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FORWARD

With well- established ambitious steps on continuing success way, IJST is coming for you all today in its new volume, the seventh volume for year 2012.

Year after year, IJST proves its strength and faithful belief in developing our scientific communities among Arab World, especially in Iraq by giving an opportunitiy to al researchers to present their fruitful achievements in main vital fields to let all world knows that we are still the first leaders in civilized scientific life, despite all the unfortunate situations or constraints.

It is my pleasure to welcome you and present you a new issue of our Journal, Volume 76, No. 1 (2012), the first issue of this year, with diversity of researches and elite experts of the Editorial Board and Advisory Group. The members of Editorial Board, the ICAST and TSTC teamwork and I hope you will find this collection of research articles useful and informative.

The journal is one of the scientific contributions offered by ***the International Centre for Advancement of Sciences and Technology*** *in cooperation with* ***Treasure Est. for Scientific Training and Consultations*** *to the science and technology community (Arab region with specific focus on Iraq and International).*

Finally, on behalf of the International centre, I would like to express my gratitude and appreciation to the efforts of the Editorial Board, Advisory group with their valuable efforts in evaluating papers and the Editorial Board Secretary for managing the scientific, design, technical and administrative aspects of the Journal and for preparing this issue for final printing and publishing.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

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ENGLISH SECTION

Evaluation of Lipid Profile and Proliferation of Bone Marrow Cells in Obese Mice Feed with Flaxseed Diets

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ABSTRACT

This research revealed the effect of flaxseed diet in body weight, organ weight and lipids profile in the serum and proliferation of bone marrow cells in mice. Twenty four mice included were exposed to three types of diets includes: 16 animals feed with high fat diet (HFD), 4 animals feed with Flaxseed diet (FSD) and 4 animals feed with basal diet (BD) for one month.

The results showed a significant increasing in body weight of animals feed with HFD and FSD in comparison to control group (BD).Furthermore increasing in liver and kidney weight in animal fed with HFD. Total cholesterol (TC) and other types of lipids in serum such as HDL-C, TG and VLDL-C were increased significantly in animal feed with HFD. In comparison with FSD and BD. Mitotic index (MI) and Blastogenic index (BI) revealed insignificant increasing depend on the type of the diets ,the highest MI and BI was determined in bone marrow of animals feed with HFD.

The obese animals (12 animals) submitted to stage two of diet for additional 30 days and divided into 3 groups includes: FSD, defatted flax seed diet (dFSD) and (BD).

In this stage two of the diets, FSD led to a significant decrease in body weight of animals, while there were insignificant difference in body weight of animals feed with dFSD and BD in comparison to obese animals .furthermore significant differences in liver and spleen weight depend on the type of diets. The highest weight of liver was recorded in animal feed with dFSD. In addition to significant differences in HDL-C, LDL-C and TG levels in serum of mice depend on the type of diet in stage two. So, FSD showed lowest LDL-C and highest HDL-C and TG after 30 day of diets. FSD revealed significant differences in MI, while it was insignificant differences in BI in comparison to other types of diet. The data demonstrate that FSD cause a decrease in body weight and improve lipid levels in the blood.

Key words: **Flaxseed, Diets, Lipid profile, Bone marrow proliferation**

الملخص باللغة العربية

تظهر الدراسة الحالية تاثير التغذية ببذور الكتان في الوزن الكلي و ووزن الأعضاء و مستوى الدهون في الدم وتضاعف خلايا نخاع عظم الفئران. تضمنت الدراسة 24 فأراً تم تغذية 16 منها بغليقة غنية بالدهون HFD و 4 فئران أعيت علقة بذور الكتان الكاملة FSD و 4 فئران اعيت علقة أساس BD و لمدة شهر واحد.

اظهرت النتائج زيادة معنوية في الوزن الكلي للفئران المغذاة بـ HFD و FSD قياسا بالعليقة BD ، فضلاً عن زيادة أوزان الكبد و الكلى في الحيوانات المغذاة بـ HFD ،كما سجلت زيادة معنوية في مستوى الكولسترول الكلي TC وبقية أنواع الدهون مثل HDL-C و TG في دم الحيوانات المغذاة HFD قياساً بمستوى الدهون في الفئران المغذاة بـ FSD و BD . في حين لم تسجل فروق معنوية في النسب المؤية لمعامل الانقسام الخلوي MI ومعامل الانقسام الأروميBI في خلايا نخاع العظم بالاعتماد على نوع التغذية ، وقد وزعت الفئران المسمنة و المغذاة بـ HFD (12 حيوان) لمدة شهر الى ثلاث مجاميع ثانوية ،الاولى أعطيت عليقة بذور الكتان الكاملة FSD و الثانية أعطيت عليقة بذور الكتان منزوعة الدهون dFSD و الثالثة أعطيت عليقة أساس BD ، وبواق 4 حيوانات لكل مجموعة . أظهرت حيوانات مجموعة FSD أنخفاض معنوي في وزن الجسم في حين لايوجد فرق معنوي في وزن الجسم بين حيوانات مجموعة dFSD و BD بالمقارنة مع وزن الجسم لدى الحيوانات المسمنة HFD ، فضلاً عن وجود فروقات معنوية في وزن الكبد و الحال في المجاميع الثلاث. أعلى وزن للكبد سجل كان لدى حيوانات مجموعة dFSD .

لوحظ وجود فروق معنوية في مستوى كل من HDL-C و LDL-C و TG في مصل الفئران في المجاميع الثلاث في المرحلة الثانية من التجربة ، وقد أظهرت حيوانات مجموعة FSD أقل مستوى للـ LDL-C وأعلى مستوى للـ HDL-C و TG بعد شهر من تناول العليقة . أظهرت حيوانات مجموعة فروقات معنوية في معامل الانقسام الخلوي MI في حين كانت الفروقات غير معنوية لمعامل الانقسام الأرومي بين هذه المجموعة و بالمقارنة مع المجاميع الاخرى.

INTRODUCTION

Flaxseed or linseed (Linum usitatissimum L.) is a common name for Linaceae family, Linum within that family. The major nutritional components of flaxseed include oil contain about 41% have been used in human and animal diets (50-55% as alpha linolenic acid, and 15-18% as alpha-linoleic acid) , viscous lignanrich fibers (mucilage) 28%, protein 21% and minerals 4% ,and carbohydrates distributed among phenolic acids, sugars, lignan and hemicelluloses. Flaxseed is the richest source of a –linolenic acid (18:3n-3), soluble and insoluble fiber, and mammalian lignan precursor secoisolariciresinol diglucoside (SDG) (1,2). The beneficial effects are mostly due to flax lipids. Flax oil is the richest plant source of linoleic (omega-6) and linolenic (omega-3) polyunsaturated fatty acids (PUFA), which are essential for humans since they cannot be synthesized in the organism and must be ingested in food.

Flax (*Linum usitatissimum* L.) is a multi-purpose crop. Flaxseeds oil, have long been used in human and animal diets and in industry as a source of oil and as the basic component or additive of various paints or polymers. Flaxseed has emerged as a healthy food owing to its beneficial effects and property of acting in the prevention of some diseases (3). Last years there has been a growing interest in the probiotic properties of flax and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (4). Today, flaxseed is used as a good source of soluble and insoluble fiber to reduce blood cholesterol and promote laxation (5). Interesting in the use of whole flaxseed and its derivatives (ground , .oil, defatted , and lignan extracts) as functional food or nutraceutical ingredients and adjuncts to a healthful diet continues to grow, as a result of the increasing body of evidence over the past 20 years, investigating the protective effects of flaxseed against a variety of chronic diseases and risk factors including breast and colon carcinogenesis, atherosclerosis, insulin dependent diabetes mellitus (IDDM) and hyperlipoproteinemias (6,7).

When and where a somatic cell divides is crucial to health, and regulation of mitosis is a daunting task; the cell cycle includes interphace and mitosis; during G_0 the cell "decides" to divide ,die or stay differentiated .Interphase includes G_1 and S phase that prepare the cell for mitosis, proteins ,carbohydrates and lipids are synthesized during G1 and more protein synthesized in G_2 .During prophase ,anaphase and telophase ,replicated chromosomes condense ,align split and distribute into daughter cells (8).

Keeping in view the medicinal and nutritional benefits of the flaxseed, the present study was designated to study the effect of flaxseed diets contain flaxseed as a basic ingredient on the body and organ weight and lipid profile in the serum and mitotic index of bone marrow cells of mice previously feeds with high fat diet.

MATERIALS AND METHODS

Defatted flaxseed preparation:

Flaxseeds were purchased from local markets then the seeds grinded to obtain powder. Flaxseed were mixed twice with n- hexane (1:3, w/v), continuously by magnetic stirrer at room temperature , first for 5 h and then the residue mixed with a new n-hexane for overnight ,finally the mixture filtered through whatman filter paper NO 1,the residue represent the defatted flaxseed flour (DFF) (9).

Experiment design:

Twenty four mice (8-10 wk old) were obtained from the animal lab of fertility center in Medicine College /Al-Nahrain University and appropriated housed. Mice were kept in the animal house at room temperature (25 C), exposed to a 12h light and dark cycle. The animals divided mainly into 3 groups and each group kept in a cage feed with the following diets for 30 days:

-First group include 16 mice feed with a high fat diet (HFD).

-Second group include 4 mice feed with whole flaxseed (FSD).

-Third group include 4 mice feed with a basal diet (BD) (as negative control)

After 30 days the animals in groups (FSD and BD) and only 4 animals from HFD group were sacrificed. The other 12 mice of HFD group are exposed to additional periods of diets (for 30 days) and re-divided into 3 subgroups feed with the following diets:

-First subgroup include 4 mice feed with a flax seed diet (HFD-FSD).

-Second subgroup include 4 mice feed with a defatted flax seed diet (HFD-dFSD).

-Third subgroup include 4 mice feed with a basal diet (HFD-BD)

All animals were allowed to have free access to water and food during the study

Animal Diets:

Four types of experimental diets (about 1 kg) were prepared from the following ingredients feed for the animal groups which include:

1-Modified High fat Diet (HFD): (10)
Casien (305 gm) , corn starch (150 gm), wheat flour (245 gm), egg Yolk (97 gm), lard (120 gm), cholesterol (3 gm), vitamin mixture (50 gm), mineral mixture (30 gm).

2-Modified whole flax seed Diet (FSD): (11)

Wheat flour (414 gm), flaxseed (346 gm),casien (150 gm),cellulose (10 gm),vitamin mixture (50 gm),mineral mixture (30 gm).

3-Modified defatted flax seed Diet (dFSD): (11)

Wheat flour (414 gm), defatted flaxseed (346 gm), casien (150 gm), cellulose (10 gm),vitamin mixture (50 gm),mineral mixture (30 gm).

4-Basal Diet (BD) (11)

Wheat flour (414 gm), corn starch (346 gm), casien (150 gm), cellulose (10 gm), vitamin mixture (50 gm), mineral mixture (30 gm). The ingredients were purchased from commercial sources and were weekly prepared in our laboratory and stored at +4 ^{o}C.

Biometry, sample and procedures:

The animals were weighted before and after the end of the program of diet. . Blood samples were obtained by puncture of the heart. Plasma samples were obtained after blood was centrifuged at 120 g for 15 min and stored at -80 ^{o}C until the lipids were analyzed .The liver, spleen and kidney were immediately excised and weighted. All the lipids were extracted by the colorimetric enzymztic method. Total cholesterol (TC) was determined with (Randox kit reagents-UK) Triglycerides (TG) were measured using (Diamond kit reagents-jordan). The high density lipoprotein (HDL-C) was determined with (Randox kit reagents-UK). Fiedewald's formula (12) was used to determine the LDL cholesterol: LDL-C = TC - (HDL-C – VLDL -C), where VLDL-C = TG/5.

Chromosomal preparation from somatic cells of mouse bone marrow:

Bone marrow cells were obtained from femur bone of mice scarified by cervical dislocation, according to Shubber *et al*., 1985,(13) the cells were fixed by fixative solution methanol: glacial acetic acid (3:1), finally cell suspension were dropped on slides from a height 30-50 cm (5-8) drops to give the chance for chromosomes to spread well, after the slides were dried, it stained with Giemsa stain and washed with distilled water.
The cells were examined under light microscope (40X), and 1000 of divided and non divided cells were counted and the percentage of cells at metaphase or blast stage was calculated for only the divided once according to the following equation:

$$Mitotic\ index\ (MI) = \frac{Number\ of\ divided\ cells}{Total\ number\ of\ the\ cells\ (1000)}$$

Statistical Analysis:

The results were analyzed using the (completely randomized design – CRD) to identify the effect of diet treatments on different criteria, the significant differences has been compared between the treatments by (least significant differences test –LSD). SAS program used for statistical analysis.(14).

RESULTS AND DISCUSSION

Effect of diets in body and organ weight of mice:

The results showed a significant increase in body weight of mice in the groups feed with HFD and group feed with FSD for a month (7.42 g. and 6.17 g respectively) in comparison with the control group (Feed with BD) (2.73 gm).
Eating fat rich foods cause an crease in body weight with the age , many research confirmed this through exposing animals to high fat diets like coconut oil and cholesterol ,lard ,egg yolk and canola oil (15,16).

Table (1): Effect of a variety of diets on body weight in stage one of experiment.

Treatments	Body wt. (gm) in Stage one	
	Before the diet (0 diet)	After the diet (30 diet)
HFD	22.04 ± 1.82	29.46 ± 1.60 b
FSD	20.83 ± 1.37	27.00 ± 1.62 b
BD	22.10 ± 1.72	24.83 ± 1.37 a
LSD value	8.92 NS	5.65 *

* $p<0.05$, NS: non significant

Our results were in agreement with the results of (17) research which include feeding 3 groups of rats with different protein rich diets for 6 months. The group feed with diet contain 20% flaxseed rich with secoisolariciresinol diglucoside -SDG shows higher body weight than the other groups (which is feed with diet contain 20% casien protein) and diet contain 20% soya protein rich with isoflavonoides).

The obese animals fed with HFD, later divided into 3 groups, each group were feed with different diet for another month; then they were weighted at the end of periods of diets as show in table 2.

The results in stage two of diet showed insignificant increasing in body weight of mice in the groups feed with BD and dFSD, while the animals in the group FSD show a significant decrease in body weight in comparison with the original body weight of obese animals in group HFD.

Table (2): Effect of additional diets in body weight of obese animals in stage two

Body wt. (gm) of animals feed with HFD for 1 month (Stage one)		
30.65 ± 1.76 a		
Body wt. (gm) of animals feed with additional diets for 1 month (Stage two)		
FSD	dFSD	BD
25.83 ± 0.69 b	31.32 ± 1.41 a	34.78 ± 2.10 a
LSD value : 5.42 *		

* p<0.05, NS: non significant

The reason for decreasing effect of flaxseed on body weight due to the hypoglycemic and hypocholestremic effect of flax seed gum. The possible reason for the hypoglycemic effect can be attributed to the reduction in the Glucose and cholesterol absorption from the gut by flax lignan complex (18) and reduction of the gastric emptying time (19).All these factors may lead to less body weight.

The flaxseed has many antinutritional factors that may cause decreasing body weight, as revealed by many researches; mucilage for example is one of these factors. The mucilage composes about 8% of the seed contains polysaccharides such as rhomonose ,fucose ,arabinozylans, zylose ,galactouronic acid and glucose. Linatine in mucilage found to cause decreasing in the productivity of animal by decreasing the amount of endogenous enzymes released from the pancreas, resulting in reduced digestion of food particles. (20).

Mucilage is an important water-soluble polysaccharide of flaxseed and has been reported to increase the viscosity of intestinal contents in broiler chickens (21) with the viscous environment causing a significant inhibition of digestion and absorption of dietary nutrients, with fat digestibility suffering the most among the macronutrients (22). Possible mechanisms involved include a reduced diffusion rate between digestive secretions (i.e., lipases and bile salts) and their substrates (23). In stage one of experiment, table 3 shows organ weights. There were no significant differences in spleen weight of animal feed with the three types of diets. While there were a significant differences in liver and kidney weight of mice feed with the three types of diets. The liver of HFD animals show higher weight than other groups, that may related to the deposition of total lipids and cholesterol in the liver, and may be indicate that the type of lipids in the diet exert an influence on liver lipid composition because the content of saturated fatty acids in the diets correspond to the layer depositions of cholesterol and triglyceride in the liver (24).

Table (3): Effect of a variety of diets on organ weights of mice in stage one and two

Treatments	**Organ wt. (gm) in Stage one**		
	Liver Wt.	**Spleen Wt.**	**Kidney Wt.**
HFD	1.79 ± 0.18 a	0.19 ± 0.06	0.28 ± 0.05 **a**
FSD	1.21 ± 0.07 b	0.12 ± 0.02	0.17 ± 0.01 **b**
BD	1.14 ± 0.11 b	0.16 ± 0.06	0.15 ± 0.02 **b**
LSD value	0.106 *	0.173 **NS**	0.112 *
Organ wt. (gm) in Stage two			
FSD	1.10 ± 0.22 **c**	0.42 ± 0.10 a	0.35 ± 0.05
dFSD	1.88 ± 0.12 **a**	0.40 ± 0.15 a	0.35 ± 0.05
BD	1.47 ± 0.15 **b**	0.25 ± 0.04 b	0.28 ± 0.06
LSD value	0.402 *	0.178 *	0.190 **NS**

* p<0.05, NS: non significant

On the other hand, the kidney weight increased significantly in the animals feed with the HFD and it was higher than the kidney weight in the animals feed with FSD and BD in stage one of experiment. Our results were in contrast with the results revealed by (25) ,in which weights of major organs, such as the liver, heart, spleen, kidney, ovary, and uterus, adjusted to body weight, were not significantly different between animals feed with flaxseed diet and animals feed with basal diet . In stage two of experiment, there were a significant increasing in spleen weight of mice in the three groups in comparison to the weight of obesed animals in stage one. , but the liver weight showed higher increasing in the animals feed with dFSD. In contrast, there was a significant decrease in liver weight of mice feed with the three types of diets in stage two of experiment in comparison with obesed animals in stage one.

The results show that the liver weight in stage two of experiment in group feed FSD showed decreasing weight of liver in comparison in group feed with HFD in stage one of experiment. (17) revealed the same results, flaxseed meal significantly lowered fat deposition in livers of both lean and obese rats compared to rat feed casein or soy protein.

Effect of diets on lipid profile in mice serum:

The result showed in table 4 a significant increasing in TC and HDL-C in serum of mice feed with the three types of diets, the highest level of TC was in HFD and FSD groups (168.3, 149.16) mg/dl respectively, furthermore a significant increasing in HDL-C in comparison with negative control animals feed with BD. While there was no significant differences in LDL-C and a significant increase in TG between the three treatments in stage one. In additional to significant differences in TG depend on types of diets. In stage two of the experiment, there was no significant differences in TC in serum of mice of the three types of diets .While there were a significant differences in HDL-C, LDL-C and TG levels between the three groups. HDL-C (Good cholesterol) level was (50.6 mg/dl) in serum of animal feed with HFD and showed a higher level of HDL-C in the animals feed with whole flaxseed (FSD) (44.36 mg/dl and dFSD (37.06 mg/dl0 in stage two, and the LDL-C (Bad cholesterol) level was higher in the animals feed with defatted flaxseed (dFSD) (103.06 mg/dl) and BD (100.53 mg/dl) in stage two while it was lower (90.6 mg/dl) in serum of animal feed with FSD. Furthermore the significant decreasing in TG in serum of animal feed with the three types of diets in stage two in comparison to TG levels in the animal feed with FSD (143.1 mg/dl) ,which revealed the highest level of TG in serum of obesed animals in HFD group . The dFSD cause more decreasing in TG level (137.13 mg/dl), while the BD couldn't reduce the TG level in stage two (157.1 mg/dl). Whole flaxseed contains approximately 41% fat and 21% protein of the seed weight. Flaxseed is particularly rich in alpha linolenic acid (approximately 57% of the total fatty acids in flaxseed), which has lipid lowering properties (26). Thus, the reduction of blood cholesterol by dietary flaxseed in these studies may be due in part to linoleic acid and alpha-linolenic acid present in whole seed. Therefore, it is highly unlikely that these fatty acids were responsible for the observed reduction of plasma cholesterol and triglyceride that was associated with flaxseed meal in the present study. The cholesterol-lowering effect reported in studies examining the effect of whole or defatted flaxseed on plasma lipids may be an effect of the protein or fiber fraction and polysaccharides alone or that fraction in combination with SDG be responsible for reduce TC and LDL-C concentrations (27).

Table (4): Effect of study treatment of diets in lipid profiles in stage one and two

Treatments	Lipid concentration (mg/dl) in Stage one				
	TC	**HDL-C**	**LDL-C**	**TG**	**VLDL-C**
HFD	168.30 ±4.88 **a**	50.60 ±7.49 **a**	87.06± 5.75	153.06 ±8.46 **a**	30.63 ±1.68 **a**
FSD	149.16 ±9.96 **b**	36.66 ±4.81 **b**	87.93± 2.81	123.00 ±12.13 **b**	24.60 ±2.40 **b**
BD	131.13 ±1.50 **c**	27.76 ±1.44 **c**	83.20± 2.51	100.73 ±2.08 **b**	20.15 ±0.43 **b**
LSD value	12.377 *	13.02 *	13.76 NS	29.85 *	5.92 *
	Lipid concentration (mg/dl) in Stage one				
FSD	163.60 ±0.91	44.36 ±2.09 **a**	90.60± 1.65 **b**	143.10 ±0.70 **b**	28.66 ±0.16 **b**
dFSD	167.53 ±0.75	37.06 ±0.67 **b**	103.06 ±1.26 **a**	137.13 ±0.53 **c**	27.40 ±0.10 **a**
BD	164.43 ±1.92	32.50 ±1.60 **b**	100.53 ±3.86 **a**	157.10 ±2.72 **a**	31.43 ±0.55 **c**
LSD value	4.51 **NS**	5.42 *	8.77 *	5.71 *	1.17 *

* $p<0.05$, NS: non significant

Our results were in agreement with the results obtained from Prasad, 1997 (28). In studies of hypercholesterolemic rabbits, Prasad showed that dietary flaxseed reduced total and LDL cholesterol and prevented hypercholesterolemic atherosclerosis. Cunnane *et al*. 1994 (29) showed that consumption of 50 grams of flaxseed/day for four weeks resulted in a small but significant reduction in LDL cholesterol in young healthy humans. Furthermore, the intake of 38–50 g flaxseed reduces serum total cholesterol. (TC), LDL cholesterol (LDL-C), apolipoprotein (apo) B, apo A-I, and increases triacylglycerol (TAG) concentrations in hyperlipidemic subjects (30). These findings suggest that the cholesterol-lowering effect may be limited to the component of protein, fiber, or plant lignans or a combination of the three. The health benefits of isolated plant lignans from flaxseed were examined recently in animal models. Those studies showed that isolated SDG from flaxseed reduced TC and LDL-C concentrations to 33 and 35% respectively in rabbits feed a high-cholesterol diet. In addition, further results showed that a lignan complex isolated from flaxseed reduced TC and LDL-C concentrations by 20 and 14% in rabbits feed a high-cholesterol diet (31).

Effect of diets on proliferation of bone marrow cells:

The results showed insignificant increasing in mitotic activity of bone marrow cells from mice feeding with the three types of diet (HFD, FSD, BD), as well as in blastogenic index, in stage one, in spite of induction in mitotic index obtained from bone marrow cells of animal feed with HFD.

In stage two of feeding animal the obesed animal revealed a significant differences in mitotic index and insignificant differences in blastogenic index of bone marrow cells obtained from the three types of diets .The highest value of MI and BI were determined in bone marrow cells of animal feed with FSD (28.47 and 29.23) % respectively followed by dFSD (Table 5), while the lowest value of MI and BI were obtained in bone marrow cells of animals feed with BD.

Induction of the MI may be related to the component of nutrients in diet used in this study. HFD contain high level of fats in addition to lipid and protein and other nutrients present in flaxseed, these components induced the proliferation of bone marrow cells (32), in addition to relationship between proteins and cyclogenase, lipogenase enzyme and its effects on inducing cell division and it does have the activity of many mitogenic agent, while the BD have little effect on proteins and enzyme that have correlation with cell division and it does have the activity of many mitogenic agent. While the induction of BI in bone marrow cells feed with HFD in compare with BD may due to the effect of fats in delayed cell division caused by the correlation of protein requirement for mitotic spindles composition during cell division (non histonic proteins) .furthermore the cell cycle is controlled by many checkpoints, telomerase, hormones and growth factor from outside the cell, and cyclins and kinases from them.

Table (5): Effect of a variety of diets in the percentage of Mitotic and blastogenic index in bone marrow cell of mice

Treatments	Stage one	
	Mitotic index (%)	**Blastogenic index (%)**
HFD	26.84 ± 0.38	28.18 ± 0.29
FSD	24.06 ± 1.02	24.79 ± 0.81
BD	22.71 ± 1.62	23.22 ± 1.22
LSD value	4.21 **NS**	6.24 **NS**
	Stage two	
FSD	28.47 ± 0.69 **a**	29.23 ± 1.62
dFSD	24.98 ± 2.76 **b**	25.73 ± 2.21
BD	22.18 ± 2.10 **b**	24.60 ± 0.32
LSD value	2.52 *	5.62 **NS**

* $p<0.05$, NS: non significant

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The effect of commercial pesticide Carbaryl on fresh water Cladoceran *Simocephalus vetulus*

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ABSTRACT

The ecological risk assessment of insecticide carbaryl occurred in the present study when cladoceran *Simocephalus vetulus* was exposed to it. The acute toxicity test of this insecticide was assessed by LC_{50}, mortality percentage and safe concentration. LC_{50} of carbaryl on *S.vetulus* were 131.8 µg/L and 95.4 µg/L during 24 hr and 48 hr respectively. Increasing the concentrations of insecticide lead to an increasing in mortality percentage. *S.vetulus* appeared to be less affected and this is observed from the values of safe concentrations. Furthermore the present study emphasizes the chronic toxicity tests for insecticide through studying different vital parameters such as: life span, survivor mean, fecundity of eggs and young, broods number and broods size, body length and molting.

الملخص باللغة العربية

تضمنت الدراسة تحديد المخاطر البيئية الناتجة من استخدام نوع من المبيدات الحشرية هو مبيد الكاربارايل (Carbaryl) و ذلك من خلال دراسة تاثيره على حياتية نوع من القشريات المائية التابعة لرتبة متفرعة اللوامس (Cladocera) و هو *Simocephalus vetulus* ضمن فترات من التعريض الحاد و المزمن لمبيد الكاربارايل على النوع *S.vetulus* و تضمن التعريض الحاد تحديد قيم كل من التراكيز المميتة لنصف العدد (LC_{50}) و تحديد النسب المئوية لهلاك الأفراد كما تم ايجاد قيم التراكيز الامينة (safe concentrations) ، و قد وجد ان قيم التراكيز المميتة لنصف العدد (LC_{50}) للنوع *S.vetulus* المعرض لمبيد الكاربارايل 131.8 ، 95.4 مايكروغرام / لتر خلال 24 ، 48 ساعة، لقد تبين من الدراسة ان الزيادة في تراكيز المبيد تؤدي الى الزيادة في النسب المئوية للهلاكات . فضلا عن ذلك درست جداول الحياة للنوع *S.vetulus* المعرضة تعريضا مزمنا للمبيد السابق ذكره و اثر هذا التعريض على بناء جدول الحياة و توقع الحياة المستقبلية. كما تمت دراسة عدد من المؤشرات التكاثرية و الحياتية خلال فترة التعريض المزمن اذ وجد ان لهذا المبيد تاثيرا سلبيا على انتاجية البيض و الافراد و قلل العدد بشكل واضح علاوة على تاثيره في حجم و عدد الحضنات. كما لوحظ انخفاض في متوسط الطول الكلي و عدد الانسلاخات و معدل البقاء.

INTRODUCTION

Water pollution caused by agricultural pesticides is a well recognized problem, as pesticides are widely produced and released into the natural environment (1).
Their effects on aquatic ecosystems may arise from chronic exposure (long-term and low concentration) as well as short-term exposure to high concentrations that can result from accident, improper use, or runoff from treated fields (2).
Organphosphorus pesticides (OPs) including carbaryl are among the most potent insecticides known and have been used throughout the world to control pests agricultural crops, forests and wetlands for more than four decades (3). Their wide use is due to high toxicity and rapid environmental degradation (4). Unfortunately, OPs lack target specificity and can cause severe and persistent population effects on aquatic non-target species, particularly invertebrates (5).
Water fleas are among the most preferred animals for toxicity test, especially Cladocera because of large body sizes and high rate of reproduction (6). They are sensitive to poor water conditions and are more commonly used for monitoring water quality (7).
Simocephalus vetulus is common Cladocerans inhabiting macrophyte-rich littoral zones of fresh water and this microcrustacean show a benthic behavior and can be an important component of zooplankton system (8). *S.vetulus* was used in this study because of its' abundance in Iraqi water, as reported by (9).

Therefore, this study aimed to:
1. Determine the acute and chronic toxicity of carbaryl in its' commercial available form under constant exposure conditions.

2. This study will aid future risk assessment of this insecticide and gives additional data to the other insecticide studies.

MATERIALS AND METHODS

Water properties of the sampling area

Some properties of water collecting from Tigris River at AL-Jadyria campus were measured including: the water temperature, pH, water conductivity, salinity (10).Also dissolved oxygen was measured by using Winkler method [9]. These parameters were measured according to chemical water quality analysis (10).

Testing water quality

Filtered river water (fresh water) was used in culturing of isolated animals and preparation of toxic solutions (11). This water was collected from Tigris river which it represented the natural habitat of these animals. Water filtered by Dual water purification system with three filter housing which filters the water in the first stage and purifies it in the second. After filtration techniques, some of water properties were checked. These parameters included:
Temperature, pH, water conductivity,salinity and dissolved oxygen were measured according to (10).

Collection and Isolation of Crustacea

Simocephalus vetulus was collected from the bank of Tigris River in AL-Jadyria campus by using zooplanktons net with meshed pores of (45-50) µm. The organisms were trapped in net container washed with water into a glass container and transported directly to the laboratory for isolation and identification. The collection or catchment area should be had no rural activity (2).

Identification and Classification of *S. vetulus*

The collected animales were identified by using the key (12).

Culturing of *S. vetulus*

The cultures were maintained in filtered river water by using the method described previously (13). The animals were reared under laboratory conditions 20±2 ºC, photoperiodicity of 16 hrs. light and 8 hr. dark which was sufficient to the growth and reproduction of the cladocera (14).

Nutrition of cladocera

The juice of two (vegetables) of Spinach and Celery were used as food for the cladocera (15).

Preparation of S. *vetulus* for the toxicity test

The gravid females of S. *vetulus* were taken out and cultured. Healthy neonates (about 24 hrs.) were always taken from the second and the following broods as recommended (16,17)

Preparation of carbaryl solutions for toxicity test

Carbaryl (trade name Sevin) 85% wp (waterable)(manufactured by Behavar chemical Co. ,packed in aluminum foil sac , Iranian origin). Purchased from Al-Meqdadia .Stock solution of carbaryl was prepared by dissolving 0.005g (equal to 5000µg) in 25ml of filtered river water to obtain 200µg/ml concentration of stock solution. The filtered river water was used in preparation of all the solutions (18). Carbaryl toxicity concentrations were (75,105,135and165) µg/L by adding (375 , 525 , 675 and 825) µL respectively completed to 1L of filtered river water .

Acute toxicity test

Acute toxicity experiments occupied the exposing of 4 groups each one consist of 10 animals with control group of 10 animals too (19). Acute exposure of carbaryl was conducted according to methods outlined previously (20). The following concentrations of carbaryl for *S. vetulus* were: 75, 105, 135, 165 µg/L. Ten either *S. vetulus* were exposed to carbaryl with 0.0 (zero) control. The characteristic that should be available in animals were used in this study were mentioned previously (21,22)

1- Mortality Percentages: Mortality percentages were calculated for *S.vetulus* which were exposed to carbaryl through 96hr of exposing (23).
2- Median Lethal Concentration (LC_{50}): Probit analysis was used to estimate LC_{50} values at regular intervals for each test in acute exposure with cladocera (immobilization) (16).
3- Safe Concentration (SC): Safe concentration was calculated by using the method described in (24).

Chronic Toxicity Tests

Life Tables:
Life tables were obtained and study according to the method described previously (23,25)

Carbaryl effectiveness in reproductive parameters of S. *vetulus:*
The following parameters were evaluated according to (9):
Fecundity of young (offspring count), fecundity of eggs (total number of eggs), Mean number of broods, mean number of brood size, onset to reproduction.

