simple_phylogeny.cgi). And PhyML 3.0 program(http://www.phylogeny.fr/version2_cgi/one_task.cgi?workflow_id=b2b03c37ce3013783dcb9d5b2ddba7cc&tab_index=3).

Results and Discussion

Similar protein of 14-3-3**γ**, domains and post-translational modifications: Protein regions and domains provide valuable information indicating possible large regions and architectures that have the same or similar function. The 14-3-3γ protein sequences were compared according to SSWIS, Pfam domain database and InterPro integrated domains and patterns database. However, Gene expression can be regulated by protein-protein interactions and post-translational modifications such as phosphorylation. Most post-translational modifications can be predicted by the presence of specific motifs. Motifs are conserved elements of a sequence alignment, which are likely to be a structural or functional region, and can be used to predict further the occurrence of similar motifs on other protein sequences. Modification phospho site are predicted to

be phosphorylated by the same kinases (Figure 1), in phosphoserine (57-232aa) and phosphotheronine(145aa). Also, Modification phospho site are shown in (Table 1).

In addition, multiple phosphorylation sites were predicted for 14-3-3γ (S38, S58, S59, S64, S65, S71, S102, S149, S150, S155, S215, S219, and S235).

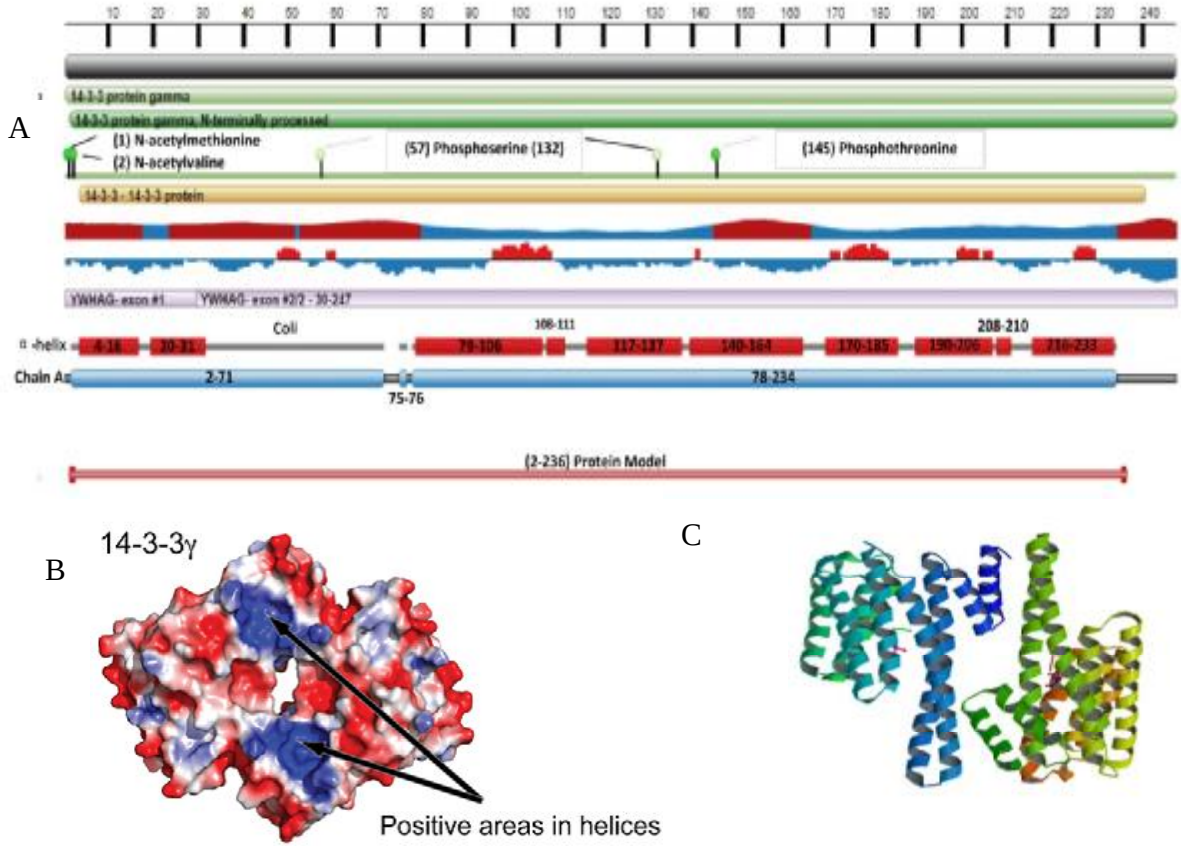
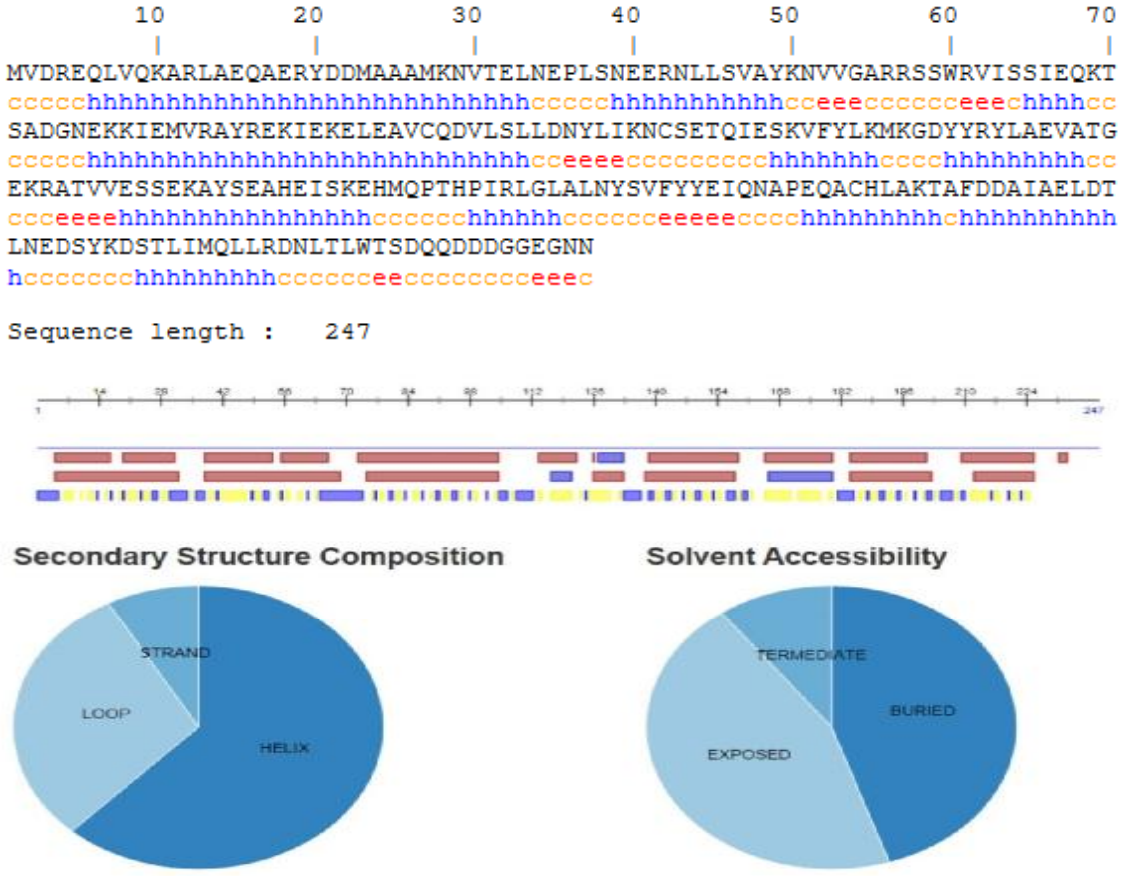


Figure 1. Schematic structure of the 14-3-3γin *Bostaurus* ,A. Domains are represented by rectangles where the 14-3-3γ protein gamma, N- terminally processed domain is in green, α- helix domains in red and chain A domains in blue. The phosphorylation site position shown in Colum green,B. predicted positive areas in helices where shown in the X-ray structures of 14-3-3γ (PDB 2B05)C. Crystal structure of the 14-3-3γ protein.

Table 1: Overview of protein 14-3-3**γ** in *Bostaurus* from NCBI

Display	Overview of Vital Regulatory Processes
Protein Name:Recommended name, Alternative name, Short name:	14-3-3 protein gamma, Protein kinase C inhibitor protein 1, KCIP-1
Protein Group Accession	P68252
Modification phospho site	NVVGARRSSW RVISSIEQKT SADGNEKKIEMVRAYREKIE KELEAVCQDV LSLLDNYLIK NCSETQIESK VFYLKMKGDYYRYLAEVATG EKRATVVESS
Molecular Function	Insulin-like growth factor receptor binding, protein domain specific binding
Biological Process	Regulation of the cell cycle, control of metabolism, apoptosis and control of gene transcription
14-3-3 regulating cell-signalling	(1) Akt-Regulated 14-3-3 Protein: The binding of 14-3-3 in an Akt-phosphorylation-dependent manner can alter the trafficking of membrane proteins either directly or indirectly, depending on whether the site of phosphorylation, and thus 14-3-3 binding, is on the membrane protein itself or on an accessory protein. (2)14-3-3 regulate Cdc25C by sequestering it to the cytoplasm, thereby preventing the interactions with CycB-Cdk1 that are localized to the nucleus at the G2/M transition. (3)Dephosphorylated BAD forms a heterodimer with Bcl-2 and Bcl-xL, inactivating them and thus allowing Bax/Bak-triggered apoptosis. When BAD is phosphorylated by Akt/protein kinase B (triggered by PIP3), it forms the BAD-(14-3-3) protein heterodimer. This leaves Bcl-2 free to inhibit Bax-triggered apoptosis. (4)14-3-3 motifs are important for the feedback regulation of Raf kinases in the ERK1/2 pathway. (5)Ribosomal S6 Kinase (RSK) regulates 14-3-3 binding to SOS1 by promoting phosphorylation of Ser1134 and Ser1161.
MW/PI	4.80 / 28252.57
Pfam IDs/ Sequence length	PF00244/247AA

Secondary structure: Not much information is known about the structural features of the 14-3-3γ. Only the translation catalytic domains structures have been solved. From the Network protein sequence analysis (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html/) and Scratch protein predictor program (http://scratch.proteomics.ics.uci.edu/).were used to predict the primary and secondary structure. 14-3-3γ secondary structure was predicted to be mainly Alpha helix (Hh):138 is 55.87%,Extended strand (Ee):24 is 9.72%, Random coil (Cc):85 is 34.41% (Figure 2). The prediction of secondary structure provides valuable information on possible structurally conserved regions which may have a particular function, with disordered regions often found to be the site for protein -protein interactions and post-translational modifications.



GOR4:
Alpha helix (Hh):138 is55.87%
Extended strand (Ee):24 is 9.72%
Random coil (Cc):85 is 34.41%

Figure.2. Secondary structure prediction of 14-3-3γ in *Bostaurus.*

Phylogeny of the 14-3-3**γ**: 14-3-3γ protein is ubiquitous and is detected in practically ten species in eukaryotes. (Fig.3) represents the phylogenetic tree depicting homology of the primary structure of different eukaryotes :*Homo sapiens* (GI: 119592210), *Bostaurus* (GI: 71153781), *Macacamulatta* (GI: 302565023), *Mus musculus*(GI: 31543976), *Pan troglodytes* (GI: 332865957),*Rattusnorvegicus* (GI: 9507245), *Pongo abelii* (GI: 68565133), *Callithrix jacchus* (GI: 532523554),*Ailuropodamelanoleuca* (GI: 301776182) and *Myotisbrandtii* (GI: 521033951).The tree was built by phylogenetic analysis of the primary structure using the phylogenetic analysis. Alignment was performed on the basis of Gblocks 0.91b matrices with parameters gap open = 10 and extension gap = 0.02 and provides similar results for both Matrices, which are displayed as a tree using the PhyML 3.0 program.

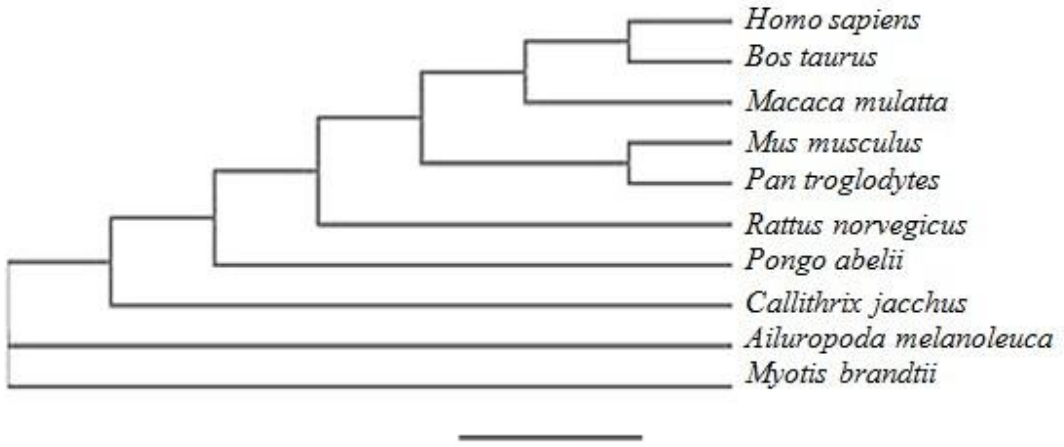


Figure 3: Phylogenetic tree of ten species of 14-3-3**γ** constructed using phylogenetic analysis (PhyML 3.0) as a database. The scale corresponds to 0.02 replacements in the ancestral polypeptide per amino acid residue.

Subcellular local prediction14-3-3**γ**: The subcellular localization of 14-3-3γis in the nucleusand cell cytoplasm in different species of eukaryotes as (Table.2).

Table 2: Subcellular localization prediction

ID	Site	Comments
143G_BOVIN	Cytoplasmic	[Uniprot]SWISS-PROT45
143G_RAT	Cytoplasmic.	[Uniprot] SWISS-PROT45:
PSB1_MOUSE	Cytoplasmic and nuclear.	[Uniprot]SWISS-PROT45
PSA3_RAT	Cytoplasmic and nuclear.	[Uniprot]SWISS-PROT45
CAH1_HUMAN	Cytoplasmic	[Uniprot]SWISS-PROT45
IFEB_ASCSU	Cytoplasmic	[Uniprot]SWISS-PROT45
CAH1_SHEEP	cytoplasmic	[Uniprot]SWISS-PROT45

Gene ontology analysis of 14-3-3γ proteins: To explore the biological functions of the 14-3-3γ binding proteins we used gene ontology software and analyses molecular function (Figure 4). Among molecular Function of 14-3-3γ, for example: transcription factor activity, protein binding, Enzyme regulator activity, Serine / Threonine Kinase Inhibitor activity and Protein Kinase C Inhibitor Activity.

Figure 4: Gene ontology analysis of Molecular function of proteins 14-3-3γ_BOVIN.

Scansite Molecular Weight & Isoelectric Point Calculator: The molecular weights here are derived from the 1997 IUPAC standard atomic weights, assuming pH = 7.0. The isoelectric points of 14-3-3γ are calculated using the algorithm from ExPASy's Compute pI/Mw program, (Table 3).We have added the option to include phosphorylation by using pKa = 2.12 for the first ionization and pKa = 7.21 for the second.

Table 3: Molecular Weight and Isoelectric Point of 14-3-3**γ**.

Phosphates	Molecular Weight	Isoelectric Point
0	28241.1913	4.80
1	28319.1553	4.75
2	28397.1193	4.70
3	28475.0833	4.66
4	28553.0473	4.61
5	28631.0113	4.57
6	28708.9753	4.53
7	28786.9393	4.48
8	28864.9033	4.44

The majority of previous studies of 14-3-3γ subunits focused on the biochemistry of the complex, mainly in lower eukaryotes. It was essential to catalogue the characteristics the 14-3-3γ subunits in higher eukaryotes. Studies aimed at the identification of the processes that 14-3-3 proteins are involved in have identified a large number of potential client proteins, suggesting that they regulate most cellular processes. Indeed, they have been implicated in regulating metabolic pathways, redox regulation, transcription, RNA processing, protein synthesis, protein folding and degradation, cell cycle, cytoskeletal organization, and cellular trafficking (32).

In this study, in *silico* analysis of the 14-3-3γ described the related sequences or pseudogenes, which are thought not to be, expressed which revealed 14-3-3γ in bovine.The crystal structures of all of the 14-3-3 proteins have been resolved, and all have the same structural organization. Moreover, the 14-3-3 residues that are keys for binding phosphoamino acids and that form the phosphoamino acid binding motif corresponds to Lys49, Arg56, Arg127, and Tyr128 of human 14-3-3 ξ, and these are completely conserved in all known 14-3-3 proteins (33).

Studies with dimerization-deficient 14-3-3 mutants revealed a significant role of dimerization for phosphorylation-dependent target binding (34). Muslin et al discovered that the recognition of a target protein by 14-3-3 proteins are dependent on a phosphoserine and identified a putative motif for 14-3-3 binding and showed that phosphorylated serine is crucial for recognition by 14-3-3 (35). It is now well established that phosphorylation of serine/threonine is a prerequisite for recognition by 14-3-3 proteins, but there is one report where phosphorylation of the target protein is not required for 14-3-3 binding (36).Phosphorylation-dependent association with binding partners forms the mechanistic basis for the fundamental role for 14-3-3 in modulating

kinase signaling pathways. A large number of 14-3-3 binding motifs have been established (33).

Protein kinase C (PKC) comprises a family of phospholipid-dependent serine/threonine kinases that regulate diverse cellular functions. On the basis of their requirement for Ca2+ and diacylglycerol and their structural characteristics, PKC isoforms involved in distinct roles in signal transduction pathways are classified as conventional (PKCα, βI,βII and γ) (37).

Althoughthe common binding motif presents on client proteins and the common phosphoamino acid binding domain of all of the family of 14-3-3 proteins, each of the family members appears to interact preferentially with specific intracellular signaling proteins.

It will probably have a role in the synthesis of milk protein. One of the recent studies found that 14-3-3γ participation in the synthesis of milk protein and cell proliferation (38), but how involved in 14-3-3γ Signal transduction pathway of milk synthesis still need further studies.

The human 14-3-3 isoforms share nearly 80% similarity of their amino acid sequence, within which there are five highly conserved blocks, as defined by Wang and Shakes (39).Since the 14-3-3γ interaction residue information was derived from the N-terminal 40 amino acids, the transformation ability of 14-3-3γ requires two specific residues(40). The secondary structure prediction was identical between both N-termini. It was found that the 14-3-3γN-termini is extremely evolutionarily conserved at the primary, secondary and 3-D structures, the few amino acid variations are enough to alter completely the prediction of the size and number of protein-binding clefts. This indicates that the different protein binding clefts might have an effect on different functions. Consistent with this is the observation that there is only a 15% overlap in the proteins that interact with 14-3-3ε and that also interact with 14-3-3 γ, -ξ, and -σ(41). Hence, the cadre of proteins that is bound by each of the 14-3-3 isoforms is distinctive, suggesting that they have different biological activities and that the specificity of their interactions with cellular proteins is unique to each isoform.

Future studies will also have to determine how the strength of patterns of functional divergence at the sequence level accurately describes real divergence at the Biomolecular level. The Insertion sequence, phylogenetic precision, and models of molecular evolution necessarily affect signals of functional variation at the level of the sequence. Some details of these signals are understood from a phylogenetic and protein perspective (42, 43).However, there is a need more detail before we understand the relationship between patterns of sequence and molecular biological characteristics that selective or neutral forces as a result of the shape of the patterns themselves(44).

Conclusion

The biological activities of 14-3-3 proteins involve protein motifs outside of the phosphoamino acid binding domain. Understanding how structure determines 14-3-3 function is crucial to understanding how they function in lactogenic.

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Construction of recombinant NDV expressing VP2 gene of vvIBDV in two insertion sites

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Abstract

The use of Newcastle disease virus (NDV) as a viral vector for vaccines has developed and reached mature stages. However, Majority of recombinant viruses as vaccine vectors are unable to achieve desired immunological efficacy because of the low expression level of protective pathogen antigens, which leads to limit the use of NDV as a live vaccine vector.VP2 protein is the major structural protein of infectious bursal disease virus (IBDV), has good antigenicity, in the host can induce neutralizing antibodies, thus VP2 gene is an important purpose of genetic research for IBDV vaccines. In this study, we successfully constructed recombinant Newcastle disease virus (NDV) expressing (vvIBDV) protein VP2 in two different locations (rClone30-VP2), between HN and F, and between HN and L. By using PCR, Hpa I andMlu I were introduced to the 5' end and 3' end of VP2 gene.Real-time PCR test results showed that rClone30-VP2 mRNA transcript levels of both recombinant viruses were significantly higher than the control group rClone30.In summary, this study used a reverse genetics system to establish NDV expressing VP2 of IBDV and proved that the expression levels of genes were higher than the control recombinant virus.It provides a new way to lay a foundation for the development of NDV-IBDV live vector vaccine.

Introduction

Newcastle disease virus is a single negative-strand RNA virus, Newcastle disease virus (NDV) can infect almost all birds, poultry is highly susceptible to infections and the disease is highly infectious and fatal. The disease is one of the most important infectious diseases of poultry, mainly in Asia, Africa, and the Americas; it is enzootic in many countries. Newcastle disease outbreaks have been a threat to the poultry industry all over the world. Depending on the virulence of the various strains of the Newcastle disease virus, it is divided into three types: weak strain, moderate virulence strains and virulent strain (1).

In China there are LaSota strains (2), goose source NA-1 (3) and ZJI strains (4) and many other viruses were successfully rescued. And after salvation virus also successfully inserted foreign genes and NDV genome has been successfully expressed, such as IBDV VP2 Gene (5), the green fluorescent protein gene (6), AIV HA gene (7). The use of vaccines against infectious diseases is typical of modern medicine success. For treating poultry, the vaccine is effective and inexpensive preventive tool. Generally speaking,

there are two main methods for producing vaccines: one is to use the engineered live attenuated vaccine; the other is a chemically inactivated virus using an inactivated vaccine. However, the traditional method of producing vaccines regarding safety, efficacy and cost many respects incomplete. Such as the inability to distinguish between vaccine strains and field strains of infection is the use of a live attenuated vaccine is a limiting factor. Traditional vaccine because of the poor results, there are security risks cannot effectively deal with antigenic variation and emerging new strains, so there is an urgent need to develop a more effective new vaccine.

With the extensive application of recombinant DNA technology, resulted in the safety, efficacy and low cost there is a significant increase of the live virus vaccine. By deleting the gene in the genome of the virus, the mutant gene can produce better safety performance; Recombinant DNA technology using a virus as a vector, expression of exogenous viral protective antigens, and the new vaccine is called vector vaccines. For a long time, scholars from various countries by means of biotechnologies developed a different expression system of recombinant avian pox virus, a recombinant HVT / MDV Recombinant avian adenovirus, recombinant NDV and other live vector vaccine have been successfully constructed (5,8,9). In addition, more and more studies showed NDV viral proteins as the expression ofthe exogenous live viral vector is a good choice (10). Recombinant strain LaSota as a poultry vaccine vector has the following advantages (5): 1, NDV attenuated vaccine has long been used for poultry vaccination, and its safety and efficacy has been proved; 2, NDV genetic material is relatively stable, there is only one serotype,recombination, and reversion of virulence between strains highly unlikely;3, the replication process is completed within the cytoplasm, from RNA to RNA, DNA, and cell genomic integration phase may not exist ; 4, NDV attenuated vaccine induces the body at the same time humoral immunity, mucosal immunity, and cellular immunity formation; 5, easy inoculation, through drinking water, spray,nasal, eye or injected in various ways to the seedlings; 6, NDV have high titers of embryo growth characteristics, relatively low production cost.

Infectious bursal disease virus (IBDV) causes infectious bursal disease of poultry (IBD), mainly against the immune organ, a direct result of the incidence of infection in chickens or death is more important cause of immune-suppression in chickens, leading to increased susceptibility to other pathogenic factors and the immune response capacity to the vaccine is reduced. IBDV belongs to double-stranded RNA virus family (Birnavirdae family), avian double RNA virus genus (Avibirnavirus). Virus-free capsule, having a single capsid. No surface protrusions, showing three-dimensional icosahedral symmetry. Virus particle diameter of about 60 mm, 32 shell particles composed of nucleocapsid, 92 morphological subunits (11).

VP2 constitutes the outer capsid of the virus, 51% percent of total protein of the virus are the main components of the viral capsid, is also the main structural protein of IBDV and functional proteins, VP2 and virus neutralizing antibody induction and recognition, the virus mutates, viruses, antigenic drift, induction of apoptosis, are closely related (14,15). VP2 of IBDV regulates cell tropism, and the molecular basis is being locked in

253, 279, 284, 330-amino acids. Precursor proteins by theprocess of post-translational modifications are classified into mature VP2, VP3, and VP4 proteins. Gene fragment encoding the VP2 protein of about 1362bp (12, 13).

Since IBDV in the external environment is more stable, long-term presence in the field of infection, so disinfection, in general, is difficult to control. The disease is mainly carried out by immunization prevention and control, use the general traditional vaccines (including inactivated vaccines and live vaccines) were immunized, but because of superior strains and mutants, immune failure often occurs, as well as poor security and other issues (16,17). In recent years, due to the development of breakthrough in genetic engineering vaccines, DNA vaccines (18,19), subunit vaccines (20,21), especially recombinant viral vector vaccines and DNA vaccines are relatively rapidly developing (22,23,24), However, these vaccines as compared with the traditional vaccines are having varying degrees of defects, has not been widely used in clinical application.

In recent years, with the rapid development of molecular biology, recombinant NDV live vector vaccine has made a significant progress. The insertion position ofthe foreign gene may also have a significant impact on its expression level, is generally believed that the 3' end of a foreign gene inserted into the higher position closer to the genome of its protein expression level. But the expression of exogenous immunogen some high protein may affect the recombinant virus in the body of infection, replication, and even genetic stability.

In this study, we have inserted VP2 gene in the recombinant NDV genome in two different positions, between F and HN, and between HN and L gene, then compared them to the recombinant NDV as a control. Real time-PCR test results showed that the expression level of Newcastle disease virus expressing VP2 gene was significantly higher thanthecontrolgroup, the two insertion site tested showed a convergent expression level ofthe foreign gene of NDV / IBDVlives vector vaccine foundation, this provides an experimental evidence for *in vivo* immunization trials evaluating vaccines.

Materials and Methods

Virus strains and plasmids: NDV LaSota Clone30 vaccine strain, IBDV B87 strain, and strain HLJ07 were kept by our laboratory.

Full-length cDNA plasmid transcription Clone30 strain, pClone30, and pVP2 plasmid constructed and saved by our laboratory.

Media and biochemical reagents: rTaq DNA enzyme, dNTP Mixture, Oligo (dT) 18, a restriction enzyme (Hpa I, Mlu I,), T4 DNA ligase was purchased from the NEB, DNA molecular weight standards λ-EcoT14 marker, DL2000 marker, cloning vector pMD18-T simple vector purchased from TaKaRa Company.

Phenol reagents were purchased from Tianjin Hao Yang Biological Products Technology Co., Ltd., RNA enzyme inhibitors (RNase Inhibitor, RNasin), reverse transcriptase (M-MLV), RNaseA, RNA extraction reagent TRIzol were purchased from Promega. Agarose particles are commercially available from SIGMA Company. The plasmid small lift kit was purchased from TIANGEN companies. DNA extraction kit, PCR

product purification kit (purchased from Qiagen Inc.) and the plasmid big lift kit was purchased from OMEGA Company.
VP2 MAb by the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences as gifts.
Primer: Amplification primers for each gene are shown bellow: (synthesized by Invitrogen Corporation).

Table (1): the primers for gene amplification

Primer name Primer sequence (5'-3 ')
Primer (P1) HpaI
5'GTTAACTTAAGAAAAAATACGGGTAGAAGCCACCATGACAAACCTGCAAGATC 3'
MluI(P2)MluI
5' ACGCGT TTATGCTCCTGCAATCTTCAGGGGAGAG 3'
Primer 3 (P3)
5'TTAAGAAAAAATACGGGTAG3'

Table (2): Primers for real Time PCR

Target gene	GenBank accession number	Primer	Sequence(5′–3′)	PCR fragment length (bp)
VP2	JN127375	Forward Reverse	5'GATGCCAACAACCGGACC3' 5'TCCCATTGCTCTGCAGTG3'	1323

The vvIBDV VP2 gene was PCR amplified: According tothe VP2 gene sequence of the plasmid, pVP2 using primer design software Primer Premier 5.0 design PCR primers P1 and P2 for cloning complete VP2 genes. Synthesized by Invitrogen Corporation.

Temperature gradient PCR method using cDNA as a template, amplified VP2 gene, PCR reaction cycle parameters: denaturation for 3min at 95ºC, 94℃ for 1min, followed by annealing temperature: 50℃, 50.4℃, 51.3℃, 52.5℃, 54.2℃, 56.4℃, 58.9℃, 61.0℃, 62.7℃, 63.9℃, 64.7℃, 65℃, time for 1min, after 72℃ for 1min, 10 cycles, 72℃ then extended for 10min. (25 μl) the mixture is as follows:

10×PCR buffer	2.5 μL
dNTPs	1.0 μL
Template	2.0 μL (10 mg)
P1 upstream	1.0 μL(10 pmol)
P2downstream	1.0 μL(10 pmol)
Taq enzyme	0.5 μL
ddH2O	17 μL

After the mixture being amplified with temperature gradient PCR, take 5 μl of the mixture and run it through a 1% agarose gel electrophoresis fragment size. Annealing

temperature was 58.9 ºC for electrophoresis analysis of VP2 gene amplification effect was good.

PCR product purification: Take 20 μl of PCR product (a concentration of approximately 100 mg / μl) were purified by a purification step with reference Qiagen PCR Purification Kit instructions. In the PCR reaction solution, add 3 volumes of Buffer PCR-A, after mixing, thetube is transferred to the preparation, the prepared tube was placed in 2 ml centrifuge tube, centrifuged at 12000 r / min for 1min, discard the filtrate. The prepared tubes set back in 2 ml centrifuge tube, add 700 μl Buffer W2, centrifuge at 12000 r / min for 1min, and discard the filtrate. Then add 400 μl Buffer W2 and wash once. The preparation of a clean 1.5 ml tube placed in a centrifuge tube, floating in the center of the membrane dropped 25 ~ 30 μl of deionized water at room temperature for 1min. 12 000 r / min centrifugation for 1min eluted DNA. Take 1 μl purified products after a 1% agarose gel electrophoresis purification result, stored in -20 ℃ for use.

vvIBDV VP2 Clone Sequencing: The purified fragment was cloned into pMD18-T Simple Vector, following the step reference of TaKaRa Company pMD18-T Simple Vector's instruction manual. Ligation reaction as follows:

pMD18-T Simple Vector	1 μl (50 mg)
purified1 PCR product	1μl （50 mg）
Solution Ⅰ	5 μl
Sterile deionized water	3 μl

A total volume of 10 μl, after mixing, incubated at 16℃ for connection overnight. The ligation was completely transformed into*E.Coli* DH5α competent bacteria, specific transformation steps are as follows: Remove the two tubes of DH5α competent bacteria from -80℃ freezer, placed on ice to make it melt. The ligation product in step 3 was added to a total of 10 μl of competent bacteria in a tube, the other tube was left without any substance as a negative control. Incubated in the ice bath for 30min, and then at 42℃ water bath heat shock for 1min, remove it quickly in an ice bath, placed on ice for 2min. The added 200 μl sterilized LB liquid medium, at 37℃ for 1h after gentle shaking, join ligation products were applied to two DH5α containing 100 μg / ml Amp of LB solid medium plate, without any substance the DH5α were applied as a control, at 37℃ for 12h culture on a solid LB medium containing 100 μg / ml Amp and Amp's free.

Several single colonies were randomly picked randomly picked, inoculated in liquid LB medium containing 100 μg / ml Amp, the amplified cultured incubated overnight, and plasmids were extracted using a plasmid kit small lift (TIANGEN Corporation). Several individual colonies were randomly selected, were inoculated in liquid LB medium containing 100 μg / ml Amp's, shacked at 37℃ overnight, cells were collected plasmid was extracted, the specific steps mentioned kit (centrifugal columnar accordance TIANGEN small companies Plasmid) The instructions.

For convenience, the recombinant plasmid was named pMD-VP2, and then identifies the correct recombinant plasmid by the Shanghai Biological Co. Sequenced. By using DNAMAN software for sequence analysis.

rClone30-VP2 fragment ligation:

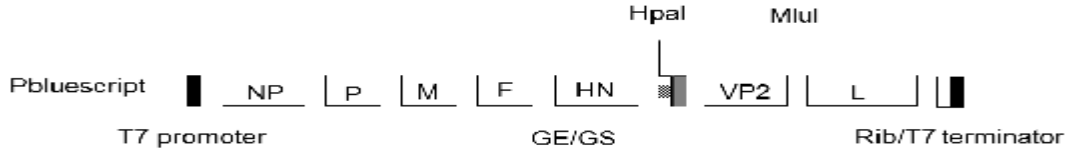
RClone30-VP2vector fragment	1.0 μL10 ng)
10× T4 Ligation Buffer	1.0 μL
T4 Ligase	0.5 μL
ddH_2O	7.5 μL

The mixture of rClone30-VP2 for ligation is as the following, total volume is (10 μL): After mixing incubated at 16°Covernight for ligation, the entire ligation product was transformed with *E.Coli* DH5 α competent bacteria with a control group, then applied to a medium containing 100 μg/ml Amp LB then inoculated in plates with solid medium, incubated at 37°C for 12~16h then the plasmid was extracted.

GE: TTAAGAAAAAA
GS: ACGGGTAGAAG

pClone30-VP2

Figure 1: Construction of recombinant NDV genome vector rClone30-VP2 between HN and F protein.



GE: TTAAGAAAAAA
GS: TACGGGTAGAA

Figure 2: Construction of recombinant NDV genome vector eClone-VP2 between HN and L protein.

The recombinant plasmid: Several single colonies were picked and inoculated in liquid LB medium containing 100 μg / ml Amp, the amplified cultured incubate overnight, plasmids were extracted with TIANGEN mentioned plasmid extraction kit, For convenience the recombinant plasmid was named rClone30-VP2, with the restriction enzyme Hpa I and Mlu I double enzyme digestion, incubated at 37°C for digestion for 2h. System mixture is as follows (10 μl):

Hpa I	0.5 μL
Mlu I	0.5 μL
10×K Buffer	1 μL
rClone30-VP2-IRES-VP2	6 μL
ddH_2O	2 μL

Applications P1 / P2 recombinant plasmid was identified by PCR cycling parameters.

Results andDiscussion

PCR vvIBDV VP2 gene amplification: IBDV VP2 gene amplification results shown in Figure 1. Figure 1 shows that in this experiment rescued pVP2 plasmid as a template, with the P1 and P2 amplified an approximately 1323 bp specific band.

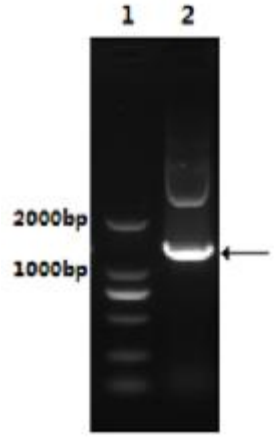


Figure 3: Amplification of VP2 gene of vvIBDV, Lane 1 DL2000 marker; Lane 2 vvIBDV VP2gene PCR product

Purification of PCR products: The PCR amplification product was digested in gel, on a 1% agarose gel electrophoresis, and correspond with the size of 1323 bp, results shown in Figure 2.

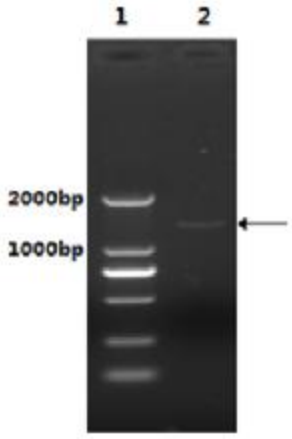


Figure 4: Gel extraction of VP2, Lane1 DL2000 marker; Lane2 recycling result.

VP2 Gene Sequencing: VP2 recovered in PCR product connected with pMD-T simple vector designated pMD-VP2, transformed into E. coli DH5α competent bacteria, screened on a solid LB medium containing 100 mg / L Amp, randomly a single colony was picked, theplasmid was identified by PCR, results to the sequenced plasmids were positive. Comparing with known gene sequence as expected, cloning success.

Because IBDV in the external environment is more stable, long-term presence in the field, so prevention and control are generally difficult to control. The disease mainly controlled and prevented through immunization, the general use of traditional vaccines (including inactivated vaccines and live vaccines) is for immunization. However, due to the presence of super strains and mutants, immune failure often occurs, as well as poor security aspects. With the breakthrough development of genetically engineered vaccines, recombinant viral vector vaccine has entered a mature stage of development. VP2 protein is encoded by IBDV A fragment ORF I IBDV major structural protein of about 40kDa and is closely related to VP2 and antigenic variation and virulence, cell tropism, pathogenicity and apoptosis (25). VP2 having multiple epitopes can induce the body to produce neutralizing antibodies, thus, VP2 genes are the main antigen gene recommended developing subunit vaccine and recombinant live virus vaccine (8, 21). Neutralizing epitopes are mainly located in the hypervariable region amino acids 206-350 (26). IBDV VP2 is an important virulence gene, VP2 of 253,279,284 amino acid is recognized virulence loci (27,28), but the three sites of the VP2 structure and function mechanism is unclear; VP2 immunogenic genetically engineered vaccine depends on different expression systems, the ability to induce protective immunity depends on the VP2 protein conformational epitopes. Many researchers have been inserted vvIBDV VP2 gene in the recombinant NDV in poultry into the body to produce a stronger immune level reached on IBDV vaccination. Previous studies showed that expression of recombinant NDV live vector vaccine vvIBDV VP2 gene of IBDV attacks can provide a certain degree of protective immunity. Therefore, this study chose to insert vvIBDV VP2 gene as a foreign gene into a recombinant NDV in two different positions.

When NDV nucleocapsid infects cells, the viral genome, and NP, P, L proteins form will transfer out of the virus into the cells at the first round of transcription initiation. First, a negative-strand RNA of virus genome works as a template, expressing positive-strand RNA transcription of various proteins. Since the RNA-dependent RNA polymerase(RdRp) during transcription can identify NDV genome GS, GE sequences encountered GS sequence to initiate transcription, transcription termination encounter GE sequence, when faced with GE, the sequence of the polymers may continue to find new GS, then gene transcription sequence start, transcription may stop leaving templates. Therefore, from the 3 'end to the 5' end of the virus geneme protein expression showed a decreasing trend. After NDV genome-inserted with a foreign gene, changing the length of the genome, but also introduce new GS, GE sequence, increases the chances of the polymers from the template, it may affect the viral replication cycle and self-proliferation efficiency.

Newcastle disease virus and other non-segmented negative-strand RNA viruses, are sharing the same transcription and replication principles, beginning at the end of each gene transcriptional regulatory sequences are conserved gene start signal (GS) and gene termination signal (GE), so that at each downstream gene transcription, is always better than less immediate area upstream of the transcription part, namely the phenomenon of polar attenuation of transcription. After inserting a foreign gene in NDV genome, not only changed the length of the genome, but also introduce new GS, GE sequence. Inserting a foreign gene near the 5 'end to ensure that the impact on virus replication is minimum and close to the 3' end is to ensure the maximum expression of exogenous genes. Considering all factors, this study inserted the VP2 gene close to the 5 'end of the viral genome between F protein and HN protein, and also between HN protein and L protein, use Hpa I and Mlu I restriction sites to insert a foreign gene, so as to ensure the virus does not replicate in a greater impact and at the same time can get the expression level of exogenous gene as much as possible.

Newcastle disease virus is a non-segmented negative-strand single-stranded RNA viruses, the genome sequence through gene start (GS), gene terminator sequence (GE) and the intergenic region (IG) The genome is divided into six separate transcription units these sequences for gene replication and transcription is essential. Both ends of the inserted gene sequences must have GS and GE sequences, genome recombinant viruses in line with the "six bases" rule. By analyzing the NDV Clone30 strain GS, GE sequence inserted foreign genes determine GS used, GE sequence. With the addition of exogenous gene GE action is to terminate before the HN gene transcription, and, therefore, choose their own GE F gene sequence TTAAGAAAAAA.

The NDV F protein structure is a very important cleavage site, it is to determine the key parts of the virulence of the virus; the F protein is located in the first 112-117 amino acid residues. Virulent strains and the strains premise F0 protein can be recognized by a wide range of host protease and cleavage, and attenuated strains F0 precursor protein must be in the presence of specific proteolytic enzymes to normal cleavage.

This study was designed to evaluate the changes in the expression level of recombinant viruses by Real time-PCR and indirect immuno-fluorescence methods, namely from the comparative gene and protein levels of those two viruses. The indirectimmunofluorescence technique(IFA), is a fluorescent labelled antibody, with antigen-antibody reaction. The technique is simple, sensitive, rapid, and low cost. Real time-PCR is added to the PCR reaction system fluorophores, fluorescent signal accumulation time monitoring throughout the PCR process, and finally through the unknown mRNA template for quantitative analysis. Its use changes the fluorescence signal in real-time detection PCR amplification reaction amplified product in each cycle the amount of changes, analysis and standard curve by Ct value (the number of cycles the sample reaches the threshold level of experience) for initial template quantification analysis can also be used for qualitative detection of a gene for rapid, non-polluting. Having high sensitivity, good reproducibility, wide dynamic range, the advantages are of

high-throughput. Therefore, the present study used quantitative Real-time PCR method to detect differences in levels of mRNA transcription.

IFA results showed that inserting VP2 gene can effectively increase VP2 protein expression more than clone-30 alone, but because of IFA cannot determine the expression level of the exogenous protein, Therefore, the present study, requires more sensitive quantitative Real-time PCR method to detect differences in levels of mRNA transcription.

Conclusion

Using reverse genetics techniques to construct and express VP2 gene of vvIBDV in (HLJ07 strain) recombinant NDV, while the constructed and expressed recombinant NDV as a control. Recombinant NDV rClone30-VP2 in different sites, infectious and reproductive capacity rClone30-VP2 had no difference and were much higher than rclone-30.

Real time-PCR test results showed rClone30-VP2 in VP2 mRNA transcript levels of both sites were significantly higher than rClone30.

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Integrating landsat images data and geographic information system to determinate changes in vegetative cover in Mesan governorate southeast of Iraq

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Abstract

This study aimed to determinate vegetation cover change in Mesan Governorate south east of Iraq by using Landsat images data and Geographic Information System (GIS) for investigation of the spatial and temporal changes. A part of Mesan Governorate, whose Area is 11895 km^2 was selected as study area. Three cloud free Landsat TM, ETM+, and LDCM scenes covering the study area were selected for analysis. Images were acquired in years 1990, 2000, and 2015 respectively. All images which mentioned above are rectified and registered in Universal Transverse Mercator (UTM) projection zone 38 N and supervised image classification system has been observed to classify the images in different land cover categories. Normalized difference Vegetation Index (NDVI), and Five land cover classes have been identified and used to determine the change in vegetation cover in study area and these classes are: Water, agricultural area, natural vegetation, bare land, and salty area. According to the results obtained from visual analysis of NDVI and statistics of classification, it was observed that negative changes have been occurred during period 1990 to 2000 and positive changes have been occurred during period 2000 to 2015. It is thought that the main reasons of this change are decreasing saline area, and increasing water bodies(Marshes). Those reasons have been led to the increase of the vegetation cover in study area during period from year 2000 to 2015.

تكامل بيانات صور القمر لاندسات ونظم المعلومات الجغرافية لتحديد التغييرات في الغطاء النباتي في محافظة ميسان جنوب شرق العراق

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الخلاصة

هدفت هذه الدراسة إلى رصد تغير الغطاء النباتي في محافظة ميسان جنوب شرق العراق باستخدام بيانات صور القمر الصناعي لاندسات ونظم المعلومات الجغرافية في التحقيق في التغيرات المكانية والزمانية. وتم اختيار جزء من محافظة ميسان له مساحة حوالي 11895 كم^2 كمنطقة الدراسة.وقد اختيرت ثلاثة من المشاهد الفضائية الخالية من الغيوم للقمر الصناعي لاندسات TM ، ETM+ و LDCM للاعوام 1990، 2000 و 2015 لتغطي منطقة الدراسة. اجريت عملية التصحيح الهندسي لكل الصور المذكورة أعلاه وفق مسقط مركاتور المستعرض العالمي (UTM)

وضمن نطاق رقم ثمانية وثلاثون شمالا وقد استخدم نظام التصنيف الموجهه لتصنيف الصور في فئات مختلفة تمثل الغطاء الأرضي.وقد استخرج الدليل النباتي بالاضافة الى تحديد خمس فئات للغطاء الأرضي لغرض استخدامها لتحديد التغير في الغطاء النباتي في منطقة الدراسة وهذه الفئات هي: المياه و الاراضي الزراعية والنباتات الطبيعية والأراضي العارية، والمالحة. ووفقا للنتائج التي استخرجت من التحليل البصري للدليل النباتي و إحصاءات التصنيف، فقد لوحظ أن التغييرات السلبية وقعت خلال الفترة من 1990 الى 2000 و التغيرات الإيجابية وقعت خلال الفترة من 2000 إلى 2015. ويعتقد أن الأسباب الرئيسية لهذا التغيير هو نتيجة التناقص في مساحة المنطقة المتملحة، وزيادة المسطحات المائية(الاهوار). وقد أدت هذه الأسباب إلى زيادة الغطاء النباتي في منطقة الدراسة خلال الفترة من العام 2000 الى عام 2015.

Introduction

Geographic Information System (GIS) is a powerful set of tools for collecting; storing, retrieving at will, transforming and displaying spatial data from the real world for a particular set of purpose. Since 1972, Landsat satellites have been providing repetitive, synoptic, global coverage of high-resolution multispectral imagery. Landsat data have potential applications for monitoring the conditions of the Earth's land surface and the environment components. An increasingly common application of remotely sensed data is for change detection. Change detection is the process of identifying differences in the state of an object or phenomenon by observing it at different times. Change detection is an important process in monitoring and managing natural resources and urban development. Satellite can repeatedly observe the wide area at once and continuously acquire the information about the ground features and environmental changes. Satellite sensors can detect the electromagnetic radiation energy reflected from the earth over a wide range of spectrum with a visible and infrared wavelength, and record it in digital image. Moreover, as most of satellite images are analyzed through computer systems, it has many advantages to acquire the various information simultaneously rather than visual interpretation (1). The objectives of this study were assessing, monitoring, and mapping the vegetation cover changes in Mesan Governorate south of Iraq. In this study, which carried out based on visual and digital procedures, various changes are identified, and were detected during years for three times. The digital images of Landsat Thematic Mapper (TM) 1990, Enhance Thematic Mapper Plus (ETM+) 2000, and Landsat Data Continuity Mission (LDCM) 2015 were used.

Study Area: The study area is a part of Mesan Governorate in the south east of Iraq. The study area bounded by the coordinate (from 632496 to 771344) Easting and (from 3445803 to 3558285) Northing in zone 38N according to UTM projected coordinate system. It covers an area of 11895 Km2. The selected area could be recognized in Figure (1), which illustrates the Iraq map with map representing the study area.

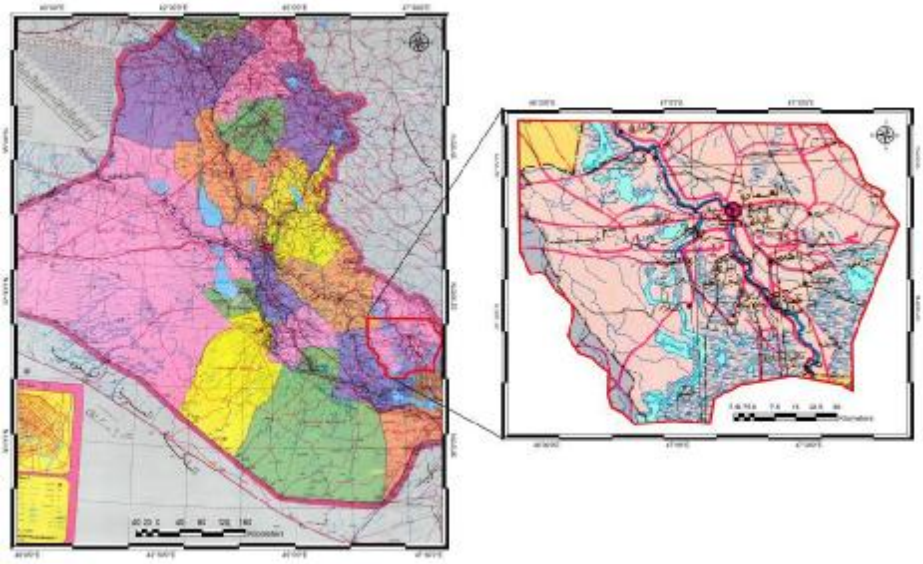

Figure (1) Iraq map with map represent the study area

Materials and Methods

Satellite Images Data: In this study three free cloud Landsat images have been used: Thematic Mapper (TM) dated March 4, 1990, Enhance Thematic Mapper Plus (ETM+) dated April 12, 2000, and Landsat Data Continuity Mission (LDCM) dated April 10, 2015.

Preprocessing:

Radiometric Correction: The Landsat images have been calibrated and converted to spectral radiance and then converted to reflectance values by using meta data files for each image.

Rectification and Registration: Rectification and registration of Landsat Thematic Mapper (TM), Enhance Thematic Mapper Plus (ETM+), and Landsat Data Continuity Mission (LDCM) imageries were based on 25 ground control points (GCP) collected from intersection of roads, and rivers, at the study area. The remotely sensed dataset was geometrically corrected in the datum World Geodetic System 1984 (WGS84) and projection UTM zone 38N using the first order (linear) of polynomial function and Nearest Neighbor rectification re-sampling, which was chosen in order to preserve the radiometry and spectral information in the imagery. Image to image registration was done in order to register the TM image dated 1990 and ETM+ image dated 2000 with geocoded LDCM image dated 2015(master or reference image). The Root Mean Square error (RMS) of the image-to-image was 0.38 for TM and 0.4 for ETM+.

