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FORWARD

With well- established ambitious steps on continuing success way, IJST is coming for you all today in its new issue of the seventh 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 all 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 7, No. 2 (2012), the second 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.

In this issue, IJST is honored to welcome a new Editorial Board member, ***Dr. Abdullah Al-Shebani*** *from Al- Kufa University, who presented great efforts in evaluating researches and gave the journal another prestigious dimension with his patiency and faithful attitudes toward IJST. On behalf of the Editorial Board members and the Editorial Board secretary, I present my deep thanking and appreciation for Dr. Abdulah.*

Also, we present a special thanking for ***Dr. Atheer Al-Douri*** *for his valuable endless support and efforts regarding the publicity of the journal in Ira, and it is our pleasure to announce that Dr. Atheer Al- Douri is the corresponding representative of IJST in Iraq.*

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 Antioxidant, Antimitotic & Antimicrobial Activity of *Urtica dioica* Leaf Extract

Hussein Oudha (1), Sundus H. Ahmed (1), Suhaila Nafiaa (2), Jinan Ali(3), Hussain A. Hussain (1) & Mageed Hameed (1)

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ABSTRACT

In this study, leaf extracts from *Urtica dioica*. have been tested for antioxidant, antimitotic and antibacterial activity. *Allium cepa* L. has been used for evaluating cytotoxicity. All extracts reduced the number of roots , length and mitotic index. All extracts shows antimitotic activities but methanol extract has found to be the most effective on reducing mitoses in root meristem cells. Methanol extract has also found effective against Gram-positive bacteria & negative bacteria.

Key words: **Urtica dioica, Antioxidant, Allium cepa L., Antibacterial, Antimitotic, Cytotoxicity,Mitotic index.**

الملخص باللغة العربية

اختبرت في هذه الدراسة الفعالية المضادة للاكسدة والانقسام الخلوي والبكتيري لمستخلصات نبات القريص *Urtica dioica* . استخدم البصل لتقييم الفعالية السمية الخلوية وتبين انخفاض في عدد وطول الجذور وعامل الانقسام . واظهرت كل المستخلصات فعالية مضادة للانقسام وان مستخلص الميثانول هو الاكثر فعالية في خفض انقسام الخلايا المرستيمية للجذر كما وجد ان له فعالية مضادة للبكتريا الموجبة والسالبة لصبغة جرام.

INTRODUCTION

Urtica dioica L. (stinging nettle) is a plant belong to the family Urticaceae. This herb is widely used in medicine for improving certain diseases such as diabet (1,2) rheumthy pertension and allergic rhinitis (3) Lipid peroxidation is an important deterioration reaction in food during storage and processing. It not only causes a loss in food quality but also is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing, and arteriosclerosis (4). The role of active oxygen and free radicals in tissue damage in such diseases, are becoming increasingly recognized (5). However, antioxidant supplements or foods rich in antioxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen (6) Recently, various phytochemicals and their effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, spices and herbs, have been intensively studied (7). An important goal of this research was to examine antioxidant , antimitotic and antimicrobial activity of *Urtica dioica leaf extract*.

MATERIALS AND METHODS

Free radical scavenger

The free radical scavenging ability of each of the plant extracts against 1,1-diphenyl-1-2-picrylhydrazyl (DPPH) free radical will be evaluated according to the method of (8).

Antimitotic activity

Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with the leaf extracts. D.W was used as a control, and for dilution leaf extracts. The test tubes were kept in an incubator at 27°C and the test samples were changed daily. After 72 h the roots were counted and their lengths were measured for each onion .bulb When the newly emerged roots measured 2.0 − 3.0 cm, they were fixed. The fixative was glacial acetic acid/absolute alcohol (1/3 v/v). The root tips were kept in the aceto alcohol solution for 24 h. After fixation, the slides were prepared for examination or the roots were transferred to%70 ethyl-alcohol and stored in a refrigerator. For examination, the root tips were put into a watch glass where 9 drops of aceto-orcein and 1 drop of 1MHCl were added and warmed over a flame of spirit lamp for 3min. These were kept at room temperature for 15- 30 min. After removing the root caps from well stained root tips, 1 mm of the mitotic zones were immersed in a drop of %45 acetic-acid on a clean slide and squashed under a cover glass. In order to spread the cells evenly on the surface of the slide, squashing was accomplished with a bouncing action by striking the cover glass with a match stick. MI were expressed in terms of divided cells / total cells. A statistical analysis was performed on total cells.

Antibacterial activity

Test Microorganisms:

Pseudomonas aeruginosa, *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris*, *Staphylococcus aureus* & *Candida albicans* , were used as test organisms.

Extract preparation: The dried and powdered plant materials ten gram from dried sample. were extracted in 100 ml solvent (Methanol, Ethanol & Water) by kept on a rotary shaker for 24 h. Then, it was filtered through Whatman No. 1 filter paper. The sample were further concentrated to dryness under using a rotary evaporator. It was dissolved in dimethyl sulfoxid and stored at 4ºC for further studies. All the obtained extracts thus was injected into empty sterilized antibiotic disc having a diameter of 6 mm in the amount of (20 µl). The controls which only 20 µl of solvents were injected to the disc. Streptomysin sulfate and Nystatin were used as standard .Preparation of microbial cultures: The bacterial strains were inoculated into nutrient broth and yeast strain inoculated in to malt extract broth for 24 and 48 h., respectivelly. In the disc-diffusion method, sterile Mueller Hinton agar for bacteria and Malt extract agar for yeast were separately inoculated with the test bacteria and yeasts 10^5 bacteria per/ml, 10^4 yeast per /ml). Disc were applied on the solid agar medium by pressing slighthly . Petri dishes were placed at 4ºC for 2 h. and then incubated at 37ºC for 24 h and yeast incubated at 25for 3-4 day, inhibition zones were measured in millimeters (9).

Data Analysis

Quantitative data would be expressed as mean ± standard deviation. Statistical evaluation of the data would be performed by using one – way analysis of variance (ANOVA) followed by Duncan's multiple range test (10).

RESULTS AND DISCUSSION

The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by an tioxidants. The maximum absorption of a stable DPPH radical in is at 517

rnn. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant phase. molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visulized as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity (10, 11). Figure (1) illustrates a significant ($P< 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of the Urtica dioica extract. All extracts almost equal DPPH scavenging activity, the scavenging effect of Methanol > Ethanol > Water extracts on the DPPH radical decreased in the order of methanol >Ethanol > water were 84.5, 82.7 and 74.5 % at the concentration of 50 µg /ml respectively. Invitro antimicrobial activities of plant extracts and standard antibiotic shown in Table (1)

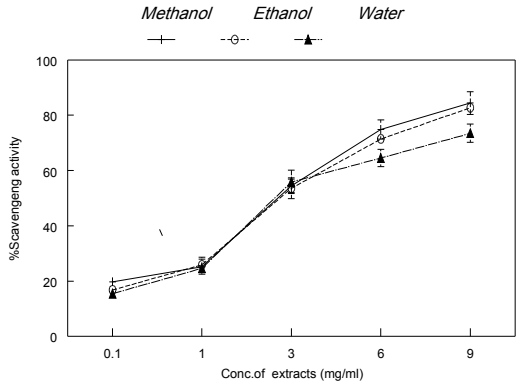

Figure (1): DPPH scavenging activity of different extracts of *Urtica dioica*

Table (1): The root length and number of Allium in presence of *Urtica dioica* plants extracts

Extract	Average root numbers (± SD)	Average root lengths mm (± SD)
Control	30.8± 3.7 [a]	22.6± 2.6 [a]
Methanol	1.9± 2.1 [b]	0.5± 1.4 [b]
Ethanol	8.6± 1.3 [c]	4.1± 3.2 [c]
Water	16.5± 2.1 [d]	9.7± 1.1 [d]

Significant at P <0.05
Values represent means of triplicate.
Values with the different alphabet along the same column a

The plant extracts showed various antimicrobial activities against the microorganism *E coli, , S. aureus, , C. albicans, K. pneumoniaeand aeruginosa*} (inhibition zone between 6- 26 mm). Methanol extract efficiency as shown: 23, 21, 10, 16, 25,26mm inhibition zone . Eethanol extracts antimicrobial activity 15, 16, 25, 20 mm while water extract inhibition zone shown 13, 0, 6, 12, 10 mm. Control disc did not show any activity against microorganism. Standard disc inhibited the growth of all the tesKmicroorganisms. These results show that there are differences in the antimicrobial effect of plant extracts, due to phytochemicial differences among extracts. (12) claimed that sensivity of microorganism to chemoterapeUtic compounds change even against different strains. In similar studies, the extracts of different plants inhibited the grpwth of some microorganisms at different ration s It suggested that all plant extracts, especially methanolic and ethanolic extracts can be used as antimicrobal agents in development of new drugs for the treatment of infectious disease . A wide variety of anticancer drugs exhibit cytotoxic effect by interfering with cell cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in Mphase. All alkylating agent, methotrexate interferes with DNA integrity and thereby exhibits strong anti-mitotic activity both in vivo and in vitro . The root length and number for control and for each extract are given in Table (2).

Table (2): Antimicrobial activity (inhibition zone) of *Urtica dioica* plant extracts

Antimicrobial	E coli	S. aureus	*K. pneumoniae*	*P aeruginosa*	C. albicans
Ethanol extract	18 ± 0. 14 [a]	13 ± 0.25 [a]	10 ± 0.28 [a]	16 ±0.26 [a]	20 ± 0.12 [a]
Methanol extract	23 ± 0. 21 [b]	21 ±0.33 [b]	16 ± 0.41 [b]	25 ±0.31 [b]	26 ± 0.23 [b]
Water extract	13 ± 0. 17 [c]	-	6 ±0.33 [c]	12 ±0.22 [c]	10 ± 0.35 [c]
Control	-	-	-	-	-
Standard	*14± 0. 30 [d]	*18 ±0.19 [d]	*15 ±0.17 [d]	*11 ± 0.43 [d]	**13 ± 0.20 [d]

**Streptomysin sulfate , ** Nystatin*
Significant at P <0.05
Values represent means of triplicate.
Values with the different alphabet along the same column

Extracts reduced significantly root number and root length when compared with control. Methanol extract was more effective on root length and number when compared with the other extracts . This results shows that the extracts from *Urtica dioica* leavs haves inhibitory effects on root growth and length in *Allium cepa*. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable di amagnetic molecule. The model of scavenging stable DPPH radical is a widely used method to evaluate antioxidant'activities in a relatively short time compare to other methods (13). ln conformity with animal and human cell cytotoxicity it was found that *Urtica dio*ica extracts have cytotoxic properties also in plant test systems. In table (3) the mitotic indexes are given for control and for each extract. It is evident that all extracts reduced the mitotic index significantly. The reduction in dividing cells in the root meristem shows the antimitotic effects of the substances that found in leavs extracts . Methanol extract was more effective on mitotic index when compared with the other extracts. In respect of this results, *Urtica dioica* leavs contains antimitotic constituents that can stop the mitosis of the cell cycle. Further more these extracts of plants showed various antimicrobial activities against the microorganism. *E. coli, , S. aureus, , C. albicans, K. pneumoniaeand against P. aeruginosa*, (inhibition zone between 6- 26 mm).

Table (3): The dividing and total cells that counted in microscopic observations and mitotic index (MI) in control and in extracts

Extract	Total cells	Dividing cells	MI(±SD)
Control	17304± 0.50 [a]	3107± 0.72 [a]	%18 ± 0.23 [a]
Methanol	10712± 0.31 [b]	134 ± 0.33 [b]	%1 ± 0.36 [b]
Ethanol	11244± 0.22 [c]	327 ± 0.28 [c]	% 2 ± 0.70 [c]
	12236± 0.36 [d]	506± 0.46 [d]	%4 ± 0.16 [d]

Significant at P <0.05
Values represent means of triplicate.
Values with the different alphabet along the same column

Methanol extract efficiency as shown: 23, 21, 10, 16, 26mm inhibition zone, Eethanol extracts antimicrobial activity was: 18, 15, 16, 25, 20 mm while water extract inhibition zone shown 13, 0, 6, 12, 10 mm. Control disc did not show any activity against microorganism. Standard disc inhibited the growth of all the test microorganisms. These results show that there are differences in the antimicrobial effect of plant extracts, due to phytochemicial differences among extracts. (13, 14) claimed that sensivity of microorganism to chemoterapeutic compounds change even against different strains. In similar studies, the extracts of different plants inhibited the growth of some microorganisms at different ratio . It would suggest that all plant extracts, especially methanolic and ethanolic extracts can be used as antimicrobal agents in development of new drugs for the treatment of infectious disease . A wide variety of anti-cancer drugs exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase. An alkylating agent, methotrexate interferes with DNA integrity and thereby exhibits strong anti-mitotic activity both in *vivo* and in *vitro*. The root length and number for control and for each extract are given in table (2). Extracts reduced significantly root number and root length when compared with control. Methanol extract was more effective on root length and numbers when compared with the other extracts . This results shows that the extracts from *Urtica dioica* haves inhibitory effects on root growth and length in *Allium cepa*. In conformity with animal and human cell cytotoxicity (15) it was found that *Urtica dioica* extracts have cytotoxic properties also in plant test systems. In table (3) the mitotic indexes are given for control and for each extract. It is evident that all extracts reduced the mitotic index significantly. The reduction in dividing cells in the root meristem shows the antimitotic effects of the substances that found in leavs extracts . Methanol extract was more effective on mitotic index when compared with the other extracts. In respect of this results, *Urtica dioica* leavs contains antimitotic constituents that can stop the mitosis of the cell cycle. Furthermore these constituents probably affects the cytoskeleton or tubulin polymerization or degradation.

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Production, purification and characterization of asparaginase from *Pisum sativum* var.Jof

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ABSTRACT

Asparaginase was extracted from plant parts of *Pisum sativum* var.Jof collected from a field crop. Asparaginase activity was detected in seeds, stems and leaves extracts. Enzyme specific activity was higher in seeds extracts (75.6 U/mg) compared with leaves extracts (73.6U/mg) and stems extracts (72.8 U/mg) respectively. Asparaginase extracted from plant seeds was purified by ion exchange chromatography using DEAE-Cellulose and gel filtration chromatography using Sephadex G-200. Purified asparaginase with specific activity of 228.8 U/mg was characterized, and it was found that the molecular weight of asparaginase was 66464 Dalton, the optimum pH for enzyme activity and stability was pH 8.5, while the optimum temperature for enzyme activity and stability was 37°C and 40°C respectively.

Key Words: **Asparginase, *pisum sativum*, chromatography.**

الملخص باللغة العربية

تم استخلاص انزيم الاسبارجنيز من اجزاء نباتية مختلفة لنبات البزاليا صنف Jof . وكانت اعلى فعالية للانزيم في البذور فقد وصلت الى (75.6U/mg) اما في الاوراق فكانت (73.6U/mg) وفي الساق فكانت (72.8U/mg) . استخدم الانزيم المستخلص من البذور للتنقية باستخدام كروموتوكرافية التبادل الايوني باستعمال DEAE-cellulase و كروموتوكرافيا فلترة الجل باستخدام sephadex G-200 . ان الانزيم المنقى كان ذو فعالية تساوي 228.8U/mg والوزن الجزيئي له كان 66464 دالتون . وكان Ph المناسب له هو 8.5 اما احسن درجة حرارة لفعالية الانزيم فكانت 37 – 40 مئوية.

INTRODUCTION

L-asparaginase (E.C.3.5.1.1) is the enzyme that catalyses the hydrolysis of the amide group of L-asparagine releasing L-aspartate and ammonia. The enzyme plays important roles both in the metabolism of all living organisms as well as in pharmacology (1). Asparagine is predominates in the transport of nitrogen in many legumes studied so far and constitutes an important source of reduced nitrogen for developing seeds (2).

L-asparaginase is a therapeutically important protein used in combination with other drugs in the treatment of acute lymphocyte Leukemia (mainly in children, Hodgkin disease, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticlesarcoma and melanosarcoma (3, 4). .

Two forms of the enzyme have been identified. A potassium - independent form is found in *L-arborous* and *L.polyphyllus* (5), and a potassium dependant form is found in *pisum sativum l.* and several other legume species, including other *lupines* species(6). According to importance of L- asparaginase in different therapeutical treatment this study was aimed to extract, purify and characterize the enzyme from plant seeds of *P. sativum.*

MATERIALS AND METHODS

Sample collection

Plant parts (leaves, stems and seeds) of *Pisum sativum* var.Jof. were collected during season 2011 from field of plant crops.

Extraction of asparaginase from different plants parts

Extraction of asparaginase from plant parts was achieved according to Chang and Farnden (7) by homogenization, 10 grams of plant parts with three volumes of 0.05 M potassium phosphate buffer, pH 8.0 containing 1.5 M sodium chloride, 1mM PMSF, 1mM EDTA, and 10% (w/v) glycerol, then centrifuged at 10000 rpm for 20 minutes. Supernatant was regarded as crude enzyme.

Enzyme assay

Asparaginase was assayed according to Nesslerization method based on the conversion of L-asparagine to Ammonia and L-asparatate, as described by Ren *et al*. (8).

Protein concentration in plant extracts and enzyme concentrates was determined according to Bradford method (9).

Purification of asparaginase

Asparaginase from seeds of *Pisum sativum* was purified by ion exchange chromatography using DEAE-Cellulose (2×23 cm) equilibrated with 50 mM potassium phosphate buffer (pH 8.0), then bound proteins were eluted with a flow rate of 3 ml/fraction by gradient concentrations of NaCl (0.1-0.5M) in 0.05 M potassium phosphate buffer solution (pH 8.0). Fractions represents asparaginase activity were collected, concentrated and applied onto gel filtration chromatography column using Sephadex G-200 (1.6×43cm), and eluted with 0.1 M potassium phosphate buffer (pH8.0) with flow rate of 20 ml/hour. Fractions represents asparaginase activity were pooled, concentrated and kept at 4°C for further analysis.

Characterization of purified asparaginase

Characterization of purified asparaginase was achieved by determining the optimum pH and temperature for enzyme activity and stability, and the activation energy according to Segel (10), while the molecular weight of asparaginase was determined by gel filtration chromatography technique using Sephadex G-200 as described by Andrews (11).

RESULTS AND DISCUSSION

Asparaginase activities were detected in plant parts of *Pisum sativum* var.Jof. Results indicated in Table (1) showed that maximum asparaginase activity (605 U/ml), specific activity of (75.6 U/mg) and total activity (4880 U) were noticed in plant seeds extracts, and more greater than in other plant parts extracts (leaves and stems). According to these results plant seeds were used for enzyme production, purification, characterization of asparaginase.

Table (1): Asparaginase activity and specific activity in the extracts of plant parts of *Pisum sativum* var .Jof.

Plant part	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific Activity (U/mg)	Total activity (U)
Seeds	10	605.0	8.0	75.6	6050
Leaves	10	500.5	6.8	73.6	5005
Stems	10	488.0	6.7	72.8	4880

Asparaginase extracted from seeds of *P. sativum* was purified by ion-exchange chromatography using DEAE-Cellulose. Results indicated in figure (1) showed that one protein peak appeared in the washing step, while there are four protein peaks were appeared after elution with gradient concentrations of sodium chloride, asparaginase activity was detected in the third peak as mentioned in table (2). The maximum asparaginase activity and specific activity in the asparaginase concentrate were 554.8 U/ml and 154.1 U/mg respectively, with 13.7% fold of purification and remaining activity of 91.7%.

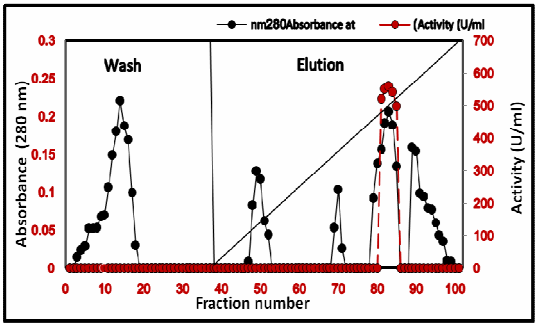

Figure (1): Ion exchange chromatography for purification of asparaginase from seeds of *P. sativum* using DEAE-Cellulose column (2×23 cm) with a flow rate of 20 ml/hour

Table (2): Purification steps of asparaginase extracted from seeds of *P. sativum*

.

Step	Volume ml)(	Activity U\ml)(	Protein conc. (mg\ml)	Specific activity U\mg)(	Total activity (U)	Purification fold	Yield %)(	Remaining activity (%)
Crude enzyme	100	605.0	8.0	75.6	60500	1	100	100
Ion exchange	15	554.8	3.6	154.1	8322	2.03	13.7	91.7
Gel filtration	20	732.4	3.2	228.8	14648	3.02	24.2	121.0

Gel filtration chromatography technique was the next step for purification of asparaginase after the ion-exchange chromatography step. Results mentioned in figure (2) showed that only one protein peak represents asparaginase activity was appeared after elution with 0.1 M potassium phosphate buffer solution.