Minimum and Maximum number of eggs/female, young/female and young/brood

The range of eggs number (min and max number) and the range of young/brood was calculated through interval(period) of surviving(23).

Mean of total length of animal and mean of longevity:

Light microscope was used for measuring the total length of experimental animals, under lens magnification (10 x). The final body length (L2) subtracted from initial body length (L1) for each animal then these results divided on the total number of animals (26).

Mean number of Molts

The mean number of molts was calculated according to (9).

Statistical Analysis

1-Probit analysis (SPSS v.13 for Windows®, SPSS Inc.) was used to estimate LC_{50} values.
2-To calculate significant differences, analysis of variance (one way- ANOVA, $P \leq 0.05$) was used to indicate the significant differences between treatments and control by using (Microsoft excel Windows ® 2007).
3-Furthermore, the uptake and adsorption of carbaryl by *S. vetulus* were observed and documented using microscope with a digital camera type (SONY DSC-W55) during the entire exposure period.

RESULTS AND DISCUSSION

Water properties of the sampling area

Some physical and chemical properties of water in the sampling area were determined. Table (1). The results occur in the range of previous study on Tigris River of AL-Jadyria campus which had been reported by (27).

Table (1): physical and chemical properties of water from the sampling area in AL-Jadyria campus from which *S. vetulus* was collected during the year 2009

Physical and Chemical properties	Range	Values Obtained
Temperature (C°)	18-20	19
Dissolved oxygen (D.O) (mg/L)	6.6-7.5	7.05
Hydrogen ion concentration(pH)	7.6 – 8.06	7.8
Electrical conductivity (µs/cm)	882 - 1098	990
Salinity (ppt)	0.54-0.68	0.61

Testing water quality

Table (2) shows the measurements of the same physical and chemical parameters that were reported for water in sampling area. The results of this table is very near or close to the results obtained in table (1) and this gives a confirmation of the suitability of using this type of water in rearing the animals.

Table (2) physical and chemical parameters of filtered river water

Physical and chemical parameters	Range	Value
Temperature ºC	18-22	20
Hydrogen ion conc. (pH)	7.9-7.97	7.93
Electrical conductivity µs/cm	980-1028	1004
Salinity (ppt)	0.6-0.63	0.61

The effect of a cute toxicity test of Carbaryl on *S.vetulus*

1-Median Lethal Concentration (LC_{50}) of *S.vetulus* exposed to carbaryl:
Median lethal concentrations (µg/L) of *S.vetulus* exposed to carbaryl were : 131.8, 95.4 in 24hr and 48hr respectively. Fig. (1, 2).
The values of carbaryl LC_{50} is higher than those reported previously (20), in that study the highest LC_{50} was 26.48 µg / L in 48 hr. Whereas in another study LC_{50} ranges were from 6 µg/L to 12.5 µg/L (28). In contrast another study found that 96hr. LC_{50} values ranged from 11.1 µg/L to 61 µg/L when stream invertebrates were exposed to carbaryl (29). Concentrations (as low as 50 µg/L) are lethal to 50% of cladoceran zooplanctons (LC_{50}) (30).

Mortality percentages of *S.vetulus* exposed to insecticide Carbaryl

Figure (3) elucidates that the mortality percentage for *S.vetulus* individuals increased with increasing carbaryl concentration during 96hr. of exposure. After 24 hr. of exposure the mortality rates were 10% and 80% in concentrations 75 µg/L and 165µg/L respectively .
The result showed that the mortality percentages increases with an increasing concentration of insecticide for the species of cladocera. This agrees with study (20) (on *D.magna*) in which they found that if the test period prolonged 72hr (3days), for example, the sensitivity of animals should increase greatly. Therefore in the present study, the acute exposure prolonged to 96hr (4days) and this proved insecticides' toxicity. In a previous study in acute exposure where no food was added they found that carbaryl have the ability to pass the animals membranes (10). The concentrations of carbaryl which were used in present study reached higher levels to be effective (165µg/L for *S.vetulus*). This may be related to the difference in the activity of carbaryl used in present study in comparison with carbaryl from other origins, or whether the diameter and morphology of particles of it may play a role, or if other factors such as surface properties are more important because carbaryl used in present study was in a powder form.
The movement behavior which was observed in *S.vetulus* included at first slowness ,then the animals became stiff and settle on the bottom of the beakers. The same thing was observed in a previous study (16).

Safe concentrations of insecticide Carbaryl to *S.vetulus*

It is observed that the values of carbaryl safe concentrations to *S.vetulus* which are 20µg/L and 15.2 µg/L
A study reported the safe concentrations of Al-Daura Refinery waste to two types of cladocera *D.magna* and *S. exspinosus* and observed that the safe concentrations for many studied locations were higher for *S. exspinosus* than for *D.magna* (24). *S.vetulus* has a comparatively smaller gut length compared to its' body size, the shorter gut passage time may save it from the toxic effects and this may give it this higher tolerant to different pollutants (34).

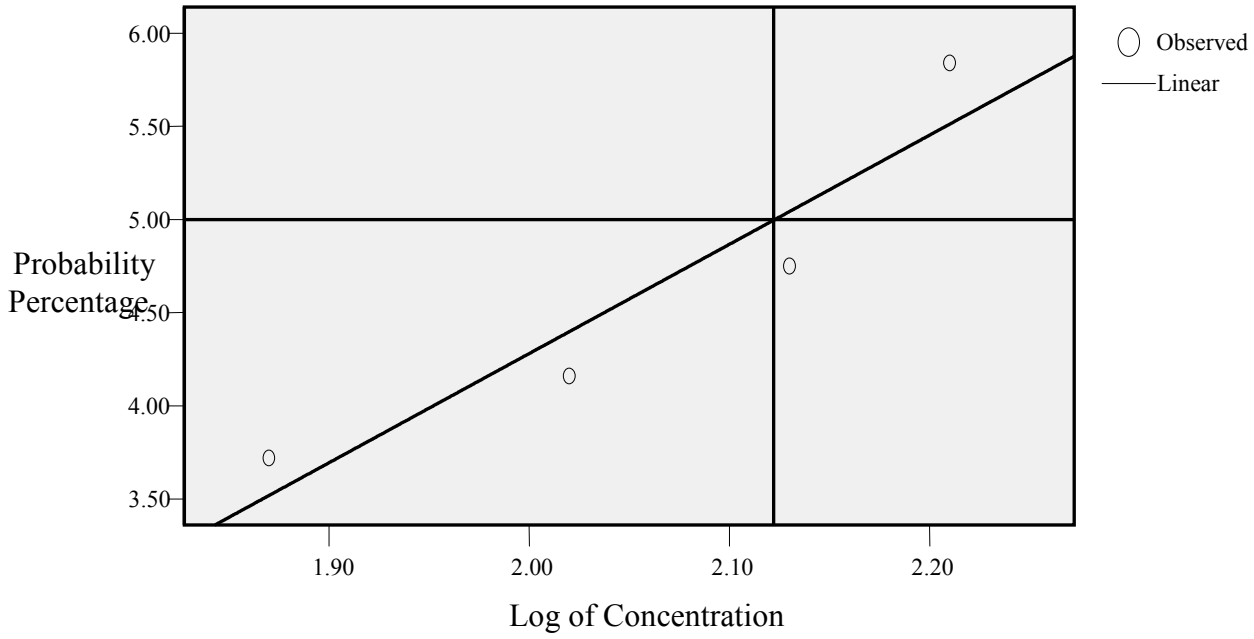

Figure (1): Toxicity curve of the insecticide carbaryl to *S.vetulus* after 24 hr. of exposure.

The effect of Carbaryl on life tables of *S.vetulus*

The life table of control appears as observed in table (3).

The life table (3) shows the control indivitual life cycle which had lasted for 63 days, the mortalities began on 10th day, and the group has the ability to reach the second half of life table. The higher expectation for future life was 38.1 days at the beginning of life table.

It was observed from table (4) that the individuals terminate their life cycle during 41 days as compared with control individuals which their life cycles lasted within 63 days. The mortalities began on the 5th day and the life table reported the mortality of 6 individuals in the middle of life table. The expectation for future life reports depletion of about 19.25 days as compared with control. The same effect and depletion were observed when another group with the same characteristics exposed to 26 µg/L of carbaryl. The mortalities began on the 5th day but 6 of the individuals didn't have the ability to reach the middle of life table. Six of individuals had died from the 5th up to the 16th day, and the expectation for future life reported depletion reached 21.6 days as compared with control group.

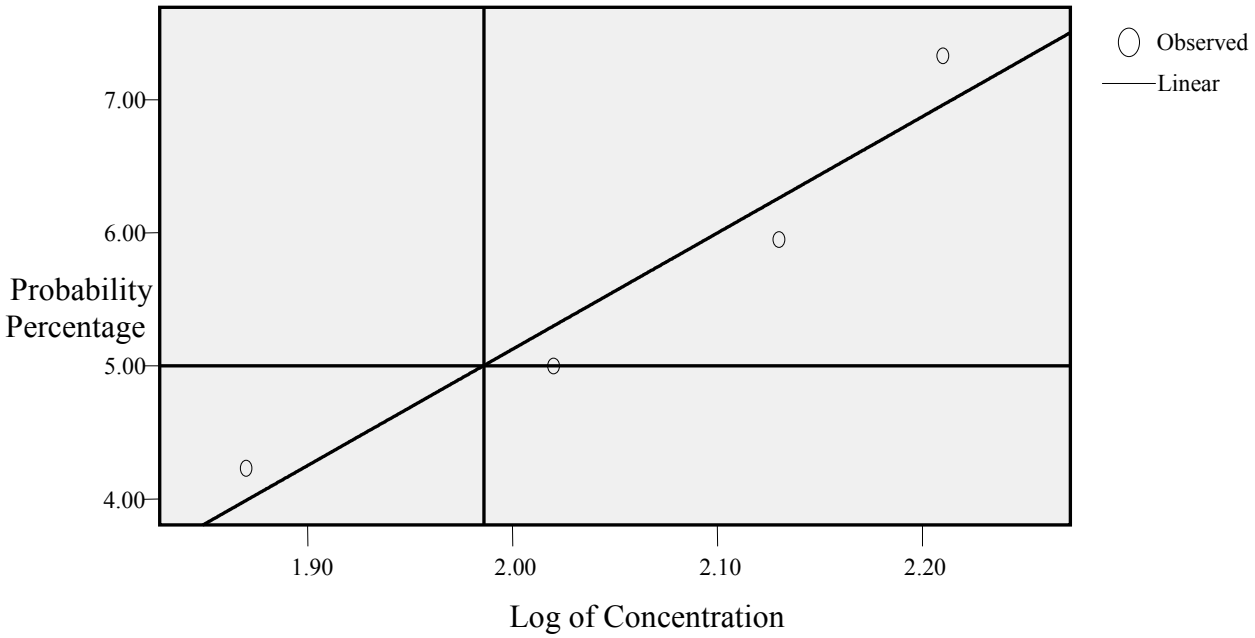

Figure (2): Toxicity curve of the insecticide carbaryl to *S.vetulus* after 48 hr. of exposure

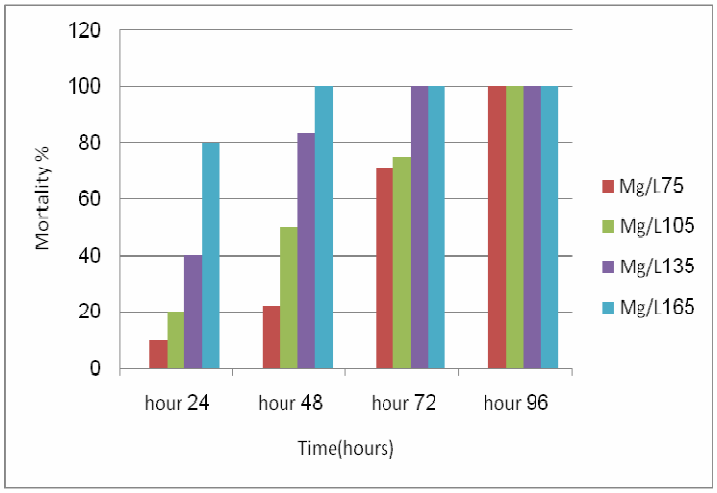

Figure (3): Mortality percentage of *S.vetulus* exposed to insecticide carbaryl

Table (3): The life table of control individuals of *S.vetulus*.

X	lx	dx	qx	Lx	Tx	ex
1	10	0	0	10	381	38.1
2	10	0	0	10	371	37.1
3	10	0	0	10	361	36.1
4	10	0	0	10	351	35.1
5	10	0	0	10	341	34.1
6	10	0	0	10	331	33.1
7	10	0	0	10	321	32.1
8	10	0	0	10	311	31.1
9	10	0	0	9.5	301	30.1
10	9	1	11.11	9	291.5	32.3
11	9	0	0	9	282.5	31.3
12	9	0	0	9	273.5	30.3
13	9	0	0	9	264.5	29.3
14	9	0	0	8.5	255.5	28.3
15	8	1	12.5	8	247	30.8
16	8	0	0	8	239	29.8
17	8	0	0	8	231	28.8
18	8	0	0	8	223	27.8
19	8	0	0	8	215	26.8
20	8	0	0	8	207	25.8
21	8	0	0	8	199	24.8
22	8	0	0	8	191	23.8
23	8	0	0	8	183	22.8
24	8	0	0	8	175	21.8
25	8	0	0	7.5	167	20.8
26	7	1	14.2	7	159.5	22.7
27	7	0	0	7	152.5	21.7
28	7	0	0	7	145.5	20.7
29	7	0	0	6.5	138.5	19.7
30	6	1	16.6	6	132	22
31	6	0	0	6	126	21
32	6	0	0	6	120	20
33	6	0	0	6	114	19
34	6	0	0	6	108	18
35	6	0	0	6	102	17
36	6	0	0	6	96	16
37	6	0	0	6	90	15
38	6	0	0	6	84	14
39	6	0	0	6	78	13
40	6	0	0	6	72	12
41	6	0	0	5.5	66	11
42	5	1	20	5	60.5	12.1

43	5	0	0	5	55.5	11.1
44	5	0	0	5	50.5	10.1
45	5	0	0	4.5	45.5	9.1
46	4	1	25	4	41	10.2
47	4	0	0	4	37	9.2
48	4	0	0	4	33	8.2
49	4	0	0	3.5	29	7.2
50	3	1	33.33	3	25.5	8.5
51	3	0	0	3	22.5	7.5
52	3	0	0	3	19.5	6.5
53	3	0	0	3	16.5	5.5
54	3	0	0	2.5	13.5	4.5
55	2	1	50	2	11	5.5
56	2	0	0	2	9	4.5
57	2	0	0	2	7	3.5
58	2	0	0	1.5	5	2.5
59	1	1	100	1	3.5	3.5
60	1	0	0	1	2.5	2.5
61	1	0	0	1	1.5	1.5
62	1	0	0	0.5	0.5	0.5
63	0	1	-	-	-	-

x :age(days) ; lx : number of surviving at each age interval ; dx : age specific mortality ; qx : mortality rate ; Lx : number of living organisms at an average ; Tx : summation of living organisms to an average ; ex : expectation for future life.

The results of chronic toxicity tests showed that carbaryl has an effect on the formation of life table for *S.vetulus* and this effect was more obvious on the expectation for future life and mortalities of the treated as compared with control group. It was observed when *S.vetulus* exposed to 22µg/L and 26µg/L of carbaryl. The reduction in the expectation for life reached 50.5% and 56.4% respectively. This reduction refers to the hyper toxicity of insecticide carbaryl despite of the lower concentration which used.

This could be explained as due to carbamate pesticides are highly toxic to non target aquatic organisms (35). In experiment with cladocera population including *D.magna*, *S.vetulus* and *Moina micrura* with 1µg/L chloropyrifos lasted 42days (36). In addition an experiment lasted 6weeks when they exposed *D.magna* to 32 µg/L of carbaryl (37).

The life cycle of the control group of *S.vetulus* terminated in 63days. This is not strange because in a study the *Daphnia* spp. has the ability to reach 110 and 114 days (38). The elevation which occurs in life expectancy number within the life table is unsurprising because in life tables, elevation had occurred in other studies (39).

Table (4): The life table of *S.vetulus* exposed to 22µg/L of carbaryl

x	lx	dx	qx	Lx	Tx	ex
1	10	0	0	10	188.5	18.85
2	10	0	0	10	178.5	17.85
3	10	0	0	10	168.5	16.85
4	10	0	0	9.5	158.5	15.85
5	9	1	11.11	9	149	16.5
6	9	0	0	8.5	140	15.5
7	8	1	12.5	7.5	131.5	16.3
8	7	1	14.2	7	124	17.7
9	7	0	0	7	117	16.7
10	7	0	0	7	110	15.7
11	7	0	0	7	103	14.7
12	7	0	0	7	96	13.7
13	7	0	0	7	89	12.7
14	7	0	0	6.5	82	11.7
15	6	1	16.6	5.5	75.5	12.5
16	5	1	20	5	70	14
17	5	0	0	5	65	13
18	5	0	0	4.5	60	12
19	4	1	25	4	55.5	13.8
20	4	0	0	4	51.5	12.8
21	4	0	0	4	47.5	11.8
22	4	0	0	4	43.5	10.8
23	4	0	0	3.5	39.5	9.8
24	3	1	33.33	3	36	12
25	3	0	0	3	33	11
26	3	0	0	3	30	10
27	3	0	0	3	27	9
28	3	0	0	3	24	8
29	3	0	0	3	21	7
30	3	0	0	3	18	6
31	3	0	0	2.5	15	5
32	2	1	50	2	12.5	6.2
33	2	0	0	2	10	5
34	2	0	0	2	8	4
35	2	0	0	1.5	6	3
36	1	1	100	1	4.5	4.5
37	1	0	0	1	3.5	3.5
38	1	0	0	1	2.5	2.5
39	1	0	0	1	1.5	1.5
40	1	0	0	0.5	0.5	0.5
41	0	1	-	-	-	-

Table (5): The life table of *S.vetulus* exposed to 26µg/L of carbaryl.

x	lx	dx	qx	Lx	Tx	ex
1	10	0	0	10	165	16.5
2	10	0	0	10	155	15.5
3	10	0	0	10	145	14.5
4	10	0	0	9.5	135	13.5
5	9	1	11.11	8.5	125.5	13.9
6	8	1	12.5	8	117	14.6
7	8	0	0	7.5	109	13.6
8	7	1	14.2	7	101.5	14.5
9	7	0	0	7	94.5	13.5
10	7	0	0	6.5	87.5	12.5
11	6	1	16.6	6	81	13.5
12	6	0	0	6	75	12.5
13	6	0	0	5.5	69	11.5
14	5	1	20	5	63.5	12.7
15	5	0	0	4.5	58.5	11.7
16	4	1	25	4	54	13.5
17	4	0	0	4	50	12.5
18	4	0	0	4	46	11.5
19	4	0	0	4	42	10.5
20	4	0	0	3.5	38	9.5
21	3	1	33.33	3	34.5	11.5
22	3	0	0	3	31.5	10.5
23	3	0	0	3	28.5	9.5
24	3	0	0	3	25.5	8.5
25	3	0	0	2.5	22.5	7.5
26	2	1	50	2	20	10
27	2	0	0	2	18	9
28	2	0	0	2	16	8
29	2	0	0	2	14	7
30	2	0	0	2	12	6
31	2	0	0	2	10	5
32	2	0	0	1.5	8	4
33	1	1	100	1	6.5	6.5
34	1	0	0	1	5.5	5.5
35	1	0	0	1	4.5	4.5
36	1	0	0	1	3.5	3.5
37	1	0	0	1	2.5	2.5
38	1	0	0	1	1.5	1.5
39	1	0	0	0.5	0.5	0.5
40	0	1	-	-	-	-

The chronic toxicity effect of carbaryl on reproductive parameters of *S.vetelus*

Toxicity of Carbaryl on the summation of produced young / brood

It is observed that the higher number of young was 15 in the second and third brood while the lower number was 6 in the eleventh brood, for control group (Table 6).
The higher number of young was 10 at first brood in a concentration of 22 µg/L and 9 at second brood in concentration of 26 µg/L. The lower number of young reached 6 and 2 in seventh and fourth brood in concentrations of 22 µg/L and 26 µg/L respectively. Also the relation between the mean of length and the summation of young was studied; the mean of length for control in the first brood was 1.47 mm and increased to reach 2.06 mm in the last brood while it reached 1.49mm and 2mm in concentration 22 µg/L. Another difference in the mean of length was reported in concentration 26 µg/L in which it reached 1.58 mm in first brood and 1.9 at fourth one. The females in 26 µg/L weren't able to produce more than 4 broods.
The explanation of these cases lies in what was mentioned by (40) that cladocerans under unaproperate conditions more energy is allocated to reproduction rather than somatic growth and production of offsprings in order to be prepared for future unaproparte conditions.

This mean that this adaptation may increase the chances for future fecundity (38). And by this mechanism the animals have the ability to cope with these different stresses and maintaining their species. It's commonly accepted that at the initial stage of adaptation to the external impacts, a quick but imperfect set of protective compensatory responses is triggered, which allow for maintaining adequate vital functions due to the enhanced exploitation of functional reserve (41).

Table (6): The influence of carbaryl on the summation of produced young and mean of length /brood of *S.vetulus*

Treatment group		1st Brood	2nd Brood	3rd Brood	4th Brood	5th Brood	6th Brood	7th Brood	8th Brood	9th Brood	10th Brood	11th Brood
Control	a	14	15	15	12	14	10	9	9	8	8	6
	b	1.47	1.5	1.52	1.8	1.88	1.9	2	2	2.02	2.02	2.06
22 µg/L	a	10	9	8	8	8	6	6	—	—	—	—
	b	1.49	1.71	1.82	1.85	1.87	1.95	2	—	—	—	—
26 µg/L	a	8	9	2	2	—	—	—	—	—	—	—
	b	1.58	1.7	1.87	1.9	—	—	—	—	—	—	—

The effect of carbaryl on fecundity of eggs and young of *S.vetulus*

In table (7), the higher number of eggs was 120 in control group, while it was 59 in concentration of 22 µg/L and the lowest number of eggs was 23 in concentration of 26 µg/L. There was loss in eggs at concentrations of 22 µg/L reach 4eggs and 2eggs at concentration 26 µg/L.

Table (8) shows the minimum and maximum number of eggs and the young of *S.vetulus* exposed to carbaryl. And this gives another confirmation about the reduction which occurred during the exposing period.

The carbaryl had an adverse effect on fecundity of *S.vetulus* as compared with control group. The same results were observed in a study on glyphosate effect to *D.magna* the sub lethal concentration of 25 mg /L and 50 mg /L cause a significant reduction in the number of neonates as compared with the control (19). In a study exposed *D.magna* to Fenvalerate (FV) insecticide with low concentrations of 1 µg /L , 3.2 µg /L, and 10 µg /L ,they observed that a reduction in the number of neonates had occurred (42) . A study found that insecticide Fipronil shows a significantly greater reduction in the number of offsprings with concentration of 2 µg /L (43).The fecundity is obviously reduced when *D.longicephala* exposed to 0.32 µg/L of Carbaryl (44). A study found that sub lethal exposure to Carbaryl 5 ppb reduced *Daphnia* spp. productivity by about 15% (6).

Table (7): Comparison of variances on fecundity of eggs, young and on mean of length, longevity (± standard deviation) between control and exposed groups of *S.vetulus* to carbaryl

Treatment group	Total number of young SD±	Total number of eggs SD±	Mean of longevity SD± (day)	Mean of total length SD± mm
Control	120±11.50 nsd	120±11.50 nsd	39.6±18.48 sd	1.07±0.42 nsd
22µg/L	55±6.60 nsd	59±7.98 nsd	20.4±12.50 sd	0.86±0.56 nsd
26µg/L	21±1.89 nsd	23±2.06 nsd	18±11.85 sd	0.59±0.48 nsd

- nsd: Significant differences (P≤0.05) are not scored by the same column.
- sd: significant differences (P≤0.05) are scored by the same column

Table (8): Toxicity of carbaryl to the min. and max. number of eggs and young/female of *S.vetulus*.

Treatment group	min. and max. number of young/female through surviving period	min. and max. number of eggs/female through surviving period	min. and max. number of young/female through one brood
Control	2 - 34	2 - 34	0 – 6
22µg/L	5 - 21	5 - 21	0 – 4
26µg/L	4 - 8	2 - 8	0 - 3

The effect of Carbaryl on the mean of broods number, mean of brood size and onset to reproduction in *S.vetulus*

Table (9) shows that the effect of carbaryl on the number of broods reached 5.4 and 2.75 at concentration of 22 µg/L and 26 µg/L respectively as compared with 5.8 for control group. The reduction in the broods size percentages were about 10.31% and 7.17% at concentration of 22 µg/L and 26 µg/L respectively, and there are an effect of carbaryl on onset to reproduction which is delayed for two days as compared with control group. Furthermore , no significant differences to be scored at $P \leq o.05$.

The results of this present effect reveal that carbaryl had an adverse effect on the number broods and number of broods size, and like these adverse effects were observed by another study when they exposed *Daphnia* spp. to sub lethal concentration of carbaryl ranged from 1 to 5 ppb, Another reduction was observed that carbaryl in concentrations 5, 10, 15 and 20 µg/L reduced clutch size of *D.pulex* (45). Another reduction in the number of broods was reported when *D.magna* was exposed to the sub lethal concentrations of imidacloprid (26). The reduction in the reproduction activity (brood no.) also reported with different pesticides and chemicals, for example the sub lethal concentrations of glyphosate suppressed the reproduction of *D.magna* (19). The same result found when *D.pulex* exposed to herbicide Diuron by which the reproduction significantly reduced at 7.7 mg/L (15). The onset to reproduction was delayed for two days when *S.vetulus* exposed to 22 µg/L and 26µg/L of carbaryl and this agrees with a previous studies (6).

The chronic effect of insecticide Carbaryl on the mean of the total length of *S.vetulus*

Table (7) shows the reduction in the mean of total length of *S.vetulus* after 41 days of chronic exposure to carbaryl, it was observed that the lowest mean of length was reported at concentration of 26µg/L in which it reached 0.59mm and the highest reported at control group of 1.07mm, therefore the mean of total length was reduced when the concentration of carbaryl was increased. There are no significant differences to be scored between control and exposing group at the level of probability $P \leq 0.05$. There are differences in the mean of total length among cladocerans control group and chronic concentration. The result of this study demonstrates that mean body length decreases with the increasing in concentration of insecticide. This reduction in the mean of total length agrees with (6) that carbaryl have an effect on the growth and development of *Daphnia* spp. Also (20) and (45) reported that carbaryl reduces the size of the mature in *Daphnia* spp. population. In previous study *D.magna* exposed to 32µg/L of carbaryl and found no significant differences to be detected between population of origin (control) and carbaryl treatment in *D.magna* body size (37).

Table (9): The effect of Carbaryl on the mean of broods, mean of brood size (± standard deviation) and the onset to reproduction for *S.vetulus*

Treatment group	Mean of Brood SD±	Mean of Brood size SD±	Onset to Reproduction (day)
Control	5.8±3.94 nsd	2.23±0.60 nsd	9
22µg/L	5.4±3.36 nsd	2±0.35 nsd	11
26µg/L	2.75±0.95 nsd	2.07±0.15 nsd	11

- nsd: Significant differences (P≤0.05) are not scored by the same column.

Table (10): Results obtained from ANOVA-one way test for *S.vetulus* molts data in the chronic experiment with carbaryl

Treatment group	Range of Molts	Mean of Molts SD±
Control	4 - 23	14.2 ± 6.57 sd
22µg/L	2 - 17	8.6 ± 5.52 sd
26µg/L	2 - 19	7 ± 6.03 sd

The effect on the mean of longevity of *S.vetulus*

The normal longevity in control was 39.6 days, where as reduced to 20.4 days at concentration of 22 µg/L, another reduction was observed at concentration of 26 µg/L which reached 18 days (Fig 4). Statistical analysis shows that there are significant differences at level of probability $P \leq 0.05$ between control and the exposed groups as in table (7).

The result of this study indicates that insecticide cause a reduction in the mean of longevity of *S.vetulus*, and this reduction increases with increasing concentrations of insecticide. The same effect was observed by (20) when carbaryl causes largest crush in the survival curve of *D.magna* which reaches 40 days under carbaryl application as compared with control which was 52 days. Fenvalerate (FV) and Diuron cause likely reduction in the survival of living organisms(15, 42)

The chronic effect on the mean of molts of *S.vetelus*

Statistically significant differences ($p \leq 0.05$) observed for carbaryl at concentrations of 22µg/L and 26 µg/L .the number of molts reached 14.2,8.6 and 7 respectively. Table (10).

However the mean of molts are reduced during the exposure to carbaryl as compared to the control group. This agrees with a previous study in which Tebufenozide and Imidacloprid affected the molting process of all arthropods (46). Vinclozolin and Methoprene (organophosphorus insecticides) which are juvenile hormones mimic, have an adverse effect on molting and another processes regulated by these hormones (47).

These figures give an illustration to the chronic effects of carbaryl on *S.vetulus*.

Therefore, we conclude the following from this study:

1. The toxicity of carbaryl to *S. vetulus* was highly affective.

2. Acute and chronic toxicity tests which were studied, indicating that adverse effects of carbaryl on *S.vetulus* and suggest that it can cause an environmental damage when used at high concentrations.

3. The chronic toxicity test of carbaryl revealed that it had noxious effects on the reproductive parameters of these cladoceran.

4. All parameters which were studied during chronic exposure provided an obvious and important reflex about the effects of carbaryl.

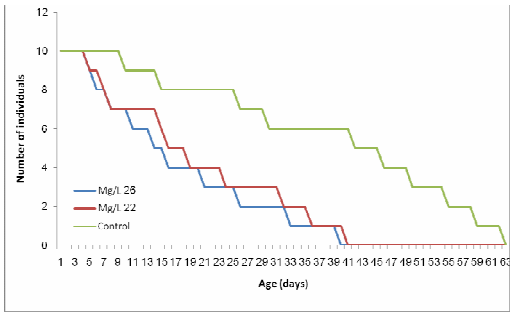

Fig.(4) : Survival curve of *S.vetulus* at control and under 22 µg/L and 26 µg/L of carbaryl

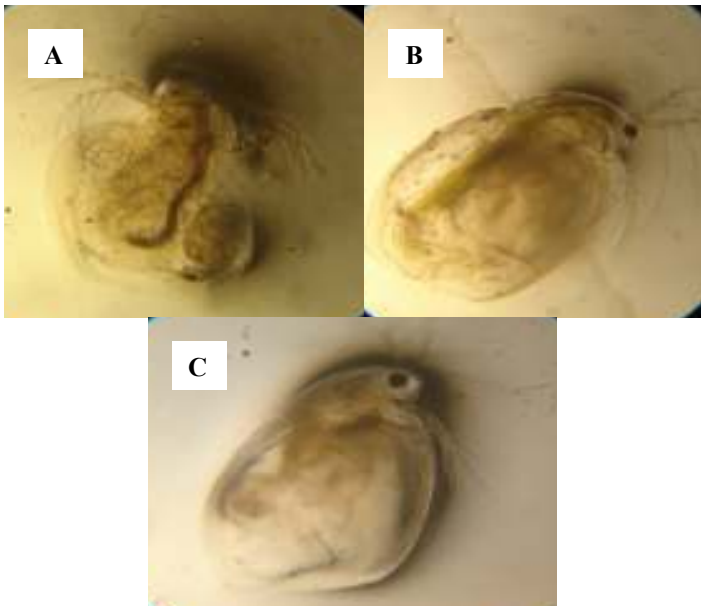

***Fig (5): S.vetulus* with their eggs or neonates after chronic exposure to carbaryl during this study.**

A: 22 µg/L carbaryl, B& C: 26 µg/L carbary

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The influence of patient's age and severity of Pulmonary tuberculosis on serum interlukin-6 levels

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ABSTRACT

Interleukin-6 (IL-6) is a proinflammatory cytokine implicated in the immunopathogenesis of tuberculosis (TB). TB is recognized worldwide as an important public health issue. To study the relationship between the age of patients with pulmonary TB and serum IL-6 levels, from the other hand, the severity of this disease with IL-6 levels. This study included 30 patients (16 female and 14 male) with pulmonary TB and 10 healthy persons (5 female and 5 male) as control group for comparison. An ELISA assay was used to quantify IL-6 in the sera. The results showed a significant increase of IL-6 levels with increase of age of patients, in (23-38) year old patients the IL-6 levels (median=17.9 pg/ml, range 12.3- 29.1), while in (50-70) year old patients (median=20.5 pg/ml, range12.4-47.2) , in comparison with controls (21-23) year old (median=2.45 pg/ml, range 1.89-2.94) $p \leq 0.013$. Additionally, IL-6 concentrations were increasing significantly with increasing the severity of TB, in score (+) of TB IL-6 concentration is (median=17.6 pg/ml, range 12.3-28.8), while in score (+++) TB IL-6 concentration is (median=27.2, range 26.9-27.1) $p \leq 0.0001$. In conclusion, Serum IL-6 levels increasing with increasing patient's age and the severity of pulmonary TB.