Normalized Difference Vegetation Index (NDVI): The most common combination of spectral bands of remotely sensed imagery for estimating green vegetation cover is the vegetation index, which employs the red (R) and near infrared (NIR) wavelengths, i.e. the Normalized Difference Vegetation Index (2):

NDVI= (NIR-R) / (NIR+R)

Many natural surfaces are about equally as bright in the red and near-infrared part of the spectrum with the notable exception of green vegetation. Red light is strongly absorbed by photosynthetic pigments (chlorophyll) found in green leaves, while near-infrared radiation either passes through or is reflected by live leaf tissue, regardless of their color. This means that areas of bare soil that have little or no green plant material will appear similar in both the red and near-infrared wavelengths, while areas with green vegetation will appear bright in the near-infrared and very dark in the red part of the spectrum. By using these wavelengths, different vegetation indices can be produced. The resulting index value is sensitive to the presence of vegetation on the land surface and can be used to address issues of vegetation type, amount and condition. The NDVI values range from -1.0 to 1.0, where areas with vegetative cover have values greater than zero, and negative values indicate non-vegetated surface features such as water, bare soil or the presence of clouds. Water bodies show negative values -1 to 0 (the fact that they have higher reflectance in red (visible) than in near infrared wavelengths. Rock and bare soil have values around zero (0 to 0.02) because they have similar reflectance values both in red (visible) and near infrared wavelengths. Vegetation has higher reflectance in near infrared than in red wavelength and as a result has positive values with higher densities of green and healthy biomass stated. The quantification of NDVI is relative and not absolute; therefore, it provides a measure of which areas of vegetation are more vigorous than other (2). Figures 2, 3, and 4 show the NDVI images in the study area for the years 1990, 2000, and 2015 respectively.

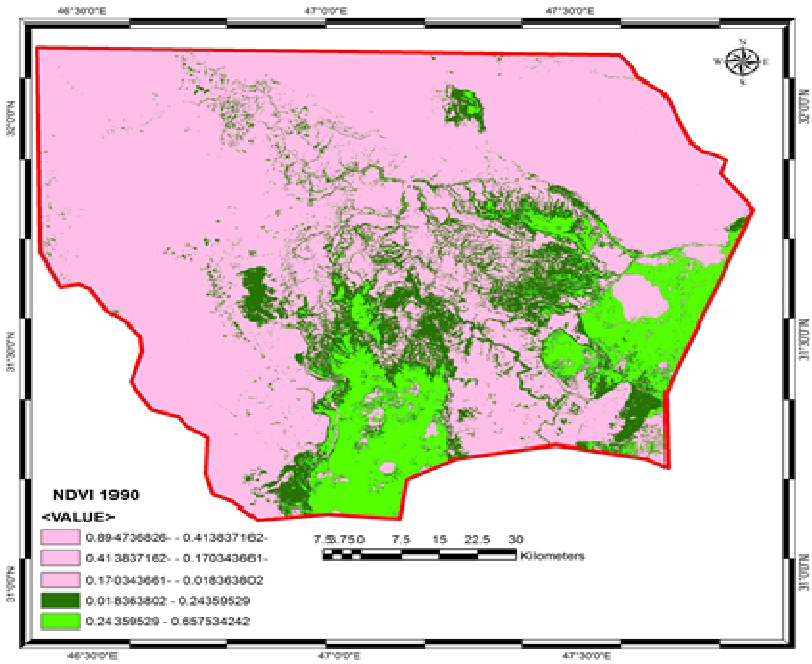

Figure (2) NDVI of study area in1990

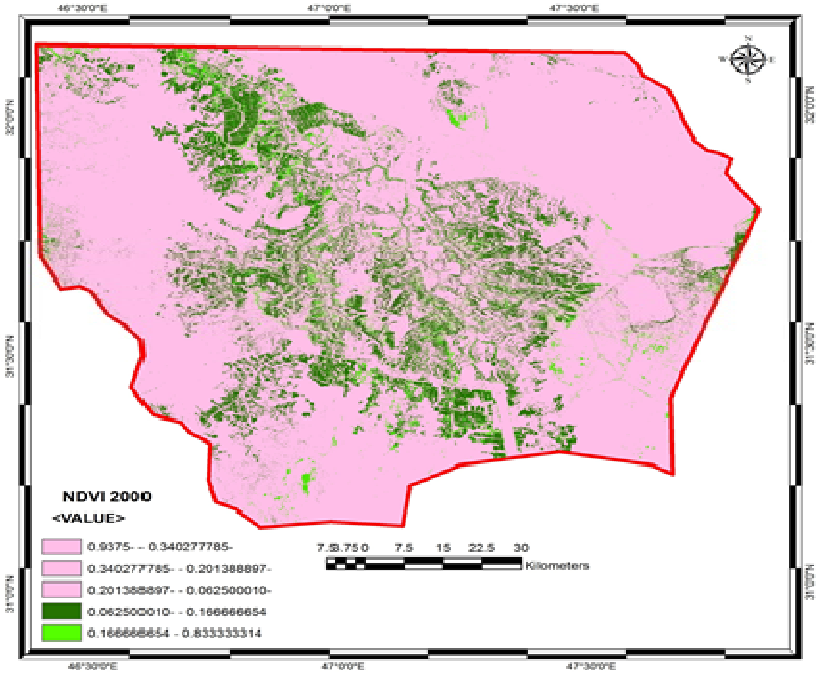

Figure (3) NDVI of study area in 2000.

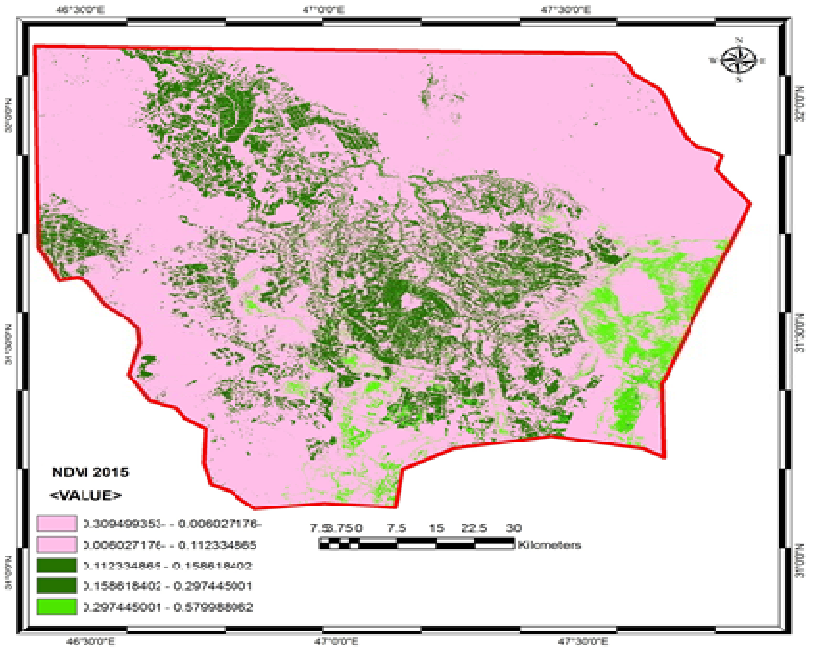

Figure (4) NDVI of study area in 2015

Change detection: In order to detect, assess, and mapping the changes of the study area during the period from 1990 to 2015, Landsat TM, ETM+, and LDCM imageries dataset were used. Change detection involves the use of multi-temporal image data sets to discriminate the changes between dates of Imaging. Change detection is the process of identifying differences in the state of an object or phenomena by observing it at different times (3). It is useful in such diverse applications as land use change analysis, monitoring of shifting cultivation, assessment of deforestation and so on. It is essential for studying change on the earth's surface. The change detection methods are grouped into seven categories: visual analysis, algebra, transformation, classification, advanced models, Geographical Information System (GIS) approaches, and other approaches (4). In this study conducted visual analysis for NDVI images and classification method for changes identification in study area. In order to provide an accurate Land cover classification of study area supervised classification of TM 1990, ETM+ 2000, and LDCM 2015 was used in this study. Locations were selected using a portable GPS device (TOPCON GRS1) during field work to collect the ground truth and to perform the field checking for the obtained results.

Supervised Classification:

Supervised classification is the process of using samples of known training data to classify the unknown identity. Knowledge of data and the desired classes are required prior to the classification process and must be obtained from ground truths, aerial photos, or maps. This method depends on the analyzer ability to delineate different characteristics of different patterns of land covers in the studied area. The training area is used for delineation of spectral characteristics of each pattern (5). The selection of appropriate training areas is based on the analyst's familiarity with the geographical area and their knowledge of the actual surface cover types present in the image. Thus, the analyst is supervising the categorization of a set of specific classes. The numerical information in all spectral bands for the pixels comprising these areas is used to "train" the computer to recognize spectrally similar areas for each class. Once the computer has determined the signatures for each class, each pixel in the image is compared to these signatures and labeled as the class it most closely digitally. Thus, in a supervised classification we are first identifying the information classes which are then used to determine the spectral classes which represent them. There are several classification algorithms can be applied in supervised classification which include parallelepiped, minimum distance, maximum likelihood and non-parametric (6). The most common and well-known supervised classification uses the maximum likelihood technique which employs a decision rule based on the probability that pixels belong to a particular class (7). The supervised classification is applied in the current study based on Maximum Likelihood Classifier Method; this method quantitatively evaluates both the variance and covariance of the category spectral response pattern when classifying an unknown pixel (8).In order to provide an accurate Land cover classification of the study area, supervised classification of TM 1990, ETM+ 2000, and LDCM 2015 was used in this study

and performed in three stages and following represent the results of supervised classification:

(1) Training Stage: Five classes were selected in the training stage that represent five land covers classes (water, natural vegetation, Agricultural land, salty area, and bare land)in research region, and the statistical measurements of these classes are computed depending on reflectance value of pixels corresponding to each class versus each of three selected TM, ETM+, and LDCM bands .Figure (5) illustrates the relationship between mean relative reflectance of different classes with TM,ETM+, and LDCM bands in terms of the spectral response curves. The spectral response patterns are considered one of the most important results, which give good information and indications about physical and geometric properties of land cover classes in the research region by means of detailed study, and analysis of spectral response curves of training classes shown in figures (5) and determination of the factors affecting it by aid of field observation.

(2) Classification Stages: The result of classification stage is represented by the map shown in figures 7, 8 and 9. These maps are produced by maximum likelihood classifier. Each class is given specific color as well as a number beside this color with summarized explanation. These maps include five classes, which represent the land cover classes in the study area.

(3) Accuracy Assessment: The classification process is not complete until its accuracy is assessed. The most common means of expressing classification accuracy is a classification error matrix. Error matrix compares, on a category-by-category basis, the relationship between known reference data (ground truth) and the corresponding results of an automated classification (9). Several characteristics about classification performance are expressed by error matrix tables (1), (2), and (3). All non-diagonal elements of the matrix represent errors of omission or commission of most classes, and several other descriptive measures can be obtained from the error matrix as the overall accuracy, probability accuracies which indicate how well training set pixels of the given cover type are classified and user's accuracies which are indicate the given cover type are classified and user's accuracies which are indicate the probability that category on the ground. The confusion matrix simply tells how well the classifier can classify the training areas and nothing more (10). The overall accuracy of the TM 1990 classification map is 88 %, ETM+ 2000 classification map is 83 %, and LDCM 2015 classification map is 89% as shown in tables (1), (2), and (3). According to the values of the overall accuracy (more than 80%) for this reason can be considered the accuracy assessment of classification has strong agreement and reliable.

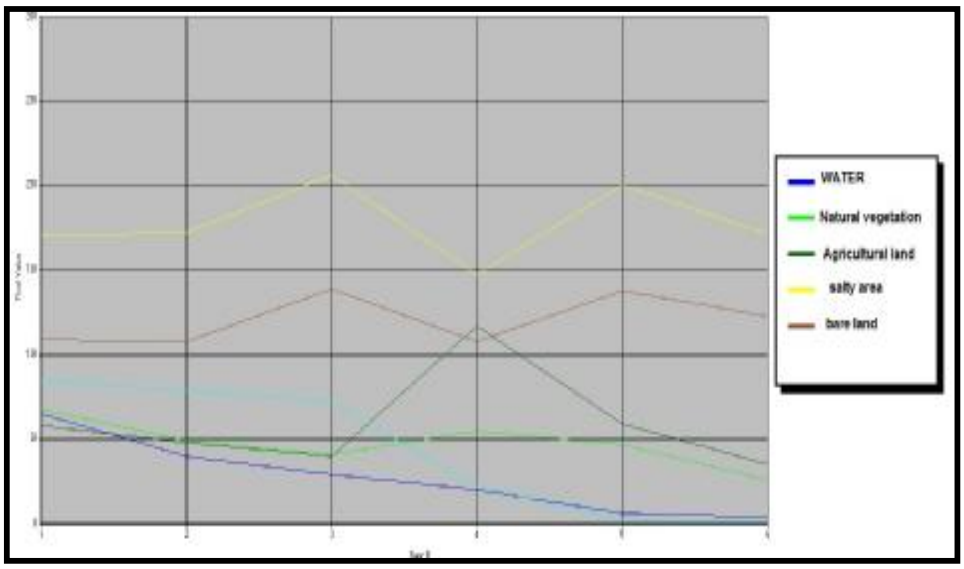

Figure (5) Spectral curves of classes

Table (1): Accuracy Assessment Report of TM 1990

Classification	users' Accuracy	Producers Accuracy
Agricultural land	15/17=88%	15/17=88%
Natural Vegetation	13/14= 93%	13/14= 93 %
Water	5/5= 100%	5/6 = 83 %
Salty Area	10/11= 91%	10/11= 91 %
Bare land	11/14= 79 %	11/13 = 85 %
Overall accuracy= (15+13+5+10+11)/61= 88%		

Table (2): Accuracy Assessment Report of ETM+2000

Classification	users' Accuracy	Producers Accuracy
Agricultural land	13/15=87%	13/15=87%
Natural Vegetation	10/12=83 %	10/11=91 %
Water	5/6=83 %	5/7=71 %
Salty Area	8/10= 80 %	8/10= 80 %
Bare land	11/14= 79 %	11/14= 79 %
Overall accuracy= (13+10+5+8+11)/57= 83 %		

Table (3): Accuracy Assessment Report of LDCM 2015

Classification	users' Accuracy	Producers Accuracy
Agricultural land	16/18=89%	16/17=94%
Natural Vegetation	12/13=92%	12/13=92%
Water	6/7=86%	6/7=86%
Salty Area	8/9=89%	8/10=80%
Bare land	13/15=87%	13/15=87%
Overall accuracy= (16+12+6+8+13)/62= 89 %		

Results and Discussion

The change analysis presented in this paper is based on visual analysis of Normalized Difference Vegetation Index (NDVI) and the statistics extracted from the three land cover maps with using GIS. From the NDVI images and table 4 in addition to figures 6 to 9 can be observed that the vegetation cover decreased through period from 1990 to 2000 and then it was increased through period from 2000 to 2015. Development change was very high in the study area through period 2000 to 2015. On the other hand, study observed the dramatic increase of the water bodies (marshes) area in the years between 2000 and 2015. It seems 3791 km2 in 1990, 1143 km2 in 2000 to become about 3259 in 2015 km2. Salinity area cover 853 km2, 1789 km2, and 1162 km2 in the year 1990, 2000, and 2015 respectively this refers to those salinity area has been increased between 1990 and 2000 and then decreased between 2000 and 2015.Bare land area covers 3488 km2, 6308 km2, and 2638 km2 in the year 1990,2000, and 2015 respectively. This area has been increased through period from 1990 to 2000, and then decreased through period from 2000 to 2015. According to the results obtained from statistics of classification above, it was observed that negative changes occurred during period 1990 to 2000 and positive changes occurred during period 2000 to 2015. It is thought that the main reasons of this change are decreasing salinity area, and increasing water area (Marshes). Those reasons have been led to the increase of the vegetation cover in study area during period from year 2000 to 2015.

Table (4): change rates of land cover during period 1990 to 2015(these values were extracted from land cover maps in figures 7,8, and 9)

classes	Area km^2			Change rate by km^2/ year	
	1990	2000	2015	1990-2000	2000-2015
Agricultural land	1149	2487	3014	133.8	35
Natural vegetation	2614	795	1195	-181.9	27
Water	3791	1143	3259	-264.8	141
Salty area	853	1789	1162	93.6	-41.8
Bare land	3488	6308	2638	282	-245

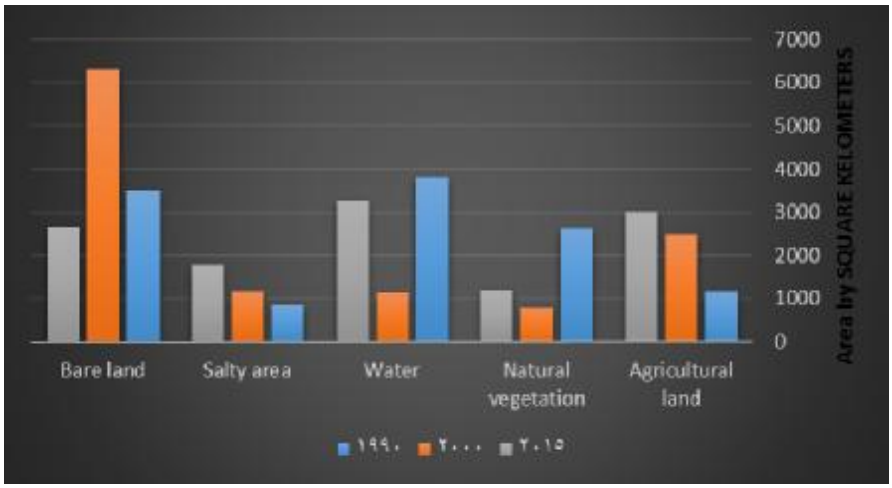

Figure (6) Trend of Land Covers Change, 1990-2015

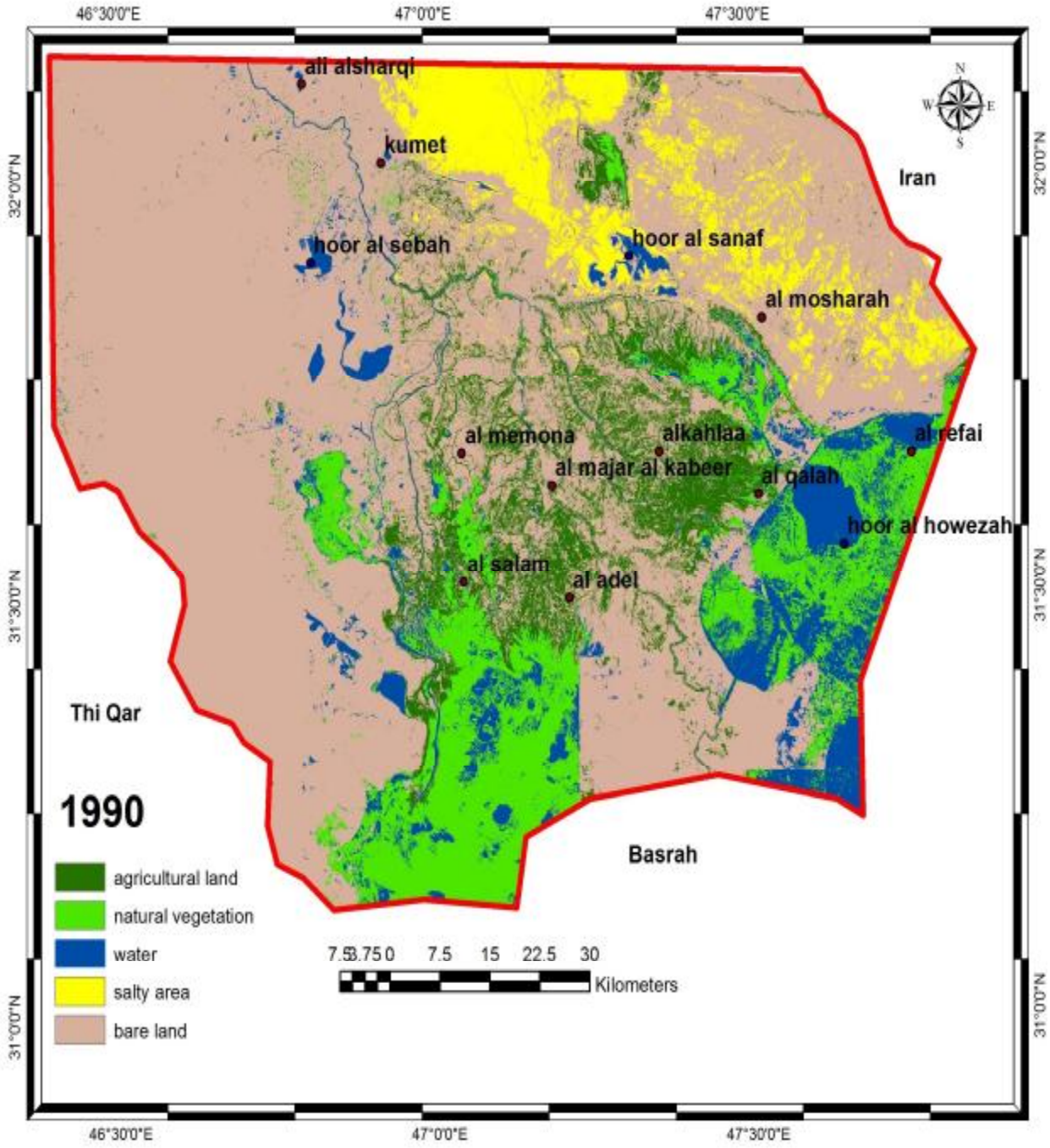

Figure (7) land cover map of study area 1990

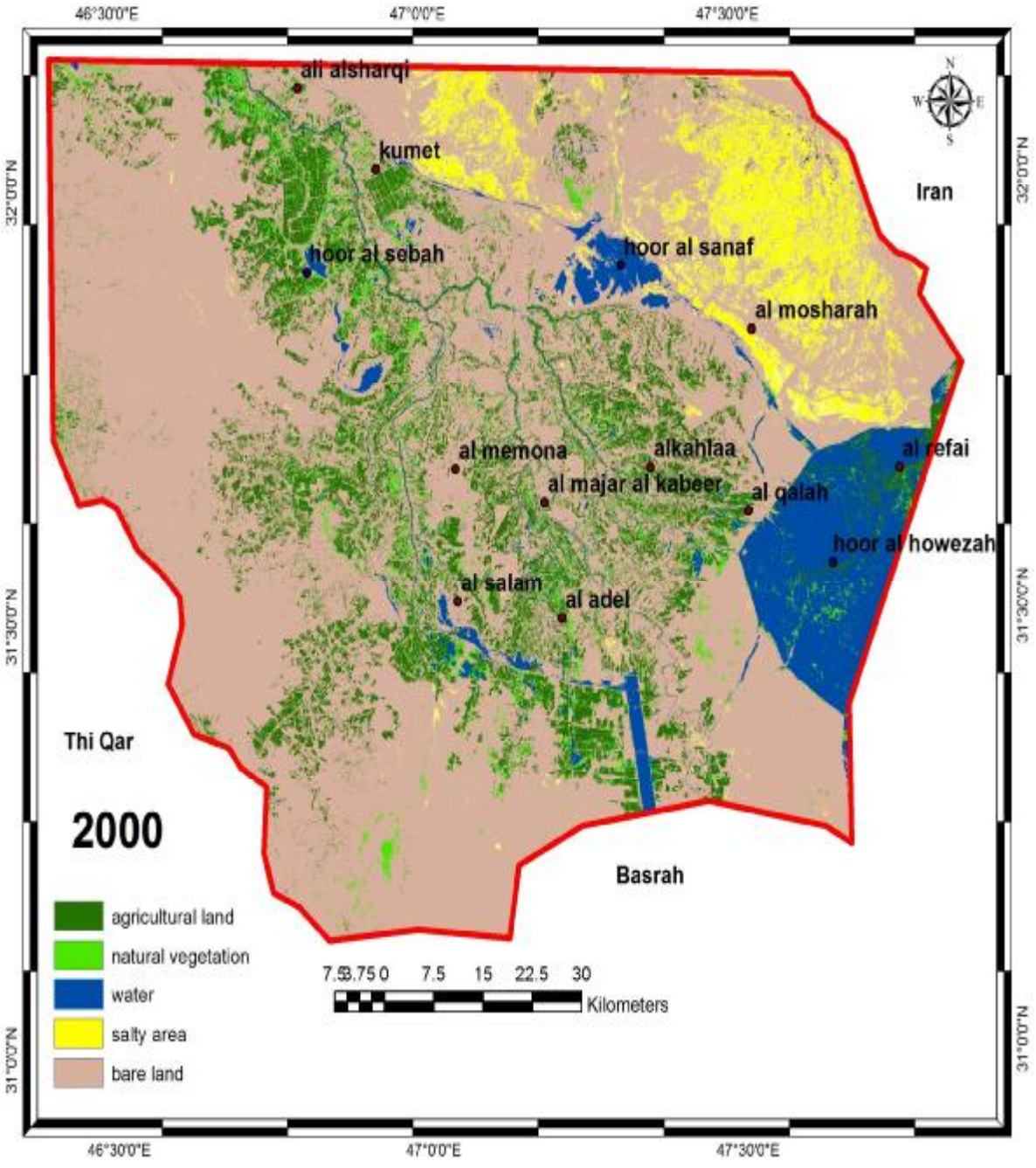

Figure (8) land cover map of study area 2000

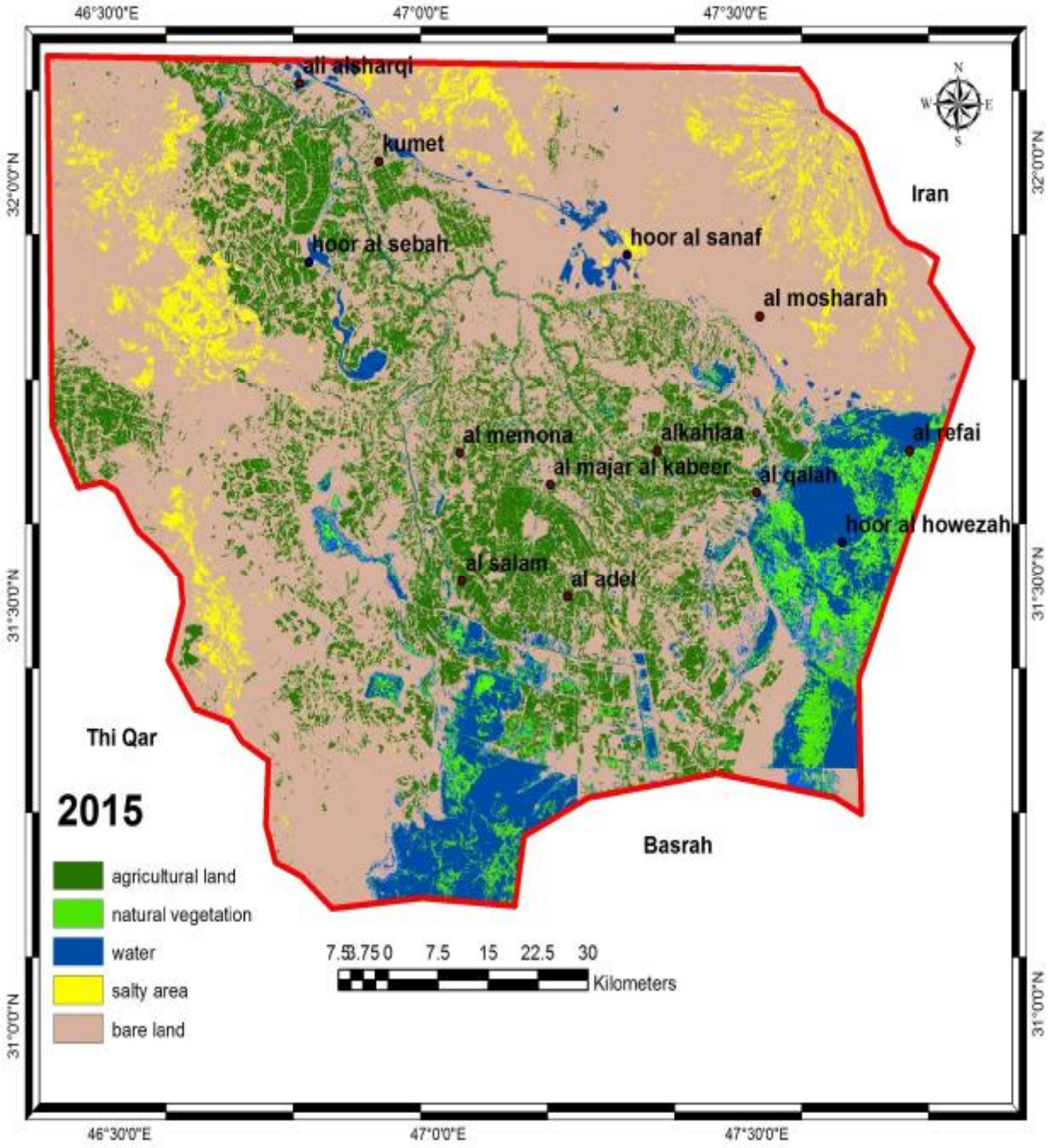

Figure (9) land cover map of study area 2015

Conclusion

1.Integrating Landsat Images Data and Geographic Information System techniques give reasonable classification for land cover in the study area.

2.The accurate and updated land cover change information is necessary for understanding main factors causes and environmental consequences of such changes.

3.Normalized Difference Vegetation Index (NDVI) is a powerful technique in characterization and mapping of vegetation cover.

4.The supervised classification process used for land cover classification shows five main land's cover classes that were identified and used to determine the change in vegetation cover in study area.

5. Based on the visual interpretation of the change maps for the period 1990 and 2015, statistical analysis, in addition to information obtained during field observations, and relevant literature, its´ clear that a significant vegetation cover changes has taken place during the addressed periods, positive change from 2000 to 2015 because of increasing of vegetation cover and water area with decreasing of bare land and salinity area.

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Effect of season on some microbial counts contaminated egg shells of some native birds in Baghdad city

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Abstract

The object of this study was to determine the effect of season on some bacterial count which contaminated egg shell of some native birds included House Sparrow, White-Cheeked Bulbul and Collared Dove. Egg samples of these birds were collected from many regions of Baghdad city during winter and summer seasons to estimate total bacterial count, coliform count, fungi count and isolation percentages of some pathogenic bacteria. Results revealed that bacterial counts on egg shells were significantly ($P<0.05$) high during winter season compared with summer season. Although bacterial counts varied among birds species, the differences were not significant. Many of zoonotic bacteria included *Listeria monocytogenes, Salmonella* sp., *Shigella* sp., *Brucella abortus* and *Campylobacter* sp. were isolated from all studied birds eggs, House Sparrow had the highest isolation percentage of these bacteria among other studied birds and *Salmonella* sp. was the highest isolation percentage among zoonotic bacterial isolates, that indicated the important role of native wild birds eggs and its hatching debris in spreading microbial contamination and some zoonotic diseases.

تأثير الموسم في أعداد الأحياء المجهرية الملوثة لقشرة بيض عدد من الطيور المحلية في مدينة بغداد

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الخلاصة

استهدف البحث الحالي دراسة تأثير الموسم في اعداد بعض المجاميع للأحياء المجهرية الملوثة لقشرة بيض بعض الطيور البرية المحلية والتي شملت العصفور الدوري (House Sparrow) والبلبل (White-Cheeked Bulbul) والحمامة الفاختة (Collared Dove). أخذت عينات من بيض هذه الطيور من عدد من مناطق مدينة بغداد خلال موسمي الشتاء والصيف وجرى تقدير اعداد البكتريا الكلية واعداد بكتريا القولون واعداد الفطريات ونسب عزل عدد من الجراثيم المرضية. بينت النتائج ان اعداد البكتريا الكلية واعداد بكتريا القولون واعداد الفطريات على سطح بيض جميع انواع الطيور المدروسة كانت مرتفعة معنويا ($P<0.05$) خلال فصل الشتاء مقارنة بفصل الصيف، وعلى الرغم من وجود تباينات في اعداد مجاميع الاحياء المجهرية بين الطيور الا ان الفروق لم تكن معنوية احصائيا ضمن نفس الموسم. تم عزل عدد من الجراثيم المرضية المشتركة وشملت *Listeria monocytogenes* و *Salmonella* sp. و *Shigella* sp. و *Brucella abortus* و *Campylobacter* sp. من كافة أنواع بيض الطيور البرية

المدروسة ، وإن أكثر نسبة عزل للجراثيم المرضية كانت من بيض العصفور الدوري مقارنة ببقية الطيور وان جرثومة *Salmonella* هي الأكثر نسبة عزل من بين الجراثيم المشتركة المعزولة ، مما يدل على وجود دور مهم لبيض الطيور البرية المحلية ومخلفات تفقيسها في نشر التلوث بالأحياء المجهرية وعدد من الأمراض المشتركة.

Introduction

Birds feces and feathers may contain different kinds of pathogens that are infectious for different species of animals and plants as well as for humans (1). From the variety of bacterial pathogens *Salmonella* sp. are the most relevant in animal feces since they can infect or contaminate nearly all living vectors from insects to mammals (2). The spectrum of pathogens found and in which concentrations depends on the origin of feces. Feces of animal origin will generally contain mostly animal pathogens or zoonotic agents.

Birds feces contained many genera of zoonotic bacteria, from a sample of 387 cloacal swabs from 364 passerines and woodpeckers. The prevalence of bacteria were as follows: *Escherichia coli* (1%), *Pseudomonas* sp. (22%), *Staphylococcus* sp. (15%), *Streptococcus* sp. (18%), and *Yersinia* sp. (1%). The prevalence of *Streptococcus* sp. was higher in omnivorous species than in granivorous species (20% versus 8%) (3).

In Iraq, the isolation of some zoonotic bacteria from different tissues of native wild birds included House Sparrow, Collared Dove and White-Cheeked Bulbul, revealed that many of zoonotic bacteria included *Listeria monocytogenes, Salmonella* sp., *Shigella* sp., *Brucella abortus* and *Campylobacter* sp. were isolated from all studied birds, House Sparrow had the highest isolation percentage of these bacteria and the content of middle intestine had the highest isolation percentage among other studied tissues, *Salmonella* spp. was the highest isolation percentage among zoonotic bacterial isolates, that indicated of important and hidden role of native wild birds in causing and spreading of zoonotic diseases (4).

The aim of this study was to determine some bacterial count groups and isolation of some pathogenic bacteria from egg shell of some wild birds included House Sparrow, White-Cheeked Bulbul and Collared Dove in Baghdad city.

Materials and Methods

Birds: Thirty five (21 during winter and 14 during summer) eggs of House sparrow, twenty six (15 during winter and 11 during summer) eggs of White-Cheeked Bulbul and Twenty seven (20 during winter and 7 during summer) eggs of Collared dove were collected from different regions of Baghdad city during summer and winter season.

Egg preparation: At sampling, egg shell microbial load were eluted by rinse method in which each egg (per replicate) were placed in sterile poly ethylene bags and carefully rinsed with 10 ml of sterile peptone water for 10 min, then several decimal dilutions were done using sterile peptone water in universal 10 ml screw caped bottles, total bacterial, coliform and fungi (molds and yeasts) counts on egg shell were done by culturing 1 ml of each decimal dilutions on Nutrient agar, MacConkey agar and Saubroud agar plates respectively (5).

Isolation of *Salmonella* sp.: A 10 ml amount of pre warmed (37°C) selenite-cystine broth was added to the 3 ml sample. Samples were mixed thoroughly before incubation. The covers of the screw-cap bottles were placed on loosely. Incubation was at 37°C for 24 hrs. The incubated samples were mixed and streaked onto Brilliant Green Bile Agar plates. The sample was streaked onto four different plates. Straw-colored colonies preliminary confirmation (6). To perform a statistical evaluation, 4 presumptive colonies were picked from each sample and inoculated the lysine-iron agar slants by stabbing method and the tubes incubated for 24 hrs at 37°C. Production of a straw-colored butt was considered a negative Salmonella reaction, and these tubes were discarded. A neutral or alkaline butt, with or without H2S production, was indicative of a presumptive positive Salmonella culture (7).

Isolation of the *Listeria monocytogenes*: After preparation of sterile media, a 2.5 ml each of a 25 ml Trypton Soya Yeast Extract Broth (HiMedia, India) was inoculated according to standard protocols, in which a portion of analytic sample was added to 9 portions of *Listeria* enrichment broth (8,9). For a homogenous distribution of the sample in the medium it was shaken for 2-3 min inside stomacher. Media so inoculated were then incubated at 30°C for 24-48 hrs. After 24 and 48 hrs enrichment processing, the culture was made on the Enrichment Listeria Broth, Modified (ELBM). The planted plates were incubated for 24-48 hrs at 35-37°C under aerobic conditions. At the end of the incubation, they were evaluated as typical colonies with a blue-green colored, smooth, convex, circular with entire margins and opaque halo of 1-3 mm in diameter while the others *Listeria* sp. appear as blue-green colonies. The typical *Listeria monocytogenes* colonies observed on the culture medium were selected and cultured separately in Trypton Soya Yeast Extract Agar (HiMedia, India). After that, the colonies were checked morphologically and their purity controlled by gram staining. Later, the separated colonies were then subjected to carbohydrate fermentation tests (mannitol, D-xylose, rhamnose), catalase activity, oxidase activity, modified motility test with triphenyltetrazolium chloride salts (Umbrella formation), esculin hydrolysis (10). The colonies isolated as *Listeria* were then subjected to β-hemolysis in a 7% sheep blood agar for species identification.

Isolation and identification of *Campylobacter* sp.: Ten ml of sample material was added into 90 ml of Bolton Broth (Campylobacter Enrichment Broth, Bury, England) and incubated at 41°C for 24 hrs in an incubator with N_2. One loopfull (10 µl) of enrichment culture was spread onto modified *Campylobacter* Charcoal Differential Agar (mCCDA) plates, which were incubated in the same conditions. In addition, one loopful (10 µl) samples was directly cultured on mCCDA (11).

Isolation of *Shigella* sp.: Samples was inoculated in *Shigella* Broth (SB) with 0.5 and 3.0 µg/ml novobiocin, were used all incubated at 37°C (SB with 3.0 µg/ml novobiocin also at 42°C) and Buffered Brilliant Green Bile Glucose Broth with 1.0 µg/ml novobiocin incubated at 37 and 42°C growth of *Shigella* sp.(12).

Isolation of *Brucella abortus*: Samples was inoculated in Trypticase Soy Broth and incubated at 37ºC for 3 days. Loopfull growth were cultured on Trypticase Soy Agar

plates and incubated at aerobic atmosphere at 37ºC for 3 days for bacterial identification (13). *Brucella abortus* like colony were performed using gram staining and identification of the biochemical profile: catalase, oxidase, citrate, indole, nitrate, motility, fermentation in TSI medium and urease (8,14).

Statistical analysis: Data were analyzed by using the General Linear Model Procedure of SAS (15). Means were compared by the Duncan's Multiple Range test at 5% probability (16).

Results and Discussion

Data obtained in Figures (1 and 2) revealed that bacterial counts on egg shells were significantly ($P<0.05$) high during winter season compared with summer season. Although bacterial counts varied among birds species, the differences were not significant.

Many of zoonotic bacteria included *Listeria monocytogenes, Salmonella* sp., *Shigella* sp., *Brucella abortus* and *Campylobacter* sp. were isolated from all studied birds eggs, House Sparrow had the highest isolation percentage of these bacteria among other studied birds and *Salmonella* sp. was the highest isolation percentage among zoonotic bacterial isolates, the isolation percentages of these zoonotic bacteria were significantly ($P<0.05$) high during winter compared with summer (Tables 1 and 2).

Free living birds including migratory species have to exploit seasonal opportunities for breeding habitat and food supplies and birds travel across national and international borders (17).

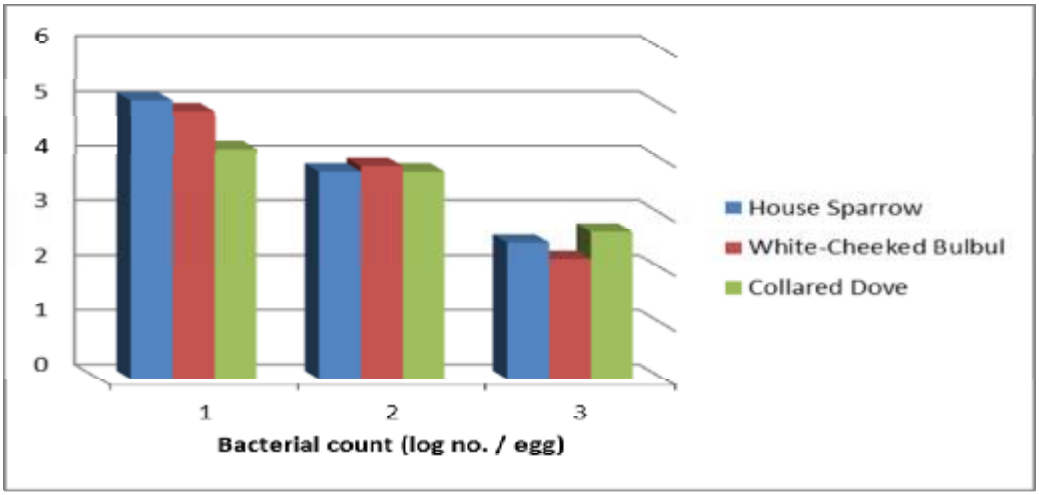

Figure (1): Total bacterial count, coliform count and fungi count of egg shell of some native birds during winter.

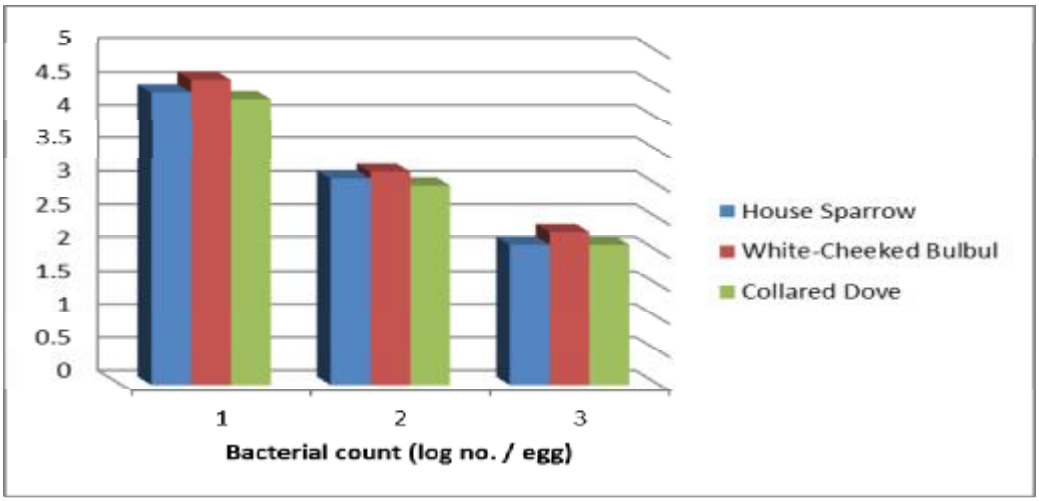

Figure (2): Total bacterial count, coliform count and fungi count of egg shell of some native birds during summer.

Table (1): Isolation of some zoonotic bacteria from eggs of some native birds in winter.

Bacteria species	House Sparrow	White-Cheeked Bulbul	Collared Dove
Salmonella sp.	21/21 % 100.0	10/15 % 66.6	18/20 % 90.0
Shigella sp.	19/21 % 90.4	13/15 % 86.6	15/20 % 75.0
Listeria monocytogenes	20/21 % 95.2	4/15 % 26.6	8/20 % 40.0
Brucella abortus	16/21 % 76.1	3/15 % 20.0	7/20 % 35.0
Campylobacter sp.	2/21 % 9.5	4/15 % 26.6	1/20 % 5.0

Table (2): Isolation of some zoonotic bacteria from eggs of some native birds in summer.

Bacteria species	House Sparrow	White-Cheeked Bulbul	Collared Dove
Salmonella sp.	14/14 % 100.0	7/11 % 63.6	5/7 % 71.4
Shigella sp.	6/14 % 42.85	3/11 % 27.2	3/7 % 42.8
Listeria monocytogenes	8/14 % 57.1	8/11 % 72.72	4/7 % 57.1
Brucella abortus	3/14 % 21.4	2/11 % 18.18	3/7 % 42.8
Campylobacter sp.	2/14 % 14.2	0/11 % 00.0	1/7 % 14.2

Avian mobility and migration are remarkable biological phenomena also there are main crucial epizootiologic factors this birds play a significant role in the ecology and circulation of pathogenic organism (18,19). They carry pathogens, even sedentary avian species can sometimes move as far as 50-100 km and nomadic bird species can transport viable pathogens to distant sites during erratic movement, that can be transmitted to domestic animals and human (19,20).

The infected bird often shed the agent, sometimes for a prolonged period while in some bird species the shedding of a pathogen is more intense and clinical signs more obvious in younger birds than in adult as in Salmonellosis (21,22).

Programmers for zoonosis control and prevention are passed on many steps that have been adopted by international health organization such as Food Agriculture Organization (FAO) and World Organization for Animal Health which know as Office International Epizooties (OIE) as prevention, control, eradication, neutralization of reservoir, reducing potential contact increasing host resistant, implementing consumer protection strategies, identifying animals appropriately, maintaining health, communication and education (23).

Salmonella sp. are the most relevant in animal feces since they can infect or contaminate nearly all living vectors from insects to mammals (2). Birds feces contained many genera of zoonotic bacteria included *Escherichia coli*, *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Yersinia* sp. (3).

Conclusion

In conclusion, this study indicated of important and hidden role of eggs and hatching egg debris of native wild birds in spreading of many genera of zoonotic bacteria.

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Effect of supplementing different levels of propolis in diet on broiler chicks performance

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Abstract

This study was conducted to evaluate the effect of dietary supplementation with propolis on broiler chicks performance at 42 day old. One hundred eighty Ross broiler chicks then reared from 1 day to 42 day old they were divided randomly to six groups (30 birds/group), feed and drinking water offered ad libitum and experimentally groups treated as follows :Group 1(G1) received propolis (0.5 g/kg feed), while Group 2(G2) received propolis (1 g/kg feed), Group 3(G3) received propolis (2 g/kg feed), Group 4(G4) received propolis (3 g/kg feed) and Group 5(G5) was control positive received (basal diet without propolis).Group 6(G6) control negative received (basal diet without propolis). Chicks were weighted weekly from 1st week to 6th week of age, feed consumption and the feed conversion ratio (g feed: g weight gain) were measured during the experimental period. The results revealed that propolis supplementation at levels of 2g/kg and 3g/kg significantly ($p<0.01$) increased growth performance parameters in broiler chicks, such as body weight, body weight gain and improved feed efficiency in most weeks of, the supplementation of propolis at 1gm/kg also affected significantly ($p<0.01$) growth parameters but in a few weeks and the addition of propolis at 0.5gm/kg (G1) non affected in most weeks or in significantly affected growth parameters.

تأثير اضافة مستويات مختلفة من البروبولس في العليقة على الاداء الانتاجي لدجاج اللحم

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير اضافة مستويات مختلفة من مسحوق البروبولس (العكبر) الى عليقة افراخ اللحم في الصفات الانتاجية لفروج اللحم استخدمت 180 طير فروج لحم نوع روز من (1-42) يوم، وزعت عشوائيا لستة مجاميع كل مجموعة تحتوي على (30) طير و كالآتي : المجموعة الأولى : أضيف بروبولس بمعدل 0.5 غم / كغم الى العليقة وعدت (G1)، المجموعة الثانية : أضيف بروبولس بمعدل 1 غم / كغم الى العليقة وعدت (G2)،المجموعة الثالثة : أضيف بروبولس بمعدل 2 غم / كغم الى العليقة وعدت (G3)،المجموعة الرابعة: أضيف بروبولس بمعدل 3غم / كغم الى العليقة وعدت (G4) ،المجموعة الخامسة : مجموعة سيطرة موجبة أعطيت عليقه اساسية بدون اضافة البروبولس وعدت(G5). المجموعة السادسة : مجموعة سيطرة سالبة أعطيت عليقه أساسية بدون اضافة البروبولس وعدت(G6) اظهرت النتائج وجود زيادة معنوية ($p<0.01$) عند اضافة مسحوق

البروبولس بمعدل 2غم / كغم و3غم /كغم في اداء النمو ويشمل وزن الجسم، الزيادة الوزنية ومعدل استهلاك العلف وتحسين معامل التحويل الغذائي اما عند اضافة اغم /كغم الى العليقة اظهرت النتائج وجود زيادة معنوية ($p<0.01$) في اسابيع قليلة اما اضافة 0.5غم /كغم فأظهرت النتائج عدم وجود زيادة معنوية في معدلات النمو في جميع الاسابيع ،ونستنتج من ذلك ان اضافة البروبولس وبمعدل 2غم /كغم و3غم/كغم كان له دور ايجابي في تحسين الاداء الانتاجي لأفراخ دجاج اللحم.