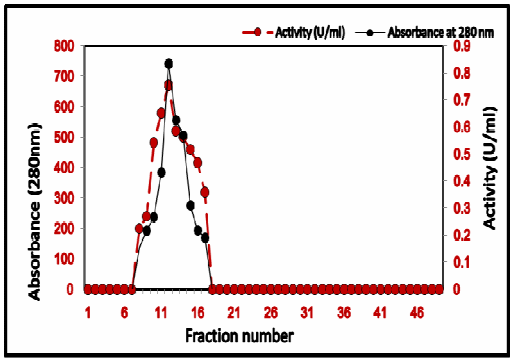

Figure (2): Gel filtration chromatography for purification of asparaginase from seeds of *P. sativum* using Sephadex G-200 (1.6×43 cm) equilibrated with potassium phosphate (pH8.0), fraction volume was 5ml at flow rate of 20 ml/hour.

Results indicated in table (2) showed that there is an increase in both activity and specific activity of the purified enzyme with a purification fold of 3.02 and an increase in the yield of asparaginase to 24.2%.

In other study, it was found that partial purification of asparaginase from chicken liver using Sephadex G-100 and Sephadex G-200 as a second step (after ammonium sulfate precipitation) gives the highest specific activity of 158.11 U/mg protein and 128 fold of purification with 17% yield (12).

Molecular weight of the purified asparaginase was determined by gel filtration using Sephadex G-200 in the presence of six standard proteins. Results indicated in figure (3) showed that asparaginase has a molecular weight of 66464 Dalton. Molecular weight of asparaginase differs according to the type of the produced genera, species and parts of plant, There was no evidence for dissociation into subunits on SDS-PAGE, and this suggest that asparaginase is a monomeric protein of molecular weight 69 KDa (6,13).

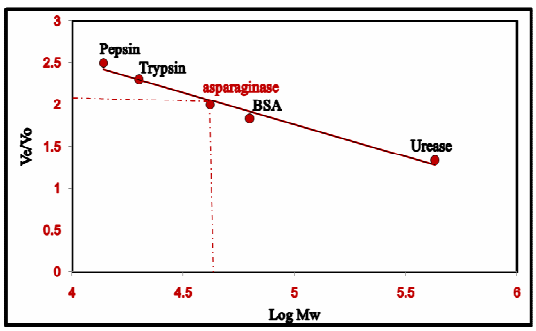

Figure (3): Selectivity curve for determining the molecular weight of purified asparaginase extracted from *P. sativum* seeds by gel filtration chromatography using Sephadex G-200 (1.6×43 cm)

Optimum pH for activity of purified asparaginase was determined by incubation with asparagine at different pH values ranging between pH4 and pH10.5. Results indicated in figure (4) showed that asparaginase was active over a wide range of pH values between 5 and 10 with a maximum activity of 632 U/ml and 622 U/ml at pH 8.5 and 8.0 respectively. Effect of pH on the rate of hydrolysis of asparagine is due to the effect of asparaginase on the velocity of enzyme-substrate complex formation and breakdown, in addition to its effect on the ionic state of the active site of enzyme, hence the change in the hydrogen ion concentration may affect the ionization state of amino acid side chains in the enzyme active site (14- 16).

On the other hand, results indicated in figure (5) showed that asparaginase was more stable at pH values ranged between 8.0 and 8.5, at this range enzyme gain both maximum activity and remaining activity, while the activity and remaining activity was lower when the enzyme was incubated at pH values less or more than optimum.

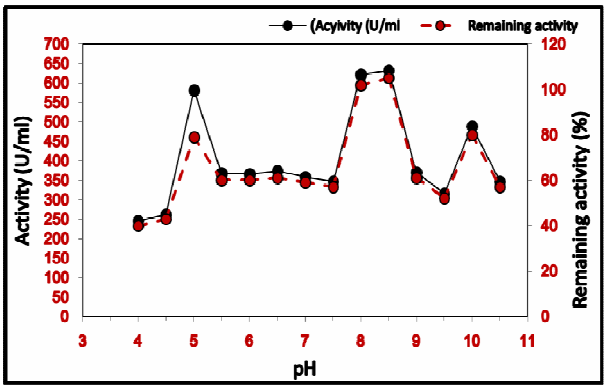

Figure (4): Effect of pH on activity of purified asparaginase extracted from seeds of *P. sativum*

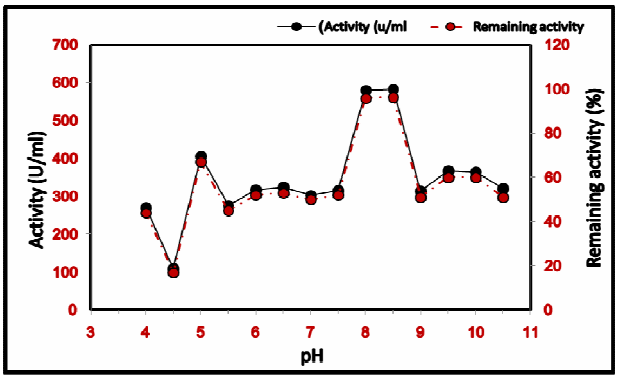

Figure (5): Effect of pH on stability of purified asparaginase extracted from seeds of *P. sativum*

Stecher *et al*., (1999) found that the tertrameric form of asparaginase remain stable at pH values ranged between 4.5 and 11.5 with slight increase at alkaline pH. Asparaginase from *S. marcescens* was more stable at the pH range between 5 and 9 (3 , 17).

Effect of temperature on the purified asparaginase was also studied. Results indicated in figure (6) showed that the optimum temperature for enzyme activity was 37°C, while the activity was decreased at less or higher than this temperature. Most enzyme reactions were found to be accelerated with the increase in temperature for a limited range.

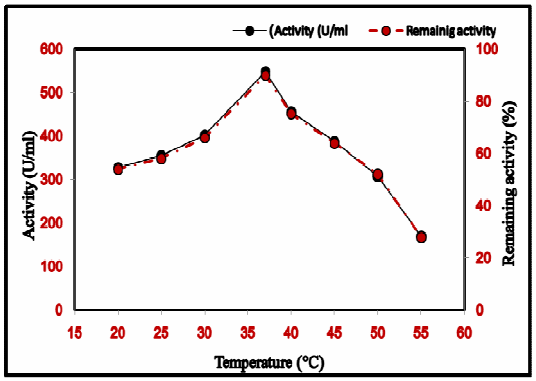

Figure (6): Effect of temperature on the activity of purified asparaginase extracted from seeds of *P. sativum*

On the other hand, it was found that asparaginase was more stable at 40°C as shown in figure (7), at this temperature enzyme gain maximum activity and remaining activity, while they were decreased when the enzyme was incubated at temperatures less or more than the optimum temperature. The increase in temperature may lead to denaturation of enzyme by destruction the three dimensional structure of protein which leads to inactivation of the enzyme at higher temperatures.

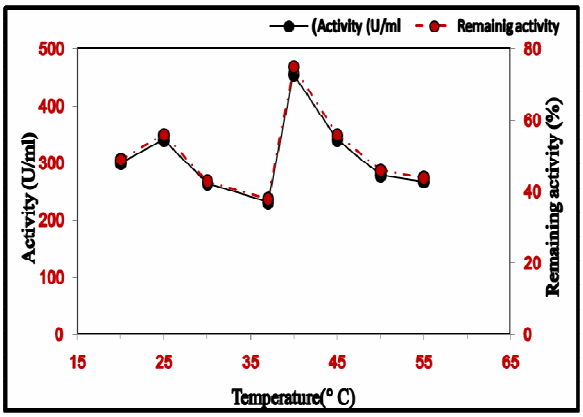

Figure (7): Effect of temperature on stability of asparaginase purified from seeds of *P. sativum*

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Green Nano particles as a disinfectant in *Iresine herbstii*

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ABSTRACT

There is an increasing demand for nanoparticles because of their various applications in many fields such as energy, chemistry, and many other machinery. This work was designed to prepare green silver nano particles from leaves of Geranium. The filtrate color was changed to light brown color. Which is an indication for nano particles, Beside the UV spectrum peak was under 500nm. These particles have similar effect than hypochlorite disinfectant in survival rate. These particles may be a substitute for hypochlorite which has health hazards.

Key Words*: green silver nano particles, disinfectant, *in vitro* culture, *Iresine herbstii

الملخص باللغة العربية

لقد زاد استعمال الجزيئات النانوية في مجالات كثيرة نتيجة الطلب على تحضير هذة الجزيئات. هذا العمل صمم لتحضير جزيئات الفضة النانوية من مستخلص اوراق الجيرانيوم. بعد تصفية المستخلص يتغير اللون من البني الفاتح الى البني الغامق. وهذا دليل على حصولنا على جزيئات الفضة النانوية, بالاضافة الى فحص المستخلص بجهاز قياس المطياف الضوئي للاشعة فوق البنفسجية تحت 500 نانوميتر, وهذة الجزيئات شبيهة بتاثيرها بمعقم هايبوكلورات الصوديوم بمعدل الزروعات غير الملوثة والنامية وهذة الجزئيات قد تكون مناسبة للاستعمال اكثر من هايبوكلورات الصوديوم لان الاخيرة لها مخاطر صحية.

INTRODUCTION

It has been noted that infection for *in vitro* plants is big problem in micropropagation, and chemical available to control these contaminations, but the efficiency of these chemical are either limited or toxic. Antibiotics are also used in controlling internal bacterial contaminations (1). However, they may affect the growth and response of explants and may induce resistance in bacteria. Therefore, they are not suggested for using in plant tissue culture techniques (1). Mercury chloride ($HgCL_2$) has been widely used to control infections in explants. HgCL2 is very toxic and should be used with high cautions (2). Such chemicals are not only toxic for the explants and peoples working in this field but they may affect the environment. Therefore, finding an effective and safe substance for decontaminated of explants is very important, and that is what this paper aim to.

The term Nano-technology was first used by Japanese scientist "Taniguchi" at the University of Japan (3).

Nano-agriculture involves the employment of nano-particles in agriculture with the ambition that these particles will impart some beneficial effects to the crops. Recently, confocal fluorescence image studies have revealed the capacity of single walled carbon nanotubes (SWNTs) to traverse across both the plant cell wall and cell membrane(4). The study also pointed out that SWNTs can serve as effective nanotransporters to deliver DNA and small dye molecules into intact plant cells. There are also some reports on other nanoparticles as smart treatment- delivery systems in plants(4).

Nano – silver is new and non – toxic material which shows high capabilities in decontamination of microorganisms, e.g. fungus, bacteria, and viruses. The detrimental effects of this chemical have been shown more than 600 microorganisms (5). This capability of nano-silver is due to release of tiny particles of silver so it is able to destroy not only bacteria, fungus but also the viruses (6).

Because of complexity in producing chemical silver Nano particles, there is a new adventure to produce these particles from plants using leaves such as *Euophorbia hirta*(7) .*Aloe vera* (8)and geranium(9) are plants which had been used intensively.

MATERIALS AND METHODS

This study was at Al-Nahrain University/ College of science/ Biotechnology department. Baghdad Iraq through 2011.

Types and media preparation: MS medium were prepared by dissolving 4.1g/l from a readymade sachets MS medium. After dissolving, this medium was supplemented with 2mg/l IAA and BA as growth regulators plus 30g/l sugar. The whole media were sterilized in autoclave at 121 °C for 15 minutes (10-11).

The preparation of green silver particles was done according to (9). 20g/l of fresh Geranium leaves were washed thoroughly and dried with filter paper No.1. These leaves were cut into small pieces then 100ml of distilled water were added. Boiled for1 minute. After boiling 5ml of the mixture were mixed with 100ml of AgNO3 [8g/l], the solution examined with UV spectrophotometer under 300-700nm (12).

Explants sterilization:

5Cm Stem cutting of Blood leaf plant *Ire*sine *herbstii* Family Araceae were sterilized in 3% hypochloride for 8 minute (positive control). Then, washed three times in sterilized distilled water 5 minutes each time. Some explants were soaked in the above nano - particles solution, the other were cultured directly in MS medium. (Negative control).

RESULTS AND DISCUSSION

The results revealed that the contamination rate was 100% in control treatment while it was reduced to about zero percent in both hypochlorite and silver nano particles at 8 and 12 minutes time intervals. Figure (1) below:

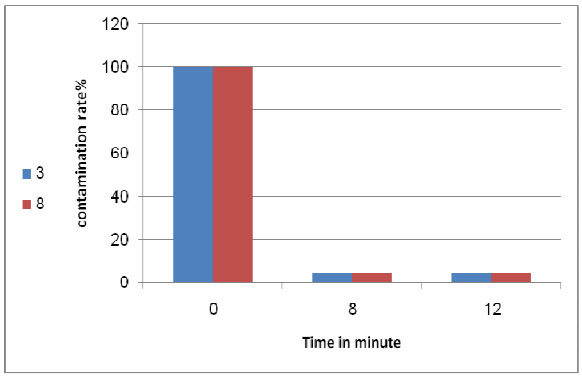

Figure (1) : Effects of silver Nano particles(in red) and sodium hypochlorite (in blue) on contamination rate% of explants

However, in figure 2 and 3 below the survival rate for explants treated with different time intervals 0,8,12 minutes for both hypochlorite and nano particles. It was found that the survival rate was about 5% in 3 minutes time will it raise up to 100% in 8 minutes time, then it was decreased in 12 minutes treatment to 40-50% in both hypochlorite and nano particles .

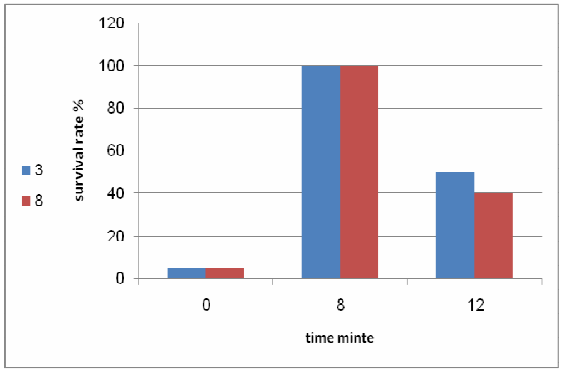

Figure (2): Effects of silver Nano particles(red) and sodium hypochlorite (blue) on survival rate%

Figure (3): survival explants after two weeks in culture

These particles were examined under UV spectrophotometer and it found their peak in 500 nm. This results was compatible with the other researchers such as (13,14).

Using hypochlorite in vitro culture makes people sensitive with some skin irritation and respiratory system problems. Therefore, looking for alternative disinfectant will be good achievement. In this paper, we are trying to use green silver particles, which we think a good substitute for old disinfectant.

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Synthesis and Characterization of metal complexes with ligands containing a hetero (N) atom and (hydroxyl or carboxyl) group

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ABSTRACT

The research includes the synthesis and identification of two types of mixed ligands complexes of M(II) Ions using amino acid **L-** proline as a primary ligand and either Nicotinamide or 8-hydroxyqinoline as secondary ligand, respectively:

a. The mixed ligand complexes of composition,$[M(pro)_2(na)_2]$.

b. The mixed ligand complexes of composition , $Na[M(pro)_2(Q)]$.

Where proline ($C_5H_9NO_2$) symbolized as pro H , Nicotinamide ($C_6H_6N_2O$) symbolized as (NA) , 8-hydroxyqinoline, ($C_9H_7NO_2$) symbolized as (8-HQ).

The ligands and the metal chlorides were brought into reaction at room temperature (37°c) in ethanol as solvent .The reaction required the following molar ratios [(1:2:2) metal:2NA:2pro$^-$] and [(1:1:2) metal:Q:2pro$^-$] with M^{+2} ions, where M = [Mn (II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and pd(II)].

Products were found to be solid crystalline complexes, which have been Characterized through the following techniques:

Melting points, Solubility, Molar conductivity. Determination the percentage of the metal in the complexes by (AAS). Spectroscopic Methods [FT-IR and UV-Vis], and. The proposed structure of the complexes using program , chem office 3D(2006) .

Key words: **Amino Acid, , 8- hydroxyqinoline, L- proline, Mixed Ligand Complexes**

الملخص باللغة العربية

يتضمن البحث تحضير وتشخيص نوعين من المعقدات المختلطة الليكاند للايونات M^{+2} باستعمال الحامض الاميني (البرولين) ليكاندا أول والليكاند (النيكوتين امايد او 8-هايدروكسي كوينولين) ليكاندا ثاني على التوالي:

1- معقدات مختلطة الليكاند بالصيغة: $[M(pro)_2(na)_2]$

2- معقدات مختلطة الليكاند بالصيغة: $[M(pro)_2(Q)]^-$.

إذ ان البرولين ($C_5H_9NO_2$) بالرمز proH النيكوتين امايد($C_6H_6N_2O$) بالرمز (na) و8-هايدروكسي كوينولين (C_9H_7NO) بالرمز (8-HQ) وذلك بمفاعلة الليكاندات مع كلوريدات العناصر باستعمال الايثانول مذيبا وفي درجة حرارة المختبروبنسب مولية [(1:2:2) (metal): 2na: 2pro$^-$] و [(1:1:2) (metal) : Q: 2pro$^-$] مع M^{+2} إذ إن M^{+2} = [Mn^{+2}, Co^{+2}, Ni^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}, pd^{+2}].

المعقدات المحضرة بلورات صلبة بعضها ملون درست من النواحي الآتية:
درجات الانصهار، التوصيلية الكهربائية المولارية، الذوبانية، تقدير النسبة المئوية للأيون الفلزي في المعقدات بوساطة مطيافية الامتصاص الذري ، الدراسات الطيفية: وتضمنت أطياف(الأشعة تحت الحمراء، الأشعة فوق البنفسجية– المرئية، مع استخدام برنامج (Chem. Office– Cs – 3D pro 2006) في رسم أشكال المعقدات

INTRODUCTION

8-hydroxyqinoline or 8-qinoline is the name most commonly used ,while its trivial name is (oxine) ,which is conventionally used for the description of chelate compounds Oxinates ,as out of seven possible hydroxyqinoles, only 8-hydroxyqinoline forms chelate with metal ions. (1). 8-hydroxyqinoline is well-characterized organic chelating ligand ,which can form covalent compounds with over 60-metal ions under controlled pH-conditions ,and its preference for transition and heavy metal cations over alkali and alkaline-earth cations is well known. (2).

8 -hydroxyqinoline has a wide variety of uses and its medicinal and agricultural Significances were discovered before the start of current (3,4). A series of mixed- ligand (saccharinato, amino acids, Iminodiacetic acid and dimethylglyoxime) complexes of Cu(II), Co(II), Ni(II) and Zn(II) were recently reported. (5-9) .Ganesh and Co-worker[10] were prepared and characterized of mixed ligand diox-ouranium(VI) and thorium (IV) complexes of 8-hydroxyquinoline as a primary ligand and amino acids such as L-proline (ProH) and 4-hydroxy-L-proline (Hyp) as secondary ligands. These complexes have been screened for their antibacterial and cyto-toxic (IC50) characteristic properties .

In this paper we reported the synthesis ,spectroscopic and structural of two types mixed ligand complexes of M(II) ions using amino acid proline as a primary ligand and either nicotinamide or 8-hydroxyqinoline as secondary ligand, respectively.

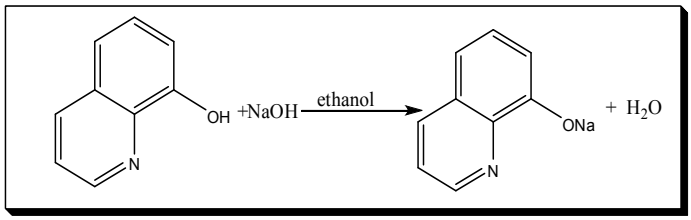
MATERIALS AND METHODS

Reagents: L-proline ,nicotinamide and 8-hydroxyqinoline were purchased from (Merck), metals chloride and solvents from (B.D.H). The reagents were applied without further purification .

b-Instruments: UV-Vis spectra were recorded on a (Shimadzu UV- 160A) Ultra Violet-Visible Spectrophotometer. FTI R- 8400S) Fourier Transform Infrared Spectrophotometer (4000-400) cm^{-1} with samples prepared as K_Br discs. Metal contents of the complexes were determined by atomic absorption technique using a Shimadzu AA 680G atomic absorption spectrophotometer. Conductivities were measured for 10^{-3}M of complexes in (DMF) at 25^{o}C using (Philips PW- Digital Conduct meter). Magnetic measurements were recorded on a Bruker BM6 instrument at 298°K following the Faraday's method . In addition melting points were obtained using (Stuart Melting Point Apparatus). The proposed molecular structure of the complexes were drawing by using chem. proposed models of the species were built with chem. 3DX (2006).