الملخص باللغة العربية

الانترلوكين−6 احد السايتوكينات التي تفرز في بدء الالتهاب ولها دور في امراضية السل الرئوي. ينتشر مرض السل على مدى واسع عالميا ويشكل احد المشاكل الصحية الهامة. اجريت هذه الدراسة لمعرفة العلاقة بين عمر المريض بالسل الرئوي ومستويات الانترلوكين−6 في مصل المريض من جهة وشدة المرض ومستويات هذا الانترلوكين من جهة اخرى. شملت الدراسة 30مريضا (16 نساء و 14 رجل)يعانون من مرض السل الرئوي وللمقارنة اخذت عينات مصل من 10 اشخاص اصحاء (5 نساء و 5 رجال). استخدمت طريقة الاليزا (ELISA) لقياس تراكيز الانترلوكين−6 في المصول. وقد اظهرت النتائج زيادة معنوية في مستويات هذا الانترلوكين في المصل مع ازدياد عمر المرضى. فقد كانت التراكيز في الفئة العمرية (23−38سنة) ذات متوسط 17,9 بيكوغرام/مل (بمدى 12,3−29,1) .اما في الفئة العمرية (50−70سنة) كانت بمتوسط 20,5 بيكوغرام/مل (بمدى 12,4−47,2) في حين كانت في مجموعة المقارنة الاصحاء (21−23سنة) بمتوسط 2,45 بيكوغرام/مل (بمدى 1,89−2,94) وعلى احتمالية≥ 0.013 . كما اظهرت النتائج ان مستويات الانترلوكين−6 قد ازدادت معنويا مع زيادة شدة المرض. ففي حالات الاقل شدة (+) كانت تراكيزه بمتوسط 17,6 بيكوغرام/مل (بمدى 12,3−28,8). اما في الحالات الشديدة للمرض (+++) فقد كانت بمتوسط 27,2 بيكوغرام/مل (بمدى 26,9−27,1) وعلى احتمالية≥0,0001 . نستنتج من الدراسة ان مستويات الانترلوكين−6 في مصل المرضى بالسل الرئوي تزداد مع ازدياد اعمار المرضى وبزيادة شدة هذا المرض لديهم.

INTRODUCTION

A third of the world's population is considered to be infected with *Mycobacterium tuberculosis* and nearly two million people die every year from tuberculosis (TB) (1). TB may develop anywhere in the body, but usually presents as pulmonary infection, ranging from mild infiltration to chronic, cavitary, and severe destructive disease (2). The success of *M. tuberculosis* as a pathogen depends upon its capacity to avoid destruction by host cells (3), and the balance between the *bacillus* and other host defense mechanisms (2). Thus varies from a 'latent' infection with no clinical symptoms to the disseminated disease (3). The immune response towards the pathogen is complex and immune parameters that confer protection against TB are not yet elucidated fully (4).

Immune recognition of *M. tuberculosis* by phagocytic cells leads to cell activation and production of cytokines. one of these cytokines, IL-6 which has both pro- and anti-inflammatory properties (5), is produced early during mycobacterial infection and at the site of infection (6-7). Lipoarabinomannan (LAM), a complex lipid glycoprotein anchored in the mycobacterial cell membrane, stimulated mononuclear phagocytes to release IL-6 in a dose-response manner (8).

The aim of this study is to determine the serum level of IL-6 in patients with pulmonary TB and relationship between this cytokine and the age of the patients, with the severity of this disease.

PATIENTS AND METHODS

Patients: This study included 30 patients with pulmonary TB (16 females and 14 males). They were defined and diagnosed as TB cases by the specialist physicians according to the clinical, chest x-rays, and direct sputum smear examinations by Ziehal-Nelseen's staining technique (9). They were consulted at Al-Kindy Teaching Hospital from November 2010- May 2011, at Baghdad city, Iraq. For comparison, 10 healthy persons (5 females and 5 males) were included.

Methods: -

The severity of the TB in patients determined by direct sputum smear staining by Ziehal-Nelseen's method and scores of +, ++, and +++ indicated for (1-9) bacterial cell/smear, (10 or more) bacterial cell/smear, and (1 or more) bacterial cell/field, respectively (9).

Serum IL-6 levels:

Blood samples (5 ml) were drawn into plan vacationers from the antecubital vein. The blood was allowed to clot for 30 minutes and centrifuged at 2000 g for 15 minutes for separation of clear serum. Sera were stored at -20 C° until analyzed. Estimation of IL-6 concentration in serum was done by ELISA method using US Biological kit, USA. It also called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to IL-6. Standards and samples are added to plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-6. IL-6 if present will bind and become immobilized and then be "sandwiched" by biotin conjugate. In order to quantization determine the amount of IL-6, Avidin conjugated to Horseradish Peroxidase is added. Only those wells that containing IL-6 biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color, and the color is measured spectrophotometrically at wavelength 450nm.

Statistical analysis:

Data were analyzed statistically using descriptive statistics Infrequencies tables, median and slandered deviation. The non-parameter Mann-Whitney test is used for measuring the differences between the groups. These were done using Mini Tab Statistical Software Program 13.20. A p-value ≤ 0.05 was considered significant.

RESULTS

Serum IL-6 levels in different age groups patients with pulmonary TB: Serum IL-6 concentrations in patients with pulmonary TB increasing significantly ($p \leq 0.013$) with increasing patient's age. As shown in table (1). in (23-38) years old patients group (n=12, 30%) the concentration of IL-6 was (median=17.9 pg/ml, range 12.3-29.1), and in (40-49) years old patients group (n=8, 20%) was (median=19.1pg/ml, range 14.6-23.9), while in (50-70) years old patients (n=10, 25%) was (median=20.5, range 12.4-47.2), in comparison with controls (n=10, 25%) healthy

persons was (median=2.45 pg/ml, range 1.89-2.94).

Table(1): Serum IL-6 levels in different age groups patients with pulmonary TB in comparison with control group.P≤0.013.

Patients age groups (year)	N (%)	IL-6 levels Median pg/ml(Min-Max)
(23-38) Group	12 (30)	17.9(12.3-29.1)
(40-49) Group	8 (20)	19.1(14.6-23.9)
(50-70) Group	10 (25)	20.5(12.4-47.2)
(21-23) Control Group	10 (25)	2.45(1.89-2.94)

Total Samples of patient's sera=30, Control's sera=10

Serum IL-6 levels and severity of pulmonary TB: As shown in table(2), there are significant increasing (p≤0.0001) between the severities of pulmonary TB indicated as (+) score for 12 (30%) patients, (++) score for 16 (40%) patients, and (+++) score for only 2 (5%) patients in addition to control group 10 (25%) healthy persons, the serum IL-6 levels are median=17.6 pg/ml (range 12.3-28.8), median=19.1 pg/ml (range 12.4-47.2), median=27.2 pg/ml (range 20.9-27.1), and median= 2.45 pg/ml (1.89-2.94) respectively

Table(2): The relationship the severity of pulmonary TB and serum IL-6 levels in patients in comparison with control group.P≤0.0001

Severity Score of TB	N (%)	IL-6 levels Median pg/ml(Min-Max)
(+) Group	12 (30)	17.6(12.3-28.8)
(++) Group	16 (40)	19.1(12.4-47.2)
(+++) Group	2 (5)	27.2(26.9-27.1)
Control Group	10 (25)	2.45(1.89-2.94)

Total Samples of patient's sera=30, Control's sera=10

DISCUSSION

Serum IL-6 levels in different age groups patients with pulmonary TB: This study revealed that there are significant differences between elevation of serum IL-6 levels and increasing patient's age. There are no such studies to compare with that result.

Usually, the development of TB is depending on:

1. The No. of TB in the inoculums and their multiplication.
2. The resistance and hypersensitivity of the host (10).

In active TB, the role of IL-6 may be predominantly negative. This supported by several facts: (i) IL-6 promote the growth of mycobacteria in peripheral blood monocytes (11, 12). (ii) IL-6 inhibits the production of TNF-α and IL-1β, which may enhances intracellular killing of microorganisms and development of granulomata (13, 14). From all that may explain the role of age become negative and causes elevation in IL-6 level.

Serum IL-6 levels and severity of pulmonary TB: The infected patients have elevated serum IL-6 levels, significantly with increasing the severity of this disease in comparison with control healthy group. There are no previous determined that relation ship; however, IL-6 levels were elevated in patients with pulmonary TB (6, 15). This result may be due to IL-6 as a proinflammatory cytokine expressed by a variety of normal and transformed cells included T cells, B cells, monocytes/macrophages, fibroblasts, hepatocytes, keratinocytes, astrocytes, vascular endothelial cells, mesangial cells, osteoblasts , and carcinomas (16). The different manifestations of infection with *Mycobacterium tuberculosis* is reflect of the balance between the bacillus and host defense mechanisms in which the quality of host defense determines outcome (2). So serum IL-6 levels may be; in part, because increasing the inflammatory cells that involved with TB. In spit of, the human host response to TB is a complex reaction (17).

CONCLUSIONS

In pulmonary tuberculosis, elevation of serum IL-6 levels depended on increasing patient's age and the severity of the disease. More researches requires to understanding the role of IL-6 in relation with other defense mechanisms and then the possibility to find new approaches in diagnosis and treatment of this disease.

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Detection of Metallo Beta-Lactamase (MβL) Production from Acinetobacter *baumannii* Isolates from Urinary Tract Infections

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ABSTRACT

Ten isolates of *Acinetobacter baumannii* were isolated from urine samples collected from patient suffering from UTI obtained from Education Labs / medical city, and Re identification according to the Api 20 E test.

Sensitivity of all isolates was tested against 23 Antibiotics. Results showed all isolates of *A. baumannii* were resistant 100% to nine antibiotics included , penicillin , ampicillin, cephalexin, carbenicillin , Amikacin , Ciprofloxacin,Gentamicin,tetracycline and amoxicillin. Detection of Beta–Lactamase by using rapid standard Iodometric assay and capillary tubes method , showed that seven isolates(70%) of *A. baumannii* were B- lactamase producing . All β- lactamase- producing *A.baumannii* isolates were also tested for their ability to produce Extended-Spectrum Beta-lactamases (ESBLs) by using two methods ,the results revealed that Screening and confirmatory tests considered the most accurate method for detection of ESBL- producing isolates, while the disk approximation was the least accurate method in detecting such enzymes. The results of metallo-β-lactamase (MBL) producing isolates test showed only one isolate (10%) of A.baumannii produce MBL enzyme.

Determination the MICs of ESBL-producer isolates against five β-lactam antibiotics showed that all three ESBL- producing *A.baumannii* isolates were highly resistant to both ampicillin and amoxicillin. They were also resistant to ceftazidime, cefotaxime as range (32to >128)(64>128)(32>128) µg/ml respectively to antibiotics above .

The result of plasmid content showed that selected isolate *A.baumannii 4* harbor two plasmid bands different in size and position. . The results of conjugation experiment revealed that genes encoding for the production of metallo β-lactamase, ESBL,reistance to several antibiotics were located on plasmid.

الملخص باللغة العربية

جمعت (10) عزلات من بكتريا *Acinetobacter baumannii* من عينات ادرار من مرضى يعانون من التهابات السبيل البولي من المختبرات التعليمية / مدينة الطب في بغداد. اعيد تشخيصها اعتمادا على Api 20 E system .

اختبرت حساسية جميع العزلات تجاه 23 مضادا حيويا,واظهرت النتائج امتلاك جميع العزلات مقاومة 100 % الى تسع مضادات حيوية تضمنت أمبيسلين، سيفاليكسين، كربنيسيلين، أميكاسين، سيبروفلوكساسين، جنتاميسين، التتراسيكلين وأموكسيسيلين. وفي اختبارانتاج انزيمات البيتالاكتاميز بطريقتي اليود القياسية السريعة والانابيب الشعرية كانت7عزلات (70%) منتجة لانزيمات البيتالاكتاميز. كما اختبرت قابلية العزلات المنتجة لانزيمات البيتالاكتاميز على إنتاج إنزيمات البيتالاكتاميز واسعة الطيف باستخدام طريقتين, وقد عد الاختبار التاكيدي .من الطرائق الاكثر دقة من طريقة الاقراص المتراصة المستخدمة في هذه الدراسة للتحري عن انتاج إنزيمات البيتالاكتاميز واسعة الطيف. أظهرت نتائج تحديد التركيز المثبط الأدنى لخمس مضادات حيوية تابعة الى البيتالاكتام أن العزلات الثلاث المنتجة كانت ذات مقاومة عالية لمضادي الامبيسيلين والاموكسيسلين, كما اظهرت مقاومة لمضادات السيفتازديم والسيفوتاكسم , اذ تراوح التركيز المثبط الادنى مابين (32–>128) و (64–>128) و (32–>128) مايكروغرام/ مل على التوالي.

اظهرت نتائج المحتوى البلازميدي امتلاك العزلة المنتخبة *A.baumannii4* حزمتين بلازميدية مختلفة في الموقع والحجم . وأشارت نتائج الاقتران إلى أن المورثات المسؤولة عن انتاج انزيمات البيتالاكتاميزالمعدنية و انزيمات البيتالاكتاميزالواسعة الطيف و المقاومة لبعض لمضادات الحيوية هي بلازميدية الموقع.

INTRODUCTION

Over the years it has been shown by numerous ecological studies that increased antibiotic consumption contributes to the emergence of antibiotic resistance AR in various bacterial genera (*1).* The noticeably increasing numbers of multi-drug-resistant microbial infections have become a serious health care problem. In particular, the appearance of multi-drug resistant strains of gram positive and gram negative bacterial pathogens (2) *Acinetobacter* species, including *Acinetobacter baumannii*, had been regarded as one of the important groups of opportunistic pathogens implicated in various infections such as pneumonia, urinary tract infection, endocarditis, surgical site infection, meningitis, and septicemia (3) .The majority of clinical *A. baumannii* isolates are highly resistant to a variety of antibiotics, including carbapenems, which are currently the drugs of choice in the treatment of the severe infections caused by this organism (4) .

Carbapenem resistance has been observed frequently in non fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacter* spp. Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes-carbapenemase (5) . Metallo-B-lactamase production is an emerging mechanism of carbapenem resistance among enteric and nonfermenting gram-negative bacilli . Five acquired M_L classes (IMP, VIM, SPM, GIM, and SIM) have been identified in various host organisms, most commonly, *Pseudomonas aeruginosa*, *Acinetobacter* species, and species of the family *Enterobacteriaceae* M_L genetic determinants are usually associated with class 1 integron structures that may reside on mobile genetic elements, such as plasmids and transposons .(6) , the present study was carried out to achieve the following aims, the prevalence of β- lactam resistance , detection of β – Lactamase ,ESBL and Metallo-B-lactamase among these 10 clinical local *Acinetobacter* isolates were study, as well as their genetic relatedness were elucidated.

MATERIALS AND METHODS

Isolation of Bacteria

Ten isolates of *Acinetobacter baumannii* were isolated from patient suffered from urinary tract infection collected from urine samples , characterized and identified in Education Laboratory / Medical City.

Antibiotic Sensitivity Test

The susceptibility of isolates to different antimicrobials were determined by Kirby-Bauer disk diffusion method (7)

Detection of β- lactamase production

Two methods were performed to detection the β- lactamase production in clinical isolates , both methods depends on detection of penicilloic or cephalosporic acid, resulted from breakdown of amide bond in β-lactam ring for each of penicillins or cephalosporins

Rapid iodometric method (8):

This test was performed for all bacterial isolates that were resistant to β- lactam

Capillary tubes method

This test was done to all isolates according to method described by (9)

Detection of ESBL production :

Two methods were performed to detection the ESBLs in clinical isolates. All bacterial isolates positive to β- lactamase production were tested for ESBLs. These tests included :

Disk approximation method:

This method was carried out as modified by (10)

Screening test for ESBLs production.

In screening test, the isolate would be considered ESBL-producer, if the inhibition zone of 3GC was as follows: Ceftazidime < 22 mm , Cefotaxime < 27 mm , Ceftriaxone < 25 mm Production of ESBLs by any of the isolates was confirmed if a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone.

Detection of metallo B-lactmase

This test was performed to one isolate that showed resistant imipeneme antibiotic (5)

Determination of Minimal Inhibitory Concentration Of ESBL-Producing Isolates (11)

Two-fold agar dilution susceptibility method was used to determine the MICs of ESBL-producer isolates against five β-lactam antibiotics(Ampicillin, Amoxicillin, Cefotaxime, Ceftazidime, and Ceftriaxone).

Total DNA Isolation (Salting out Method)

The method described by (12) was used in this study with some modification to isolate both plasmid and chromosomal DNA of Acinetobacter *baumannii isolate*.

Bacterial conjugation : (13)

Acinetobacter baumannii 4 isolates which resist to Imipenem were selected (as donor cells) for studying bacterial conjugation with *E. coli* (as recipient cell) which resist to Tobramycin .

RESULTS AND DISCUSSION

Bacterial Isolates

These isolates were identified by using a commercial identification system (Api 20 system) according to the instractions of the manufactures.

Antibiotic sensitivity Test :

All *Acinetobacter* isolates were tested for their sensitivity toward twenty three antibiotics discs using disk diffusion method. All isolates were found to be resistant to at least 9 antibiotics tested. Hence all the isolates were considered to be multidrug resistant. (Table 1)

These isolates were 100 % resistant to some antibiotics related to Beta- lactam group such as penicillin , ampicillin, cephalexin, carbenicillin , Amikacin , Ciprofloxacin,Gentamicin,tetracycline and amoxicillin (figure 1). It was found that major mechanism of resistance in gram- negative bacteria causing clinically significant infection is the expression of β-lactamases, of which there are several classes including plasmid-encoded and chromosomally encoded enzymes (14). It was also clear from figure (1) that 70 % of *A.baumannii* isolates were resistant to piperacillin , and (60 %) were resistant to cefixime.

These results are in agreement with those results being reported by other researchers who found that chromosomally encoded resistance to first and second generation cephalosporins in gram negative bacteria has been emerged in many hospitals (15, 16)

The present study revealed the high level of resistant to third generation cephalosporins (3GC) among *A.baumannii* which were resistant to cefotaxime , ceftriaxone , ceftazidime in percentage(60% ,70% ,90% respectively.

These results are in agreement with (17) who found that a high level of antibiotic resistance in *Acinetobacter* were resistant to the antibiotics tested including the third generation cephalosporins.

The therapeutic strategies to control infections due to *A.baumannii* isolates has to be carefully formulated, and since most of the isolates were sensitive to imipenem and azteronam except *A.baumannii* 4 showed resistant to it, it might serve as the drug of choice for the treatment of infections caused by *Acinetobacter* strains.

Results of figure (2) showed the isolates were highly resistance to others antibiotics groups as shown in table (1) such as gentamycin , tetracyclin , Ciprofloxacin and amikacin (100%) , they were moderately resistant to Trimethoprim and chloramphenicol (50 %) and Norfloxacin (60%) to highly resistant to tobramycin (90 %) (figure 3).

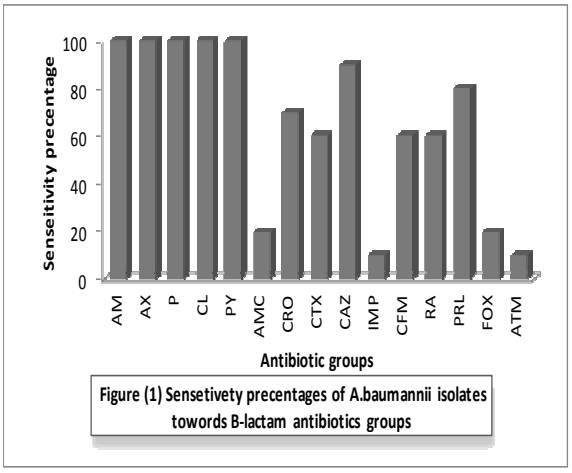

Figure (1) Sensetivety precentages of A.baumannii isolates towords B-lactam antibiotics groups

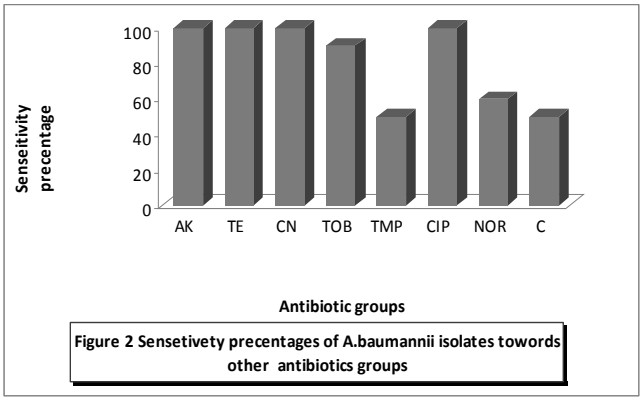

Figure 2 Sensetivety precentages of A.baumannii isolates towords other antibiotics groups

Table (1): Sensitivity of *A.baumannii* isolates towards antibiotics groups

Strains	AMC	AC	AM	P	CL	CAZ	CTX	CRO	ATM	IPM	CFM	RA	PRL	FOX	PY	C	NOR	TE	TMP	CIP	CN	AK	TOB
A.baumannii 1	R	R	R	R	R	S	S	R	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R
A.baumannii 2	S	R	R	R	R	R	R	R	S	S	R	R	R	S	R	R	S	R	R	R	R	R	R
A.baumannii 3	S	R	R	R	R	R	S	S	S	S	S	S	S	S	R	S	S	R	S	R	R	R	R
A.baumannii 4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
A.baumannii 5	S	R	R	R	R	R	S	S	S	S	S	S	S	S	R	S	R	R	S	R	R	R	R
A.baumannii 6	S	R	R	R	R	R	R	R	S	S	R	R	R	S	R	R	S	R	S	R	R	R	R
A.baumannii 7	S	R	R	R	R	R	R	R	S	S	R	S	R	S	R	S	R	R	R	R	R	R	R
A.baumannii 8	S	R	R	R	R	R	R	R	S	S	R	R	R	S	R	S	S	R	R	R	R	R	R
A.baumannii 9	S	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R
A.baumannii 10	S	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R	S	R	R	R	R

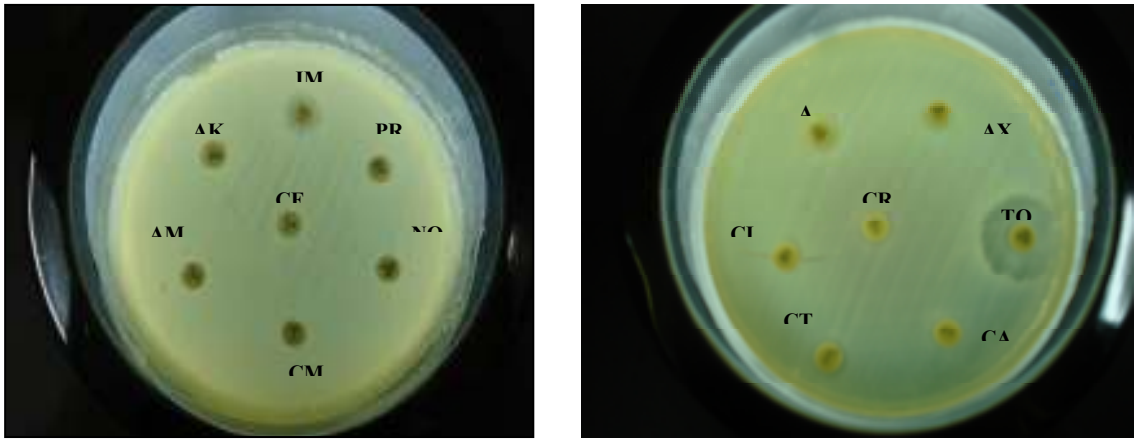

Figure (3) : Susceptibility test for *Acinetobacter baumannii* 4

(18) refers to The susceptibility of 19 isolates of *Acinetobacter baumannii* to imipeneme (100%) and resistance 100% to (Cotrimoxazol , Nitrofurantoin , Carbenicillin, Tetracycline , Aztreonam , Ciprofloxacin , Cephalothin) .
(19) referred to outbreak of multidrug-resistant Acinetobacter baumannii in US service members injured in Iraq as a result of military operations in Iraq.

Detection of β- Lactamase Production

Rapid iodometric method and capillary tubes methods were used for detection of β- lactamase production in β- lactam resistant *Acinetobacter baumannii* isolates.
The results showed that 7 isolates from *A.baumannii* (70 %) gave positive reaction with rapid iodometric method and acidometric method. (figure 4), (figure 5)
According to high ratio of resistance (100 %) to more than antibiotics that related to **β-**– lactam group this indicate the β-lactamase-producing isolates have an enzymatic mechanism of resistance represented by the production of β-lactamases.
Many studies reported that carrier rates of Gram-negative bacteria increase during staying in hospital, and of persons outside hospitals especially in patients receiving antibiotic treatment (20).The transfer of bacteria from the hospital environment would appear to be the most likely source involved (21).

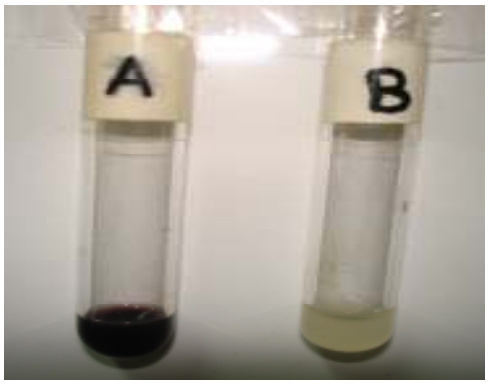

Figure (4):**Detection of β-lactamase production in isolates by iodometric assay**

A - isolate A. bauamnnii 3 (negative)
B- isolate A. bauamnnii 4 (Positive)

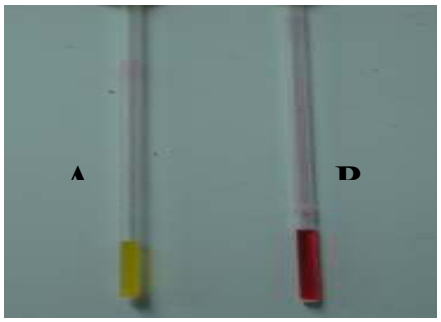

Figure (5):**Detection of β-lactamase production in isolates by capillary tubes method.**

A- Isolate *A. bauamnnii* 4 (Positive)
B- Isolate *A. bauamnnii* 3 (negative)

Such β-lactamase detection tests do not provide any information about what type of β-lactamase is present. A side from chromogenic methods, β-lactamase production can be detected by various biological methods, which depend on the β-lactamase produced by one organism . More useful biological methods are the double-disk tests used to examine the synergy between clavulanate and ceftazidime to detect ESBLs (22 ; 23).

Detection of Extended-Spectrum β-Lactamases:

Disk Approximation Method
In this test, results were determined depending on enhancement of the inhibition zone between a beta- lactam disks (CTX, CAZ, CRO and AZT) and augmentin disk (amoxicillin-clavulanate – 20/10 µg/ml), as indication for the presence of an ESBL.
From seven β- lactamase producing isolates, only four (57%) isolates of *A. baumannii* were ESBL–producers ,these were *A. baumannii* 2 , *A. baumannii* 4, *A. baumannii* 6 ,and *A. baumannii* 8 . (figure 6).
(17) reveled that ESBL production in *Acinetobacter* was 28 percent in india and vary from 46 per cent in Turkey to 54.6 per cent in Korea .
Clavulanic acid inhibits a wide range of β-lactamases, including the plasmid-mediated enzymes such as TEM and SHV which are wide-spread among the Enterobacteraceae. ESBLs are also highly susceptible to inhibition by clavulanic acid (24).

Using this test, results of the present study showed that the prevalence rate of ESBL-mediated resistance to third-generation cephalosporins (3GC) was 57 % to *A.baumannii* .

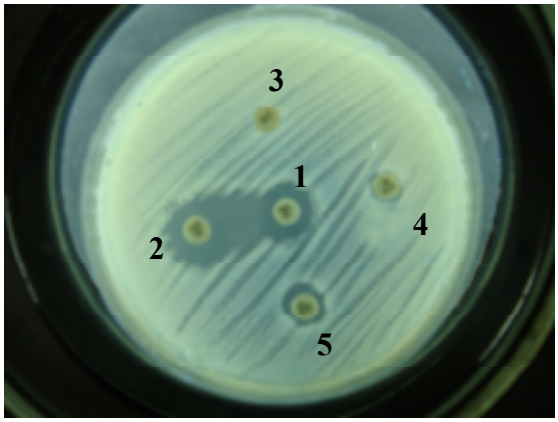

Figure (6): Detection of ESBL production in *A. bauamnnii* 4 **by disk approximation method**

1- Amoxicillin-clavulanate disk (20/10 µg).
2- Ceftazidime disk (30 µg).
3- Cefotaxime (30 µg)
4- Ceftriaxone (30 µg)
5- Aztreonam (30 µg)

Many clinical laboratories have problems detecting extended-spectrum beta-lactamases (ESBLs) and plasmid-mediated AmpC beta-lactamases. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to the therapeutic failures (25).

Screening and confirmatory tests recommended by NCCLS

Results in table (2) showed that three isolates (42.8)% were produce ESBL which detected by this method. (Figure 7).

These isolates were found to produce ESBLs, and they are considered as non- susceptible to all penicillins, cephalosporins (including expanded-spectrum cephalosporins) and aztreonam, regardless of the susceptibility test (26 , 27).
Especially feared are epidemic hospital infections caused by multi resistant strains particularly that produce ESBLs . ESBLs are usually plasmid mediated. Since these plasmids are easily transmitted among different members of the Enterobacteriaceae, accumulation of resistance genes results in strains that contain multiresistant plasmids(23).
Prevalence of ESBL enzymes has been increasing in many parts of the world. Infections caused by ESBL producing isolates are difficult to treat, because they confer resistance to all currently available β-lactam agents, except imipenem, and in some cases piperacillin–tazobactam . (28). In addition, ESBL production is usually associated with resistance to other classes of antimicrobial agent, such as aminoglycosides and fluoroqui-nolones (29).

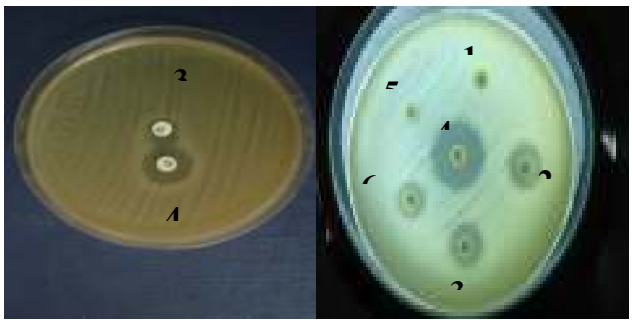

Figure (7):Detection of ESBL production in *A.baumannii 4* by confirmatory tests recommended by NCCLS
1- Cefotaxime disk (30 µg). 2- Cefotaxime disk (30 µg) with clavulanic acid . 3 – Ceftazidime disk (30 µg) 4 -Ceftazidime disk (30 µg) with clavulanic acid . 5- Ceftraxone disk (30 µg) 6– Ceftraxone disk (30 µg) with clavulanic acid .

MICs Determination of ESBL –producing Isolates

The MIC values were based on break point recommended by (27) for estimation of the response.
Results in table (2) showed that all the three ESBL- producing *Acinetobacter* isolates were highly resistant for both ampicillin and amoxicillin with concentrations reached to 2048 , and the lowest MIC for the isolates was for Cefotaxime and Ceftriaxone as it ranges (32 - 128) µg/ml **.** On the other hand the MIC values of cefotaxime and Ceftriaxone for 2 isolates of *A. baumannii* was 32 µg/ml, which were less than the break point value (≥ 64 µg/ml). Results from a local study showed that gram-negative enteric rods were 100 % resistant to ampicillin and amoxicillin (30).