Introduction

Over the years, antibiotics have been added to poultry feed for improving growth performance, stabilizing the intestinal micro flora and preventing some specific pathogenic microorganisms (1) antibiotics had been used to improve feed utilization efficiency in poultry, but after the ban imposed in the European Union because of the potential development of antibiotic resistant human pathogenic bacteria after long-term they were replaced by nature feed additives ,including., herbs(2), Various natural products have been substituted for antibiotics over the last several years in attempts to improve immune system function in fighting pathogens in humans and animals, these natural products have less side effects and desirable in food (3).Propolis was one substance which were naturally produced in many plants (4,5). Propolis was resinous, was dark green or brown in color, and has a pleasant fragrance of poplar buds, honey, wax and vanilla, although it can also have a bitter taste (6) it has been shown to be a non-specific immune stimulant (7). It contains a various of substances including phenolic compounds, such as flavonoids, aromatic acids and their derivatives, esters, alcohols and terpenoids (8), and rich in was flavonoids (9,10) . Many factors affect propolis composition, such as collecting location, time and the plant source (11, 12). Propolis has been shown to be effective against a various bacteria (13, 14, 15) and against colonization of gastrointestinal tract with *Salmonella spp* (16), viruses (17), fungi (18), and molds (19). It also has antioxidant properties (20, 21) Propolis seems to be an effective natural alternative to antibiotic growth promoters. Research results (22, 23) suggest that propolis has a beneficial on daily gains, feed intake and conversion in different animal species, including poultry. In a study by (24), chickens fed 250mg propolis per kg feed were characterized by significantly higher body weights and lower feed intake per kg body weight gain, compared with birds receiving diets without propolis supplements. In an experiment by (23), a combination of flower pollen and propolis at a ratio of 2.5:1 used as a feed additive increased the body weights of chickens by nearly 10% in comparison with the control group. However, (25), found no significant difference in performance or slaughtering traits in Japanese quail receiving 6 or 12 ml /kg propolis ethanolic extract. On other hands , (26) reported that propolis - supplemented diets at levels of 500 or 2000 ppm did not significantly improve body weight, feed intake, or feed conversion in male broilers, whereas supplementation at 4000 ppm in starter diet or in both the starter and grower diets significantly decreased final body weight and total feed intake of male broiler. Also, (27) found that supplementation with 0, 40, 70, 100, 400, 700 and 1000mg kg-1 of oil extract of propolis did not affect broiler performance. Ether extract of propolis (100, 250, 500 and 750 mg/kg) added to the diet resulted in significantly reduced body weight and reduced

weight gain with no effect on feed intake, feed efficiency, or carcass characteristics in Ross broilers (28) In view of the above, the objective of this study was to determine the effect of dietary supplementation with propolis on the productive performance in broiler chickens.

Materials and Methods

This study was done in poultry farm of Animal Resources Department at the College of Agriculture / Baghdad University .One hundred and eighty Ross broiler chicks were reared from 1 day to 42 day old they were divided randomly to six groups (30 birds/group), feed and drinking water offered ad libitum and experimental were groups treated as follows. Group 1(G1) received propolis (0.5 g/kg feed), while group 2(G2) received propolis (1 g/kg feed) , Group 3(G3) received propolis (2 g/kg feed) ,group 4(G4) received propolis (3 g/kg feed) and group 5(G5) was control positive received (basal diet without propolis but vaccinated).Group 6(G6) control negative received(basal diet without propolis and non-vaccinated The chicks were received all required vaccinations. The composition of the basal diet is presented in Table (1) the birds were fed a starter diet until 20 days of age, followed by a finisher diet from 21 to 28 days. Each group was fed its own diet. Chicks were weighted weekly from 1stweek to 6th week of age, feed consumption and the feed conversion ratio (g feed: g weight gain) were measured during the experimental period.

Table (1) Composition of experiment's diets prepared in this study.

Constituents	Percentages of ingredients in starter diet	Percentages of ingredients in final diet
Plant Protein (40 % protein)	5 %	5 %
Soybean meal (48 % protein)	25 %	24 %
Yellow corn	39 %	45 %
Wheat	28 %	22 %
Sun flower oil	1 %	2 %
Dicalcium phosphate	1 %	1 %
Minerals and Vitamin mixture	1 %	1 %
Chemical composition		
Crude protein (%)	21.94	20.07
Metabolized energy (kcal / kg)	2921.9	3038.2
Calcium (%)	0.84	0.84
Available phosphorus (%)	0.42	0.42
Lysine (%)	1.20	1.02
Methionine +Cysteine (%)	0.82	0.78

Provided per kg of diet :vitamin A :22000IU , D3:60 , E:60mg , B1:60mg , B2:140mg , B6:80mg , B12:700mcg , Biotin:2.00mcg , Folic acid:20mg , Vitamin E K3:5mg , Choline

chloride: 7.5mg, Cu:200mg, Mn:1.6mg, Zn:1.2mg, Fe:1.0mg, I:20mg, Se:5mg, Calculated composition of experimental diets according to (29).

Statistical Analysis : Data were subjected to analysis of variance (30) and significant means were separated by Duncan-multiple test (31).

Results and Discussion

The effect of dietary supplementation with propolis on Body weight(BW) of broilers is present in Table(2) the results showed that in 2nd week the BW of G3 and G4 were significantly ($p \leq 0.01$) higher than G6(control negative), in 3rd week the body weight ofG2,G3and G4 were significantly($p \leq 0.01$) highly compared with another groups. in 4th week the body weight of G4 was significant($p \leq 0.01$) highly compared with G5 and G6 and another groups and the BW of G5 was significant($p \leq 0.01$) lower than all groups.

In 5th week the body weight of G4 and G3 were significant($p \leq 0.01$) highly compared with G5, G6 at last week the BW of G4 and G3 were significant($p \leq 0.01$) highly compared with G5, G6 and another groups .while the effect of dietary supplementation with propolis on Body weight Gain(BWG) of broilers is present in Table (3) the results showed that in 2nd week the BWG of G4 was significant ($p \leq 0.01$) highly compared with G5(control positive)and G2,in 3rd week the weight gain of G4was significant ($p \leq 0.01$) higher than G5, G6 and G1followed by G2 and G3,at 4th week also the weight gain of G4was significant ($p \leq 0.01$)higher than G5,G6 and another groups and in significant with G3,at 5th also the weight gain of G4was significant ($p \leq 0.01$) higher than G5, G6 and another groups ,at last week the weight gain of G4 stay highly significant ($p \leq 0.01$) than G5, G6 and insignificant higher than G2, G3 and G1. the effect of dietary supplementation with propolis on feed Intake (FI) is present in Table (4) the results showed that in 2nd the FI of G3 and G4 was significantly ($p \leq 0.01$) higher than all groups, at 3rd , 5th and 6th weeks the FI of G4 was significantly ($p \leq 0.01$) higher than all groups and in significant with G3.

The effect of dietary supplementation with propolis on feed conversion is present in Table(5) In 2nd week the FC of G4,G3and G1was significantly ($p \leq 0.01$)better than G6 and G2,in 3rd , 4th and 5th weeks the fc of G4 was significantly ($p \leq 0.01$)better thanG5andG6 followed by G2,G3and G1 at last week the fc of G3andG4 followed by G2and G1 was significantly ($p \leq 0.01$)better than G5andG6 .

Table(2):Show effect of fed diets containing different levels of propolis on the body weight (Mean ±SE)

Groups weeks	G1	G2	G3	G4	G5	G6
1	95.70 ± 3.0 abc	91.10 ± 1.0 c	99.20 ± 2.0 ab	103.40 ± 1.0 a	93.00 ± 3.0 bc	96.20 ± 2.0 abc
2	264.30 ± 12.9 a	263.90 ± 5.3 a	280.80 ± 22.0 a	262.60 ± 0.20 a	252.0 ± 3.0 a	238.80 ± 9.0 a
3	530.0 ± 10.0 bc	705.0 ± 25.0 ab	690.0 ± 30.0 ab	855.0 ± 75.0 a	435.0 ± 95.0 c	415.0 ± 45.0 c
4	425.0 ± 50.0 bc	435.0 ± 5.0 bc	570.0 ± 1.0 ab	700.0 ± 60.0 a	390.0 ± 90.0 c	360.0 ± 40.0 c
5	750.0 ± 70.0 b	715.0 ± 65.0 b	775.0 ± 15.0 b	940.0 ± 60.0 a	750.0 ± 10.0 b	530.0 ± 10.0 c
6	340.0 ± 60.0 ab	430.0 ± 30.0 ab	390.0 ± 90.0 ab	470.0 ± 10.0 a	220.0 ± 70.0 b	240.0 ± 60.0 b

Small letters between groups (raw) denoted significant differences (p≤0.01).G1 basal diet+0.5mg/kg propolis,G2 basal diet + 1g/kg propolis , G3 basal diet + 2g/kg propolis ,G4 basal diet + 3g/kg propolis,G5 basal diet and G6 basal diet SE = standard error.

Table(3):Show effect of fed diets containing different levels of propolis on the weight gain of broilers (Mean ±SE)

Groups Weeks	G1	G2	G3	G4	G5	G6
1	135.70 ± 12.9 a	131.10 ± 0.30 a	139.20 ± 2.8 a	142.40 ± 14.8 a	133.0 ± 2.0 a	136 20 ± 4.0 a
2	400.0 ± 10.0 ab	395.0 ± 5.0 ab	420.0 ± 20.0 a	405.0 ± 15.0 a	385.0 ± 5.0 ab	375.0 ± 5.0 b
3	930.0 ± 10.0 bc	1100.0 ± 20.0 ab	1110.0 ± 10.0 ab	1260.0 ± 60.0 a	820.0 ± 100.0 c	790.0 ± 50.0 c
4	1500.0 ± 20.0 b	1535.0 ± 15.0 b	1535.0 ± 5.0 b	1620.0 ± 20.0 a	1210.0 ± 10.0 c	1490.0 ± 10.0 b
5	2250.0 ± 50.0 ab	2250.0 ± 50.0 ab	2310.0 ± 10.0 a	2370.0 ± 30.0 a	2150.0 ± 50.0 bc	2020.0 ± 20.0 c
6	2470.0 ± 20.0 b	2490.0 ± 10.0 b	2700.0 ± 100.0 a	2840.0 ± 20.0 a	2490.0 ± 10.0 b	2450.0 ± 50.0 b

Small letters between groups (raw) denoted significant differences (p≤0.01).G1 basal diet+0.5mg/kg propolis,G2 basal diet + 1g/kg propolis , G3 basal diet + 2g/kg propolis ,G4 basal diet + 3g/kg propolis,G5 basal diet and G6 basal diet SE = standard error.

Table(4):Show effect of fed diets containing different levels of propolis on the feed consumption of broilers (Mean ±SE)

Groops / Weeks	G1	G2	G3	G4	G5	G6
1	180.0 ± 3.3 a	180.0 ± 3.3 a	183.33 ± 16.6 a	183.33 ± 16.6 a	176.66 ± 16.6 a	176.66 ± 16.6 a
2	200.0 ± 3.3 b	200.0 ± 3.3 b	250.0 ± 16.6 a	250.0 ± 16.6 a	200.0 ± 16.6 b	190.0 ± 16.6 b
3	500.0 ± 3.3 bc	500.0 ± 3.3 bc	533.0 ± 16.6ab	550.0 ± 16.6a	490.0 ± 10.0 bc	480.0 ± 16.6 c
4	800.0 ± 3.3 a	850.0 ± 3.3 a	850.0 ± 6.6 a	833.3 ± 6.6 a	816.6 ± 16.6 a	850.0 ± 33.3 a
5	900.0 ± 5.0 bc	900.0 ± 6.6 bc	933.3 ± 6.6 ab	966.6 ± 16.6 a	890.0 ± 6.6 bc	850.0 ± 10.0 c
6	900.0 ± 3.3 bc	900.0 ± 3.3 bc	966.6 ± 6.6 ab	1033.3 ± 16.6 a	910.0 ± 16.6 bc	850.0 ± 16.6 c

Small letters between groups (raw) denoted significant differences (p≤0.01).G1 basal diet+0.5mg/kg propolis,G2 basal diet + 1g/kg propolis , G3 basal diet + 2g/kg propolis ,G4 basal diet + 3g/kg propolis,G5 basal diet and G6 basal diet SE = standard error.

Table(5): Show effect of fed diets containing different levels of propolis on the feed conversion of broilers (Mean ±SE)

Groops / Weeks	G1	G2	G3	G4	G5	G6
1	1.88 ± 0.02 a	1.97 ± 0.01 a	1.84 ± 0.03a	1.77 ± 0.03 a	1.90 ± 0.02 a	1.83 ± 0.04 a
2	0.75 ± 0.01 c	0.95 ± 0.03 a	0.79 ± 0.03 c	0.75 ± 0.01c	0.79 ± 0.02 c	0.89 ± 0.02 a
3	0.94 ± 0.003 b	0.70 ± 0.01 d	0.79 ± 0.02c	0.62 ± 0.02 e	1.12 ± 0.01a	1.15 ± 0.04a
4	1.88 ± 0.01 c	1.95 ± 0.01 c	1.49 ± 0.007d	1.19 ± 0.009 e	2.09 ± 0.01b	2.36 ± 0.07a
5	1.20 ± 0.11b	1.25 ± 0.02 b	1.20 ± 0.01b	1.02 ± 0.04c	1.18 ± 0.008 b	1.60 ± 0.04 a
6	2.64 ± 0.07 c	2.47 ± 0.01 c	2.09 ± 0.01 d	2.19 ± 0.04 d	4.13 ± 0.03 a	3.54 ± 0.06 b

Small letters between groups (raw) denoted significant differences (p≤0.01).G1 basal diet+0.5mg/kg propolis,G2 basal diet + 1g/kg propolis , G3 basal diet + 2g/kg propolis ,G4 basal diet + 3g/kg propolis,G5 basal diet and G6 basal diet SE = standard error.

In this study, the effects of different levels of propolis on productive performance parameters were investigated, the addition of propolis at 2g/kg (G3)and 3g/kg(G4) increased growth performance parameters significantly in broiler chicks, such as body weight , body weight gain and improved feed efficiency in most weeks, while the addition of propolis at 1gm/kg also affected significantly growth parameters but in a few weeks and the addition of propolis at 0.5gm/kg(G1)non affected in most weeks or in significantly affected growth parameters, this results is in agreement with (33,37) who found that the propolis has a positive effect on the broiler body weight gain It may be that, in addition to antioxidants activity, the components of propolis powder contributed antimicrobial properties, resulting in better intestinal health and improved digestion and absorption (16,7). Chemical analyses of propolis have shown that it is rich in vitamins and minerals (23) and contains large amount of flavonoids and proteins (12), which may improve weight and feed efficiency in chicks. The highest values of the feed intake were found in the G4and the lowest feed intake value was found in the control group this result confirms the results of(34) who used propolis to feed broiler and they were found that the feed intake was increased, also our study in agreement with(15) who tested propolis on rabbits they were found that the feed intake was increased. This positive effect can be probably attributed to quicker digestion and passage of the nutrients through the digestive system which will have been emptied earlier and feed intake will have been promoted. Due to the active ingredients of this natural product, the formation of more stable intestinal flora digestive system is a consequence of better digestion (37) The present findings show that the feed conversion ratio (FCR) in the experimental groups was improved compared to the control group, this results in agreement with (6,33,9) who was found that the propolis increased the (FCR) on broiler chickens. The reason which led propolis to the improve the growth performance on the broiler because, the propolis has improve the digestive utilization of iron and the regeneration efficiency, furthermore, (1), noted adverse effect of propolis on body weight and feed intake from the present study, From this study, it can be concluded that propolis supplementation in the diet had positive effects on enhancing productivity and the best effect occurred by addition 2g/kg and 3g/kg t on the broiler growth performance which led to increases broiler body gain and feed intake and while FCR was decreased.

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Effect of cold and hot aqueous extract of garlic on the reproductive performance of immature male mice until puberty

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Abstract

This study was investigated the role of garlic extracts on the reproductive functions, via the development of immature male mice (25 days old) until puberty. Immature male mice were divided into 3 groups (n=25). Group 1 "control" was daily administrated with tap water. Group 2 was daily administrated with cold aqueous garlic extract. Group 3 was daily administrated with hot aqueous garlic extract. Each group was randomly consisted of 5 subgroups (n=5/ subgroup) and administrated for different periods i.e, 1, 2, 3, 4 and 5 weeks respectively. Animals were scarified after 24 h from last treatment. Our findings elucidated that, cold and hot aqueous garlic extracts, when administrated at 25 days old (Immature period) have different impact depending on the duration of its administration as follows: The treatment for 1 and 2 weeks have no capability to precocious of the testicular tissue at these of immature periods. The treatment for 3 and 4 weeks (premature periods) has detrimental effect on the testicular structural and function. "i.e. a significant ($P<0.05$, $P<0.01$, $P<0.001$) reduction in testis weights and ($P<0.01$, $P<0.001$) in seminiferous tubule diameters and it caused a highly significant increment ($P<0.001$) in the percentage of damaged seminiferous tubules in comparison with controls and the degenerative traits in the I.T as atrophy, vastness in interstitial space, congestion blood vessels, hemorrhage, edema, also a significant ($P<0.01$, $P<0.005$, $P<0.001$) decrease in the Leydig cells numbers as well as a significant ($P<0.05$, $P<0.01$) decline in the serum T levels in comparison with controls". The treatment for 5 weeks (puberty period) leads to sever defect and disruption of steroidogenesis and spermetogenesis. So the administration of garlic extracts caused a significant ($P<0.05$, $P<0.01$) decrement in the serum FSH levels while led to a significant ($P<0.05$) increment in the serum LH levels.

تأثير مستخلص الثوم البارد والحار على الاداء التناسلي لذكور الفئران غير البالغة حتى سن البلوغ

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الخلاصة

بحثت هذه الدراسة دور مستخلص الثوم في الوظائف التكاثرية خلال تطور ذكور الفئران غير الناضجة (عمر 25 يوماً) حتى سن البلوغ. قسمت ذكور الفئران غير البالغة الى 3 مجاميع عدد كل منها 25 ذكر.

المجموعة الاولى استخدمت كسيطرة وجرعت يومياً بماء الحنفية.
المجموعة الثانية جرعت يومياً بمستخلص الثوم المائي البارد.
المجموعة الثالثة جرعت يومياً بمستخلص الثوم المائي الحار.
تتألف كل مجموعة بصورة عشوائية من 5 مجاميع ثانوية (5 ذكور/ مجموعة ثانوية) جرعت لفترات مختلفة أي (اسبوع واسبوعان وثلاثة اسابيع واربعة اسابيع وخمسة اسابيع) على التتابع.
تم التضحية بالحيوانات بعد 24 ساعة من اخر معاملة.
وضحت نتائجنا امتلاك المستخلصين المائيين البارد والحار للثوم عند تجريعها للذكور غير البالغة (عمر 25 يوماً) تأثير مختلف اعتماداً على مدة التجريع وكالاتي:ان المعاملة لأسبوع واسبوعان ليس لها القدرة على تبكير نضج النسيج الخصوي وأظهرت المعاملة لثلاثة واربعة اسابيع (فترتي قبل البلوغ) تأثير ضار في التركيب والوظيفة الخصوية متمثل بانخفاض معنوي ($P<0.001,P<0.01,P<0.05$) في اوزان الخصى وانخفاض ($P<0.001,P<0.01$) في اقطار النبيبات ناقلة المني وزيادة معنوية عالية ($P<0.001$) في النسبة المئوية للنبيبات المتضررة بالمقارنة مع السيطرة ، بالاضافة الى السمات التنكسية في النسيج الخلالي للخصية والمتمثلة بالضمور واتساع الاحياز الخلالية واحتقان الاوعية الدموية والنزف والاديما اضافة الى الانخفاض المعنوي ($P<0.001,P<0.005,P<0.01$) في اعداد خلايا ليدك ومستوى هرمون T مقارنة بمجموعة السيطرة. كما أدت المعاملة لخمسة أسابيع الى ضرر حاد وتعطيل لتكوين الستيرويدات والانطاف. وسببت حصول انخفاض معنوي ($P<0.01,P<0.05$) في مستوى هرمون FSH ، بينما ادى الى ارتفاع معنوي($P<0.05$) في مستوى هرمون LH.

Introduction

Garlic (*Allium sativum*) is a perennial herb plant and belongs to family Alliaceae which included onion, leek and chive. It is origin in central Asia but now it cultivated in many countries all over the world(1). Garlic is a rich source of sulfur compound, it is contain more than 33 sulfur compound at least (2).One of most biologically active is allicin which also provides garlic's distinct order (3). *A. sativum* contains high levels of vitamin A, vitamin B, vitamin C, phosphorous, potassium, Sulphur, selenium, magnesium, germanium, sodium, iron, manganese and trace iodine (4). In addition many compounds have been identified from garlic such as alkaloids, flavonoids, tannin and saponin (5).

Garlic have therapeutic properties it have been used as antihyperlipidemic(6), antidiabetes (7), antimicrobial (8),(9) reported that the water soluble compound which is unique to aged garlic extract induces apoptosis in human prostate, breast and cancer cells.

There are many conflicting studies about the effect of garlic on the male reproductive function. Some these indicated to its positive effects. As the study of Al-Bekairi *et al.* (10) who reported an increases in accessory organ weights and epididymis of administrated male mice aqueous garlic extract. Also, Oie *et al.* (11) recorded the elevation in T levels of rats that fed with protein diet pillared with o.8 g of garlic powder. Additionally, it was reported that garlic had recovery effect on testicular function in testicular hypogonodisim male mice (12). And Assayed *et al.* (13) recorded that, garlic extract was often more efficient in its protective action for reproductive toxicity than vitamin C in male rats. While other studies have indicated that, garlic caused impaired testicular function and spermicidal effect (14). And a significant reduction in serum T levels by crude garlic, and in spermatic fluid parameters (15, 16 and 17).

Our study was designed to investigate the pursued of chronic treatment from immature period until premature and puberty periods, and it is aimed to evaluate the role of garlic extracts in acceleration the testicular maturation post 1 and 2 week

of treatment, and to purse its influence on the testicular potency from immature periods then undergoing with premature period until to reach puberty period.

Materials and Methods

Preparation of garlic extract: Garlic was purchased from local markets in Baghdad, cold and hot aqueous garlic extracts were prepared according to(13) and (18) respectively.

Animals: Seventy five immature male BALB/C mice (25 day old, weighing 11g -14g) were maintained on 14L:10D and temperature 21-25°C,in the animal house of college of education for pure sciences/ Ibn Al-Haitham, university of Baghdad .

Animals were divided randomly into 3 main groups (25 animal/ group). Group 1 was administrated with 0.1 ml of tap water orally by gavage needle considered as control. Group 2 was gavage orally with 0.1 ml of 250 mg cold aqueous extract/ kg.b.w. Groups 3 was gavage orally with 0.1 ml of 250 mg hot aqueous extract/ kg.b.w. Each of the three groups were further subdivided into as follows:

1- Five male mice were administrated for 1 week.
2- Five male mice were administrated for 2 weeks.
3- Five male mice were administrated for 3 weeks.
4- Five male mice were administrated for 4 weeks.
5- Five male mice were administrated for 5 weeks.

The body weights of mice were recorded daily until the end of last administration week. Animals of all subgroups were sacrificed post 24 h from the last administration.

Blood collection and hormonal assay: Blood samples from mice were collected by cardiac puncture and transferred into sterilized sample tube. Samples were allowed to clot at room temperature and centrifuged at 2500 rpm for 10 min. Sera were collected and stored at 15 ˚C for hormonal assay.

For assessment of T, LH and FSH serum levels particular kits provided by Bio MERIEUX-France and MiniVIDIS apparatus were used, according the method of radioimmune assay (RIA) (19).

Study of histological changes : Testes were dissected out and weighted, then fixed in Boin's solution. And they were sectioned at 5 μm by using routine histological technique and stained with hematoxylin and eosin of 60 cross section of round shape seminiferous tubule were used in order to calculate its diameters and germ cells of seminiferous epithelium such as spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids, Sertoli cells. In addition, interstitial tissue were examined to recorders the mean number of Laydig cells.

Statistical analysis: Data were expressed as meant $\pm$ Standard Error of Mean (SEM). Statistical significance between the various groups were determined using ANOVA(20).

Results and Discussion

Results of current study confirmed that, the two kinds (cold and hot) of garlic aqueous extract, have no enhancement or accelerating the spermatogenesis and uncapable to advancement of the maturation of testicular tissue in immature male mice. But it was caused appearance a few of disruption in seminiferous epithelium (i.e., apparition of detached germ cell in lumen of few seminiferous tubules section

and narrow crack between germ cells), when they administrated at immature period (25 days old) for 1 and 2 weeks. Whereas there were not significant(P> 0.05) changes in the mean numbers of all types of germ cell and Leydig cells (Figures 1, 2, 3, 4, 5 and 6). On the contrary, Sinohara *et al.* (21) mentioned that, the assumed appearance of spermatozoa in the seminiferous of testes should be seen at 35 days old, our data elucidated that, at the end of two weeks (39 days old) of administration, the spermatogenesis was arrested at round spermatids, and the elongated spermatids or sperms were not shown in the seminiferous tubules compared to controls (Figures 10, 11 and 12).

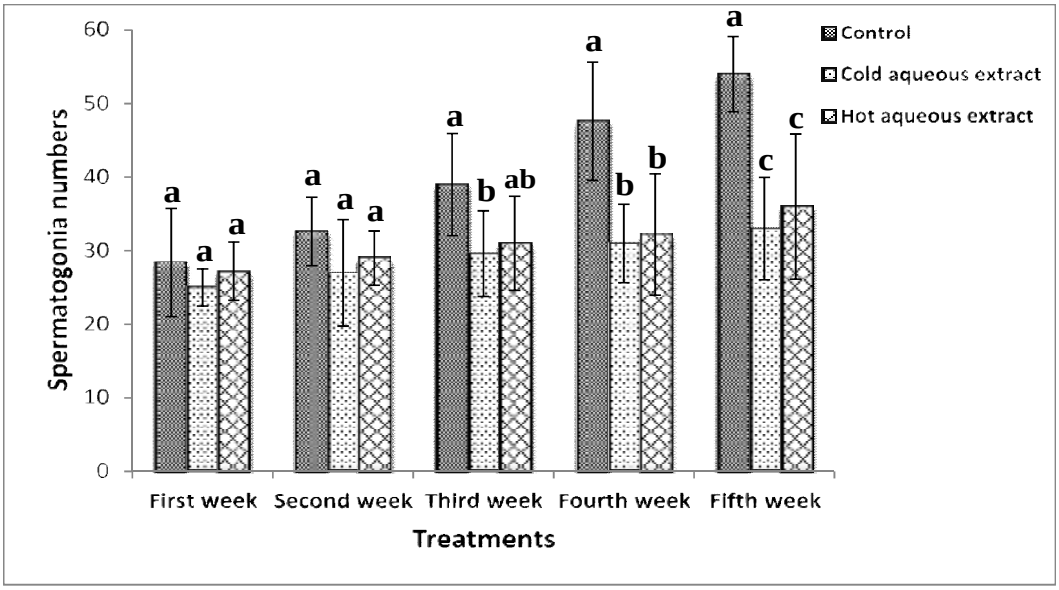

Figure -1- Effect of cold and hot aqueous extract of garlic on spermatogonia numbers. Data given as Mean ± SEM.

b Indicates significant difference from control at (P<0.05).

c Indicates significant difference from control at (P<0.01).

ab Indicates non-significant difference from control and cold aqueous extract(p>0.05).

Similar litters Indicate non-significant difference from control (p>0.05).

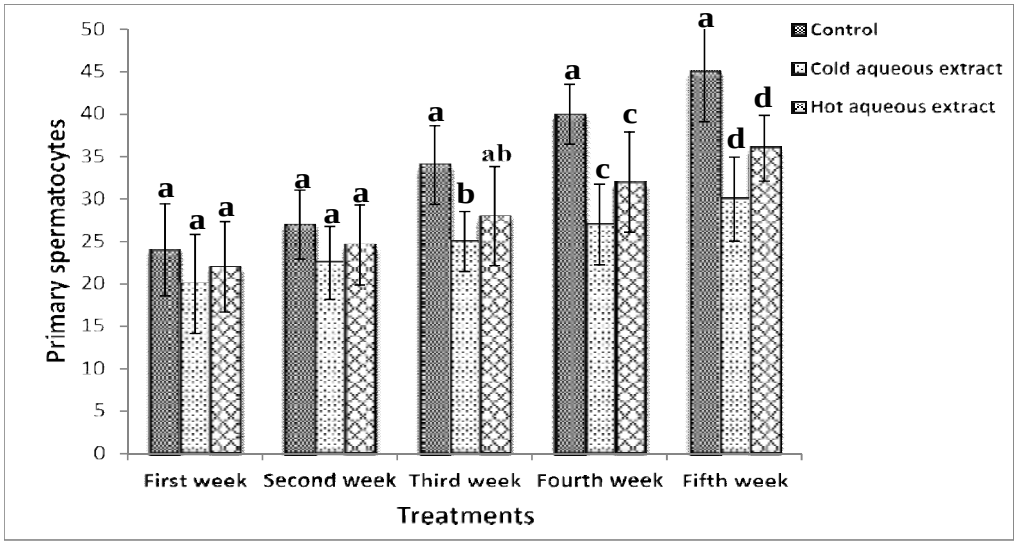

Figure -2- Effect of cold and hot aqueous extract of garlic on primary spermatocytes. Data given as Mean ± SEM.

b Indicates significant difference from control at (P<0.05).

c Indicates significant difference from control at (P<0.01).

d Indicates significant difference from control at (P<0.005).

ab Indicates non-significant difference from control and cold aqueous extract(p>0.05).

Similar litters Indicate non-significant difference from control (p>0.05).

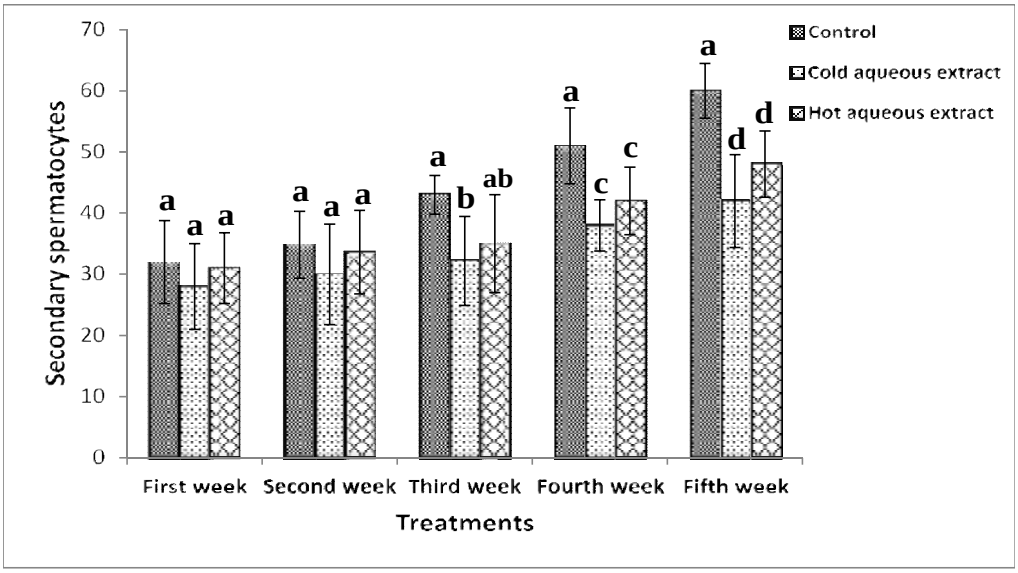

Figure -3- Effect of cold and hot aqueous extract of garlic on secondary spermatocytes. Data given as Mean ± SEM.

b Indicates significant difference from control at (P<0.05).

c Indicates significant difference from control at (P<0.01).

d Indicates significant difference from control at (P<0.005).

ab Indicates non-significant difference from control and cold aqueous extract(p>0.05).

Similar litters Indicate non-significant difference from control (p>0.05).

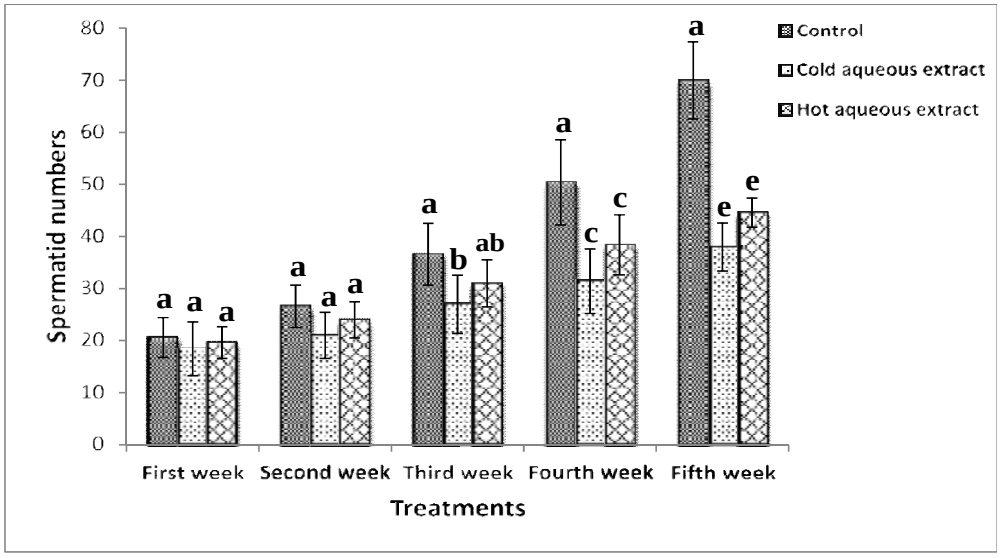

Figure -4- Effect of cold and hot aqueous extract of garlic on spermatid numbers. Data given as Mean ± SEM.
b Indicates significant difference from control at (P<0.05).
c Indicates significant difference from control at (P<0.01).
e Indicates significant difference from control at (P<0.001).
ab Indicates non-significant difference from control and cold aqueous extract(p>0.05).
Similar litters Indicate non-significant difference from control (p>0.05).

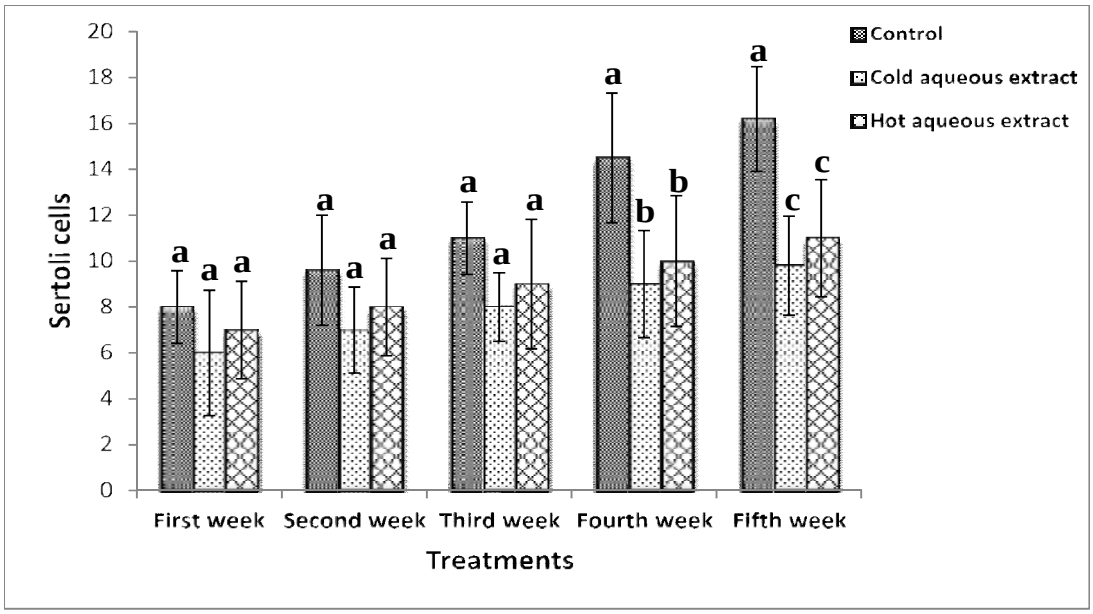

Figure -5- Effect of cold and hot aqueous extract of garlic on Sertoli cells. Data given as Mean ± SEM.
b Indicates significant difference from control at (P<0.05).
c Indicates significant difference from control at (P<0.01).
Similar litters Indicate non-significant difference from control (p>0.05).

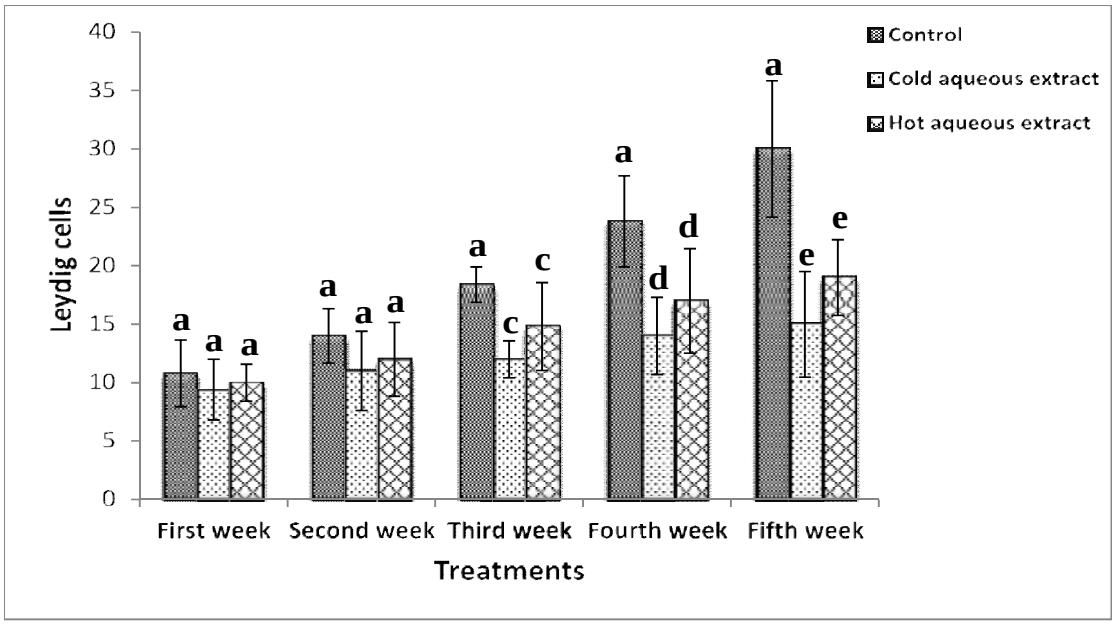

Figure -6- Effect of cold and hot aqueous extract of garlic on Leydig cells. Data given as Mean ± SEM.
c Indicates significant difference from control at (P<0.01).
d Indicates significant difference from control at (P<0.005).
e Indicates significant difference from control at (P<0.001).
Similar litters Indicate non-significant difference from control (p>0.05).

Results of this study were consistence with non-significant (P> 0.05) alternations in the mean of testis weights, seminiferous tubules diameters and the percentage of damaged seminiferous tubules compared to control (Tab 1, Fig 13,14). This impact of administration of garlic extracts for these two periods is attributable to its unable to activate of hypothalamus- pituitary- testis axis, which explained the non-significance (P> 0.05) changes in FSH, LH and T hormone levels compared to controls (Fig.15,16,17).

While, our findings revealed that, the continuity oral administration of 2 kinds of garlic extracts into male mice for 3, 4 and 5 weeks since immature i.e., juvenile period (25 day old) until premature i.e., peripuberty period (46 and 53 days old) and mature i.e., pubertal period (60 day old), caused a significant (P<0.05, P<0.01, P<0.001) reduction in testis weights and(P<0.01, P<0.001) in seminiferous tubule diameters compared to controls (Figure 13). And it caused a highly significant increment (P<0.001) in the percentage of damaged seminiferous tubules in comparison with controls (Figure 14).

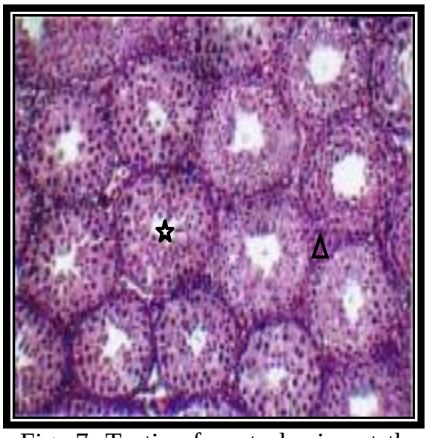

Fig 7. Testis of control mice at the end of 1st w.k of treatment with tap water (T.W). Note normal of interstitial tissue (I.T) and seminiferous tubules (S.T) (H&E, 100X).

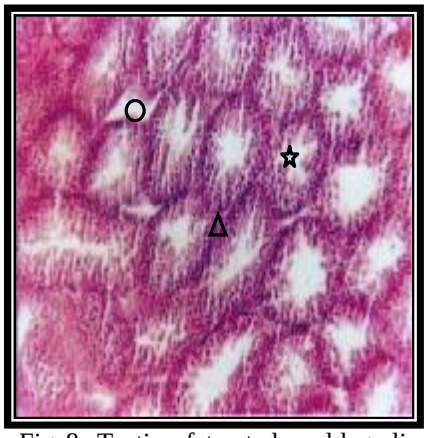

Fig 8. Testis of treated cold garlic extract (CGE) mice at the end of 1 w.k Note normal of I.T and S.T with a few crack, (H&E, 100X).

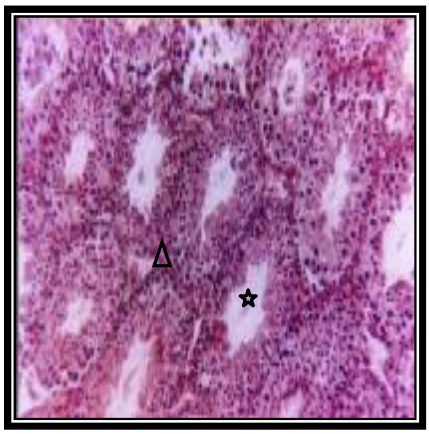

Fig 9.Testis of treated hot garlic extract (HGE) mice at the end of 1 w.k Note normal of I.T and S.T, (H&E, 100X).

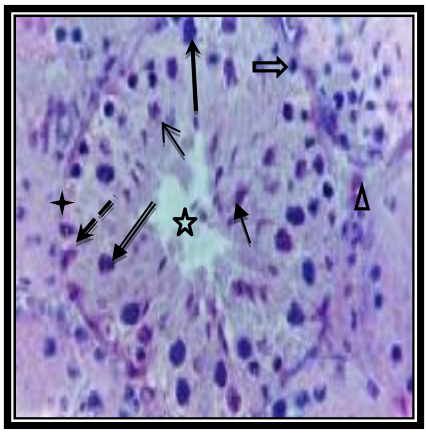

Fig 10. Testis of control mice at the end of 2ed w.k of treatment with T.W. Note normal of (I.T) and (S.T) ,Spematogonia ,primary spermatocyte, secondary spermatocyte , sertoli cell , round and early elongated spermatid, ,basement membrane (B.M.) (H&E, 400X).

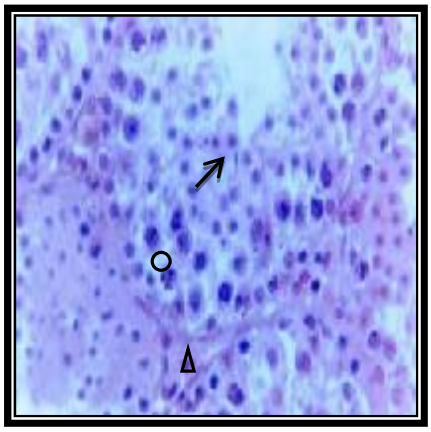

Fig 11. .Testis of treated CGE mice at the end of 2ed w.k Note normal of I.T with a few vacuole presented, absence of elongated spermatids, appearance just round spermatids and a few crack between germ cells (H&E, 400X).

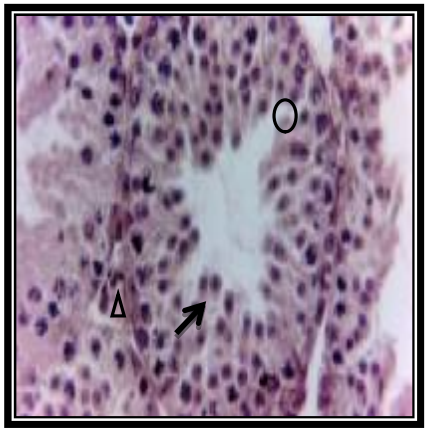

Fig 12. Testis of treated HGE mice at the end of 2ed w.k Note normal of I.T, absence of elongated spermatids, appearance just round spermatids and a few crack (H&E,400X).

Obviously, our data have shown that, garlic extracts have induced direct harmful effect on the interstitial tissue triggering to its degeneration. The degenerative traits are included atrophy, vastness in interstitial space, congestion blood vessels, hemorrhage, edema, also a significant ($P<0.01$, $P<0.005$, $P<0.001$) decrease in the Leydig cells numbers in comparison with controls (Fig. 6). But these degenerative features were strongly severe and advanced at end of 5th week (puberty period when belong 60 days old) of treatments. These degenerative changes indicated that cold and hot garlic extracts have targeted all components of this tissue then, to induce this impairment. This passive effect of garlic extracts might due to presence of some toxic chemical ingredients in garlic. By Oomen *et al.* (22)

who found that, raw crushed of garlic is high in allicin, a powerful bioactive compound that induced activation of various types of $CASP_3$ i.e. $CASP_8$ and CASP, which, involved in apoptosis of several cancer cells. Therefore, in our study the cell death of Leydig cells which caused its numbers reduction may due to apoptosis.

Consequently; the declining in testis weights could be attributed to the degenerative triats in testicular tissue i.e., both of interstitial tissue (testicular interstitial) and semiserious epithelium. And this reduction in testis weight was disagree with the results of Kasuga *et al.* (12) who recorded no change in the testis weight of administrated mature male rats with 4 ml/ k.g. bw of garlic extract for 13 days. And it was disagree with the results obtained by Assayed *et al.* (13) who observed that, the weight of testis was an increased significantly in administrated mature male rats with 500 mg/ b.k. bw of garlic extract with vitamin C.

Table (1): Effect of cold and hot aqueous extract of garlic on testes weight (mg/100 g.b.w).

Experimental group / Period of administration	Weight of testes Mean ± SE			Probability
	Control	Cold aqueous extract	Hot aqueous extract	
First week	126±12.5 A	146±12.9 A	158±17.6 A	N.S.
Second week	177±26.6 A	158±25.7 A	169±13.6 A	N.S.
Third week	211.6±4.04 A	184±8.37 B	190±22.3 B	*
Fourth week	253±37.07 A	193±10.22 B	215±23.53 B	**
Fifth week	292±27.78 A	230±9.14 B	254±18.53 B	***

Data given as Mean ± SEM
***Indicates significant difference from control at (P<0.05).**
****Indicates significant difference from control at (P<0.01).**
*****Indicates significant difference from control at (P<0.001).**

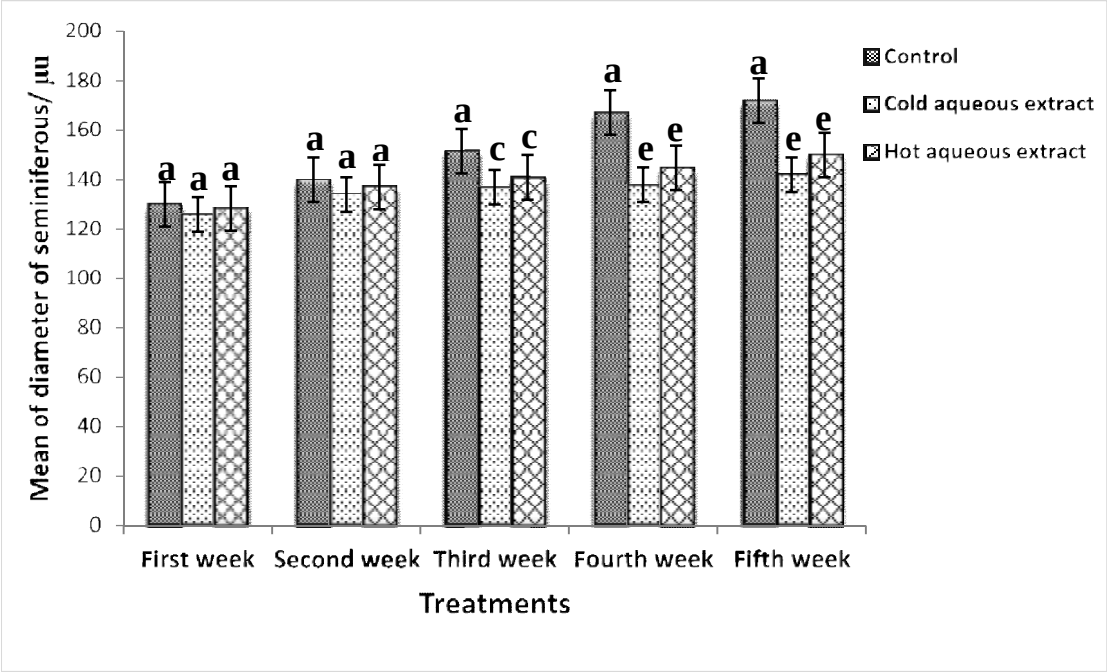

Figure -13- Effect of cold and hot aqueous extract of garlic on Mean of diameter of seminiferous. Data given as Mean ± SEM.

c Indicates significant difference from control at (P<0.01).

e Indicates significant difference from control at (P<0.001).

Similar litters Indicate non-significant difference from control (p>0.05).