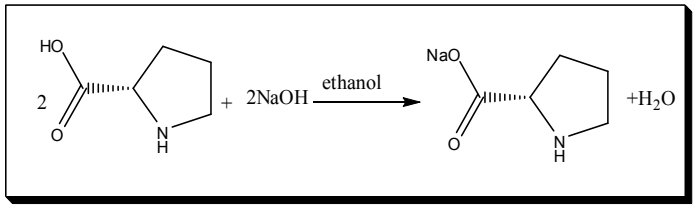
Synthesis method:

A) Sodium-8-oxyqinoliney (8Q): dissolve [0.145 gm,1mmol]8- H ydroxyquinoline(QH)with[0.04gm1mmol)] sodium hydroxide in (10ml) ethanol was deprotonated according to the following reaction: Scheme (1)



Scheme (1): Synthesis of the sodium-8-oxyqinolinate

B) Sodium prolinate (Pro^{-} Na^{+}): L-proline [0.230 gm (2mmol)] was dissolved in 10 ml ethanol and added to 10 ml of ethanolic solution containing [0.08 gm (2mmol)] of the sodium hydroxide , the solution was deprotonated according to the following reaction:



C) Nicotinamide solution: Nicotinamide[(0.112 gm) 1m.mol] was dissolved in 10 ml ethanol.

D) Synthesis of complexes: An aqueous solution of the metal salt was added to the solution of the ligand in ethanol respectively using stoichiometric amounts [(1:2:2)(metal):2NA:2pro^{-}] and [(1:1:2) metal:Q:2pro^{-}] molar ratio , the mixture was stirred for half an hour at room temperature, crystalline precipitates observed.

The resulting precipitates were filtered off , recrystallized from ethanol and dried at room temperature.

RESULTS AND DISCUSSION

Products were found to be solid crystalline complexes, which have been characterized through the following techniques: Solubility, Molar conductivity. Determination the percentage of the metal in the complexes by (AAS). Spectroscopic Method [FT-IR and UV-Vis], Biological effects for some complexes were investigated and Program [Chem. office .CS. Chem. 3D pro 2006 was used. The Physical properties listed in Table (1). All the complexes are colored, non-hygroscopic, and appears as powders with high melting points .They are not soluble in water. All complexes dissolved in DMF and DMSO solvents. The atomic absorption measurements Table (1) for all complexes gave approximated values for theoretical values.

Table (1). Analytical data and some physical properties of the complexes

Compounds	M.wt	Color	M.p°c (de)°c	*M.C $\mu S.cm^{-1}$	Metal% theory	Metal % Exp.
$[Mn(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	549	White	299(dec)	13.27	10.43	11.24
$[Co(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	530.19	Pale- Brown	277(dec)	15.15	11.11	12.4
$[Ni(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	530.96	Pale- blue	290(dec)	29.11	11.55	11
$[Cu(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	535.71	Pale- blue	>300 (dec)	19.18	11.84	11
$[Zn(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	537.63	White	>300(dec)	17.43	12.17	11
$[Cd(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	584.6	White	>300 (dec)	25.58	19.22	21
$[Pd(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	578.6	Red	>300 (dec)	32.67	18.38	20
$Na[Mn(C_9H_6NO)(C_5H_8NO_2)_2]$	450.25	Yellow	270 -300 (dec)	68.1	12.19	14
$Na[Co(C_9H_6NO)(C_5H_8NO_2)_2]$	454.25	Green wish - Brown	>300 (dec)	71.6	12.98	14.8
$Na[Ni(C_9H_6NO)(C_5H_8NO_2)_2]$	454.11	Green	280(dec)	83.31	12.92	11.92
$Na[Cu(C_9H_6NO)(C_5H_8NO_2)_2]$	458.77	Greenwish-Brown	>300 (dec)	77.7	13.85	14.21
$Na[Zn(C_9H_6NO)(C_5H_8NO_2)_2]$	460.69	Yellow	290(dec)	86.3	14.18	15.88
$Na[Cd(C_9H_6NO)(C_5H_8NO_2)_2$	507.40	White	245(dec)	88.2	22.15	23.66
$Na[Pd(C_9H_6NO)(C_5H_8NO_2)_2]$	501.4	Red	292(dec)	73.4	21.8	23.9

M.C = Molar Conductivity , (de) = decomposition

The molar conductance values of the complexes in DMF at 10^{-3} M concentration are found to be (13.27. -32.67) $\mu S.cm^{-1}$, for complexes of composition $[M^{II}(Pro)_2(NA)_2]$ indicating their non-electrolytic nature while the complexes of composition $Na[M^{II}(Pro)_2(Q)]$(68.1 -- 88.2) $\mu S.cm^{-1}$are found to be correspond to 1:1 electrolytes.(11)

The analytical data shown in Table (1) confirmed the following molar ratios [(1:2:2)(metal):2NA:2pro$^-$] and [(1:1:2) (metal):Q:2pro$^-$] with M^{+2} ions.

The infrared spectra of various mixed ligand complexes synthesized are compiled in tables (2& 3) figures (1-5).The infrared spectra of these complexes in comparison with free ligands show characteristic band positions, shifts and intensities, which can be correlated to monodentate nicotinamide [2,13] binding and bidentate (Proline acid and 8-hydroxyqinoline) chelation. The deprotonated ligand (Proline acid) to (Prolinate ion) (Pro$^-$) by using (Na OH) coordinated to metal ions as bidentate ligand through the oxygen atom of the carboxylate group (-COO$^-$) and the nitrogen atom of the imine group(NH). The nicotinamide coordinated as a monodentate through the nitrogen of the pyridine group.

Table (2) : FT-IR spectral data of mixed ligand complexes of composition,[$M(pro)_2 (NA)_2$]

Compound	NH_{2asy} str	NH str	CH (py) str	C=O str(amid)	NH_2 (am)	C-Ncyc	(C-C) str (py)	C=N Str (am)	O=CN Bend (am)	υ(-COO⁻) asym	υ(-COO⁻) sym	M-N	M-O
Nicotinamide	3368vs	3161s	3060sh	1679vs 1697sh	1618vs	-	1423vs 1123m	1395vs	736vs	-	_	_	_
Proline	-	3056	-	_	_	1473 946vs	_	_	_	1600-1624	1450s -1377	_	_
$Mn(NA)_2(pro)_2$	3394s	3309s	3190vs	1666vs	1624 vs	1577w 941w	1436m	1396s	759m	1624s 1600m	1446m 1396s	590br-m	459w
$Co(NA)_2(pro)_2$	3402s	3194m	2924m	1670vs	1614vs	1580w 941w	1458s 1199m	1320m	752vs	1665 1610	1446s 1396s	582sh-m	418m
$Ni(NA)_2(pro)_2$	3371vs	3174	2924m	1677m	1616vs	1456m	1458s 1199w	1326m	756m	1705vs-1654w	1388 1476	520m	439m
$Cu(NA)_2(pro)_2$	3402vs	3309w	2360 vs	1668m	1623vs	1485w 931m	1406s 1157m	1383m	775m	1701s 1624s	1477 1377vs	543s	430s
$Zn(NA)_2(pro)_2$	3332br	2981m	2921S	1670m	1614vs	941s	1396s	1330m	797s	1666 -1624s	1396s m1330	568m	450m
$Cd(NA)_2(pro)_2$	3394s	3263m	2981m	1630m	1622vs	1577m 941m	1430w	1379m	759m	1705vs 1658m	1446S 1396s	590m	459w
$Pd(NA)_2(pro)_2$	3394vs	3159s	2924m	1636m	1618vs	1567m 937w	1488s 1166w	1354s	771s	1623-1509	1457vs 1377vs	540s	448m

Sym: symmetric, asy: asymmetric, am: amide, py: pyridine, o.p: out of plane,str: stretching,v.s: very strong, s: strong, m: medium, w: week, sh: shoulder.

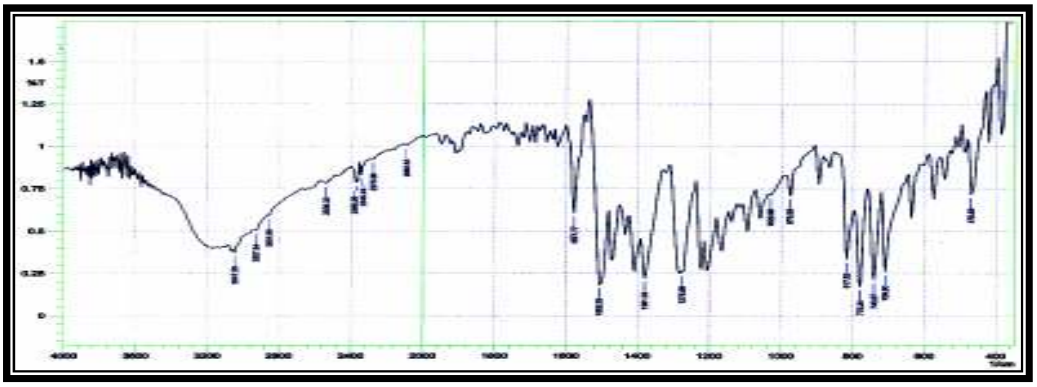


Figure (1): FT-IR spectrum of ($C_9H_7NO_2$)

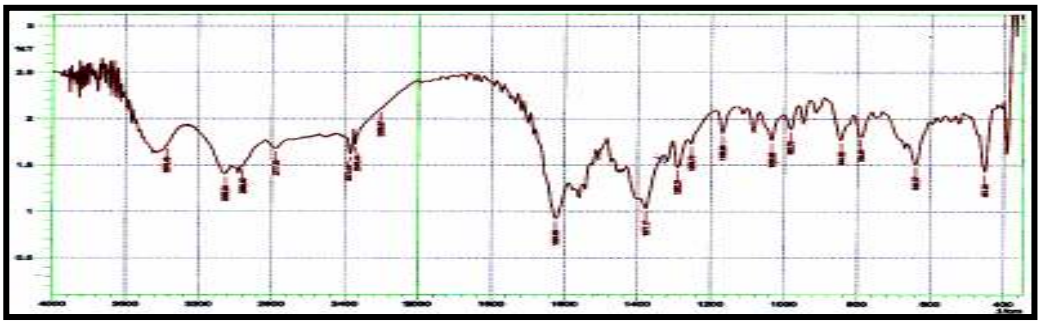


Figure. (2) FT-IR spectrum of ($C_5H_9NO_2$)

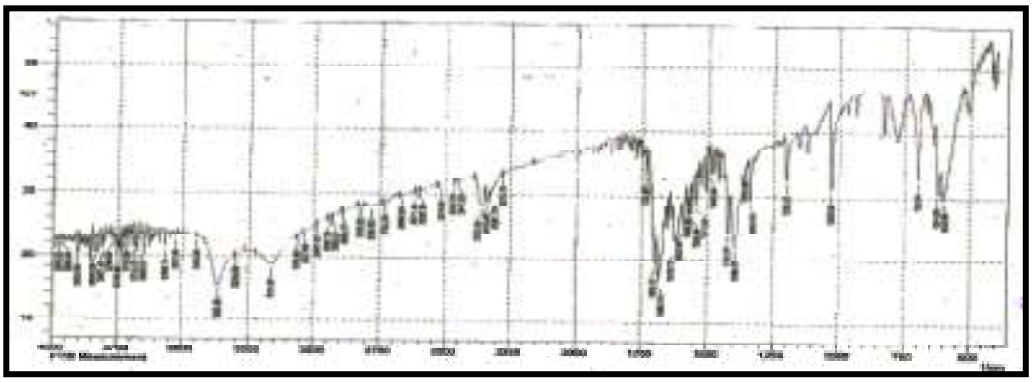


Figure (3) FTIR spectrum of ($C_6H_6N_2O$)

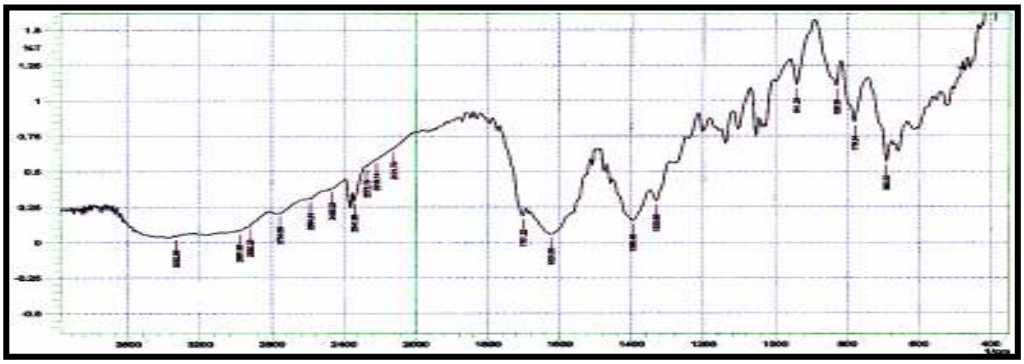


Figure (4): FTIR spectrum of [$Zn(C_6H_6N_2O)_2(C_5H_8NO_2)_2$]

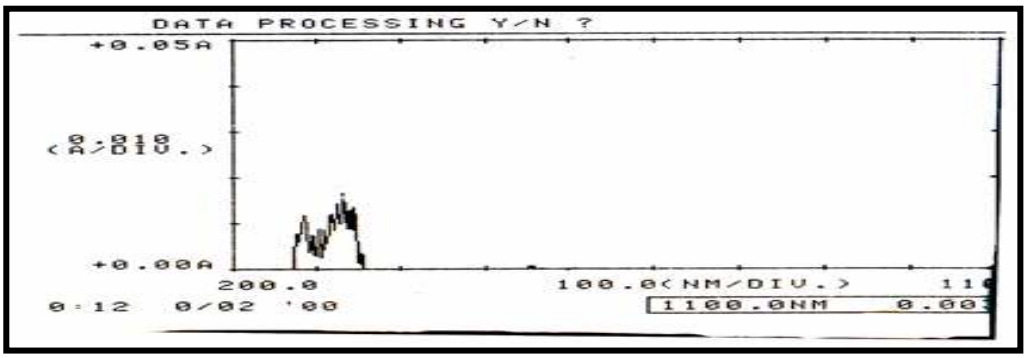


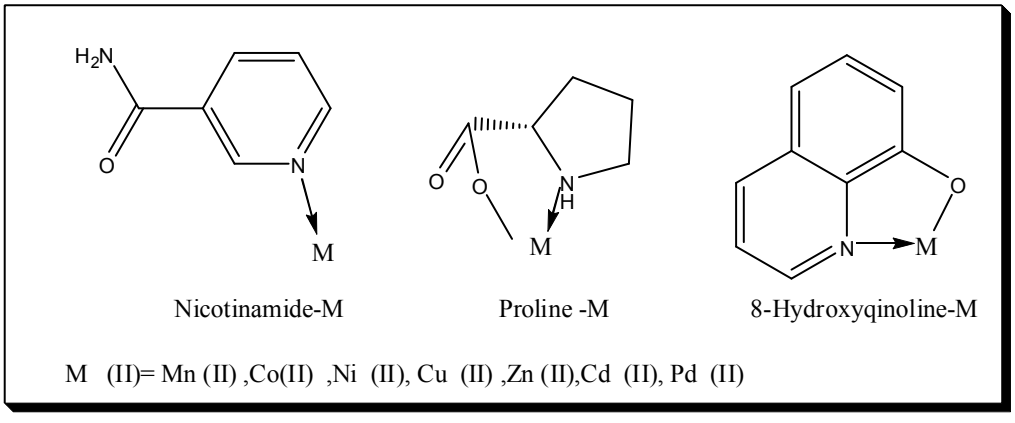
Figure (5): UV-vis absorption spectrum of the ($C_5H_9NO_2$)

Table (3) : FT-IR spectral data of mixed ligand complexes of composition Na [M(pro)$_2$(Q)]

Compounds	NH sym str	CH$_{(py)}$ str	(CH)$_{cycl}$	υ (C=N)	υ (C=C)	υ(C–O)	υ ∂(C-O)	υ(-COO$^-$) asym	sym	M-N	M-O
8-HQ	-3240 3047br			1577	1508						
Na[Mn(pro)$_2$(Q)]	3174s	3136s 2854w	2974m	1620w	1573vs	1283m	457m	1465s	1323s	640 m	450 m
Na[Co(pro)$_2$(Q)]	3194br-s	3136 m 2854w	2924w	1590s	1570s	1269m	505m	1465 vs	1377vs	648 m	450w
Na[Ni(pro)$_2$(Q)]	3352	3278 br-s	2927s	1620vs	1520w	1284m	497m	1427vs	1377s	645 m	420w
Na[Cu(pro)$_2$(Q)]	3356s	3062 m	2924w	1581vs	1504vs	1289vs	468s	1469vs	1377vs	632 m	420 m
Na[Zn(pro)$_2$(Q)]	3356s	3052 m	2924sh-m	1581vs	1508m	1284m	505s	1496vs-1462 vs	1373vs-1315vs	645 m	420w
Na[Cd(pro)$_2$(Q)]	3194br-s	3190 m	2924sh-	1573vs	1500s	1284m	505s	1496s-1419m	1384vs-1315vs	640 m	436w
Na[Pd(pro)$_2$(Q)]	3194br-s	3055 m	2927w	1620m	1577vs	1273m	597vs	1496s-1465vs	1384vs-1323vs	630 m	436w

Sym: symmetric, asy: asymmetric, str: stretching, vs: very strong, s: strong , m: medium, w:week,

The deprotonated ligand (8-hydroxyqinoline) to oxyqinolinate ion (Q$^-$) by using (NaOH) coordinated to metal ions as bidentate ligand through the oxygen atom of the hydroxyl group (–HO), and the nitrogen atom of pyridine group (15-17) as shown in scheme (2)



Scheme (2) The ligands coordinated

The infra red spectrum of free ligand (proline)exhibited a strong band around 3056 cm^{-1} corresponds to the stretching vibration of υ(N-H) +υ(O-H), while another strong absorption band at (1600-1624) cm^{-1} is appeared which could explained as $\upsilon(COO^-)_{asym}$ where the $\upsilon(COO-)_{symmetric}$ stretching vibration of proline or sodium proline was assigned band at (1450-1377) cm^{-1} (18). The infra red spectrum of free ligand (Nicotinamide) exhibited a strong band around (3368) cm^{-1} that corresponds to the stretching vibration of υ $(N-H_2)$ .An important feature of infra-red spectra of metal complexes with 8-HQ is the absence of the band at (3240 - 3047) cm^{-1} due to the O-H stretching vibration of the OH group of HQ (18) Figure (1). This observation leads to the conclusion that the complex formation takes place by deprotonation of the hydroxyl group of HQ moiety .Charles et al. (19) reported that for several metal complexes with HQ, the υ (C-O) band is observed at 1120 cm^{-1}. The position of this band varies depending on metal complex under study. A strong υ (C-O) band observed at ~ 1104 cm^{-1} indicates the presence of oxine moiety in the complexes coordinated through its nitrogen and oxygen atoms as uni negative bidentate ligand (17,18). The υ (C=N)mode observed at 1577cm^{-1}in the spectra of free HQ ligand is found to be shifted to lower wave number i.e. ~1500-1497 cm^{-1} in the spectra of complexes. A negative shift in this vibration mode on complexation indicates the coordination through the tertiary nitrogen donor of HQ. Some new bands of weak intensity observed in the regions around (520-648) $cm^{-1}$and (418-552) cm^{-1} may be ascribed to M-N and M-O vibrations, respectively[17,18]. It may be noted that, these vibration bands are absent in the spectra of the ligands.

The electronic spectra:

The electronic spectra of all compounds (Ligands and complexes) are listed in Table (4) Figures. (6-8) together with the proposed assignments and suggested geometries (19, 20)

The (UV–Vis) spectra of the ligands and their complexes were studied in order to elucidate the spatial arrangement of the ligands around the metal ion.

On the basis of the above analysis (AAS, molar conductance, FT–IR, UV–Vis) spectra. The existence of hexa coordinated $M(pro)_2(NA)_2$. and Na $[M(pro)_2(Q)]$.