Table (2) : Initial screening test for ESBL–producing *Acinetobacter* Isolates

No. of β-lactamase Producers isolates	Inhibition zone (mm)					
	Cefotaxime (≤ 27)	Cefotaxime + clavulanic acid	Ceftazidime (≤22)	Ceftazidime + clavulanic acid	Ceftriaxone (≤ 25)	Ceftriaxone + clavulanic acid
A.baumannii 1	24	28	21	21	21	28
A.baumannii 2	27	27	R	R	23	23
A.baumannii 4	R	R	13	16	R	R
A.baumannii 6	21	22	18	20	25	25
A.baumannii 7	R	15	13	20	R	11
A.baumannii 8	23	23	R	R	24	25
A.baumannii 9	20	26	14	20	21	28

(18) determined in her study the Minimum inhibitory concentration (MIC) of 8 antibiotics (Ciprofloxacin , Gentamicin , Tobramycin , Amikacin , Cefotaxime , Pipracillin , Pipracillin + Tazobactum , Cefipeme) against 19 1solates of *A. baumannii*, which they were (4 – 512 , 8 – >1024 , 4 – >1024 , 4 – 256 , 16 – >1024 , 8 – 256 , 8 –512) µg /ml respectively .

Resistance of gram-negative enteric rods to first generation cephalospo-rins, was due to the plasmid-mediated β-lactamases TEM-1, TEM-2, and SHV-1. (31 , 32). High MIC values of Acinetobacter isolates in the present study suggests that ESBL enzymes are endemic in study area. Furthermore, epidemiological studies are necessary to determine whether such isolates exist in the community, or remain largely confined to environmental samples (from which they were obtained) or hospitals where they produce nosocomial infections.

Some researchers in Iraq (30) mentioned that gram- negative enteric rods, were resistant to amoxicillin, cephalexin, and cefotaxime depending on the break point values recommended formerly by (33). In fact, these isolates should be regarded sensitive to these antibiotics according to the recent break points recommended by NCCLS (33 ; 34).

Table (3): MICs of number of β- lactam antibiotics for ESBL-producing *by Acinetobacter* isolates.

Isolate	MIC (µg/ml) of :				
	AMP (≥32µg /ml)	Amx (≥32µg /ml)	CTX (≥64µg /ml)	CAZ (≥32µg /ml)	CRO (≥64µg /ml)
A.baumannii 4	2048	2048	> 128	> 128	> 128
A.baumannii 7	1024	1024	32	64	32
A.baumannii 9	128	512	32	64	32

*** Numbers between backets refer to break points recommended by NCCLS (2003b).**

AMP,Ampicillin; Amx, Amoxicillin; CTX, Cefotaxime; CAZ, Ceftazidime; CR0, Ceftriaxone

Detection of metallo-β-lactamase (MBL) producing isolates

The detection of metallo-β-lactamase (MBL) producing isolates is necessary to prevent their dissemination. Frequency of MBLs producing strains among multidrug resistant (MDR) *Acinetobacter* species was evaluated by using imipenem-EDTA disk method. One isolate of *A.baumannii* , which showed resistant to imipenem were checked for MBL production,. MBL was produced by *A. baumannii* 4 , The average zone diameter difference between imipenem disk and imipenem plus EDTA disk for MBL-positive isolate was 25 mm (Figure 8). .In addition ,the present study demonstrate the MBL *A. baumannii* producer isolate also showed resistance to other important groups of antibiotics including third generation cephalosporin, aminoglycoside and quinolone .

In majority ,the result indicate that the percentage of MBL producer isolate *A. baumannii* was 10%

and this was agreement with the study (35)who showed that 22.2% of *Acinetobacter* spp isolated from urine were MBL-producing .`
Metallo-beta-lactamases have been reported worldwide, especially in Asia and western Europe, and confer resistance to all beta-lactams except aztreonam (36) , also the study in the Middle East have been reported of infection caused by carbapenem-resistant *A. baumannii* strains having occurred in the United Arab Emirates, Qatar, Iran and Bahrain (37)
It is important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics. Finally, to understand the epidemiology, there is a need of genetic analysis and also the typing of metallo-β-lactamase enzymes.(38).

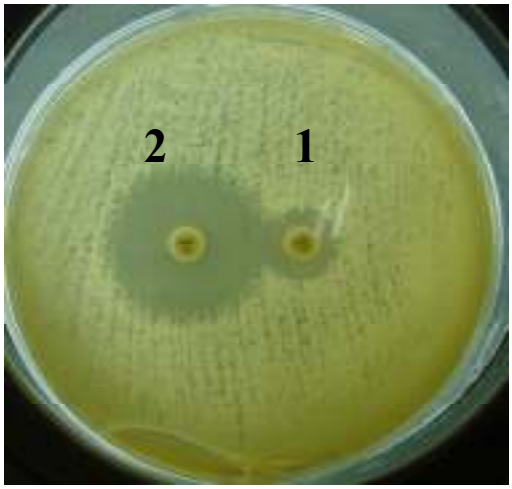

Figure (8):Detection of Metallo-B-Lactamase production in *A.baumannii 4* by

1- Imipenem disk (10 µg).
2- Imipenem disk (10 µg) with EDTA

Isolation of Plasmid DNA :

A. baumannii 4 isolate was selected and detected for their plasmid profiles . The results showed that the isolate *A.baumannii* 4 possesses two small plasmid. (figure 9), this isolate was also β-lactamase producer and resistant to most antibiotic that were used in this study especially the β-lactam antibiotic related to 3GC, such as ceftazidime ,ceftraxone and cefotaxime which are markers for the presence of extended-spectrum β-lactamases. They were also rapid β-lactamase producers within few seconds , in addition the ability of isolate *A. baumannii* 4 to produce Metallo-B-lactamase (MBL).
The production of β-lactamase as well as resistance to β-lactam antibiotics may indicate that these properties may be carried on plasmid that was detected in this isolate.

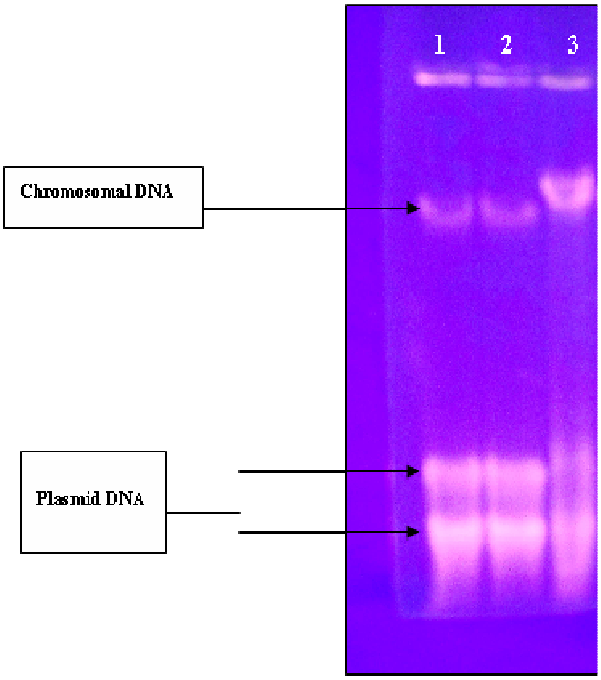

Figure (9) : Agarose gel electrophoresis of plasmid DNA from isolates of *A. baumannii 4* with agarose concentration (0.7%),voltage 5volt/cm,during 1.5hr

Lane 1 and 2 : plasmid profile of isolate. *A baumannii* 4
Lane 3 : plasmid profile of isolate *E.coli*

Bacterial Conjugation

The bacterial conjugation was carried out in order to detect the role of plasmid in transferring of drug resistance as well as metallo β-lactamase. one ESBL producing isolate (*A.baumannii 4*) used which harbor more than one plasmid band, this isolate was considered as donor cells (resistant to imipenem) and the isolate *E. coli* (resistant to tobramycin) as recipient.
Results of Table (4) revealed that the conjugation between the *E. coli* and *A.baumannii* 4 isolate was successful . The conjugation frequency for this transconjugants was ranged from 0.9 x 10^{-5} in isolate *E. coli* .
The transconjugants expressed their antibiotic resistance when they were able to grow in selective medium containing tobramycin (at final concentration of 10 µg/ml) and imipenem (at final concentration of 10µg/ml). The acquisition of tobramycin resistance in recipient cell indicated that this property was plasmid-mediated. These transconjugants were also able to give positive result in metallo β-lactamase production test.

Table (4) :Results of conjugation between metallo ESBl- producing *Acinetobacter* isolate with the *E. coli* isolate .

Isolate	Total No. of		Conjugation frequency
	Transconjugants	Recipient cells	
A. baumannii	2.6×10^{4}	2.8×10^{9}	0.9×10^{-5}

figure (10) shows results of bacterial conjugation of isolate *A.baumannii* 4 and *E coli* which revealed that the plasmid band were transferred from donor to recipient cells. Lane A represents the standard strain *E. coli*,. Lane C represents plasmid profile of metallo and ESBL-producing isolate *A.baumannii* 4 , which possesses two small plasmid . some plasmid were transferred to the recipient cell (lane B) during conjugation, conferring metallo β-lactamase and ESBL production trait to the recipient cell .

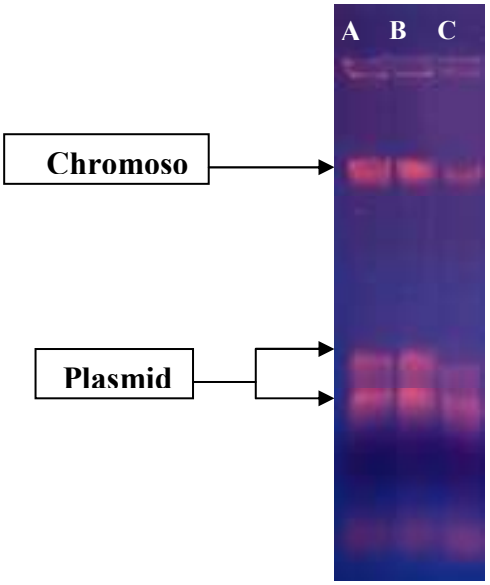

Figure (10) : Agarose gel electrophoresis of plasmid DNA from isolates of *A.bauamnnii* and their transconjugants (in *E. coli*) with agarose concentration (0.7%),voltage 5volt/cm,during 1.5hr Lane A : DNA content of *E. coli* isolate Lane C :Plasmid profile of *A.bauamnnii 4* isolate. Lane B : plasmid profile of transconjugant resulting from conjugation between *A.bauamnnii 4* with *E. coli* isolate .

CONCLUSION

It can be concluded from the results above, that the expression of metallo β-lactamase, ESBL, production, were plasmid-encoded, transferred to the recipient isolate , which received these properties. It is probably that metallo β-lactamase production property , in the clinical isolate, were carried on a non-conjugative plasmid that was transferred by mobilization, by the aid of first plasmid, to the recipient cell. It is also important to know, that the particular plasmid carrying particular property, can be detected by performing transformation process. Because of their transfer among bacterial genera as well as their facilitating transfer of non-conjugative plasmids, the conjugative plasmids are considered very dangerous, being able to confer resistance to β-lactam and very large numbers of other antibiotics. Gene transfer may occur across a very broad host range, such as between Gram-negative and Gram-positive bacteria (39) . (6) refer to metallo β- lactamase genetic determinant are usually associated with class 1 integron structures that may reside on mobile genetic elements ,such as plasmids and transposons .

In another study , Acinetobacter strains were examined for their ability to transfer their resistance to aminoglycosides and third-generation cephalosporins, and their production of beta-lactamases and it was found that similar strains harbor a self-transferable multiresistant plasmid (80 kb) with similar EcoRI and HindIII restriction patterns.This plasmid encodes an extended-spectrum beta-lactamase which confers high- level resistance to third-generation cephalosporins and aztreonam (40) .

The transconjugants in the present study, were detected for their ability to produce β- lactamase enzyme in order to prove that this property is a plasmid-mediated. Results revealed that the transconjugants were able to produce metallic β-lacatamase enzyme .

Trаnsconjugants were also tested using antibiotic susceptibility test to detect the type of plasmid-encoded resistance transferred during conjugation. Results showed that the transconjugants have acquired multiple resistance for different antibiotics compared with the original donor cells.

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Efficacy of Ceftazidime Plus Amikacin as an Initial Empirical Therapy For Adult Patients With Febrile Neutropenia

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ABSTRACT

Empirical broad-spectrum antibiotic therapy remains the cornerstone of treatment for febrile neutropenic patients. This approach has been shown to significantly reduce the morbidity and mortality from severe infection in particular gram-negative bacteraemias.

The aim of this study was to evaluate the efficacy of ceftazidime plus Amikacin as empirical therapy in patients with febrile neutropenia

This study was non-randomized prospective trial conducted in the national center of hematology in Baghdad/ Iraq from May 2009 to March 2010.Fifty-four Adult patients who were treated for acute lymphoblastic leukemia or acute myeloid leukemia and presented with febrile neutropenia included in this study. At start of study all patients received ceftazidime 2g twice daily plus amikacin at a dose of 15 mg / kg twice daily(maximum 20 mg/kg). If fever persisted, a second-line therapy with meropenem was administered. Teicoplanin was given for gram positive isolates or for unremitting fever after 48 hours, if clinically indicated. Amphotericin B was added after 4 days if fever and neutropenia persisted.

Eighty-nine episodes of fever and neutropenia were evaluated in 54 patients. Nineteen (21.3%) of the episodes were documented microbiologically, while 27(30.3 %) of episodes were clinically documented, and 43(48.3 %) were episodes labeled as fever of unknown origin. 35(39.3%) of total episodes showed response to ceftazidime plus amikacin, while 54(60.6%) showed no response and required treatment modification. In all cases of persistent fever, the antibiotics were changed to meropenem within 72 hours. The predominant pathogens isolated in this study were gram-negative organisms (63.1 %).

This study concluded that the combination therapy of ceftazidime plus amikacin is not effective as an initial empirical therapy for adult patients with febrile neutropenia.

Key words: ceftazidime, amikacin, febrile neutropenia

الملخص باللغة العربية

ان العلاج التجريبي بالمضادات الحيوية واسعة الطيف يبقى هو حجر الاساس في علاج المرضى الذين يعانون من حمى نقص عدلات الدم، وقد تبين أن هذا النهج قد قلل كثيرا في معدلات الاعتلال والوفيات الناجمة عن الالتهابات الحادة خاصة المسببة من البكتيريا السالبة.

هدفت الدراسة إلى تقييم فعالية مركب ceftazidime مع Amikacin علاج تجريبي للمرضى من قلة العدلات في مستوى الدم. حيث أجريت في المركز الوطني لأمراض الدم من مايو 2009 إلى مارس 2010، وشملت المرضى البالغين الذين كانوا يعانون من سرطان الدم الليمفاوي الحاد أو سرطان الدم النخاعي الحاد والذين يعانون من نقص شديد في عدلات الدم، وأعطي المرضى cftazidime بجرعة 2 جم مرتين يوميا بالإضافة إلى جرعة من Amikacin قدرها 15 ملغ/ كغم مرتين يوميا وكحد أقصى 20 ملغ/كغم إذا استمرت الحمى، ويعطى خط العلاج الثاني الميروبينام، أما علاج التايكوبلانين فأعطي للمرضى الذين عانوا من بكتيريا موجبة أو للحمى المتواصلة بعد 48 ساعة، وغذا استدعى ذلك سريريا، وأضيف علاج الأمفوتيراسين B في حال استمرار الحمى لمدة اربعة ايام دون تحسن.

جرى تقييم 89 نوبة من حمى مع نقص عدلات الدم في 54 مريض . وقد تم توثيق19 نوبة (21.3%) من النوبات بالطرق الميكروبيولوجية، في حين تم توثيق 27 نوبة سريريا (30.3٪) ،و 43 نوبة (48.3 %) وصفت بأنها حمى مجهولة المنشأ .وأظهرت هذه الدراسة ان (39.3 %) 35 من مجموع النوبات قد استجابت للسفتازديم مع الأميكاسين، في حين أن(54 %) 60.6لم تبد استجابة وتطلب تعديل العلاج .في جميع حالات الحمى المستمرة ، تم تغيير المضادات الحيوية للكل الى مضاد الميروبينم في غضون 72 ساعة .كانت مسببات المرض المعزولة الغالبة في هذه الدراسة هي البكتيريا السالبة الجرام (63.1%)

خلصت هذه الدراسة الى ان جمع علاج السفتازديم مع الامكاسين ليس ذو فعالية كبيرة لعلاج الحمى مع نقص عدلات الدم لمرضى سرطان الدم الحاد.

INTRODUCTION

Empirical broad-spectrum antibiotic therapy remains the cornerstone of treatment for febrile neutropenic patients. This approach has been shown to significantly reduce the morbidity and mortality from severe infection in particular gram-negative bacteraemias (1). The guidelines of the Infectious Diseases Society of America (IDSA) published in 2002 recommend either monotherapy with cefepime, ceftazidime, a carbapenem, or duotherapy with an antipseudomonal ß-bactam antibiotic in combination with an aminoglycoside as empirical antimicrobial therapy in febrile neutropenic patients.(2)

Patients with leukemia who become deeply neutropenic as a result of intensive myelosuppressive chemotherapy are at high risk of developing life-threatening infections, and unless they are treated at the first sign of infection, the rate of mortality is high.(3) Combinations of antibiotics, for example, a third generation cepholosporin plus an aminoglycoside, have been preferred as they may provide broad coverage, have high levels of bactericidal activity, and have potential synergistic effects, and there is the possibility that they protect against the development of resistance. Predominantly, the combination of ceftazidime plus amikacin has been established as a standard regimen. (4) Despite extensive clinical studies since the 1970s, no single empirical therapeutic regimen for the initial treatment of febrile patients with neutropenia has been recommended. The results from previous studies are often not comparable, because the definitions of infectious diseases and the criteria used to assess the response to therapy vary considerably (5, 6). This study was designed to evaluate the efficacy of the standard combination regimen of ceftazidime plus amikacin as empiric treatment in leukemic patients with febrile neutropenia.

PATIENTS AND METHODS

Eligible patients for the study included all febrile neutropenic adults who had been treated for either acute lymphoblastic leukemia or acute myeloid leukemia, from May 2009 to March 2010, at the national center of hematology, Almustansiriya University in Baghdad / Iraq.

All patients were more than 16 years of age with fever (≥38.5ºC once or ≥38ºC at least twice after an interval of four hours) and neutropenia (absolute neutrophil count [ANC] ≤0.5 109/L) were included. All patients enrolled in this study were informed about the investigation details of the study and gave their written informed consent. To include a patient additional times in the scheduled regimen therapy of this study, at least 7 days had to pass after recovery from the prior episode. Patients were excluded if they had been exposed to antibacterial agents (apart from those on prophylactic regimens) within the preceding 48 hours. Patients with end-stage underlying disease in whom bone marrow recovery was not anticipated, with a serum creatinine >250 mmol/L, severe hepatic disease – defined as an increase (>5 times the upper limit of normal) in the level of serum aspartate aminotransferase, or serum alanine aminotransferase. had a known hypersensitivity to one of the study drugs.

Prior to the initiation of therapy, a complete history was obtained, physical examination done by specialist phsician, routine chest X rays, and extensive routine laboratory tests including a urine culture and two sets of blood cultures (from different venipunctures) were performed. Other cultures were taken as clinically indicated. Follow-up studies including hematological analysis, blood chemistry, coagulation tests, and urinalysis were performed as required by the study protocol. Bacteria were isolated and identified by standard techniques at the Department of Microbiology of Alyarmouk hospital or Department of microbiology of the national center of hematology and were tested for antimicrobial susceptibilities by the Kirby-Bauer disc diffusion method according to recommendations of the National Committee for Clinical Laboratory Standards.(7)

The Febrile episodes were divided into 3 groups: a) microbiologically defined infection, when bacteraemias were verified or cultures showed growth from a site of infection; b) clinically defined infection, when a suspected site of infection such as cellulitis was identified without microbiological confirmation; and c) fever of unknown origin (FUO) or unexplained fever, when no site was identified and no microbiological evidence of infection was found according to previously-published definitions(8).Responses to therapy were divided into 3 categories: a) success without modification – if fever and clinical signs and symptoms (whenever present) resolved and the infecting microorganism (whenever isolated) was eradicated without recurrence of the signs and symptoms of the primary infection for at least 5 days after completion of the initial regimen; b) success with modification – if the initial infection was successfully eradicated with the primary therapy, but a second infection arose that fell outside the spectrum of the initial regimen and thus required the addition of another

antimicrobial (i.e., antifungal, antiviral, or antiparasitic); c) failure – if there is the lack of response requiring addition to, or change in the initial regimen or death due to infection. Total success referred to success with or without modification

Patients received intravenous ceftazidime (2 g every 12 hours i.v over 10 minutes) plus amikacin at 15 mg/kg/day, (maximum 20 mg/kg/day) twice daily infusion over 30 minutes. Patients were re-evaluated at 48 hours after the initiation of the antibiotics. In cases of non-response, i.e. persistent fever > 38.0 ºC, or clinical deterioration, the antibiotics were changed to meropenem. When a resistant pathogen was isolated, the antibiotic therapy was individually adapted, depending on the antimicrobial susceptibility test results of the isolated strain. Amphotericin B was added at 96 hours if fever and neutropenia persisted. Patients responding to the antibiotics would continue to receive the antibiotics until resolution of fever for at least four consecutive days. Thereafter, the antibiotics were discontinued, regardless of the duration of neutropenia. Teicoplanin was added for gram-positive isolates or for unremitting fever after 48 hours, if clinically indicated.

Nephrotoxicity and hepatotoxicity were defined as a rise in serum creatinine, transaminases, bilirubin, or alkaline phosphatase by at least twice the upper limit of the normal range. Blood chemistries were measured at least three times per week during the antibiotic therapy. Assessment of ototoxicity was done at the bedside, evaluating for vertigo with vomiting, nystagmus, and tinnitus as signs of inner ear dysfunction. Adverse effects were recorded and assumed to be probably antibiotic-related, if they occurred in the absence of other toxic agents or predisposing factors. The study data were analyzed using the Statistical Package for Social Science for Windows version 15

RESULTS

Eighty-nine episodes of fever and neutropenia were evaluated in 54 patients. Nineteen (21.3%) of the episodes were documented microbiologically, while 27(30.3 %) of episodes were clinically documented, and 43(48.3 %) were episodes labeled as fever of unknown origin. Only 35(39.3%) of total episodes showed response to ceftazidime plus amikacin, while 54(60.6%) showed no response and required treatment modification. In all cases of persistent fever, the antibiotics were changed to meropenem within 72 hours. The predominant pathogens isolated in this study were gram-negative organisms (63.1 %).

Table 1 shows the characteristics and demographic data of the 89 evaluable episodes in leukemic febrile neutropenic patients. Of the 89 episodes, 19 (21.3%) were regarded as MDI with or without bacteraemia, 27 (30.3%) as CDI, and 43 (48.3%) as FUO. The most frequent CDI was cannula site infection (9/27), then chest infection (8/27), followed by urinary tract infection (5/27), mucositis (3/27). Otitis media (2/27), and of the 19 episodes of MDI, 12 were gram-negative bacteria, and 7 were gram-positive bacteria. Table 2 shows the bacteria isolates with the results of antimicrobial susceptibility testing as follows : 4/7 (57%) of gram-positive bacteria (one not tested) showed in vitro susceptibility to ceftazidime plus amikacin respectively, while 8/12 (67%) of gram-negative bacteria showed in vitro susceptibility to ceftazidime plus amikacin.

The response rate achieved without a need for treatment modification was 35(39.3%) of total episodes, while 54(60.6%) showed no response and required treatment modification. The causes of failure in 54 (60.6%) episodes included, persistent or relapsing fever after 48 hours in 35 (39.3) episodes, culture and sensitivity results in 8 (9%) episodes, fungal sepsis in eight (9%) episodes, perianal abscess in one (1.1%) episode, and death due to overwhelming septicaemia in 2 episode (2.2%). Overall, second-line therapy with carbapenem was chosen to treat 38 (70.3%) out of 54 episodes, a triple antibiotic combination (piperacillin-tazobactam plus amikacin and teicoplanin) was chosen in twelve episodes (22.2%), and a combination of two antibiotics and antifungal (piperacillin-tazobactam plus amikacin and antifungal) was chosen in four episodes (7.4%). 12 febrile episodes that received carbapenem needed addition of empirical antifungal treatment due to persistent fever. Mild gastrointestinal intolerance occurred in 15 out of 89 (13.4%) episodes. Transient skin rash occurred in one patient. Side effects were generally mild and did not lead to any discontinuation of treatment. During this study, neither nephrotoxicity nor hepatotoxicity was detected.

Table (1): clinical characteristic of patients and febrile episodes

	Patients	Febrile episodes
Total no.	54	89
Median age (years)(range)	24(15-72)	
Gender(male/female)	51/38	
Cause of neutropenia no. (%)		
Acute lymphoblastic leukemia	22(40.7)	37(41.5)
Acute myeloid leukemia	32(59.2)	52(58.4)
G-CSF added no. (%)		
Yes	50(92.5)	
No	4(7.5)	
Prophylaxis antibiotic no. (%)		
Yes	51(94.4)	
No	3(5.6)	
Median ANC at presentation x 10^9 (range)		0.3(0.1-0.5)
Episode classification no. (%)		
Microbiologically documented		19(21.3)
Clinically documented		27(30.3)
Fever of unknown origin		43(48.3)

G-CSF:granylocyte-colony stimulating factor; ANC:absolute neutrophil count

Table(2) Causative microorganisms isolated from 19 febrile episode

Microorganism	No. of isolates	Ceftazidime sensitive	Amikacin sensitive
Escherichia coli	3	1/3	3/3
Staphylococcus aureus	2	1/2	2/2
Pseudomonas aeroginosa	3	2/3	3/3
Klebsiella pneumoniae	1	1/1	1/1
Enterobacter spp.	2	1/2	2/2
Bacillus	1	1/1	1/1
Streptococcus pneumoniae	2	1/2	1/2
Enterococcus faecalis	1	1/1	1/1
MRSA	1	n/a	n/a
Acinetobacter spp.	1	1/1	1/1
Moraxella	2	2/2	2/2
total	19		

NA: not available; MRSA: methicillin-resistant *Staphylococcus aureus*

DISCUSSION

The use of broad-spectrum antibiotics in combination with aminoglycosides as empirical treatment for febrile neutropenic episodes has been well-documented in various published studies (9).In our center, the combination of ceftazidime and amikacin has been the standard empirical antibiotics therapy. However, a rising number of resistant gram-negative and positive pathogens to the third generation cephalosporin were alarming after its prolonged use or irrational use.

Ceftazidime was considered promising therapeutic option for empirical treatment as a component of combination therapy. It has a wide range of activity against gram-positive pathogens and gram-negative pathogens. (10). this study, was designed to evaluate the efficacy of this combination as empirical therapy for febrile neutropenic patients. In this study, in vitro susceptibility test showed that only 57% of gram positive and 67% of gram negative was sensitive to ceftazidime plus amikacin which agreed with the study of Cometta *et al* in which they stated that sensitivity for gram positive and negative for this combination were 35%, 62% respectively. Regarding response to treatment, this study showed that this combination has inferior results (treatment failure 60.6%) in which the antibiotics were changed to carbapenem within 72 hours in contrast to other studies which showed higher response rate to ceftazidime plus amikacin and the probable cause for this difference is the irrationals use of antibiotic here in our hospital with no précised recommendation to decrease the emergence of antibiotic resistance (12) Comparing the results from the present study with those of other studies may be inappropriate, mainly because of considerable variations in the duration of neutropenia, the definitions of infectious diseases, the criteria used to assess the response to therapy, and the local patterns of infection and antibiotic susceptibilities (13,14)

In the past decade, many cancer centers had experienced a major change in the etiology of bacterial infections occurring in neutropenic patients. While gram-negative bacteria were predominant in the 1970s and early 1980s, gram-positive bacteria have recently increased in frequency, and seem to have largely superseded gram-negative bacilli in many institutions (4,11,15). Contributory risk factors for the increased incidence of gram-positive infections in neutropenic cancer patients include the frequent presence of oropharyngeal mucositis following intensive cytotoxic chemotherapy, altered skin integrity due to long-term indwelling vascular catheters, or cannula and the use of quinolone prophylaxis (16) In contrast to previous studies that reported predominance of gram-positive organisms isolated in neutropenic cancer patients , gram-negative bacteria were predominant in the present study, accounting for 63.1% (12/19) of monomicrobial infections. This may in part be attributable to avoiding the use of quinolone prophylaxis in our patients with limited number of patients in our unit use a central venous line. In fact, a more parsimonious use of quinolone prophylaxis has already been accompanied by a reversal of the gram-positive shift, with the re-emergence of a larger number of gram-negative infections in several centers (17)

In this study, antimicrobial modifications were required in 60.6% of 89 episodes. As in other studies, the modification of antimicrobial therapy in our study was more often indicated in patients with MDI or CDI than in FUO. About 39.3% of the treatment modification performed in this study was due to persistent fever longer than 48 hours after initiation of antimicrobial therapy. This could be due to the more stringent definitions of failure of empirical therapy used in this trial. In many trials, the evaluation of response is usually performed at 72 hours. (18)

Safety profiles of this combination were generally well tolerated and adverse effects, when present, were mild, easily tolerated, and reversible.

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Table (3) : comparison of previous studies with the present study

Author	year	No.of patients With FN	Results of study	Predominant Micro-organism
Cometta et al	1994	696	Pipracillin+amikacin more Effective than ceftazidem+ Amikacin	Gram +ve
Giamarellou et al	2000	263	Monotherapy not indicated in FN, ceftazidem + amikacin had inferior result in FN with Gram – ve bacteria	Gram +ve
Serefhanoglu et al	2006	60	Ceftazidem, pipracillin, and meropenem when combined with amikacin are equally effective	Gram – ve
Mamidah et al	2007	55	Cefepime + amikacin had good efficacy and tolerance	Gram – ve
Alwan et al	2011	54	Ceftazidem+ amikacin had inferior result	Gram – ve

In conclusion, ceftazidime when combined with amikacin was not equally effective as empirical treatment for high-risk febrile neutropenic patients. The predominant pathogens isolated in this study were gram-negative organism

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Microbiological Study of Drinking Water Supply in Al Yaduda Well in Jordan

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ABSTRACT

Jordan regards as one of the poorest country in water resources, most water supply from wells. Al Yaduda well is important supply with fit water for the capital Amman city. Water contamination by pathogenic bacteria is the major problem in developing countries. Several outbreaks causes by water contaminants had been reported in Jordan and neighborhood countries. For this purpose, 50 samples from drinking water supply from Al Yaduda well were collected and assessed by using lactose fermentation broth for MPN to exclude Coilform bacteria, MacConkey , Sabouraud media and nutrient agar to identify bacterial isolates. 26 percent of water samples were identified having bacterial growth. The identified types of bacteria were namely genera, *E. coli*, *S.aureus*, Bacillus species and yeast.

الملخص باللغة العربية

تعد المملكة الاردنية الهاشمية من الدول الفقيرة بمصادر المياه، فمعظم المياه يتم التزود بها من الآبار، ويعتبر بئر اليادودة من أهم مصادر تزويد المياه الصالحة للاستهلاك للعاصمة عمان.

يشكل تلوث المياه بالبكتيريا المرضة مشكلة حرجة للدول النامية، وقد تم تسجيل الكثير من الاكتشافات حول مسببات تلوث المياه في الاردن والدول المجاورة.

ولهذا الهدف، فقد تم جمع 50 عينة من المياه من بئر اليادودة المزود لمياه الشرب وتم عالجتها باستخدام Lactose Fermentation Broth لقياس MPN لاستبعاد بكتيريا Coliform ، كما تم استخدام الأوساط التالية: MacConkey , Sabouraud media and nutrient agar لتحديد أنواع البكتيريا المعزولة من العينات.

وقد أظهرت نتائج الدراسة أن نسبة 26% من عينات الماء وجد بها نمو بكتيري، كما ظهرت أنواع البكتيريا التالية من العينات : *E. coli*, *S.aureus*, Bacillus species , yeast

INTRODUCTION

Water contaminated with microbiological and chemical constituents can cause a variety of disease. Water intended for human consumption should be safe, patable and aesthetically pleasing. Water sources have different qualities influenced by natural or anthropological pollution. The major contaminated water pollutants are pathogenic microorganisms, metals and chemicals which make the water non drinkable (1, 2). Every 8 seconds, a child dies from water related disease around the globe (3). 50% of people in developing countries suffer from one or more water-related diseases. 80% of diseases in the developing countries are caused by contaminated water(3). Now days the concern is increasing due to outbreaks of cryptosporidiosis (4, 5), hepatitis (6), giardiasis (5) and coliform bacteria (7, 8). Coliforms are not a single type of bacteria, but a grouping of bacteria that includes many strains, such as E. coli. They are ubiquitous in nature, and many types are harmless. Therefore, it is not definitive that coliform bacteria will cause sickness (9, 10). The occurrence of Coliforms bacteria in the water indicate that water possible contaminated, the total coliforms are standard by which microbial contamination is measured (9). Therefore, the presence of Coliform bacteria does not necessarily mean that pathogenic microbes are also present. However, if large Coliform quantities are detected, the presence of other microbes should be checked (11).