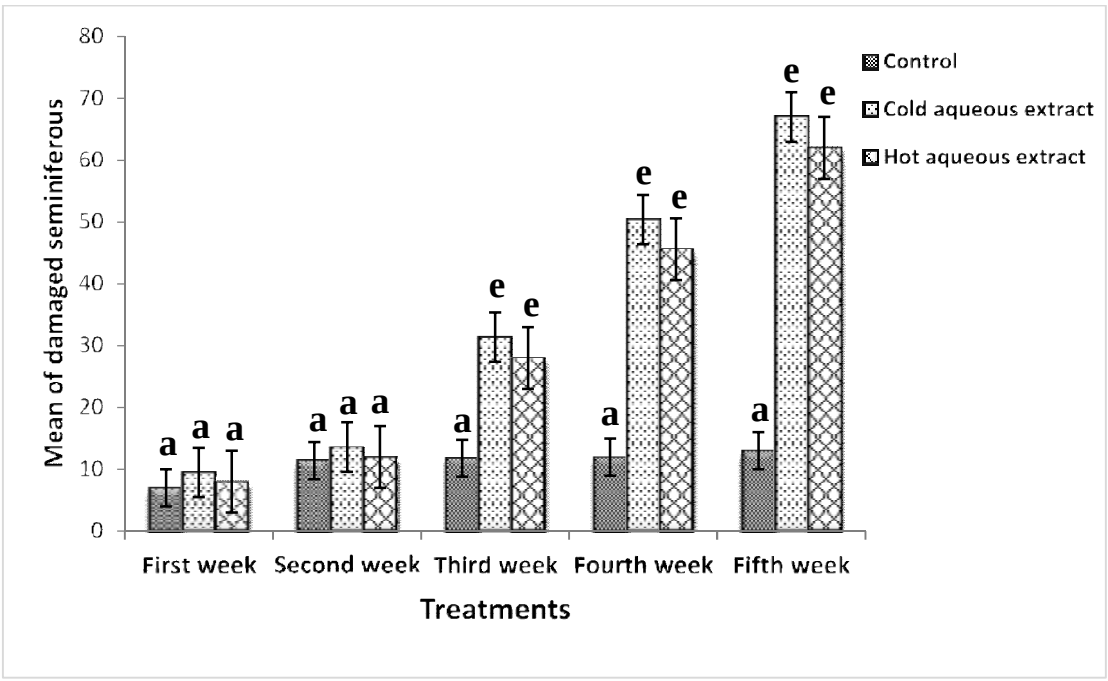

Figure -14 - Effect of cold and hot aqueous extract of garlic on Mean of damaged seminiferous. Data given as Mean ± SEM.

e Indicates significant difference from control at (P<0.001).

Similar litters Indicate non-significant difference from control (p>0.05).

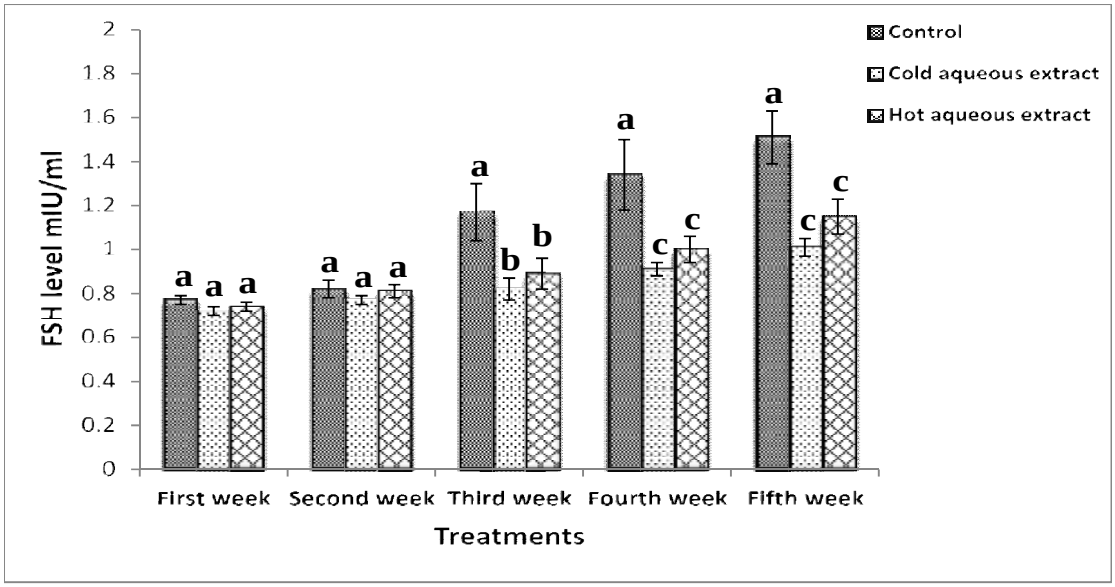

Figure -15- Effect of cold and hot aqueous extract of garlic on FSH levels. Data given as Mean ± SEM.

b Indicates significant difference from control at (P<0.05).

c Indicates significant difference from control at (P<0.01).

Similar litters Indicate non-significant difference from control (p>0.05).

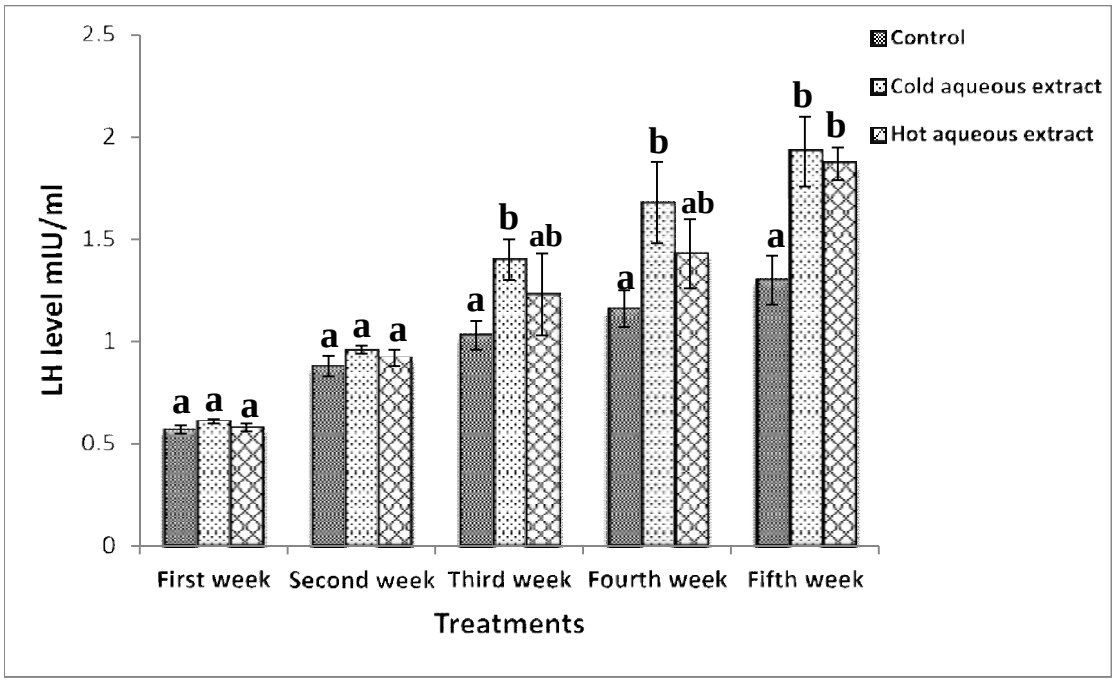

Figure -16- Effect of cold and hot aqueous extract of garlic on LH levels. Data given as Mean ± SEM.

b Indicates significant difference from control at (P<0.05).

ab Indicates non-significant difference from control and cold aqueous extract(p>0.05).

Similar litters Indicate non-significant difference from control (p>0.05).

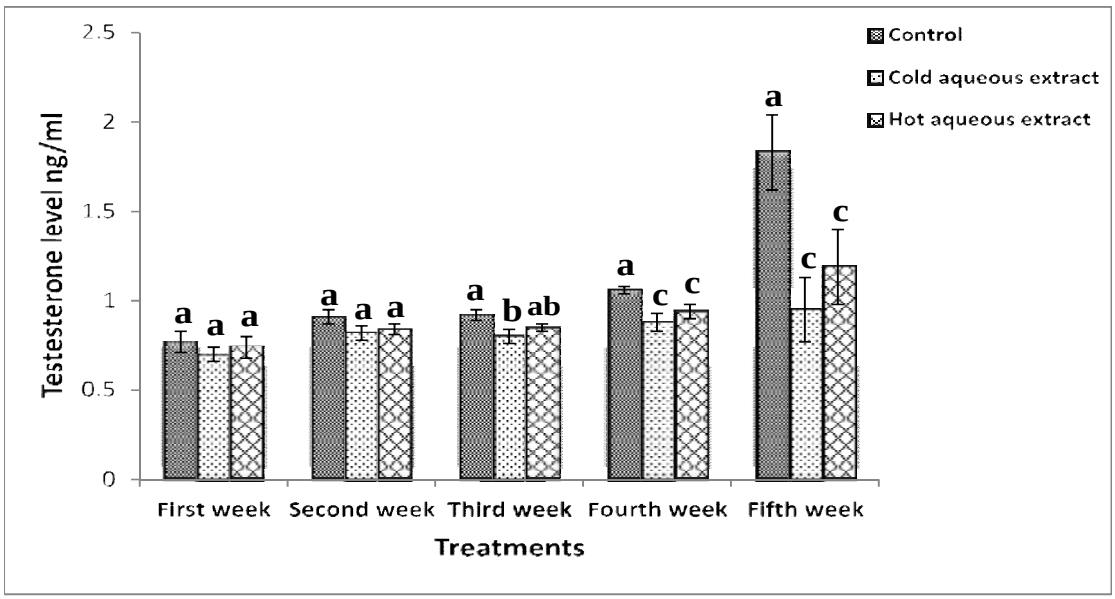

Figure -17- Effect of cold and hot aqueous extract of garlic on Testosterone levels. Data given as Mean ± SEM.

b Indicates significant difference from control at (P<0.05).

c Indicates significant difference from control at (P<0.01).

Similar litters Indicate non-significant difference from control (p>0.05).

These discrepancies with our result may be attributed to the differences in the method of garlic preparation, dosage, age, species and the strain of animals undergo in the experiment.

According to the study of Linston *et al.* (23) who reported, the balance between pro-apoptotic molecules and anti-apoptotic molecules levels determines the fate of the cell towards the executioner step of the death process. The garlic extracts may be enhancement the imbalance between pro and anti-apoptotic molecules triggering to the death of Leydig cell. And the damaged Leydig cell is comported with the study of Hammami *et al.* (16) who proved that the cell death in Leydig cells was a result of apoptosis process in adult male rats when fed with 10% and 15% crude garlic for 30 days. Also our result is in agreement with study of Embomoyi *et al.* (24) who reported that, green garlic can harms the Leydig cells.

Meanwhile, the garlic extracts might have caused direct harmful impact on the Leydig cells function via deactivation of its enzymes that responsible for steroidogenesis like 3B-hydroxysteroid dehydrogenase. As, it was reported by Yang *et al.* (25), who indicated that 3B-hydroxysteroid dehydrogenase which consider Leydig cell marker. On other hand, despite there is no direct contact between Sertoli cells and Leydig cells, Sertoli cells are necessary for maintenance of adult Leydig cell population, and it considered critical regulator for androgen secretion. Some recent studies indicated that, Sertoli cell-derived factors that are required to maintain the Leydig cells and may act directly or indirectly via proportion of testicular peritubular myoid cells(PTMC). Whereas Welsh *et al.* (26) have been shown that, PTMC regulates Leydig cell function. Also Chen *et al.* (27)revealed that Sertoli cells have

been postulated to secret factors which stimulate Leydig cell function such as desert hedgehog(DHH).

As, in male mice the Leydig cells continue to require the Steroli cells in order to prevent degeneration Rebource *et al*.، (28). Therefore, in this study, the indirect passive effect of garlic extract on the Leydig cells population could be attributed to the reduction and regression in Steroli cells as recorded. A huge destruction in interstitial tissue by garlic extracts administration for 3 periods leads to sever inhibition in steroidogenesis as evidenced in hormonal assay in our study.

The continuance of the garlic extracts administration from 25 days old until the end of 3rd, 4th weeks indicate to garlic possession for harmful effect on the seminiferous tubules too, and this effect was also sever and advanced at the end of 5th week (at reaching puberty period). The damaged seminiferous epithelium marked with the appearance of cracks, detaches between germ cells (Sloughing), necrosis in various germ cells and disappearance of basement membrane, that surround and shored to the seminiferous tubules (Fig. 22,23, 25, 26).

In addition, a significant decline in the mean numbers of all spermatogenic cells and Sertoli cells were recorded. The disadvantage in the seminiferous epithelium may arise as a result of the circulatory breakdown as noticed in the defective interstitial tissue that, it means non-reaching of nutrient and necessary substance into Sertoli cell, then deadliness it.

At the currentness study degenerative changes in the seminiferous epithelium at the end of 3 periods of garlic extracts administration was consistence with the lower in the serum FSH and T levels as recorded in this study. Given FSH has direct action in the Sertoli cells via initiation and maintenance of its proliferation. As several previous researches had demonstrated the importance role of FSH for Sertoli cells. Whereby it was known that, in rodents the primary role of FSH in spermatogenesis is stimulation of Sertoli cells proliferation during puberty development (29). And the differentiation of Sertoli cells is controlled by FSH in addition to many factors(30). As well as, it was mentioned that, FSH released by the pituitary stimulates the Sertoli cells of testis which give support and nourishment to developing spermatozoa (31).

Meanwhile, the damage of Sertoli cells in treated male mice for 3 periods may be reflected on the germ cells leading to appearance of regression in all types of germ cells including spermatogonia as we have obtained. And accordance, what is known about the redounded and potently role of the Sertoli cells by several previous study.

Where, it was known that Sertoli cell number largely determines of the number of germ cell and enhance male fertility (32). As the damaged Sertoli cells leads to the impairment of basal junction, that divided the seminiferous tubule in to basal and adluminal compartment. And Verhoen *et al.(*33) revealed that, this specialized tight junction had formed by Sertoli cells at the onset of puberty. As, the decline in these hormones levels may have negative effect in the gap communicating junction which leads to defect in the seminiferous epithelium. Hence it was reported that, adhesion junctions which have critical role in spermatogenesis and adhering between Sertoli cells and spermatids (34).

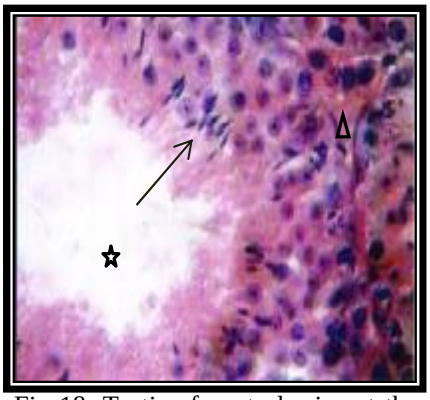

Fig 18. Testis of control mice at the end of 3rd w.k of treatment with T.W. Note normal I.T and S.T ✯ with present of late elongated spermatids, ↗ (H&E, 400X).

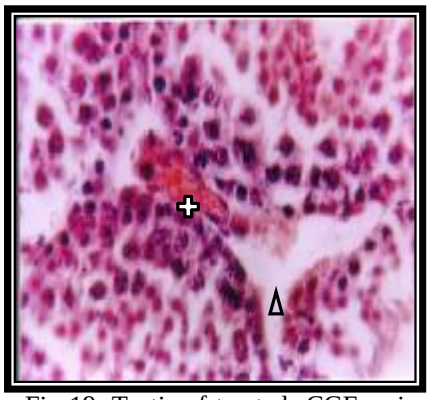

Fig 19. Testis of treated CGE mice at the end of 3rd w.k Note the recedes of I.T , conjestion , vastness of I.T space and absence of mature elongated spermatids and B.M, (H&E ,400X).

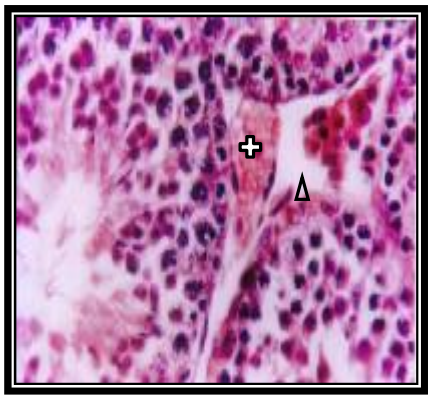

Fig 20. Testis of treated HGE mice at the end of 3rd w.k. Note the same observation (H&E, 400X).

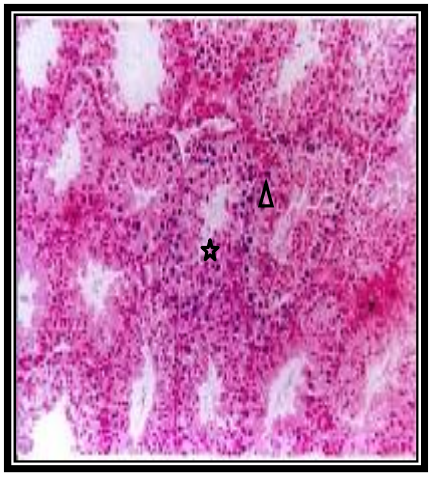

Fig 21. Testis of control mice at the end of 4th w.k of treatment with T.W .Note normal I.T and S.T ✯, (H&E, 100X).

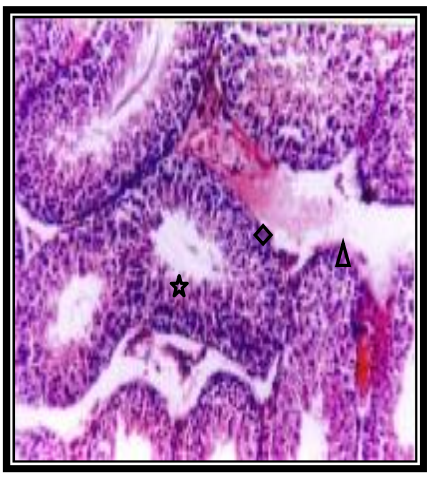

Fig 22. Testis of treated CGE mice at the end of 4th w.k Note regression in I.T and S.T with hemorrhage in I.T (H&E, 100X).

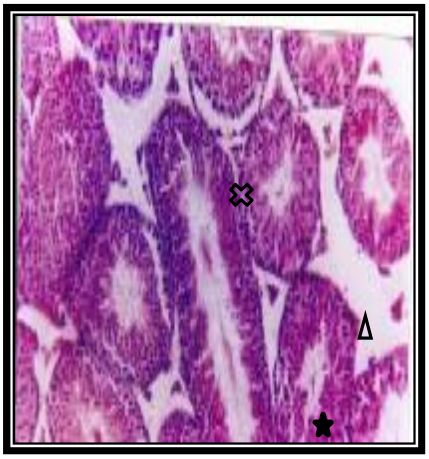

Fig 23. Testis of treated HGE mice at the end of 4th w.k Note regression in I.T and S.T ✯ with also appearance of spermatogonia sloughing and necrosis (H&E,100x) .

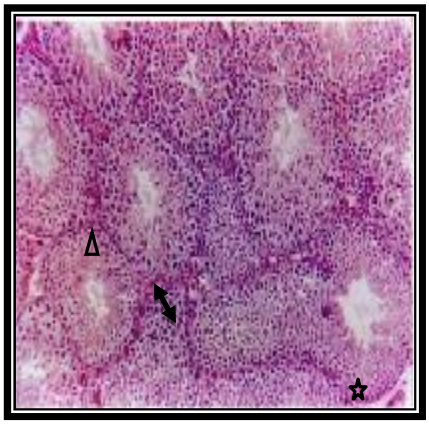

Fig 24. Testis of control mice at the end of 5th w.k of treatment with T.W .Note normal I.T and S.T ✯ with ↔ presence of mature spermatozoa, (H&E ,100X).

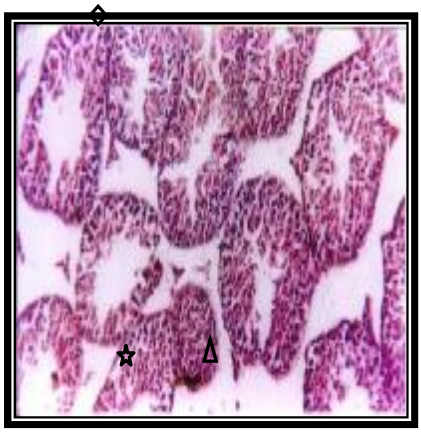

Fig 25. Testis of treated CGE mice at the end of 5th w.k Note several degeneration in I.T and abnormal S.T ✯ with necrosis, disappear of B.M absence of mature spermatozoa, (H&E , 100X).

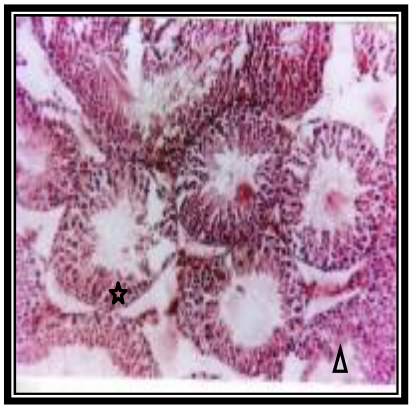

Fig 26. Testis of treated HGE mice at the end of 5th w.k Note the same observation (H&E, 100X).

And in this study the lower in FSH and T levels may affected negatively on the seminiferous epithelium via decrease the production of cell adhesion molecule by Sertoli cells. Hence both FSH and T were a significantly increased the Sertoli cell's adhesion cellular of (N-cadherin) *in vitro* (35). As the previous studies indicated to the positive correlation between FSH and germ cells number. Wherein the prime role of FSH controls on spermatogonia proliferation and it could partly support the initial phase of spermatogenesis up to the level of round spermatid (36). And it was reported that, FSH was increased germ cell numbers by several fold by increasing the complement of spermatogonia and spermatocyte (37). We have suggested that, the damaged spematogonia in our study may due to the direct passive effect of decreased FSH levels on it.

On the other hand, the decline in T levels in the present study has negative effect on the seminiferous epithelium in treated male mice for 3 period, because T hormone is requested for performing of meiotic division during spermatogenesis. Where it was reported that, the elevation of T levels has important role in the primary and secondary spermatocyte and turned into spermatid (38). Also, it was shown that T hormone is more important to support spermiation (39). As it was found that T may initiates and maintenance some degree of complete spermatogenesis in apparent absence of FSH (36). Also Hammami *et al.* (16) attributed the germ cell death process to the decrease in T production in treated adult male rats with 10% and 15% crude garlic for 30 days.

As it was reordered that, the T deficiency caused impairment of sperm production (40). Thus the decrement of T hormone could be explained the absence of mature sperms in the lumen of seminiferous tubules at the end of 5th week of administration i.e. at puberty period.

The detrimental effect of garlic on the germ cells could be ascribe to the saponin, that is one of identified of chemical compounds [16]. Accordance Obianime *et al.* (41) who suggested that, it has harmful effects on sperms. Signaling that administration of garlic extracts since immature periods until premature and puberty period have induced a huge defect on spermatogenesis lead to expiry it.

Our finding revealed that garlic extract have no impeded effects on the hypothalamic-pituitary axis because the elevation of LH levels despite the decrement in FSH levels post the end of 3 periods of administration (Fig 15,16). This result is accordant with the study of Hummami *et al.*, who recorded the decrement in FSH levels with increment in LH levels in male rats fed with 15% and 30% of crude garlic for 30 days (15).

D'souza *et al.* (42) also noticed that increased estradiol production has been shown to be associated with decreased plasma FSH without effect on LH production. The increment of LH levels in our study might be related to the noradrenaline hormone based on the result of Oi *et al.* who reported that injection of 10 mMol/L of diallyl disulfide one of the active component of garlic led to the elevation of noradrenaline hormone levels which, enhanced the pituitary to secret LH (11). Our finding suggest that, the FSH levels decline may due to an increase of inhibin which produces by remanent Sertoli cells as refer by the study of Hammami *et al.* (15). In addition, it is well know that a decrease T production induces an increase inhibin

production that, in turn a decrease of FSH levels. Additionally, our study have elucidated that, garlic extracts administration for along 3 periods has negative effect on the serum T levels. The decline in T levels is resulted of a huge destruction in interstitial tissue and considerably to decline in Leydig cell numbers. Also it may be resulted by altering the androgen hormone synthesis of remanent Leydig cells via disruption of enzymes and coenzymes which, involved in the androgens biosynthesis and secretion, Wherein D'cruz and Mathur (43) indicated that, the garlic may be inhibit of cytochrome P450 which participate in the steroid synthesis pathway. According to Kabbaj *et al.* (44)who reported that the interstitial tissue cholesterol is necessary for synthesis of T hormone, huge destruction in interstitial tissue means the reduction in cholesterol levels which might to be related to the decline T levels in our study.

In conclusion cold and hot aqueous extracts at immature period dose not precocious the testicular maturation when they administration in immature male mice for 1 and 2 week and they have negative effect which lead to disruption of steroidogenesis of spermatogenesis when administration for 25 days old until 60 days old (puberty period) while these garlic extracts have no influence on hypothalamic –pituitary axis.

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Effect of additional levels of butyric and citric acids or their combination to the broiler ration on some histological and biochemical characteristics of meat chicken

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Abstract

This trial was conducted at the poultry farm of Animal Resources- Dept., Agric. Coll., University of Al-Qasim Green from 31/10- 12/12/2013 to study the effect of two levels (1 and 1.5 g/kg feed) for each of butyric and citric acid and their combination to broiler ration on histological and blood biochemical characteristics of meat chicken.420 (one day age) of Rose-308 broiler chicks were used in this study. At age of 8 days these chicks were randomly allocated into 7 treatments (60 chicks per each one), then each treatment was subdivided into 4 replicates (15 chicks per each). The results indicated that treatments T4(Citric acid 1g/kg) T5 (Citric acid 1.5 g/kg) T6 (Citric and Butyric acid 0.5 g/kg)and T7 (citric and Butyric acid 0.75 g/kg) were superior (P<0.01) compared to other treatments in villus height , on the other hand they are superior (P<0.01) in villus width compared to T1(Control) and T3 (Butyric acid 1.5 g/kg) whereas, treatments T5 (Citric acid 1.5 g/kg) and T6(Citric and Butyric acid 0.5 g/kg) were superior (P<0.05) as compared with treatments T1 (Control),T2 (Butyric acid 1g/kg) and T3 (Butyric acid 1.5 g/kg) in crypts depth. Regarding serum biochemical characteristics, results showed that treatment 7 was superior (P<0.05) compared to treatment 2 in the concentration of glucose. T_4 (citric acid at rate of 1g/kg of diet) was superior (P<0.05) compared to treatment 3 in the concentration of triglycerides; however, no significant differences were shown among treatments in cholesterol concentration.

تأثير إضافة مستويين مختلفين من الحامضين العضويين البيوتريك والستريك وخليطهما في العلائق على بعض الصفات النسيجية والكيمياحيوية لفروج اللحم

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الخلاصة

أجريت هذه التجربة في حقل الطيور الداجنة التابع لقسم الثروة الحيوانية / كلية الزراعة / جامعة القاسم الخضراء للفترة من 2013/10/31 ولغاية 2013/12/12 لغرض معرفة تأثير إضافة الحامضين العضويين (حامض البيوتريك وحامض الستريك بالتراكيز 1 و1.5غم/كغم علف لكل منهما) بشكل منفرد أو خلطهما مع بعض إلى العلائق على بعض الصفات النسيجية والكيمياحيوية لفروج اللحم إذ أستخدم في هذه التجربة 420 فرخ فروج لحم غير مجنس بعمر يوم واحد من سلالة Rose- 308 وزعت بصورة عشوائية عند عمر8 يوم إلى 7 معاملات بواقع (60 طير) لكل معاملة قسمت إلى أربع مكررات ولكل مكرر 15 طير . فقد أشارت النتائج إلى تفوق المعاملات T7,T6,T5,T4معنوياً (P<0.01) على المعاملات T3,T2,T1 في طول الزغابات ولوحظ تفوق المعاملات T7,T6,T5,T4 معنوياً (P<0.01) على المعاملتين T3,T1 في عرض الزغابات إما عمق الخبايا فقد تفوقت معنوياً

(P<0.05) المعاملتين T6,T5 على المعاملات T3,T2,T1 .إما بالنسبة للصفات الكيمياحيوية للسيرم فقد لوحظ تفوقاً معنوياً (P<0.05) للمعاملة T7 على المعاملة T2 في نسبة الكلوكوز كما تفوقت المعاملة T4 معنوياً (P<0.05) على المعاملة T3 في تركيز الكليسيريدات الثلاثية ولم تلاحظ أي فروق معنوية في تركيز الكولسترول بين طيور المعاملات المختلفة .

Introduction

Organic acids are an important component of modern nutrition strategies rather than antibiotic treatment since they have antibacterial effect (1,2.3.4). There are many organic acids in nature exist as a natural component in animal and plant tissues, it can be formed as a result of bacterial fermentation of carbohydrates in the gut, and can be formed into a human and animal metabolic intermediate compounds in the citric acid cycle (Krebs cycle) and the outputs of some proteins (5). (6)reported that using of butyric acid in poultry diets play an important role in the development of intestinal epithelial tissue. They also lead to increase the length of intestinal villus (7).

Addition of organic acids improves digestion and thereby increasing digestibility of energy and hence affects level of glucose and triglyceride in the blood (8,9). (10) demonstrated that the addition of organic acids in different level works to reduce pH of the digestive leading to improve ability of small intestine to absorb minerals such as calcium, phosphorus which transfer through bloodstream, thus increase minerals concentration in the blood and affects its composition. Therefore, the present study aims to determine the effect of additional levels of butyric and citric acids in the ration of broiler chicken and effect on some histological and biochemical characteristics.

Materials and Methods

This study was conducted at the poultry farm of the Department of Animal Resources/College of Agriculture-University of Al-Qasim Green, from 31/10/2013 to 12/12/2013. Chicks were supplied by Babylon private in Babylon province. 420 of Rose-308 unsexed broiler chicks were used in this study which included addition of butyric and citric acids (98% purity) levels to the experimental diets to investigate their influence on some histological and blood biochemical characteristic.

In the first chicks ages (from 1 to7 days age) , starter feeding was offered without addition of organic acids. In the last age (from 8 to 42 days age) the treatments were added. Chicks weight was recorded at 8 days followed by random allocation into 28 bins (including 7 treatments with 4 replicates) with 15 chicks in relatively equal weight per each. Organic acids levels were added to the diets separately or in combination, as follows:

Treatment T_1 (control, without addition of acids).
Treatment T_2 (butyric acid at rate of 1g/kg of diet).
Treatment T_3 (butyric acid at rate of 1.5 g/kg of diet).
Treatment T_4 (citric acid at rate of 1g/kg of diet).
Treatment T_5 (citric acid at rate of 1.5 g/kg of diet).
Treatment T_6 (butyric acid at rate of 0.5 + citric acid at rate of 0.5 g/kg feed).
Treatment T_7 (butyric acid at rate of 0.75 + citric acid at rate of 0.75 g/kg feed).

Chicks were raised on 5 cm sawdust. Water was provided by inverted plastic fountains and feeds were offered by cylindrical feeders. Continuous lighting system for 23 hours/day was used throughout the experiment.

Starter diet was offered to chicks from 1-21 days followed by finisher diet from 22-42 days table (1). Organic acids were added by hand mixing with small quantity of feed which was gradually increased until the homogeneity required between the diet ingredients had been achieved. Diets were packed individually in labeled sacs according to experimental treatments, tightly closed to maintain the effectiveness of organic acids.

Percentage of ingredients of experimental diets used in the current study and calculated chemical composition are shown in tables 1 and 2 respectively.

Table 1- experimental feed components percentage [-1].

Ingredients	starter diet%	growth diet %
Yellow corn	38.93	43.93
Wheat rods	20	20
Soy bean meal[2] (SBM)	33	26
Concentrated animal protein[3]	5	5
Sun flower oil	2	4
Limestone	0.7	0.7
Table salt	0.3	0.3
Methionine	0.05	0.05
Choline	0.02	0.02
Total	100	100

Table 2- The calculated chemical composition

Items	starter diet %	growth diet %
Crude protein % (CP)	22.65	20
Metabolizable energy, ME (kcal/kg)	2960	3150
Digestible methionine	0.51	0.42
Choline	1.540	1.400

(1) Chemical composition was calculated according to (11).
(2) SBM used was Argentinian source, 48% CP and 2230 kcal/kg as ME.
(3) Concentrated animal protein (Claire), Dutch origin imported by the Almowafaq company, 40% CP, 5% crude fat, 2% crude fiber, 6.5%, calcium, 4% available phosphorus, 3.85% lysine, 3.70% methionine,% 4 methionine + cysteine, 2.3% sodium, 2100 kcal/kg ME, and contains a mixture of vitamins and minerals ensure the needs of birds. Phytase enzyme 15000 enzyme unit/kg concentrate, choline chloride 5000 mg/kg concentrate.

The studied characteristics:

1- Histological characteristics:

A. Prepares of tissue sections:

After the slaughter of birds (male and female) for each replicate, tissue samples were taken in the shortest possible time to keep intact. Sections of 2 cm long were taken from jejunum, the second part of the small intestine (jejunum). Contents of sections were removed and washed with tap water and prepare as described by (12) as follows:

1. Fixation: the selected pieces were put in glass container filled with 10% neutralized formalin for 48 hours.
2. Washing: pieces were washed with tap water for 1 hour to get rid of the residual of installer solution.
3. Dehydration: this process was performed by exposure of samples to increased concentrations of ethanol (50, 70, 80 and 90%) for 1 hour and to 100% absolute alcohol for 2 hours to remove water from a radar model because water is a good media for the growth of various bacteria and fungi.
4. Clearing: this process was performed by 2 phases exposure of samples to pure zaylol solution, 1 hour per each phases. To make the fabric transparent and to allow the passage of light beam emitted from the microscope lamp to facilitate the screening process.
5. Infiltration: tissues were then immersed in 2 bathrooms of paraffin wax for two hours per each and at 57 and 60 C° respectively, to ensure the penetration of wax material in the fabric parts in order to facilitate the process of cutting the fabric.
6. Blocking and embedding: paraffin wax was used in a 60 C° oven, and then metal molds were used for casting to get wax molds containing samples of fabric and then left for 24 hours to dry.
7. Trimming: this step was done if the template is larger than the required size and to maintain the knife of rotary microtome from quick consumption.
8. Sectioning: the templates were cut using rotary microtome to obtain sequential cross sections with thicknesses of 5 micron. Slides were transferred to water bath with a temperature of 50-45 C° to eliminate folds formed in sections during cutting. Sections were then fixed on glass container containing light tinge of 1: 1 of egg white and glycerin plus a few thymol crystals to prevent the growth of fungi and bacteria and finally slices were dried and kept in the laboratory for 24 hours with a temperature of 25 C° away from dust.
9. Staining: since wax is opaque and non-transparent material and may prevent penetration of dye in the tissues, wax was removed before staining using zaylol for 3 minutes. Staining was then done using Hematoxylin and Eosin Harries dye. slides were then passed through a descended concentrations of ethanol (100, 90, 80, 70, 60 and 50%), for 5 minutes per each concentrations to recover the aquatic media that removed from the fabric, because most tinctures give full interaction in the aqueous hydrated media only. Slides were then washed with distilled water for the same duration. Tissue sections were colored with hematoxylin for 2-5 minutes and then washed in running tap

water for 5 minutes and then exposed to the acidified alcohol to remove the excess dye. Slides were then placed in distilled water for 3 minutes and textiles were colored by eosin for 2-3 minutes and exposed for 3 minutes to zaylol for clearing.

10. Mounting: the colored slides were covered using slide covers made by the D.P.X (Distrene Plasticizer xylene) produced by Fluka chemicals, slides were then left on hot surface at 37 C° to accelerate the desiccation.

B-scan radar sections Histological sections were examined by using Olympus BH2 compound microscope of Japanese origin. Textile sections were photographed by digital camera linked to computer. The fine scale of the lens eyepiece (ocular micrometer) with power zoom of 400x was used to measure histological characteristic of the jejunum sections including length of villi , width and depth of the villus Crypts in microns (10^{-3} mm). Fine scale of the stage (stagemicrometer) was used to calibrate the lens eyepiece as described by (13). Length of villus was measured from the top of villus till the crypt depth that linked with. Crypt depth is defined as the depth or distance between adjacent villus immersions (14.15,16,12). Depth of villus was measured as a distance from mid height of villus or from the point of its curvature where it was divided (12,16,17). (12) (18) estimated the depth from the base of the villus. In a current study depth of villus was estimated as a mean of both measurements. All histological characteristics were expressed as mean of 10 measurements.

2: Blood biochemical characteristics:

Blood glucose, cholesterol and triglycerides were measured during the 6th week at the end of the experiment. At time of slaughtering blood samples were collected and placed into non coagulant containing tubes. Serum was separated by centrifuge and serum glucose, cholesterol and triglycerides concentrations were measured photo metrically using a MindrayBs-120 spectrophotometer.

Data were analyzed according to complete random design (CRD) and the differences between means were compared by using Duncan test (1955). Statistical Analysis Software System- (19) was used to perform statistical analysis.

Results and discussion

1-Effect of butyric and citric acids levels addition on height, width of villus and depth of crypts villus in the jejunum: Table 3 revealed that there are no significant differences between birds in treatments 7, 6, 5 and 4, which were superior ($P<0.01$) compared to treatments 2, 1 and 3. The mean lengths of the villus were 810, 855, 815, 805, 600, 712 and 610 micron for treatments 7, 6, 5, 4, 3, 2 and 1 respectively. Regarding depth of villus, statistical analysis of data showed no significant differences among birds of treatments 7, 6, 5 and 4, but they were superior ($P<0.01$) compared to treatments1 and 3. Birds of treatments T1 (Control)T2(Butyric acid 1 g/kg) and T3 (Butyric 1.5 g/kg) were not significantly differed in width of villus which was 180, 192, 148 185, 163, 178 and 165 micron for treatments T7(Citric and Butyric acid)T6 (Citric and Butyric acid 0.5 g/kg)T5(Citric acid 1.5 g/kg)T4 (Citric acid 1g/kg) T3 (Butyric acid 1.5 g/kg)T2 (Butyric acid 1 g/kg) and T1 (Control) respectively. Results also revealed that though there are no significant differences among birds of treatments T1(control)T2(Butyric acid 1 g/kg)T4(

Citric acid 1 g/kg)T5(Citric acid 1.5 g/kg)T6 (Citric and Butyric acid 0.5 g/kg) and T7(Citric and Butyric acid 0.75 g/kg) in depth crypt (140, 148, 150, 160, 160 and 155 micron respectively), they are all significantly (P<0.05) superior in comparison with birds of T 3 (Butyric acid 1.5g/kg) (126 micron).

Development of villus length and breadth and depth of crypts as a result of addition of organic acids may be due to the increase of growth of the mucous layer lining the bowel and stimulating proliferation of gastrointestinal cells and improve sustainable cells (20). (21) reported that the addition of organic acids have improved the length of villus in the small intestine and the doubling of the cells lining the digestive tract. Increase the length of villus in the small intestine can be also attributed to the low growth of bacteria in the intestines and the role of the virtual layer of the intestine, which act as a natural barrier to bacteria and toxins in the intestinal Lumen cavity thus reducing preparation and reduce the incidence of diseases and infections of the mucous layer of the intestinal epithelial layer cells and this in turn affects the depth of crypts and improves the length and width of the villus and improve digestion and absorption of nutrients (22,23.24).

Table (3): Effect of addition of butyric and citric acids in height and width villus and depth of crypts in the jejunum (micron).

Treatment	Mean length and width of villus and depth of crypts in the jejunum (micron) ± SE		
	Length villi	Width villi	Depth crypt
T_1	610^{c} ±16.70	165^{b} ±7.19	140^{b} ±8.46
T_2	712^{b} ±19.36	178^{c} ±7.44	148^{b} ±8.70
T_3	600^{c} ±12.04	163^{b} ±9.33	126^{c} ±6.82
T_4	805^{a} ±21.30	185^{a} ±9.77	150^{ab} ±6.74
T_5	815^{a} ±15.09	184^{a} ±11.20	160^{a} ±8.31
T_6	855^{a} ±13.61	192^{a} ±11.39	160^{a} ±8.29
T_7	810^{a} ±16.72	180^{a} ±7.52	155^{ab} ±7.93
The moral level	**	**	*

*Different letters vertically refers to significant differences between the treatments under the probability level of (P<0.05).

**Different letters vertically refers to significant differences between the treatments under the probability level of (P<0.01).

T_1 control treatment without addition, T_2 addition of butyric acid at level of 1g/kg), T_3 addition of butyric acid cat level of 1.5 g/kg), T_4 addition of citric acid at level of 1 g/kg), T_5 addition of citric acid at level of 1.5 g/kg), T_6 addition of a mixture of butyric and citric acids at level of 0.5 g/kg for each, T_7 addition of a mixture of butyric acid and citric acids at level of 0.75 mg/kg + 0.75 g/kg for each).

The increased numbers of beneficial bacteria leads to fewer infections in the digestive tract of the bird through the expulsion of harmful bacteria and this helps the development of digestive system in particular the intestine where nutrient absorption and also development in villus width (4), but he did not notice a change in the depth of

crypts. Organic acids involved in cell membrane integrity and interfere in the process of transfer of food and metabolism of energy and this is due to the sour influence of organic acids (9). (25) pointed out that the addition of organic butyric acid has a role in the development of epithelial tissue lining the small intestine and this has improved the length of villus and crypts in the jejunum depth so adding acid butyric are very important, especially in small birds which be unprotected by antibiotics. And may be due to the role manos (poly saccarides) who works to prevent harmful bacteria from going in the intestinal lining and this improves intestinal health by increasing the length of villus and t giant cells (26,27), this result is not consistent with finding of (28) who did not notice the impact long the villus and depth of crypts.

2-Effect of addition of butyric and citric acids on some blood biochemical characteristics:

Table 4 shows the effect of addition of organic acids to broiler diet on some blood biochemical characteristics including glucose, triglycerides and cholesterol. Results revealed that birds of treatment 7 was superior ($P<0.05$) in serum glucose concentration as compared with those of treatment2 (334.50 vs. 250.00 mg/dl). However, there were no significant differences in this blood constituent among other treatments. Mean serum glucose concentrations in blood withdrawn from birds of these treatments were, 318.50, 311.00, 278.50, 360.00 and 300.00 mg/dl for treatments 1, 3, 4, 5 and 6 respectively. Results also showed that birds of treatment 4 were superior ($P<0.05$) in serum triglycerides concentration as compared with those of treatment 3, whereas, no significant differences were observed among other treatments in that blood constituent. Blood triglycerides concentrations were 116, 100, 59, 185.5, 94, 102 and 122.5 mg/dl for treatments 1, 2, 3, 4, 5, 6 and 7 respectively. Regarding serum cholesterol concentration, results of biochemical characteristics revealed that there were significant differences among treatment due to addition of organic acids.

Addition of organic acids improve digestion and thereby increasing digestibility of energy and this may affects the percentage of blood triglyceride concentrations (8,9). (10) reported that the addition of organic acids reduced the pH of the digestive leading to absorb more minerals such as calcium, phosphorus, these travels through the bloodstream, thus increasing the concentration of minerals in the blood serum, which affects its components.

Result concerning cholesterol concentration in a current study does not agree with that obtained by. (29) where they observed a moral difference ($p<0.05$) in the concentration of cholesterol due to addition of butyric acid, however, triglyceride concentrations were not affected significantly. (30) referred to a moral difference in cholesterol and triglycerides ($p<0.05$) as a result of addition of butyric at rate of 0.2% but did not note any significant differences in the proportion of glucose. However (10) didn't notice differences in blood cholesterol when they added acetic, citric or lactic acids to the drinking water.

Table 4-Effect of addition of butyric and citric in the some biochemical characteristics.

Treatment	Mean concentration of serum glucose, triglycerides and cholesterol mg/dl ± SE		
	Glucose	Triglycerides	Cholesterol
T_1	318.50^{ab} ±11.50	116.00^{ab} ±33.00	137.50 ±0.50
T_2	250.00^{ab} ±35.00	100.00^{c} ±36.00	139.50 ±46.50
T_3	311.00^{ab} ±7.00	59.00^{b} ±5.00	110 ±7.50
T_4	278.50^{ab} ±16.50	185.50^{a} ±55.50	162.50 ±21.50
T_5	306.00^{ab} ±14.00	94.00^{ab} ±36.00	154.00 ±21.50
T_6	300.00^{ab} ±20.00	102.50^{ab} ±3.00	124.00 ±9.00
T_7	334.50^{a} ±32.50	122.50^{ab} ±9.50	165.00 ±18.00
The moral level	**	**	*

*Different letters vertically refers to significant differences between the treatments under the probability level of (P<0.05).
**Different letters vertically refers to significant differences between the treatments under the probability level of (P<0.01).
N.S non-significant.
T_1 control treatment without addition, T_2 addition of butyric acid at level of 1g/kg), T_3 addition of butyric acid cat level of 1.5 g/kg), T_4 addition of citric acid at level of 1 g/kg), T_5 addition of citric acid at level of 1.5 g/kg), T_6 addition of a mixture of butyric and citric acids at level of 0.5 g/kg for each, T_7 addition of a mixture of butyric acid and citric acids at level of 0.75 mg/kg + 0.75 g/kg for each).

Our results agree with (31) who didn't notice differences in cholesterol but noted difference in glucose due to addition of butyric acid at level of 2% and 3% to initiator, growth and final diets. (4) also did not observed differences in cholesterol and glucose concentrations when they added butyric and formic acid at rate of 2% and 3% to broiler feeds (32), (33) and (34) stated that the addition of organic acids have a positive effect on the blood serum by increasing digestibility of nutrients due to increased acidity as well as increase the secretion of pancreatic enzymes that stimulate the absorption of calcium, phosphorus, magnesium and zinc.

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Purification and characterization of lectin from *Acinetobacter baumannii* isolated from urinary catheter samples and its effect on some pathogenic bacteria

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Abstract

Twenty eight isolates of *Acinetobacter baumannii* were obtained from urinary catheters samples, one of the isolates was used to lectin production. Purification of lectin was achieved by 45%saturation ammonium sulfate followed by ion exchange chromatography on DEAE cellulose column and gel filtration chromatography sephadex G100 with purification fold 2.793, molecular weight 58 KDa, it is an acidic protein with highest stability at pH 6.5. Divalent ions did not effect on its activity and it was stable under heating till 80°C.The antibacterial activity of lectin against some pathogenic bacteria was studied and these bacteria included *Listeria monocytogenes*, *Enterococcus faecalis*, *Serratia marscenes, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Streptococcus mutans* and *Klebsiella pneumonia*. The lest value of MIC was 8 µg/mland for MBC was 16µg/ml. and the pure was higher affected than the crude lectin and highest effect was at concentration of 1000 µg/ml except of *B. subtilis* which was totally resist for the crude and purified lectin at both concentrations 250 and 125 µg/ml, in addition to *K. pneumonia* which was totally resist to the crude lectin at both concentration 250 µg/ml and 125 µg/ml and pure lectin at 125µg/ml.

عزل وتنقية اللكتين من *Acinetobacter baumanii* المعزولة من عينات القسطرة البولية وتأثيرها على بعض أنواع البكتريا المرضية

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الخلاصة

عُزلت ثمانية وعشرون عزلة *Acinetobacterbaumanii* من عينات القسطرة البولية ، وأستخدمت عزلة واحدة لأنتاج اللكتين . تمت تنقية اللكتين بواسطة الأشباع بـ 45% كبريتات الأمونيوم وكروموتوكرافيا التبادل الأيوني بأعمدة سليلوز DEAE وكروموتوكرافيا الترشيح بالهلام بأستخدام sephadex G100 وبلغت تنقيته 2793 مرة ووزنه الجزيئي 58 كيلودالتون وتبين أنه بروتين حامضي وأعلى أستقرارية له عند pH 6,5 . لم تؤثر الأيونات ثنائية التكافؤ على فعالية اللكتين وكانت ثابتة تحت تأثير درجات الحرارة حتى 80ْم . دُرست فعالية اللكتين ضد بعض أنواع البكتريا المرضية والتي شملت *Listeria monocytogenes* و *Enterobacter faecalis* و *Serratia marscenes* و *Pseudomonas aeruginosa* و *Staphylococcus aureus* و *Streptococcus pyogenes* و *Bacillus subtilis* و *Streptococcus mutans* و *Klebsiella pneumoniae* . كانت أقل قيمة للـ MIC 8 مايكروكرام/مل وللـ MBC 16 مايكروكرام/مل . كان اللكتين النقي أكثر تأثيراً من اللكتين الخام وأعلى تأثير له عند تركيز 1000مايكروكرام/مل ، ما عدا *B. subtilis* الذي كان مقاوماً وبشكل كلي للكتين الخام والنقي

عند التركيزين 125 و 250 مايكروكرام/مل . بالأضافة الى *K. pneumoniae* الذي كان مقاوما وبشكل كلي للكتين الخام عند التركيزين 125 و 250 مايكروكرام/مل واللكتين النقي عند تركيز 125 مايكروكرام/مل .

Introduction

Acinetobacter species are strictly aerobic gram-negative cocco-bacilli, ubiquitous, free living bacteria (1). The most site of infection is the respiratory tract. However, infection in the blood, urinary tract andother positions has also been mentioned (2). They are usually an opportunistic pathogens, and in the last years have been stated to cause a number of outbreaks of nosocomial infections in hospitalized patients like Septicemia, Pneumonia, Wound sepsis, Endocarditis, Meningitis and Urinary tract infection (UTI)(3). The colonization of *Acinetobacter baumannii* to different host tissues related to adhesion factors. These virulence factors called lectins that found on the surface and the capsule of bacteria can bind to the glycol-conjugates that were be on other cell surfaces (4). Lectins are proteins with specificity for simple sugar, a sequence of sugars or their glycosidic linkages (5). These proteins are ubiquitous in nature, and found in animals, plants, bacteria, viruses, and fungi(6). As a result for their chemical composition, they have become a useful tool in many fields such as immunology, cell biology, molecular biology, membrane structure, pharmacology, cancer research, clinical chemistry and genetic engineering (7).

As long as many researches about lectins from plant sources, we aimed in this research to study the effect of lectin from *A. baumanniii* isolated from urinary catheters on some pathogen bacteria.