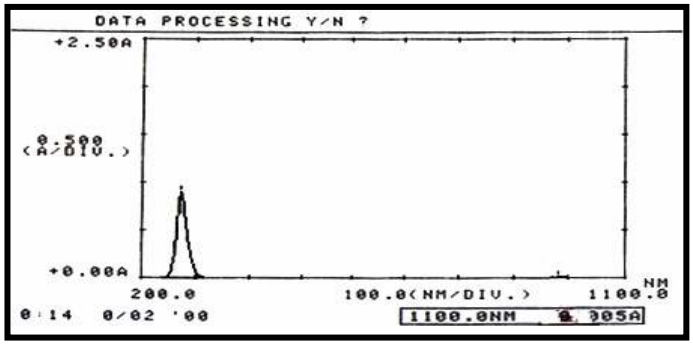


Figure (6) UV-vis absorption spectrum of the $(C_6H_6N_2O)$

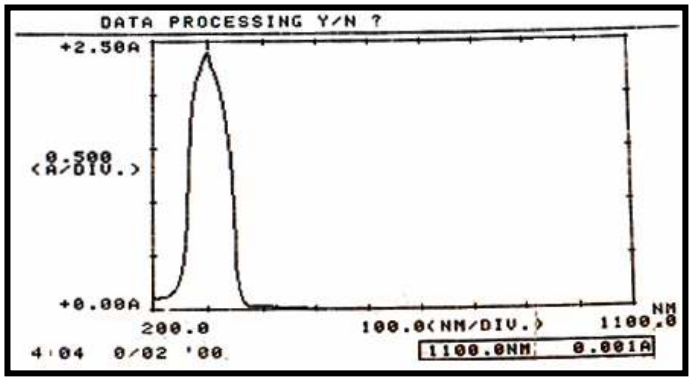


Figure (7) UV-vis absorption spectrum of the (C_9H_7NO)

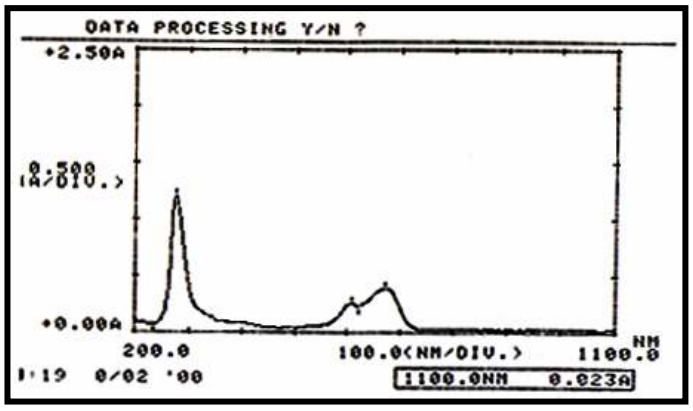


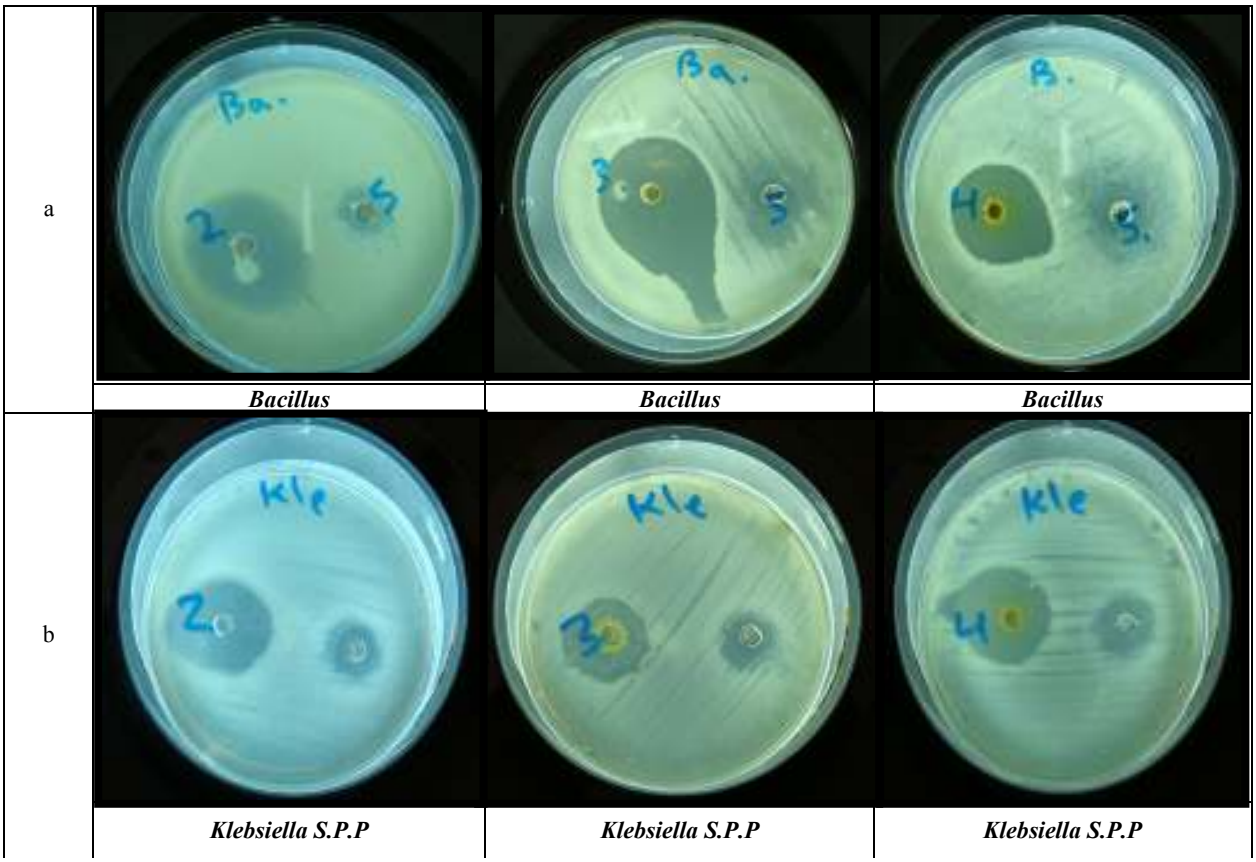
Figure (8) UV-vis absorption spectrum of the $[Co(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$

Table (4). Electronic data for the ligands and there complexes

Compounds	Bacillus	Klebsiella S.P.P	Staph S.P.P
Control(DMF)	10.1	10	9
$[Ni(pro)_2(na)_2]$	19.1	15.9	11.1
$[Co(pro)_2(na)_2]$	25.4	15.9	19.1
$Na[Cu(pro)_2(oxine)]$	20.5	14.9	25.6
$Na[Pd(pro)_2(oxine)]$	20.2	18.1	20.2

Proposed molecular structure

Studying complexes on bases of the above analysis , the existence of Hexa coordinated $[M(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$ and $Na[M(C_9H_6NO)(C_5H_8NO_2)_2]$, where M = [Mn (II), Co(II), Ni(II),Cu(II), Zn(II), Cd(II) and pd(II)].A proposed models of the species were built with chem. office program [21]. Figure (9)



c			
	Staphylococcus S.P	***Staphylococcus S.P***	***Staphylococcus S.P***
	$[Co(pro)_2(NA)_2]$	**$Na[Cu(pro)_2(oxine)]$**	**$Na[Pd(pro)_2(oxine)]$**

Figure (9) shows the antimicrobial activity of complexes (appear the inhibition zones against some pathogenic bacteria) **(a)** ***Bacillus*** **(b)** ***Klebsiella S.P.P*** **(c)** ***Staphylococcus S.P***

Table (5) Showed the inhibition circle diameter in millimeter for the bacteria after 24 hour incubation paid and 37°C for some complexes

Compounds	λ(nm)	$\bar{\upsilon}$ (cm^{-1})	ABS	$\varepsilon_{(max)}$ $L.mol^{-}.cm^{-1}$	Assignment
Proline	332	30120	0.150	150	$n\rightarrow\pi^*$
Nicotineamide	276	36238	0.620	629	$\pi\rightarrow\pi^*$
8-Hydroxyqinoline	280 301	35714 33222	1.880 2.407	1880 2407	$n\rightarrow\pi^*$ $\pi\rightarrow\pi^*$
$[Mn(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	279	35842	1.377	1377	Ligand field
$[Co(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	276 608 672	36231 16447 14880	1.200 0.262 0.391	1200 262 391	Ligand field $^4T_{1g}\rightarrow{}^4A_{2g}$ $^4T_1g\rightarrow{}^4T_2g(P)$
$[Ni(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	280 390 623	35714 25641 16051	1.624 0.616 0.437	1624 616 437	Ligand field $^3A_{2g}\rightarrow{}^3T_{2g}(P)$ $^3A_{2g}\rightarrow{}^3T_{1g}$
$[Cu(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	299 439 815	33444 22779 12269	1.885 1.48 0.065	1885 148 65	Ligand field $^2B_{1g}\rightarrow{}^2A_{2g}$ $^2B_{1g}\rightarrow{}^2B_{2g}$
$[Zn(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	273 344	36630 29069	0.959 0.210	959 210	Ligand field C.T
$[Cd(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	277	36101	1.240	1240	Ligand field
$[Pd(C_6H_6N_2O)_2(C_5H_8NO_2)_2]$	300 412	33333 24271	2.332 1.331	2332 1331	Ligand field C.T
$Na[Mn(C_9H_6NO)(C_5H_8NO_2)_2]$	279 345 411 850	35842 28985 24330 11764	1.378 1.872 1.766 0.032	1378 1872 1766 0032	Ligand field $^6A_{1g}\rightarrow{}^4T_{1g}(D)$ $^6A_{1g}\rightarrow{}^4E_{1g}{}^4A_{1g}(G)$ $^6A_{1g}\rightarrow{}^4T_{2g}(G)$
$Na[Co(C_9H_6NO)(C_5H_8NO_2)_2]$	301 420	33222 23809	1.91 1.84	1931 1840	Ligand field $T_{1g}(F)\rightarrow{}^4T_{2g}(P)$
$Na[Ni(C_9H_6NO)(C_5H_8NO_2)_2]$	276 328	36231 30487	1.192 1.645	1192 1645	Ligand field $^3A_{2g}\rightarrow{}^3T_{2g}(F)$

	388 525	25773 19047	1.100 2.20	1100 220	$^3A_{2g}\rightarrow{}^3T_{1g}(F)$ $^3A_{2g}\rightarrow{}^3T_{1g}(P)$
$Na[Cu(C_9H_6NO)(C_5H_8NO_2)_2]$	281 412 688	35587 24271 14534	1.470 1.426 0.031	31 1426 1470	Ligand field $^2B_{1g}\rightarrow{}^2A_{2g}$ $^2B_{1g}\rightarrow{}^2B_{2g}$
$Na[Zn(C_9H_6NO)(C_5H_8NO_2)_2]$	276 332 412	36231 31055 24271	1.188 2.295 1.786	1188 2295 1786	Ligand field Ligand field C.T
$Na[Cd(C_9H_6NO)(C_5H_8NO_2)_2]$	301 390	33222 25641	2.286 1.105	2286 1105	Ligand field C.T
$Na[Pd(C_9H_6NO)(C_5H_8NO_2)_2]$	288 440	34722 22727	1 .629 1.993	1629 1993	Ligand field C.T

Biological activity

The antibacterial activity of the synthesized some complexes was determined in vitro using paper disc method (agar plate diffusion method) against three pathogenic microorganism viz., Staphylococcus aureus (Gram +ve) , Klebsiella S.P.P and Bacillus cereus (Gram +ve).

The solvent used was dimethyl sulfoxide (DMSO)and sample from 1 to 200 µg/ml were used. The plates were incubated for 24 hours at 37 C^0 (22,23). The zone inhibition of bacterial growth were measured in mm depending upon the diameter as shown in Table (5) Figure (9).

Complexes have been observed that the metal complexes have a high activity than ligand against same organisms under the identical experimental condition.

It is evident from the above data that the antibacterial activity significantly increased on coordination. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity. Coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system.(24,25).

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Duel-infection of Hepatitis B Virus and Hepatitis D Virus (HDV) in Iraqi Hemodialysis Patients

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ABSTRACT

The HBV infection is highly endemic in many developing countries. Iraq is among the countries of intermediate hepatitis B endemicity. The HBV and HDV Duel-infection may occur and played an important role in the progression of chronic liver damage in patients. the prevalence and incidence rates of HBV and HDV are still high among patients undergoing maintenance hemodialysis in the less developed countries .The present study aimed to evaluate the prevalence of co-infection with HBV and HDV among Iraqi Hemodialysis patients.The thirty eight patients with HBV infection on hemodialysis and fifty patients with HBV infection by other mode of transmission (control group) who admitted to Hepatology and Gastroenterology teaching Hospital in Baghdad during October 2009 to April 2010 were included. HBsAg, IgM anti-HBc and anti-HDV was detected using ELISA kit. According to the manufacturers' instruction. The results duel infection with HBV and HDV in Hemodialysis patients with positive-HBsAg carriers was (52%) while those patients with positive-HBsAg who has the infection by other mode of transmission duel-infection with HBV and HDV was (7.4%).

Key words: **duel-infection, Hemodialysis, risk factor, HBV, HDV**

الملخص باللغة العربية

العديد من الدراسات أظهرت إن الإصابة بفيروس الكبد "ب" متوطن في الدول المتقدمة والنامية منها العراق هو من هذه الدول التي تعاني من توطن الإصابة بالالتهاب الكبد الفيروسي "ب".
قد تحدث الإصابة المزدوجة بفيروس الكبد من النوع "ب"و "د" والتي لها دور مهم في تطور التهاب الكبد المزمن في حالة الإصابة المزدوجة. إن معدل الإصابة بالتهاب الكبد الفيروسي من النوع "ب" و "د" باقية مرتفعة بين مرضى غسيل الكلية في الدول الأقل تطورا وقليلة في الدول المتطورة.
تهدف هذه الدراسة الى تقييم معدل انتشار الإصابة المزدوجة لدى مرضى غسيل الكلية بفيروس التهاب الكبد من النوع "ب" و "د".
لقد تم دراسة ثمانية وثلاثون مصابا بالتهاب الكبد الفيروسي من النوع "ب" لمرضى غسيل الكلية بالإضافة إلى دراسة مجموعة السيطرة والتي تضم خمسون مصابا بالتهاب الكبد الفيروسي نوع "ب" والذين انتقلت إليهم العدوى بطرق أخرى. أجريت الدراسة في مستشفى الجهاز الهضمي وأمراض الكبد التعليمي / بغداد للفترة منذ شهر تشرين الأول لسنة 2009 ولغاية شهر نيسان 2010, حيث تم فحص عينات الدم باعتماد الطريقة الإنزيمية والارتباطية لوجود الأجسام المضادة الكاملة لالتهاب الكبد الفيروسي من النوع "ب" و"د".
أظهرت الدراسة إن الإصابة المزدوجة بفيروس الكبد "ب" و"د" لدى مرضى غسيل الكلية كانت (52%) بينما الإصابة المزدوجة بفيروس الكبد "ب" و"د" عند المرضى الذين انتشرت إليهم العدوى بطرق أخرى كانت (7.4%).

INTRODUCTION

HBV infection in Hemodialysis patients varies among different localities and correlates with the endemicity in the general population of the region. HBV prevalence has decreased in many countries in general population and Hemodialysis patients. Improvement of people's knowledge about risk factors, national vaccination programs for neonates and vaccination of high risk groups are responsible for these decrease in prevalence of HBV infection in general population (1). Iraq is among the countries of intermediate hepatitis B endemicity However, the prevalence and incidence rates of HBsAg positivity are still high among patients undergoing maintenance hemodialysis in the less developed countries (2).

Humans are reservoir and vector; spread by direct contact including exchange of body secretion, recipient of contaminated blood products, and percutaneous injection of virus. Hepatitis B in dialysis patients, prevalence is higher than standard tests for hepatitis B would suggest some kidney dialysis patient's contract hepatitis B virus (HBV) during the course of their treatment, possibly from other members of the dialysis population with occult HBV. People with occult HBV test negative for HBV surface antigen (HBsAg) but positive for HBV-DNA, which is detected through sensitive tests not typically performed on dialysis patients.

Transmission of HDV is similar to HBV, via blood and blood fluids containing the virus, and infection occurs by parenteral routs (3). Hemodialysis patients may acquire HDV infection as they are at risk of hepatitis B infection. The information on the epidemiology of HDV infection in the dialysis patients is limited. This may be in part related to limited use or availability of delta testing. In European countries, such as France, Sweden, and the USA, HDV infection is restricted to high risk group of drug addicts and has decreased during recent years (4). There are some reports of acute and fulminant hepatitis or symptom-free transmission in dialysis patients with HBV and HDV infections (5, 6). Delta virus is of particular potential concern in hemodialysis units where segregation of HBsAg positive patients to minimize hepatitis B transmission to susceptible patients may facilitate the transmission of delta agent. HDV infection is not important in developed countries, but may be a major risk for fulminant hepatitis in hemodialysis patients with HBV infection in developing countries.

The chronic infection (Super-infection) with HBV and HDV can lead to life threatening conditions such as cirrhosis, liver failure and hepatocellular carcinoma (7).Co-infection of HBV and HDV (simultaneous infection with the two viruses) result in both acute type B and acute type D hepatitis. The incubation period depends on the HBV titer of the infecting inoculum. Depending on the relative titers of HBV and HDV (8).

PATIENTS AND METHODS

Thirty eight adult patients on Hemodialysis male and female with acute hepatitis (AH) or chronic liver disease, such as chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC) who admitted to Hepatology and Gastroenterology teaching Hospital in Baghdad during October 2009 to April 2010 and fifty patients with HBV infection who had infected by other mode of transmission (control group) were included. They were established by positivity for surface antigen HBsAg and HCV detection was done by serology may have detected. From each individual included in this study, 5-10 ml of blood was drawn by venepuncture using disposable syringes.The sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20◦C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test. The serological tests were performed using commercially available ELISA kits according to the instructions provided in the manufacturer's manual. HBsAg and IgM anti-HBc was detected using ELISA kit, (Biokit, Spain) and IgM anti-HDV by using ELISA kit, (Biokit, Spain).

RESULTS

Table (1) shows that a total percentage of co-infection in Hemodialysis patients was (52), the table also shows total (38) patients on Hemodialysis mean age (mean±SD) was (44.1±11.69) years, the male: female ratio was 3.74: 1.0. The mean age of control group (mean±SD) was (38.97±12.6) years; of them were male: female ratio was 2.1:1.0.

It was found that most of Hepatitis B patients on Hemodialysis were in chronic stage twenty-one patients with a percentage (55%) of all patients on Hemodialysis, table (1) shows there were fourteen patients (36%) had co-infection of HBV with HDV at chronic stage

While acute stage found in four patients (10.5%) and no patient had co-infection, cirrhosis stage in seven patients (18.3%) and four patients (10.5%) with Duel-infection of HBV and HDV, primary hepatocellular carcinoma (H.C.C.) stage in six patients (15.7%) with three patients (7.9%) had Duel-infection of HBV and HDV.

Table (1) : Distribution of Hepatitis B virus and Hepatitis D virus Duel-infection in patients on Hemodialysis according to the stages of disease

Stage of HBV Infection	No. of patients	Percentage %	No. of patients with HDV Duel-infection	Percentage
AH	4	10.5%	---	---
CH	21	55.5%	14	36.8%
LC	7	18.3%	4	10.5%
HCC	6	15.7%	3	7.9%
Total	38	100%	21	55.2%

The total percentage of Duel-infection in control group was (7.4%) as in table (2). Distribution of Hepatitis B patients in control group according to the stages of disease was in table (2) that shows the most of HBV were in chronic stage (74%) and two patients (5.4%) with Duel-infection of HBV and HDV. In acute stage there were ten patients (20%) and no patient had co-infection. Patients in cirrhosis stage were two patients (4%) one of them (2%) was having co-infection HBV and HDV. Primary hepatocellular carcinoma stage was in one patient (2%) without Duel-infections.

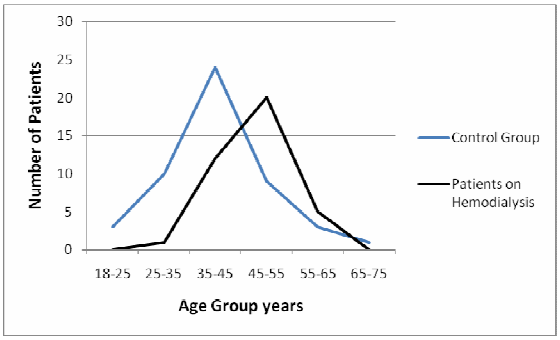

Figure (1): Distribution of Hepatitis B virus patients on Hemodialysis and control group according to the age

Table (2): Distribution of Hepatitis B virus and Hepatitis C virus Duel-infection in control group according to the stages of disease

Stage of HBV Infection	No. of control group	Percentage %	No. of patients with HDV Duel-infection	Percentage
AH	10	20%	---	
CH	37	74%	2	5.4%
LC	2	4%	1	2%
HCC	1	2%	----	
Total	50	100%	5	7.4%

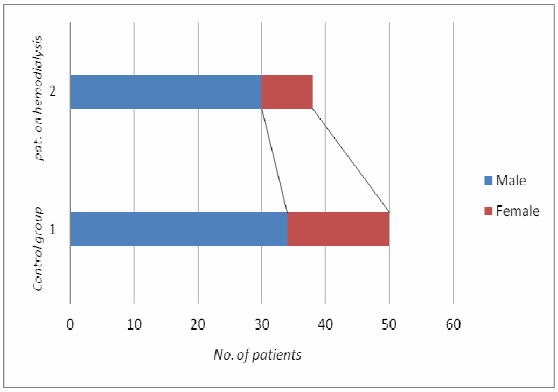

Figure (2): Distibution of Hepatitis B virus patients on Hemodialysis and control group according to the Gender

DISCUSSION

Table (2) shows that in control group the prevalence of anti-HDV antibodies among HBsAg-positive carriers was (7.4%). This finding was disagreed with the finding of Maria (2003) who found that (<0.5%) of patients with HBV had Duel-infection with HDV (9) while these results was agreed with other study that found (5-8) of patients with HBsAg –positive had Duel-infection with HDV (10, 11). These finding could be explained by that, the difference of geographical area, improved hygienic conditions, and the using of vaccination may lead to these results.