During 2007 thousands of Jordanian has been suffered from illnesses related to water contamination in village and town across the Kingdom (12,13). Again, at the summer of same year, nearly 1000 people from village near northern city of Mafrag suffered from severe diarrhea and high fever due to contamination of water with Cryptosporidium (13). Several outbreaks occur during the recent years due to contamination of water supply, but the health authorities tried to blame a small local restaurant for selling spoiled food.

Jordan is considered as one of the four most water scarce countries in the World (14,15). The limited water resources are exposed to pollution. Population growth is expected to increase the pressure on available water resources. Conventional water resources in Jordan consist of groundwater and surface water. Twelve groundwater basins have been identified in Jordan (16).

Al Yaduda well supply almost western Amman area located at Airport road, were not subjected to such study for detection of unpleasant microbes in the water. The goal of this study is to detect microorganisms in chlorinated water distributed to restaurants, hotels and houses.

MATERIALS AND METHODS

Sample Collection

Fifty Samples were collected for microbiological quality analyses. Sampling was done aseptically into sterile plastic bottle. All the samples were properly labeled and accompanied by complete and accurate data (including the date and time of collection, and the type of source of the sample) of each sample. Prior to filing, the sample bottles had rinsed out two or three times with water to be collected. Microbiological analyses were conducted within 3 hours after the collection of samples (17).

The Most Probable Number (MPN)

The presumptive test is a specific for detection of coliform bacteria. Measured amount of water to be tested, sample was applied to lactose fermentation broth (17,18) containing an inverted gas vial (Dirham tube) and incubated at 37 ^{0}C for 24 hours. Because these bacteria are capable of using lactose as carbon source while other enteric organisms are not, their detection is facilitated by use of this medium (18).

Isolation and identification of microorganisms

MPN culturing tubes show turbidity was selected for cultivation on Nutrient agar, Sabourada and MacConkey agar. After 24 hours of incubation at 37C, colonies of different colors from different samples were isolated. Then these colonies from each sample were counted and then picked up and streaked on nutrient agar plates to obtained pure culture. Distinguishably, there were three different color colonies Yellow smooth colonies, Creamy, large rounded colonies and small pink colonies. Colonies were examined after incubation on plates for 24 hours at 37C. Biochemical tests were performed to identification of bacteria (19, 20).

Protozoa test: A drop of water from Al-Yaduda well sample put on a clean slide than adds a drop of iodine to the slide of water sample and covered with cover slide. The sample was examined under microscope (5).

RESULTS AND DISCUSSION

The current study reveals the contamination of chlorinated drinking-water supplies from Al_Yaduda well in Amman region of Jordan. This finding confirm the previous studies done in Zarqa area (9) and different areas in Jordan (2). Out of 50 samples, only 13 samples show positive reaction when examined by MPN in table (1). This percent regards as high percent (26%) and gives cause for consideration alarm, because the source of contamination belong to faecal coilform and other bacteria, on other hand that need intensive efforts to prevent contamination of ground water.

Table (1). The MPN of water samples taken from Al- Yaduda Well

Sample No.	No. of positive tubes out of 5	MPN + / 100	Sample No.	No. of positive tubes out of 5	MPN + / 100
Y1	0	<1.1	Y26	0	<1.1
Y2	1	1.1	Y27	0	<1.1
Y3	0	<1.1	Y28	0	<1.1
Y4	1	1.1	Y29	1	1.1
Y5	0	<1.1	Y30	0	<1.1
Y6	0	<1.1	Y31	0	<1.1
Y7	1	1.1	Y32	0	<1.1
Y8	0	<1.1	Y33	0	<1.1
Y9	0	<1.1	Y34	0	<1.1
Y10	0	<1.1	Y35	0	<1.1
Y11	0	<1.1	Y36	1	1.1
Y12	0	<1.1	Y37	0	<1.1
Y13	3	3.6	Y38	1	1.1
Y14	2	2.4	Y39	0	<1.1
Y15	2	2.4	Y40	0	<1.1
Y16	0	<1.1	Y41	0	<1.1
Y17	0	<1.1	Y42	0	<1.1
Y18	1	1.1	Y43	0	<1.1
Y19	0	<1.1	Y44	0	<1.1
Y20	1	1.1	Y45	0	<1.1
Y21	0	<1.1	Y46	0	<1.1
Y22	0	<1.1	Y47	0	<1.1
Y23	0	<1.1	Y48	1	1.1
Y24	1	1.1	Y49	0	<1.1
Y25	0	<1.1	Y50	0	<1.1

MPN + : most probable number, , 1*:turbid with gas, 1**:gas, 1***: turbid

Bacterial growth were shown on MacConkey agar, that's mean coliform bacteria were detected. Most isolates were grown on nutrient

agar, mainly gram positive bacilli and cocci. Yeast organisms colonies were detected on Sabourad agar (Table 2). The detection of gram positive bacilli and cocci are indigenous microorganisms in water (1, 17, 19). No protozoa were detected from Al- Yaduda well.
Coliforms are used to indicate the quality of drinking water. A large number of different bacteria, including: *Escherichia, Enterobacter, Klebsiella, Serratia, Citrobacter and Proteus* belong to the total Coliform group. The fecal Coliform group is an under-group of the total Coliforms and has fewer bacteria (10, 18).

Table (2): Identification of Microorganisms isolated from Al Yaduda well

Sample code	Colony morphology	Gram stain	Identification
Y2	Yellow smooth colonies	Gram positive rod	*Bacilli*
Y4	Creamy colonies	Gram positive oval Gram positive rod	*Yeast* *Bacilli*
Y7	White colonies	Gram positive oval	*Yeast*
Y13	Creamy, large rounded colonies	Gram positive oval Gram positive cocci	*Yeast* *Staphylococci*
Y14	Pinkish colonies on MacConkey media	Gram negative rod	*E.coli*
Y15	Pinkish colonies on MacConkey media	Gram negative rod	*E.coli*
Y18	Yellow smooth colonies	Gram positive rod	*Bacilli*
Y20	Yellow smooth colonies	Gram positive rod	*Bacilli*
Y24	White colonies	Gram positive oval	*Yeast*
Y29	Creamy, large rounded colonies	Gram positive cocci	*Staphylococci*
Y36	Pinkish colonies on MacConkey media	Gram negative rod	*E.coli*
Y38	Small white colonies	Gram positive oval	*Yeast*
Y48	Pinkish colonies on MacConkey media	Gram negative rod	*E.coli*

The results demonstrate that collectively all water sources are poor in quality and there is a health risk to use this water without purification, so there is an urgent need to purify water before use, especially drinking water sources need more attention.
Non- point source delivers pollutants indirectly through environmental changes. An example of this type of water is when fertilizer from a field is carried into a stream by rain, in the form of run-off which in turns affects aquatic life. The technology exists for point sources of pollution to be monitored and regulated, non-point sources are much more difficult to control. Pollution arising from non-point sources for a majority of the contaminants in streams and lakes.
An ideal water works management ensures that water supplied for public distribution should be free from pathogenic organisms, undesirable taste and odors should be clear, palatable of reasonable temperature, neither corrosive nor scale-forming in pipes and should be free from minerals which could produce undesirable physiological effects.

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Fas Expression in Colorectal Cancer Tissue of Iraqi Patients and its Correlation to Some Clincopathological Parameters

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ABSTRACT

Colorectal cancer is one of the important public health problems in Iraq. It's considered as the 7th among the commonest 10 cancers by site according to the Iraqi cancer registry. Studying the fas positive expression in colorectal cancer patients and its correlation to some clincopathological parameters was our agenda.

In this study, 50 colorectal cancer samples (paraffin embedded sections) were used to detect fas protein positive expression using Immunohistochemical technique .The correlation between fas positive expression with site, type and grading was also included . The results showed that there was no significant difference ($p<0.05$) between the fas protein positive expression of the cancerous tissue compared to the resection margin which explained as an immune evading mechanism done by the colorectal cancerous cells while in grading there was a significant difference ($p<0.05$) in the correlation between the well and moderately differentiated cancerous cells and not the poorly differentiated one , which may be due to the expression of the mutant type of the concerned protein.

In conclusion, the positive expression of the fas protein and its correlation to some clincopathological parameters can be used as a tool for the prognosis of the colorectal cancer cases.

الملخص باللغة العربية

يعتبر سرطان القولون والمستقيم من المشاكل الصحية المهمة في العراق حيث يقع في المرتبة السابعة من حيث الترتيب للتسجيل السرطاني في العراق. كان هدف الدراسة هو معرفة مستوى التعبير الموجب لبروتين fas في مرضى سرطان القولون والمستقيم ومدى علاقتها ببعض المقاييس السريرية – المرضية.

جمعت 50 عينة لمرضى سرطان القولون والمستقيم لدراسة مستوى التعبير الموجب لبروتين الfas باستخدام فحص كيميائي نسيجي مناعي immunohistochemistry لقوالب الشمع الخاصة بهم وكذلك ارتباط هذا التعبير بموقع ونوع ودرجة التمايز لخلايا هذا السرطان .

أظهرت النتائج فرقا غير معنوي($P<0.05$) في مستوى التعبير الموجب لبروتين fas بين النسيج السرطاني و الحدود المقطوعة والذي قد يكون احد آليات هرب النسيج السرطاني من الخلايا المناعية المعنية بالقضاء عليه ، بينما درجة التمايز للخلايا السرطانية أظهرت فرقا معنويا($P<0.05$) بين الخلايا ذات التمايز الواضح وذات التمايز المعتدل والذي قد يكون سببه أن الجين للfas بروتين قد حدثت به طفرة .

استنتجنا من خلال هذه الدراسة أن قياس التعبير الموجب لبروتين ألfas بالإضافة إلى ارتباطه ببعض مقاييس السريرية– المرضية ممكن إن يستخدم كعامل تقييم مال المرض.

INTRODUCTION

Colorectal cancer is an important public health problem. There are nearly one million new cases of colorectal cancer diagnosed world-wide each year and half a million deaths (1). In Iraq, it's the 7th among the commonest 10 cancers by site according to the Iraqi cancer registry (2).

Fas is a 45,000 transmembranous glycoprotein that is a member of the tumor necrosis factor/nerve growth factor receptor family and is known to be expressed in a variety of cells, including activated T cells and tumor cells (3–4). FasL is a 31,000 type II transmembranous protein that is known to initiate apoptosis in activated T lymphocytes by binding to the Fas transmembranous glycoprotein (4).

In addition to the immune system, the apoptotic cell death of hepatocytes, intestinal epithelial cells, kidney cells, and ovarian cells has been known to be mediated through this Fas-FasL system. Moreover, expression of FasL has also been demonstrated in various types of tumor cells such as human melanoma, hepatocellular carcinoma, esophageal carcinoma, and colonic carcinoma. These findings thus suggest that the Fas- FasL system is involved in the immune privilege of cancerous tissues due to a Fas counterattack against Fas-expressing TILs (5).

The epithelial layer of the normal colonic mucosa expresses Fas protein at high levels, from the bottoms of the crypts to the luminal surface (6). A functional role for Fas receptors in the colon has not yet been demonstrated, as the Fas ligand has not been detected in the colon, except in subsets of lymphocytes in the lamina propria. Expression of Fas protein in the colon is progressively reduced during the transformation of normal epithelium to benign neoplasms, adenocarcinoma and, ultimately, to metastases (7).

The extent of loss was related to the stage of the disease, as only 10% of adenomas exhibited reduced Fas expression compared with 88% of carcinomas. Total absence of Fas was most common in non-mucinous and metastatic tumors. If Fas is required for apoptosis of the colonic epithelial cells, it is possible that loss of Fas activity can contribute to the reduction in apoptotic capacity of colonic carcinomas. (8).

Our aim in this study is to correlate between positive fas expression and some histopathological parameters like: site, type and differentiation in Iraqi colorectal cancer patients.

MATERIALS AND METHODS

Fifty colorectal cancer cases were collected from both digestive system diseases and liver teaching hospital and Baghdad teaching hospital in 2008.

Patients data were taken from the Histopathological reports that was written by a professional histopathologist which concern: site, type and differentiation of the tumor and resection margin which was the comparison to the cancerous tissues and confirmed to be free of malignancy.

Adequate thin paraffin embedded sections (5µm thick) of tumor and resection margins were prepared on positively charged slides for the immunohistochemistry technique which was done by using anti fas monoclonal antibodies from DAKO(Denmark) .

The streptavidin biotin indirect method was employed along with DAB (3 3´diaminobenzidine tetrahydroclorid) as chromogen and the sections were counterstained with hematoxylin. Cytoplasmic staining was accepted as positive for fas protein.

To define the ratio of positivity, 10 fields of each slide was studied under high power magnification (40x) and the percentage of Cytoplasmic positivity for fas was graded as the Shibakita method(9).

Statistical analysis was performed using ANOVA (2 way) with significant difference ($P<0.05$).

RESULTS

Fas positive Expression:

There was no significant difference ($P<0.05$) in the positive of fas expression of the cancer tissue compared to resection margin (Table1) (Figure 1&2).

Table (1): The positive fas expression of the cancer tissue compared to resection margin

	Tissues Fas expr. % Mean+SE	
	Tissue cancer	Res.marg
Positive	A* 13.20±5.53	A 9.16±4.46

* *Differences A, B are significant (P<0.05) to compare rows.*

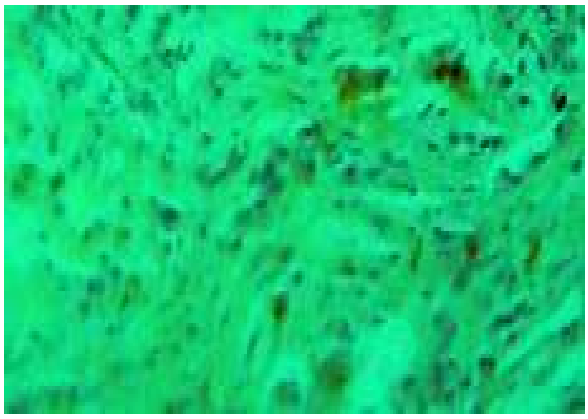

Figure (1): Immunohistochemical staining in colorectal cancer sections. Fas cytoplasmic positive Immunostaining by peroxidase/DAB (brown) counterstained with hematoxylin 40x

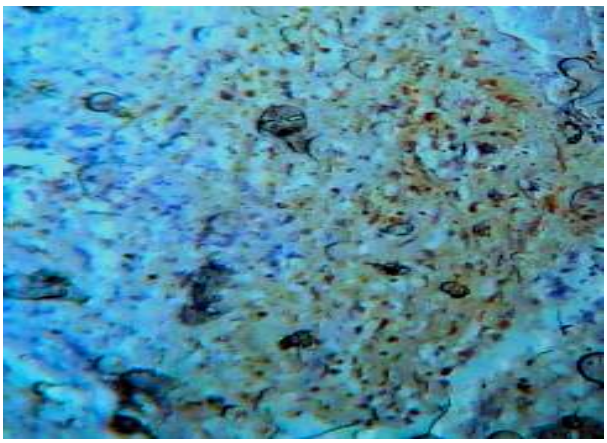

Figure (2): Immunohistochemical staining in colorectal cancer sections. Fas cytoplasmic positive Immunostained by peroxidase/DAB (brown) counterstained with hematoxylin 40x.

Correlation between positive fas expression and the site of the colorectal cancer site:

There was no significant difference (P<0.05) in the positivity of the fas expression between the cancerous tissue and resection margin inside all colon and rectum anatomical sites (Table 2).

Table(2): The positive fas expression in all anatomical sites.

	Tissues Fas expression % Mean+SE Site of Tumor													
	ASC.		CECUM		DESC.		H.FLEX		RECTOSIG.		RECTUM		SIGMOID	
	Ca.	R.m.	Ca.	R.m.	Ca.	R.m.	Ca.	R.m.	Ca.	R.m.	Ca.	R.m.	Ca.	R.m.
+ve Fas	A 7.00 ± 2.12	A 0.0 ± 0.0	A 13.5 ± 1.36	A 7.87 ± 1.44	A 13.6 ± 1.64	A 12.00 ± 1.45	A 12.20 ± 1.01	A 11.4 ± 5.81	A 4.00 ± 1.93	A 0.0 ± 0.0	A 15.64 ± 1.28	A 12.00 ± 7.10	A 14.14 ± 3.23	A 10.43 ± 4.66

Differences A, B are significant (P<0.05) to compare rows, Ca: cancer; R.m.: resection margin.

There was no significant difference (P<0.05) in positive fas expression of the cancerous tissue in all anatomical sits except the ascending and rectosigmoide colon which showed significant difference (P<0.05) compared with the other sites while in the resection margin tissue only the hepatic flexure and the descending colon showed significant difference (P<0.05)(table 3).

Table (3): The correlation between different anatomical sites and fas positive expression.

		Tissue Fas expression % Mean+SE Site of Tumor						
		ASC.	**CECUM**	**DESC.**	**H.FLEX**	**RECTOSIG**	**RECTUM**	**SIGMOID**
Ca.	**Positive**	A 7.00 ± 2.12	B 13.50 ± 1.36	B 13.60 ± 1.64	B 12.20 ± 1.01	A 4.00 ± 1.93	B 15.64 ± 1.28	B 14.14 ± 3.23
R.m.	**Positive**	A 7.0 ± 0.0	A 7.87 ± 1.44	B 12.00 ± 1.45	B 11.40 ± 5.81	A 7.3 ± 0.0	A 7.00 ± 7.10	A 8.43 ± 4.66

Differences A, B are significant (P<0.05) to compare rows, Ca: cancer; R.m.: resection margin.

Correlation between positive fas expression and the colorectal cancer type:

There was no significant difference (P<0.05) in positive fas expression between the cancerous tissue and resection margin inside all the 3 types presented in this study (Table 4).

Table(4): The positive Bcl-2 expression in all types of colorectal cancer.

	Tissues Fas expression % Mean+SE Cancer type					
	AC		**MUCINOUS**		**SIGNET RING**	
	cancer	**R.m.**	**Ca.**	**R.m.**	**Ca.**	**R.m.**
Positive Fas	A 13.49+5.26	A 8.32+4.17	A 12.20+6.35	A 12.80+6.76	A 13.00+2.52	A 7.33+2.70

Differences A, B are significant (P<0.05) to compare rows, Ca: cancer; R.m.: resection margin

While There was no significant difference (P<0.05) in positive fas expression of the cancerous tissue and the resection margin tissue between those 3 types of colorectal cancer(table 5).

Table (5): The correlation between all types of colorectal cancer according to the fas positive expression.

		Tissues Fas expression % Mean+SE Cancer type		
		AC	MUCINOUS	SIGNET RING
Ca.	Positive	A 13.49+5.26	A 12.20+6.35	A 13.00+2.52
R.m.	Positive	A 8.32+4.17	A 9.80+6.76	A 7.33+2.70

Differences A, B are significant (P<0.05) to compare rows, Ca: cancer; R.m.: resection margin.

Correlation between positive fas expression and the grading of the colorectal cancer cells

There was no significant difference (P<0.05) in positive fas expression between the cancerous tissue and resection margin in the 3 different type of differentiation (table 6).

Table (6): The positive Bcl-2 expression in all colorectal cancer grades.

	Tissues Fas expression % Mean+SE Grading					
	MD		PD		WD	
	Ca.	R.m.	Ca.	R.m.	Ca.	R.m.
Positive	A 14.11±5.63	A 10.28±5.15	A 12.30±6.53	A 6.30±3.28	A 7.25±4.50	A 6.25±2.50

Differences A, B are significant (P<0.05) to compare rows, Ca: cancer; R.m.: resection margin, WD: well differentiated, MD: moderately differentiated, PD: poorly differentiated, ca: cancer; R.M.: resection margin.

There was significant difference (P<0.05) in positive fas expression of the cancerous tissue between the poorly differentiated and both well and moderately differentiated while there was no significant difference (P<0.05) in positive fas expression of the resection margin tissue in the 3 different type of differentiation(table 7).

Table (7): The correlation between all colorectal cancer grades concerning positive fas expression.

		Tissues Fas expression % Mean+SE Grading		
		MD	PD	WD
Ca.	Positive	A 14.11±5.63	AB 12.30±6.53	B 7.25±4.50
R.m.	Positive	A 10.28±5.15	A 6.30±3.28	A 6.25±2.50

Differences A, B are significant (P<0.05) to compare rows, Ca: cancer; R.m.: resection margin, WD: well differentiated, MD: moderately differentiated, PD: poorly differentiated, ca: cancer; R.M.: resection margin.

DISCUSSION

Fas-FasL system is one of the human body's defense mechanisms against uncontrolled cancerous cell growth(10). However, most of the tumors escape from the host immune attack by imitating themselves as immune-privileged sites by either overexpression FasL or down-regulating Fas.(9,11,12,13,14,15,16,17,18,19).

So, our results can be explained more deeply according to the genetic point of view, The size and structure of cell populations are controlled by a balance between the rates of cell renewal and death. Physiological cell death normally occurs as a series of distinct morphological and biochemical events termed apoptosis (20).In the colon, apoptosis contributes to the homeostasis of the epithelial layer of the mucosa, which has a rapid rate of cell turnover (21). Apoptosis is also responsible for the removal of colonocytes with potentially oncogenic DNA damage. Recent studies on bile acids, which are genotoxic and normally induce apoptosis of colonocytes, have reported a reduction in the ability of these agents to induce apoptosis in the normal mucosa of colorectal cancer patients (22-23).Resistance of colonocytes to apoptosis may allow hyperproliferation,accumulation of oncogenic mutations and prevent deletion of malignant cells by chemotherapeutic agents. Abnormal expression of the apoptosis-related genes p53 and bcl-2 had been reported in both benign and malignant colon tumors (24-25), however, other molecular mediators of resistance to apoptosis remain to be identified.

Fas mRNA is expressed constitutively in normal colonic mucosa, but Fas gene transcription was reduced in the majority of tumors analyzed. Losses or rearrangements of chromosomes are common in tumor cells and, when accompanied by an inactivating point mutation in the remaining allele, can abrogate the expression of tumor-suppressor genes. We found Fas gene rearrangements and allelic losses to be rare events in colon cancer, suggesting that other mechanisms are responsible for reduced Fas expression in these tumors (26).

Fas gene transcription was reduced in the majority of the informative colon tumors. Fas deletion is not the only mechanism for loss of Fas gene expression. In the tumors of patients with LOH, disruption of the remaining wild-type Fas allele by a point mutation or epigenetic mechanisms may explain the complete loss of Fas expression (26).

The lack of detectable deletions or other rearrangements in the Fas gene in colonic tumors suggests that the gene may be transcriptionally silenced during colon cancer progression. There are recognition sequences in the Fas promoter for a number of transcriptional proteins including p53, c-myb and NF-KB (27); the loss of any of which may prevent Fas expression. The promoter region of Fas is GC rich and contains a number of CpG dinucleotides. De novo methylation of CpG islands often occurs in tumors and has been associated with epigenetic silencing of tumor-suppressor genes. Future studies in this laboratory will examine the patterns of CpG methylation in the Fas gene promoter in colonic carcinomas, as a possible mechanism by which Fas gene transcription is reduced (27). Recently, cellular FLICE-like inhibitory protein (cFLIP) has been identified as an endogenous inhibitor of Fas- or other receptor-mediated apoptosis and its altered high expression has a suspected association with tumor development or progression (28). In an effort to investigate the prevalence of $cFLIP_L$ alterations in colon carcinomas and their possible implications for the progression of colon cancers, $cFLIP_L$ expression was analyzed in adenocarcinomas and adenomatous polyps of colon, with matched normal tissues, at RNA and protein levels, by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry (29).

$cFLIP_L$ transcripts were constitutively expressed in colon cancers and expression levels were significantly higher in carcinomas than in normal tissues. Overexpression of $cFLIP_L$ protein was found exclusively in carcinoma cells in all matched sets analyzed and approximately three-fold induction was detected in cancer cells. The expression of $cFLIP_L$ protein was not significantly altered in adenomatous polyps compared with normal tissues (29).

Most of the articles concerning positive fas expression and colorectal tumor site, type and grading association mentioned no correlation

(27-29),While in our study the correlation existed between different sites especially between the Ascending colon and rectosigmoide which can be explained that the cancers at or below the peritoneal reflection (rectosigmoide/rectum) are more aggressive compared with those above the reflection(colon) and the worse prognosis for patients with lesions in the right colon (30). While grading showed significant difference ($p<0.05$) between well differentiated and moderately differentiated cancerous cells in fas expression which can be explained that it's the expression of the mutant type of fas protein and not the wild type.

CONCLUSION

We conclude that the positive expression of the fas protein and its correlation to some clincopathological parameters can be used as a tool for the prognosis of the colorectal cancer cases.

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Skin Cancer and Cutaneous Blastomycosis

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ABSTRACT

Blastomycosis is a pulmonary disease caused by inhaling spores of the dimorphic fungus *Blastomyces dermatitidis*, occasionally, the fungi spread hematogenously, causing extrapulmonary disease. Cutaneous blastomycosis are common on the face, extremities neck and scalp as the infection spreads from the lungs to other parts of the body. Skin neoplasms are skin growths with differing causes and varying degrees of malignancy.. Basal-cell carcinoma is the most common type of skin cancer. This study was conducted to relate between cutaneous blastomycoses and skin cancer, we found that there were two cases of blastomycoses with basal cell carcinoma.

Key words: Blastomycosis, skin cancer

الملخص باللغة العربية

مرض blastomycosis يكتسب من خلال استنشاق الهواء الملوث بسبورات الفطر الثنائي الشكل. المرض الجلدي يحدث عادة في الوجه والاطراف والرقبة وفروة الراس وينتشر عن طريق الرئتين او بواسطة الدم. سرطان الدم يحدث نتيجة النمو المفرط ولاسباب كثيرة وبدرجات مختلفة. سرطان الخلايا الجلدية القاعدية هو من الامراض الشائعة. من خلال هذه الدراسة تم الربط بين المرضين الجلديين ووجدت هنالك حالتان من الاصابة بالنوعين في نفس الوقت.

INTRODUCTION

Blastomycosis is an infection caused by inhaling microscopic particles (spores) produced by the fungus *Blastomyces dermatitidis.* Infection appears to begin in the lung and, in acute cases, may resolve without spread to other organs, infection is usually progressive, either in the lung or in sites of hematogenous spread. Skin, bone, and the genitourinary tract are the most common sites of hematogenous dissemination (1). In North America, the endemic area for blastomycosis includes Ohio–Mississippi River valleys (extending into the middle Atlantic and Southeastern states), Northern Midwest, Upstate New York
Southern Canada. Infection also occurs in the Middle East and Africa(2).

Cutaneous blastomycosis are common on the face, extremities neck and scalp as the infection spreads from the lungs to other parts of the body one or many lesions may develop, lesions begin as papules, pustules or as subcutaneous nodules, within weeks to months the lesions develop into ulcers and form crusty sores, over a period of months to years lesions grow larger and heal to form raised wart-like scars, lesions may cover much of the face causing severe disfigurement, and irreversible scarring often occurs (3). The skin lesions caused by primary cutaneous blastomycosis are similar in appearance to those caused by disseminated pulmonary infection (4).

B. dermatitidis has been found in moist soil with a low pH, especially soil that is enriched with organic debris such as decaying vegetation, rotting wood and animal excreta (high nitrogen content)(5; 6; 7; 8; 9). Acquisition of blastomycosis usually occurs by inhalation of airborne spores and in rare cases by traumatic inoculation through the skin (10; 4). Exposure to soil has been the most commonly identified risk factor for infection during outbreaks (11).

Basal-cell carcinoma is the most common type of skin cancer. It rarely metastasizes or kills. However, because it can cause significant destruction and disfigurement, it is still considered malignant by invading surrounding tissues. Statistically, approximately 3 out of 10 Caucasians may develop a basal-cell cancer within their lifetime(12). About two thirds of basal-cell carcinomas occur on sun-exposed areas of the body. One-third occur on areas of the body that are not exposed to sunlight, emphasizing the genetic susceptibility of basal-cell cancer patients (13), leads to an increase of free radicals in the skin, if applied in too little quantities and too infrequently(14) . Patients present with a shiny, pearly nodule. However, superficial basal-cell cancer can present as a red patch like eczema. Infiltrative or morpheaform basal-cell cancers can present as a skin thickening or scar tissue – making diagnosis difficult without using tactile sensation and a skin biopsy. It is often difficult to distinguish basal-cell cancer from acne scar, actinic elastosis, and recent cryodestruction inflammation(15).

MATERIALS AND METHODS

Patients:

Skin biopsies were obtained from one hundred patients with verrucous, ulcerative and noduler lesions were collected from patients attended Al-Kadhumyia teaching hospital and Baghdad teaching hospital to Dermatology department, from. Thirty six (36) were females and sixty four (64) were males, with a mean age of 48.66 years (ranging between 10-80 years old). And the median that divided the age into two equal parts was 47.
Fifty two (52) of patients with ulcerative lesions, forty six (46) with verrucous and two (2) with noduler ones.The diagnosis was established by clinical examination, done by Dermatologist.

Controls:

Controls included skin debris of 10 apparently healthy individuals were randomaly selected from patients attended Al-Kadhumyia teaching hospital to Dermatology department as (8 women and 2 men) with a mean age of 30.3 years (range between 20-40 years old).

Baseline data:

Base line data were obtained from routine history & clinical examination the informations arranged in a questionnaire including: name, age, sex, type of lesion and site of lesion.

Samples collection:

Samples collaction were included skin biopsies from patient and skin debris from control, skin biopsies have been taken from the site of lesion(16) then divided into two parts, one put in 10% formalin and send for histopathology to make paraffin embedded tissue (blocks) Tissue skin biopsies were embedded in paraffin then sectioned for staining with periodic acid Schiff stain (PAS)

and the other part put in sterile normal saline and immediately cultivated on a suitable media (Sabouraud's Dextrose Agar).

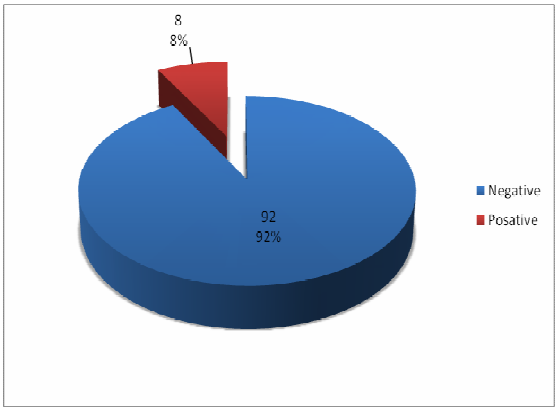
RESULTS

A total of one hundred patients had been included in the present study, their ages were ranging from 10 to 80 years, with a mean of (48.66 ± 0.278 years), and the median divided the age into two equal parts was 47. Males were 64 and females were 36 (64.00% and 36.00% respectively) had been distributed to eight which are the more frequent age groups between (31-40) .
Controls included skin debris of 10 apparently healthy individuals, their ages were ranging from 20 to 40 with a mean of (30.30 ± 2.088 years). Only two males had been included in this group, table (1) .

Table (1): Age of individuals involved in the study

Persons	No.	Age		
		Minimu	Maximum	Mean± Std.Error
Patients	100	10.00	80.00	48.66 ± 0.278
Control	10	20.00	40.00	30.300± 2.088

Only 8(8%) cultivated skin biopsies out of 100 gave positive results (growth of *Blastomyces dermatitidis)*, figure (1).



Figure(1): Distribution of Blastomycotic patients according to the skin biopsies culture results

According to the type of lesion, the patients distributed into three groups of clinical presentations which are :-
a. verrucous lesions
b. ulcerative lesions
c. noduler lesions

The most frequent types of lesions were the ulcerative ones, figure (2 and 3).