Materials and Methods

Isolation of *Acinetobacter baumannii*: Seventy one samples of urinary catheters were collected and only twenty eight isolates of *A. baumannii* were diagnosed by VITEK-2system.

Isolation of Pathogenic Bacteria: Mouth, sputum, urine and stool swabs in addition to blood cultures were collected from different clinical cases and several bacterial samples were isolated and diagnosed by VITEK -2 system. These bacterial isolates included *Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae, Listeria monocytogenes, Pseudomonas aeruginosa, Serratia marscenes, Staphylococcus aureus, Streptococcus mutans* and *Streptococcus pyogene.*

Three isolates of each species were included in this study.

Screening for Lectin Production by *Acinetobacter baumannii*: All *A. baumannii* isolates were grown separately in Luria Bertaini broth at 37°C for 24h. andlectin activity was checked by hemagglutination assay followed by monitoring the protein content.

Quantitative Hemagglutination Assay for *Acinetobacter baumannii* isolates: The quantitative screening was done in microtiter plate according to Mirelman and Ofek (8) as following: serial two fold dilution of bacterial suspension 50µl in microtiter plate with 0.02M phosphate buffer saline (PBS) pH 7.2 was mixed with the same volume of a 3% suspension of rabbit and human erythrocytes in the same buffer and incubated at 37°C for 2 h. The activity was expressed as hemagglutination units (HU). One HU was identified as the inverse of the highest dilution still capable of causing agglutination.

Extraction of Crude Lectin: *A. baumannii* isolate (No.7) was grown in brain heart infusion broth at 37°C for 24h. The cells were collected by centrifugation at 8000 rpm

for 30 min., washed twice and re-suspended in 0.02M PBS pH 7.2 . Cells were disrupted by glass beads for 50 min. at 4°C using vortex. Whole residual cells and cell membrane fragments were removed by centrifugation at 8000 rpm for 20 min. and then fractionated with ammonium sulfate at a concentration 45% and the precipitation obtained after centrifugation at 8000 rpm for 30 min. was suspended in 0.02M PBS pH 7.2 for 24h. Thus the viscous extract obtained was the crude lectin. The hemagglutination activity and the protein concentration were measured and was subjected to further processing (9).

Purification of *Acinetobacter baumannii*lectin: The dialyzed protein was introduced to a DEAE cellulose column (2.5 × 20) cm previously standardized with the buffer(0.02M PBS pH 7.2) and eluted with a salt gradient containing (0.1-0.5) M NaCl. The fractions that revealed significant peak of activity were mixed together and applied to sephadexG-100 column previously standardized with the buffer (0.02M PBS pH 7.2). Protein concentration and hemagglutination activity were measured (9).

Estimation of Hemagglutination Activity: Hemagglutination activity of the purified lectin was determined according to Correia and Coelho (1995) (10) using rabbit and human A^+,B^+,AB^+ and O^+ type erythrocyte suspension (2.5% v/v:50µl) treated with gluteraldehyde. HA was defined as the lowest lectin concentration able to promote erythrocyte agglutination.

Estimation of the Protein: Protein was estimated according to Lowry et al. (1951) (11) using bovine serum albumin as a standard.

Characterization of *Acinetobacterbaumannii*lectin:

Effect of Temperature and pH on Lectin Activity: The effect of temperature and pH on lectinhemagglutination activity was evaluated. One ml of crude lectin was incubated at different temperatures (20, 30, 40, 50, 60, 70, 80, 90, and 100)°C for 30 min. while the effect of pH on HA was evaluated by incubation crude lectin for 45 min. at 25°C in selected buffers: 10mM citrate phosphate pH3-6.5, 10mM sodium phosphate pH 7 and 7.5 and 10mM Tris-HCl pH 8, 8.5, 9, 9.5 and 10(12).

Effect of Metal Ions on Lectin Activity: The activity of purified lectin was determined in the presence of 10mM of each of Ca^{++}, Mn^{++}, Mg^{++} and also after dialysis against 10mM EDTA (13).

Molecular Mass Determination: The molecular mass of the lectin was determined by gel filtration on sephadexG-150 (0.75×100 cm) using lysozyme, trypsin inhibitor and α-amylase as reference proteins (14).

MIC and MBC: Pathogenic Bacteria isolates were cultured in nutrient broth and incubated under continuous shaking at 37 °C overnight. The culture concentrations were turbidimetrically adjusted at 600 nm to 10^5–10^6 colony forming units (CFU)/mL. *A. baumannii* purified lectin was diluted (1:1024) in a microtiter plate containing nutrient broth (50 µl per well). Subsequently, 20 µl of bacterial suspension was applied to each well and the plate was incubated at 37 °C for 24 h. After incubation, the optical density at 490 nm (OD490) was measured using a spectrophotometer for microplates. The assays were performed in triplicate. The minimal inhibitory concentration (MIC) corresponded to the lowest lectin concentration able to inhibit the growth of 50% or more of microorganisms relative to the negative control (15). Thereafter, aliquots (20 µl) of each well in which inhibitory activity was observed were transferred to petri

plates containing nutrient agar. The plates were incubated at 37 °C for 24 h. The minimal bactericidal concentration (MBC) corresponded to the lowest concentration of lectin able to reduce the number of CFU to 0.1% relative to the negative control.

Antibacterial Activity Assay: Agar well diffusion method was used to detect antimicrobial activity of lectin produced by *A. baumannii* against the pathogenic bacterial isolates at different concentrations (1000, 500, 250, 125) μg/ml according to Batdorj (16).

Statistical Analysis: Results were explained as the mean ± standard deviation (SD). The intergroup variation was determined by one way analysis (ANOVA) $P< 0.05$ using state statistical software.

Results and Discussion

Quantitative Hemagglutination Assay: The hemagglutination activity in microtiter plate was revealed in all *A. baumannii* isolates. The results showed that human erythrocytes A^+, B^+, AB^+ and O^+ and rabbit erythrocytes gave hemagglutination activities, and the isolate *A. baumannii* S7 showed higher hemagglutination value against rabbit erythrocytes than O^+ human blood group, while the remaining blood groups showed lower values. These differences revealed that the lectin is discrete in its specialty to carbohydrates or glycoproteins on erythrocytes (12).

Hemagglutinating activity is the most commonly used test for the determination of lectin in a sample because of the simplicity of method and ease visualization of agglutination. Aliquot of sample is serially diluted in microtitration plate before addition of erythrocytes and hemagglutinating activity (titer) is defined as the reciprocal of the highest dilution of sample promoting full agglutination of erythrocytes. The hemagglutinating activity occurs when the lectin binds to carbohydrate on erythrocyte surface forming a network among them; sometimes lectin is not detected due to steric hindrance in the lectin-carbohydrate reaction and previous enzymatic treatment of erythrocytes is needed to occur hemagglutination (17). The hemagglutination test allows estimation of lectin stability at pH and temperature values and thus can estimate the conditions to be applied in the biotechnological application of lectin (18).

Extraction and Purification of *Acinetobacterbaumannii*lectin:Lectin was purified up to 2.793 folds, the overall yield and activity are summarized in Table (1).

Lectin from *A. baumannii* was purified by DEAE cellulose with a yield of 40% and purified to 1.25 followed by purification with sephadexG-100 with a yield of 14% and purified to 2.793 fold. Another study on lectin from locally isolates of *A. baumannii* was purified by ammonium sulfate precipitation to 1.28 fold with a yield of 48% followed by DEAE-sephadex and it was purified to 3.18 fold with a yield of 44% followed by sephadexG-75 purification to 4.66 fold with a yield of 36% (9).

Effect of Temperature and pH on Lectin Activity: Hemagglutination activity was heat stable up to 80°C with total loss of activity after heating to 90°C as shown in Figure (1) and the highest value was at pH 6.5 as shown in Figure (2).

Table (1): Steps of Lectin Purification from *Acinetobacterbaumannii*

Purification Steps	Volume(ml)	Protein Con.(mg/ml)	Hem.Activity(U/ml)	Specific Activity(U/mg)	Total Activity(HU)	Purification Fold	Yield(%)
Crude Extract	100	3.25	406	124.923	40600	1	100
DEAE cellulose	40	2.58	812	314.728	32480	1.25	40
SephadexG-100	14	1.56	1038	665.38	14532	2.793	14

The compact globular structures, molecular aggregation and glycosylation in general result in high structural stability of lectins (19,20); high temperature is a powerful denaturing factor leading to protein unfolding through breaking of hydrogen bonds that keep protein structure and heated lectins can or not lose their biological characteristics (18). It was revealed that *Sophoraalopecuroides* seeds lectin (SAL) was less susceptible to variations in temperature and pH than the lectins of *Astragalus mongholicus* and *Curcuma longa* (21,22,23). BfLlectin from *Bauhinia forticata* seeds was an acidic protein and its highest HA value was at pH 6 (12) which was closely to our results. The HA of LEL from fresh fruity bodies of shiitake mushroom *Lentinulaedodes*was stable up to 60°C and decreased at 80°C and was totally inactivated at 90°C, while the HA was unaffected by pH (24) and these results were different to ours.

Effect of Metal Ions on Lectin Activity: Lectin did not require divalent cations for its activity since extensive dialysis against 10 mM EDTA pH 6.5 followed by dialysis against plain buffer to remove the EDTA did not affect the hemagglutination activity and there was no increase in HA at addition of 10 mMCa^{++}, Mn^{++} and Mg^{++} .

The hemagglutinating activity of SAL required Mn^{++}; this is consistent with a lectin from *C. longa* (21). Similar results were revealed by Silva et al.(2012) (12) for the BfLlectin which its HA was not dependent on metal ions.

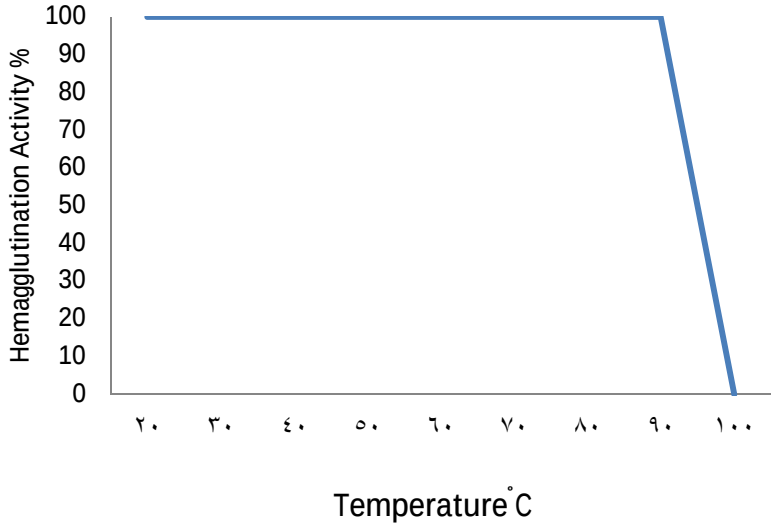

Figure (1): Effect of Temperature on Hemagglutination Activity of *A. baumannii* Lectin

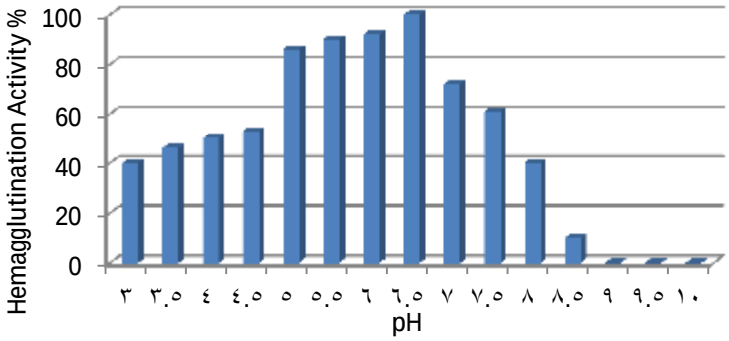

Figure (2): Effect of pH on Hemagglutination Activity of *A. baumannii* Lectin

MIC and MBC:

Table (2): Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of *A. baumannii* S7 purified lectin

Bacterial Isolate	MIC (µg/ml)	MBC (µg/ml)
Bacillus subtilis no. 1	256	512
Bacillus subtilis no. 2	8	18
Bacillus subtilis no. 3	32	64
Enterococcus faecalis no. 1	256	512
Enterococcus faecalis no. 2	32	64
Enterococcus faecalis no. 3	512	ND
Klebsiellapneumoniae no. 1	ND	ND
Klebsiellapneumoniae no. 2	ND	ND
Klebsiellapneumoniae no. 3	1024	ND
Listeria monocytogenes no. 1	8	16
Listeria monocytogenes no. 2	32	128
Listeria monocytogenes no. 3	16	32
Pseudomonas aeruginosa no. 1	512	1024
Pseudomonas aeruginosa no. 2	16	32
Pseudomonas aeruginosa no. 3	1024	ND
Serratiamarscenes no. 1	32	128
Serratiamarscenes no. 2	16	64
Serratiamarscenes no. 3	8	16
Staphylococcus aureus no. 1	512	1024
Staphylococcus aureus no. 2	8	16
Staphylococcus aureus no. 3	32	64
Streptococcus mutans no. 1	512	1024
Streptococcus mutans no. 2	1024	ND
Streptococcus mutans no. 3	ND	ND
Streptococcus pyogenes no. 1	1024	ND
Streptococcus pyogenes no. 2	256	1024
Streptococcus pyogenes no. 3	ND	ND

ND: Not Determined

Molecular Mass Determination: The molecular mass of the lectin as determined by gel filtration on sephadexG-150 was 58 KDa. Eghianruwa (24) determined the molecular mass of LEL by gel filtration which was assessed to be 71 KDa. Olivera (25) showed that the molecular mass of Euni SL lectin isolated from *Eugenia uniflora* seeds was 67 KDa.

Antibacterial activity on Gram-positive and Gram-negative bacteria occurs through the reaction of lectin with components of the bacterial cell wall including teichoic acid and lipoteichoic acid, peptidoglycans and lipopolysaccharides (18). A thermo stable lectinEuni SL lectin*Eugenia unifloras*eeds lectinshowed distinctive non-selective antibacterial activity (25); the lectin strongly inhibited the growth of *S. aureus, P. aeruginosa* and *Klebsiella*sp. with MIC of 1.5 µg/ml while was less effective in inhibiting the growth of *B. subtilis*, *Streptococcus* sp. and *E. coli* (MIC of 16.5 µg/ml). Bactericide activity was mainly detected for *S. aureus, P.aeruginosa* and *Klebsiella*sp. (MBC of 16.5 µg/ml); the authors suggested the application of lectin for clinical microbiology and therapeutic uses.

Antibacterial Activity Assay: As shown in Table (3), lectin produced by *A.baumannii* had antibacterial activity against all the bacterial isolates used in this study as crude and purified at different concentrations and the pure was higher affected than the crude lectin and highest effect was at concentration of 1000µg/ml except of *B. subtilis* which was totally resist for the crude and purified lectin at both concentrations 250 and 125µg/ml, in addition to *K.pneumoniae*which was totally resist to the crude lectin at both concentration 250µg/ml and 125µg/ml and pure lectin at 125 µg/ml.

Table (3): Antibacterial Activity of Crude and Purified Lectin on Some Pathogenic Bacteria

Bacterial Isolates	Control	Inhibition Zone (Mean ± SD)							
		Crude Concentration(µg/ml)				Purified Concentration (µg/ml)			
		1000	500	250	125	1000	500	250	125
Bacillus subtilis	0±0	15.22±1.07 a	9.11±0.86a	0±0	0±0	20.48±0.95 a b	14.82±1.22 a b	0±0	0±0
Enterococcus faecalis	0±0	26.61±0.97 a	20.88±1.55 a	16.32±1.32 a	11.59±1.69 a	31.14±1.11 a b	26.73±0.68 a b	22.17±1.14 a b	19.81±0.89 a b
Klebsiellapneumoniae	0±0	19.11±1.62 a	12.81±1.47 a	0±0	0±0	17.96±1.02 a b	16.13±0.18 a b	11.32±1.42 a b	0±0
Listeria monocytogenes	0±0	31.26±0.18 a	27.78±1.33 a	24.44±1.49 a	19.59±0.78 a	35.11±1.66 a b	33.53±1.02 a b	28.68±1.33 a b	22.64±1.93 a b
Pseudomonas aeruginosa	0±0	24.8±1.12a	19.52±1.27 a	14.13±0.95 a	9.66±1.29a	27.9±0.41a b	24.71±0.36 a b	18.9±0.12a b	13.22±0.47 a b
Serratiamarscenes	0±0	32.48±1.34 a	27.15±1.77 a	23.16±0.39 a	17.08±1.22 a	35.61±1.75 a b	31.83±1.61 a b	28.42±1.11 a b	23.17±0.98 a b
Staphylococcus aureus	0±0	25.77±1.33 a	21.03±0.19 a	18.09±1.52 a	16.1±0.23a	29.08±0.85 a b	25.11±0.73 a b	22.81±1.02 a b	19.37±1.39 a b
Streptococcus mutans	0±0	22.18±1.09 a	17.46±1.83 a	14.27±1.69 a	9.53±0.18a	27.11±1.88 a b	23.54±0.79 a b	18.91±0.96 a b	13. 06±1.08a b
Streptococcus pyogenes	0±0	29.16±0.96 a	25.17±1.38 a	18.75±1.02 a	10.26±0.88 a	34.81±1.57 a b	27.65±1.38 a b	21.27±1.09 a b	14.55±0.74 a b

The study on SAL revealed that SAL absent of antibacterial activity against *Klebsiella ozaena*, *A. baumannii*, *Shigella flexneri*, *K. pneumoniae*, *Pseudomonas pseudoalcaligens*, *Proteus mirabilis*, *Shigella sonnei* and *Escherichia coli* (23).While Euni SL lectin showed a distinguish nonselective antibacterial activity (25).

Conclusion

Lectin of *A. baumannii* was thermostable up to 80°C and kept its HA at a wide range of pH and the highest one was at pH 6.5 while it did not require any metal ions. Lectin had antibacterial activity against both gram positive and negative bacteria so it can be used for the applications of clinical microbiology.

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Effect of some derivatives quinoline prepared on some pathogenic bacteria species compared with the 2-hydrazinobenzothiazole

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Abstract

We're preparing some chemical compounds which included a 2-methyl quinoline-4-carboxylic acid, ethyl-2-methyl quinoline-4-carboxylate, 2-methyl quinoline-4-carbohydrazide and 2-hydrazinobenzothiazole and study its effect on gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) , and gram positive (*Bacillus cereus* and *Staphylococcus aureus*) using well diffusion method. The result was the effectiveness of the compounds 2-methylquinoline-4-carbohydrazide and 2-hydrazinobenzothiazole on all species bacteria tested. The minimum inhibitory concentration (MIC) in 2-methylquinoline-4-carbohydrazide is (15-25) µg/ml and in 2-hydrazinobenzothiazole is (5-25) µg/ml.

تأثير بعض مشتقات Quinoline المحضرة على بعض الانواع البكتيرية المرضية بالمقارنة مع 2-Hydrazinobenzothiazole

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الخلاصة

حضرت بعض المركبات الكيميائية والتي شملت 2-methyl quinoline-4-carboxylic acid و ethyl-2-methyl quinoline-4-carboxylate و 2-methyl quinoline-4-carbohydrazide و 2-hydrazinobenzothiazole وتم دراسة تأثيرها على البكتيريا السالبة لصبغة غرام وهي *Echerichia coli* و *Pseudomonas aeruginosa* و *Klebsiella pneumoniae* والموجبة لصبغة غرام و التي شملت *Bacillus cereus* و *Staphylococcus aureus* باستخدام طريقة الانتشار في الحفر وكانت النتيجة فعالية المركبين 2-methylquinoline-4-carbohydrazide و 2-hydrazinobenzothiazole على جميع انواع البكتيريا المفحوصة. وتم حساب التركيز المثبط الادنى Minimum inhibitory concentration (MIC) لهذين المركبين ولكل الانواع البكتيرية المستخدمة في التجربة فكان 2-methylquinoline-4-carbohydrazide بين (15-25) مايكروغرام/ مل وفي 2-hydrazinobenzothiazole بين (5-25) مايكروغرام/مل.

Introduction

Bacteria is divided in to harmful and useful , the harmful bacteria cause disease to human and animal include *Pseudomonas aeruginosa* , *Bacillus cereus* , *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumoniae* . *Pseudomonas aeruginosa* which cause pneumonia, urinary tract infection , otitis , burns and wounds infection. Also production

of lethal toxins(1),(2),(3). *Pseudomonas aeruginosa* resistance to many antibiotics(4). *Bacillus cereus* is another type of bacteria cause eye infection, otitis , meningitis , endocarditis ,bacteremia in weak human immunity, also production toxin(5),(6).(7). The type of bacteria *Escherichia coli,* is normally and not cause diseases, but some strains cause food poisoning, Intestinal infection, urinary tract infection, meningitis in infants(8). *Staphylococcus aureus* bacteria cause different diseases in the human body and resistance to different groups of antibiotics, especially beta lactam and aminoglycoside , this bacterial considers cause of nosocomial infection(9). *Klebsiella pneumoniae* is one of the most important pathogens, which when inhalation it, cause damage in lung lead to bloody sputum, also considers cause of nosocomial infection(10),(11). the mechanisms of antimicrobial resistance in bacteria are : enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics. Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome(12). Because bacterial resistant to antibiotics, we prepared another compounds as antibacterial and study the effect of this compounds on bacteria. The compounds is derivatives from Quinoline which act as antibacterial in spite of not isolate it from organisms but chemically prepared (13) and compared with 2-Hydrazino Benzothiazole.

Materials and Methods

Prepared compounds: Prepared four compounds as stated at (14),(15),(16). The first compound is 2-methyl quinoline-4-carboxylic acid ($C_{11}H_9NO_2$) dissolved in water at concentration 0.025 M (4675000 μg/ml), the second compound is ethyl-2- methyl quinoline-4-carboxylate ($C_{13}H_{13}NO_2$) dissolved in ethanol to reach concentration 0.025 M (5375000 μg/ml), this compound is one of the ester compounds ,the third compound is 2-methyl quinoline-4-carbohydrazide ($C_{11}H_{11}N_3O$) dissolved in ethanol to reach concentration 0.025 M(5025000 μg/ml) , this compound is one of the hydrazide compounds , the fourth compound is 2-hydrazinobenzothiazole ($C_7H_7N_3S$) which derivatives from 2-Mercaptobenzothiazole dissolved in ethanol to reach concentration 0.025 M (8400000 μg/ml) ,this compound is one of the hydrazide compounds too.

Biological activity; We depended (17) method, for prepared compounds in antimicrobial activity, we chose five type of bacteria wich are *E.coli* , *Staphylococcus aureus* , *Bacillus cereus* , *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, this bacteria activated in nutrient agar and used Mueller Hinton agar media in antimicrobial activity test by chemical compounds which are 2-methyl quinoline-4-carboxylic acid,ethyl-2-methyl quinoline-4-carboxylate,2-methyl quinoline-4-carbohydrazide and 2-hydrazinobenzothiazole , with alcohol 95 % as a control. After cultured bacteria 24 hours to activated, take bacterial colony and diluted with peptone water to reach $1.5 * 10^8$ cell/ml, take swab from it and streaking on agar at every directions, and make pits by cork screw to obtain pits with 6 millimeter in diameter, this pits filled with prepared compounds and incubated aerobically in 37 c at 24 h, after that observed the inhibition zones around the pits.

Minimum inhibitory concentration (MIC): It has been determined MIC by turbidity method as in (18) method , add 10 ml nutrient broth to each tubes and autoclaved after that add 0.1 ml from concentration (500, 1500, 2500, 3500) μg/ml to each of two compounds 2-methyl quinoline-4-carbohydrazide and 2-hydrazinobenzothiazole to reach to the end concentration (5, 15, 25, 35) μg/ml in tube with positive control (bacteria without compounds) and negative control (compounds without bacteria) , after that added 0.1 ml from bacterial suspension and incubated in 37 c in (18-24) h and recorded results by depended on turbidity which measured by spectrophotometer.

Results and Discussion

Study of biological activity: Heterocyclic compounds represent one of the most active classes of compounds possessing a wide spectrum of biological activities, including antibacterial, antifungal, and other biological activities (19, 20, 21, 22, 23, 24), where depended the infusion in agar by pits to compare between Quinoline derivatives and thiazole hydrazide on bacteria gram positive and negative, show in Figure (1) and Table(1).

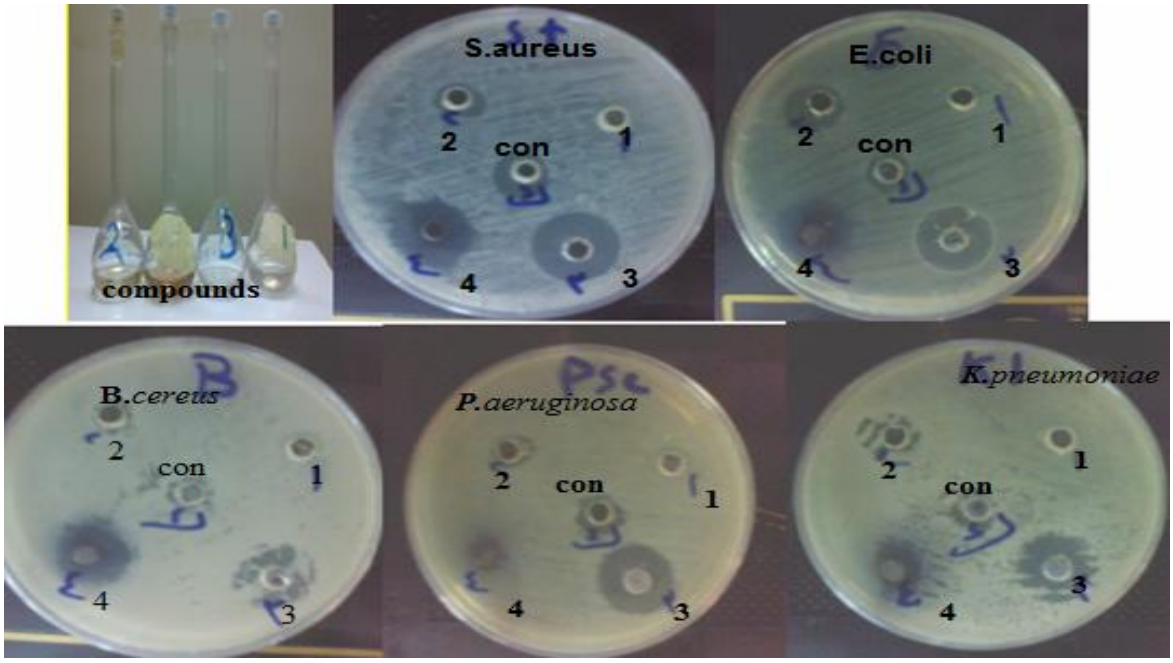

Figure (1): the effect of prepared chemical compounds on types of bacteria and inhibition zone

2-methyl quinoline-4-carboxylic acid 1=
2=ethyl-2- methyl quinoline-4-carboxylic acid
3=2-methyl quinoline-4-carbohydrazide
4=2-hydrazinobenzothiazole
con= is ethanol control

Table (1): inhibition zone diameters of chemical compounds against different type of bacteria

Bacteria	2-methyl quinoline-4-carboxylic acid (mm)	ethyl-2-methyl quinoline-4-carboxylic acid (mm)	2-methyl quinoline-4-carbohydrazide (mm)	2-hydrazinobenzothiazole (mm)	Ethanol Control (mm)
Staphylococcus aureus	-	10	20	20	12
E. coli	-	12	19	15	11
Bacillus cereus	-	8	15	17	8
Pseudomonas aeruginosa	-	10	18	7	11
Klebsiella pneumoniae	-	11	18	13	10

(-) no inhibition zone

Observed through table, 2-methyl quinoline-4-carboxylic acid is (0) mm against any type of studied bacteria , the ethyl-2- methyl quinoline-4-carboxylic acid is (8-12) mm and ethanol control is (8-12) mm , 2-methyl quinoline-4-carbohydrazide is active against all bacteria in this research its hydrazide compound derivative from quinoline is (15-20) mm, and 2-hydrazinobenzothiazole is active against all bacteria toois (7-20) mm.
Because the activity of 2-methyl quinoline-4-carbohydrazide and 2-hydrazinobenzothiazole, we took the MIC of each two compounds on all bacteria, show Table (2)

Table (2): MIC of 2-hydrazinobenzothiazole and 2-methyl quinoline-4-carbohydrazide on different types of bacteria

Bacteria	2-hydrazinobenzothiazole (µg/ml)	2-methyl quinoline-4-carbohydrazide (µg/ml)
Staphylococcus aureus	15	25
E.coli	15	25
Bacillus cereus	5	15
Pseudomonas aeruginosa	25	25
Klebsiella pneumoniae	25	25

2-hydrazinobenzothiazole was active more than 2-methyl quinoline-4-carbohydrazide against *Staphylococcus aureus* , *E.coli* and *Bacillus cereus* , and equal in activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* , the MIC of 2-hydrazinobenzothiazole is (5-25) μg/ml and to 2-methyl quinoline-4-carbohydrazide Is (15-25) μg/ml , this results agreement with (25) in activity of 2-methyl quinoline-4-carbohydrazide against *Staphylococcus aureus* , *E.coli* and *Klebsiella pneumoniae* In a study of (25) he prepare this compounds:

2-methyl-N'-((E)-phenylmethylidene) quinoline-3-carbohydrazide (C18H15N3O)
2-methyl-N'-((E)-(4-methylphenyl) methylidene) quinoline-3-carbohydrazide (C19H17N3O)
N'-((E)-(4-methoxyphenyl)methylidene)-2-methylquinoline-3-carbohydrazide (C19H17N3O2)
2-methyl-N'-((E)-(2-nitrophenyl) methylidene) quinoline-3-carbohydrazide (C18H14N4O3)
N'-((E)-(4-chlorophenyl) methylidene)-2-methylquinoline-3-carbohydrazide (C18H14N3OCl)
N'-((E)-(4-fluorophenyl) methylidene)-2-methylquinoline-3-carbohydrazide (C18H14N3OF)
N'-((E)-(4-hydroxy-3-methoxyphenyl)methylidene)-2-methylquinoline-3-carbohydrazide (C19H17N3O3)

The MIC of this compounds in *Staphylococcus aureus* about (66.66-166.66) μg/ml

In *E. coli* (8.33-66.66) μg/ml , in *Klebsiella pneumoniae* (6.25-166.66) μg/ml and in *Bacillus subtilis* (10.41-166.66) μg/ml

and agreement with (26) in activity of 2-hydrazinobenzothiazole against *Staphylococcus aureus* , this result is good because low toxic of thiazole compounds when used via oral and veins (27) , the thiazole nucleus appears frequently in the structure of various natural products and biologically active compounds, notably thiamine (vitamin-B), antibiotics such as penicillin, micrococcin (28)

It is therefore concluded the hydrazide compounds are active against all studied bacteria in different concentration , this activity return to effective group in hydrazide (C(O)NH-NH2-) which replace the carboxylic group (-C(O)OH) (29).

The chromosome of bacteria is composed of helical double-stranded DNA and contains 60 to 70 spatial regions of organisation, termed domains of supercoiling. Each domain is about 20 mu long, attached to an RNA core and is organised by supercoiling which occurs quite independently of the DNA coiling in any other domain. Supercoiling is controlled by the enzyme DNA gyrase, which introduces transient breaks into both DNA strands of each domain, removes about 400 turns from its DNA helix, then reseals the DNA so locking in the supercoiling. This supercoiled state is essential to the well-being of bacteria as it enables them to accommodate their chromosome (1300 mu long) within the confines of their cell envelope (2 mu X 1 mu). The target site of action of the quinolone antibacterial agents is DNA gyrase and its inhibition by them sets off a

complex series of events which ultimately causes bacteria to die. However, the bactericidal action of nalidixic acid and most other quinolones can be abolished if protein synthesis is inhibited by chloramphenicol, and perhaps not surprisingly the same is true if RNA synthesis is inhibited by rifampicin. With ofloxacin and ciprofloxacin the situation is more complicated because protein or RNA synthesis inhibition does not completely abolish their bactericidal effects. Hence ofloxacin and ciprofloxacin exhibit a qualitative difference from most other quinolone antibacterial agents in that they possess an additional mechanism of killing bacteria that is not possessed by the older, lesser active drugs. Mammalian cells possess an enzyme which resembles bacterial DNA gyrase in that it cuts double-stranded DNA in a similar manner. However, the mammalian enzyme does not possess any supercoiling action nor is it susceptible to inhibition by the quinolone antibacterials, which can hence be used to inhibit bacteria in man without harm to the latter(30).From the present study it is concluded that the hydrazide compounds are most effective against pathogenic bacteria.

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Microfacies study of Tanjero Formation from Rawandoz section north of Iraq

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Abstract

Tanjero Formation studied at Rawandoz section near Rawandoz city, north Iraq. Its type section near Sirwan village about 15km south of Halabcha town, Erbil Governorate, north Iraq. 19 samples of carbonate rock bed units includes limestone, marly limestone and shale, has been collected and thinned section to analyze them by polarized microscope to identify petrographic components, digenetic processes and fossil contents. According to Dunham classification, Wilson model and Flugel, the carbonate rock bed units of Tanjero Formation were subdivided into microfacies: 1-Pelagic mudstone facies, 2-Biogenic three Packstone facies and 3-Bioclastic wackestone facies. Microfacies analysis shows that the environment of deposition of the lower part is deep shelf margin, while the middle and upper parts is deposited at deep shelf environment of deposition.

دراسة السحنات الدقيقة لتكوين تانجيرو من مقطع راوندوز شمال العراق

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الخلاصة

تمت دراسة تكوين تانجيرو في مقطع راوندوز بالقرب من راوندوز شمال العراق. ان طبيعة المقطع بالقرب من قرية سيروان بحدود 15 كم جنوب مدينة حلبجة في محافظة اربيل شمال العراق. تم جمع 19 نموذج من صخور الكاربونات تضمنت وحدات طباقية من الحجر الجيري والحجر الكلسي (مارلي) و الاطيان وعملت منها مقاطع او شرائح رقيقة من اجل فحصها بالمجهر المستقطب وللتعرف على المكونات الدقيقة والمتحجرات المختلفة. حسب تصنيف "دنهام" ونظام "ويلسون وفلوكال فان الوحدات الطباقية الكاربونية لتكوين تانجيرو ينقسم الى عدة سحنات دقيقة : 1 – سحنات من الحجر الطيني البلاجي، 2 – سحنات حجر مرصوصة بايوجينية و 3 – سحنات حجرية ذات محتوى من متحجرات الهياكل العظمية (بايوكلاستية). وبين تحليل السحنات الدقيقة للبحث ان التركم او الترسبات بيئية .

Introduction

Tanjero Formation firstly described by (1) in (2), (Figure 1). It was comprises thick sequence of sandstones, mudstones and some rock bed units of conglomerate in addition to few beds of limestone. The type section near Sirwan village about 15 Km south Halabcha town, Erbil Governorate, north Iraq (Figure 1). *Planktonic foraminiferal* study shows deep environment of deposition (3) and (4) during Campanian-

Maastrichtian. Studied formation crop out along narrow belt that extended about 300 km north west– south east, from Halabcha on Sirwan River to Arkosh village near Iraq-Turkish border. Tanjero Formation are subdivided into two units (1): the lower part consist of predominantly open sea *globigerinal* marlstone and rarely marly Limestone and silty marly limestone. The upper part consists of silty marls, siltstone, sandstone, conglomerates and sandy or silty detrital limestones. (5) subdivided it into 8 divisions according to lithology and characters. Upper contact of Tanjero Formation with Kolosh Formation is unconformable and shows some basal conglomerate between them. Lower contact with other formations show conformable contact particularly with Balampo Formation on the Azmur anticline sections near Sulimaniya, and with Shiranish Formation at Rawandoz and Dokan sections. The upper and lower parts were deposited at shallow marine environment while the middle part deposited at deep basin environment (6). The aim of this study is to identify the microfacies of carbonate rock bed units and determines the environment of deposition.

Materials and Methods

Nineteen samples were collected from Tanjero Formation at Rawandoz section, its thickness about 1210 meters, north Iraq. 19 samples of carbonate rocks include limestone, marly limestone and shale (Figure 2) has been thinned sections and examined by polarized microscope to identify petrographic components, diagenetic processes and fossils contents.

Results and Discussion

According to the petrographic composition and fossils contents, and by using (7) in (8) and (9) (Figure 3); Tanjero Formation has been subdivided into three microfacies: Pelagic mudstone facies, biogenic packstone facies, and fine bioclstic wackestone facies:

1- Pelagic mudstone facies: it is appeared at the lower part of the formation ,and composed of plantonic *foraminifera* within micrite, such as *Globotruncana* sp. (Plate1-1), *Globigerina* sp., *Gastropoda* and shell fragments (Plate 1-2). The Abundance of micrite and rare of cement resulted from low energy current that cannot remove micrite to deposit cement within pores (10). There is spary calcite within some *globigerinal* chamber (Plate 1-3). Involved facies equivalent to (S.M F.3) within the zone (F.Z A1) deep sea wave and below the euphotic zone in quite oceanic deep water environment.

2- Biogenic packstone facies: It is consists of contact grains of skeletal grain such as *Milliolid* plate (1-4), *Globigerina* (Plate 2-1) and shell fragments; as well as non-skeletal grains such as *cortoid* (Plate 2-2) and intraclast, mentioned grains found within matrix of micrite. Intraclast derived from the same basin of deposition and transported by water currents (11). *Cortoid* refers to high energy of shallow marine environment in which micrite covers are grows (12), in addition to bacteria, fungi, and algae. The later organisms produce mucilage to fix the grains together and prevent dissolution during transgression when the energy reduces and the depth become deeper. Dolomite is the main minerals scattered in the matrix, in addition to Pyrite in which deposited in the fossil chambers (Plate 2-3). Calcite cement is the main diagenetic processes. Involved

facies is equivalent to (S.M.F.4) within (F.Z.3). Toe-of-slop apron (Deep shelf margin below wave base and barely Oxygen level).

3- Fine bioclastic wackestone facies: It is composed mainly of micrite that contains planktonic *forminifera* such as *Globigerin*a and *Globotruncana* which refers to quite deep marine environment (13). The most important diagenetic processes are calcite cement deposition and authigenic pyrite which filled *Foraminifer*al chambers in addition to dolomite rhomboid (Plate 2-4). The presence of dolomite in the microfacies is rich in skeletal grains and shells are reflects secondary diagenetic processes (14). Above mentioned microfacies equivalent to (S.M.F.9) within (F.Z.2) which deposited in the deep shelf, below fair weather wave base, but within reach of the extreme storm wave.

Conclusions

Carbonate rocks of Tanjero Formation were classified into three microfacies : 1- Pelagic mudstone facies, 2- Biogenic packstone facies, and 3- Fine Bioclastic wackestone facies. Mentioned microfacies refers to deep marine depositional environment. The lower part is deposited at deep see; middle part is deposited at deep shelf margin; and upper part is deposited at deep shelf below fair weather wave. Deposition of microfacies 1- pelagic mudstone over the microfacies the microfacies 2- biogenic packstone refers to transgression, while the deposition of microfacies 2- biogenic packstone over the microfacies 3- Fine bioclastic wackestone refers to regression.

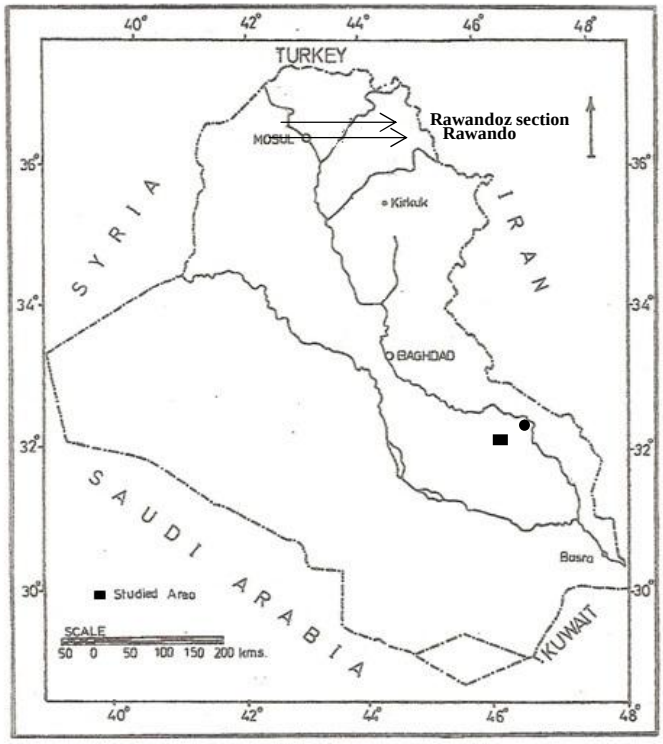


Figure (1): Map description Tanjero Formation in Erbil.

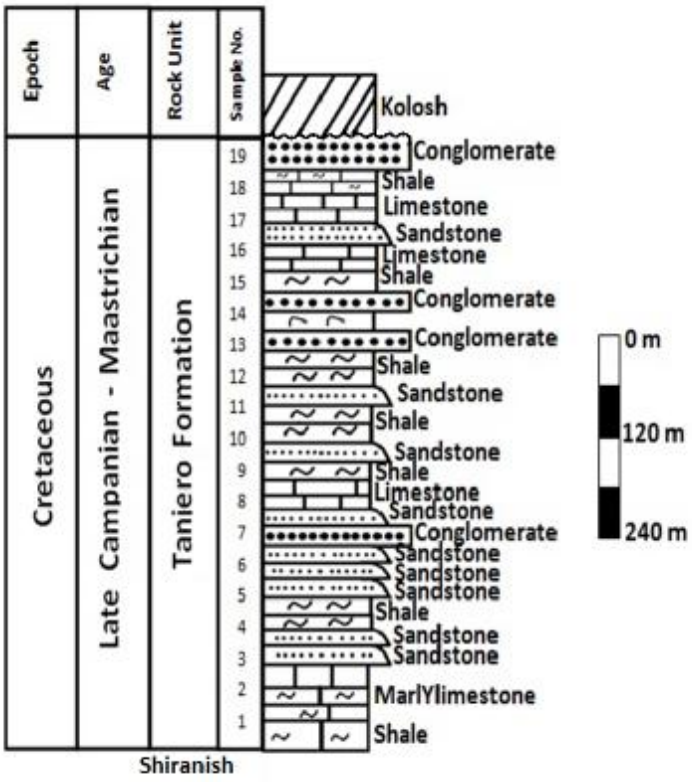


Figure (2): Petrographic description of the Tanjero Formation at Rawandoz section north Iraq.

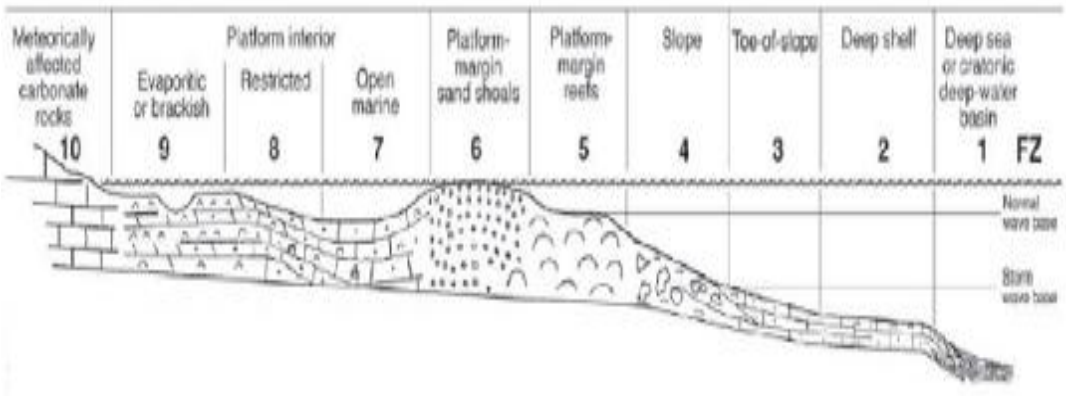
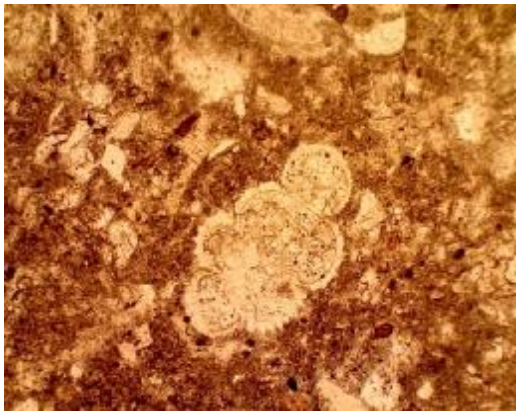
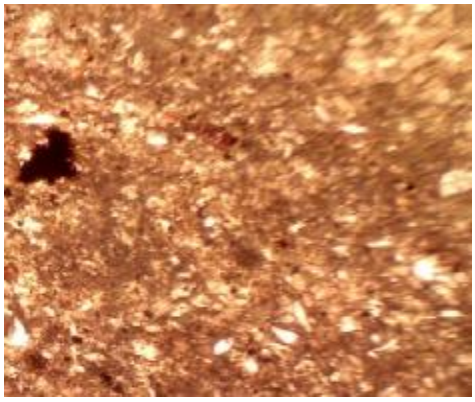


Figure (3): Rimmed carbonate platform: The standard facies zones modified after Wilson model (Wilson, 1975).

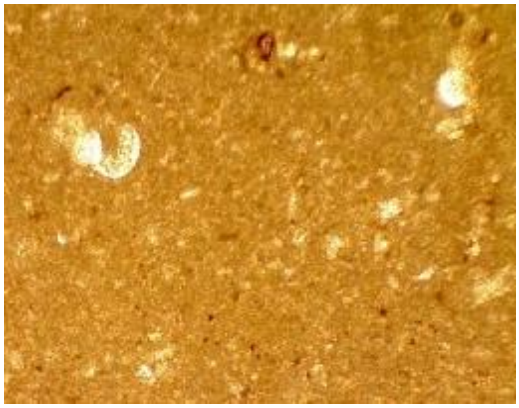
(Plate-1)



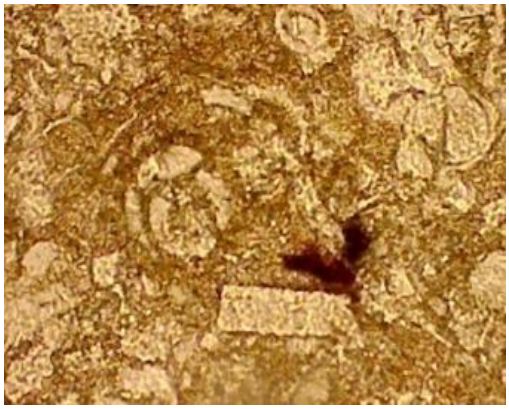
1
Globotruncana sp. In Mudstone facies 40x



2
Shell fragment filled with calcite 40x

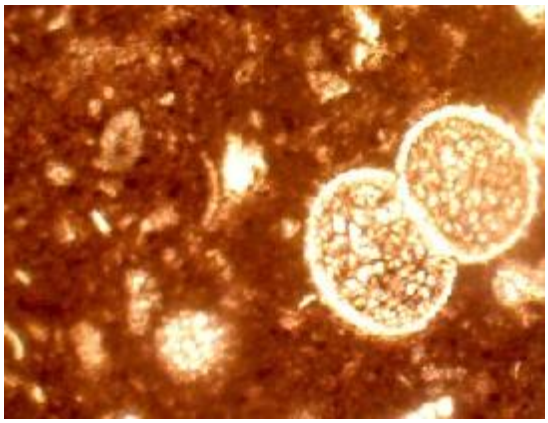


3
Fossils filled with calcite-cement 40x

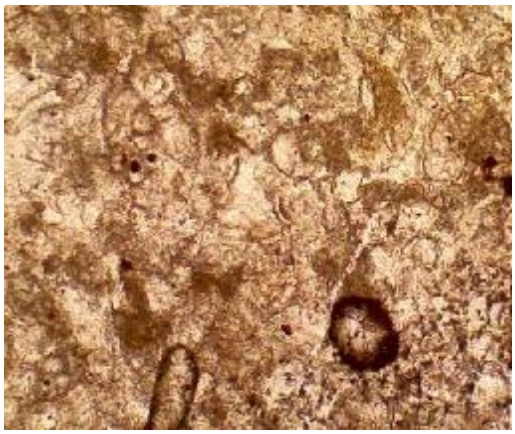


4
Some species of *milliolid* in micrite matrix 100x

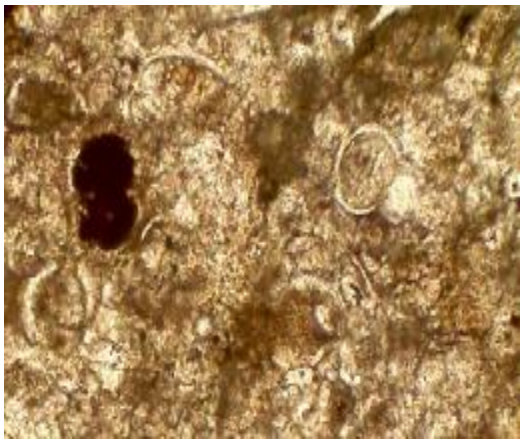
(Plate-2)



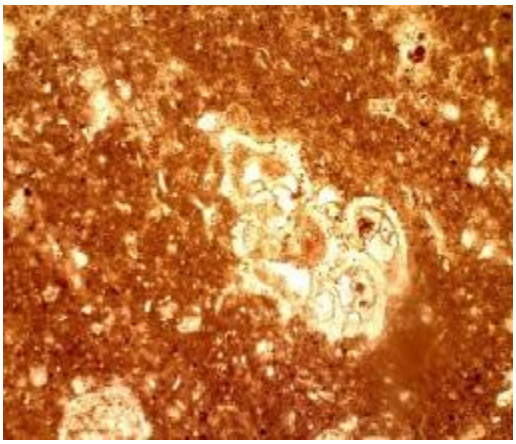
1
Globigirina sp. in Tanjero Formation 40x



2
Cortoid in packstone facies 100x



3
Pyrite filled in the chamber from fossils 100x



4
The Chambers of the Globotrucana sp. filled with rhomboid dolomite 100x

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Assessment of students' knowledge about pulmonary tuberculosis disease in college of health and medical technology, Baghdad, Iraq

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Abstract

A cross-sectional study was made with sample size (335) students (168) male and (167) female from college of health and medical Technology/ Baghdad during period from (15 November 2014 to15 March 2015) to evaluate the knowledge of the students about pulmonary tuberculosis. The study included all stages and departments of the college, using a questionnaire format including (stage, department, sex and age for the student) and other questions about the disease. This study found that students in (fourth, third and second) stages have good information about causative agent of TB and have percentages in arrangement (30.1%, 22.1%, 23.9%). This study show the smoking is have the highest percentage among other risk factors which cause TB (84.5%).