Control group (table 2) showed that the most of Hepatitis B virus patients were in chronic stage (74%) of all HBV patients. These results were agreed with the results of (1) and (13) who found (86.4%) and (74.75%) of patients with HBV were on chronic stage of the disease (12, 13). The high rate of Duel-infection of HBV with HDV in chronic stage of Hepatitis B infection may be due to the greater chance of risk factor of Dule-infection.

Table (1) shows that total percentage of Duel-infection in Hemodialysis patients was (55.2%) which was higher than the total percentage of Duel-infection in control group (7.4%). This finding agreed with the result of Maria (2003) who found that Duel-infection with HBV and HDV was (57%) among Hepatitis B virus on Hemodialysis patients(m) this finding could be explained by that the highest risk factor for transmission of HDV among HBsAg positive patients in Iraq was Hemodialysis history (13) .

Table (1) also showed the higher rate of co-infection with HBV and HDV among patients on cirrhosis stage (10.5%) and primary hepatocellular carcinoma stage patients (7.9%) comparative with the rate of Duel-infection among these stage in control group patients that were (2%) of Duel-infection among cirrhosis stage patients the table also showed that no patients among primary hepatocellular carcinoma had Duel-infection with HBV and HDV, this results were agree with other study that showed co-infection with HBV and HDV is characterized by accelerated progression of liver disease and great incidence of cirrhosis than HBV monoinfection (14-17). That result could be also explained by that Hepatitis D virus HDV, a defective RNA virus that requires a helper function of HBV for packaging and transmission play an important role in the progression of chronic liver damage in patients chronically infected with HBV (18, 19).

CONCLUSION

Co-infection with HBV and HDV in Hemodialysis patients with HBsAg-positive carriers is higher than other mode of transmission in Hepatitis B virus Iraqi patients.

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The effects of Hemodialysis treatment In CRF patients on the level of Antioxidant parameter

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ABSTRACT

This study was conducted to study the effect of HD session for CRF patients on the level of Antioxidant parameters include Ceruloplasmin (CP), Uric acid (UA), Albumin (Alb), Malonildialdehyde (MDA) and Glutathione (GSH),The results show that a significant elevation of MDA,CP,UA ($P<0.001$)and significant reduction of GSH and Alb ($p<0.001$).

Key words: **Reactive oxygen species (ROS), Free radicals (FR), Ceruloplasmin (CR), Malonildialdehyde (MDA), Glutathione enzyme.**

الملخص باللغة العربية

يتضمن البحث دراسة تأثيرالديلزه ألدمويه لمرضى العجز الكلوي المزمن على مستوى مانعات الاكسده المتواجد في الجسم والتي تشمل (السيريولوبلازمينCP,ألبومينAlb,كلوتاثايونGSH,حامض اليوريكUA،مالونايلديهايد MDA),أظهرت النتائج وجود ارتفاع معنوي في مستوى السيريولوبلازمين و مالونايلديهايد وحامض اليوريك وبمستوى احتماليه ($p<0.001$)لدى مجموعه المرضى عند مقارنتها مع مجموعه السيطرة في حين أظهرت النتائج وجود انخفاضا معنويا في مستوى الألبومين وال كلوتاثايون وبمستوى احتماليه ($p<0.001$)لمجموعه المرضى عند مقارنتها مع مجموعة السيطرة.

INTRODUCTION

Antioxidants represent a first line body defense against oxidative stress produced by generation of free radicals and reactive oxygen species (ROS), that can neutralize free radicals by accepting or donating one acess of neutralizing a free radical molecules to non free radical molecule (1).

Oxidative stresses define an imbalance between formation of reactive oxygen spices (ROS) and ant oxidative defenses mechanisms. In view of the profound biological effects of (ROS), in recent years numerous clinical and experimental studies focused on detective of singe of oxidative stress in renal patients. There is good evidence indicating that uremia in general is associated with enhanced (2),and treatment of uremic patients with HD has been suggested to particularly contribute to oxidative stress and reduced antioxidant level in these patients (3).loss or deficiency of antioxidant activity (e.g. vitamin E deficiency) could also contributed to enhanced oxidative stress in uremia .

Free radicals are molecules or molecule fragments with unpaired electron (4) those molecules are highly reactive, so they easily capture an atom to form other reactive molecules starting a chemicals chain reaction.

Free radicals (FR) vary in their reactivity, but most of them are reactive and unstable (5)

The major deleterious effect of intracellular free radical generation is lipid peroxidation, lipid peroxidation is the oxidative deterioration of poly unsaturated lipids under influence of free radicals, wich has several biochemical effects, and lipid peroxidation is a free radical process comprised of three principle events initiation ,propagation cycle and termination(6)

The most important protective antioxidant in human plasma is Ceruloplasmin, Uric acid, Albumin, Vitamin C, E, Transfering and other antioxidant Ceruloplasmin (CP) is mainly responsible for serum antioxidant activity Its active phase reactant protein found in various body fluid, protects tissues from damage caused by free radicals in the foci of inflammation where it is found. It's an alpha-2-glycolprotein with enzymatic activity containing six copper atoms per molecule (7).

The major physiological role of (CP) is the oxidation (Fe+2)to (Fe+3)allowing iron incorporation into transferring(8) Uric acid a major protective antioxidant against nitrogen dioxide and hypochlorous acid (HOCL)(9)it has a potential for chelating iron and copper rendering them unreactive and thus inhibiting lipid peroxidation

MATERIALS AND METHODS

Fifty five patients with end stage renal disease undergoing HD treatment attending General Hospital of Ramadi city, Hemodialysis unit, age between (17-60) years from both sex .

(5cc) blood specimens was taken from each patient, these samples were used to pool serum, pool serum were used for determine antioxidant which include serum Malondialdehyde (MDA),Erythrocyte glutathione (GSH),Ceruloplasmin (CP),Uric acid(UA)and Albumin (Alb) by using special kit for enzymatic and photometric methods .A total of 30 health individual from both sex were including as control group .

Statistically data were analyzed with (SPSS) system which analyzed mean value, standard deviation and study (T-test) and (P-value).

RESULTS AND DISCUSSION

Oxidative stress:

Antioxidant parameters include measuring GSH, CP, MDA, UA and Alb, for this study the value of Mean and ±SD for each one of antioxidant levels to patient and control group are showing in table (1)

DISCUSSION

Dialysis patients are subjected to an oxidative stress resulting from the dialysis sessions (8, 10).In present study GSH level in (CRF) patients highly significant decrease than control group ($p<0.001$) this finding indicate a significant higher in peroxidation process in HD-patients the healthy subjects, Reduction (11).Reduced (GSH) is on of the most important scavengers of free radicals in red blood cell membrane (12)

Table (1) Mean and SD and P-value for some antioxidant parameter in patients and control group

	Patient group		Control group		
Variables	**Mean**	**±SD**	**Mean**	**±SD**	**P-value**
GSH(mmol\L)	0.53	±0.05	0.93	±0.08	<0.001
CP(mmol\L)	9.01	±1.01	4.58	±1.01	<0.001
MDA(µmmol\L)	1.79	±0.14	0.44	±0.14	<0.001
UA(mg\dl)	7.5	±3.52	4	±1.16	<0.001
Alb(mg\dl)	3.2	±2.77	4.9	±62.2	<0.001

Current studies preset varying results regarding the relation between antioxidant ,oxidant and element levels in HD patients (13).90%or more of total serum copper is found in CP(14),high levels occur in cupper intoxication ,our study there were significant elevation in CP in patients group with control group,CP can act as antioxidants ,it scavenges damaging particles in the body known as free radicals ,and these occurs naturally in the body and can damage cell walls, antioxidants can neutralize free radicals and may help prevent some of the damage they cause(15).

In our study the level of plasma (MDA) in patient was higher than in control group ($p<0.001$) and this result consistent with different studies (15,16,17) but not with (18).elevated of uric acid level for patient group as compared with control group may provide a compensatory mechanism to contract oxidative damage related to atherosclerosis and ageing in humans(18).Due to progressive loss of the GFR, patients with renal disease have decrease renal clearance of uric acid and higher serum uric acid level .

In this study there is highly significant increase in albumin compared with control group and this result may b due to its action as acute phase reactant which increased with any inflammatory process and reflect the underlying inflammatory process in CRF (19)

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The Relationship between Information System strategy & Information Technology strategy

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ABSTRACT

This study aimed at clarifying the relationship between information system strategy and information technology strategy. To achieve the object of the study, it attempted content analysis methodology to reveal literature that discusses concepts and theories of ISs and Its, information strategy, information management strategy, information technology strategy and change management implementation strategy. Then, clarifies the relationship between information system strategy and information technology strategy. The most important findings were Information system strategy and information technology strategy fall under the business strategy, and ways to deliver strategy , to support or to shape competitive strategy of the organization , the IS strategy is an essential element responsible for delivering the technology used for application systems, and it should be used synonymously with business strategy. Meanwhile, the IS strategy should reflect the strategic view of the business executives regarding the role that IS should play and this should encompass how to invest and utilize IS for strategic goal .The study recommended that the IS strategy must be a plan for gaining and maintaining competitive advantage and IT used to get competitive advantage , then both ISs and ITs are used synonymously with business strategy to gain competitive advantage and to achieve strategic goals.

الملخص باللغة العربية

هدفت الدراسة الى توضيح العلاقة بين استراتيجية نظم المعلومات واستراتيجية تكنلوجيا المعلومات. لتحقيق هدف الدراسة اعتمدت هذه الدراسة منهج تحليل المحتوى من خلال مراجعة المفاهيم والنظريات لأستراتيجية نظم المعلومات واستراتيجية تكنلوجيا المعلومات, واستراتيجية المعلومات, واستراتيجية ادارة المعلومات, واستراتيجية تكنلوجيا المعلومات, واستراتيجية ادارة وتنفيذ التغيير. ثم وضحت العلاقة بين استراتيجية نظم المعلومات واستراتيجية تكنلوجيا المعلومات. من النتائج المهمه التي توصلت اليها الدراسة ان استرايجية نظم المعلومات واستراتيجية تكنلوجيا المهلومات, تأتي تحت استراتيجية المنظمه, وهما منهجان لتطبيق الاستراتيجية لدعم وتشكيل الاستراتيجية التنافسية للمنظمه. كما ان استراتيجية نظم المعلومات هي العنصر المهم المسئول عن تنفيذ التكنلوجيا المستخدمة في النظم, ويجب ان يستخدم بشكل متزامن مع مع استراتيجية المنظمة. بكل الاحوال استراتيجية نظم المعلومات يجب ان تشكل النظرة الاستراتيجية للأدارة التفيذية من خلال الاهتمام بدور نظم المعلومات, وهذا يشتمل كيف تستثمر نظم المعلومات في الاهداف الاستراتيجية.

اوصت الدراسة بضرورة ان تكون استراتيجية نظم المعلومات خطه لكسب وتحقيق الميزة التنافسية, وتكنلوجيا المعلومات ايضا يجب ان تستخدم للحصول على الميزة التنافسيه, لذا استراتيجية نظم المعلومات واستراتيجية تكنلوجيا المعلومات تستخدم بشكل متزامن مع استراتيجية المنظمة للحصول على الميزة التنافسية وتحقيق الاهداف الاستراتيجية.

INTRODUCTION

In every conceivable aspect, the need for an Information System which consist of information technology infrastructure ,data , application system and personnel who deploys IT to deliver the information and communications services in any organization (1). The presence of an information system will help propagate a more secure and accessible means to company information using the appropriate technology . However, more essential to having a perfect strategy to effectively guide and build information and technology systems.

The current study aims to discuss the relationship between information system strategy, information technology strategy and business strategy, mention to different meaning for ISs and ITs in order to achieve the main goal of this study regarding the relationship between information system strategy and information technology strategy.

Information system Strategy and business strategy:

From the literature review it's obvious that every business should have a business strategy - this is a long-term plan which shows the direction the business is taking (2). The business strategy provides an agreed set of objectives for the business, it is important because it allows resources to be targeted, and also because it allows the shareholders, customers, banks and employees (the "stakeholders" of the business) to see that the business is taking account of their interests.In order to respond to environment challenges organizations must integrate their information systems, and link subsystem together to provide access from any location with capability of sharing information across the organization. The organization go toward a wide use of IS/IT. And because of ever increasing cost of IT ,this had led to a need for an executive level manager called Chief Information Officer (CIO) to assist senior executive to oversee overall information management of the organization , the CIO share The development of IS strategies with senior manager to manage overall effective use of all the IS/IT investments, alignment between business needs and IS/IT strategies, competitive advantage from business opportunities on strategic use of IS/IT, appropriate resources and competencies for successful IS/IT deployment.

Building on Porter's definition of strategy as 'the route to competitive advantage that will determine and sustain performance'(3), we can define an ISS as the analysis of the role that information systems can play in helping business units or companies to define a route to competitive strategy by making appropriate investment in Information Technology(4). IS related-decision are contingent on the chosen role of IS (push or support of business initiatives) agreed upon by the top management and CIO. While business strategy consider as large umbrella and this plan include group of initiatives to optimize the performance and what will they need to deliver them, for example increasing the efficiency of existing business process by transforming information system from manual to electronic methods Require information technology to implement them (4).

Pearlson and Saunders, had mentioned that IS strategy must complement business strategy, when IS support business goal, the business appears to be working well. It will inevitably require resources and it may require a change in working practices within the organization. and while the IT strategy is concerned with the planning, introduction and use of IT resources for the benefit of the whole organization, the information system will benefit from IT resources. Therefore The Information Systems Strategy and IT strategy are parts of the business strategy. Then we can conceptualize the relationship between IS strategy and IT strategy as illustrated in figure (1):

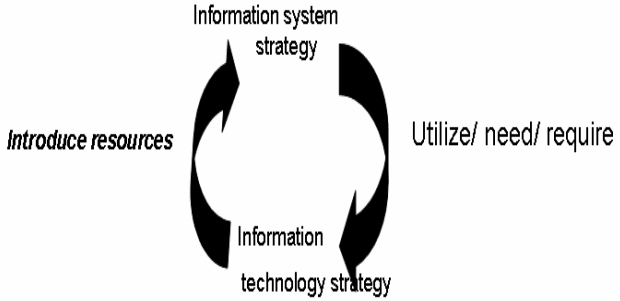

Figure (1) the relationship between information system strategy and information technology strategy. Source: David , Suzanne , and Matthew, (2003) Integrating operations and information strategies in e-business. European management journal, 21(5), 632

Although there is no general agreement on the constituents of the information system strategy, the information management literature, for the most part, is keen to differentiate between information system and information technology .*Information systems strategy* is seen as being concerned with meeting the information needs of organizational members and other stakeholder (5), it support the organization and business processes with the information they needs to execute the business strategy successfully. So Business strategy which is A well-articulated vision of where a business seeks to go and how it expects to get there *drives* IS strategy. And IS should clearly support defined business goals and objectives.

The function of a IS strategy itself is best described by Wilson (1989) who states that: An information system strategies bring together the business aims of the company (6), An understanding of the information needed to support those aims, and the implementation of the computer systems to provide that information, it is a plan of a development of systems toward some future vision of the role of information systems in the organizations.

This definition is qualified by Reponen who argues that: An IS strategy is something which is essentially a planning process in the minds of the decision makers, users and developers of the systems. It is supported with written reports and plans, but they are of secondary importance (7). Information system strategies is the plan of an organization used in providing information services and allows the company to implement its business strategy , it can itself affect and affected by changes in a firm's business and organization's strategies because IS touch every part of a business operation . The essential point toward understanding the relationship between information system strategy an information technology strategy is that IS strategy has four distinct components : the information strategy, the information technology strategy, the information management strategy and the change management/ implementation strategy(8), as illustrated in figure (2) .

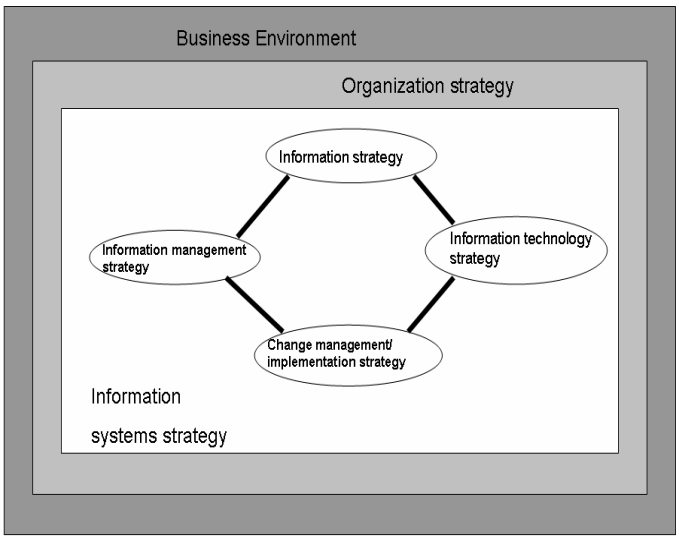

Figure (2): Component of IS strategy Source: Reponen, T. (1993) Strategic information systems - a conceptual analysis. Journal of Strategic Information Systems, 2(2), 100-104.

The Information Strategy

The information strategy acts as the linchpin between the organization strategy and the IS strategy. It answers the questions: what information is required? and where is the information required to support the primary tasks, or key goals, of the organization strategy. It also questions the appropriateness of the critical assumptions behind the organization strategy in light of the changing environment and changing perceptions (9).

Information strategy is seen as an organization's unified blueprint for capturing, integrating, processing, delivery, and presentation of information in a clean, consistent, and timely manner. All information in an organization should meet a certain standard for quality. It should be delivered consistently across the organization, i.e., asking for the same information in different divisions should yield the same result, and users and applications shouldn't have to wait long to get their requested information. Establishing an information strategy for any organization is important because information is asset and has a value. The value of information is in it accessibility and accuracy. Information that is not accessible on demand, and/or is not accurate is not an asset (9).

The information technology strategy is, for Galliers, of secondary importance: it is concerned with applications and platforms, the 'nuts and bolts' of how to provide the information. Thus, it is concerned with the technological infrastructure necessary to fulfill the requirements of the information strategy (10). An information technology strategy is concerned with what technology (i.e. The computer hardware) and technology systems (i.e. the computer software, programs, operating systems , applications… etc) including interconnecting telecommunications network are needed in order that the ISS can be realized (3) . The target of the strategy can be either the forms of technology being used or the people who are using it. There is a principle from a business expert that an IT strategy has to focus on the strategy by creating and measuring the value of the business from the perspective of the investment put into the employment of IT. Some strategies have focused on the ability of a company to spend on IT. Another focus has been how employees of a company can use the technology to create value for the organization. The best way to come up with a strategy is to have both groups in managerial positions from the business and IT departments come together to design a plan. This helps to see the goals of the business along with how the IT department can accomplish this (10).

Developing IT strategy is a critical organizational process, this process well become more important as strategic necessity of IT increases. According to John P. Glaser The IT strategy should be (11):

- The IT strategy often derived directly from a thorough review of organizational strategies and plans but *IT strategies based on continuous improvement of core operations and information management needs*; There are a small number of core operational processes and information management tasks that are essential for the
  Effective and efficient functioning of the organization. The organization assesses the performance of processes and develops plans to improve performance of these processes. The organization defines core information needs, identifies the gap between the current status and its needs, and develops plans to close those gaps. These plans often will point to an IT agenda. As a result IT strategies may be derived from the organization's strategy, but not always. There can be ongoing efforts to improve processes, regardless of the specifics of the organization's strategic plan. Also *IT strategies determined by examining the role of new information technologies;* This approach involves determining whether new IT capabilities enable the organization to consider new approaches or significantly alter current approaches to its strategies. the organization examines new applications and new technologies and tries to answer the question, "Does this application or technology enable us to advance our strategies or improve our core processes in new ways?" For example, applications that support the communication between a physician and his or her patient through the Internet might enable the organization to think of new approaches to providing care to the chronically ill patient. Holding up new technologies in the spotlight of organizational interest can lead to decisions to invest in the new technology.
  The IT strategies derived by assessment of strategic trajectories; that is Organizational and IT strategies invariably have a fixed time horizon and fixed scope. These strategies might extend two to three years into the future, outlining a bounded set of initiatives to be undertaken in that time period. Assessment of strategic trajectories asks the question, "What do we think we will be doing after that time horizon and scope? Do we think that we will be doing very different kinds of things, or will we be carrying out initiatives similar to the ones that we are doing now?".
- The IT strategy should be dominated by a focus on defining needed application systems ; The centerpiece of any IT strategy is an inventory of applications that need to be acquired and implemented. Applications are where the IT rubber meets the organizational road. However, the IT

strategy needs to go well beyond the definition of applications. Application sourcing and uniformity , infrastructure characteristics such as reliability, agility, supportiveness , and efficiency, data standardization Strategies which In general, strategies focus on acquiring new types of data, defining the meaning of data, determining the organizational function responsible for maintaining that meaning and quality, integrating existing sets of data, and identifying technologies used to manage, analyze, and report data , The IT Staff Issues that encompass the analysts, programmers, and computer operators who daily manage and advance information systems in an organization. This IT staff strategy focus on acquiring new skills, organizing the IT staff, sourcing the IT staff and solidifying the characteristics of the IT group, such as innovative, service-oriented, and efficient. , and the way an organization views IT are all essential elements of the IT strategy(11)

- The IT strategy is better if it is developed by using a rigorous methodology; Methodologies can be helpful in developing an IT strategy. These approaches can make the process more rigorous, politically inclusive, comprehensive, and more likely to produce a set of desired outcomes (11).