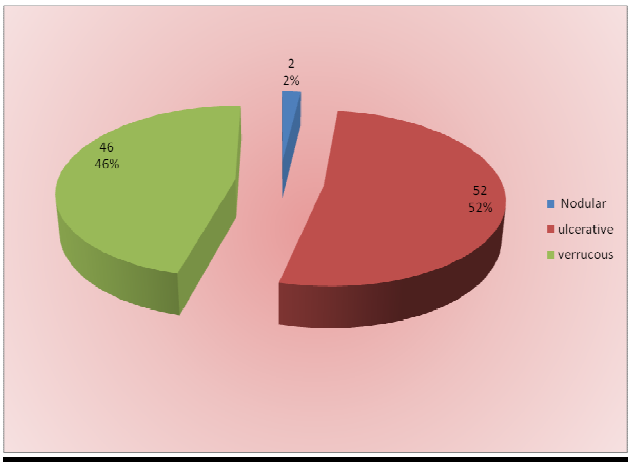
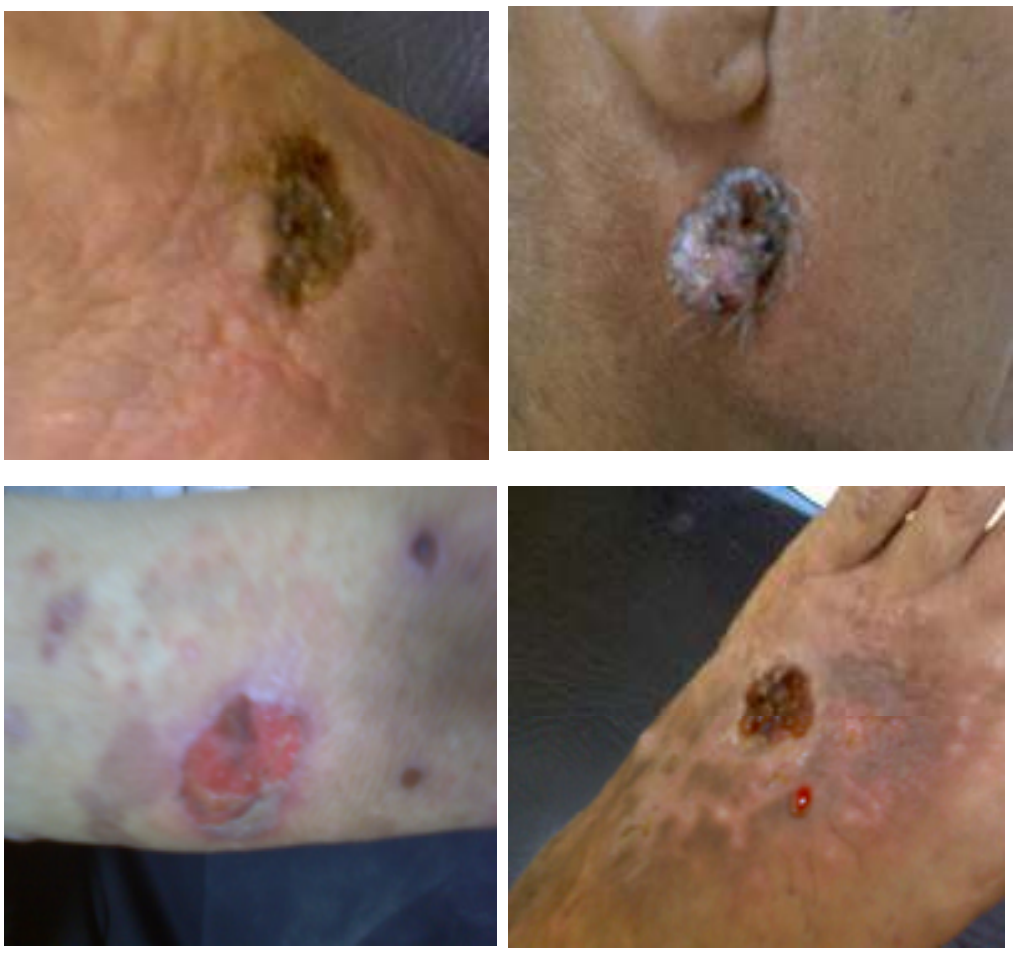


Figure (2): Clinical presentation frequency in patient' s group

Figure (3) shows different types of *Blastomyces dermatitidis* according to the type and the site of infection.



Figure(3) : Gross appereance of different lesions from different sites of skin. A (ulcerative lesion in foot). B (verrucous lesion in face). C (ulcerative lesion in abdomen). and D (ulcerative lesion in foot).

According to histopathology only eight samples (8%) out of 100 were positive, revealed (budding yeasts), which were the same of that culture results.

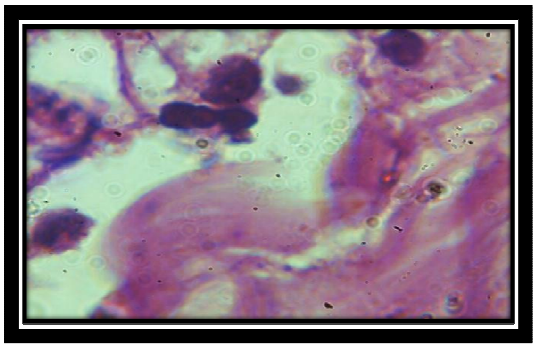


Figure (4): Histopathological preparations with (PAS)reveales the characteristic budding yeasts of *B.dermatitidis* in human skin tissue.

A 51 and 75 years old males with verrucous and ulcerative lesions, respectively, one in the face and the other on his leg, both showed skin cancer (basal cell carcinoma) superimposed with blastomycosis.

DISCUSSION

Blastomycosis is caused by a thermally dimorphic fungus (*Blastomyces dermatitidis*), found in soil especially soil that is enriched with organic debris such as decaying vegetation, rotting wood and animal excreta (high nitrogen
content) (11; 17; 5; 18, 19). This disease has also been referred to as Gilchrist's Disease, North American Blastomycosis, Chicago Disease and Namekagon River Fever(20). Primary Cutaneous Blastomycosis is rare (21; 22).

Skin neoplasms are skin growths with differing causes and varying degrees of malignancy. The three most common malignant skin cancers are basal cell cancer, squamous cell cancer, and melanoma, each of which is named after the type of skin cell from which it arises (23). Basal cell carcinomas are present on sun-exposed areas of the skin, especially the face. They rarely metastasize and rarely cause death. They are easily treated with surgery or radiation. usually presents as a raised, smooth, pearly bump on the sun-exposed skin of the head, neck or shoulders. Sometimes small blood vessels can be seen within the tumor. It is often mistaken for a sore that does not heal(23). This study revealed that a patients with basal cell carcinoma and infected with blastomycosis in the same time, this is the first cases detected in Iraq and there were no such results were obtained from the publishments to compare with, it was not known whether the patients acquired the infection before skin cancer or later on, the plausible explanation for this, that cancerous patients mainly are immunesuppresed therefore they will be superimposed with any infectious agents, such as blastomycosis.

CONCLUSION AND RECOMMENDATIONS

1. Only 8(8%) out of 100 patients were infected with *Blastomyces dermatitidis,* which improved by culture and histopathologically.
2. Only 51 and 75 years old males with verrucous and ulcerative lesions, were developed basal cell carcinoma.
3. We recommend further studies to correlate this disease with other dermatological disorders.

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Total phenol determent and antimicrobial and anti-inflammatory activity of the extracts of *Tagetes patula* L. flowers

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ABSTRACT

In the current study the locally grown plant *Tagetes patula* L. (Jafari) was studied for its phytochemical and antimicrobial properties. Folins-Ciocalteau reagent assay was used to estimate the phenolic content of *Tagetes patula* L. extracts. The methanol extract showed higher total phenolic content followed by ethanol extract. The efficacies of methanol and ethanol extracts were tested against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebseilla pneumoniae*, *Escherichia coli*, and *Candida albicans* with different concentrations (*in vitro*). It was applied topically on laboratory rabbit induced skin burn. Methanol extract showed antibacterial and healing effect as compared with the skin ointment Silver sulfadaizine on rabbit skin burned with drops of concentrated HCl (*in vitro*).

Keywords: *Tagetes patula* L.; Total phenol; antimicrobial activity; methanolic & ethanolic extracts; Silver sulfadaizine.

الملخص باللغة العربية

في الدراسات الحديثة درس النبات الجعفري النامي محلياً لصفاته النباتية الكيميائية وكمضاد ميكروبي .استخدم كاشف فولنك لتحيدد محتويات الفينول في متخلصات الجعفري. لوحظا ان مستخلص المثانول اكثر فينول كلي من مستخلص الايثانول. اختبرت كفاءة المستخلصين الميثانولي والايثانولي ضد *Staphylococcus aureus* و *Pseudomonas aeruginosa* و *Klebseilla pneumoniae* و *Escherichia coli* و *Candida albicans* بتراكيز مختلفة (خارج الجسم). وطبق بشكل موضعي على الأرانب المختبرية ألمصابه بحروق جلدية. واظهرت النتائج بان المستخلص الميثانولي كمضاد للبكتريا وعند مقارنته بمرهم علاج الجلد السلافيدزن الفضي على الارانب المحترقة بحامض الكلويك المركز (خارج الجسم).

INTRODUCTION

Tagetes species are grows worldwide, and *Tagetes patula* L. (French marigold) from Asteraceae family is endemic of South America for its medicinal properties and as a flowering ornamental plant. In addition, it has been cultivated in Europe, Iraq, India, China, Argentina, and Colombia (1, 2). The plant is rich in flavonoids, terpenoids, flavonoids, alkaloids, polyacetylenes and fatty acids which have been the subject of studies by different researchers (3, 4). It is used in folk medicine, which is approved for treating gastrointestinal diseases; however, some studies have shown other effects including antimicrobial and larvicidal activities by many plants of this species. It has also been used as antimicrobial, antihelminthic and antispasmodic remedy (5, 6).

Antibacterial activity of its flavonoids has been reported against microorganisms like *Staphylococcus aureus* (7). Enzyme inhibition, antioxidant and cytotoxic effects have also been reported for flavonoids. This paper reports the chemical composition, antimicrobial activity and anti-inflammatory effect of different extracts of *T. patula* L plant parts (8). In induced burn in rabbits.

MATERIALS AND METHODS

Tagetes flowers were collected from public garden in Baghdad city, Iraq. Flowers were air dried at room temperature and grinned into powder by using coffee grinder (9).

Microorganisms

Staphylococcus aureus(ATCC 25923), *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli*(ATCC 25992), *Klebseilla pneumoniae*(ATCC23357) and *Candidas albican* were used in this study.

Laboratory animals

Healthy rabbits were used in this study for induced burn.

Preparation of plant extracts

-Methanolic extraction

A quantity of 25g of dried homogenized flowers were mixed with 125ml of methanol (100%) and left at room temperature for 72 hrs, filtered through a filter paper (Watmann No.1), concentrated for a proper volume using a rotary evaporator at 40 ^{0}C.

-Ethanolic extraction

Aquantity of 25g of dried homogenized flowers were mixed with 125ml of ethanol (100%) and left at room temperature for 72 hrs, filtered through a filter paper (Watmann No.1), concentrated for a proper volume using a rotary evaporator at 40 ^{0}C.

Determination of total phenolic content

The total phenol was determined using the Folins-Ciocalteau method. 0.5ml of *T. patula* extracts was taken in test tubes and 5ml of Folins-Ciocalteau reagent and 4ml of aqueous sodium carbonate were added to the tubes. The tubes were kept at density was measured 745 nm using spectrophotometer (10).

Total phenolic contents were determined as a gallic acid eguivalent (GAE) based on Folins-Ciocalteau calibration curve using gallic acid (ranging from 0 to 250 mg/l) as the standard and expressed as mg gallic acid per gramof dry sample (11).

Antimicrobial assays

Disc diffusion method was used to determine the antimicrobial activities of the plant extracts (12). Aliquot of 0.1ml of each of the plant extracts was dropped on a 10mm oval filter paper disc and incubated for 15 minutes to dry at 45ºC.
The tryptone soya agar medium was mixed well and 20ml was poured into Petri-dishes. The surface of the medium was swabbed with 0.1ml of a suspension containing 1.5×10^{8}CFU/ml of the pathogenic bacteria (*S. aureus, P.aeruginosa*, *K. pneumoniae*, and *E.coli*) and 1.5×10^{3}CFU/ml of the pathogenic yeast (*C. albicans*).The dried extracts were impregnated on the surface of the inoculated agar plate by a dispenser. Then incubated for 24 hrs at 37^{o}C. The inhibitory zone arund the diac was measured and expressed in mm. a positive control was also assayed to check the sensitivity of the tested organisms using the following antibiotic: Sulbactam-Ampicilin®(10Mg/10Mg), Cefoperazone®(75Mg), Netilmicin®(30Mg), Aztreonam®(30Mg) and Sporalim®(25Mg). It was measured with a ruler at 24hrs intervals. The analytical grade solvents considered for the extraction were used as negative control experiments (13).

Induction of burns

The skin of rabbits were shaved using a surgical razor and left for 24 hrs, three drops of concentrated HCl were topically dropped carefully on the shaved skin. Then the skin burned with Benzene flame. Rabbits were housed separately under sterile conditions in isolated room.

Topical application of treatments

The two experimental animal groups were treated twice daily one of those groups was treated by Jafari ointment, while the other one treated the antibiotic Flamazine. The diameter of the burns was recorded daily to determine the healing period of induced burn. The granulations of tissues of rebuilt layers were observed. Time course for hair growth was recorded. Healing ability of burns was measured by drawing the wound.

Measurement of wound area

The progressive changes in wound area were measured in mm at 3 days intervals. Progressive decrease in the wound size was monitored periodically.

Statistical analysis

The relative wounded area healing was compared with controls using one- way analysis of variance (ANOVA) followed by Duncan´s Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The total phenol content of *T. patula* methanol and ethanol extracts were estimated by the Folins-Ciocalteau method as 75.8mg GAE/gm of methanol extract and 60.2mg GAE/mg of ethanol extract. The content of total phenols was higher for methanol then that for ethanol extract. Plant extracts showed varying degrees of antimicrobial activity on the microorganisms tested as shown in table 1.
The conventional antibiotics in table (1), showed superior activity than methanolic and ethanolic extracts this may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of manufacturing techniques and procedures, herbal medicinal products are prepared from plant and animal origins, most of the time subjected to contamination and deterioration. In addition that, the storage of extracts likes any other pharmaceuticals requires special conditions of humidity, temperature and light (14, 15).
Generally, the MeOH extracts were more effective than the EtOH extracts on the growth of pathogens. The less effect of the EtOH extracts may be due to the quality and quantity of the active compounds extracted by the solvent. While MeOH flower extract showed an inhibitory action against staphylococcal activity as well as against *P. aeruginosa*. It also showed pronounced activity against the fungus *C. albican*, thus demonstrating a broad spectrum activity against organisms. Both extracts were active against *E.coli* which is a gram negative bacterium; the MeOH extract gave the highest activity (27.0mm). *E.coli* is an intestinal bacterium that causes traveler's diarrhea, urinary tract infection and dysentery in man. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (16). *C. albican* was more sensitive to the MeOH extract recoding an inhibition zone 18.0mm. This may be attributed to the presence of flavonoids and tannins, which are known to have antibacterial and antifungal properties (17).

Table (1): growth of microorganisms under investigation as affected by different concentrations of *T. patula* L. extracts

Treatment			Diameter of inhibition zone (mm)				
			S. aureus	*P.aeruginosa*	*E. coli*	*K.pneumoniae*	*C.albicans*
Control negative			0.00	0.00	0.00	0.00	0.00
Concentration of methanol extract (mg/ml)		12.5	10 ± 0.06	0.00	0.00	0.00	9.6 ± 0.06
		25	14 ± 0.18	0.00	0.00	Slight inhibition	12 ± 0.13
		50	25 ± 0.88	13.3 ± 0.18	Slight inhibition	17 ± 0.63	19 ± 0.88
		75	20 ± 0.88	10.5 ±0.06	0.00	19 ± 0.88	15.2 ± 0.20
Concentration of ethanol extract (mg/ml)		12.5	10 ± 0.63	0.00	0.00	0.00	
		25	12.2 ± 0.19	0.00	0.00	0.00	16.9 ± 0.03
		50	17 ± 0.41	12.1 ± 0.13	0.00	9.4 ± 0.02	20.2 ± 0.65
		75	18 ± 0.26	10.6 ± 0.55	Slight inhibition	10.2 ± 0.06	18 ± 0.32
Control positive (antibiotic)	SAM	10Mg/10Mg	35 ± 6.92	/	/	/	/
	CEF	75Mg	/	28 ± 2.79	/	/	/
	NET	30Mg	/	/	23 ± 2.97	/	/
	AZT	30Mg	/	/	/	20 ± 2.17	/
	SP	25Mg	/	/	/	/	30 ± 3.45

- / : not used
- SAM:Sulbactam-Ampicilin®, CEF: Cefoperazone®, NET: Netilmicin® , AZT: Aztreonam® , and SP: Sporalim®
- Values are means of eight sample reading ± SD

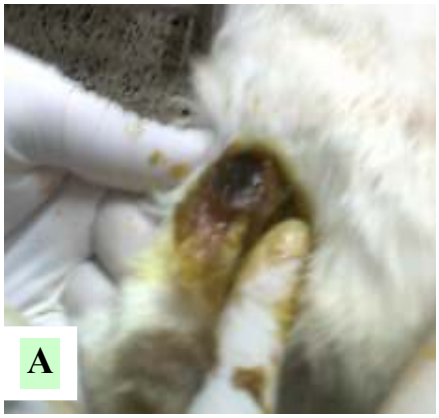
Table (1) appears that methanol extract is more efficient than ethanol; the reason for this may be due to the compounds already extracted by methanol particularly phenolic compounds. The site and number of hydroxyl groups on the phenol are thought to relate to their relative toxicity to microorganisms, with that increased hydroxylation results in increased toxicity. Jawety *et al.,* [18] described the mechanism thought to be responsible for phenolic toxicity against microorganisms to membrane disruption, binding or adhesion making complex with cell wall, inactivation of enzymes, and binding to proteins.

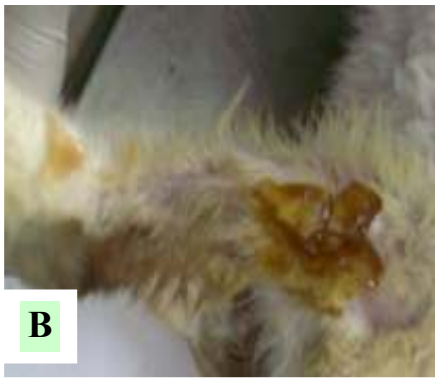
Results of wound healing on rabbit skin (Table 2) indicated a healing potential for the methanol extract that was comparable to Silver sulfadiazine. On the other hand, results showed that there were significant differences ($P \leq 0.05$) between untreated group and treatment groups within 12 days. After 6 days of treatment with methanolic extract the group was better than those treated with ethanolic and Silver sulfadiazine since animals showed fully-grown regenerated epidermis at the 12th day after treatment.

Methanol extract showed a good potential for acceleration of burn wound healing in rabbits (Figure 1). These effects might be due to several mechanisms including an increasing collagen synthesis and rate of epithelialization as a result of the anti-inflammatory, an antimicrobial, and a moisturizing action (19, 20).

Table (2): percentage of wound healing size (mm) control and treated rabbits after four time periods

Treatment	Wound healing % on days			
	3	6	9	12
Control	30.67±0.08	40.81 ±0.30	50.05±0.04	61.33 ± 0.34
Methanol extract	52.23± 0.32	79.53 ± 0.43	91.33± 0.15	100± 0.15
Ethanol extract	42.04± 0.21	61.05 ± 0.09	70.32± 0.26	85.04 ± 0.34
Zinc Oxide	48. 56± 0.33	60.23 ± 0.34	78.02±0.33	89.32 ± 0.21





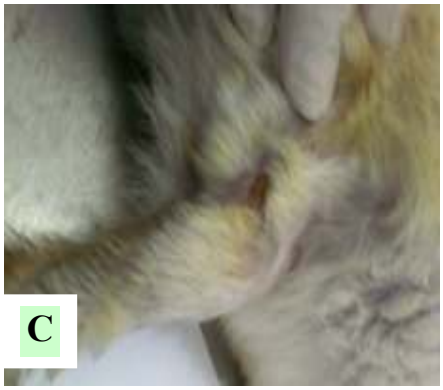


Figure (1): Morphological repair of the skin burned by concentrated HCl and treated with methanol extract of *T. patula* L. [3rd day of infection (A), 6th day of treatment (B), 12th day of treatment (C)].

Our results in this study indicate that total phenol compounds in *T. patula* L. posse's antimicrobial activities and may contribute to the therapeutic effect of this medicinal herb. Further studies are required to characterize the antioxidant properties of *T. patula* flower extracts, and study the interactions it with other types of drugs which are commonly used in the treatment of cancers.

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The Reference Values of Blood Urea and Creatinine in Iraqi Racing Horses

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ABSTRACT

The aim of the present work was to determine serum urea and creatinine concentrations to assess kidney function in racing horses at Equestrian Club, Baghdad, Iraq. Blood samples were collected from the jugular vein of 104 clinically healthy racing horses (males and females, Arabian and Crossbred), aged between 2-8 years divided into three major subgroups: < 4 , 4 , and > 4 .
The results showed that the range and mean values ± standard error (SE) of serum urea and creatinine concentrations were as follows : serum urea 8.21– 24.99 mmol/L and 14.75 ± 0.37 mmol/L , creatinine 70.7–176.8 µmol/L and 126.15 ± 2.42 µmol/L respectively. However, there was no significant difference in serum urea concentration between the related studied groups. While serum creatinine values showed a significant difference between less and more than 4 years age subgroups ($P<0.05$). Moreover, the values increased with age ($r=0.20$).
In conclusion, this study records normal range reference values and the mean± SE of serum urea and creatinine to assess renal function in clinically healthy Iraqi racing horses.

Key Words: Iraqi racing horses, serum urea, serum creatinine*.*

الملخص باللغة العربية

هدف البحث الحالي قياس تراكيز اليوريا والكرياتينين في مصل الدم وذلك لتقييم وظيفة الكلية في خيول السباق الموجودة في نـــادي الفروسية، بغداد، العراق. جمعت عينات الدم من الوريد الوداجي من 104 خيول سباق سليمة ســـريريا (ذكـــور وأنـــاث، عربيـــة، ومضربة)، تراوحت أعمارها بين 2 – 8 سنوات قسمت الى ثلاث مجاميع حسب العمر : <4 ، 4 ، >4) .

أظهرت النتائج أن المدى وقيم المعدلات ± الخطأ القياسي لكل من تراكيز اليوريا والكرياتينين في المصل كانت كما يـــأتي : يوريـــا المصل 8.21 – 24.99 ملي مول/لتر و 14.75 ± 0.37 ملي مول/لتر ، والكرياتينين 70.7 – 176.8 مايكرومول/لتر و 126.15 ± 2.42 مايكرومول/لتر على التوالي. على أية حال، لا توجد هناك فروق معنوية في مستويات يوريا الدم بين المجاميع التي تمـــت دراستها. بينما أظهرت قيم الكرياتينين فروقاً معنوية بين المجاميع العمرية ذات الأعمار اقل وأكثر من 4 سنوات وبمستوى معنويـــة ($P<0.05$). علاوةً على ذلك أظهرت النتائج ازدياد القيم مع تقدم العمر ($r= 0.20$).

نستنتج مما سبق أعلاه، أن هذه الدراسة قد سجلت المدى القياسي كقيم مرجعية وكذلك المعدل ± الخطأ القياسي لليوريا فـــي مـــصل الدم والكرياتينين في خيول السباق العراقية السليمة سريريا .

INTRODUCTION

Hematologic researches made on the racing horses in resting period and also during an exercise period were routinely done to get an optimum condition and better performance to the racehorses (1). Many parameters used for assessment of a healthy racehorse such as : plasma cholesterol , triglyceride, total protein, albumin, creatinine, and urea (2).

Creatinine is a byproduct of the breakdown of creatine. It is most commonly used in Veterinary Medicine as an indirect indicator of renal glomerular filtration rate and to thereby estimate renal function (3).

Serum creatinine concentrations vary based on a multiple factors including animals' diet, muscle mass, exercise, ability of kidney to excrete creatinine, and gender (4).

Urea is formed in the liver from ammonia (shown below in the equations) and is mainly excreted by the kidney. Once formed, urea diffuses freely throughout all body fluids, but the major to excrete is via kidney, for this reason, urea consider as a barometer of renal function (4),(5). Urea appears in the glomerular filtrate in the same concentration as is found in the blood, this filtration process does not require energy. Some urea is passively reabsorbed from the tubules back into the blood (6), (7).

$$NH_3 + H_2CO_3 \rightleftharpoons NH_4^+ + HCO_3^-$$

$$2NH_4^+ + CO_2 \longrightarrow 2H^+ + Urea + H_2O$$

Precisely, serum creatinine concentration is more specific than blood urea concerning to renal function in horses and cows, because gastrointestinal excretion or absorption do not occur (3).

However, blood urea nitrogen (BUN) and creatinine should be considered together since they both help to give a clear picture of healthy kidney. BUN sometimes is affected by other conditions and can be altered by dehydration, blood pressure and others (6).

In Iraq, there is very little information about racing horses in regard to renal function or blood parameters related to it, rather than there were nothing recorded about serum creatinine and / or blood urea. For this reason, this research was done so as to determine the standard levels (reference values in Iraq) of both serum creatinine and blood urea as parameters to evaluate renal function in Iraqi racing horses.

MATERIALS AND METHODS

Blood samples were drawn into plain tubes by jugular vein puncture of 104 clinically healthy race horses (41 males and 63 females), among these horses 31 of them were Arabian and 73 Crossbred. All horses were aged between 2 – 8 years old, they subdivided into three subgroups (<4 , 4 , >4) age groups. These horses were kept in Equestrian Club, Baghdad, Iraq, and this research was done during the period from February to May 2010. Serum was separated routinely after centrifugation for 5 minutes at 3000 rpm (8).

The sera were used directly for measurement of urea and creatinine concentrations. Blood urea was determined according to (9), while serum creatinine was measured by colorimetric method of (10).

SAS program was used for statistical analysis. Data were subjected to Analysis of Variance (ANOVA) and significant means were compared by T- test at a level ($P<0.05$).

RESULTS

The serum values of measured urea and creatinine for horses independent of any subdivision are presented in (Table 1), while the values in (Table 2) are presented according to the following factors : sex, breed and age , with related number of horses for each factor.

Table (1) : Serum urea and creatinine concentrations for Iraqi race horses (Range and Mean ± SE).

Parameters	No. of horses	Range	Mean ± SE
Serum Urea mmol/L	104	8.21 – 24.99	14.75 ± 0.37
Serum creatinine µmol/L	104	70.7 – 176.8	126.15 ± 2.42

The mean of serum urea concentrations in healthy Iraqi racing horses was 14.75±0.37 mmol/L ranged from (8.21-24.99) mmol/L as shown in (Table 1). There were no significant differences in serum urea concentrations between males and females: 15.02±0.54 mmol/L and 14.61±0.50 mmol/L, Arabian and Crossbred: 15.27±0.69 mmol/L and 14.56±0.43 mmol/L respectively, and <4 , 4 , >4 years : 14.46±0.61 mmol/L, 13.92±0.56 mmol/L, and 15.69±0.66 mmol/L respectively (Table 2).

Table (2) : Serum urea and creatinine concentrations expressed according to sex, breed, and age factors (Mean ± SE).

Factors	Subgroup	No. of horses	Parameter (Mean ± SE)	
			Serum Urea mmol/L	Serum creatinine µmol/L
Gender	Males	41	15.02±0.54 a	123.67±4.11 a
	Females	63	14.61±0.50 a	127.65±2.99 a
Breed	Arabian	31	15.27±0.69 a	125.25±4.01 a
	Crossbred	73	14.56±0.43 a	126.44±2.97 a
Age	< 4 years	30	14.46±0.61 a	118.44±4.65 a
	4 years	33	13.92±0.56 a	126.62±3.99 ab
	> 4 years	41	15.69±0.66 a	130.98±3.78 b

The differences in letters vertically refer to presence of significant value at (P<0.05).

DISCUSSION

It is clearly shown that the normal values for both blood urea, and creatinine concentrations were displayed in Tables 1 and 2 , and there is no significant differences between subgroups except in those related to age subgroups. An interpretation for these results may be related to healthy kidneys; good diet intake may improve renal function especially water intake, this refers to well management process as well as excellent public health and renal diseases were avoided. All attempts to get a reason for aged cases may be diseases and this may lead to renal malfunction due to high protein degradation, which finally resulted in urea yield, which is first formed in the liver. Urea contains nitrogen and when excesses their quantity they become toxic to body and must be removed by kidney. Any renal failure (completely or partially) due to any cause leads to increase urea and creatinine levels in urine as well as in blood because of filtration from glomeruli.

The results of the present study revealed normal ranges and means for both creatinine concentrations and blood urea in Iraqi racing horses when compared with other studies internationally and / or when compared with neighbor countries; for example, the mean values of creatinine concentration and blood urea were (97.24±0.07) µmol/L and (12.71±0.67) mmol/L respectively in a research applied to 9 racehorses in a study by (11) in Turkey. He found no significant differences between values obtained during pre-exercise to the horses.

Another study performed by (12) in Brazil on 12 racehorses fed on different types of food, they found that means of creatinine concentration and blood urea were 114.9 µmol/L and 9.75 mmol/L respectively, also they found no significant difference between the values in his study and it is nearly what was I found in this study.

Internationally, (13) recorded reference mean levels of creatinine concentr-ations and blood urea, which were 110-170 µmol/L and 3.5-7.0 mmol/L in the horses respectively, while, (14) registered standard levels of mean values for serum creatinine concentrations and blood urea, as 77-175 mmol/L and 3.7-8.8 mmol/L in horses respectively. These mentioned values above were somehow in accordance with the results of creatinine obtained in this study with slight differences but do not reach to significant level (P<0.05).

Globally, (15) listed reference mean values in Thoroughbred mares for both creatinine concentrations and blood urea, which were 115 µmol/L and 17.7 mmol/L respectively; these data were accepted by internationally

mentioned values as well as agreed with our study with slight non-significant differences in both values.

An investigator (16) in Texas (USA) was studied on thirty adult mares, they were deprived of access to feed and water for 6 hrs, blocked by age, sex, breed, and body condition score, he found that the mean values for both blood urea and, creatinine concentrations influenced and they were affected positively, he found significant differences in both values ($P<0.0001$).

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قسم الدراسات العربية

ARABIC SECTION

تأثير الليزر (هليوم – نيون) في فعالية انزيمي الاميليز والبروتييز في بذور وبادرات الباقلاء

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الملخص باللغة العربية

هدفت الدراسة الى معرفة تاثير الليزر (هليوم – نيون) في فعالية انزيمي الاميليز والبروتييز لبذور وبادرات نبات الباقلاء ***Vicia faba*** حيث عرضت بذور نبات الباقلاء لاشعة ليزر(الهليوم – نيون) بطاقات مختلفة شملت (20 و 50) ملي واط / ملم^2 وبطول موجي 632.8 نانومتر وبمسافات (30 و40 و60) سم عن المصدر المشع وبفترات زمنية مختلفة شملت (1.15 و2.5 و4) دقيقة لكل بعد اومسافة ، لتحديد تاثير الليزر على الفعالية النوعية لانزيمي الاميليز والبروتييز .

بلغت اقصى فعالية نوعية لكلا الانزيمين عند تشعيع بذور الباقلاء بطاقة اشعاع مقدارها 50 ملي واط / ملم^2 عند البعد 40 سم وبزمن تشعيع 1.15 دقيقة ، حيث قدرت الفعالية النوعية لانزيم الاميليز للبذور بـــ 12 وحدة / ملغم بروتين و14 وحدة / ملغم بروتيـــن للبادرات وبلغت الفعالية النوعية لانزيم البروتييز 15 وحدة / ملغم بروتين للبذور و17.2 وحدة / ملغم بروتين للبادرات ، مقارنة بتشعيع البذور بطاقة اشعاع مقادرها 20 ملي واط / ملم^2 ، بلغت اقصى فعالية نوعية لكلا الانزيمين عند بعد 30 سم عن المصدر المشع بفترة زمنية مقدارها 4 دقائـــق وبلغت الفعالية النوعية لانزيم الاميليز عندها 4.9 وحدة / ملغم بروتين للبذور و 5.3وحدة / ملغم بروتين للبادرات وبلغت 17 وحدة / ملغم بروتين للبذور و16.6 وحدة / ملغم بروتين للبادرات لانزيم البروتييز . تبين النتائج ان الفعالية الانزيمية تزداد بزيادة طاقة الاشعاع نتيجة لزيادة الطاقة الداخلية للبذور والبادرات ما يعزز من الفعالية الانزيمية ويعجل من عمليات الايض الخلوية .