تقييم مدى معرفة الطلبة بمرض السل الرئوي في كلية التقنيات الصحية والطبية / بغداد / العراق

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الخلاصة

اجريت دراسة مقطعيه لعينه مكونة من (335) طالب منهم (186) من الذكور و (167) من الإناث من كلية التقنيات الصحية والطبية – بغداد ، للفترة من (15 ، تشرين الثاني 2014 ولغاية 15 ، آذار 2015) لتقييم معارف الطلبة حول مرض السل الرئوي ، شملت الدراسة كافه المراحل والأقسام وهي (قسم التحليلات المرضية و قسم التخدير و قسم صناعه الأسنان و قسم الأشعة و قسم البصريات و قسم العلاج الطبيعي و قسم صحة المجتمع) باستخدام استمارة استبيان منظمه تتضمن (المرحلة ، القسم ، الجنس ، العمر) وأسئلة أخرى حول المرض و عوامل خطورته . بينت الدراسة ان طلبة المراحل(الرابعة، الثالثة، الثانية) لديهم معلومات جيده بخصوص المسبب المرضي للسل الرئوي وبنسبة (30.1% ، 22.1 % ، 23.9%) على التوالي. كما بينت الدراسة الحالية ان للتدخين النسبة الأعلى من بين عوامل الخطورة التي يمكن ان تؤثر في حصول السل الرئوي وبنسبة 84.5% .

Introduction

Tuberculosis, commonly caused by *Mycobactrim tubercolosis* .TB, is a contagious and an often severe airborne disease caused by a bacterial infection. TB typically affects the lungs, but it also may affect any other organ of the body. It is usually treated with a

regimen of drugs taken for 6 months to 2 years, depending on the type of infection .TB is caused by the infectious agent known as *Mycobacterium tuberculosis* (1). TB infection: the TB germs in the body, but inactive. In most cases, the body's defenses to control this germs. However, these spores can remain in the body inactive spores. TB: It is possible, even after many years covert from non-active TB germs to active spores, so when weaken the body's defenses. This may be due to the age, serious illness , suffering from a painful experience, misuse of alcohol and drugs or HIV infection and other reasons (2,3,4). The systemic clinical signs of tuberculosis include fever, malaise, weight loss, a variety of hematologic abnormalities, metabolic disorders, and neuropsychological manifestations. TB usually develops slowly, symptoms might not begin until months or even years after initial exposed to the bacteria (3,5). In some cases the bacteria infect the body but don't cause any symptoms, which is known as latent TB. It is called active TB if the bacteria cause symptoms (6). TB is primary an airborne diseases. The bacteria are spread to person in tiny microscopic droplets when a TB suffer cough, sneezes, speak, sing, or laughs. Only people with active TB can spread the disease to other (7). Tuberculosis usually attacks the lungs but can also affect other parts of the body. (8). Tuberculosis has been associated with malnutrition. Serum cholesterol was significantly lower in tuberculosis patients and got worse in homeless patients who were prone to starvation (9). Worldwide, TB is responsible for more than ((1.5)) million deaths every year, with an estimated rate of ((13.7)) million prevalent cases of TB in ((2007)), ((206 per 100.000 population)) (10). Therefore, despite recent progress, TB remains an important global public health problem (11).

Materials and Methods

Methods of the study : This study is a cross sectional study . Included ((335)) students ((168)) male and ((167)) female from the college of Health and Medical Technology / Baghdad divided in to three groups according to age. The students from all stages and departments of the college

Data collection: starting from ((15, November 2014 to 15, March 2015)).

The data collection were carried out through designing questionnaire including (stage,department ,sex and age for the student) and other questions about the pulmonary tuberculosis disease, some of these items were scored according to three levels-Likert scale as (3) for Yes, (2) for No, and (1) for I don't know (12,13,14) .

Data analyzed: it is analyzed through the use of SPSS (Statistical Process for Social Sciences) version 20.0 application statistical analysis system and Excel application. Descriptive and data analysis (Frequencies, Percentages), person's correlation coefficients, *MCP*, and *P. value* .A *P.value* of less than (0.05) was considered statistically significant.

Results and Discussion

Table number (1) showed that a highest percentage was among female at age group (18 – 21) year 39.1% , While the lower percentage also among female was at age group more than (25) year was (0.6%) . Highly significant association between age and gender .

Table (1): Distribution of students' knowledge according to age and gender.

Age	Gender				Total	
	Male		Female			
	No.	%	No.	%	No.	%
18 – 21	65	19.4	131	39.1	196	58.5
22 – 25	87	26.0	34	10.1	121	36.1
> 25	16	4.8	2	0.6	18	5.4
Total	168	50.1	167	49.9	335	100.0

MCP = 0.000 HS , HS (Highly significant), No.(number), % (Percentage), MCP(Mean Monte Carlo).

Table number (2) showed that a highest percentage was for *M. Tuberculosis* 30.1% in the fourth stage .While the lower percentage was for *M. leprae* 0% in fourth stage . Highly significant association between stage and causative agent.

Table (2): Distribution of students' knowledge about the causative agent according to the study level stages.

Stage		Causative agent			Total
		M.tuberculosis	*S.typhi*	*M.leprae*	
First	Count	32	20	10	62
	% of T	9.6%	6.0%	3.0%	18.5%
Second	Count	80	8	4	92
	% of T	23.9%	2.4%	1.2%	27.5%
Third	Count	74	3	1	78
	% of T	22.1%	9%	3%	23.3%
Fourth	Count	101	2	0	103
	% of T	30.1%	6%	0%	30.7%
Total	Count	287	33	15	335
	% of T	85.7%	9.9%	4.5%	100.0%

MCP= 0.000 HS , HS (Highly significant)

Table number (3) showed that a highest percentage was for smoking 84.5%. While the lower percentage was 5.4%. For diabetes that highest percentage was 36.1%, while lower percentage was 31.3%. For malnutrition that highest percentage was 63.3%, while lower percentage was 14.9%. For contact that highest 82.7%, While lower percentage was 8.1%. For alcohol that highest percentage was 62.1%, while lower percentage was 15.5%. For cortisone drug use that highest percentage was 45.7%, while lower percentage was 16.1% . For infection with HIV that highest percentage was 79.4% , while lower percentage was 5.1% . For family size that highest percentage 81.5% , while lower percentage was 2.4% .For low weight 49.6% , while lower percentage was 20.9% .

Table (3): Distribution of students' knowledge about the causes .

Causes		No.	%
Smoking	yes	283	84.5
	no	18	5.4
	I don't know	34	10.1
Total		335	100.0
Diabetes	yes	121	36.1
	no	109	32.5
	I don't know	105	31.3
Total		335	100.0
Malnutrition	yes	212	63.3
	no	73	21.8
	I don't know	50	14.9
Total		335	100.0
Contact	yes	277	82.7
	no	31	9.3
	I don't know	27	8.1
Total		335	100.0
Alcohol	yes	208	62.1
	no	52	15.5
	I don't know	75	22.4
Total		335	100.0
cortisone drugs use	yes	153	45.7
	no	54	16.1
	I don't know	128	38.2
Total		335	100.0
Infection with HIV	yes	266	79.4
	no	17	5.1
	I don't know	52	15.5
Total		335	100.0
family size(crowding)	yes	273	81.5
	no	54	16.1
	I don't know	8	2.4
Total		335	100.0
Low Weight	yes	166	49.6
	no	99	29.6
	I don't know	70	20.9
Total		335	100.0

No.(number) , % (Percent).

Table number (4) showed that a highest percentage was for chest pain 86.9% , 83.3% For cough, 64.2% For fever, 66.3% weakness, 79.1% poor appetite, 86.3% For tiredness . While night sweating77% and indisposition80.6%

Table (4): Distribution of students' knowledge about the signs and symptoms.

Signs and symptoms		No.	%
cough 2 weeks	yes	279	83.3
	no	15	4.5
	I don't know	41	12.2
Total		335	100.0
Fever 2 weeks	yes	215	64.2
	no	52	15.5
	I don't know	68	20.3
Total		335	100.0
Weakness	yes	222	66.3
	no	50	14.9
	I don't know	63	18.8
Total		335	100.0
poor appetite	yes	265	79.1
	no	32	9.6
	I don't know	38	11.3
Total		335	100.0
chest pain	yes	291	86.9
	no	24	7.2
	I don't know	20	6.0
Total		335	100.0
Tiredness	yes	289	86.3
	no	25	7.5
	I don't know	21	6.3
Total		335	100.0
night sweating	yes	258	77.0
	no	32	9.6
	I don't know	45	13.4
Total		335	100.0
Indisposition	yes	270	80.6
	no	20	6.0
	I don't know	45	13.4
Total		335	100.0

MCP = 0.000 HS, HS (Highly significant)

Table number (5) showed that a highest percentage was for droplet transmission 28.7% in fourth stage .While the lower percentage was for blood 1.8 % in third stage. Highly significant association between stage and transmission .

Table (5): Distribution of student knowledge about the transmission and stage.

Stage	Transmission				Total	
	droplet		blood			
	No.	%	No.	%	No.	%
First	51	15.2	11	3.3	62	18.5
second	78	23.3	14	4.2	92	27.5
Third	72	21.5	6	1.8	78	23.3
fourth	96	28.7	7	2.1	103	30.7
Total	297	88.7	38	11.3	335	100.0

P.value = 0.07 , HS (Highly significant)

Table number (6) showed that a highest percentage was for diagnosis by sputum 24.8% in fourth stage, while the lower percentage was for diagnosis by blood 1.2% in first stage .

Table (6): Distribution of students' knowledge about the methods of diagnosis.

Stage		Diagnosis			Total
		urine	Sputum	Blood	
First	Count	7	51	4	62
	% of T	2.1%	15.2%	1.2%	18.5%
Second	Count	5	72	15	92
	% of T	1.5%	21.5%	4.5%	27.5%
Third	Count	7	59	12	78
	% of T	2.1%	17.6%	3.6%	23.3%
Fourth	Count	13	83	7	103
	% of T	3.9%	24.8%	2.1%	30.7%
Total	Count	32	265	38	335
	% of T	9.6%	79.1%	11.3%	100.0%

MCP= 0.17 , NS (Non-significant), MCP(Mean Monte Carlo).

Table number (7) showed that the students thought that the highest percentage of infection was among male 70.7%, while the lower percentage of infection was among female 29.3%. Also , this study showed that a highest percentage was for urban 65.7%, While the lower percentage was for rural 34.3%.

Table (7): Distribution of students' knowledge about the gender infection and occurrence.

Variables		No.	%
Gender infection	Male	237	70.7
	Female	98	29.3
	Total	335	100.0
Occurrence	Urban	220	65.7
	Rural	115	34.3
	Total	335	100.0

Table number (8) shows that a highest percentage was for answer yes 27.5% in fourth stage . Highly significant association between stage and can cure after infection .

Table (8): Distribution of students' knowledge in different stage about the cure after infection .

Stage		can be cure after infection			
		Yes	No	I don't know	Total
First	Count	39	13	10	62
	% of T	11.6%	3.9%	3.0%	18.5%
Second	Count	74	8	10	92
	% of T	22.1%	2.4%	3.0%	27.5%
Third	Count	67	5	6	78
	% of T	20.0%	1.5%	1.8%	23.3%
Fourth	Count	92	6	5	103
	% of T	27.5%	1.8%	1.5%	30.7%
Total	Count	272	32	31	335
	% of T	81.2%	9.6%	9.3%	100.0%

P. value = 0.003 , HS (Highly significant)

Table number (9) shows that a highest percentage was for answer yes 22.7% in fourth stage, while the lower percentage was for I don't know 2.4% in third stage . significant association between stage and live after cure .

Table (9): Distribution of students' knowledge in different stages about the ability of the patients live after cure .

Stage		can be live after cure			Total
		Yes	No	I don't know	
First	Count	35	17	10	62
	% of T	10.4%	5.1%	3.0%	18.5%
Second	Count	53	16	23	92
	% of T	15.8%	4.8%	6.9%	27.5%
Third	Count	53	17	8	78
	% of T	15.8%	5.1%	2.4%	23.3%
Fourth	Count	76	13	14	103
	% of T	22.7%	3.9%	4.2%	30.7%
Total	Count	217	63	55	335
	% of T	64.8%	18.8%	16.4%	100.0%

P. value = 0.02, S (significant)

Table number (10) showed that a highest percentage was for answer yes 26.3% in fourth stage, while the lower percentage was for I don't know 3 % in first stage . No significant between stage and can be dead from T.B.

Table (10): Distribution of students' knowledge about the dead from T.B.

Stage		can be dead from T.B			Total
		Yes	No	I don't know	
First	Count	55	6	1	62
	% of T	16.4%	1.8%	3%	18.5%
Second	Count	78	6	8	92
	% of T	23.3%	1.8%	2.4%	27.5%
Third	Count	69	5	4	78
	% of T	20.6%	1.5%	1.2%	23.3%
Fourth	Count	88	8	7	103
	% of T	26.3%	2.4%	2.1%	30.7%
Total	Count	290	25	20	335
	% of T	86.6%	7.5%	6.0%	100.0%

MCP= 0.67 , NS (Non-significant) at level (P≤0.05).

Tuberculosis (TB) has remained a major health problem worldwide, most noted in developing countries such as the Philippines around eight million new cases are diagnosed yearly, while more people are dying daily. In terms of the number of cases, Southeast Asia carries the biggest burden of disease (15).In Iraq TB also remained main problem many studies show this real (16).Throughout the course of the data analysis , the distribution of the student characteristics had revealed that the male is the highest rate about (50.1%) while female (49.9%).The highest percentage was among female at age group (18 – 21) year about (39.1%) , while the lower percentage also among female was at age group more than 25 year was (6%) also in male the highest percentage at age group (22 – 25) year about (26%) and the lower percentage among male was at age group more than 25 year was (4.8%), this show in table number (1) and this agreement with (15,17).In 2013.9 wounded Balsel million people and 1.5 million people died as a result of this Almrd.vi in 2013, it is estimated that 550,000 children infected with TB worldwide in 2013, it is estimated that 480,000 people have been infected Yalsl MDR(18).In 2014, more than 6,500 cases of TB were reported in England. Of these, around 4,700 affected people who were born outside the UK. It's estimated that around one-third of the world's population is infected with latent TB. Of these, up to 10% will become active at some point (6).

Regarding Causative agent of TB the highest percentage of students knowledge was (85.7%) for *M. Tuberculosis*, (30.1%) in the fourth stage and the lower percentage was (9.6%) in first stage .While the lower percentage was (4.5%) for *M. leprae* ,about (3.0%) for the first stage and the lower percentage for *M. leprae* was (0%) in fourth stage this show in table number (2). Highly significant association between stage and causative agent, this agreement with (14) .

Table number (3) shows that a highest percentage for knowledge about risk factors was for smoking (84.5%), the second percentage for contact with patient was (82.7%).While (36.1%) for diabetes, malnutrition was(63.3%). For alcohol that highest percentage was (62.1%), for cortisone drug use that highest percentage was (45.7%) , while infection with HIV the percentage was (79.4%) ,the family size that highest percentage (81.5%) and the low weight was (49.6%) this agreement with (14,19).

Regarding the most sign of TB shows that highest percentage was for chest pain as the most diagnostic signs 86.9% and this result in consistence with (14). The highest percentage of knowledge about the mode of transmission was for air droplet 28.7%, and this agreement (15,16) . Regarding diagnosis of TB highest percentage was for diagnosis by sputum 24.8% and this agreement with D.S hashims (14,15). Regarding gender who most infected with TB the highest percentage was for male infection 70.7% . And this agreement with (14,19) . Regarding the occurrence of TB according to this study was highest percentage for urban 65.7%. And this result agreement with (14). We find the students' knowledge about cure from TB highest percentage was for answer yes 27.5 % . And this agreement with (15).

The students' knowledge about live after cure highest percentage was for answer yes 22.7% . And this agreement with (15).

The highest percentage knowledge about dead from TB was for answer yes 26.3% . And this not agreement with (15) , Because Development of health care for patient , proper protective measure and avialable of good treatment in researcher city . so that we need to increase the knowledge of people especially students of medical and health collage to provide good prevention to community in the future.

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Synthesis of silver nanoparticles by sol-gel method, study antibacterial activity and toxicity to lymphocyte

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Abstract

In this study, we propose a simple approach to prepare silver nanoparticles. The silver nanoparticles were synthesized by chemical reduction of $AgNO_3$ in the presence of $NaBH_4$ as reducing agent in water at room temperature. The structure and average grain size of prepared particles were characterized by X-ray powder diffraction (XRD) and scanning electron microscope (SEM). Based on proposed method, nearly spherical shape nanoparticles with a mean diameter of 48 nm were obtained. The results shows that silver nanoparticles synthesized by sol-gel method has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 18mm for *E. coli* and *Klebsiella oxytoca*, 17mm for *Proteus mirabilis* and *Pseudomonas aeruginosa*, 19mm for *Streptococcus* sp. and *Enterobacter cloacae*, 20mm for Bacillus *sp.* and *Staphylococcus aureus*. The results of effect of silver nanoparticles on the lymphocytes viability show there is no significant difference between treatments and control after 24hrs of treatment, where the other treatment periods gives significant difference ($P \leq 0.05$). The results obtained from analysis of antimicrobial activity and toxicity of these silver nanoparticles ensures that they are safe to be applied for therapeutic agent for human microbial infections.

تخليق منمنمات الفضة بطريقة سول-جل ودراسة فعاليتها المضادة للجراثيم وسميتها للخلايا اللمفاوية

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الخلاصة

في هذه الدراسة ، فإننا نقترح طريقة بسيطة لتحضير منمنمات الفضة. تم تخليق منمنمات الفضة بوساطة الاختزال الكيمياوي لنترات الفضة AgNO3 في وجود NaBH4 كعامل مختزل بالماء في درجة حرارة الغرفة. درست خصائص تركيب ومعدل حجم المنمنمات المحضرة بوساطة حيود الاشعة السينية (XRD) والمجهر الالكتروني الماسح (SEM). واستنادا إلى الطريقة المقترحة، تم الحصول على منمنمات كروية الشكل تقريبا يبلغ معدل قطرها 48 نانومتر. أظهرت منمنمات الفضة المحضرة بطريقة سول-جل انها تمتلك فعالية مضادة للجراثيم المختبرة واستدل علي ذلك من خلال قطر منطقة التثبيط. حيث كان قطر التثبيط 18 ملم للاشريكية القولونية و الكليبسيلا اوكسيتوكا، 17 ملم للمتقلبة الرائعة و الزائفة الزنجارية، 19 ملم للعقدية س.و الأمعائية المذرقية، 20 ملم

للعصوية س. والمكورات العنقودية الذهبية. اظهرت نتائج تأثير منمنمات الفضة على فعالية الخلايا اللمفاوية انه لا يوجد فرق معنوي بين المعاملات والسيطرة بعد 24 ساعة من المعاملة، بينما بقية اوقات المعاملات اعطت اختلافات معنوية عند مستوى ($P \leq 0.05$). وكشفت نتائج تحليل الفعالية المضادة للجراثيم والسمية لمنمنمات الفضة انها امنة لاستخدامها كعامل علاجي للمايكروبات التي تصيب الانسان.

Introduction

Nanomaterials have a long list of applicability in improving human life and its environment (1). The term *nano* is adapted from Greek word meaning dwarf. When used as a prefix, it implies 10^{-9}. A nano meter is one billionth of a meter, or roughly the length of three atoms side by side. A DNA molecule is 2.5nm wide, a protein approximately 50nm and a flu virus about 100nm. A nanoparticle is a microscopic particle with less than 100nm size atleast in one dimension and atleast one property different from their bulk counterpart (2). Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. Several well characterized bulk materials have been found to possess most interesting properties when studied in the nanoscale. There are number of factors responsible for property change at nanoscale such as high aspect ratio, ineffective gravitational force and significant van der Wall's force. This effect is extremely robust and as little as 1gm of AgNPs is known to impart antibacterial properties to hundreds of kilograms when used in bulk form (3).

Metal nanoparticles are intensively studied due to their unique optical, electrical and catalytic properties. A large spectrum of research has been done to control the size and shape of nanoparticles which is crucial in tuning and optimizing their physical, chemical and optical properties. Various techniques such as chemical reduction, electrochemical reduction, sol-gel, laser ablation, photochemical reduction etc have been developed to synthesize nanoparticles. Recently biological methods are being investigated for the synthesis of nanoparticles (4 and 7).

The sol-gel process is a wet-chemical technique used to prepare metals nanoparticles. In this process, the sol (or solution) evolves gradually towards the formation of a gel-like network containing both a liquid phase and a solid phase. Typical precursors are metal alkoxides and metal chlorides, which undergo hydrolysis and polycondensation reactions to form a colloid. The basic structure or morphology of the solid phase can range anywhere from discrete colloidal particles to continuous chain-like polymer networks (8 and 9).

The present study aims to synthesize of silver nanoparticles by Sol-Gel method and study their efficacy the antimicrobial activity against multidrug resistance bacteria and cytotoxicity against lymphocytes.

Materials and Methods

Collection of pathogens: The pathogenic bacteria such as *E.coli, Proteus mirabilis, Klebsiella oxytoca, Streptococcus sp., Enterobacter cloacae, Bacillus sp., Pseudomonas aeruginosa,* and *Staphylococcus aureus used* for the antimicrobial activity were collected from microbiology diagnosis laboratory, Al-Numan hospital and from microbiology diagnosis laboratory, Central Public Health Laboratory, Baghdad.

Synthesis of silver nanoparticles: Silver nanoparticles were synthesized according to (10) by reducing $AgNO_3$ (99.5%) with strong as well as weak reducing agents in the presence of ultrasonic waves. In a typical synthesis, 50 ml of 0.1 M silver nitrate (AgNO3) was taken in a conical flask assembled within an ultrasonic cleanser. 50 ml of 0.1 M sodium borohydride $NaBH_4$ (97%) was added drop wise to the aqueous solution of AgNO3 in the presence of ultrasonic waves. On complete addition of sodium borohydride, the resulting mixture became grey in color which was further irradiated (ultrasonically) for 12 h during which the grayish precipitate started to settle at the bottom of the flask. The suspension obtained was then centrifuged at 8000 rpm and the precipitate washed three times with double distilled water to remove any water soluble impurity, then precipitate was dried in an oven at 50 °C for one hour.

Characterization of silver nanoparticles:

1- X-Ray Diffraction (XRD) analysis: Resulting solution of the developed nanoparticles of silver was centrifuged at 10,000 rpm for 30 min. The solid residues of Ag NPs were washed twice with double distilled water and then dried at 80ºC to obtain powder Ag NPs used for X-ray powder diffraction measurements. The powder X-ray diffraction (XRD) patterns were recorded on (Shimadzu XRD-6000) with copper radiation (Cu K_α, 1.5406 Å) at 40 kV and 30 mA.

2- SEM Analysis of Silver Nanoparticles: Scanning Electron Microscopic (SEM) analysis was done using (Inspect S 50) SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

Determination of antimicrobial activity: Antibacterial activity of the silver nanoparticles by Sol-Gel Method was evaluated by the cup plate agar diffusion method (11). The bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto Mueller Hinton agar (MHA, Oxoid) plates. A sterile cork borer was used to make well (6 mm in diameter) on the MHA plates. Aliquots of 100 µl of silver nanoparticles solution were applied in one of the wells in the culture plates previously seeded with the test organisms. The cultures were incubated at 37ºC for 24 h. Antimicrobial activity was determined by measuring the zone of inhibition around each well excluding the diameter of the well.

Determination of Cell Viability: The cytotoxicity and viability effect of silver nanoparticles against Lymphocytes was studied by the method (12). Lymphocytes were isolated from whole blood using Ficoll Hypaque density gradient method and incubated with different concentrations of silver nanoparticles (0 µl, 10µl and 50µl) at different time intervals of 24 hrs, 48hrs, 72hrs and 1 week.

Statistical analysis: Data are presented as mean ± standard error (S.E.), and differences between means were assessed by ANOVA-one way test followed by Duncan test, in which $p \leq 0.05$ was considered significant. The analyses were carried out using the statistical package SPSS version 13.

Results and Discussion

Powder X-ray diffraction: Silver Nanoparticles are widely used in day-to-day products such as lotions, ointments, toothpastes, toys, socks, etc. These day-to-day products utilized by humans in daily life could result in exposure to silver nanoparticles unknowingly thereby resulting in deposition of these particles inside the body causing harm to the normal cells. This provides an insight to study the effects of these particles on human cells.

The X-ray diffraction patterns of the Ag-NPs synthesized by the Sol - Gel method using $AgNO_3$ and $NaBH_4$ as the reducing agents are shown in (Figure-1). All the reflections correspond to pure silver metal with face centered cubic symmetry. The reflections were indexed as 111, 200 and 220 with the corresponding 2θ values of 38.090, 44.267 and 64.413 respectively (JCPDS 04-0783). The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks were broad indicating that the crystallite size is very small. The average particle size of Ag-NPs can be calculated using the Debye - Scherrer equation (13).

$D = K \lambda / \beta \cos\theta$

Where K is the Scherrer constant with value from 0.9 to 1 (shape factor), λ is the X-ray wavelength (1.5418 Å ,β is the width of the XRD peak at half-height and θ is the Bragg angle and D is the grain size. From the Scherrer equation, the average crystallite size of Ag-NPs is 48 nm (Figure- 1).

We have reported a fast method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of $NaBH_4$ this method named Sol-Gel method. The XRD results suggested that the crystallization of the silver nanoparticles. The reflections were indexed as 111, 200 and 220 with the corresponding 2θ values of 38.090, 44.267 and 64.413 respectively (JCPDS 04-0783) (Figure- 1). The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks were broad indicating that the crystallite size is very small. From the Scherrer equation, the average crystallite size of Ag-NPs is (48 nm). These finding are in agreement with the results of several studies (14 and 15 and 16) in which found the size of silver nanoparticles synthesized by sol-gel method was at the range 25-50 nm.

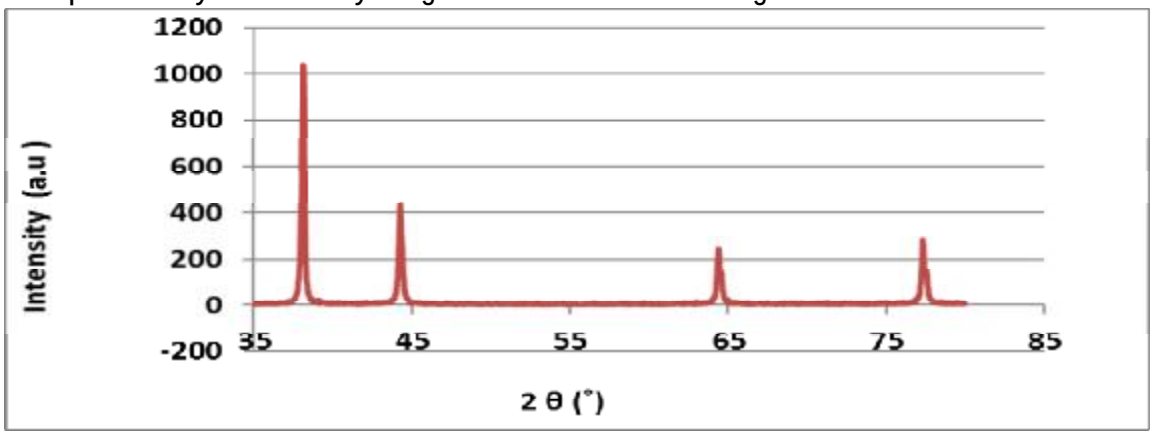

Figure 1. X ray diffraction of Silver nanoparticles synthesized by Sol-Gel Method

SEM analysis of Silver nanoparticles: Figure (2) shows SEM image of silver nanoparticles, it reveals that silver nanoparticles were spherical and particles form cluster. It is easy to notice that the examined particles consist of a number of smaller objects of 0.5 μm to few micrometers in size. However, we did not manage to examine the structure of the observed nanoparticles because of difficulties connected with getting higher magnification. In Figure (3), a standard EDX spectrum recorded on the examined sample is shown. In the middle part of the presented spectrum a strong peak located at 3 KV. This maxima is directly related to the silver characteristic line L. The maximum located on the left part of the spectrum at 0.2 kV clearly comes from carbon. Quantitative analysis proved high silver contents (100%) in the examined samples the result shown in Fig (3) and table (1).

The results shows SEM image of silver nanoparticles. It reveals that silver nanoparticles were spherical and particles form cluster figure (2). A standard EDX spectrum recorded on the examined sample is shown. In the middle part of the presented spectrum a strong peak located at 3 KV. This maxima is directly related to the silver characteristic line L. The maximum located on the left part of the spectrum at 0.2 kV clearly comes from carbon. Quantitative analysis proved high silver contents (100%) in the examined samples the result shown in Figure (3) and table (1), these results are in agreement with (15 and 16).

The data in Table (2) and Figure 4 and 5 shows that silver nanoparticles synthesized by Sol-Gel Method has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 18 mm for *E. coli* and *Klebsiella oxytoca*, 17mm for *Proteus mirabilis* and *Pseudomonas aeruginosa*, 19mm for *Streptococcus sp*. and *Enterobacter cloacae*, 20mm for Bacillus *sp.* and *Staphylococcus aureus*.

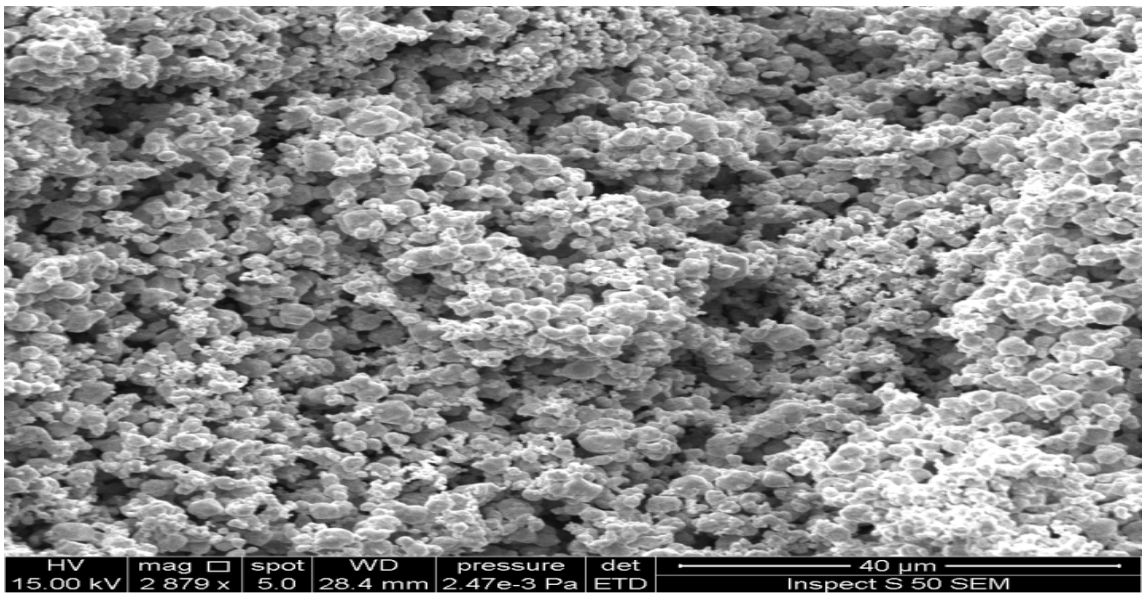

Figure 2. SEM micrographs of silver nanoparticles synthesized by Sol-Gel Method

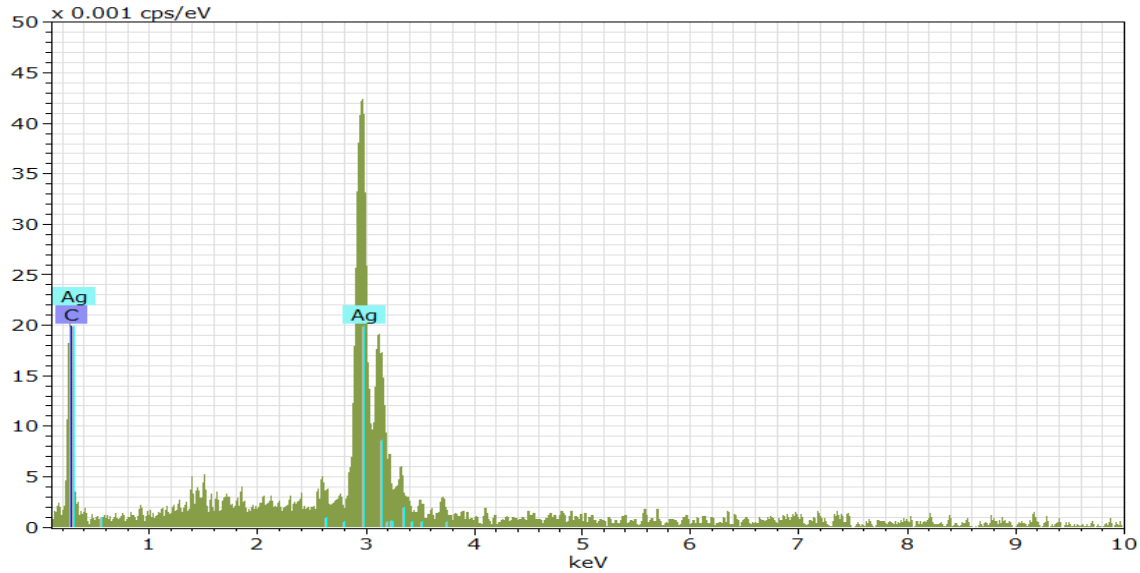

Figure 3.EDX characteristic spectrum obtained for silver powder

Table (1): The elements in silver nanoparticles

Element	AN	series	[wt.%]	[norm. wt.%]	[norm. at.%]
Carbon	6	K-series	0	0	0
Silver	47	L-series	66.40724888	100	100
		Sum:	66.40724888	100	100

Table (2): The inhibitory activity of the Ag-NPs synthesis by Sol-Gel method against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)*

Isolated bacteria	Inhibition Zone
	Ag-NPs
Escherichia coli	18
Proteus mirabilis	17
Klebsiella oxytoca	18
Streptococcus sp.	19
Enterobacter cloacae	19
Bacillus sp.	20
Pseudomonas aeruginosa	17
Staphylococcus aureus	20

* Zone of inhibition, including the diameter of the cup plate method (6.0 mm) .The recorded value is mean value of 3 replicates.

Figure 4. The antibacterial effect of Silver nanoparticles synthesis by Sol-Gel method using the test bacterium *Klebsiella oxytoca* and *Staphylococcus aureus*

Figure 5. The antibacterial effect of Silver nanoparticles synthesis by Sol-Gel method using the test bacterium *Escherichia coli* and *Bacillus* sp.

The results of the present study shows that silver nanoparticles synthesized by sol-gel method has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 17 mm for *E. coli*, *Enterobacter sp*., and *Pseudomonas aeruginosa*, 14mm for *Proteus sp*., 16mm for *Klebsiella oxytoca*, 15mm for *Streptococcus sp*. and *Staphylococcus aureus*, 18mm for *Bacillus sp.* Table (2) and Figures (4 and 5). Our interpretation of these results, the silver nanoparticles synthesized by sol-gel method has a mechanism to kill bacteria.

The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles (17 and 18 and 19). In contrast, Sondi and Salopek-Sondi, (20) reported that the antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticles, and was closely associated with the formation of 'pits' in the cell wall of bacteria. Then, Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. However, because those studies included both positively charged Ag ions and negatively charged Ag nanoparticles, it is insufficient to explain the antimicrobial mechanism of positively charged Ag nanoparticles. Therefore, we expect that there is another possible mechanism. Amro *et al*. (21) suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins (22). Also, Sondi and Salopek-Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with Ag nanoparticles (20). Although their inference involved some sort of binding mechanism, still unclear is the mechanism of the interaction between Ag nanoparticles and component(s) of the outer membrane. Recently, Danilczuk and co-workers (22) reported Ag-generated free radicals through the ESR study of Ag nanoparticles. We suspect that the antimicrobial mechanism of Ag nanoparticles is related to the formation of free radicals and subsequent free radical–induced membrane damage.

Cytotoxicity of Silver Nanoparticles: Viability assay is vital step in toxicology that explain the cellular response to a toxicant. Also, they give information on cell death, survival, and metabolic activities. The results of viability assays demonstrated there is no significant difference between treatments and control after 24h of treatment. Whereas the other treatment periods gives significant difference ($P \leq 0.05$) between treatment and control table (3).

Table (3): Cytotoxicity of Silver Nanoparticles synthesized by Sol-gel method on Human Lymphocytes

Concentration	Cell vitality (%) mean ± S.E.			
	24h	48h	72h	1 week
Control	67.05 ± 14.52A	44.65±18.87BC	38.37±11.32C	27.10±11.99D
10µl	57.33 ± 11.35A	44.22±15.57AB	36.28±15.81BC	24.99±18.01C
50µl	59.49 ± 17.28A	43.63±16.12B	44.62±15.41B	26.73±12.76C

Different letters represent significant difference ($p \leq 0.05$) between means of columns, while similar letters represent no significant difference ($p > 0.05$) between these means (Duncan test)

Our experiments unveiled the *in vitro* cytotoxic effects of silver nanoparticles that were screened against human lymphocytes. The results of viability assays demonstrated there is no significant difference between treatments and control after 24h of treatment. Whereas the other treatment periods gives significant difference ($P \leq 0.05$) between treatment and control table (3). We have exploited the microscope based assay to study the effect of AgNPs. The toxicity of silver nanoparticles showed a concentration and time-dependent drop in viability of human lymphocytes as compared to the controls (cells not treated with silver nanoparticles), signifying time and concentration dependent toxicity. The result is in proportion of viable lymphocytes expressed in integral percentage. These finding are in agreement with several studies (23and 24 and 25). These data indicate that AgNPs inhibits cell viability in Lymphocytes in a dose and time-dependent manner and anti-proliferative activities against Lymphocytes.

Our results support the hypothesis that Ag nanoparticles can be prepared in a simple and cost-effective manner and are suitable for formulation of new types of bactericidal materials.

Conclusions

From the results of the present study we concluded the sol-gel method can be used to produce large scale production of silver nanoparticles for their use in various biomedical and biotechno-logical applications.

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Effect of supplementing different levels of pumpkin seed oil in the diets of spent laying Japanese quail (*Coturnix coturnix japonica*)

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Abstract

The aim of this study was to investigate the effect of dietary supplementation of pumpkin seed oil (0, 1, 2 and 4 %) on performance, egg quality, some biochemical values of older Japanese quail (*Coturnix coturnix japonica*). A total of 128 laying quail hens at forty weeks of age were randomly and equally assigned into four treatments (n = 32). Each treatment was replicated four times (containing 8 birds each). Dietary supplementation of pumpkin seed oil had no significant effect on body weight, egg weight, shell weight, yolk weight, albumen weight, egg width, egg length and shell thickness. Supplementation of 1 and 2% pumpkin seed oil improved feed intak, egg mass, feed conversion ratio, egg production and egg number. The addition of pumpkin seed oil to the laying quail feed led to a significant increase in yolk height. The addition of 2 and 4% pumpkin seed oil to the laying quail feed led to a significant decrease in serum cholesterol and triglyceride concentration, whereas, serum total protein, albumin, globulin, urea and creatinine concentration was not significantly effected in supplemented quails. It can be concluded that the addition of pumpkin seed oil, especially at a level of 2%, had positive effects on performance values, serum parameters concentrations and quality criteria of egg.

تأثير مستويات مختلفة من زيت بذور اليقطين في علائق السمان الياباني (*Coturnix coturnix japonica*) في نهاية فترة انتاج البيض

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الخلاصة

هدفت هذه الدراسة لمعرفة تأثير مستويات مختلفة من زيت بذور اليقطين (pumpkin seed oil) (0 ، 1 ، 2 و 4 %) في الاداء الانتاجي ، نوعية البيض المنتج وبعض المعايير الكيماحيوية للدم في السمان الياباني عند نهاية فترة انتاج البيض . استخدم 128 طير بعمر 40 اسبوعاً ووزعت عشوائياً الى اربع معاملات بواقع (32 طير لكل معاملة) وبواقع اربع مكررات (8 طير لكل مكرر). بينت النتائج عدم وجود تأثير معنوي لإضافة زيت بذور اليقطين في علائق التجربة في وزن الجسم ، وزن البيضة ، وزن القشرة ، وزن الصفار ، وزن البياض ، عرض البيضة ، طول البيضة وسمك القشرة . اظهرت النتائج تحسناً معنوياً في كمية العلف المستهلكة ، كتلة البيض المنتج ، معامل التحويل الغذائي ، انتاج البيض وعدد البيض المنتج عند المستويين 1 و 2 % من زيت بذور اليقطين . ادت اضافة زيت بذور اليقطين الى العلائق الى زيادة معنوية في ارتفاع الصفار. حصل انخفاض معنوي في تركيز الكولستيرول والكليسيريدات الثلاثية عند المستوين 2 و 4 % من زيت بذور اليقطين، بينما لم تتاثر تراكيز البروتين الكلي ،

الالبومين ، الكلوبيولين ، اليوريا والكرياتنين معنوياً عند المستويات المختلفة من زيت بذور اليقطين. نستنتج من هذه الدراسة ان اضافة 2 % من زيت بذور اليقطين كان له تأثير ايجابي في قيم الاداء الانتاجي ، تراكيز معايير مصل الدم والصفات النوعية للبيض المنتج.

Introduction

After the use of most antibiotic growth promoters as feed additives has been banned by the European Union due to cross-resistance against pathogens and residues in tissues (1), the possibility of using new natural alternative additives instead of antibiotics in animal diets is being recently used. Some plants, containing various essential oils , have been used as alternative remedies by some researchers. Vegetable oils, also called essential oils are aromatic, oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root). Pumpkin seed oil (PSO) is rich in β-carotene and Vitamin E, which had powerful antioxidant and profound protective actions against tumor , and it has been recognized for several health benefits such as prevention of the growth and reduction of the size of prostate, reduction of bladder and urethral pressure and improving bladder compliance, alleviation of diabetes by promoting hypoglycemic activity, and lowering level of gastric, breast, lung, and colorectal cancer (2, 3). There was increasing interest in the role of antioxidant vitamins like β-carotene in neutralizing free radicals and overtly aggressive oxygen species (4). Pumpkins are highly nutritious and it's seeds contained 41.59% oil and 25.4% protein , L-tryptophan, n-6 and n-3 fatty acids and a very high concentration of vitamin E (5,6). Several studies have reported the chemical composition and oil characteristics of the pumpkin seed from different origins and varieties (5, 7, 8, 9) . Up to 60.8%, of the PSO is from the fatty acids, oleic (up to 46.9%), linolenic (up to 40.5%) and palmitic and stearic (up to 17.4%), the ratio of monounsaturated to polyunsaturated acids from 0.60 to 0.75 g (7). Stevenson (8) studied several pumpkin cultivars (*Cucurbita maxima* D.) for their seed oil content, fatty acid composition and tocopherol content. The oil content ranged from 11 to 31%. Total unsaturated fatty acid content ranged from 73 to 81 %, the a-tocopherol content of the oils ranged from 27 to 75 mg/g, while c-tocopherol ranged from 75 to 493 mg/g. The four fatty acids presented in significant quantities are linoleic (39.84%), oleic (38.42%), palmitic (10.68%) and stearic (8.67%) acids (5) . Pumpkin has been considered beneficial to health because it contains various biologically active components such as polysaccharides, p-aminobenzoic acid, fixed oils, sterols, proteins and peptides (10). Pumpkin seed oil has antimicrobial components and is effective against a lot of bacteria. Several *In vitro* studies reported significant antibacterial and antifungal properties of pumpkin seed oil (11, 12). Pumpkin seed oil increases elasticity of blood vessel walls and strengthens them, thus helping in the treatment of atherosclerosis (13). Several investigators reported that using PSO in chickens diets improved performance , feed conversion efficiency, egg weight, egg production, egg quality and reduce the cost of feed and mortality (14, 15,16, 17). Celik (18) reported that inclusion of PSO in laying diets significantly lowered the egg yolk cholesterol content, besides produce eggs enriched in unsaturated acids particularly of oleic and linolenic

acids. On other hands, Hajati (14) indicated that supplementation of diets with 5g/ kg pumpkin seed oil in broiler diet reduced mortality and it did not have any adverse effect on the bird's performance, however cholesterol and triglyceride concentrations in plasma decreased at 49 days of age. The intake of apricot or pumpkin kernel oil at the level of (1g/Kg body weight), has been showed significantly lower levels of total cholesterol, total triglycerides, low density lipoprotein-cholesterol, as well as high levels of high density lipoprotein-cholesterol and total protein in comparison with the hypercholesterolemic group in rats (19) From this given background, the objective of this study was to determine the effect of dietary supplementation with pumpkin seed oil on the productive performance in laying quails. We also examined whether pumpkin seed oil supplementation has effects on egg quality, total serum cholesterol, triglycerides, urea and creatinine concentration during the late laying period of Japanese quail.

Materials and Methods

This study was conducted at the Quail Farm, Department of Animal Resources, College of Agriculture, University of Basra during the period from 15/ 11/ 2014 to 24/ 1/ 2015. A total of 128 Japanese quail hen (*Coturnix coturnix japonica*) at forty weeks of age were individually weighed. The initial body weight was comparable at the beginning of the experiment. The birds were randomly assigned into 4 treatments, each of 4 replicates (cages) with eight laying birds. The birds were allowed free access to feed and water. All the birds were fed corn and soybean meal based diets formulated to meet the nutrient requirements of laying quail hens. Ingredients and chemical composition of diet were shown in Table (1).The following 4 dietary treatments were used: Group not supplemented with additives served as control T1, while T2 , T3 and T4 were represented control diet supplemented with 1% or 2 and 4 % pumpkin seed oil , respectively. The birds were housed in stainless-steel wire cages (45 ×70 × 75cm) in an experimental house on a 17–h lighting schedule. The experiment was terminated when the birds were 48 weeks of age. The live body weights of birds were recorded at the beginning and at the end of the study. Egg production was recorded daily and feed consumption , cumulative egg number, egg mass and egg weight were recorded at two weekly intervals. Feed efficiency was calculated by determining the amount of feed consumed per one kg of egg. Egg shape index was estimated by dividing eggs width to egg length . Egg , yolk , shell and albumen weights were obtained at the third day of every two weekly intervals by using scale. Shell thickness of eggs were measured using a special micrometer. Four quails per treatment were randomly selected at the end of the experiment to determine blood serum parameters. Blood samples were collected in tubes without heparin for biochemical assays, and centrifuged (3000 rpm, 15 min, 25°C) to obtain plasma. Serum samples were stored at –20 ºC until analyzed for total protein and albumin by a colorimetric method using a commercial kits (Biolab AS, France). Serum globulin was calculated by subtraction from total proteins. Blood serum cholesterol, triglycerides, urea and creatinine concentration were determined according to the methods of (20) using commercial kits (Biolabo AS, France). All data were

analyzed by using SPSS program software (2012). Significant treatment means were assessed using the Least Significant Difference (LSD) test at ($P < 0.05$) (21).

Table (1): The ingredients and composition of basal diet fed to quail hens.