IT strategy should be based on a derivation of needs from the organization's strategy. After all, IT is a tool of which the value is based on its ability to support organizational plans and activities. However, this derivation is not the only approach for identifying important IT investments. The IT agenda can be significantly influenced by efforts to improve core organizational processes and information needs, the opportunities created by new technologies, and a discussion of strategic trajectories, then the relationship between them is that Information technology strategy is one of the component of the information system strategy (11).

The Information Management Strategy (How organization should run IS and IT activities***)***

The Information Management Strategy is concerned with how the information services are organized for the different facets of organization (i.e., centralized, distributed, out-sourced) and policy issues such as who gets access and what level of access they receive. IM strategy deals with the management of the entire information systems function .This strategy focus on the relationships between specialists and users, and between the corporate level and divisions or business units .it is concerned with the management controls for IS, management responsibilities ,performance measurement and management process (10).

The Change Management/ Implementation Strategy will identify what organizational change will be needed for the information systems strategy to be successful and when it will be implemented and by whom. Importantly, those who will implement the strategy should be involved in its formulation and specific plans and budgets should be drawn into the process here.(management process, people, organization structure ,etc)(10) .

CONCLUSION

Information system strategy and information technology strategy which are falls under the business strategy are ways to deliver strategy , to support or to shape competitive strategy of the organization , the IS strategy which deal with the information system application and the IT strategy which is essential element or main component in IS strategy responsible for delivering the technology used for application systems *should* be used synonymously with business strategy. Meanwhile the IS strategy should reflect the strategic view of the business executives regarding the role that IS should play and this should encompass how to invest and utilize IS for strategic goal .

From the discussion above we can say that IS strategy is a plan that aims to identify the required IS assets , including personnel (e.g. IS staff and its capabilities) ,structure (e.g. IS processes) monetary resources (IS budget) and technologies (e.g. IS application and

infrastructure) and to allocate the existing IS assets in efficient way .IS plan covers both the purpose of IT strategy to direct the efficient and effective management of IS resources and IM strategy to develop technology polices and architecture .

Because IS strategy is a plan for gaining and maintaining competitive advantage and IT related to using IT to get competitive advantage , then both ISs and ITs are using synonymously with business strategy to gain competitive advantage and achieve strategic goals.

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Anti-HCV-Positive in HBsAg-Positive Patients with Chronic Liver Diseases

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ABSTRACT

Hepatitis B and hepatitis C are common infections and therefore it is not uncommon to see patients co-infected with both of these viruses. The combination of both infections appears to lead to an increased risk for liver damage as well as an increased risk for the development of hepatocellular carcinoma. Evaluate the prevalence of dual infection with HBV and HCV among Iraqi patients with chronic liver diseases. Three hundred fifty adult patients male and female with chronic liver disease, they were admitted to Hepatology and Gastroenterology teaching Hospital in Baghdad. All these patients were assessed serologically. Co-infection with HBV and HCV in chronic liver diseases patients was (11.14%). The mean age for co-infection with HBV and HCV was 41.18±15. The most of co-infection of HBV and HCV were in Hepatocellular Carcinoma with percentage of 60% of all co-infected patients. The suspected major risk factors, for transmission of co-infection was found that (37.46%) due to Hemodialysis history.

Key words: **co-infection, risk factor, HBV, HCV**

الملخص باللغة العربية

إن الإصابة بالتهاب الكبد الفيروسي من النوع "ب" و"س" هو من الإصابات الشائعة , ولذلك فليس من غير الشائع أن نرى مريضا يحمل إصابة مزدوجة بالفيروسين . أن الإصابة المزدوجة بالفيروسين عادة ما تقود إلى الزيادة في خطورة الإصابة بتلف الكبد ,كما تزيد من خطورة تطوره الى سرطان الكبد. ان هذه الدراسة تهدف إلى تقييم معدل انتشار الإصابة المزدوجة بالتهاب الكبد الفيروسي من النوع "ب" و"س" بين المرضى العراقيين المصابين بالتهابات الكبد المزمنة. لقد تم دراسة ثلاثمائة وخمسون مريضا بالغا من المرضى الراقدين في مستشفى بغداد التعليمي لإمرض الكبد والجهاز الهضمي حيث شملت الدراسة كل من الذكور والإناث المصابين بالتهابات الكبد الفيروسية و تم إخضاع جميع المرضى للفحوص السريرية والمختبرية بالطرق الانزيمية والارتباطية لوجود الاجسام المضادة الكاملة لفيروس الكبد "ب" وفيروس الكبد "س".أن الإصابة المزدوجة بالتهاب الكبد الفيروسي نوع "ب" و"س" لإمراض الكبد المزمنة كانت (11.14%) .أن اغلبيه حالات الإصابة المزدوجة كانت في أثناء الإصابة بسرطان الكبد وبمعدل (60%)من مرضى الإصابة المزدوجة. كما وجد ان عامل الخطورة الاكبر للعدوى والاصابة بالفيروسين "ب" و"س" كانت لدى مرضى غسيل الكلية حيث كانت (37.46%) فقد وجد ان غسيل الكلى كان من اهم العوامل لانتقال الفيروسين.

INTRODUCTION

Approximately 350 million people are infected with HBV worldwide, and the World Health Organization (WHO) estimates that approximately 170 million people are infected with HCV. HBV and HCV infection account for a substantial proportion of liver diseases worldwide. Because the two hepatotropic viruses share same modes of transmission, co-infection with the two viruses is not uncommon, especially in areas with a high prevalence of HBV infection and among people at high risk for parenteral infection. The exact number of patients infected with both HCV and HBV is unknown (1).

Dual infection with HBV and HCV is not uncommon in geographic areas where a high endemic level of both infections is reported, such as Southeast-Asia and Mediterranean (2-4).The primary concern with HBV/HCV co-infection is that it can lead to more severe liver disease and an increased risk for progression to liver cancer (HCC). Moreover, the number of confected patients is likely higher than is usually thought (5).

Hepatitis B and hepatitis C are common infections and therefore it is not uncommon to see patients co-infected with both of these viruses. The combination of both infections appears to lead to an increased risk for liver damage as well as an increased risk for the development of hepatocellular carcinoma. Studies have shown that both viruses can inhibit the replication of the other virus. Of note, in most cases, HCV predominates over HBV (6).

PATIENTS AND METHODS

Three hundred fifty adult patients, male and female with chronic liver diseases such as chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC), were admitted to Hepatology and Gastroenterology teaching Hospital in Baghdad from October 2009 to December 2010. They were established by biochemical evaluation (Total serum bilirubin TSB, Alanin aminotransferase ALT and Aspartate aminotransferase AST), positivity for surface antigen HBsA and negativity for HBc IgM, chronic hepatitis B patients (HBc IgM – CHB). The patients were submitted for historical and clinical evaluation using special questionnaire, from each individual included in this study; 5-10 ml of blood was drawn by venepuncture using disposable syringes. The blood was placed in plastic disposable tubes, it was left to stand at room temperature (20-25◦C) to allow it to clot, then the sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20◦C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test. The serological tests were performed using commercially available ELISA kits according to the instructions provided in the manufacturer's manual. HBsAg and IgM anti-HBc was detected using ELISA kit, (Biokit, Spain). The HBsAg confirmatory test uses the principle of specific antibody neutralization to confirm the presence of HBsAg in human sera. In the test, a human serum containing anti-HBs (the confirmatory reagent) is incubated with the sample in solution. If HBsAg is present in the sample, it is bound by its specific antibody and blocked from binding to the antibody coated to the microplate well. A sample is confirmed as true HBsAg positive if the reduction in absorbance of the neutralized ample is at least 50%, and the non- neutralized ample absorbance is equal to or greater than the assay cut-off. Detection of anti-HBc by ELISA test for qualitative determination of IgM antibodies to Hepatitis B virus core antigen (anti-HBc IgM) in human serum or plasma. The assay is an antibody-capture, non-competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibody to human IgM. The blood samples were detected for HCV by bioelisa HCV 4.0 is an immunoenzymatic method in which the wells of a microplate are coated with recombinant antigens representing epitopes of HCV: Core, NS3, NS4 and NS5. Serum or plasma samples are added to these wells. If antibodies specific for HCV are present in the sample, they will form stable complexes with the HCV antigens on the well.

RESULTS

The distribution of HBV patients according to the sex was found that males were more than females with a ratio of (3.3:1).while the distribution of dual infection patients according to the sex was (3.8:1).

Table (1) shows the number of patients with HBV and HCV among chronic liver diseases patients, they were 39 patients in a ratio of 11.14%. The table also shows the distribution of Hepatitis B patients and co-infection of HBV and HCV according to their age. It was

found that the age of patients with Hepatitis B was ranged between 21-75 yrs. with a mean age of 39.39±15 and the age of patients with co-infection was ranged between 25-70 yrs with a mean age of 45.18±15**.** Also it was found that most age group infected with Hepatitis B was recorded with (30-40 years) with a percentage of 32%, and age group infected with co-infection of HBV and HCV was (40-50) with percentage of 46.15%.

It was found that most of co-infection of HBV and HCV were in Hepatocellular Carcinoma with percentage of 60% of all co-infected patients. Co-infected of HBV and HCV patients were in Cirrhosis with a percentage (36.36%) of all co-infected patients; while chronic stage found in (30.85%).

Table (1): Age Distribution of Hepatitis B patients and co-infection of HBV and HCV

Age Group (Years)	No. of patients with HBV	Percentage%	No. of patients with co-infection of HBV and HCV	Percentage%
20-30	55	15.71	2	5.12
30-40	112	32.00	15	38.46
40-50	87	24.85	18	46.15
50-60	58	16.57	3	7.69
60-70	29	8.28	1	2.56
>70	9	2.57	-	-
	350	**-**	**39**	**11.14**

Table (2) also showed a percentage of (94%) in Chronic stage, (3.14%) of Cirrhosis (2.56%) of Hepatocellular Carcinoma in Hepatitis B virus patients as showed in table (2).

Table (2) :Distribution of Hepatitis B Patients According to the Stages of Disease

Stages of HBV Infection	No. of patients	Percentage%	No. of patients with co-infection of HBV and HCV	Percentage%
Chronic	329	94	29	30.85
Cirrhosis(LC)	11	3.14	4	36.36
Hepatocellular Carcinoma (HCC)	10	2.56	6	60
Total	**350**	**100**	**39**	

Table (3) shows the suspected major risk factors for transmission of co-infection of HBV and HCV. It was found that (27.46%) due to Hemodialysis history and (25.64%), (23.7%), (13.69) and (10.12) due to Frequent paranteral drug administration, Previous blood transfusion, family history and previous surgery respectively. The suspected major risk factors for transmission of HBV infection was found that (22.85%) due to Frequent paranteral drug administration and (21.71%) due to Previous blood transfusion, (18.28%) due to and Hemodialysis history and (14.85%), (12.28%), due to family history, previous surgery respectively.

Table (3): Major Risk Factors for Transmission of HBV and HCV Co- Infection

Mode of Transmission of HBV virus	No. of patients	Percentage%	No. of patients with co-infection of HBV &HCV	Percentage%
Previous blood transfusion	76	21.71	9	23.07
Frequent parenteral drug administration	80	22.85	10	25.64
Hemodialysis history	64	18.28	11	27.46
Family history of Positive HBsAg	52	14.85	5	13.12
Previous surgery	43	12.28	4	10.69
Unknown Source	35	10.00		
			39	

DISCUSSION

The studies show that 5% of the world population has chronic Hepatitis B (7). As other developing countries, HBV infection is one of the most common infections in Iraq (8, 9). Because HBV and HCV infection have the same mode of transmission, the dual infection is not uncommon in countries where a high endemic level of the two infection (1). The infections with chronic hepatitis B virus and hepatitis C virus are more common in male than female with a ratio of (3.3:1) as well as the dual infection with HBV and HCV with a ratio of (3.8:1). This difference could be explained by that the risk factors of hepatitis viruses like alcohol intake are more common in male than female which may enhance the development of liver diseases (10).

Table (1) shows the number of patients with HBV and HCV among chronic liver diseases patients, they were 39 patients in a ratio of 11.14%. This finding agrees with Irene (2011) who found that Chronic HCV co-infection occurs in 7–15% of patients with chronic HBV infection (11). Other studies found that dual infection with hepatitis B virus and Hepatitis C virus is a common infection in areas where a high endemic level of both infections, the prevalence is around 10-20% in patients with chronic HBV infection (2-4). Table (1) also shows patients with hepatitis B virus whom were ranged between 21-75 yrs. with a mean age of 39.39±15, are infected with a percentage of 32% within the range of 30-40 yrs. These results coincide with previous studies done in Iraq; such as: Youssif (1998) who reported that the infection targeted patients with mean age of 42 years, Al-Waysi (2005) with mean age of 45 years, and Watheq, (2011) with mean age of 38 yrs. Also, several studies in the world agreed with this study's results like Chakraborty *et al*. (2005) who reported a mean infection age of 35.6 years, Hajiani *et al*. (2009) who found it 37 years, and Abed El-kader *et al*. (2010) who reported 39.2 years, while some studies were recorded a lower mean age as Gaetano *et al*. (2010) who reported 27years for hepatitis B patients in Southern Italy. This difference may be due to the different source of infection or to environmental and geographical distribution (12-18). In addition, the table shows the age of

patients with co-infection was ranged between 25-70 yrs with a mean age of 45.18±15, has a co-infection rate of 46.15% within 40-50 years. In other studies that agree with this study, it was found that the dual infection ratio increased in the patients who had more than 42 years (19).

Table (2) shows that co-infection with HBV and HCV was 60% of Hepatocellular Carcinoma patients, 35.36% of Cirrhosis patients, and 30.85% of chronic stage patients. This study agrees with other studies that found the dual infection is more aggressive with greater incidence of liver cirrhosis and hepatocellular carcinoma than HBV or HCV alone (20, 21). In Italian study, 29% of patients with dual-infection were cirrhotic (20). Another study in KSA found that the liver cirrhosis and hepatocellular carcinoma were more common in patients with dual with HCV and HBV infection than the diseases were found in patients infected with HCV only (22).

Table (3) demonstrate the similarity between chronic hepatitis virus and dual infection with HBV and HCV in terms of risk factors for transmissions, it shows that transmission risk factors of chronic hepatitis B virus patient's and dual infection patients are the same, as agreed with other studies that totally found that the risk factors of dual infection are similar to those of single infection of the two viruses because the two viruses have the same mode of transmission (1).

The hemodialysis history is an important mode of transmission of HBV and dual infection in addition to frequent parenteral drug administration and blood transfusion, as shown in table (3). This finding agrees with Stark *et al*. (1997) who reported that HBV is transmitted through unsafe injections and transfusions by inoculation of even a small amount of the virus (23). An Iraqi study by Watheq (2011) showed that the blood transfusion is an important mode of transmission of HBV in addition to frequent parenteral drug administration and hemodialysis history, but also reported that the important mode of transmission is blood transfusion which may be due to including acute HBV and CHB in his study while this study includes chronic hepatitis B virus only.

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Antibacterial activity of raw and commercial Honey against clinical pathogenic bacteria

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ABSTRACT

Antibacterial activity of some raw and commercially available honey was tested against Gram-positive bacteria Staphylococcus aureus and Streptococcus spp. and Grame negative bacteria, Shigella spp., Salmonella spp., Pseudomonas aeruginosa, and Escherichia coli. Both types of raw honey (RH1 and RH2) showed antibacterial activity against test organisms with the zone of inhibition ranging from 18 to 45 mm and from 22 to 47 mm respectively. Also both types of commercials honey (SH3 and LH4) showed antibacterial activity against test organisms with the zone of inhibition ranging from 15 to 48 mm and from 17 to 49 mm respectively. Raw honey possesses more inhibitory activity against Streptococcus spp., Salmonella spp., and S. aureus than commercially available honey. On the other hand, commercially available honey possesses more inhibitory activity than raw honey against Shigella spp., Pseudomonas aeruginosa, and Escherichia coli. . MIC values of raw honeys RH1 and RH2 for tested organisms was 750 µg/mL. Where as MIC of commertial honeys SH3 and LH4 was 1000 and 1250 µg/mL respectively. The potency of both types of raw and commercial honey at 100% concentration was found to be higher than all other concentrations tested. However, no effect was observed at concentration of 6.25% v/v honey in the case of both raw and commercial honey samples. However, when compared the results of some standard antibiotics to antibacterial activity of the raw and commercial honeys extracts, it was observed that the inhibitory activity of the extracts of tested microorganisms was greater than of some standard antibiotics used in the study.

Keywords: **Antibacterial activity; Raw and processed honey; MIC; Antimicrobial susceptibility.**

الملخص باللغة العربية

اختبرت الفعالية ضد البكتيرية لبعض العسل المحلي والتجاري المتوفر ضد البكتريا الموجبة لصبغة كرام *Staphylococcus aureus.* و *Streptococcus spp* والسلبية لصبغة كرام *Shigella spp.*، *Salmonella spp.* ، *Pseudomonas aeruginosa*، *Escherichia coli*. اظهرت النتائج بان كلا النوعين من العسل (RH1 و RH2) لها فعالية مايكروبية ضد البكتريا الممرضة مع قطر تثبيط تراوح ما بين 18 الى 45 ملم ومن 22 الى 47 ملم بالترتيب. كما اظهر كلا نوعي العسل التجاري (SH3 و SH4) فعالية مايكروبية ضد البكتريا الممرضة مع قطر تثبيط بلغ 15 الى 48 ملم و 17 الى 49 ملم بالترتيب. و بينت النتائج بان العسل المحلي اظهر فعالية مايكروبية عالية ضد بكتريا *Streptococcus spp.* ، *Salmonella spp.* و *S. aureus* من العسل التجاري. ومن جانب اخر دلت النتائج على امتلاك العسل التجاري فعالية مايكروبية اعلى من العسل المحلي ضد *Shigella spp.*، *Pseudomonas aeruginosa.* و *Escherichia coli* . كما لوحظ من خلال النتائج بان التركيز المثبط الادنى للعسل المحلي (RH1 و RH2) ضد البكتريا الممرضة بلغ 750 مايكروغرام/ مل, في حين بلغ التركيز المثبط الادنى للعسل التجاري (SH3 و SH4) 1000 و 1250 مايكروغرام / مل بالترتيب. واشارت النتائج الى ان قوة التأثير لنوعي العسل المحلي والتجاري بلغت اعلاها عند التركيز 100%. في حين لم يلحظ اية فعالية مايكروبية لنوعي العسل عند التركيز 6.25%. وكما وجدت من خلال مقارنة النتائج بان الفعالية التثبيطية لكلا النوعين من العسل المحلي والمستورد اكبر من بعض المضادات الحياتية المستخدمة في الدراسة.

INTRODUCTION

Honey is a popular sweetener and a common household product throughout the world. It is nonirritant, nontoxic, available and cheap. It has been used from ancient times as a method of accelerating wound healing. Traditional importance and use of honey as therapeutics has been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago, Reference (origin) cited at Maryann, (2000) (1). Honey has been used since ancient times for the treatment of some respiratory diseases and for the healing of skin wounds. It has been proposed that the healing effect of honey could be due to various factors such as high osmolarity, acidity and particularly hydrogen peroxide which is formed from the oxidation of glucose by the enzyme glucose oxidase, during the period when honey is ripening. Glucose oxidase originates from the hypopharyngeal glands of honeybees (2).

In general, all types of honey have high sugar content but a low water content and acidity, which prevent microbial growth. Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects (3). Besides its antimicrobial properties, honey can clear infection in a number of ways, including boosting the immune system, having anti-inflammatory and antioxidant activities, and via stimulation of cell growth (4). Honey has a well established usage as a wound dressing in ancient and traditional medicines. In recent times this has been re-discovered, and honey is widely used as a topical antibacterial agent for the treatment of wounds, burns and skin ulcers. Several types of bacteria, commonly involved in wound infections like *Escherichia coli. Staphylococcus aureus, Proteus mirabilis*, *Klebsiella* spps., *Streptococcus faecalis* and *Pseudomonas aeruginosa*, are susceptible to the antibacterial activity of honey regardless to their resistance to antibiotics [5].

A large number of honeys are available in the Iraqi. These honeys are either locally produced or imported from different countries. The purpose of the present study was to evaluate antibacterial activity of four types of honey against six human pathogenic bacterial cultures such as *Shigella spp., Streptococcus spp., Salmonella spp., Pseudomonas aeruginosa, Staphilococcus aureus, Escherichia coli.*

MATERIALS AND METHODS

Honey samples

Four kinds of honeys were used in this study. Two honey samples are raw honey (RH1 and RH2) were collected from Baghdad and Al-Basra Governorate respectively in Iraqi. While two commercial available honeys San Francisco and Langnese (SH3 and LH4) respectively was bought from the market. Honey samples were stored at 4ºC in the dark until analyzed.