ABSTRACT

The study aimed to see the effect of the laser (helium - neon) in the activity of the amylase and protease enzymes for seeds and seedlings of ***Vicia faba*** plant.

The ***Vicia faba*** seeds are offered to the (helium - neon) laser in different energies included (20 and 50) mW/mm^2 with wavelength 8.632 nm and distance (30, 40 and 60) cm from the radioactive source and the different time periods (1.15, 2.5 and 4) minutes after each dimension or distance, to determine the effect of laser on the specific activity of amylase and protease enzymes.

The maximum activity of both enzymes are reached at the irradiation of seeds with 50 mW/mm^2 when the distance of 40 cm and the time period of irradiation was 1.15 minutes, the specific activity of amylase enzyme of the seed was estimated by 12 units / mg protein and 14 units / mg protein for seedlings and the specific activity of protease enzyme was 15 units / mg protein for the seeds and 17.2 units / mg protein for seedlings,

compared with irradiation of seeds with 20 mW/mm^2, the maximum specific activity of both enzymes were reached at 30 cm from the source of radioactive when period of time was 4 minutes, the specific activity of amylase enzyme equal to 4.9 units / mg protein for seeds and 5.3 units / mg protein for the seedlings and 17 units / mg protein for the seeds and 16.6 units / mg protein for the seedlings to the protease enzyme.

The results show that the activity of enzyme increases with radiation energy due to the increasing internal energy of the seeds and seedlings to enhance the enzyme activity and accelerates cellular metabolism.

المقدمة

تعد الباقلاء من المحاصيل البقولية الرئيسية المهمة التي تزرع بشكل زراعة مروية ، موطنها الأصلي هو آسيا الغربية وشمال أفريقيا ، عرفته الصين منذ عام 2800 ق.م ، وكذلك في أوروبا (في إيطاليا وفرنسا وإسبانيا) ومن ثم تأهلت زراعته وانتقلت من أوروبا إلى أمريكا الشمالية ، تحتوي الحبوب الجافة على المواد التالية:
28% من وزنها بروتين ، 48% نشاء ، 3% دهن ، 2% كلوكوز ، 3% أملاحاً معدنية (بوتاسيوم ، فوسفور ، حديد... الخ) ، 16% مواد أخرى (ماء ، ألياف ... الخ) ، فضلا عن احتواءها على السليلوز بنسبة 7.1-11% وعلى مواد نتروجينية 25- 30% وعلى مواد غير نتروجينية 45-48% بالإضافة إلى الأحماض الأمينية النباتية المتعددة كحامض الأسبارتيك وحامض الثريونين وحامض الكلوتاميك والبرولين والكلايسين والفالين والألانين والليوسين والمثيونين والهستدين وأحماض أخرى وكلها يحتاج إليها الجسم بمقادير معينة ، كما استعمل في تغذية الخيول والبغال والماشية بعد جرشه وخلطه مع المواد العلفية الجافة كمصدر للبروتين من أجل تسمينها وإدرارها للحليب (1).
يعتبر الضوء واحد من العوامل البيئية الأكثر أهمية حيث يلعب دورا حاسما في عملية التمثيل الضوئي للنباتات (Photosynthesis) مثل التوجه الضوئي (Phototropism) ، التخليق الضوئي (Photomorphogenesis) والتمثيل البايولوجي الضوئي (Photobiosynthesis) المعروفة منذ عقود ، فمن الواضح أن استجابة البذور للضوء تكون بمجموعة متنوعة من التفاعلات المعقدة التي تتأثر بمدة التعرض للضوء وشدته وكذلك نفاذية الضوء من غلاف البذور التي تعتمد على الطول الموجي للضوء المستعمل (2) . اشار كبير من الدراسات التجريبية على مدى السنوات الأخيرة إلى ان تعريض البذور الجافة لاشعة الليزر بطول موجي (632.8 نانومتر) كان محفز لكثير من العمليات البايولوجية ، كما احدث تغييرات في الخصائص الكهروكيميائية والخصائص البايوكيميائية (3) .
يمكن تصنيف انواع الليزر الى ليزارات الحالة الصلبة (Solid - state lasers) مثل النيوديميوم Neodymium والإيتريوم Yttrium والألومنيوم Aluminum والعقيق Garnet و XeCl وهي تستخدم أساسا في العلاج الطبي (4) ، وليزر الاكسيمر (Excimer lasers) ، وليزرات الصبغة السائلة (Dye lasers) وليزرات أشباه الموصلات (Semiconductor lasers) ، وليزرات الحالة الغازية (Gas - state lasers) (5) ، في حين أن هذا الأخير مثل He-Ne ليزر وCO_2 ليزر قد استخدم في المحاصيل لمعرفة تأثيره فيها (6) . ويكون الخرج الليزري اما نبضي الموجة Pulsed wave اومستمر الموجة Continuous wave .
لقد بينت الدراسات السابقة ان جرعة مناسبة من ليزر هليوم – نيون وليزر ثاني اوكسيد الكاربون (موجة مستمرة) لديها اثر ايجابي في تسريع نمو النباتات وايضها (7) ، وتحسين تركيز البروتينات والفعالية الانزيمية (8) ، وقد بينت الدراسات السابقة ايضا ان الليزر لايقوم فقط بحماية خلايا البذور من التلف الناتج من التعرض للـ (UV) ، وانما يقوم ايضا باصلاح التلف في البادرات الناتج من التعرض للاشعة الفوق البنفسجية UV (9) .

تهدف الدراسة الى معرفة ثاثير الليزر (هليوم – نيون) بطاقتيه (20 و50) ملي واط / ملم^2 وبطول موجي 632.8 نانومتر وبقطر حزمة 1.5 ملم على الفعالية النوعية لانزيمي الاميليز والبروتييز في بذور وبادرات نبات الباقلاء خلال اوقات زمنية مختلفة.

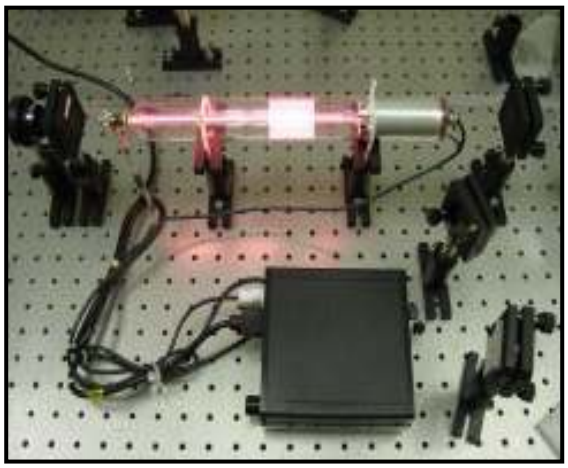
المواد وطرائق العمل

المواد المستخدمة في الدراسة:
1- تم الحصول على بذور الباقلاء من احد الاسواق المحلية في مدينة بغداد.
2- استعمل ليزر هليوم – نيون في الدراسة بطاقتين هي 20 ملي واط / ملم^2 و50 ملي واط / ملم^2 وبطول موجي 632.8 نانومتر وبقطر حزمة 1.5 ملم لكلا الطاقتين في قسم الفيزياء – كلية العلوم/ جامعة بغداد.

طرائق العمل:

1- تشعيع بذور الباقلاء

اختيرت مجموعة من بذور الباقلاء الخالية من اي تسوس او تشوه وعقمت باستعمال مادة كلوريد الزئبقيك $HgCl_2$ بتركيز 0.05% ، بعدها غسلت هذه البذور لمدة 30 دقيقة بماء الحنفية الجاري ثم جففت بالظل.
عرضت البذور المعقمة والمجففة واحدة تلو الاخرى الى الليزر باوقات زمنية مختلفة شملت (1.15 و2.5 و4) دقيقة وبمسافات مختلفة عن المصدر المشع (الليزر) تمثلت بـ (30 و40 و60) سم ولكلا طاقتي الليزر باستخدام ثلاث مكررات لكل زمن ، تم اجراء كافة الاختبارات الخاصة بالتشعيع في قسم الفيزياء- كلية العلوم / جامعة بغداد باستعمال جهاز التشعيع كما في (الشكل-1).



(الشكل -1) جهاز هليوم

2- تنمية البذور

نميت بذور الباقلاء بوضعها على قطعة من الشاش المرطبة داخل عبوة زجاجية وبثلاث مكررات لكل فترة زمنية للابعاد المستخدمة ولكل طاقة بدرجة حرارة 25 درجة مئوية وبنسبة رطوبة 70% .

3- تحضير المستخلص الخام للبذور والبادرات

طحنت بذور وبادرات الباقلاء المشعة باستعمال الطاحونة الكهربائية ، وفصلت الخلايا باستخدام جهاز الطرد

المركزي المبرد بسرعة 6000 دورة / دقيقة لمدة 20 دقيقة ، وعد الراشح الناتج مستخلصا خاما للبذور والبادرات لقياس فعالية انزيمي الاميليز والبروتييز وتركيز البروتين .

4- قياس الفعالية الانزيمية وتركيز البروتين :

- **انزيم الاميليز**

قدرت فعالية انزيم الاميليز وفق الطريقة الموصوفة من قبل Lin وجماعته (10) التي تعتمد على التحلل المائي للنشأ (مادة التفاعل المحضرة بتركيز1% في دارئ فوسفات البوتاسيوم بتركيز 0.1 مولاري وبدالة حموضة تساوي 7) الى السكريات المختزلة المتحررة بفعل الانزيم وبالاعتماد على المنحنى القياسي للمالتوز، تعرف وحدة الفعالية الانزيمية بأنها كمية الانزيم التي تؤدي إلى تحرير 1 مايكرومول من السكريات المختزلة (كالكلوكوز والمالتوز) في الدقيقة الواحدة عند ظروف التقدير.

- **انزيم البروتييز**

قدرت فعالية انزيم البروتييز في المحاليل الناتجة وفق الطريقة الموصوفة من قبل Brock وجماعته (11) التي تعتمد على تحلل الكازائين (مادة التفاعل المحضرة بتركيز 1% في دارئ فوسفات الصوديوم بتركيز 0.1 مولاري وبدالة حموضة تساوي 7) الى الببتيدات والاحماض الامينية المكونة له بفعل الانزيم ، تعرف وحدة الفعالية الانزيمية (وحدة) على انها كمية الانزيم اللازمة لزيادة الامتصاصية بمقدار0.01 بالدقيقة الواحدة تحت الظروف القياسية.

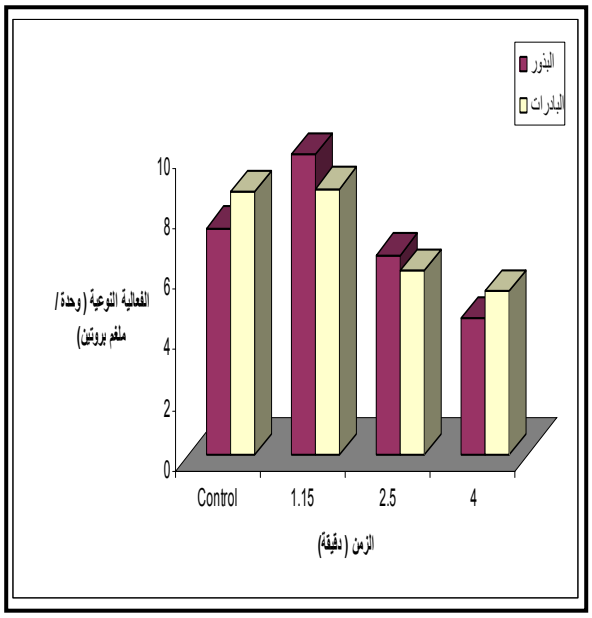
اما طريقة تقدير البروتين فقد قدرت وفق الطريقة الموصوفة من قبل Bradford (12) بالاعتماد على المنحنى القياسي لالبومين المصل البقري واستعمال صبغة الكوماسي الزرقاء G-250 عند طول موجي 595 نانوميتر.

النتائج والمناقشة

1- تأثير طاقة الليزر 50 ملي واط / ملم^2 بابعاد مختلفة في فعالية انزيمي الاميليز والبروتييز لبذور وبادرات الباقلاء *Vicia faba* :

- تأثير طاقة الليزر عند البعد 30 سم

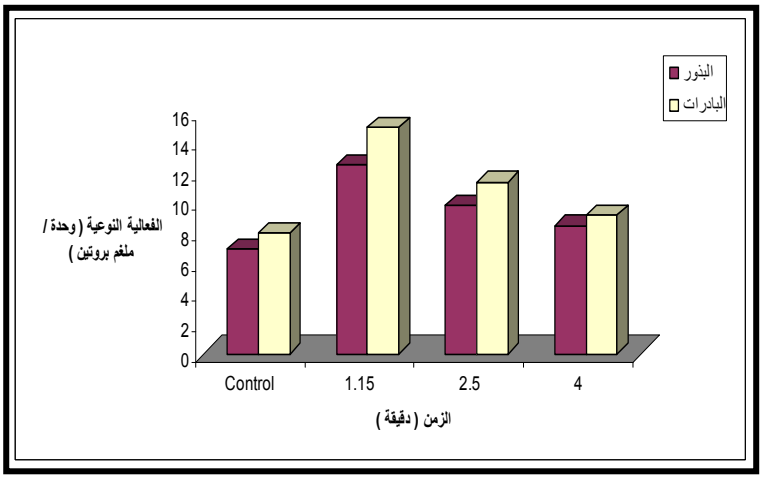
لوحظ تأثير واضح لطاقة الليزر المشع بمقدار 50 ملي واط / ملم^2 عند تعريض البذور لمدة 1.15 دقيقة عند البعد 30 سم في الفعالية الانزيمية للاميليز ، حيث تبين النتائج ان الفعالية النوعية لانزيم الاميليز قد بلغت 9.9 وحدة / ملغم بروتين للبذور و8.8 وحدة / ملغم بروتين للبادرات بعدها اخذت الفعالية النوعية بالانخفاض بزيادة زمن التعريض لليزر للفترتين 2.5 و 4 دقائق حيث وصلت الفعالية النوعية للانزيم حوالي 4.5 وحدة / ملغم بروتين للبذور و5.3 وحدة / ملغم بروتين للبادرات بعد 4 دقائق من التعريض مقارنة مع معاملة السيطرة التي امتلكت فعالية نوعية 7.5 وحدة / ملغم بروتين و8.7 وحدة / ملغم بروتين للبادرات (الشكل-2) ، لوحظ انخفاض في الفعالية النوعية للانزيم في البادرات مقارنة مع البذور المشعة ، كانت هذه النتائج مقاربة الى نتائج الباحثان Ouf و abdel-Hady اللذان بينا ان زيادة وقت تعريض البذور للتشعيع قد يؤدي الى حدوث تلف في البذور مؤدية الى تثبيط عملية الانبات بينما وجدا ان عملية انبات البذور تتحفز عند تعريض البذور دقيقة واحدة للمصدر المشع (13).



(الشكل – 2) تأثير طاقة الليزر (50 ملي واط / ملم^2) في فعالية انزيم الاميليز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 30 سم

كذلك درس تأثير طاقة الليزر المشع بمقدار 50 ملي واط / ملم2 باوقات زمنية مختلفة شملت (1.15 ، 2.5 ، 4) عند البعد 30 سم على الفعالية الانزيمية لانزيم البروتييز (الشكل-3) ، بينت النتائج ان اقصى فعالية نوعية كانت ايضا عند تعريض البذور لمدة 1.15 دقيقة للمصدر المشع اذا بلغت الفعالية النوعية 12.5 وحدة / ملغم بروتين للبذور و15 وحدة / ملغم بروتين للبادرات ثم اخذت الفعالية النوعية بالانخفاض باطالة زمن التعريض للمصدر المشع حتى اصبحت 8.5 وحدة / ملغم بروتين للبذور و9.2 وحدة / ملغم بروتين للبادرات عند الدقيقة الرابعة من زمن التعريض ، كذلك لوحظ ان الفعالية النوعية للبروتييز في البادرات كانت اعلى مقارنة مع قيم الفعالية النوعية للبروتييز للبذور المشعة.

بينت الكثير من الدراسات ان التاثير الحراري لليزر على الانسجة الحية هي عملية معقدة تنتج من ثلاث ظواهر مميزة هي تحويل الضوء الى حرارة ، نقل الحرارة وتفاعلها مع الانسجة ، والتي ترتبط مع درجة الحرارة وزمن التسخين (14).

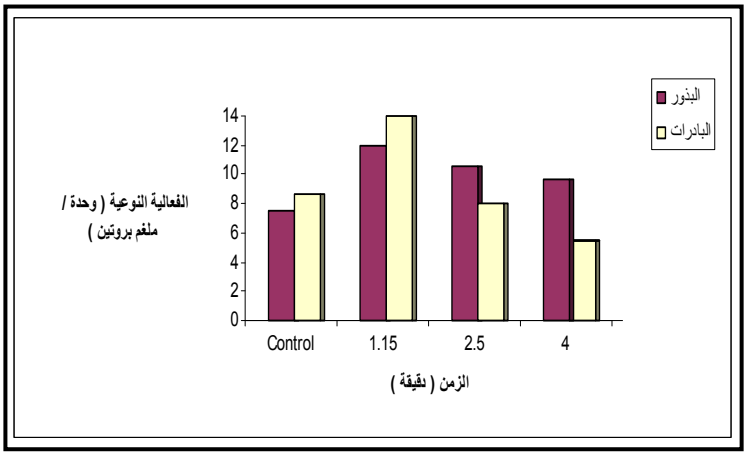


(الشكل –3) تأثير طاقة الليزر (50 ملي واط / ملم^2) في فعالية انزيم البروتييز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد30 سم

- تأثير طاقة الليزر عن البعد 40 سم

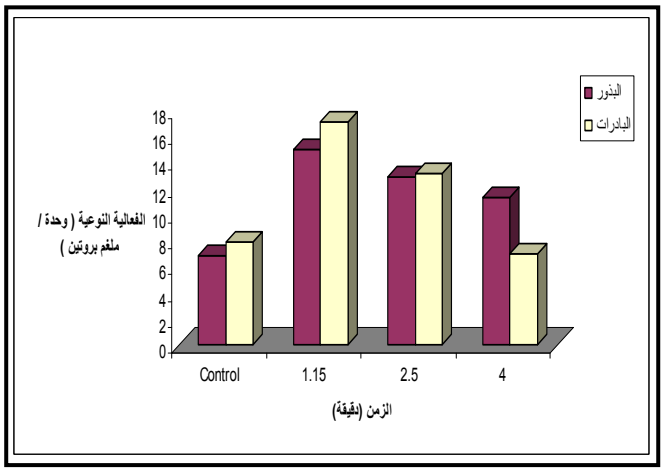
درس تأثير طاقة الليزر المشع بمقدار 50 ملي واط / ملم^2 باوقات زمنية مختلفة شملت (1.15 ، 2.5 ، 4) عند البعد 40 سم في الفعالية الانزيمية لانزيم الاميليز (الشكل-4) ، حيث لوحظ ان تعريض البذور لليزر لمدة 1.15 دقيقة قد رفع الفعالية النوعية للاميليز ، حيث كانت الفعالية النوعية اقصاها وبلغت 12 وحدة / ملغم بروتين و14 وحدة / ملغم بروتين لكلا من البذور والبادرات على التوالي ، ثم بدأت الفعالية بالانخفاض عند زيادة فترة التعريض لمدة 2.5 و4 دقيقة .

بينت الدراسات السابقة ان تاثير الليزر المشع على الخلايا الحية يكون من خلال كمية طاقة الليزر المستخدمة وفترة التعرض لتلك الطاقة حيث ان الارتفاع المعتدل بدرجة الحرارة لعدة درجات حرارية والذي قد يتراوح بين (41 - 44) درجة مئوية لبضع دقائق قد يؤدي الى موت الخلية الحية نتيجة حدوث تغير في العمليات الانزيمية (14).



(الشكل -4) تأثير طاقة الليزر (50 ملي واط / ملم^2) في فعالية انزيم الاميليز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 40 سم

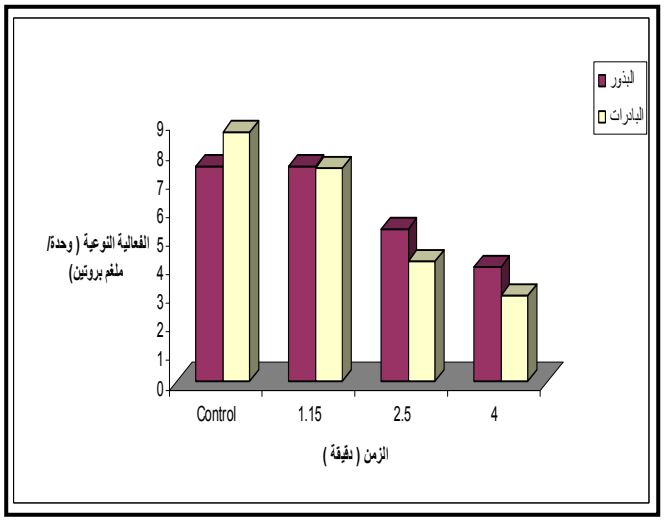
بينما اختبر تأثير طاقة الليزر المشع بمقدار 50 ملي واط / ملم^2 باوقات زمنية مختلفة عند البعد 40 سم في الفعالية الانزيمية للبروتييز (الشكل-5) ، واظهرت النتائج ان اعلى فعالية انزيمية لانزيم البروتييز كانت بعد 1.15 دقيقة من زمن التعريض لليزر المشع اذا بلغت الفعالية النوعية 15 وحدة / ملغم بروتين للبذور و17.2 وحدة / ملغم بروتين للبادرات وانخفضت هذه الفعالية بزيادة زمن التعريض لليزر عند الدقيقة 2.5 و4 . بينت دراسات سابقة ان تعريض اي نسيج حي لاشعة الليزر فان النسيج يقوم بامتصاص الطاقة الضوئية وتحويلها الى طاقة حرارية ثم التفاعل مع النسيج خلال زمن التعرض الابتدائي وكلما زاد زمن التعرض كلما احدثت تلك الحرارة اثار مسخ على ذلك النسيج (15) ، كذلك اشار الكثير من الباحثين الى ان عمل ضوء الليزر مماثل تماما لعملية التمثيل الضوئي في النباتات حيث ان فوتونات الضوء المنبعث من الليزر تخترق عميقا في النسيج وتتفاعل لتخليق جزيئات الادينوسين ثلاثي الفوسفات (ATP) وتقوم هذه الجزيئات بنقل الطاقة من موقع تفاعل الى اخر في جميع الخلايا الحية. ان زيادة عدد هذه الجزيئات يزداد بزيادة ضوء الليزر الممتص وبالتالي زيادة الطاقة التي تسرع من اخذ المواد الغذائية والتخلص من الفضلات (15).



(الشكل -5) تأثير طاقة الليزر (50 ملي واط / ملم^2) في فعالية انزيم البروتييز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 40 سم

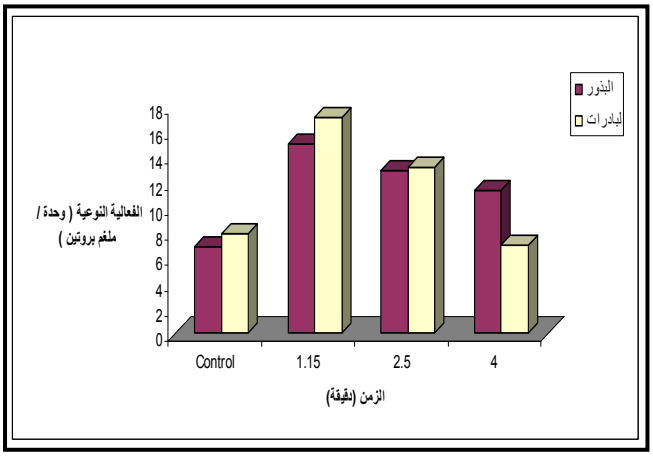
- تأثير طاقة الليزر عن البعد 60 سم

لوحظ ايضا تأثير الليزر في فعالية انزيم الاميليز عند البعد 60 سم ولفترات زمنية مختلفة حيث ازدادت الفعالية النوعية بعد 1.15 دقيقة من التعريض لتصل الى حوالي 7.5 وحدة / ملغم بروتين لكلا من البذور والبادرات مع انخفاض واضح لهذه الفعالية عند زيادة وقت التعريض لليزر المشع (الشكل -6) ، ان الطاقة الحرارية لاشعة الليزر الممتصة من قبل نبات الباقلاء تعمل على زيادة نشاط الخلايا النباتية للنبات ، ومن المهم جدا تحديد زمن التعرض للمصدر المشع حيث ان زيادة فترة التعريض تؤدي الى انتشار الحرارة في كافة اجزاء البذرة وبالتالي حدوث تحطيم لمكونات البذورة او الخلية الحية وبالتالي تؤثر على العمليات الايضية والفعاليات الانزيمية لكثير من الانزيمات ، اما اذا كان زمن التعريض لحرارة المصدر المشع قليل فان تاثيره لايكون كافيا لحدوث اي تغيير ايجابي في العمليات الايضية والفعاليات الانزيمية (15).



(الشكل -6) تأثير طاقة الليزر (50 ملي واط / ملم^2) في فعالية انزيم الاميليز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 60 سم

كذلك الحال بالنسبة لتأثير هذه الطاقة من الليزر عند البعد 60 سم على الفعالية النوعية لازيم البروتييز في بذور وبادرات الباقلاء عند الزمن 1.15 دقيقة (الشكل –7) . تؤكد هذه النتائج ان بعض الفعاليات الانزيمية تتأثر بزمن تعريض الاشعاع للبذور ، فضلا عن بعد المصدر المشع عن البذرة حيث ان طاقة الليزرتؤدي الى احداث تغيرات في الطاقة الداخلية للبذور والتي تؤدي الى تعزيز الفعالية الانزيمية او تثبيطها وزيادة سرعة ايض الخلية (3).

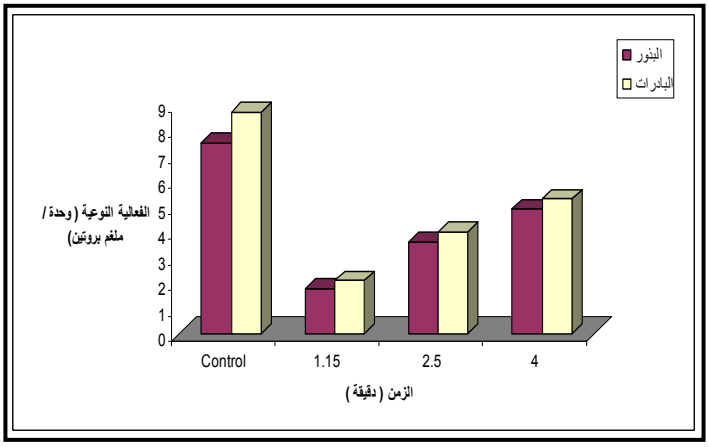


(الشكل –7) تأثير طاقة الليزر(50 ملي واط / ملم^2) في فعالية انزيم البروتييز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 60 سم

2– تأثير طاقة الليزر20ملي واط / ملم^2 بابعاد مختلفة في الفعالية النوعية لانزيمي الاميليز والبروتييز لبذور وبادرات الباقلاء *Vicia faba* :

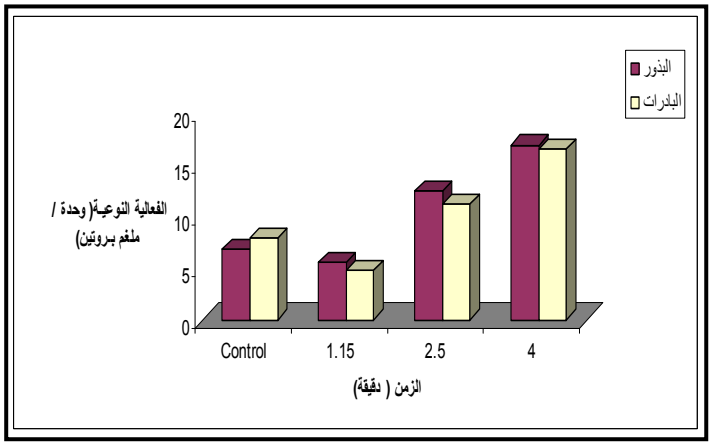
– تأثير طاقة الليزر عند البعد 30 سم

لوحظ حدوث زيادة تدريجية في الفعالية الانزيمية مع تزايد فترة التعريض للمصدر المشع اذا كانت اقصاها عند الدقيقة الرابعة من فترة التعريض لليزر حيث بلغت 4.9 وحدة / ملغم بروتين للبذور و5.3 وحدة / ملغم بروتين للبادرات ولكن قيم هذه الفعالية كانت اوطأ من قيمتها في عينات السيطرة غير المعاملة او غير المعرضة لليزر (الشكل –8) ، كانت هذه النتائج مقاربة الى ماجاء به Chen وجماعته (16) ، حيث وضحوا بان الطاقات الواطئة من الليزر والتي تتراوح بين (2–12) ملي واط تعمل على تحفيز بسيط في عمل الخلايا الحية وزيادة فعاليتها الانزيمية بدرجة بسيطة.



(الشكل –8) تأثير طاقة (20 ملي واط / ملم^2) في فعالية انزيم الاميليز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 30 سم

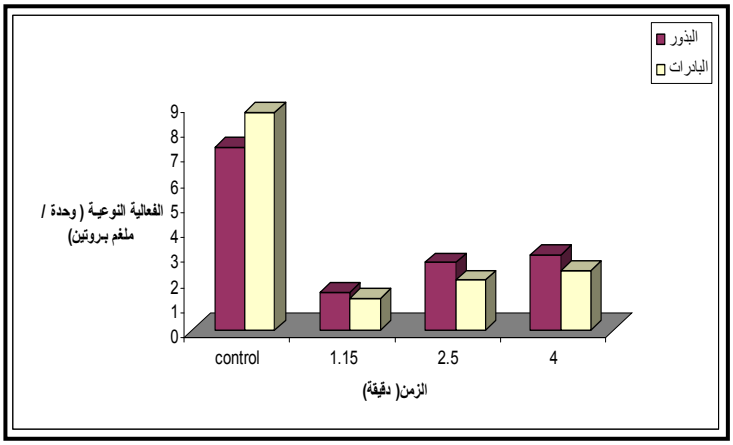
وبينت النتائج ايضا ان افضل فعالية نوعية للبروتييز كانت بعد تعرض البذور لاربع دقائق من الاشعاع اذا بلغت الفعالية النوعية 17 وحدة / ملغم بروتين للبذور و16.6 وحدة / ملغم بروتين للبادرات مقارنة بمستوياتها الواطئة جدا خلال زمن اشعاع مقداره 2.5 و1.15 دقيقة ، قورنت النتائج مع نموذج السيطرة اذا بلغت الفعالية النوعية الانزيمية فيها 6.9 وحدة / ملغم بروتين للبذور و8 وحدة / ملغم بروتين للبادرات (الشكل– 9) بينت دراسات سابقة ان تاثير الاشعاع على الخلايا الحية يختلف اعتمادا على نوع الليزر المشع وعلى شدة وفترة التعرض وعلى نوع الخلايا الحية (17).



(الشكل–9) تأثير طاقة الليزر (20 ملي واط / ملم^2) في فعالية انزيم البروتييز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 30 سم

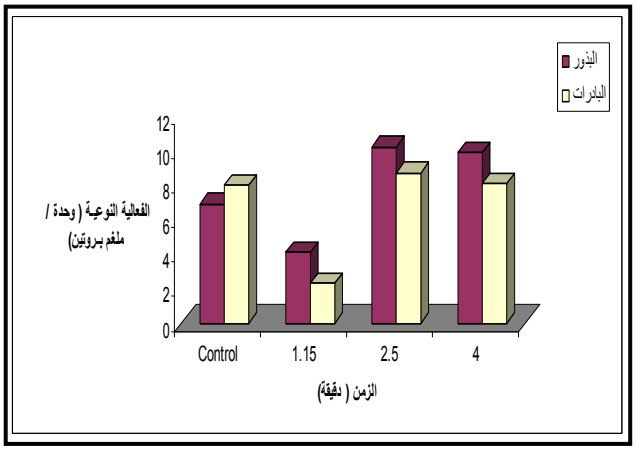
– تأثير طاقة الليزر عند البعد 40 سم

تم تعيين الفترة الزمنية المثلى اللازمة لزيادة الفعالية النوعية لانزيم الاميليز من خلال تعريضه الى فترات زمنية مختلفة شملت (1.15 ، 2.5 ، 4) دقيقة وببعد 40 سم عن المصدر المشع ، اظهرت النتائج المبينة في (الشكل– 10) انخفاضا واضحا في الفعالية النوعية للاميليز عند الاوقات المختلفة للتعريض (1.15 و2.5 و 4) دقيقة ، اذا لم يتجاوز 3 وحدة / ملغم بروتين للبذور المشعة والبادرات عند الزمن 4 دقائق مقارنة مع قيمة هذه الفعالية في نموذج السيطرة التي وصلت الى حوالي 9 وحدة / ملغم بروتين للبادرات ، يتضح من النتائج ان الجرعات الواطئة من الليزر (20 ملي واط / ملم^2) يكون لها تاثيرا عكسيا في الفعالية الانزيمية حيث تعمل الجرعات القليلة على حدوث ضرر او عطب يمكن اصلاحه (18).