Ingredient and composition	%
Yellow corn	53
Wheat bran	04
Soybean meal (44%)	27
Protein concentration	7
Oil plant	2
Limestone	5.4
Dicalcium phosphate	1.0
Vitamin / mineral premix	0.30
Common salt	0.30
Total	100
[1]ME (Kcal /Kg) diet	2896
Crude protein %	20.63
Ether extract %	3.87
Crude fiber %	3.77
Calorie : protein ratio	140.38
Calcium %	2.91
Phosphorus available %	0.43
Methionine %	0.33
Lysine %	1.09
Methionine + Cystine %	0.66

[1]ME (Kcal /Kg) diet = Metabolizable energy

Results and Discussion

The effects of dietary supplementation with pumpkin seed oil (PSO) on body weight are shown in Table (2). It was observed that treatment the laying quail with PSO have no significant effect on live body weight (L.B.W.) during the period of the experiment. The absence of a response to the dietary inclusion of PSO on the body weight of laying quail ($P < 0.05$) in this experiment confirmed the findings of Pal (22) who found no significant effect of 4 %PSO supplement level on the body weight of laying hens after the 4 wk of feeding period and with Guclu (23) who found no significant differences of different oil sources on body weight of laying quail. Al-Daraji (24) found no significant effect of sesame oil or seeds supplement on the body weight of laying quail. Celik (18) found no significant differences of dietary pumpkin seed oil on body weight of laying hens. Our results agreed with Martinez (25), who reported that levels up to 10 % of pumpkin (*Cucurbita maxima)* seed meal in the feedstuff of laying hens, had not harms effects on

the productive performance, health and viability of the birds. Table (2) also showed that feeding different levels of PSO supplementation significantly ($P < 0.05$) affected feed intake (FI) during the experimental period. Laying quail birds that fed PSO had consumed more feed as compared with the control groups. The increase in feed intake with the addition of PSO could be due the stimulation effect of essential oils toward appetizing and digestive process in birds (1). On the other hands, pumpkin seed oil has become a recognized source of phenolic compounds, fatty acid and antioxidant vitamins such as carotenoids, tocopherol and tocotrienols (8, 26, 27). Nworgu (28) indicated that the level of 60 and 120 ml of fluted pumpkin leaves extract / liter of water caused significant improvement in feed intake and feed conversion ratio of broilers during the late dry season. Furthermore, broiler feeding 1 - 3 min with heat-treated fluted pumpkin (*Telfaria occidentali*) leaves extract had significant improvement in weight gain, feed conversion ratios of broiler during early rainy season in humid tropical environment (29). Shurmasti (30) found no beneficial effect of dietary Pumpkin seed oil and canola oil supplementation on the growth performance and productive traits of broiler chickens. The results of feed conversion ratio (FCR) revealed that laying quail birds supplemented with PSO at levels 1% and 2% had better ($P<0.05$) FCR value as compared with other groups, whereas, there was no significant difference in FCR between birds fed 4 % of PSO and control group. The better FCR of birds fed 1% and 2% PSO suggests that those levels may have higher stimulated effects on digestive tracts of laying quail birds and increase the utilization efficiency of feed nutrients and as result increase egg mass which was reflected in the improvement of feed conversion ratio. It was observed that adding Pumpkin seed oil didn't affect laying hen in terms of FI and FCR (18,22). Broiler fed 80 ml of fluted pumpkin (*Telfaria occidentalis*) leaves extract/liter of water had significantly the highest weight gain and the best feed conversion and protein efficiency ratios, but had not effects on feed intake (31). Gaafar (32) showed that supplementing with 2.5 g pumpkin seed oil and 2.5 g black seed oil /kg diet together improved feed conversion ratio in growing rabbits. Our results confirmed the results of Tabari (17), who showed that the combination of 0.5 g/kg nettle root extract and 0.5 g/ kg pumpkin seed oil had a positive impact on FCR of broiler chickens. These result are agreement with the observations of previous researchers (15,28,29). Results also denoted that adding PSO to laying quail diets resulted in significant ($P < 0.05$) increase concerning egg mass, hen day egg production (%) and number of eggs during the experimental periods, while egg weight was not differed statistically among the groups during the study (Table 2). It can be speculated that the improvement in productive characteristics probably resulted from the highly unsaturated fatty acid contents of PSO especially oleic (up to 46.9 %) and linoleic (up to 40.5 %) acid (3, 7). El–Yamany (33) concluded that enrichment the quail diet with high linoleic and linolenic acids proved a higher economic efficiency without any adverse effect on the performance and improve the physiological parameters.

Table (2): Fed diets containing different levels of pumpkin seed oil on the performance of laying quail at 40-48 week of age (mean ± SE)

Laying quail performances	Dietary Pumpkin seed oil (%)				Significance.
	0	1	2	4	
Initial body weight (g)	175.09 ± 1.68	175.08 ± 2.71	176.38 ± 2.72	174.71 ± 3.83	N.S
Final body weight (g)	215.77 ± 16.65	193.50 ± 8.59	209.73 ± 12.66	182.17 ± 13.76	N.S
Feed intake (FI) (g/bird/8 weeks)	990.45 [b] ± 18.24	1139.41 [a] ± 17.90	1004.23 [a] ± 15.55	1068.96 [a] ± 19.58	*
Egg mass (EM) (g/bird/8 weeks)	717.86 [b] ± 48.23	1004.71 [a] ± 57.51	1136.58 [a] ± 66.96	742.42 [b] ± 12.56	*
Feed conversion ratio (FI/ EM)	1.66 [c] ± 0.34	1.35 [ab] ± 0.45	0.97 [a] ± 0.14	1.53 [c] ± 0.52	*
Hen day egg production (%)	13.12 [b] ± 1.84	18.19 [a] ± 1.52	19.63 [a] ± 1.40	13.07 [b] ± 1.10	*
egg weight (g)	12.26 ± 0.19	12.57 ± 0.46	12.52 ± 0.63	12.47 ± 0.48	N.S
Number of eggs (g/bird/8 weeks)	63.00 [b] ± 3.63	87.33 [a] ± 1.72	94.25 [a] ± 6.73	62.75 [b] ± 3.37	*

[a,b,c]: Means in the same row with no common superscript are different significantly ($p < 0.05$)
N.S: none significant.

In the diet of laying hens a pumpkin seed oil has become successfully alternative of soy oil up to 4 % when added to the ration without effect on the productive performance, egg quality, as well as it improve yolk index and increase the proportion of unsaturated fatty acids (n-3 and n-9) in eggs and caused reduction in cholesterol (18). A marked improvement in the number of eggs laid was observed in breeder Turkey hens treated with50 ml okra seeds extracts/liter of water and 50 g pumpkin seed powder extracts / kg of feed (15). While there were no differences among the groups in terms of egg shell weight, yolk weight, albumen weight, egg width, egg length, egg shell thickness and shape index, there were significant differences between the results of the groups regarding yolk height ($P < 0.05$) (Table 3). Our findings are similar to the results of Celik

(18), except for the egg shell thickness in laying hens, and with Al–Daraji (24) who revealed that, feeding laying quails with diets containing sesame oil (0.5 % and 1 %) or seeds (1 % and 2 %) recorded the best results as concerns total hen-day egg production, cumulative egg number, egg mass, feed conversion ratio and enhancing reproductive performance when compared with control group. In addition, results of Herkel, (16) differed from the results of our present study, in terms of average egg weight in laying hens. Results of blood biochemical parameters are presented in Table (4).

Table (3): Fed diets containing different levels of pumpkin seed oil on egg quality traits (Mean ± SE) of laying quail

Laying quails performances	Treatment				Significance
	Control (0 %)	Pumpkin seed oil (1 %)	Pumpkin seed oil (2 %)	Pumpkin seed oil (4 %)	
Shell weight (g/ egg)	1.03 ± 0.095	0.93 ± 0.092	0.92 ± 0.61	0.98 ± 0.059	N.S
Yolk weight (g/ egg	4.89 ± 0.26	4.98 ± 0.16	4.91 ± 0.35	4.24 ± 0.12	N.S
Albumen weight (g/ egg)	6.17 ± 0.50	6.60 ± 0.33	6.94 ± 0.51	7.25 ± 0.35	N.S
Yolk height	26.95 [b] ± 1.22	27.53 [b] ± 1.43	30.22 [a] ± 0.94	27.38 [b] ± 1.78	*
Egg width (mm)	26.24 ± 0.27	26.88 ± 0.45	26.43 ± 0.35	26.16 ± 0.51	N.S
Egg length (mm)	33.09 ± 0.34	32.56 ± 0.19	33.82 ± 0.34	33.14 ± 0.70	N.S
Shape index (%)	80.47 ± 1.44	82.57 ± 1.45	78.16 ± 0.78	79.03 ± 2.33	N.S
Shell thickness (mm)	0.35 ± 0.020	0.34 ± 0.035	0.36 ± 0.012	0.41 ± 0.057	N.S

[a,b,c]: Means in the same row with no common superscript are different significantly ($p < 0.05$)
N.S: none significant.

Table (4): Some blood characteristic at 48 weeks of age of quail fed Pumpkin seed oil (mean ± SE)

Parameters	Treatment				Significance.
	Control (0%)	Pumpkin seed oil (1%)	Pumpkin seed oil (2%)	Pumpkin seed oil (4%)	
Total Protein ($g\ dL^{-1}$)	5.25 ± 0.17	5.41 ± 1.46	5.13 ± 0.56	5.73 ± 0.34	N.S
Albumin ($g\ dL^{-1}$)	1.73 ± 0.19	1.77 ± 0.74	1.71 ± 0.39	1.85 ± 0.30	N.S
Globulin ($g\ dL^{-1}$)	3.52 ± 0.11	3.63 ± 0.71	3.42 ± 0.08	3.88 ± 0.24	N.S
Cholesterol ($mg\ dL^{-1}$)	256.21^{a} ± 20.07	234.92^{a} ± 17.29	164.89^{b} ± 14.78	164.08^{b} ± 20.70	*
Triglycerides ($mg\ dL^{-1}$)	262.79^{a} ± 10.47	254.25^{b} ± 7.31	248.99^{c} ± 10.09	247.79^{c} ± 8.27	*
Urea ($mg\ dL^{-1}$)	13.41 ± 4.80	16.33 ± 5.01	12.45 ± 3.99	13.01 ± 1.04	N.S
Creatinine ($mg\ dL^{-1}$)	1.40 ± 0.16	1.38 ± 0.16	1.32 ± 0.26	1.68 ± 0.34	N.S

^{a,b,c}: Means in the same row with no common superscript are different significantly ($p < 0.05$)
N.S: none significant.

The differences in concentrations of total protein, albumin, globulin, urea and creatinine among groups were not significant, whereas, the total serum cholesterol (TC) and triglyceride (TG) concentrations were decreased significantly ($P < 0.05$) when pumpkin seed oil was included in the diets of laying quail. The reduction in the levels of TC and TG when the birds supplemented with pumpkin seed oil may reflect the beneficial effects of PSO in the diets, as PSO has a high content of linoleic acid (42.6 %), with good amount of oleic acid (35.3 %) (34). Oleic acid was reported to reduce total plasma cholesterol, and LDL-cholesterol (34,35). Also, it has been found that vitamin E in pumpkin prevent oxidative damage in the cell by preventing the oxidation of unsaturated fatty acids in cell membrane (36), or it may be attributed that low cholesterol levels due to the role of fatty acids particularly Omega-3 by its secretion to

intestine and biliary oxidation (37). As well as, vitamin E strongly affects cholesterol metabolism in the intestine (38). In chickens, supplementation with mixed antioxidant vitamins such as tocotrienols has resulted in significant reductions in total serum cholesterol and LDL-cholesterol in hypercholesterolemic group (39). Phytosterols present in the pumpkin seed oil are also being studied for their role in lowering cholesterol levels (3). Our results of blood parameters agreed with those obtained by Hajati (14) and Miraghaee (40), who recorded that PSO significantly decreased serum levels of TC and TG in plasma and in the serum of broilers. Also, Shobana and Jayachitra (41) reported that ethanolic extract of *Cucurbita maxima Duchesne* (L) (200 mg/ Kg/ B.W.) and high fat diet treated rats significantly decreased the level of serum total cholesterol and triglycerides as compared to the unsupplemented high fat diet fed rats. The intake of apricot or pumpkin kernel oil at the level of (1 g/ Kg/ B.W.), has been showed significantly lower levels of total cholesterol, total triglycerides, low density lipoprotein-cholesterol, in comparison with the hypercholesterolemic group in rats (19). In laying hens, Martinez (25) found reduction in the harmful lipids and increment in serum essential fatty acids when including four levels of pumpkin (0, 3.3, 6.6 and 10 %) (*Cucurbita maxima*) seed meal in the diets, not affecting the principal productive indicators. In contrast to our results Nworgu (29) noted that broiler chicken served 120 ml/L of fluted pumpkin leaves extract had the highest value of cholesterol and urea in blood serum.

Conclusion

Considering the effects of different levels of PSO on feed intake, feed conversion ratio of the birds, egg mass, hen day egg production, number of eggs and some biochemical parameters in blood sera, it appears that 2 % level of PSO gave the best result and should be used in the diets of quail during late period of egg production .

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Effect of SNP G565A of growth hormone receptor gene on the productive and physiological performance in broiler chicken

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Abstract

In this study two commercial broiler breeds were used (Cobb500 and Hubbard-F15) to detect the SNP G565A in the fifth intron of chicken GHR gene and investigate its effect on the productive and physiological performance in broiler chicken. PCR- RFLP method was used to identify this SNP. The results revealed that three genotypes were found when using of *Eco72I* restriction enzyme wild genotype GG, hetero GA and mutant AA. Highly significant difference ($p<0.01$) was found between the distribution of the different genotypes, the genotype GA had the highest percentage followed by GG then AA and allele G had the superiority over allele A in both broiler breed also significant differences ($P<0.05$) were found in the means of weekly live body weight and weekly weight gain in both breeds, and there was significant effect ($p<0.05$) of the SNP G565A on the means of carcass weight, dressing percentage and dressing percentage with giblets and relative weights of back, heart, liver and gizzard of Cobb500 only as the genotype GA gave the highest mean followed by AA and then GG. The SNP G565A was affect significantly ($P<0.05$) on the mean of blood serum total protein concentration of Cobb500 at 14 and 42 days of age and on the blood serum cholesterol concentration of Hubbard- F15 at 42 days of age only.

تأثير تعدد المظاهر الوراثية (G565A) لجين مستقبل هرمون النمو على الاداء الانتاجي والفسلجي لفروج اللحم

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الخلاصة

استخدم في هذه الدراسة هجينان تجاريان من فروج اللحم (Coob500 وHubbard F-15) لغرض الكشف عن تعدد المظاهر الوراثية للانترون الخامس لجين مستقبل هرمون النمو و المتمثل بالطفرة A565G و التحري عن تأثيره في الاداء الانتاجي و الفسلجي لفروج اللحم .استخدمت طريقة PCR-RFLP للكشف عن الطفرة A565G عن طريق استخدام انزيم القطع *Eco*721I و تم الحصول على ثلاث طرز وراثية طبيعي GG و هجين GA و طافر AA . لوحظ وجود فرق عالي المعنوية $p<0.01$ في توزيع الطرز الوراثية اذ شكل الطراز GA اعلى نسبة تلاه الطراز GG ومن ثم AA كما تفوق التكرار الاليلي للاليل G على الاليل A في كلا هجيني الدراسة كذلك لوحظ وجود تاثير معنوي ($P<0.05$) في معدل الوزن الحي الاسبوعي و الزيادة الوزنية الاسبوعية لكلا هجيني التجربة، واثر تعدد الطرز الوراثية لجين مستقبل هيمون النمو(G565A) معنويا ($P<0.05$) في معدل وزن الذبيحة ، نسبة التصافي ، نسبة التصافي مع الاحشاء و الاوزان النسبية للظهر ، القلب ، الكبد و القانصة لهجين ال Cobb500 اذ سجل الطراز

GA اعلى معدل تلاه الطراز AA و من ثم GG كما اثر تعدد المظاهر الوراثية (G565A) معنويا (P<0.05) في معدل تركيز البروتين الكلي لمصل الدم في ال Cobb 500 بعمر 14 و 42 يوم كما اثرت معنويا (P<0.05) في تركيز كولسترول مصل الدم لل Hubbard-F15 بعمر 42 يوم فقط.

Introduction

Molecular markers, revealing polymorphisms at the DNA level, are now key players in animal genetics. Presence of various molecular biology techniques lead to produce them and to the various biological implications some can have a large variety exists, from which choices will have to be made according to purposes (1). The application of genetic selection methods in the poultry industry has resulted in increased growth rate and carcass quality (2,3). The fundamental genetic nature is very complex and most economic traits in poultry shown continuous variation (4). Also, somatotropic axis genes plays an important role in chicken growth and development and this axis consists of essential components like growth hormone (GH) and growth hormone receptor (GHR) (5).

Growth hormone receptor (GHR), consists of 608 amino acids with a 16 amino acid signal peptide and a 24 amino acid (238—261) and it is one of member of the cytokine receptor super-family membrane spanning domain (6,7). By binding growth hormone receptor on the cell membrane surface, growth hormone stimulates cellular transfer signal to induce the synthesis and secretion of insulin-like growth factors, which accelerate the growth and differentiation of muscle cells (8). Unlike the mammalian counterpart chicken growth hormone receptor gene comprised 9 exons (with exon 3 absent) and 8 introns (7,9), and has been mapped to Z chromosome (10,11). Three forms of GHR mRNA have so far been found in chicken liver with lengths of 4.3, 3.2 and 0.8 kb, respectively, attributable to the presence of different poly (A) signal sites in chicken growth hormone receptor gene (9,12). The purpose of this study was to detect the G565A SNP in the fifth intron of growth hormone receptor (GHR) gene and to investigate their association with some productive and physiological traits in both Cobb500 and Hubbard (F-15) breeds of broiler chicken.

Material and Method

This study was conducted at the poultry farm of animal resources department – Agriculture College of Baghdad University. Two hundred day-old chicks were randomly housed in pens measuring 300 cm wide x 350 cm x 250 cm high (Coob500 and Hubbard separately) and marked with wing tags. Birds were kept indoor, on sawdust litter, in accordance with standard production technology. Commercial diet was provided *ad libitum*. While fresh drinking water was made available at all times. At day one of age water with sugar 0.5gm/litter, vitamin C 50gm/litter were provided. Newcastle B1 spraying at day one of age and then *Lacota* strain was used in drinking water at (10, 20, 30 and 37) day of age respectively , and the chicks were vaccinated in drinking water with Gambaro (*Locard* strain) at 12 and 22 day of age .

Five ml of blood were collected from the brachial vein of all chicks under the study. These samples were putted in EDTA tubes and kept in freezer (-18 ºC) for DNA isolation

(Molecular genetic studies), then the blood collected at 14 and 42 day of age, serum was obtained by putting the blood samples in a clean dry plain plastic tube and then was allowed to clot at 37 ºC for 30 minutes before being centrifuged. The tubes were centrifuged at 6000 rpm for 5 minutes; then the serum was collected and kept in freezer (-18 ºC) until it was used for serum biochemical analysis and total genomic DNA isolated from the whole frozen blood which was collected in EDTA anticoagulant tubes for molecular studies and it was applied using genomic DNA purification kits (Promega, USA), blood volume was reduced to 20 microliters and cell lysis buffer increased to 500 microliters because of of all the blood cells of chicken are nucleated and contained DNA and proteins levels in chicken blood higher than mammals blood . The primers was supplied from Alpha DNA/Canada, as lyophilized powder of different picomols concentrations F-5′ CCCTTCCATTATGCATTTTATC 3′ and R-5′ GGGGGTACACTCTAGTCACTTG 3′ gene bank (AJ506750) according to [5].

PCR reaction: The PCR reaction was performed in 0.2ml tubes by mixing master mix reagents in final volume of 20 µl. The amplification was performed in a TECHNE (T-C 5000) thermal cycler and the reaction mixture was prepared according to the procedure that suggested by the manufacture company (BIONEER, Korea) using 75-90 ng/ µl of DNA and 0.8 µl of primers and then complete the PCR reaction volume to 20 µl by distilled water finally reaction mixture vortexes thoroughly. PCR mixture without DNA template was used as a negative control. Thermal cycle with the following profile: Initial denaturation at 94 ^{o}C for 4 minutes, 35 cycles of 94 ^{o}C for 30 seconds,54 ^{o}C for 30 seconds,72 ^{o}C for 30 seconds and a final elongation at 72 ^{o}C for 5 minutes

Eight microliters PCR products were digested with 3 units of *Eco72I* at 37 ^{o}C overnight. Restriction pattern were visualized in a 1.5% agarose gel electrophoresis stained with Ethidium bromide .

Measurement of productive performance: Weekly live body weights were individually recorded in grams for each chick during the six week experimental period and then weekly body weight gains in grams were calculated for each chick under study. At 42 day of age, 15 male and 15 female for each hybrids randomly chosen were weighed and slaughtered after 12 hours of starvation, immersed in 53°C water for 2 minutes, and plucked in a rotary drum, chickens heads and legs were removed. Then liver, gizzard, heart were collected, weighed, and calculated as a percentage of carcass weight and dressed carcass were weighed and calculated as percentage of live body weight. Each carcass was cut into breast muscle, back, thigh muscle, drumstick, wings and neck. All weights were recorded and calculated as a percentage of live body weight [13].

Measurement of blood serum biochemical parameters: Blood serum GH concentration was measured by using ELISA Kits provided from CUSABIO Company and the rest physiological parameters (total protein, cholesterol, triglycerides and glucose) concentrations were measured by an automatic biochemical analyzer (Accent 200-Poland) following the instructions of the corresponding reagent kit.

Statistical analysis: Data were statistically analyzed using the program Statistical Analysis System -SAS [14] to study the effect of growth hormone (GH) gene

polymorphism for each line (Mathematical models I and II) in various characteristics (productivity and physiological traits), and compared the significant differences between the averages using the Duncan test [15] polynomial.

Results and Discussion

Detection of Enzyme Digestion by agarose electrophoresis (RFLP):The PCR products (571 bp) which underwent restriction digestion with *Eco 72I* enzyme (GAC/GTG) in order to detect SNP G565A in the intron five of growth hormone receptor gene and it was able to cut this site of the wild genotype GG. The following fragments sizing patterns were observed by agarose gel electrophoresis (Figure 1).

1. Wild type GG: *Eco72I* was cut the sequence to show two fragments in agarose gel electrophoresis (531 bp ,45 bp) and the 45 bp fragment did not seen (Figure 1, lane 10 and 11).

2. Heterozygous GA: *Eco 72I* was cut the sequence to show two fragments in agarose gel electrophoresis (576 bp , 531 bp and 45 bp did not seen) (Figure 1, Lane 3,5,6,9,12,13,14 and 15).3. Homozygote AA: No cleavage of the whole 576 bp segment by *Eco72I* (Figure 1,Lane 2,4,7and8).

The results of present study are similar with previous study of(5) on the Chines chicken.

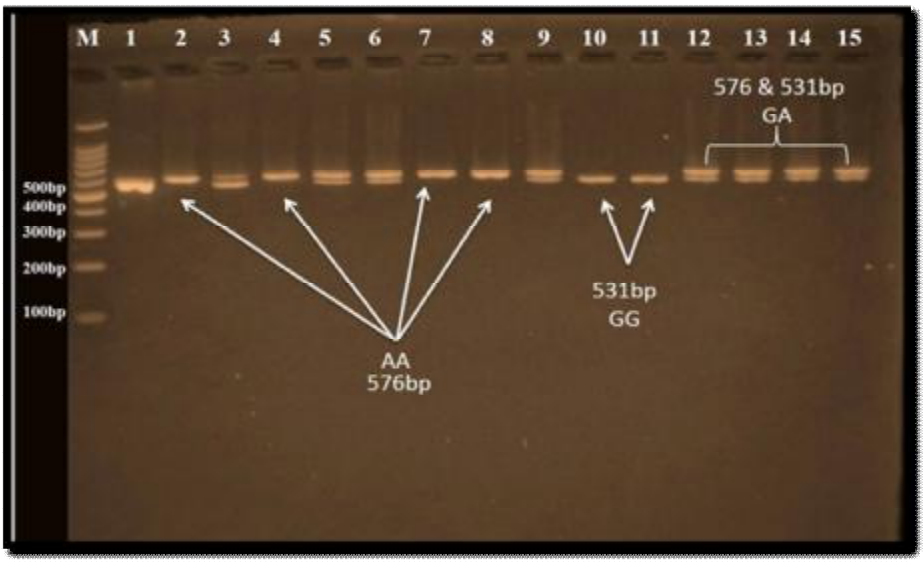

Figure (1). PCR product digested with *Eco72I* electrophoresis on 2%. Lane M: DNA ladder (100-1000). Lane1: PCR product . Lane 10 and 11 : Wild type GG genotype 531bp,45bp. Lane3,5,6,9,12.13.14 and 15: heterozygote GA genotype 576 bp, 531bp and 45bp. Lane 2,4,7and 8: Homozygote AA 576 bp .The (RFLP) products were agarose gel at 5 volt/cm^2 for 1hour. Visualized under U.V light after stain with Ethidium Bromide.

Distribution of growth hormone receptor gene genotypes in Cobb500 and Hubbard (F-15) broiler chicken:Table (1) showed high significant differences between the various genotypes (P<0.01), genotype GA followed by GG , and then AA amounted in Cobb500 42.35 , 35.29 and 22.35 % respectively and in Hubbard 42.11 , 29.47 and 28.42 % respectively.

Table (1). Distribution of growth hormone receptor gene polymorphism (No. and %) in Cobb500 and Hubbard F-15 broiler chickens

Genotype	Cobb 500	Hubbard F-15
	No. (%)	No. (%)
GG	30 (35.29)	28 (29.47)
GA	36 (42.35)	40 (42.11)
AA	19 (22.35)	27 (28.42)
Total	85 (100)	95 (100)
Chi-square value (χ^2)	8.207 **	7.963 **
** (P<0.01)		

Allele frequency of growth hormone receptor gene in Cobb500 and Hubbard (F15) broiler chicken: The results of Table (2) showed that allele G was dominant over allele A amounted 0.56 and 0.44 for Cobb500 respectively and in Hubbard 0.51 and 0.49 respectively.

Table (2). Allele frequency of growth hormone receptor gene in Cobb500 and Hubbard F-15 broiler chickens

Genotype	Cobb500	Hubbard F-15
G	0.56	0.51
A	0.44	0.49
Total	100%	100%

Effect of growth hormone receptor polymorphism (G565A SNP) on the mean weekly live body weight: The Table (3) showed non-significant differences between growth hormone receptor gene genotypes of mean live weight for weeks 1 and 2 of the Cobb500 and Hubbard. While there were significant increases (P<0.05) in the mean of live weight of the genotype GA third week amounted 935.83 g for Cobb500 and 923 g for Hubbard and followed by the genotype AA in the two hybrids was 916.05 g for Cobb500 and 886.29 g for Hubbard. The GG genotype recorded lower mean of live weight in the third week amounted to 876.66 g for Cobb500 and 870.35 g for Hubbard. In the fourth week, there were significant differences (P<0.05) as the genotype GA recorded highest mean of live weight for Cobb 1539.86 g followed by genotypes AA and GG 1466.05 and 1393.67 g respectively. The hybrid Hubbard genotype GA scored a significant increase (P<0.05) on the other genotypes, followed by AA and then GG 1475, 1417.15 and 1365.79 g respectively. In the fifth and sixth weeks, observation showed

significant increase (*P*<0.05) in the mean of live weight for Cobb500 genotype GA 2031.94 and 3745.83 g respectively while there were no significant differences between the genotypes AA and GG. Either the Hubbard genotype GA recorded significant increase (*P*<0.05) in the mean of live weight 2039.88 and 2695.38 g respectively, followed by AA 1945.56 and 2585 g respectively and then GG 1863.21 and 2474.82 g respectively .

Table (3). Effect of growth hormone receptor gene polymorphism on weekly live body weight (g)

Body weight (gm/week)	Cobb500			Hubbard F-15		
	GG	GA	AA	GG	GA	AA
1	a 166.16±3.0	a 164.44±2.5	a 168.94±3.7	a 153.03±2.5	a 158.75±2.3	a 155.92±2.6
2	a 415±8.3	a 426.52±7.1	a 438.42±9.0	a 432.5±6.6	a 451.25±6.7	a 437.40±7.1
3	b 876.66±12.3	a 935.83±15.1	ab 916.05±14.7	b 870.35±12.5	a 923±12.3	ab 886.29±13.7
4	c 1393.67±18.0	a 1539.86±25.1	b 1466.05±30.3	b 1356.79±20.7	a 1475±23.0	ab 1417.15±27.1
5	b 1845.50±24.4	a 2031.94±36.4	b 1916.58±43.4	b 1863.21±31.9	a 2039.88±34.9	ab 1945.56±40.7
6	b 2437.17±41.5	a 2745.83±57.4	b 2505±49.2	b 2474.82±44.1	a 2695.38±49.2	ab 2585±00.0

Means with the different superscripts of each hybrid within each row are significantly different (P< 0.05)

Effect of growth hormone receptor polymorphism (G565A SNP) on the mean of weekly weight gain: Results of Table (4) referred that the growth hormone receptor gene polymorphism did not affect significantly (*P*<0.05) in the weekly weight gain of weeks 1and 2 for the Cobb500 and Hubbard. But in the third week the genotype GA for Cobb500 had significant increase (*P*<0.05) amounted 509.30 g on the other genotypes and there were no significant differences between other genotypes. As with Hubbard, the effect of polymorphism was similar to what found in Cobb500 while the genotype GA recorded a significant increase (*P*<0.05) amounted 471.75 g, then followed by the other genotypes. The fourth week showed that there were significant increases (*P*<0.05) of Cobb500 genotype GA compared to the other genotypes amounted 604.02 g, On the other hand genotypes GA and AA recorded significant increase (*P*<0.05) against the genotype GG for Hubbard amounted 552,530 and486.42 g respectively , and did not show any significant effect of the different genotypes in the mean of weekly weight gain of Hubbard and Cobb500 in the fifth week. Sixth week results showed a significant increase (*P*<0.05) of the genotype GA over the genotypes GG and AA for Cobb500 amounted 713.88, 591.66 and 588.42 g respectively .But in the Hubbard there were no significant differences between the different three genotypes for this week.

Table (4). Effect of growth hormone receptor gene polymorphism on weekly weight gain (g)

Weight gain (gm/week)	Cobb500			Hubbard F-15		
	GG	GA	AA	GG	GA	AA
1 *	a 91.83±2.71	a 89.02±2.12	a 94.47±2.72	a 88.03±2.01	a 91.37±2.27	a 91.40±1.95
2	a 248.83±6.85	a 262.08±6.47	a 269.47±6.10	a 279.46±4.58	a 292.50±5.13	a 281.48±5.36
3	b 461.66±7.63	a 509.30±9.30	b 477.63±8.05	b 437.85±7.01	a 471.75±7.17	a 448.88±7.93
4	b 517±9.25	a 604.02±12.56	b 550±17.35	b 486.42±11.97	a 552±12.85	a 530.85±16.34
5	a 451.83±13.30	a 492.08±21.95	a 450.52±22.00	a 506.42±21.30	a 564.87±19.23	a 528.40±24.15
6	b 591.66±30.45	a 713.88±37.80	b 588.42±44.65	a 611.60±26.58	a 655.50±27.37	a 639.44±29.69

Means the with different superscripts of each hybrid within each row are significantly different (P< 0.05)
*weight gain was measured from age 3 -7 days.

Effect of growth hormone receptor polymorphism (G565A SNP) on the live and carcass weight ,dressing and dressing with giblets: The results of table (5) indicated the significant effect (*P*<0.05) of polymorphism the growth hormone receptor gene in live weight of Cobb500, genotype GA had the highest mean of live weight was 2808.93 g and there were insignificant differences between the genotype AA and GG live weight 2561.9 and 2448.75 g respectively. On the other hand it had been observed a significant increase (*P* <0.05) in the mean of carcass weight as GA genotype amounted 2117.50 g followed by genotype AA 1991.3 g then genotype GG 18853.57 g. Dressing percentage increased significantly (*P* <0.05) to genotype GA amounted 77.62 % followed by the genotypes AA and GG 75.67 and 75.40 % respectively, without significant effect between AA and GG genotypes. The dressing percentage with giblets significantly increased of genotype GA 81.68 % followed by genotypes AA and GG 80.06, 79.50 % respectively, significant differences were not observed in the growth hormone receptor gene polymorphism to all of the above traits for Hubbard hybrid.

Effect of growth hormone receptor polymorphism (G565A SNP) on relative weight of carcass cuts and giblets: It was evident from the table (6) there were non-significant differences between the various genotypes in the mean of relative weights of (thighs, drum stick, wings, neck and breast) of the Cobb500 and Hubbard. The genotype GG affected significantly (*P*<0.05) on the mean of back relative weight amounted 23.84 % followed by GA, and then AA 20.72 and 20.21 % respectively, and there were non-significant differences between the various genotypes in the back relative weight of the Hubbard, and found a significant increase (*P*<0.05) of heart relative weight in the Cobb500 for the genotype GA amounted 0.54 % followed by GG and AA 0.44 and 0.44 % respectively , and there were no significant differences between the three genotypes in

the Hubbard. There was a significant increase (P<0.05) of the liver relative weight in the Cobb500 for genotype GA amounted 2.20 % followed by AA and GG 2.15 and 2.14 % respectively, and there were non- significant differences in the Hubbard . In the Cobb500 genotype GA was recorded significant increase (P<0.05) in the mean of Gizzards relative weight amounted1.55 % followed by AA and then GG 1.37 and 1.23 % respectively. While the various genotypes were not significantly effect in Hubbard.

Table (5). Effect of growth hormone receptor gene polymorphism on live and carcass weight, dressing and dressing with giblets

Traits	Cobb500			Hubbard F-15		
	GG	GA	AA	GG	GA	AA
Live weight (g)	b 2448.75±69.15	a 2808.93±64.6	b 2561.9±53.25	a 2601±59.11	a 2742.70±83.8	a 2518±70.8
Carcass weight (g)	b 1853.75±56.8	a 2117.50±48.1	ab 1991.3±62.1	a 2064.30±52.1	a 2168.46±73.9	a 2018±66.49
Dressing (%)	b 75.40±0.36	a 77.62±0.81	b 75.67±0.37	a 79.34±0.84	a 78.97±0.39	a 80.16±16
Dressing with giblets (%)	b 79.50±0.33	a 81.68±0.80	ab 80.06±0.38	a 83.35±0.44	a 83.08±0.32	a 84.30±1.63

Means with the different superscripts of each hybrid within each row are significantly different (P< 0.05)

Table (6). Effect of Growth hormone receptor gene polymorphism on relative weight of carcass cuts and giblets

Traits (%)	Cobb500			Hubbard F-15		
	GG	GA	AA	GG	GA	AA
Thighs	a 13.88±0.16	a 14.24±0.28	a 13.07±0.75	a 14.31±0.50	a 14.14±0.60	a 15.18±0.48
Drum stick	a 11.83±0.44	a 12.11±0.27	a 11.14±0.37	a 12.80±0.33	a 13.55±0.18	a 12.84±0.42
Wings	a 9.58±0.39	a 9.52±0.24	a 9.15±0.17	a 9.65±0.26	a 9.78±0.17	a 10.53±0.28
Neck	a 5.18±0.57	a 6.10±0.31	a 5.21±0.47	a 5.25±0.16	a 5.56±0.19	a 5.37±0.35
Back	a 23.84±0.91	b 20.72±0.54	b 20.21±0.77	a 22.01±0.45	a 22.72±0.44	a 21.74±0.94
Breast	a 37.36±0.97	a 36.00±0.64	a 35.88±1.14	a 32.93±0.48	a 32.25±0.62	a 31.90±0.80
Heart	b 0.44±0.04	a 0.54±0.02	b 0.44±0.01	a 0.53±0.23	a 0.54±0.01	a 0.56±0.03
Liver	b 2.14±0.04	a 2.20±0.01	b 2.15±0.10	a 2.25±0.05	a 2.24±0.09	a 2.28±0.09
Gizzard	b 1.23±0.06	a 1.55±0.04	ab 1.37±0.60	a 1.46±0.08	a 1.31±0.04	a 1.46±0.09

Means with the different superscripts of each hybrid within each row are significantly different (P< 0.05)

Effect of growth hormone receptor gene polymorphism on some serum biochemical parameters of Coob500: The Table (7) showed there were significant differences (*P*<0.05) between the effect of the various genotypes of GHR gene on total protein concentration at the 14 and 42 days of age, where the genotype GG recorded the highest concentration amounted 3.4 g / dl, followed by each of the genotypes AA and GA amounted 3.1 and 2.9 g / dl, respectively at 14 days of age. While the genotypes AA and GG had the highest concentration amounted 3.6 and 3.4 g/dl followed by GA amounted 3.0 g/dl at 42 days of age. From the other side there was no significant effect of GHR gene polymorphism on the rest of the physiological traits under study.

Table (7). Effect of growth hormone receptor gene polymorphism per age in some serum biochemical parameters of Cobb500

Traits	14 days			42 days		
	GG	GA	AA	GG	GA	AA
GH (pg/ml)	a 2171±3.13	a 2166.1±6.4	a 2173.5±1.6	a 1827±1.68	a 1825.1±1.2	a 1724±100.0
GHR (mg/l)	a 0.99±0.01	a 0.98±0.01	a 0.97±0.01	a 1.28±0.01	a 1.28±0.01	a 1.28±0.01
Total protein (g/dl)	a 3.4±0.1	b 2.9±0.08	ab 3.1±0.08	a 3.4±0.05	b 3.0±0.07	a 3.6±0.29
Triglyceride (mg/dl)	a 122±0.23	a 121.6±0.28	a 121.7±0.33	a 66.9±0.43	a 65.6±0.56	a 65.7±0.67
Cholesterol (mg/dl)	a 121.3±0.4	a 121.3±0.41	a 122.2±0.48	a 114.6±2.43	a 112.2±0.3	a 112.7±0.33
Glucose (mg/dl)	a 229.7±0.21	a 229.8±0.22	a 229.8±0.29	a 228.7±0.29	a 228.7±0.16	a 228.9±0.63

Means with the different superscripts within each row per age are significantly different (P< 0.05)

Effect of growth hormone receptor gene polymorphism on some serum biochemical parameters of Hubbard F-15: The results of Table (8) pointed to insignificant differences between the various genotypes of GHR gene on the (GH, GHR, total protein , triglycerides ,cholesterol and glucose) concentrations of blood serum during the 14 days of age. While the genotype AA had significant increased (*P*<0.05) in cholesterol concentration at age 42 days of age amounted 121.7 mg/dl, followed by GA and GG genotypes amounted 120.3 and 119.5 mg / dl , respectively.

Table (8). Effect of growth hormone receptor gene polymorphism per age in some serum biochemical parameters of Hubbard F-15

Traits	14 days			42 days		
	GG	GA	AA	GG	GA	AA
GH (pg/ml)	a 2031±142.6	a 2170.1±2.68	a 1973.8±200.5	a 1825.9±2.25	a 1824.8±0.98	a 1824.4±1.32
GHR (mg/l)	a 0.97±0.01	a 0.97±0.01	a 0.99±0.01	a 1.3±0.01	a 1.3±0.01	a 1.3±0.01
Total protein (g/dl)	a 3.4±0.14	a 3.2±0.07	a 3.3±0.22	a 3.3±0.16	a 3.1±0.12	a 3.2±0.12
Triglyceride (mg/dl)	a 123±0.24	a 122.9±0.19	a 122.8±0.47	a 60.3±0.27	a 60.7±0.34	a 60±0.20
Cholesterol (mg/dl)	a 120.6±0.49	a 125.1±1.8	a 124.5±2.4	a 119.5±0.53	ab 120.3±0.51	b 121.7±0.93
Glucose (mg/dl)	a 230±0.31	a 229±0.28	a 230±0.24	a 228.6±0.29	a 228.7±0.16	a 228.9±0.06

These results are similar with the results were obtained from previous studies of (5,16) when they used *Eco72I* to detect the G565A SNP in the intron 5 of GHR gene. On the other hand the results of this study are different from the previous results of (17) who referred to absence of allele A of the G565A SNP in the GHR gene when he used *Eco721* to detect this SNP.

The compatible and Incompatible between present and previous results confirms the relationship between the number of SNPs and the selection programs in the commercial flocks to improve the productive performance and these selection programs lead to more mutations in the future between different type of breeds and hybrids and The results of this study are similar to the previous study of (5)who indicated the dominance of allele G over allele A of the G565A SNP in the fifth intron of GH receptor gene in the Chines chicken.

The results of this study are similar to the previous results of (18,19) that mentioned the GHR gene polymorphism had direct effect on broiler and laying hens productive performance and growth. Also (17) found that GHR gene polymorphism had significant effect on the carcass quality characteristics of the different breeds of the Chinese chicken, such as breast and thigh muscle fiber diameter and abdominal, subcutaneous fat. The presence of 2 SNPs in the fifth intron of GHR gene caused by the intensive selection in broiler commercial flocks(20). There were 33 SNPs in the GHR gene of chicken and mostly distributed in the non-coding regions, on the other hand the SNPs in GHR gene led to presence or absence of a poly(A) signal in intron 2, was found to influence egg production and live body weight in broiler (5).

In a study of (21) on Chinese chicken pointed to the existence 55 SNPs of the GHR gene by amplified 8908 bp fragments covered most of the cDNA for GHR gene and found that the G663177A SNP may affected the rate of body weight and subcutaneous fat of different chicken's ages. This significant differences in the mean of weight gain

may be because of the crucial role of the GHR gene in influencing the growth, according to the number of mutations were happened (18) . These results are similar with previous study of (21) who referred to the significant effect of the GHR gene polymorphism on hatching body weight at different ages and carcass weight of four breeds of chicken (White Leghorn, WRR broiler,Taihe Silkies and Xinghua). The main reason of significant increase of weight gain in older ages as compared with earlier ages in broiler related to GHR mRNA expression decline in the liver after hatching directly, and then increased sharply with age until maturity and this increase of GHR mRNA expression in the liver to be accompanied by an increase in the level of GH mRNA and IGF-1 mRNA in the liver, and the abundance of the IGF-1 leading to an increase in the growth of skeleton and muscles (12).

The significant differences of present study caused by the importance role of GHR gene and the effect of its polymorphism in response of the GH, IGF-1 effectiveness and their impact on the growth of the skeleton and muscles, as well as GHR polymorphism may be led to increase hepatic GHR mRNA expression, accompanied by an increase of IGF-1 mRNA in the liver, this affected positively on the mean of live body weight and increase the number and size of muscle cells (12). These results were agreed with previous study of (21) who showed the SNP G6631778A in the GHR gene which diagnosed by restriction endonuclease enzyme *Hin6I* was significantly affected hatching weight , mean of weekly body weight and dressing percentage. The different effects of GHR gene polymorphism between study hybrids was due to a difference of GHR gene expression as a result of the intensive selection for commercial flocks.

Decrease of GA and AA genotypes back relative weight may be caused by superiority of these genotypes over genotype GG in the mean of live body weight and this increase was often accompanied by a decrease of weight parts that did not contain the muscles and the back of them. The GA genotype superiority of the genotypes GG, AA status in the relative weight of the heart, liver and gizzard is attributable to the superiority of this genotype in the mean of body weight, led to a significant increase in the relative weight of some of the edible parts of the body.

The results of this study were consistent with the study of (17,21) they pointed to the crucial role of GHR gene polymorphism in the most productive traits ,because of its direct effect on gene expression of GHR mRNA in the target tissue , affecting gene expression of GH mRNA in the liver and various members of the body, leading to increase the impact of growth hormone in the target tissue. The reason the significant differences in the total protein levels was the direct effect of GHR protein on growth hormone activity, which was a crucial factor in the increase of protein biosynthesis to reduce proteolysis or both, and to reduce protein oxidation leading to net increase in protein accretion (22) and the significant increase in cholesterol concentration at 42 days of age caused by the direct impact of GHR mRNA expression on GH mRNA expression in the target tissue and this effect led to control of lipids synthesis and metabolism in blood serum .The age and the secretion pattern of GH are essential factors that determine its concentration in blood serum .The activity of GH in lipids

metabolism through the activation of acetate corporation in liver lipids, led to lipogenic effect (23). The difference of selection programs in commercial flocks (broiler and layer) led to presence of new mutations (SNPs) in the gene map of these flocks in the future.

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Effect of various metals nanoparticles (Ag NP, Ni NP, Co NP, Cu NP and Fe NP) on different antibiotic resistance pathogens

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Abstract

The objective of this research study the effect of metals nanoparticles (Ag NP, Ni NP, Co NP, Cu NP and Fe NP) on different antibiotic resistance bacteria and fungi. This research has been in a privet laboratory in the period from 31/05/2015 till 20/06/2015. Use three multidrug resistant bacterial strains *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* sp. and one multidrug resistant fungal strain *Candida* sp. by using Agar Mueller Hinton as medium to antibiotic sensitivity test. The significant effect was clear in the antibiotics sensitivity test where it was put different concentration of metal nanoparticles solutions (silver, copper, iron, nickel and cobalt) US origin (5 microliter to 25 microliter) individually on five disks and the 6th disk(antibiotic) on the surface of microbial culture plate and was used as control. this test prove all nanoparticles possess antimicrobial effect through measure the diameter of inhibition zone for growth of the bacteria and fungus, taking into consideration the minimum inhibitory concentration of nanoparticles to growth of the bacteria and fungus. Therefore, this study opened the new door to find the magic cure against the multidrug resistant bacterial strains.

تأثير مختلف الجسيمات النانوية المعدنية (الفضه، النحاس، النيكل، الحديد و الكوبالت) على الميكروبات الممرضة المقاومة للمضادات الحيوية

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الخلاصة

هدف هذا البحث دراسة تأثير الجسيمات النانوية المعدنية (الفضة، النحاس، النيكل، الحديد والكوبالت) على الممرضات البكتيرية والفطرية المقاومة للمضادات الحيوية . اجريه هذا البحث في مختبر اهلي خاص خلال الفترة من 2015/05/31 ولغاية 2015/06/20 تم استخدام ثلاث سلالات بكتريه ممرضة مقاومة للمضادات الحيوية الستافيلوكوكس (المكورات العنقودية) وبكتريا الاشيريشيا (القولونية) و الكليبسيلا (عائلة البكتيريا المعوية) وسلالة فطرية الكانديدا (المبيضات) المقاومة للمضادات الحيوية في أغار مولر هنتون لاختبار الحساسية للمضادات الحيوية وكان التأثير واضح وكبير في اختبار التحسس للمضادات الحيوية حيث وضعت احجام مختلفة من محاليل الجسيمات النانوية المعدنية (الفضة، النحاس، الحديد، النيكل والكوبالت) ذات المنشأ الامريكي من (5 ميكروليتر الى 25 ميكروليتر) بشكل منفرد على خمسة اقراص والقرص السادس كنترول (مضاد حيوي) حيث اثبت هذا الاختبار ان كل الجسيمات النانوية تمتلك تأثير مضاد ميكروبي وذلك من خلال قياس قطر المنطقة المثبطة لنمو الميكروب مع الاخذ بالاعتبار الحد الادنى من التركيز لجسيمات النانو المثبطة للميكروبات و هذه الدراسة فتحت باب جديد للعثور على علاج سحري ضد سلالات البكتيريا والفطريات المقاومة للأدوية المتعددة .

Introduction

Nanotechnology these days plays a vital role, that is, science is moving towards it in many branches: information, energy, environmental, medical technologies. Because of the quantum size effect of nanoparticles that is different from the bulk, nanoparticles' physical and chemical properties qualified them to be used in many applications in the electronic, chemical and mechanical industries, drug carriers, sensors, magnetic and electronic materials (1). Our field in this research is the application of nanoparticles in medicine, due to the increased number of deaths and hospitalizations because of increased bacterial resistance to multiple antibiotics within both gram positive and gram negative microorganisms, and the continuing emphasis on health-care costs (2). Health concern moving towards metal oxide nanoparticles as a an effective and efficient antibacterial therapeutic and diagnostic methodologies and techniques (3). Metallic nanoparticles which have unique physicochemical characteristics due to their high specific surface area (4). and also a unique adsorption properties because of different distributions of reactive surface sites that can be functionalized with various chemical groups to increase their affinity towards target compounds (5). Metallic oxide nanoparticles are prepared and stabilized by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction (6). and these days via green chemistry route (4). Most of previous studies concentrated on the antibacterial activity of the famous nanoparticles represented by silver followed by ZnO and CuO (7). However, there is less concerns of the other nanoparticles like CoO. Cobalt oxide nanoparticle displays structural, magnetic, electronic and catalytic properties (8). but in last few decades CoO is used as bactericides for water disinfection (5). So, this property enables cobalt oxide nanoparticles to be used as antibacterial activity (7). examined the antibacterial activity of cobalt oxide nanoparticles, nickel oxide NPs, zinc oxide NPs, copper oxide NPs, iron oxide NPs and titanium dioxide NPs against *E. coli* using two methods: culturing in liquid media containing one of these nanoparticles and electro spraying the NPs directly onto bacterial surface(9). The results indicate a significant cell death when *E. coli* was exposed directly using electrospray exposure method to oxidized nickel, zinc and cobalt species; but no antibacterial properties from titanium, iron and copper oxide (10).

Staphylococcus aureus is a gram positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies (11).

Escherichia coli (*E. coli*) is a gram-negative, facultative anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E.coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally

responsible for product recalls due to food contamination *E. coli* is expelled into the environment within fecal matter. (12, 13, 14).

Klebsiella sp. is a genus of nonmotile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. *Klebsiella* species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens. *Klebsiella* species are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens (15).

Candida sp. is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans, however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease(Candidiasis When it affects the mouth, it is commonly called thrush When it affects the vagina, it is commonly called a yeast infection (16).

Materials and Methods

Medium: Muller-Hinton agar: is a microbiological growth medium that is commonly used for antibiotic susceptibility testing.

Microbial culture: *E. coli*, *staphylococcus aureus, , klebsiela* ssp and *candida* ssp for different antibiotic resistance (was purchased from American Type Culture Collection (ATCC)).

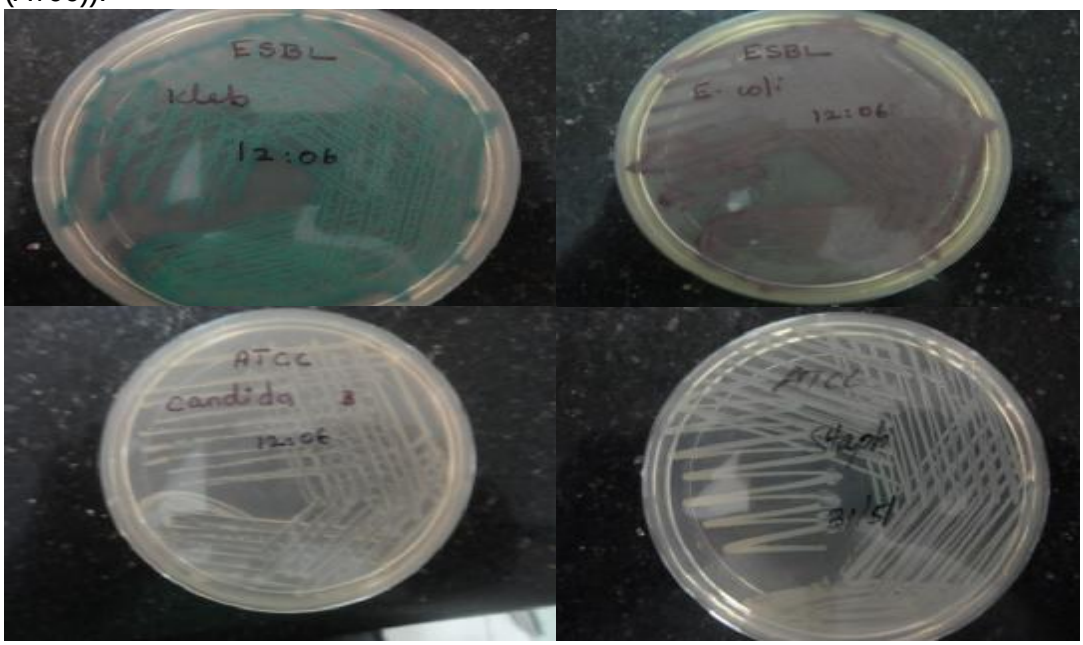

Figure1: Showing the *E. coli*, *staphylococcus aureus, candida, and klebsiela* growing on Muller-Hinton agar media.