Bacterial isolates

Six bacterial isolates which are pathogenic to human were obtained from Department of Biology and Biotechnology - College of Science – University of Baghdad. They include Gram negative *Pseudomonas aeruginosa, E.*

coli, *Salmonella spp, Shigella spp.* and Gram positive *S. aureus and Streptococcus spp.* These bacteria were isolated from patients' from hospitals and identified by standard methods. Pure cultures of isolates were preserved at 4°C on nutrient agar slants.

Preparation of inoculums:

The bacterial isolates were inoculated in sterile nutrient broth and were incubated at 37 ºC for 24 hours. The turbidity of the broth was matched with McFarland's standard solution to obtain a bacterial inoculums of 1.5 $\times 10^8$ (colony forming unit) CFU/ml (6).

Susceptibility test

The antibacterial susceptibility was determined by Kirby and Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute Guidelines using commercially available antimicrobial discs. The following antibiotics were used: Amoxicillin (10 µg), tetracycline (10 µg), chloromphenicol (30 µg), and Ciprofloxacin (5 µg). (1)

Antimicrobial Activity

The antimicrobial activity of different samples of honey against different pathogens was tested using method described by (7). Test materials were prepared by diluting each honey sample (RH1, RH2, SH3, and LH4) in sterilized, distilled water at different dilutions concentration net honey i.e. 100%, 75%, 50%, 25%, 12.5%, and 6.25%. Muller Hinton Agar (MHA) plates were prepared. A loop full (4mm in diameter) of the prepared bacterial suspensions (1.5 x 10^8 CFU/ml) were separately applied to the centre of a sterile Muller Hinton agar plate and spread evenly using a sterile cotton wool. Wells were made on the inoculated plate using a sterile well borer (6mm in diameter). Then 100 microliters of different concentrations of honey were dispensed and inoculated at 37°C for 20 hours and the zones of inhibition around each well were observed and the diameter of zones were reported. This experiment was performed in duplicate and the average data was recorded.

Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration of honey that is able to inhibit the growth of tested pathogenic bacteria. A series of test tubes were prepared, each test tube was added with 5 ml of Muller Hinton broth and calibrated with 100 µL of microbial suspension. Different concentration of honey ranging from 250 µg/ml to 1500 µg/ml was added to each test tube. The wide dilution range was considered to compare the efficacy. The tubes were incubated at 37°C for 24h. After incubation, turbidity of each tube was visually inspected; clear test tube indicated break point. The tube containing the least concentration of extracts showing no visible growth was considered as MIC. This experiment was performed in duplicate to ensure the reproducibility of the results (8).

RESULTS AND DISCUSSION

In general, all types of honey have high sugar content as well as low water content and acidity, which prevent microbial growth. Osmotic effect, effect of pH and hydrogen peroxide are represented as an "inhibition" factor in honey. Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their variable antimicrobial effects (1).

Four honey samples activities were tested in current study against pathogenic bacteria, and the differences in average diameter of the inhibition zones were showed in (Table 1 and 2). The results for various activities for the antibacterial activity of various honey samples on different bacterial isolates showed that, the growth was inhibited at varying concentrations. The honey samples were tested at concentrations of 100%, 75%, 50%, 25%, 12.5%, and 6.25% against tested pathogenic bacteria. RH1, RH2, SH3 and LH4 honey samples showed no activity at 6.25% concentration for all the tested isolates (Table 1). RH1 honey sample showed activity against *Shigella spp*. and *S. aureus* at honey concentration of 12.5%, where as RH2 honey sample showed activity against *Salmonella spp., P. aeruginosa andS. aureus* at the same concentration. Both raw honey RH1 and RH2 showed antibacterial activity against all tested pathogenic bacteria at concentration of 25% and higher.

The results of commercial honey SH3 and LH4 are represented in Table 2. Both honey

samples showed antibacterial activity against *Salmonella spp. and P. aeruginosa* at concentration of 12.5%, where as SH3 honey sample at concentration 25% inhibited the growth of *Streptococcus spp., Salmonella spp. and P. aeruginosa*. Both SH3 and LH4 honeys revealed antibacterial activity against all tested pathogens at concentration of 50% and higher. Diameter of zone of inhibition increases with increase in concentration of honeys samples. Both types of raw honey (RH1 and RH2) showed antibacterial activity against test organisms with the zone of inhibition ranging from 18 to 45 mm and from 22 to 47 mm respectively. Also both types of commercial honey (SH3 and LH4) reviled antibacterial activity against test organisms with the zone of inhibition ranging from 15 to 48 mm and from 17 to 49 mm respectively. The results in Table 1 and 2 also showed that both raw honeys possesses more inhibitory activity against *Streptococcus spp., Salmonella spp., and* S. aureus than commercially available honey. On the other hand, commercially available honey possesses more inhibitory activity than raw honey against *Shigella spp., Pseudomonas aeruginosa, and Escherichia coli.* The results also indicated that the potency of both types of raw and processed honey at 100% concentration was found to be higher than all other concentrations tested. The results was agreement with that found by (4) they found that raw and processed (Indian) honeys showed antibacterial activity against *P. aeruginosa, E. coli, Salmonella lspp. S. aureus, Bacillus subtilis* and *Enterococcus faecalis* with ZOI ranging from 6.94 to 37.94. Also the results in the present study supported by the results obtained by (2) they prepared different concentration of 100%, 75%, 50%, 40, 25%, and 6.25% of New Zealand named Manuka honey. Undiluted honey samples inhibited the growth of all the tested pathogenic strains and the concentration of 50% and higher also inhibited the growth of all strains with different ZOI. The variation of susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the honey sample. It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally leads to leakage of ions from the cells (9) Our results also resembles that of others (1,7 and 10) who found that honey inhibited the growth of *S. aureus, E. coli, Shigella* spp. and *Pseudomonas* sp. and 100% concentrated honey is more effective than other concentrations (1).

Effects of Minimum Inhibitory Concentrations (MIC)

Minimum inhibitory concentrations (MIC) for six tested bacterial cultures are showed in Table 3 and 4.

Results in Tables 3 and 4 showed that the MIC values of raw honeys RH1 and RH2 for tested organisms, such as *Shigella spp., Streptococcus spp., Salmonella spp,, P. aeruginosa, S. aureus, and E. coli* was 750 μg/mL. Where as MIC of commercial honeys SH3 and LH4 was 1000 and 1250 μg/mL respectively. The MIC value indicates the inhibitory concentration at which honey showed no visible growth of any test organisms. The results obtained was closely to that observed by (4) pointed that the MIC values for raw honey sample for five tested organisms such as *Escherichia coli Pseudomonas aeruginosa, Salmonella spp,B. subtilis, and S. aureus* were 625 µg/ml.

Table (1): Antibacterial activity of raw honeys against six pathogenic bacteria

Bacterial isolates	Mean of inhibition zone (mm)											
	Raw Honey RH 1						Raw Honey RH 2					
						Honey	Con.	(v/v %)				
	100	75	50	25	12.5	6.25	100	75	50	25	12.5	6.25
Shigella spp.	39	36	25	20	20	-	33	27	20	25	-	-
Streptococcu sp	45	40	13	10	-	-	47	32	28	20	-	-
Salmonella sp.	43	40	26	20	-	-	35	32	32	23	30	-
P. aeruginosa	43	42	23	23	-	-	37	35	35	36	36	-
S. aureus	42	42	42	22	18	-	43	42	36	37	22	-
E. coli	40	32	22	18	-	-	39	30	28	20	-	-

(-) indicates no antibacterial activity

Table (2): Antibacterial activity of processed honeys against six pathogenic bacteria

Bacterial isolates	Mean of inhibition zone (mm)											
	SH 3 Honey						LH 4 Honey					
						Honey	Con.	(v/v %)				
	100	75	50	25	12.5	6.25	100	75	50	25	12.5	6.25
Shigella spp.	40	37	-	-	-	-	45	30	30	27	-	-
Streptococcu sp	38	35	30	25	-	-	25	38	35	35	-	-
Salmonella sp.	42	42	39	35	17	-	34	40	40	30	14	-
P. aeruginosa	48	40	36	35	15	-	49	49	37	31	17	-
S. aureus	35	35	24		-	-	40	32	28	24	-	-
E. coli	45	25	20	-	-	-	43	35	-	20	-	-

(-) indicates no antibacterial activity.

Table (3): Minimum inhibitory concentration of raw honeys against six pathogenic bacteria

Bacterial isolates	Minimal inhibition concentration											
	Raw Honey A						Raw Honey B					
						Honey	Con.	(µl/ml)				
	250	500	750	1000	1250	1500	250	500	750	1000	1250	1500
Shigella spp.	+	+	-	-	-	-	+	+	-	-	-	-
Streptococcu spp	+	+	-	-	-	-	+	+	-	-	-	-
Salmonella spp.	+	+	-	-	-	-	+	+	-	-	-	-
P. aeruginosa	+	+	-	-	-	-	+	+	-	-	-	-
S. aureus	+	+	-	-	-	-	+	+	-	-	-	-
E. coli	+	+	-	-	-	-	+	+	-	-	-	-

(+) indicates turbid test tubes; (-) indicates clear test tubes

Table(4): Minimum inhibitory concentration of processed honeys against six pathogenic bacteria

Bacterial isolates	Minimal inhibition concentration											
	San francisco	Commertially	Available	Honey C			Langnese	Commertially	Available	Honey D		
						Honey	Con.	(µl/ml)				
	250	500	750	1000	1250	1500	250	500	750	1000	1250	1500
Shigella spp.	+	+	+	-	-	-	+	+	+	+	-	-
Streptococcu sp	+	+	-	-	-	-	+	+	+	+	-	-
Salmonella sp.	+	+	+	-	-	-	+	+	+	+	-	-
P. aeruginosa	+	+	+	-	-	-	+	+	+	+	-	-
S. aureus	+	+	-	-	-	-	+	+	+	+	-	-
E. coli	+	+	-	-	-	-	+	+	+	+	-	-

(+) indicates turbid test tubes; (-) indicates clear test tubes

Results in Table (5) also showed that test organisms exhibited resistance to some standard antibiotics used in this study. The test organisms *Salmonella spp. and S. aureus* used in this study were resistant to ciprofloxacin (5 μg), and Amoxicillin (10 μg). The results showed that all positive controls had zone of inhibition (ZOI) higher than 12 mm causing sensitivity to microorganisms. When comparing ZOI values of positive control with raw and commercial honeys extracts (Table 1 and 2), it was observed that most of the extracts showed ZOI value more than 12 mm. However, when compared to antibacterial activity of the raw and processed honeys extracts, it was observed that the inhibitory activity of the extracts of tested microorganisms was greater than those of standard antibiotics. The results were in agreement with (4 and 11) who observed that the inhibitory activity of the honey extracts of *E. coli, Salmonella spp*, and *P. aeruginosa* (Gram negative) was greater than those of standard antibiotics, ciprofloxacin and tetracycline.

Table (5): Antibacterial activity of standard antibiotics against bacterial isolates

Bacterial isolates	Antibiotics (µg/disk) Zone of inhibition (mm)			
	A_{10}	T_{10}	CiP_{30}	$Ciprof_5$
Shigella spp.	-	-	-	22
Streptococcu	-	-	-	22
Salmonella spp.	12	-	25	13
S. aeruginosa	30	-	17	25
S. aureus	25	42	25	12
E. coli	-	-	-	28

A10 = Amoxicillin (10 µg), T10 = tetracycline (10 µg), CiP30 = chloromphenicol (30µg), Ciprofloxacin (5 µg). Values are the mean of duplicate determinations.
(-) = represent no zone of inhibition.

Dilution of honey was observed by (10) who found that honey samples inhibited the growth of *S. aureus* even at 50% dilution. Undiluted honey samples also inhibited the growth of *Staphylococcus uberis, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*, although to a lesser extent.

The antimicrobial activity of different honey concentrations (100, 75, 50, 25, 12.5 and 6.25% v/v) against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (UCR 2902), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC25922), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 19116) and *Aspergillus niger* was evaluated. They obtained for the microbiological characterization of honey showed that 91% of samples had counts equal or lower than 1,0 x10 (1) cfu g^{-1} (10).

The variations of the activity of different honey was attributed to the previously mentioned factors which influenced the antibacterial activity as osmotic properties of honey; honey pH or activity of glucose oxidase; hydrogen peroxide, non peroxide Substances(11), Presence of propolis which contain flavonoid and volatile antibacterial substances (12).

Also the antibacterial activity of honey samples from different sources against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was studied. The results showed that majority of the honey samples (75%) generally inhibited the bacteria tested. The honey samples which were obtained from Zmir showed more effectiveness as inhibitors against *P. aeruginosa*, *E. coli*, and *S. aureus* (13).

Collectively, our findings indicate that the different levels of raw and commercially imported samples of honey at different concentrations, 25, 50, 75% and 100% showed antibacterial activity against a broad spectrum of pathogenic bacteria with variable degrees.

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Culture independent method for identification of *Lactobacilus acidophilus* directly from Aushari cheeses using Polymerase Chain Reaction

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ABSTRACT

Lactobacillus acidophilus is a Gram-positive bacterium that is associated with fermented dairy products. Method for identifying these bacteria directly without isolation and culturing the bacteria, by application of molecular tools that depend on Polymerase Chain Reaction (PCR) was used in this study. The method of DNA extraction from Aushari cheese directly was successful and gave amplifiable DNA for PCR experiments without any inhibitor for these kinds of reactions.PCR reaction was applied using ribosomal RNA (rRNA) and its encoding genes as primers specific for *L. acidophilus*. Three pairs of specific PCR primers were used, the amplified product indicated by three major bands (approx. 150-200bp, 300-400bp and 785 bp in size). The primers were shown to be specific for *L. acidophilus* genome, since no PCR product was obtained when genomic DNA from other bacteria, were used. This PCR assay provides a more rapid, specific and sensitive alternative to conventional culture methods for the identification of *L. acidophilus*

الملخص باللغة العربية

يعد ال Lactobacillus acidophilus من البكتريا الموجبة لصبغة غرام والتي تكون مقترنة بمنتجات الالبان ومن ضمنها جبنة الاوشاري(البيستا) المصنعه محليا في اقليم كوردستان .تم استعمال التفاعل التضاعفي االمتكرر لسلسة ال DNA او ال PCR (polymerase chain reaction)المعتمدة على استخدام بادئات متخصصة للنوع وذلك لتشخيص بكتريا ال *L. acidophilus* من جبن الاوشاري بدون عزل البكتريا وتنقيتها وذلك لتشخيص الانواع البكتيرية الموجودة في هذا النوع من الجبن والمسوؤلة عن النكهة والطعم الموجودة فيه .ولتحقيق هذا الهدف تم استخلاص الDNA من العزلات النقية لهذه السلالات من البكتريا وبعد ذلك تم تطبيق ال PCR باستخدام بادئات متخصصة قسم منها تستهدف جزء من جينات الرايبوسوم والتي يطلق عليه 16Sوالتي تكون متخصصة لبكتريا ال *L. acidophilus* واظهرت النتائج حزم متضاعفة وبالحجم المتوقع وهو (150 , 350 ,785) زوج قاعدي عند استعمال كل بادئ على حدة . تم التاكد من تخصص تلك البادئات لبكتريا *L. acidophilus* وذلك بظهور نفس الحزمة عند استعمال الDNA المعزول من السلالة النقية للبكتريا وعدم ظهور تلك النتيجة عند تطبيقها على انواع بكتريا اخرى.وان تقانة التفاعل التضاعفي االمتكرر لسلسة ال DNA تعد طريقة دقيقة و سريعة و والبديل الامثل للطرق التقليدية المستخدمة في تشخيص بكتريا ال*L. acidophilus* والتي تستغرق وقت وجهد ليس بالقليل ويمكن تطبيقها على انواع اخرى من البكتريا وخصوصا التي تظهر صعوبة في تنميتها والتي تستغرق عملية عزلها وتنقيتها فترة طويلة .

INTRODUCTION

Aushari (Pesta) cheese is a type of traditional ripening cheese that is very common in Kurdistan region, the identification of strains deals with ripening cheese is very important, in order to know the accurate strain that are responsible of the last flavor of these kinds of cheese. Classically, the microbial ecology of Cheeses has been studied by using traditional microbiological analysis based on the use of phenotypic test (1). However, this approach is not always appropriate to study complex microbial communities, because different species within a genus can demonstrate the same fermentation patterns and growth requirements (2). Also, different culture conditions and the diversity of strains can create difficulty in reproducing the results of phenotypic tests (3). Molecular techniques have allowed the precise and rapid identification and typing of bacterial strains, providing new ways to check for their presence and monitor their development (4). Among molecular techniques that have improved the discriminatory power of identification, Polymerase Chain Reaction (PCR) finds wide application in detection of lactobacilli (5) Integral to the efficacy of a PCR reaction is the choice of primers and the target gene(6) .A variety of PCR methods are now available for the identification of bacterial strains. Variations in the lengths and sequences of rRNA intergenic spacer regions (ISRs)and Random Amplified Polymorphic DNA (RAPD) analysis have been successfully applied to the lactobacilli, including *L.acidophilus* (7).One of the most widely applied approaches deals with the use of 16S rRNA and its encoding genes as target molecules. PCR amplification of 16S rRNA gene-targeted primers have successfully been used for the detection and identification of Lactobacillus species (8,9) , the aim of this study was: to develop an easy and fast procedure to accurately identify at the species level *L.acidophilus* directly from Aushari cheese using molecular methods that based on PCR.

MATERIALS AND METHODS

Atotal of 21 fresh Aushari cheese samples were collected from different villages of Sulemania and Erbil governorates, then10g of each sample were weight aseptically and homogenized in 90 ml of distilled water.

DNA extraction from cheese:

It was done according to (10) by mixing 0.5 ml of homogenate cheese with 0.5 ml of sterilized water, and 200 µl of the aqueous phase mixture was transferred to a new tube and vortexed well. Then, 400 µl of lysis buffer, 50 µM Tris-HCl, 25 µM EDTA, pH 8.0) was added to the mixture After vor-texing for 1 min, the mixture was centrifuged at 15,000 rpm for 5 min. The pellet was resuspended in 200 µl of lysis buffer containing glycogen at a final concentration of 0.03 **l**g/ µl and 4 µl of proteinase K (2 mg/ml) was then added to the suspension. After incubation for 1 h at 37°C, 300 µl of Sodium Iodide (NaI) solution (NaI in 50 mM Tris-Cl, 25 mM EDTA, pH 8.0) and 500 µl of isopropanol were added to the suspension and then centri- fuged at 15,000 rpm for 5 min.The pellet was washed with 35% isopropanol, dried for a short time, and then suspended in 20 µl of sterilized water for PCR.

To determine DNA concentration an aliquot 10 µl of DNA sample was added to 90 µl of TE buffer mixed thoroughly then optical density (O.D.) was measured using a spectrophotometer at wavelength of 260 nm. The DNA concentration in the solutions was calculated according to the following formula:-

DNA concentration (µg /µl) = [OD_{260} X 1000 X 50 µg/ml] / 1000

Theoretically O.D. =1 at 260 nm corresponds to approximately 50µg/ml for double- stranded DNA. The ratio between the readings at 260nm /280nm provides estimate of the purity of nucleic acid (11).This was performed for each of the samples.

PCR amplification

The oligonucleotide primers used in this study were obtained from (Notengham, England) and are listed in Table 1. species-specific primer were applied for the identification of the *L. acidophilus*. one set of primers for 16 SRNA were designed from the nucleotide sequence data depending on primer 3 program and the other pairs depend on previously puplished paper . PCR reactions were performed with 1×PCR buffer without $MgCl_2$; 2.5 mM $MgCl_2$; 50ng DNA, 0.3 µM each primer; 0.25 mM (each) dNTP; 25 U/ml Taq DNA polymerase (sigma). PCR reactions were carried out in PCR thermal cycler((Biotech,U.K) . Amplification consisted of 35 cycles: 1 min at 94°C, 1 min ,1 min at 55°C, and 1 min at

72°C. The amplified products were electrophoresed in a 1% agarose gel and were subsequently visualized by UV illumination after ethidium bromide staining. (11)

Table (1): represents the primers used in this study

Primers	Sequences 5' → 3'	Molecular weight	References
16S1(sense)F	AGSTGAACCAACAGATTCAC	780bp	(12)
16S2(antisense)R	ACTACCAGGGTATCTAATCC		
Lac1F	AGCAGTAGGGAATCTTCCA	150 bp	13)(
Lac2R	ATTYCACCGCTACACATG		
Laci01F	GACCGCATGATCAGCTTATA	350bp	(14)
Laci02R	**AGTCTCTCAACTCGGCTATG**		

RESULTS AND DISCUSSION

DNA extractions from cheeses

Suitable yields of genomic DNA were obtained using with average yields between 50-95Ug/ ml (figure 1).The purity was also found good ,ranging between 1.7-1.8 , This result prove that the method used in this study was successful and gave amplifiable DNA for PCR experiments without any inhibitor for these kinds of reactions.