(الشكل –10) تأثير طاقة الليزر (20 ملي واط / ملم^2) في فعالية انزيم الاميليز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 40 سم

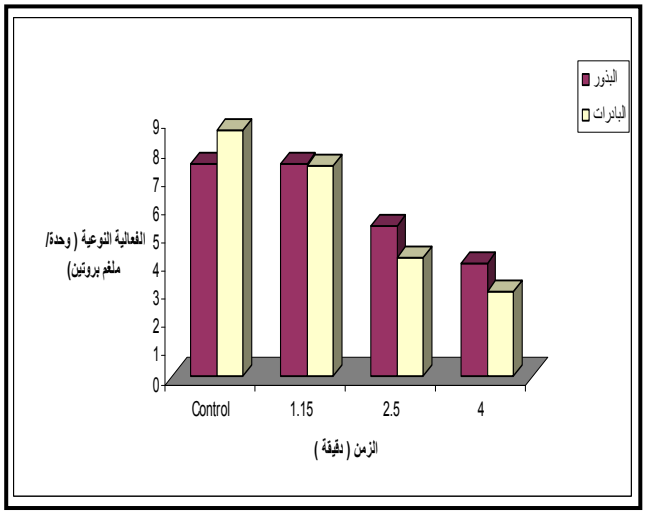
بينما لوحظ انخفاض في الفعالية النوعية للبروتييزعند الدقيقة 1.15 من تعريض بذور الباقلاء لليزر بطاقة 20 ملي واط / ملم^2 اذ بلغت حوالي 4.1 وحدة / ملغم بروتين للبذور و2.3 وحدة ملغم بروتين للبادرات مع زيادة ملحوظة للفعالية قد بلغت 9.9 وحدة / ملغم بروتين للبذور و8.1 وحدة / ملغم بروتين للبادرات عند الدقيقة الرابعة من زمن التشعيع (الشكل –11) .هنالك علاقة بين طاقة الليزر والخلايا الحية وهذه العلاقة تعتمد على خصائص الليزر من ناحية طوله الموجي وشدته وشكله عند سقوطه على المادة المراد علاجها (19).



(الشكل –11) تأثير طاقة الليزر (20 ملي واط / ملم^2) في فعالية انزيم البروتييز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 40 سم

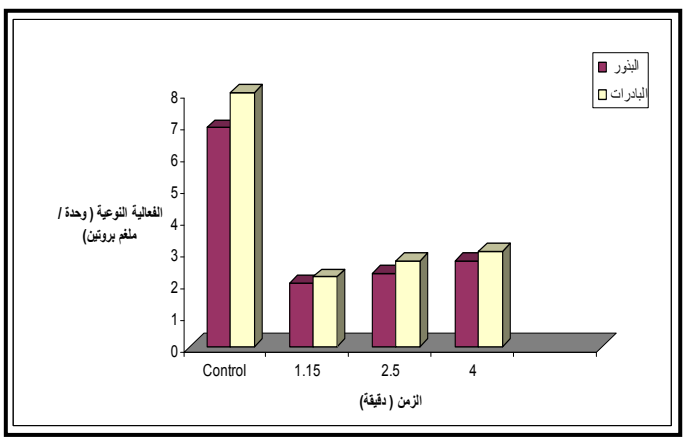
– تأثير طاقة الليزر عند البعد 60 سم

اختبر تأثير طاقة الليزر المشع بمقدار20 ملي واط / ملم^2 باوقات زمنية مختلفة شملت (1.15 ، 2.5 ، 4) عند البعد 60 سم على الفعالية النوعية لانزيم الاميليز ، لوحظ من خلال النتائج انخفاض ملحوظ في الفعالية النوعية للبذور المشعة والبادرات عند اوقات التشعيع الثلاثة اذا بلغت 1.8 للبذور و1.99 للبادرات عند زمن التشعيع 4 دقائق مقارنة مع نموذج السيطرة التي وصلت الى حوالي 9 وحدة/ ملغم بروتين للبادرات (الشكل –12) . ان تاثير التداخل بين ضوء الليزر والانسجة الحية ينجم عنه تاثيران الاول يسمى بالتاثير الضوئي التحطيمي Photo-destructive influence وفيه يكون للتاثيرات الديناميكية والحرارية والكيميائية للضوء تاثيرا محطما للانسجة الحية ، والتاثير الثاني هو تاثيرا ضوئيا فيزياويا وتاثيرا ضوئيا كيماويا ، عندما تمتص الانسجة الحية ضوء الليـــزر فـــان ذرات وجزئيات ذلـــك النسيج ســـوف تظهـــر تفاعلا ضوئيأ فيزياويـــا وكيمياويـــا (20) .



(الشكل –12) تأثير طاقة الليزر (20 ملي واط / ملم^2) في فعالية انزيم الاميليز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 60 سم

اما بالنسبة لتاثير الليزرعلى الفعالية النوعية للبروتييز فقد لوحظ انخفاض ملحوظ في الفعالية النوعية للبروتييز اذا بلغت 3 وحدة / ملغم بروتين للبادرات عند الدقيقة الرابعة من زمن التشعيع مقارنة مع نموذج السيطرة اذا بلغت 8 وحدة / ملغم بروتين في البادرات (الشكل–13). بينت دراسات عدة ان هناك نوع من التاثيرات البايولوجية بين ضوء الليزر المنبعث والانسجة الحية وهو مايسى بالتاثير الصفري Zero-influence وهو ان المادة البايولوجية لاتتاثر خصائصها بعد التعرض للضوء المشع (20).



(الشكل –13) تأثير طاقة الليزر (20ملي واط / ملم^2) في فعالية انزيم البروتييز لبذور وبادرات نبات الباقلاء بعد اوقات زمنية مختلفة عند البعد 60 سم

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عزل وتنقية أنزيم البيروكسيديز من الفجل الأحمر

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الملخص باللغة العربية

أكدت النتائج أن أعلى فعالية نوعية للأنزيم تركزت في المستخلص الخام لجذور نبات الفجل فقد بلغت 253.4 (وحدة / ملغم بروتين) بأستخدام دارئ خلات الصوديوم ذو تركيز 0.2 مولار ورقم هيدروجيني 5.7 محلولا لأستخلاص الأنزيم. تم تنقية انزيم البيروكسيديز بثلاثة خطوات تضمنت الترسيب بكبريتات الامونيوم بنسبة اشباع (30-90%) تلتها خطوة تبادل أيوني بأستخدام عمود (DEAE-Cellulose) حيث بلغ عدد مرات التنقية 7.10 مرة وبحصيلة أنزيمية 44.03% أعقبتها خطوة الترشيح الهلامي للأجزاء غير المرتبطة بالمبادل في عمود هلام السيفادكس G-100 فقد تم الحصول على عدد مرات تنقية 11.83 مرة وبحصيلة أنزيمية 18.76%.

ABSTRACT

The results indicated the highest peroxidase specific activity was in the turnip root horseradish 253.4 unit/mg protein.The sodium acetate 0.2 M buffer solution at pH 5.7 was found the sutable solution . The enzyme was purified into three steps including precipitation by ammonium sulfate with (30-90%) saturation followed by Ion exchange chromatography on DEAE-Celluloes which showed 7.10as fold of Purification and 44.03% as enzymatic yield than step of Gel filtration using Sephadex G-100 column, this steps gave 11.83 as fold of purification and 18.76% as enzymatic yield

المقدمة

يعد أنزيم البيروكسيديز من الأنزيمات الواسعة الأنتشار في المملكة النباتية فضلا" عن تواجده في المملكة الحيوانية والأحياء المجهرية (1) .يحفز أنزيم البيروكسيديز أكسدة العديد من مواد الأساس المانحة للهيدروجين مثل المركبات الفينولية والأمينات الأوروماتية والهيدروكوينون ومشتقات البنزدين ، بوجود بيروكسيد الهيدروجين H_2O_2 كمادة أساس مستقبلة للهيدروجين (2) ، حيث ينتقل الهيدروجين من المادة المانحة للهيدروجين الى المادة المستقبلة للهيدروجين (3) .يستخدم انزيم البيروكسيديز بشكل واسع في المجالات الطبية والتحليلية والصناعية والغذائية اذ يدخل أنزيم البيروكسيديز في الفحوصات المناعية الأليزا (ELISA) Enzyme linked immunosorbent assay لأمتلاكه ثباتية عالية عند أرتباطه بالأجسام المضادة أذ تؤدي تفاعلاته الى تكوين نواتج ملونة يستدل منها على كمية المستضدات في العينة المراد تحليلها وبدرجة عالية من الدقة (3) ، كما أستعمل الأنزيم في تحديد كمية حامض اليوريك والكلوكوز والكالكتوز في السوائل الحيوية (4) بأستخدام الطرائق الأعتيادية او بوساطة المتحسسات الحيوية (Biosensors) (5) ،أضافة الى أستخدامه في تقنية وصمة ويسترن Westren Blotting التي تستعمل في التحليلات المناعية(6).

ينتمي أنزيم البيروكسيديز إلى المجموعة الأولى(أنزيمات الأكسدة والاختزال Oxidoreductase) و عرف بـ (donor:hydrogen – peroxide oxidoreductase;EC1.11.1.7).يعد أنزيم البيروكسيديز من الأنزيمات المعدنية(7) إذ يحتوي الأنزيم على ذرة حديد في موقعه الفعال . ترتبط مجموعة بروتوبورفرين الحديد بالأحماض الامينية الموجودة بالموقع الفعال أرتباطاً تناسقياً قوياً (8) ، وبسبب وجود مجموعة Protohemin ، تقاس الامتصاصية لأنزيم البيروكسيديز على أطوال موجية مختلفة كالاطوال 275 نانوميتر ،403 نانوميتر , 497 نانوميتر ، 641.5 نانوميتر. والوزن الجزيئي للأنزيم يقدر بـ 40.000 دالتون (9). وتوجد تقنيات متنوعة لتنقية الأنزيمات او البروتينات بصورة عامة تعتمد على معايير مختلفة كاختلاف في كثافة الشحنة ، الحجم ، شكل الجزيئة والتخصص في الارتباط بمجاميع معينة والتي تشتمل على الترسيب بالأملاح والمذيبات العضوية وكروموتوغرافيا التبادل الأيوني Ion exchange chromatography ، الترشيح الهلامي Gel filtration ، الهجرة الكهربائية Electrophoresis ، والترشيح الفائق Ultrafiltration ، الديلزة Dialysis كرموتوغرافيا الالفة Affinity chromatography (10,11) ، و من النادر الحصول على الأنزيم بصورته النقية من تطبيق خطوة تنقية واحدة (12) . أعتمدت عدة تقنيات لتنقية أنزيم البيروكسيديز ، جميعها تعمل على زيادة نقاوة الأنزيم وبحصيلة انزيمية عالية .

المواد وطرائق العمل

المواد الكيمياوية :-

حامض الهيدروكلوريك (HCl) ، هيدروكسيد الصوديوم (NaOH) كلوريد الصوديوم (NaCl) ، كبريتات الصوديوم (Na_2SO_4) ، خلات الصوديوم ، متعدد الكلايكول اثيلين-6000 ، حامض الخليك، الكحول الاثيلي ، الترس (Tris-HCl) ، كلايسين (Glysine) ، فوسفات الصوديوم احادية الهيدروجين (Na_2HPO_4) ، فوسفات الصوديوم ثنائية الهيدروجين NaH_2PO_4 ، ازايد الصوديوم Sodium Azide ، متعدد الأثلين كلايكول - 6000.من شركة BDH،الكوايكول Guaiacol ، بيروكسيد الهيدروجين H_2O_2 من شركة Fluka. ،سيفادكس G-100 (Sephadex G-100)، داي اثيل امينو اثيل سليلوز (DEAE-cellulose) من شركة Pharmacia.

الأجهزة المستخدمة والشركة المجهزة:

مطياف ضوئي Spectrophotometer من شركة Shimadzu – uv (Japan)، جهاز جامع الأجزاء Fraction Collector من شركة MRK / England ، مازج Vortex من شركة Whirli mixer . TM/FSA/USA

طريقة العمل:

تم تهيئة النماذج الخاصة بمصادر الأنزيم من جذور الفجل ، أجريت الخطوات اللازمة لتنظيف و تقطيع الجذور الى قطع صغيرة مكعبة الشكل ذات أبعاد 1×1 سم تقريباً وحفظها بالمجمدة و بدرجة حرارة - 8 م^0 لحين الأستخلاص . فقد تم تحضير المستخلص الخام بنسبة أستخلاص 4:1 (وزن : حجم) من وزن 4 غم من العينة المعدة للأستخلاص ثم سحقت بأستخدام هاون خزفي Morter مبرد لمدة خمسة دقائق ثم اضيف 20 مللتر من دارئ فوسفات الصوديوم بتركيز 0.2 مولار ورقم هيدروجيني 7.0 ، ونبذ المزيج بسرعة 5000 دورة /دقيقة لمدة 15 دقيقة وبدرجة 4 م^0 ثم رشح الرائق خلال ورق ترشيح. وتم تقدير الفعالية الانزيمية حسب طريقة واتكر (13) في تقدير فعالية أنزيم البيروكسيديز المستخلص وفق الظروف المثلى للأستخلاص باستخدام دارئ خلات الصوديوم بتركيز 0.2 مولار برقم هيدروجيني 5.7 .واستخدمت الطريقة اللونية من أضافة 5 مللتر من مزيج التفاعل لدارئ خلات الصوديوم بتركيز 0.2 مولارورقم هيدروجيني 5.0 في خلية المطياف الضوئي ، فقد صفر الجهاز أولاً على المحلول الكفأ (مزيج التفاعل) باستخدام طول موجي 436 نانوميتر ، وبدأ التفاعل بأضافة 200 مايكروليتر من المستخلص الأنزيمي الخام ثم مزج جيدا بالتقليب السريع للخلية الضوئية أعلى وأسفل ومتابعة التغيير في الأمتصاصية الضوئية لمدة 3 دقائق على الأقل بأستعمال ساعة توقيت ،ثم حسبت الفعالية (14) .و أستخدمت الطريقة المطلقة لتقدير تركيز البروتين في

المستخلصات الأنزيمية والتي تعتمد على قراءة الأمتصاص الضوئي على طول موجي 235 و 280 نانوميتر (15) ، حيث رسب البروتين باضافة 3 مللتر من محلول حامض الخليك ثلاثي الكلور الكلور(TCA) 5% الى 3 مللتر من المستخلص الأنزيمي ثم نبذ المزيج بجهاز النبذ المركزي بسرعة 5000 دورة /دقيقة لمدة 15 دقيقة ، أهمل الرائق وأذيب الراسب بكمية مناسبة من محلول هيدروكسيد الصوديوم بتركيز 0.05 مولار بأستخدام المازج ثم قرأت الأمتصاصية للمحلول على طول موجي 280,235 نانوميتر بأستخدام المطياف الضوئي بعد تصفيره على المحلول الكفأ Blank (0.05 مولار من هيدروكسيد الصوديوم) ثم أحتسب تركيز البروتين .

نقي أنزيم البيروكسيديز من جذور الفجل بخطوات أشتملت على تركيز الأنزيم بأملاح كبريتات الأمونيوم أعقبتها عملية ديلزة الناتج المتكون من الترسيب بالأملاح. ركز أنزيم البيروكسيديز بأستخدام تقنية الديلزة ضد متعدد الأثيلين كلايكول –6000 الجاف لغرض امتصاص نسبة الماء الموجودة وتركيز الانزيم .وبأوزان كمية معينة من كبريتات الأمونيوم للوصول الى نسبة الأشباع 30% ثم فصل الراسب المتكون بالنبذ المركزي بسرعة 8000دورة/دقيقة لمدة نصف ساعة وأهمل الراسب المتكون ثم رفعت نسبة أشباع الرائق الى90% بأضافة كمية معينة من كبريتات الامونيوم وبنفس الطريقة السابقة ثم ذوب الراسب المتكون بكمية قليلة من الماء المقطر ثم قدرت الفعالية الأنزيمية وفقا" للطريقة الموصوفة سابقا وتركيز البروتين كما تم ذكره أعقبت خطوة الترسيب عملية ديلزة للمحلول الأنزيمي ضد الماء المقطر لمدة 4 ساعات مع عدة تبديلات للماء المقطر للتخلص من أملاح كبريتات الامونيوم المتبقية مع النموذج . ثم خطوة تبادل أيوني بالمبادل الأيوني (DEAE- cellulose Diethylaminoethy cellulose) على عمود فصل (1.6 × 20) سم ثم جرت عملية موازنة المبادل بالمحلول فوسفات الصوديوم الدارئ بتركيز 5 ملي مولار ، أضيف 3 مللتر من االمحلول الأنزيمي الناتج من خطوة تركيز الانزيم على عمود المبادل الأيوني المحضر والذي سبقت موازنته بمحلول فوسفات الصوديوم الدارئ بتركيز 5 ملي مولار ورقم هيدروجيني 8.0 وبسرعة جريان 30 مللتر / ساعة حيث تمت متابعة الأمتصاصية الضوئية للأجزاء المفصولة على طول موجي 280 نانوميتر لحين الوصول الى الخط الصفري Base line بعدها جرى أسترداد البروتينات المرتبطة بالمبادل الأيوني بالمحلول دارئ فوسفات الصوديوم بتركيز 5 ملي مولار ورقم هيدروجيني 8.0 و تدرج ملحي خطي من (0-1) مولار من كلوريد الصوديوم NaCl .ثم قيست الأمتصاصية الضوئية على طول موجي 280 نانوميتر للأجزاء المستردة (Elution) لحين الوصول الى الخط الصفري Base line ثم قدرت الفعالية الأنزيمية وتركيز البروتين للأجزاء المنفصلة في خطوتي الغسل Wash والأسترداد Elution بعدها جمعت الاجزاء الفعالة وقيس حجمها ثم قدرت الفعالية الأنزيمية وتركيز البروتين ثم حفظت في ظروف مبردة لحين الأستخدام .

ثم تلتها خطوة ترشيح هلامي باستخدام هلام Sephadex G-100 .في عمود زجاجي ذو ابعاد (1.5 × 35) سم ثم جرت عملية موازنة العمود بسرعة جريان 15 مللتر / ساعة الى اليوم التالي .

أضيف المحلول الأنزيمي الناتج من خطوة التبادل الأيوني (الأجزاء المستردة غير المرتبطة) على عمود الترشيح الهلامي سيفادكس G-100 ثم جرت عملية الموازنة والأسترداد بوساطة محلول خلات الصوديوم الدارئ بتركيز 0.2 مولار ورقم هيدروجيني 5.7 وبسرعة جريان 15 مللتر / ساعة وبواقع 2 مللتر / جزء وتمت متابعة الأمتصاصية الضوئية للأجزاء المنفصلة على طول موجي 280 نانوميتر ثم قدرت الفعالية الأنزيمية للأجزاء المنفصلة ، بعدها جمعت الأجزاء الفعالة وقيس حجمها وقدرت الفعالية الأنزيمية لها وتركيز البروتين، بعدها أجريت عملية ديلزة المحلول الأنزيمي ضد الماء المقطر لمدة 4 ساعات ، ركز المحلول الأنزيمي بمتعدد الاثلين كلايكول –6000 ثم وزع في قناني صغيرة (Vials) وحفظ في ظروف مبردة لحين اجراء عملية التجفيد.

النتائج والمناقشة

أجريت عدة خطوات تنقية للأنزيم المستخلص من جذور الفجل الاحمر شملت هذه الخطوات تركيز المستخلص الخام للأنزيم بوساطة أملاح كبريتات الأمونيوم أعقبتها خطوة تبادل أيوني بالمبادل DEAE-Cellulose ثم خطوة ترشيح هلامي بهلام Sephadex G-100. و تعتبر عملية الترسيب بالأملاح المتعادلة من العمليات الضرورية في تنقية الأنزيمات للتخلص من البروتينات غير المرغوب فيها والمتواجدة مع الأنزيم وتقليص حجم المستخلص الخام والحصول على الأنزيم بدرجة من النقاوة ،فهناك العديد من الأملاح تكون فعالة في ترسيب البروتينات وأكثرها استخداما كبريتات الأمونيوم بسبب ذائبيتها العالية فضلا عن إنعدام تاثيرها على البروتينات (7) فيحدث الترسيب بالأملاح بفعل معادلة الشحنات المتواجدة على سطح البروتين والأخلال بطبقة الماء المحيطة بجزيئات البروتين مما يؤدي ذلك الى أنخفاض ذائبيتها وترسبها وتسمى هذه العملية Salting out. أستخدمت كبريتات الأمونيوم وبنسبة اشباع (30-90)% لتركيز أنزيم البيروكسيديز فقد حققت هذه الخطوة تنقية جزئية للأنزيم بلغت 2.55 مرة وبحصيلة أنزيمية مقدارها 78.3% مع ارتفاع الفعالية النوعية لتصل 787 (وحدة/ملغم بروتين) كما مبين في جدول (1) .

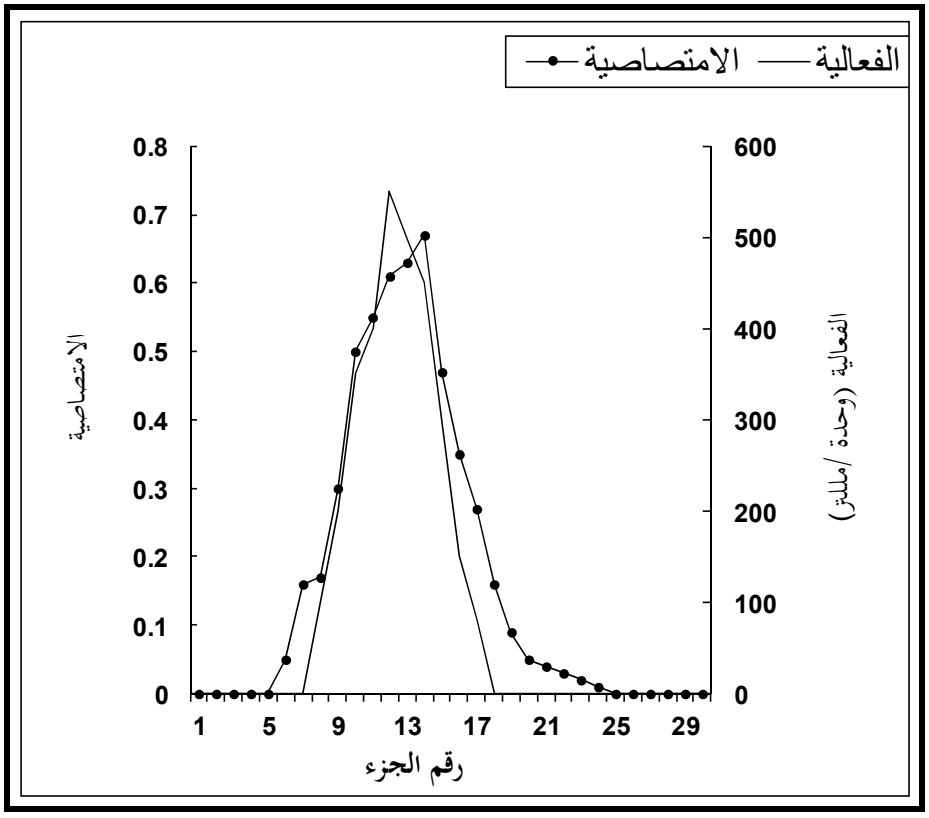
جدول (1) خطوات تنقية إنزيم البيروكسيديز من جذور الفجل الأحمر

خطوات التقنية	الحجم (مللتر)	الفعالية الحجمية (وحدة/مل)	تركيز البروتين (ملغم/مل)	الفعالية النوعية (وحدة /ملغم)	الفعالية الكلية (وحدة)	عدد مرات التنقية	الحصيلة %
المستخلص الخام Crud extraction	132	109.0	0.43	253.4	14388	1	100
الترسيب بكبريتات الأمونيوم بنسبة إشباع(30-90)%	8	1409.4	1.79	787	11278	3.10	78.3
التبادل الايوني بعمود DEAE- cellulose الغسل (Wash)	22	288.0	0.16	1800	6336	7.10	44.03
الترشيح الهلامي في عمود SephadexG-100	9	300.0	0.10	3000	2700	11.83	18.76

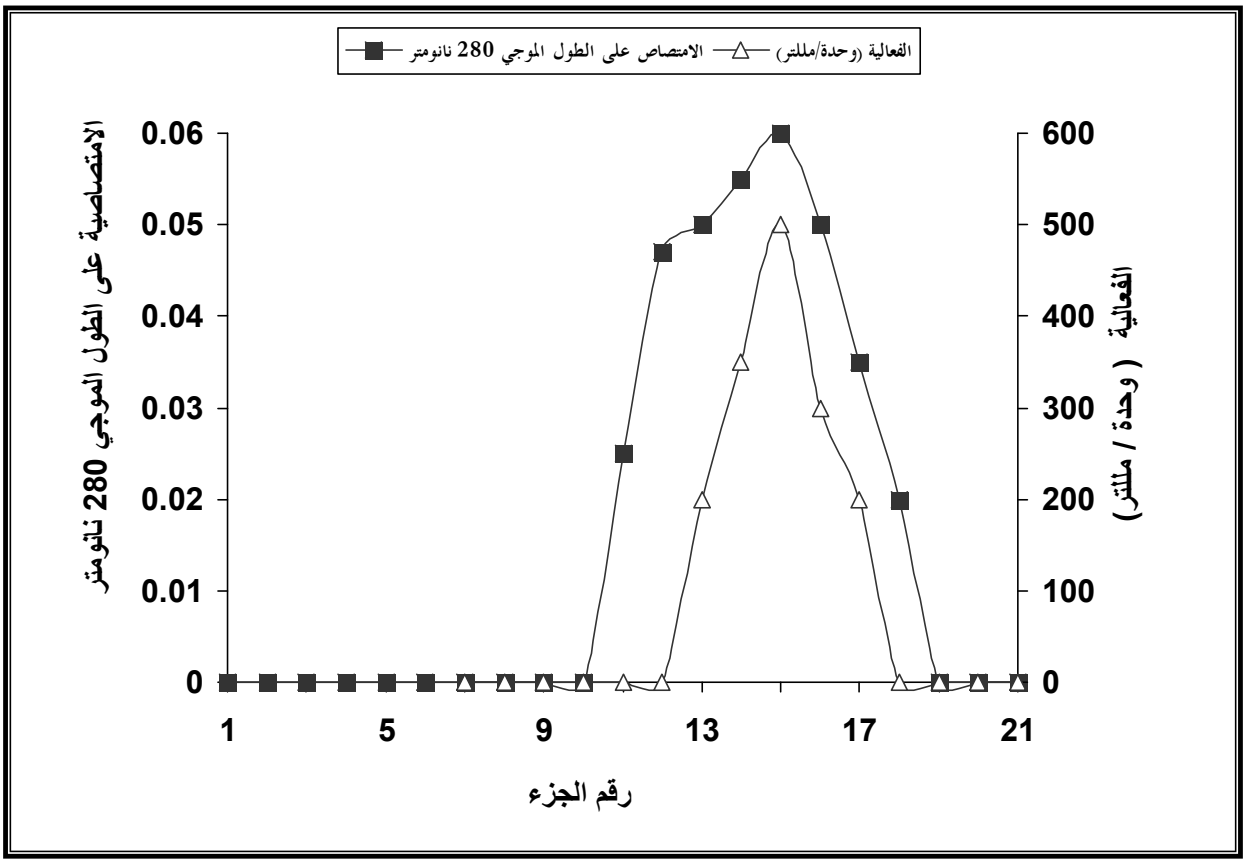
أظهرت النتائج المبينة في الشكل (1) التي تم الحصول عليها من خطوة الغسل (Wash) ظهور قمة واحدة من البروتين والفعالية الأنزيمية و بشكل متطابق تقريباً مما يدل على أن هذه الفعالية قد تركزت في أجزاء الغسل وأن الأنزيم يحمل محصلة شحنة موجبة مشابهة لشحنة المبادل الأيوني ضمن الظروف المستخدمة قيد التجربة جعلته لايرتبط بالمبادل وقد تم الحصول بهذه الخطوة على عدد مرات تنقية 7.10 وبحصيلة أنزيمية 44.03% مع أرتفاع تلت خطوة التبادل الأيوني أمرار المحلول الأنزيمي الناتج من الخطوة السابقة على عمود الترشيح الهلامي Sephadex G-100 . قيست الأمتصاصية الضوئية على طول موجي 280 نانوميتر بعدها جمعت الأجزاء الفعالة وقدرت الفعالية النوعية لها فكانت مساوية 3000 (وحدة/ملغم) بعدد مرات تنقية 11.83 مرة وبحصيلة أنزيمية18.76% كما في جدول رقم (1).

في الفعالية النوعية فكانت مساوية لـ 1800 (وحدة/ملغم بروتين) وكما هو موضح في الجدول (1) ، أما خطوة الأسترداد فقد لوحظ ظهور ثلاث قمم للبروتين بعد أستردادها بالتدرج الملحي الخطي وأن قيم الفعالية النوعية للأجزاء المستردة كانت منخفضة جدا قدرت بـ 1.63(وحدة/ملغم بروتين) لذلك تم أهمالها ولم تستكمل الدراسة عليها .

لقد ظهرت قمة البروتين للترشيح الهلامي مطابقة تماما لقمة الفعالية الأنزيمية وكما موضح في الشكل (2) إذ تعد هذه الخطوة دليلا على نقاوة الأنزيم ، ثم جرى ديلزة الأنزيم وتركيزه ووزع في قناني صغيرة Vail وحفظ تحت التبريد لحين تجفيده وتحويله الى صلب للحفاظ عليه اطول فترة ممكنة .



شكل رقم (1) يوضح النتائج في خطوة الغسل لفصل الانزيم بالتبادل الايوني



الشكل(2) يبين نتائج تنقية الانزيم بكروماتوغرافيا الترشيح الهلامي

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INSTRUCTIONS FOR AUTHORS

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* Materials and Methods
* Results and Discussions
* References (Use numbering in the text instead of full references.
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time they appear. A list of keywords (up to six) must be included after the abstract for indexing purposes. Words that appear in the title should not be repeated in the keywords.

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The text should be divided into sections with the headings: Introduction, Materials and Methods, Results, and Discussion. Subheadings within sections except introduction can be used to clarify their contents. Introduction and Discussion sections may contain present tense to convey generally accepted information. Materials and Methods and Results are normally written in the past tense.

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The introduction should define the problem and provide sufficient information to explain the background but there is usually no need for a comprehensive literature survey. The objectives should be stated but it should not contain a summary of the results.

2. Materials and Methods

Sufficient detail must be provided to allow the work to be repeated. This section should contain the experimental protocols and the origin of materials, tissue, cell lines, or organisms.

3. Results

The Results section should be in logical order presenting the experimental results. Please do not include any interpretations, inferences, arguments or speculations in this section.

4. Discussion

The authors should interpret their results clearly and suggest what they might mean in a larger context. Please do not repeat the information provided in the Results section.

Acknowledgements

Assistance received from funding agencies and colleagues should be acknowledged in this section.

References

Published or "in press" articles may be included in the reference list. Unpublished studies should be referred to as such or as a personal communication in the text. Citations of references should use square brackets, e.g. [1,3,5-7]. The lists of references, tables or figures should be numbered consecutively staring from 1. The references should be contain the last names and initials of up to four authors, year of publication, title of the paper, and the title of the journal. These should be followed by the volume and page numbers. References to books should include the title of the book, the year of publication, the publishing company and the place of publication. *Some examples are given below.*

Smith PF, Patel KR, Al-Shammari AJN. 1980. An Alde hydro-Phosphoglycolipid from Acholeplasma granularum. Biochem.Biophys. Acta 617: 419-429

Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

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Example

Department of Health: The Interdepartmental Working Group on Tuberculosis 1999. The Prevention and Control of Tuberc1ilosis in the United Kingdom [Online] [accessed 2000 September]. Available from URL http://www.doh.gov.ukltbguide1.htm

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