Nanoparticals: Silver nanoparticle (Ag NP),Nickel nanoparticle (NiNP), Iron nanoparticle (Fe NP), Cobalt nanoparticle (CoNP) and Copper nanoparticle (CuNP) all Provided in powder form by American elements the material science manufacture.

Table 1: Showing the product data of Nanoparticles (Ag, Ni, Fe, Co and Cu)

Nanoparticals Formula	Average Particle Size nm	Molecular Weight	Appearance Colure	Appearance Form	CAS Number	Purchased From
Ag NP	40 nm	107.87	Yellow	Liquid	730807	Sigma-Aldrich Us
Ni NP	20 nm	58.69	Blue	Powder	7440-02-0	American Elements Us
Fe NP	50 nm	55.85	Black	Powder	7439-89-6	American Elements Us
Co NP	28 nm	58.93	Grey	Powder	7440-48-4	American Elements Us
Cu NP	25 nm	63.65	Brown	Powder	7440-50-8	American Elements Us

Antibiotics: Gentamycin, Cephalosporin (cephalexin) and Amikacin. All used as ready antibiotic discs All was purchased from HiMEDIA Laboratories(India).

Table 2: Showing the product data of ANTIBIOTIC (Gentamycin, Cephalosporin and Amikacin)

Antibiotics name	Concentration mcg/disc	Symbol	purchased from
Gentamycin	10mcg/disc	GEN	HiMEDIA Laboratories(India)
Cephalosporin	30mcg/disc	CN	HiMEDIA Laboratories(India)
Amikacin	10mcg/disc	AK	HiMEDIA Laboratories(India)

Inoculum: Growth method or direct colony suspension with turbidity comparable to 0.5 McFarland Standard.
Incubation:16 to 18 hours at 35°C in an ambient air incubator.
Testing conditions for Kirby Bauer disc diffusion method**:** Muller-Hinton agar obtained from Hi-Media was used as culture medium. It was sterilized by autoclaving. About 15-20 ml of medium was poured into Petri dishes of 9cm size, with aseptic precautions to give a thickness about 4mm.With the help of straight wire 3-4 identical colonies were picked up from the microbial culture plate and were inoculated into 5ml of nutrient broth. The broth was incubated at 35°C for 3-4 hours, so as to obtain moderate turbidity if necessary turbidity was adjusted to 0.5 McFarland standards. A sterile cotton swab was dipped in this broth and excess fluid was removed by gently squeezing the swab on the inner sides of test tube. Using this swab. streak was made on entire Mueller-Hinton agar plate in all directions and rotating the plate every time. The plates were dried for

15 minutes with lid in place. Whatman paper discs were applied with aseptic precautions. Discs were deposited with centers at least 24 mm apart. The plates were incubated at 35°C in ambient air for 16 to 18 hours (17).

Minimum Inhibitory Concentration (MIC) by agar dilution method**:** On the surface of each microbial culture plate 5 Discs were applied with aseptic precautions and the nanoparticles were deposited on the disc. The different volume of nanoparticles solution of silver,nickel, iron, cobalt, Copper was placed individually on disc. Antibiotic disc was placed as 6th disc on the surface of microbial culture plate and was used as control. All the plate was incubated in a vertical position for 24h at 37°C, with the cover plate lined with filter paper to prevent moisture. After incubation the presence of bacterial growth inhibition halo around the samples were observed and their diameter in millimeters was measured. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297) (18).

Reporting of MIC: The lowest concentration of nanoparticles dilution which could inhibit the growth of the strains is taken as minimum inhibitory concentration of the nanoparticles for the strain.

Results and Discussion

In the test of antimicrobials sensitivity in vitro following results were obtained:

I) Effect of different nanoparticles on different microbial strains:

Table (3): Showing the Effect of different nanoparticles on *E. coli* and used Cephalosporin(CN) as ready antibiotic discs (concentration 30mcg/disc) and Gentamycin(GEN) as ready antibiotic discs (concentration -10mcg/disc), Average diameter of zone of inhibition observed 24 hours incubation at 37°C for standard cultures.

S. NO	Concentration of different Nanoparticales (µl)	Inhibition zone for Ag NP (mm)	Inhibition zone for Cu NP (mm)	Inhibition zone for Co NP (mm)	Inhibition zone for Fe NP (mm)	Inhibition zone for Ni NP(mm)
1	5 µl	R*	20	20	20	15
2	10 µl	R	22	20	24	20
3	15 µl	12	24	24	24	22
4	20 µl	15	25	24	26	26
5	25 µl	18	26	24	28	28
6	Antibiotic disc	CN-R	GEN-28	GEN-28	GEN-28	GEN-28

*R: Resistance: showing no inhibition zone.

Table (4): Showing Effect of different nanoparticles on *Staphylococcus aureus* and used Amikacin(AK) as ready antibiotic discs (concentration - 10mcg/disc) , Average diameter of zone of inhibition observed 24 hours incubation at 37°C for standard cultures.

S. NO	Concentration of different Nanoparticales (µl)	Inhibition zone for AgNP (mm)	Inhibition zone for CuNP (mm)	Inhibition zone for CoNP (mm)	Inhibition zone for FeNP (mm)	Inhibition zone for NiNP (mm)
1	5 µl	R	20	20	22	20
2	10 µl	R	24	20	24	22
3	15 µl	R	26	24	26	26
4	20 µl	10	28	26	28	27
5	25 µl	20	30	28	30	30
6	Antibiotic disc	AK- 28	AK- 28	AK-28	AK- 28	AK- 28

*R: Resistance: showing no inhibition zone

Table (5): Showing Effect of different nanoparticles on *klebsiela* and used Cephalosporin(CN) as ready antibiotic discs (concentration 30mcg/disc) and Gentamycin as ready antibiotic discs (concentration -10mcg/disc), Average diameter of zone of inhibition observed 24 hours incubation at 37°C for standard cultures.

S. NO	Concentration of different Nanoparticales (µl)	Inhibition zone for AgNP (mm)	Inhibition zone for CuNP (mm)	Inhibition zone for CoNP (mm)	Inhibition zone for FeNP (mm)	Inhibition zone for NiNP (mm)
1	5 µl	R	R	R	R	R
2	10 µl	R	20	R	21	23
3	15 µl	R	24	24	26	24
4	20 µl	12	24	24	26	24
5	25 µl	14	26	24	28	30
6	Antibiotic disc	CN- R	GEN-29	GEN-29	GEN-29	GEN-29

*R: Resistance: showing no inhibition zone

Table (6): Showing Effect of different nanoparticles on *candida* and used Cephalosporin(CN) as ready antibiotic discs (concentration 30mcg/disc), Average diameter of zone of inhibition observed 24 hours incubation at 37°C for standard cultures.

S. NO	Concentration of different Nanoparticales (µl)	Inhibition zone for AgNP (mm)	Inhibition zone for CuNP (mm)	Inhibition zone for CoNP (mm)	Inhibition zone for FeNP (mm)	Inhibition zone for NiNP (mm)
1	5 µl	R	R	R	R	R
2	10 µl	20	21	23	21	23
3	15 µl	24	26	24	26	24
4	20 µl	24	26	24	26	24
5	25 µl	26	28	30	28	30
6	Antibiotic disc	CN-30	CN-30	CN-30	CN-30	CN-30

*R: Resistance: showing no inhibition zone

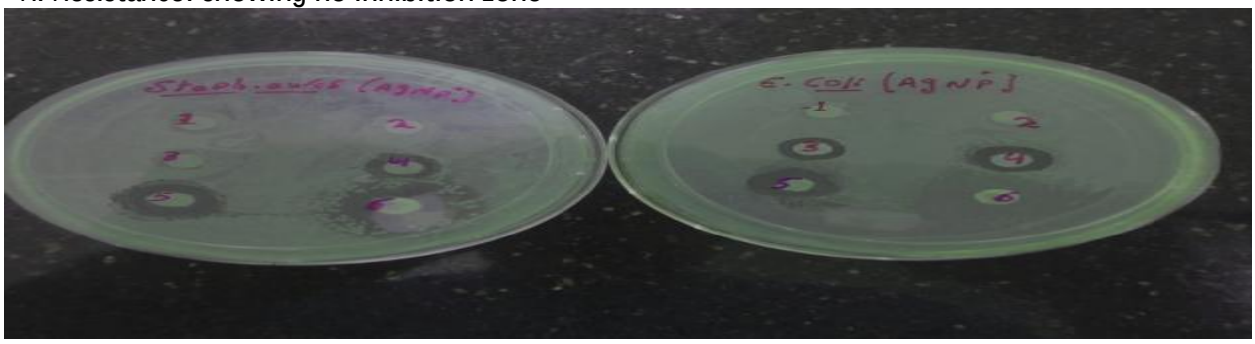

Figure 2: Shows the inhibition zone for *S. aureus* and *E. coli* when incubat with different concentration of Ag NP with Cephalosporin(CN), Gentamycin(GEN) and Amikacin(AK) as ready antibiotic discs.

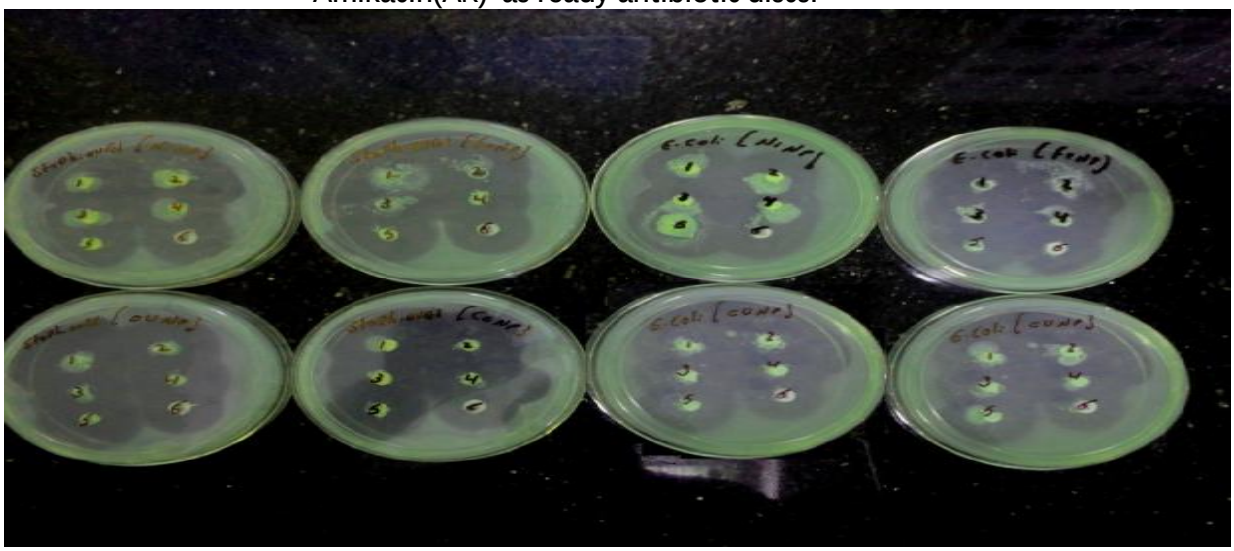

Figure (3): Shows the inhibition zone for *S. aureus* and *E. coli* when incubated with different concentration of Ni NP, Co NP, Cu NP and Fe NP with Cephalosporin(CN) and Amikacin(AK) as ready antibiotic discs.

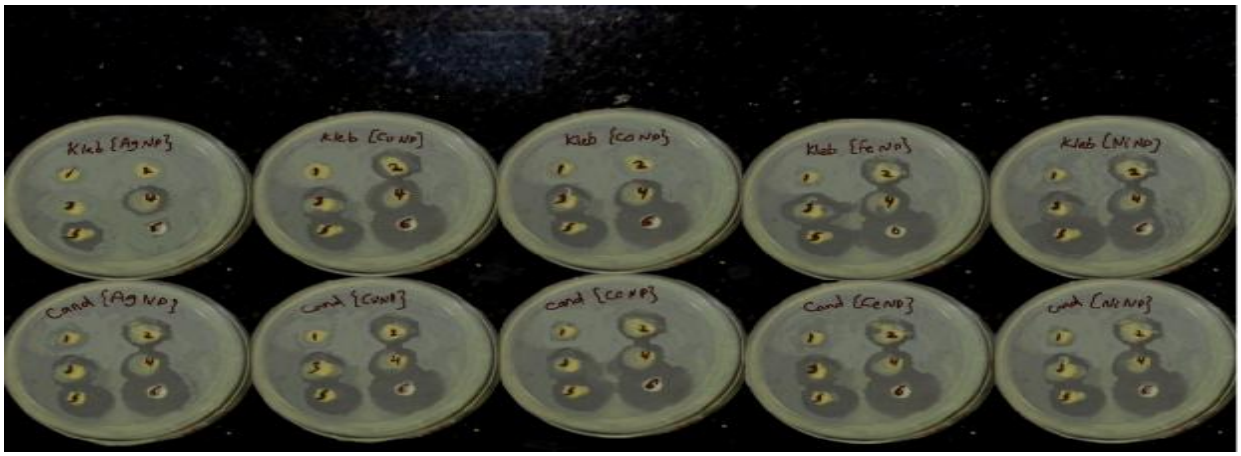

Figure (4): Shows the inhibition zone for *klebsiela* and *candida* when incubated with different concentration of Ag NP, Ni NP, Co NP, Cu NP and Fe NP with Cephalosporin(CN) and Gentamycin(GEN) as ready antibiotic discs.

In the above experiment, when different nanoparticles (Ag, Ni, Co, Cu, Fe) were tested against various strains of bacteria (*E. coli, S. aureus, klebsiela* ssp)and against a fungi (*candida* ssp), all the nanoparticles showed to possess antimicrobial property at different concentration. In the test of sensitivity nanoparticles showed the formation of inhibition zone.

In Table (3) case of *E. coli* at higher concentration i.e. 25 µl almost all the nanoparticles showed inhibition zone, with FeNP and NiNP showing the highest diameter of 28mm. MIC for Fe, Cu, Co & Ni was found to be 5µl but for Ag it was 15 µl Table(A). Thus we can say that for *E.coli* CoNP, CuNP and FeNP are most effective with MIC 5 µl and diameter for zone of inhibition 20mm, 20mm and 20mm respectively. Among Ag, AgNP is more effective at MIC 15 µl with diameter of zone of inhibition as 12mm (Figure 2,3)

In Table (4) case of *Staphylococcus aureus* also at higher concentration i.e. 25µl almost all the nanoparticles showed inhibition zone, with CuNP, FeNP and NiNP showing the highest diameter of 30 mm. MIC for Fe, Cu, Co & Ni was found to be 5 µl but for Ag it was 20 µl. Hence *Staphylococcus aureus* is more resistance against Ag compared to *E.coli*. Thus it can be concluded that for *Staphylococcus aureus* CoNP , CuNP , NiNP and FeNP are most effective with MIC 5 µl and diameter for zone of inhibition 20mm, 20mm , 20mm and 22mm respectively. Among Ag, AgNP is more effective at MIC 20 µl with diameter of zone of inhibition as 10mm (Figure 2, 3)

In Table(5) case of *Klebsiela* also at higher concentration i.e. 25 µl almost all the nanoparticles showed inhibition zone, with NiNP showing the highest diameter of 30 mm. MIC for Fe, Cu, & Ni was found to be 10 µl, for Co it was 15 µl and for Ag it was 20 µ1. Hence *Klebsiela* more resistance to nanoparticles at very low concentration compared to *Staphylococcus aureus* and *E. coli*.with the data it's clear that for *Klebsiela* NiNP is most effective with MIC 10 µl and diameter for zone of inhibition 23mm. Among

Ag, AgNP is more effective at MIC 20 µl with diameter of zone of inhibition as 12mm. (Figure 4).

In Table(6) case of *Candida* also at higher concentration i.e. 25 µl almost all the nanoparticles showed inhibition zone, with NiNP & CoNP showing the highest diameter of 30mm. MIC for Fe, Cu, Ni, Co & Ag was found to be 10 µl. Thus it can be concluded that for *Candida* and CoNP& NiNP are most effective with MIC 10 µl and diameter for zone of inhibition 23mm. Among Ag, AgNP is more effective at MIC 10 µl with diameter of zone of inhibition as 20mm. (Figure 4)

It is well known that nanoparticles of inorganic metals have strong antimicrobial effects and many investigators are interested in using these inorganic nanoparticles as antimicrobial agents. These inorganic nanoparticles have a distinct advantage over conventional chemical antimicrobial agents or antibiotics. The most important problem caused by the chemical antimicrobial agents is multidrug resistance and so these nanoparticles can be easily used because of their antimicrobial property (1). Among all other elements silver is known to have broad-spectrum antimicrobial activity against bacteria, viruses and eukaryotic microorganisms (7). With the above results, it is obvious that there is effective antimicrobial activity of AgNPs against *E. coli* and *S. aureus*. Apart from silver other nanoparticles such as CuNPs, NiNPs, CoNPs etc also showed good result against different microorganisms when used in higher concentration. Even for fungi which have a thick cell wall these nanoparticles were effective. While the mechanism of the inhibitory effects of nanoparticles on microorganisms is partially known. Some studies have reported that the positive charge on the nanoparticle ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles (6). In contrast, Sondi and Salopek-Sondi reported that the antimicrobial activity of silver nanoparticleson Gram-negative bacteria was dependent on the concentration of Ag nanoparticle(19), and was closely associated with the formation of “pits” in the cell wall of bacteria. The antimicrobial effect of silver is dependent on superficial contact, in that silver can inhibit enzymatic systems of the respiratory chain and alter DNA synthesis(2). Thus the mechanism of inhibition is not clearly understood.

The bactericidal action of copper may occur in different ways, both in interstitial space and within cells(10). This metal has the ability to accept or donate their electrons resulting in a high catalytic oxidation and a high potential reduction. Thus it is possible that the copper ions alter the protein existing within the cells of bacteria, impairing its normal functions. Our study clearly demonstrates the bactericidal action of copper on various types of bacteria .

Nickel-containing enzymes are well known in the bacterial world. They represent a good model to study nickel bio metabolism, transport systems and nickel binding proteins(21). The precise mechanism of action of nickel nanoparticle requires further investigation. In our study other nanoparticles such as cobalt and iron also showed antibacterial effect but exact mechanism of their action was very difficult to understand and require further detailed study on it.

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Evaluation hydroxyurea treatment effect on the metabolic products levels, and GST activity, substrate concentration effect on the kinetic parameters of GST in pre, post-treatment patients with MPN

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Abstract

Levels of three metabolic products (uric acid, urea, and creatinine), glutathion S Transferase activity Blood cell components, were determined in the sera of the pre, and post- treatment MPN patients with hydroxyurea as compared to control. The influence of the treatment on glutathion S transferase(GST) kinetic parameters was examined by using concentration effect of both substrate (GSH, CDNB) in the sera of pre-treatment MPN with (HU) and healthy control. Results revealed insignificant differences in the level of metabolic products as compared to control. HU showed significant effect of the uric acid in post-treatment patients as compared to control. GST activity showed insignificant deceases in pre ,and post- treatment patients as compared to control, with high differences in post-treatment patients . Km of GST in pre-treatment in both concentration of (GSH, CDNB) were higher than that in control group, while the Vmax was higher in CDNB concentration.

تقييم تاثير علاج الهيدروكسي يوريا على مستويات النواتج الايضية ، فعالية انزيم الكلوتاثايون-اس- ترانسفيريز(GST)، دراسة تاثير التركيز على حركيات الانزيم (GST) عند مرضى ال MPN قبل وبعد العلاج

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الخلاصة

تم قياس مستويات ثلاث نواتج ايضية (حامض اليوريك ، اليوريا و الكرياتينين)، فعالية انزيم الكلوتاثيون -اس- ترانسفيريز (GST)، مكونات خلايا الدم في امصال المرضى المصابين بال MPN قبل وبعد العلاج بالهيدروكسي يوريا مقارنة بالمجموعة المقارنة . لقياس تاثير العلاج على حركيات ال (GST) تم دراسته باستخدام طريقة تاثير تركيز كلتا مادتي الاساس (GSH, CDNB) في امصال امرضى المصابين قبل العلاج بالهيدروكس يوريا (HU) وعند الاصحاء. اظهرت النتائج اختلافات غير معنوية في مستويات النواتج الايضية في مجموعة المرضى مقارنة بمجموعة الاصحاء. كما اظهرت الدراسات تأثيرا معنويا للعلاج بالهيدروكس يوريا على مستويات حمض اليوريك في امصال مجموعة المرضى بعد العلاج مقارنة بالمجموعة الضابطة . لوحظ انخفاض غير معنوي في فعالية ال (GST) في مجموعتي المرضى مقارنة بالمجموعة الضابطة مع تسجيل اعلى انخفاض في مجموعة المرضى بعد العلاج. دراسة حركيات ال (GST) اظهرت ارتفاع Km في مجموعة المرضى قبل العلاج في تركيزي ال (GSH, CDNB) مقارنة بالمجوعة الضابطة ، الا ان السرعة القصوى Vmax كانت اكثر فقط في تركيز ال .CDNB .

Introduction

Myeloproliferative neoplasm (MPN) is a class of chronic hematologic disorders in which there is to describe excess production of blood cells by mature hematopoietic stem cells in the bone marrow(1). MPN is characterized by boon marrow hyper cellularity with effective hematopoietic maturation and more numbers of granulocytes, RBC, WBC, or platelets is raised in the peripheral blood. Reservation of increased blood cells and proliferation of abnormal hematopoietic cells are common causes for splenomegaly and hepatomegaly, despite an insidious onset each MPN has the potential to undergo a gradually progress that terminates in marrow failure due to myelofibrosis, then, invalid hematopoiesis or transition to an acute stage. Disease progression as may increase organ a megaly, increasing or decreasing blood counts, myelofibrosis that are evidence of genetic evolution (2).

Hydroxyurea(HU) is used for the treatment of Myeloproliferative Neoplasms. HU ((CH4N2O2) Carbamohydroxamic Acid). The World Health Organization's (WHO) considered this drug in the List of Essential Medicines, a type of the most important medication needed in a basic health system (3) . Because its effect on the bone marrow, regular monitoring of the full blood count is vital, also early response to possible infections. In addition, renal function, uric acid and electrolytes, as well as liver enzymes, are commonly checked (4) . Moreover, because of this, severe anemia and neutropenia are contradicted. The side-effects of Treatment are; drowsiness, nausea, vomiting and diarrhea, constipation, mucositis, anorexia, stomatitis, bone marrow toxicity (doselimiting toxicity, abnormal liver enzymes, creatinine and blood urea nitrogen (4).

Hydroxyurea has a complexes mechanism include converted it to a free radical nitroxide (NO) in vivo and the metabolism in the liver with The excretion of the total by 80%, transported by diffusion into cells where the M2 protein subunit of ribonucleotide reductase have it quenches the tyrosyl free radical at the active site, inactivating the enzyme and DNA synthesis is selectively inhibited by the entire replicas complex, including ribonucleotide reductase, resulting cell death in S phase and synchronization of the fraction of cells that survive. Hydroxyurea inhibit the Repair of DNA damaged by chemicals or irradiation (5).

Glutathione -S- transferase is a member of glutathione system, and antioxidant enzymatic repair systems that repair biomolecules damaged by ROS and RNS. GSTs catalyze the coupling of glutathione (GSH) to a wide variety of electrophilic compounds) xenobiotics, endogenous and exogenous) (6). Three glutathione S transferase isoenzyme have been appear to inactivate the function of kinase protein implicated in the MAPK pathway by interfering with kinase role in cell signaling cascade (7).

This study aimed to evaluate the level of metabolic products (uric acid, urea, creatinine), GST activity, and the kinetic of GST in newly diagnosed and post-treated patients with MPN with Hydroxyurea.

Materials and Methods

Selection of patients and healthy volunteers: A total of (40) patients screened of having MPN who were attending to (Iraqi national center of hematology / Al-

Mustansiriyah university) from the beginning of August 2015 up to the med of February 2016. An age range from (30-72 years) recorded in this study, with (8) females and (32) males. MPN patients were classified into two groups, group(A) : new suspected (20)patients(pre-treatment), group (B): post-treated (20)patients. Questioner diagnosis of MPN was depend on regards of complete blood picture, smoking , patient s family history, previous disease, blood section, physical examination include abdominal examination for palpable spleen and liver, treatment & doses (from patients medical data). On the other hand a total (10) of apparently healthy individuals were included in this study as healthy control group, age (30-72 years) with (6) males and (4) females.

Blood Sampling: Blood sample were obtained from each individual 4 ml, by venipuncture using disposable syringes. Five ml of blood was placed in (activator clotting tubes), after allowing the blood to clot at room temperature, the blood was separated by centrifugation for 8min at 4000 rpm, and serum was divided into aliquots (250 µl) and stored at -80C ° till examination. 1ml of blood was placed in EDTA tube for Complete Blood Picture.

Chemicals: Phosphate buffer saline (PBS, BDH, England); 1-chloro-2, 4-dinitrobenzene (CDNB , Hopkins and Williams, UK), reduced glutathione (GSH, Sigma chemicals, USA); ethanol (Fluka, Germany). Uric acid kit, Urea kit , and Creatinine kit (Biosystems; Barcelona, Spain).

Complete Blood Picture: Blood cell components includes:- Hb,PCV, RBC ,WBC, Platelet count,MCV,MCH,MCHC and RDW, done by cell-dyn automated by taken 100 µl of whole EDTA blood .

Metabolic products measurements: Uric acid, urea, and creatinine were measured by using spectroscopic method according to the instruction supplied by the manufactured company. Cecil CE 72000 spectrophotometer (France) was used.

GST activity and Kinetic parameters: Serum Glutathione S transferase activity was measured by using artefactual substrate (CDNB), and reducing glutathione (GSH)[8]. The overall reaction mixture was 1100µl. 900µl of [PBS (pH 6.5), 0.10 M GSH, 0.1 M CDNB], 100 µl of enzyme source. Bland was set by using the exact mixture but the enzyme source. The absorbance was measured at 340nm for five minutes.

Kinetic parameters amounts (Km and Vmax) of Crude GST were determined for GSH, and CDNB. GST activity was measured for a range of GSH (80-600) µM and fixed concentration of CDNB (400 µM), and a range of CDNB (80-600) µM and fixed concentration of GSH (400 µM). Km and Vmax were calculated by using Microsoft Excel 2016.

Results and Discussion

Demographic Distribution: Demographic distribution (age, gender) of MPN groups (pre, and treated) and control group is illustrated in table (1). Mean ±SD of age for both pre- treatment and post- treatment groups patients were (53.5 ± 2.34) with range (30-72) years, (56.00 ± 1.71) with range (39-71) years respectively. These two groups showed a significant difference from control group ($p<0.05$). this results were in agreement with (Srdan Verstovsek. et al. (2015)) (9) who establish that age is divorced

in MPN patients (median, ≤ 65; range (30–80) years, as shown in the results a significant difference was observed in MPN post-patients as compared to control($p<0.05$).

Table (1): Distribution of the studied groups was according to age and gender

Parameters		Pre-treatment Group No.=20		Post treatment Group No.=20		Control group No.= 10		P. value
		N.	%	No.	%	No.	%	0.015*
Age (years) <40		3	15.6 %	1	3.5 %	3	35.0 %	
40 — 59		10	49.5 %	9	46.4%	6	60 %	
60 >		7	35.2 %	10	50.0%	1	5 %	
Mean ± SE (Range)		53.5 ± 2.34 (30-72)		56.00 ± 1.71 (39-65)		44.33 ± 3.24 (30-61)		
Gender	Male	13	62.8 %	9	42.8 %	5	48 %	0.232
	Female	7	37.2 %	11	57.1 %	5	52 %	
duration of disease				1.4 M	7.1 %	--		
				1 y.	21.4 %			
				7 Y	46.4 %			
				10Y	21.6 %			
				12Y	3.5 %			

Note: *: significant using student T- test at 0.05 level of significance, **: > 0.01 level of highly significance.

Both male and female were affected by MPN as shown in (table -1). As shown in this table male are more affected with MPN than female in pre-treatment group with ratio (1.7:1). This result is in contrast with (10) who demonstrated a male to female ratio in patients with MPN (1.8:1)[10] . On the other hand post-treatment patients with MPN allocation results showed that female are higher that male with ratio (0.75:1), this result is in covenant with (11) who established insignificant differences in male to female ratio (1:1.44) in treated patients with MPN.

As for control group , the age range(30 – 61) years with male to female ratio was (0.66:1), no palpable spleen was found in this group.

Palpable Spleen: One of the diagnostic sign of subject suspected with MPN is splenomegaly according to WHO 2008 diagnostic criteria. Table (2) revealed that palpable spleen is observed in (44%) of pre-treatment patients group.

Table (2): Distribution of the studied groups was according to Splenomegaly

Parameters		Pre-treatment Group No.= 20		Post treatment Group No.=20		Control group No.= 10		P. value
Splenomegaly	Yes	9	44 %	14	70.1 %	--	--	0.010**
	No	11	56.8 %	6	29.8 %	--	--	

Note: *: significant using student T- test at 0.05 level of significance, **: > 0.01 level of highly significance.

A significant difference was observed between to patients group ($P < 0.01$) as shown in (table-2). This results in in full agreement with (12), who concluded that palpable spleen was observed in (43.8%) of newly diagnosed patients with MPN. As for post-treatment group, the most common site of extra medullary hemopoesis is the spleen. The percentage of palpable in this group was (70.1%), which they were previously diagnosed with splenomegaly and were treated with hydroxyurea(13).

Haematological Determinations (blood cell indices): Significant differences were observed ($P>0.05$) in the mean of (PLAT count and WBC) in both patients groups as compared to control (table - 3). HGb, RBC, PCV means showed revealed highly significant differences ($P>0.01$) between the two patients groups as compared to control healthy group.

This finding is matching with that identified by(13), who was mentioned the (haematocrit >45 in both sex), leucocytosis (WBC count >10.5 $x10^9/L$), or thrombocytosis (platelets<450x $10^9/L$), in pre-treatment group patients (13). Moreover, in post treatment group were found results lower than group one , this may due to hydroxyurea was used to decrease elevated blood cell counts until the MPN patient's (haematocrit is stabilize at less than 45%, WBC count <11.0 $x10^9/L$), platelets <400 x $10^9/L$), These findings are coincided with that confirmed by(14) , both researcher clearly specified a highly significant differences in blood cell indices which are similar with the present study finding ($P < 0.01$) between two groups MPN patients and control group.

Biochemical Determinations in The Sera of Two Groups MPN Patients & Controls:

Metabolic products (Uric acid (UA), Urea(U), and Creatinine (Cr)) determinations in the sera of two groups MPN patients & controls: Metabolic products Uric acid, Urea, and creatinine levels were determined in the sera of the three studied groups. As shown in (table-4) an insignificant increment were observed in the levels of theses metabolic products in patients groups as compared to control group (Figure 1,2,3).

Table(3):- complete blood count of myeloproliferative neoplasm patients in different studied groups and healthy control group

Parameters		Pre-treatment Group / No.= 51	Post treatment Group / No.= 28	Control group / No.= 20	P. value
WBC ($\times 10^9$/L)	Mean ± SEM	12.00 ± 0.97	9.66 ± 1.24	7.33 ± 0.26	0.012*
	Range	(4.29 - 28.48)	(4.93 - 32.09)	(4.2 - 9.79)	
RBC ($\times 10^6$/µl)	Mean ± SEM	7.10 ± 0.35	5.85 ± 0.22	5.82 ± 0.10	0.001**
	Range	(2.8 - 9.42)	(2.77 - 7.24)	(4.26 - 5.27)	
HGB (g/dl)	Mean ± SEM	14.56± 0.62	13.86 ± 0.33	13.54 ± 0.19	0.001**
	Range	(7.3 - 22.2)	(11 - 17.4)	(12.4 - 14.7)	
PCV (%)	Mean ± SEM	49.26 ± 1.33	41.64 ± 1.76	40.76 ± 0.38	0.0001**
	Range	(15.4 – 69)	(28.6 - 54.8)	(38.7 - 42.9)	
PLAT ($\times 10^9$/l)	Mean ± SEM	410.80 ± 47.76	389.92 ± 44.00	236.25 ± 27.85	0.023*
	Range	(83 – 1124)	(59 – 985)	(180 – 323)	

Note: *: significant using student T- test at 0.05 level of significance, **: > 0.01 level of highly significance.

Table (4): Metabolic products (Uric acid (UA), Urea(U), and Creatinine(Cr) determinations in the sera of two groups MPN patients & controls

Metabolic products	Patients	Control	p value
Uric acid	9.71 ± 4.25	13.02±4.09	0.0539
Urea	36.90 ± 12.36	36.82 ±14.84	0.6499
Creatinine	1.043 ±0.158	1.0043 ±0.23	0.8519

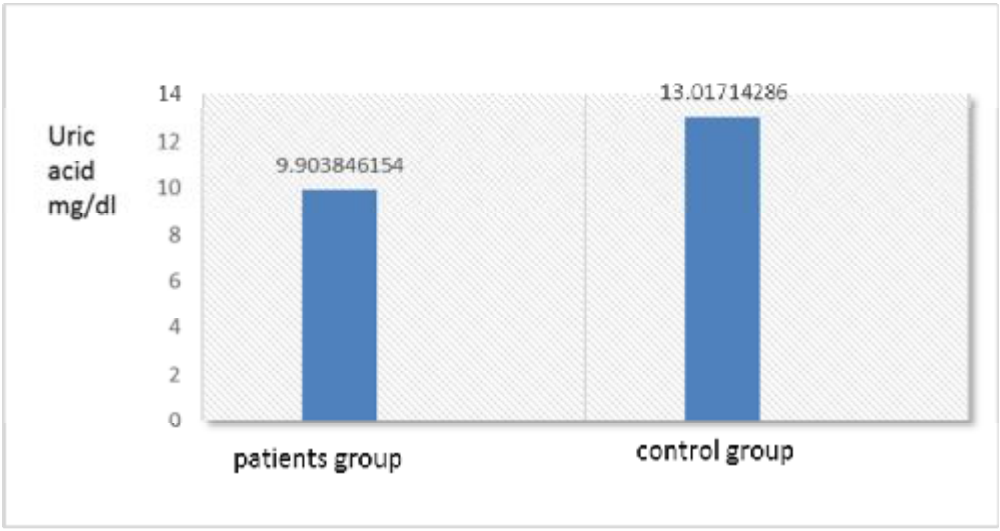


Figure (1): Uric acid levels in patients group with MPN and control

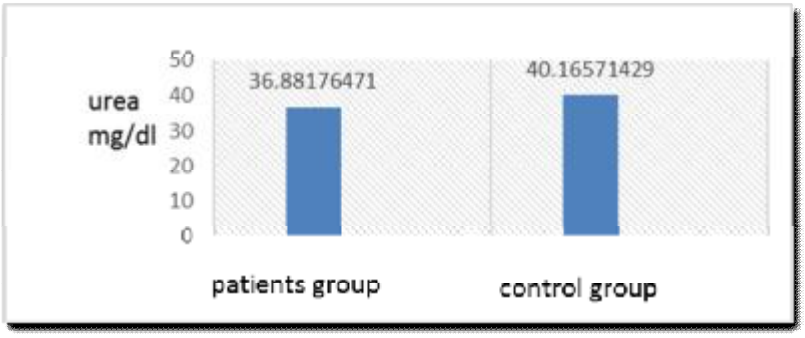


Figure (2): Urea levels in the patients and control groups

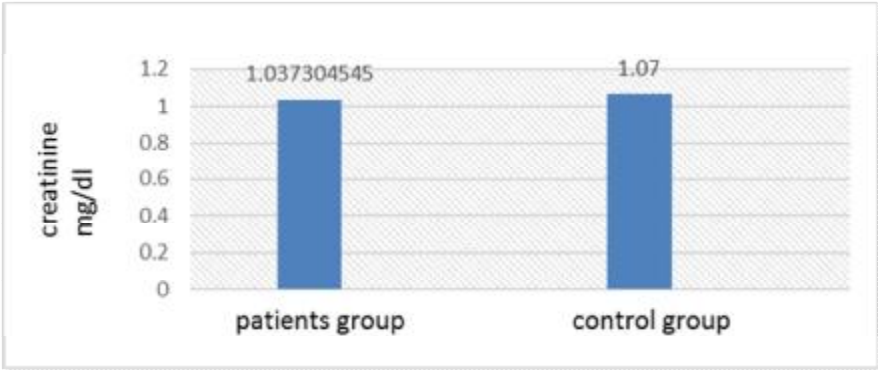


Figure (3) : Creatinine levels in both patients and control groups

To show the influence of treatment with hydroxyurea of the level of metabolic treatment, patients group was subdivided for two groups: group (1) pre-treatment patients group, and post-treatment patients group. Table (5) shows the levels of the metabolic products for those groups as compared to control group.

Table (5) : Metabolic products levels in pre, post treatment MPN patients, and control group

Metabolic products	Pre-treatment	Post - treatment	Control	p value
Uric acid mg/dL	10.39 ± 3.88	9.021 ±2.89 *P Value* 0.0322	13.02±4.09	0.7266
Urea mg/dL	36.88 ±8.51	36.913 ±16.27	36.82 ±14.84	0.1359
Creatinine mg/dL	0.898 ±0.400 P Value= 0.526	1.188 ±0.476	1.0043 ±0.23	0.7471

Results in (Table-5) showed an insignificant increment in the level of uric acid in pre-treatment group as compared to post--treatment group. On the other hand, a significant decrement was observed in uric acid level in post- treatment patients ($p<0.05$) as compared to control . This result was agreed with (15) who reported that uric acid level would stay under level in patients treated with hydroxyurea.

An insignificant increase was observed in the level of urea and creatinine levels respectively as compared to control group. This results were in agreement with (15) who reported that the increment in urea, and creatinine levels in the sera of patients treated with(HU) could lead to chronic renal failure in treated patients which can be diagnosed by the decrease in the evaluated GFR. Hydroxyurea, which inhibits ribonucleotide reductase would cause an increment in creatinine levels and jaundice(15).

Glutathione-S- transferase activity in the sera of patients and healthy group.

Glutathion-S_transferase(GST) activity were determined in the sera of both patients and control groups as shown in table (6)

Table (6): Activity of GST in the pre, post treatment group, and control group

	Patients			P value
Subgroups	Pre-treatment	Post-treatment	Control	
GST (U/L)	134.23 ±80.82 *P Value* =0.2557	112.50± 86.48 *p Value* = 0.0963	221.87±160.30	0.3376

As showed in (Table-6) an insignificant decrement in the activity of GST patients groups as compared to control group. This results were in agreement with (16) who adduced an insignificant differences between cancer patients and control . Although this decrement could be due to oxidative stress mechanism, or the mutation in one of the three gene the codes to GST as a result of elevation in ROS or RNS in cancer cell. On the other hand it disagreed with (17) how reported an overexpression of GST genes in different types of tumor to regulate MAPK/ERK pathway (a spectrum of protein in the cell that transfer illusion from the receptor on the cell surface to the nucleic acid (DNA) in the nucleus of the cell) (18). GST could also involve in chemotherapeutic resistance development (17).

Kinetic Studies of GST in the Sera of pre-treatment and control groups: Glutathione -S-Transferase catalyze reaction between GSH and CDNB to yield two product or more (fig 4)(19)

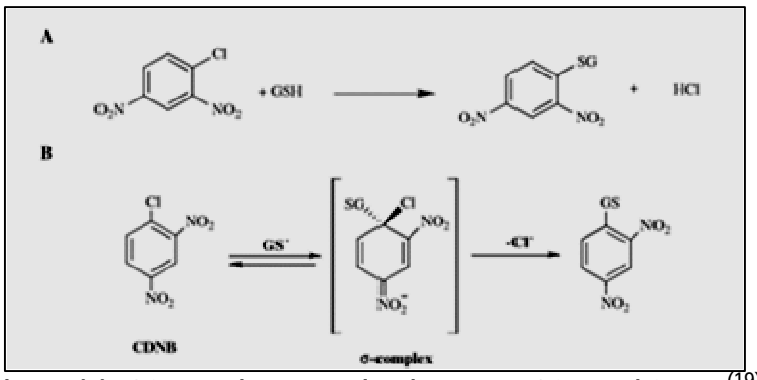


Figure (4): GST catalyze reaction between GSH and CDNB [19]

GST has the ability of nucleophilic displacement of an halogen (4 A). GSH can react directly with halogen as a electrophile (3). In this reaction a nucleophilic aromatic substitution occurs which involves a short – lived sigma -complex intermediate (Meisenheimer complex intermediat through an addition -elimination reaction (Figure 4 B).

Substrate effect of on GST activity :

1. GSH concentration effect: The relevance between GST activity and GSH concentration were shown in fig(5,6) for both pre-treatment and healthy control groups. Initial velocity of the enzyme was plotted against GSH concentration , the curve were nearly rectangular hyperbolic. The initial velocity increased gradually in a way of concentration dependency up to maximum concentration of the GSH subsequently reaching saturation area. The double reciprocal in correspondence with Line-weaver-Burk analysis were plotted for both groups (Figure 9,10).
2. CDNB concentration effect: The relevance between GST activity and CDNB concentration were shown in fig(7,8) for both pre-treatment and healthy control groups. Initial velocity of the enzyme was plotted against CDNB concentration , the curve were nearly rectangular hyperbolic. The initial velocity increased gradually in a way of concentration dependency up to maximum concentration of the CDNB

subsequently reaching saturation area. The double reciprocal in correspondence with Line-weaver-Burk analysis were plotted for both groups (Figure 11,12).

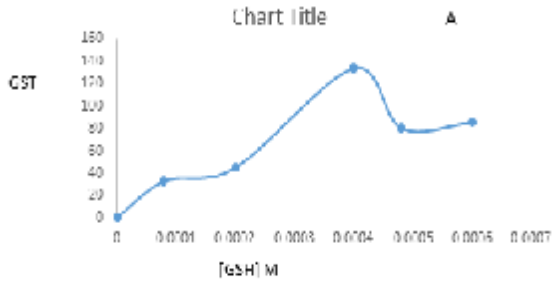


Fig (5) : Distinction of kinetic of the human GST with different concentration of GSH and fixed concentration of CDNB: A: In control group

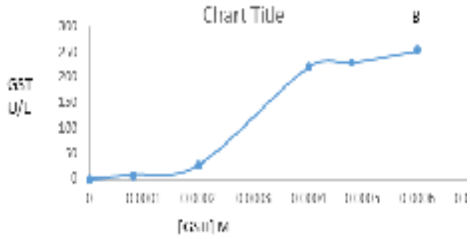


Fig (6) : Distinction of kinetic of the human GST with different concentration of GSH and fixed concentration of CDNB: **B**: pre-treatment group

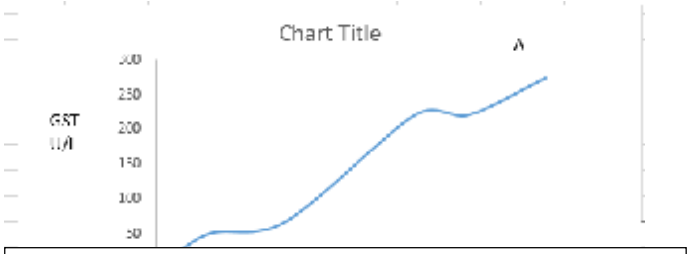


Fig (7) : Distinction of kinetic of the human GST with different concentration of CDNB and fixed concentration of GSH: A: In control group

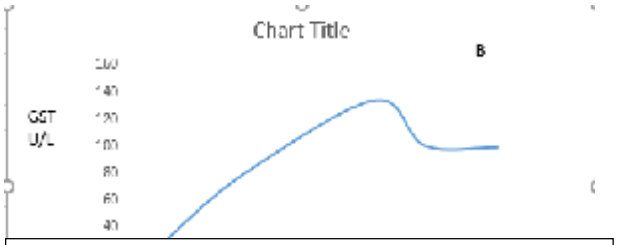


Fig (8) : Distinction of kinetic of the human GST with different concentration of CDNB and fixed concentration of GSH: B: In control group

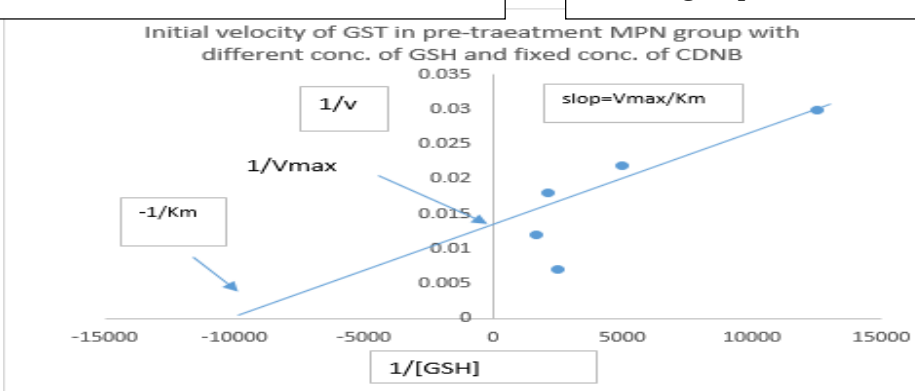


Figure (9): Reciprocal plot for GST in pre-treatment MPN patients with different concentration of GSH and fixed concentration of CDNB

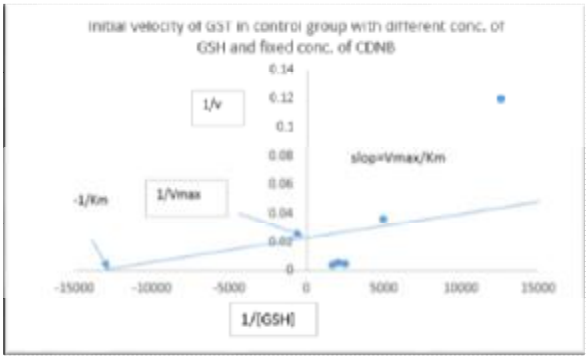
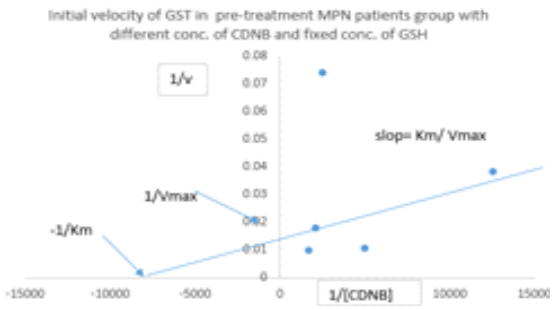


Fig (10) : Reciprocal plot for GST healthy control group with different concentration of GSH and fixed concentration of CDNB



Fig(11): Reciprocal plot for GST in pre-treatment MPN patients with different concentration of CDNB and fixed concentration of

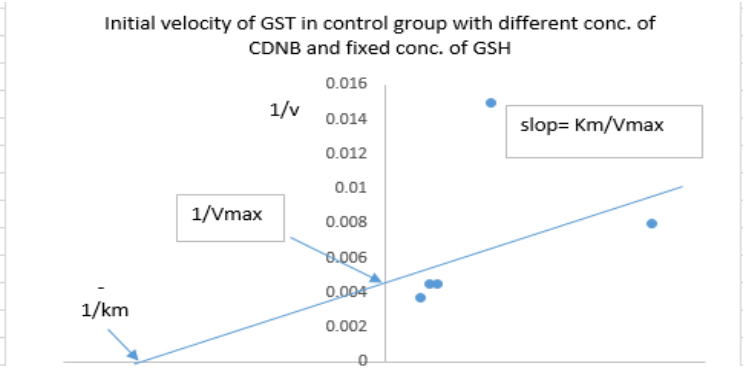


Figure (12): Reciprocal plot for GST in control group with different concentration of CDNB and fixed concentration of GSH

Table (7):Kinetic parameter of GST enzyme for both pre-treatment and healthy control : concentration effect of GSH, and CDNB

	Pre-treatment MPN group		Control group	
Different conc. Of GSH fixed conc.of CDNB	Km M	*V max* Mole.litter-1.min-1	Km M	V max Mole.litter-1.min-1
	0.0001	76.92	0.00008	55.55
Different conc. Of CDNB fixed conc. of GSH	Km M	Vmax Mole.litter-1.min-1	Km M	Vmax Mole.litter-1.min-1
	0.00018	125	0.0001	217.78

Kinetic values (Km and Vmax) of GST for GSH and CDNB were calculated from reciprocal plotted data , table (7). For pre-treatment group in both concentration of GSH, and CDNB , Km were increased which revealed an decrease in the affinity of GSH for both substrates. This could be due to the free radical that causes an uncompetitive inhibition for the enzyme through conformational changes induced by the free radical(20) . The free radical hypothesis was approved by Cheng(21) who reported that hydrogen peroxide lead to the formulation of disulphide bond (intra and intersubunites) between specific cysteine residues in the GST amino acid primary structure causing inhibition in GST activity. As for Vmax of GST, the Vmax for CDNB as a substrate is higher than that for GSH for both groups under study.

The researcher didn`t find any references that approve the results that were found in this study, other studies measured GST activity in some other vertebrate(22 and 23) .

The eminent conclusions from the present study are: treatment with hydroxyurea affect the level of uric acid significantly, in parallel a decrement in the activity of GST in post-treated MPN patients. An increment in the Km of GST in pre-treatment MPN patient cloud be due to the inhibitory effect of free radicals in enzyme environment.

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