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.

Figure (1): represent the results of DNA extraction method from the cheese on gel electrophoresis which performed on 1 % Agarose gel and run at 90 Volt /cm for one hours , Lane 1 was standard molecular weight marker 50Ug/ml and lanes 2-7 represent the DNA extracted from seven samples of *Aushri cheese* which collected from deferent governorates *of* kurdistan

Application of PCR

The success of PCR assays for reliable identification of lactobacilli both at genus and species level is chiefly dependent upon the designing of the primers targeted against specific genes ,with the increasing number of primers, the manufacturer of a bacterial dairy product is faced with the challenge of selecting the most suitable primer set. One of the target

gene used in this study was the 16srRNA gene because it s very effective for species identification of lactobacilli, and other genus of bacteria (3). The three set of primers produce three amplified bands in an expected size150-200bp ,300-400bp and 785 bp in size for each primer respectively and figure 2 represents the PCR result of one set of primers. The primers were shown to be specific for *L. acidophilus* genome, since no PCR product was obtained when genomic DNA from other bacteria, were used as test species such as *L. fermentum* , *L. delbrueckii subsp. lactis* .(Results not shown)

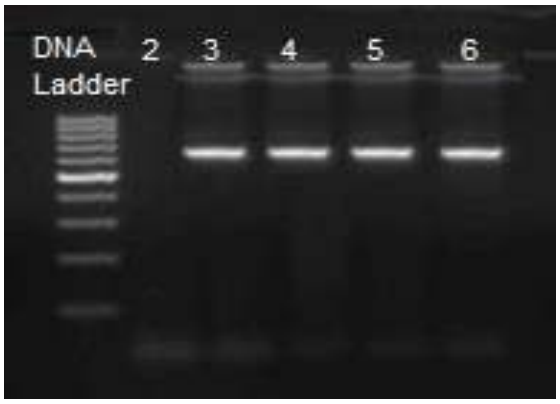

Figure (2): represent The amplification products of *PCR* using the primer targeting the 16SrRNA gene . Lane 1. 1kb DNA ladder Lanes3, 4, 5 show the presence of a band specific for *L.acidophillus* with molecular size of 785 bp using the DNA extracted from three samples of cheeses . and 6 represent the DNA extracted from pure culture of *L.acidophillus* .Electrophoresis was performed on 1.2% Agarose gel and run with 90 volt for one hour.

16srRNA PCR is new molecular technique for the microbial community analysis, that do not require isolation of the microorganisms ,it was very promising method and called culture-independent method ,it means direct application of molecular biology techniques for bacterial identification without the need of cultivation. Cocolin and Ercolini (2008) provide a complementary picture of the population obtained using culture-based techniques when applied to the analysis of milks and dairy products. The use of culture-independent methods can avoid the biases related to the cultivation of the microorganisms. Since the microorganisms are detected by targeting the DNA and/or the RNA extracted directly from the cheese sample, problems relating to the lack of growth of some populations, or the need of selective, enrichments for the recovery of stressed or injured cells, do not interrupt the results.

Although DNA-based used successfully in identification of bacteria. However, these molecular approaches have several limitations, including the design of adequate primers, and the possibility that DNA isolation; amplification might be biased by certain strains and sequences. Nevertheless, these methods provide an overview of the diversity to reality of microorganisms present in a particular sample. They provide qualitative and possibly semi-quantitative information, which should be complemented by quantitative PCR to obtain accurate results. This PCR assay provides a more rapid, specific and sensitive alternative to conventional culture bacteria .It can be used for detection of inaccuracies in labeling of species contained within commercial probiotic products .Application of other species specific PCR for identification techniques such as recA gene was recommended.

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Detection of Epstein Barr virus DNA by In Situ Hybridization in Gastric Cancer Patients

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ABSTRACT

Epstein Barr virus (EBV) is a member of Herpesviridae families and the well known cause of the worldwide common infectious mononucleosis. An increasing number of studies have suggested causal relationship between (EBV) and primary gastric adenocarcinoma. In this study we investigate the expression of (EBV) in gastric adenocarcinoma using (EBV-DNA) detection kit and we try to correlate the clinical and the pathological parameters with (EBV) expression. Among 30 randomly selected cases of gastric adenocarcinoma, there were 17 male and 13 female with male to female ratio 1.3:1 (EBV) was detected in (26.6%) cases, male to female 1:1. The mean age for the entire group was 55.8 ± 13.39 where as the mean age for patients with EBV-GC was 56 ± 17.7 yrs. (EBV) positive expression was slightly predominant in the distal third of the stomach, more frequent in the intestinal subtype, distributed between grade II and III. Statistical analysis shows no significant correlation regarding all the above parameters. Our conclusion that there is an association between (EBV) infection and gastric cancer.

***Key words*: Epistein Bar virus, DNA, In situ hybridization gastric Ca.**

الملخص باللغة العربية

Epstein Barr Viurus هو عضو من عائلة ال Herpesvirida وهو معروف عالميا كأحد اسباب تكثر وحيدات النواة في الدم ، أن الدراسات المتزايدة أقترحت علاقة سببية بين EBV والسرطان الغدي المعوي الاولي. في هذه الدراسة قمنا بالتحري عن EBV في السرطان الغدي المعوي باستعمال عدة التحري عن EBV –DNA كما حاولنا ايجاد العلاقة بين وجود EBV والعلامات السريرية والمرضية.

من بين 30 حالة للسرطان الغدي المعوي المختارة عشوائيا كانت هنالك 17 من الذكور و13 من الاناث وبنسبة 1.3: 1 تم تشخيص EBV في (26.6%) حيث كانت نسبة الذكور الى الاناث 1:1. ان المعدل العمري للمجموع الكامل 13.39 ± 55.8 سنة بينما كان معدل اعمار المرضى الحاملين لل EBV في السرطان الغدي المعوي هو 17.7 ± 56 سنة.

اظهر التحليل الاحصائي عدم وجود فروقات معنوية بالاعتماد على المقاييس المذكورة اعلاه. ان استنتاجنا هو وجود ارتباط بين الاصابة ب EBV والسرطان المعدي.

INTRODUCTION

Epstein Barr virus (EBV) has a unique place in the lymphocrypto virus genus of the gamma subfamily of the large herpes virus family, it has been linked to the etiology of several cancer including gastric carcinomas (1,2). (EBV) has been strongly associated with a particular variant of gastric carcinoma with lymphoid stroma , initially described by Watanab et. al., 1976. At the beginning of the 1990s, several investigators could categorically demonstrate traces of the virus in the neoplastic gastric cells by molecular methods, since then epidemiological, clinical, pathological and molecular studies have been conducted, allowing researches to become aware of the behavior of (EBV) associated gastric cancer. At present it is widely accepted that (EBV) is responsible for at least few cases of gastric cancer although the subtle mechanism of malignant transformation remained unclear. The presence of clonal episomal forms of the virus in the neoplastic epithelial cells suggests that infection occurs before neoplastic transformation. (3) The major clinicopathological features of (EBV) associated gastric carcinoma are male predominance and predisposition to upper two-thirds of stomach (4). Histologically, they are more frequently observed in moderately differentiated or poorly differentiated adenocarcinomas than in other histological types (5). In the affected cells, (EBV) nuclear antigens, latent membrane proteins and small non poly-adenylated RNAs were expressed latent infection. The program of viral gene expression in the malignant gastric epithelial cells corresponds to latency I, represented by Burkitts' lymphoma (6). In Iraq, there is no large scale study regarding the association between gastric carcinoma with (EBV) infection, so we conducted this study for the detection of (EBV) prevalence in gastric carcinoma by in situ hybridization technique and study the relationship among different clinicopathological parameters (age, sex, anatomical site, gross appearance, histological subtype, stage and grade) and the (EBV) expression in gastric cancer.

Aim of study:

1. To find whether there is an association between gastric carcinoma with EBV infection.
2. To study the relationship among different clinico-pathological and demographic parameters and EBV expression in gastric cancers.

MATERIALS AND METHODS

A retrospective study conducted on 30 gastric carcinoma specimens from gastrectomy operation (17 males and 13 females), their ages ranged from 30-80 years. Archival paraffin-embedded blocks were obtained from Department of Histopathology of the Medical City Teaching Laboratories, during the period between January 2000 – January 2007. The information's about these cases were recovered from the histopathological reports, archival files and documents regarding the site of the tumor, age, sex, stage and grade. No data was available regarding previous history of infection with infectious mononucleosis for those patients. For each paraffin blocks, 2 sets reprehensive sections at different thickness level were prepared. One stained with heamatoxylin and (H & E), the other sections (on positively charge slides) prepared for in situ hybridization procedure.

RESULTS

Patient characteristics:

A total number of 30 cases of gastric cancer were studied. Among the 30 cases; males patients represent (56.7%) whereas females represent (43.3%) as shown in Table (1). The male : female ratio was 1.3:1.

The mean age was 55.8 ± 13.39 years (range: 30 – 80 years) for all patients, with a peak age in 60 years. 73.3% of them, their age were above 50 years as shown in Figure (1)

Table (1): Sex distribution of the collected cases

Sex	Number	Percentage (%)
Male	17	56.7
Female	13	43.3
Total	30	100

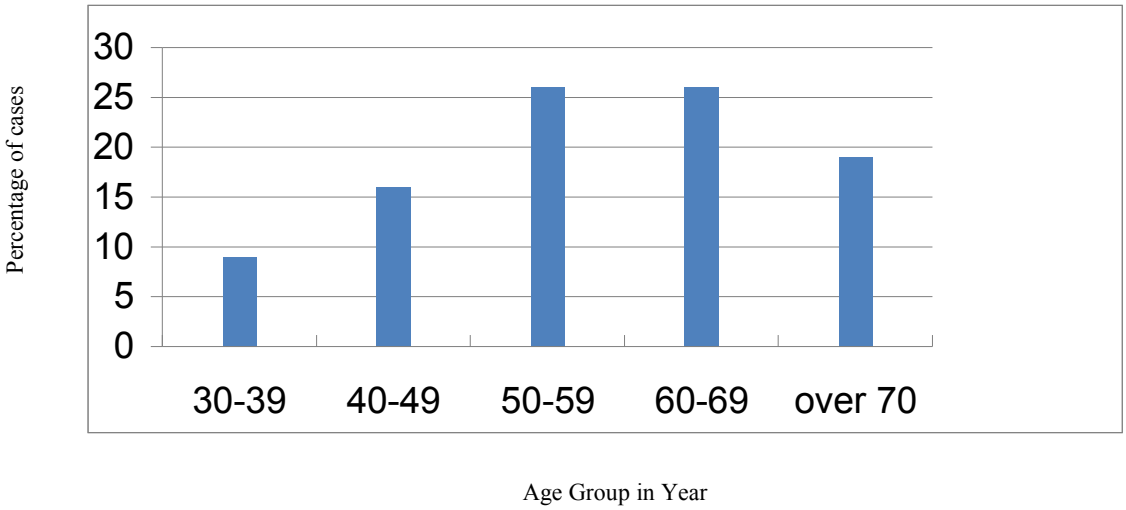

Figure (1): Age distribution of the collected cases

Correlation between (EBV) prevalence (using EBV DNA detection kit) and various clinicopathological factors

As shown in Fig. (1), (26.7 %) of cases express positive dark blue signals as irregular dumped, diffuse or mixed pattern in their nuclei. The normal non neoplastic gastric mucosa surrounding the area of adenocarcinoma, lymphocyte, and stromal cells do not express positive hybridization as in Fig. (2)
Table (2) reveals that (EBV) positive cases distributed throughout wide range of age groups with mean age 56 ± 17.7 years. The highest percentage (9.99%) was in age group above 70 years. The p value was 3.54 which are of no statistical significance.

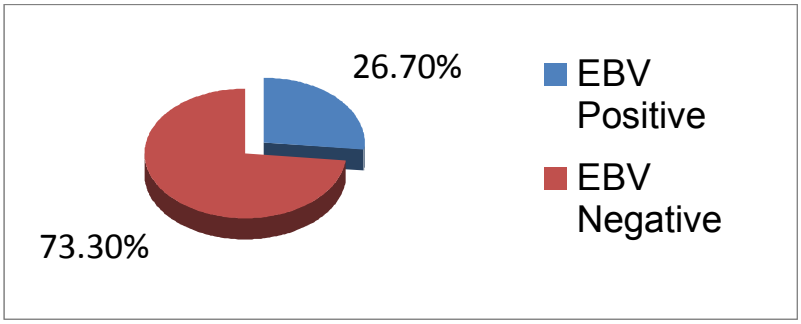

Figure (2): EBV expression in the collect cases

Table (2) EBV expression in relation to age

Age group	EBV expression				Total
	Positive		Negative		
	No. of cases	%	No. of cases	%	
30-39	1	3.3	2	6.7	3
40-49	2	6.7	3	10	5
50-59	1	3.3	7	23.3	8
60-69	1	3.3	7	23.3	8
Over 70	3	10	3	10	6
Total	8	26.7	22	73.3	30

EBV positive cases are equally distributed between males and females as shown in table (3) p value was 0.198 which is of no statistical significance.

Anatomical site of primary tumor:

As shown in Figure (3) 19.41% (EBV) adenocarcinoma cases were seen in lower third of the stomach control and pyloric there was no statistical significance between EBV positive expression with regard to anatomical site of the adenocarcinoma p value was 0.56.

Histological type:

The expression of EBV was higher in intestinal subtype of gastric cancer according for (16.7%) of total (EBV) positive cases while (10%) at EBV positive cases were of diffuse subtype as shown in Figure (4). P value was 0.089 which is of no statistical significance.

Table (3): EBV expression in relation to sex

Sex	EBV expression				Total
	No. of cases + ve	%	No. of cases – ve	%	
Male	4	13.3	13	43.3	17
Female	4	13.3	9	30	13
Total	8	26.7	22	73.3	30

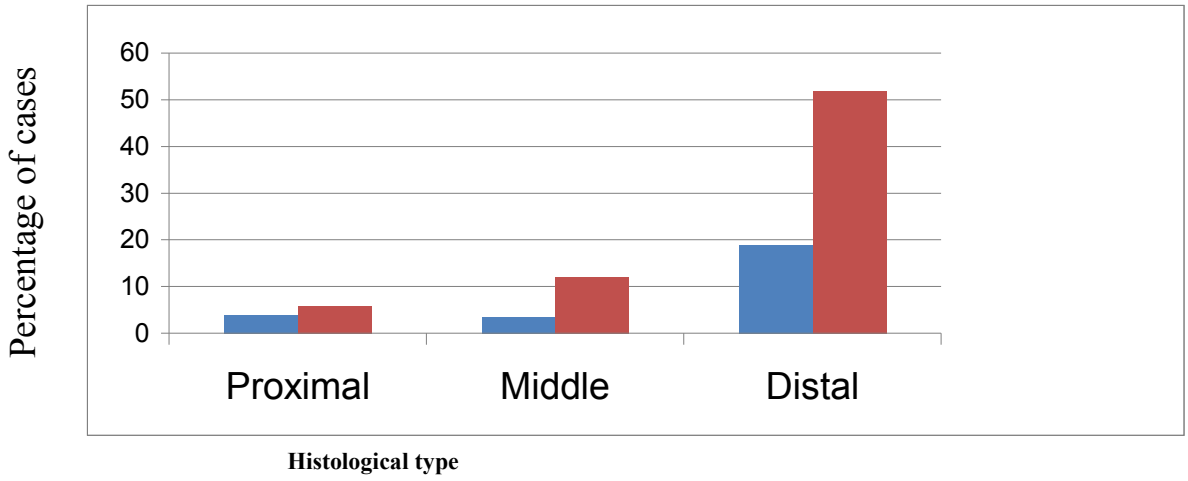

Figure (3): Distribution of patients with EBV (+ve) – blue and EBV (- ve) – red expression according to anatomical site

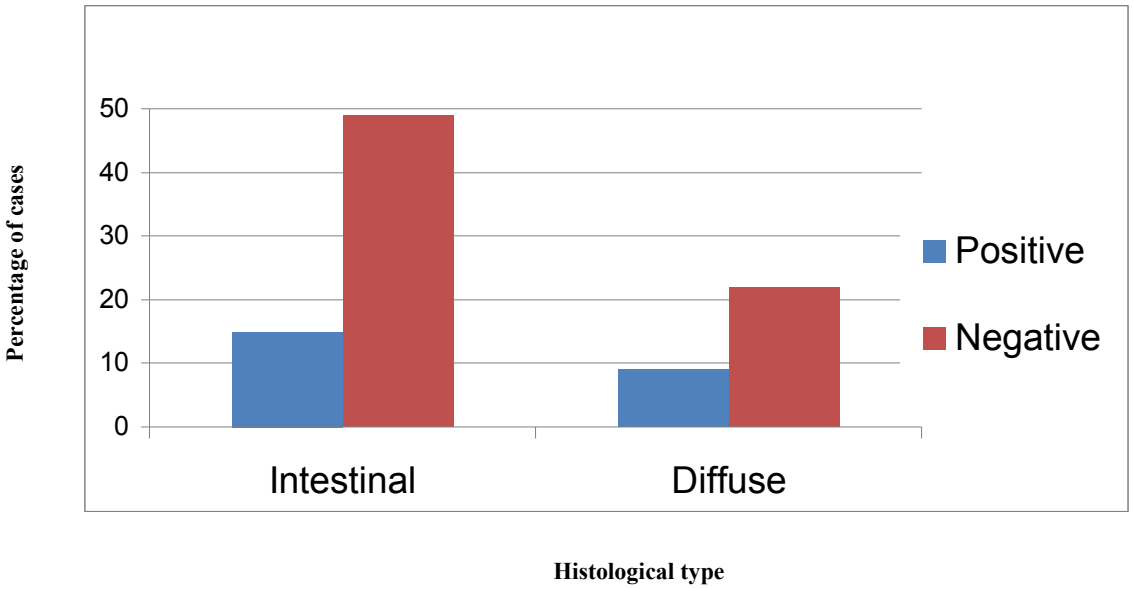

Figure (4): Distribution of patients with EBV positive and EBV negative expression according to histology type

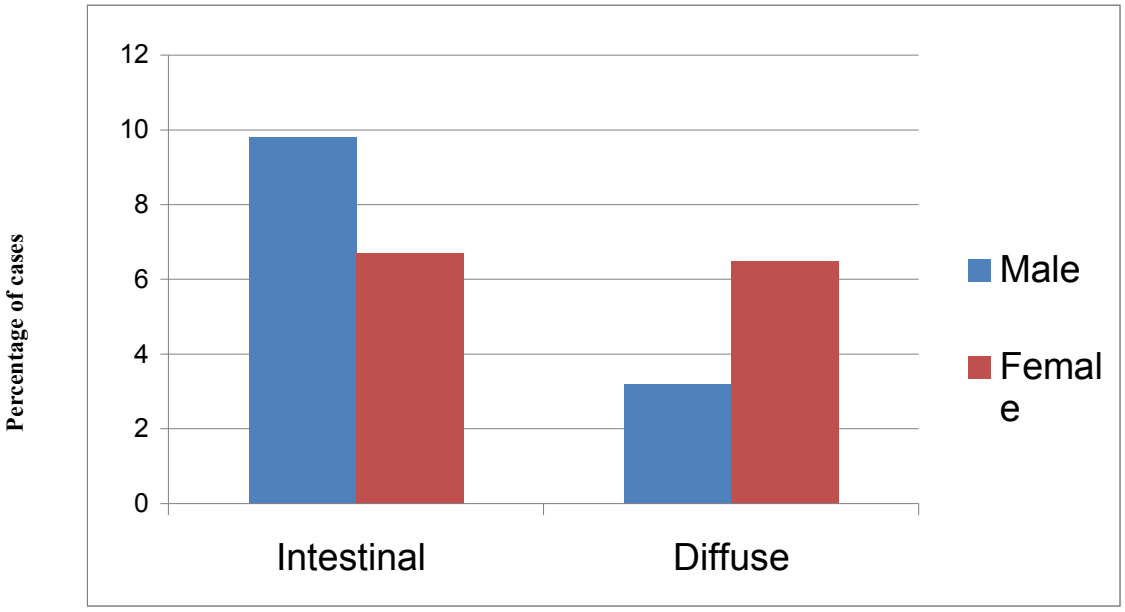

Figure (5): Distribution of patients with EBV positive expression according to Histology type and Sex

As shown in figure (5) the positive cases in females were equally distributed between the histological types, while 10% of male who express (EBV) were of intestinal type. P value was 0.53 which was of no statistical significance.

Tumor grade:

Expression of EBV was equally distributed in both moderate & poorly differentiated adenocarcinoma, while none of EBV positive cases were seen in well differentiated adenocarcinoma. P value was 1.07 which reflects no statistical significance as shown in figure (6).

Tumor stage:

15% of EBV positive cases were in stage IIIA as shown in figure (7) p value was 2.07 which is of no statistical significance.

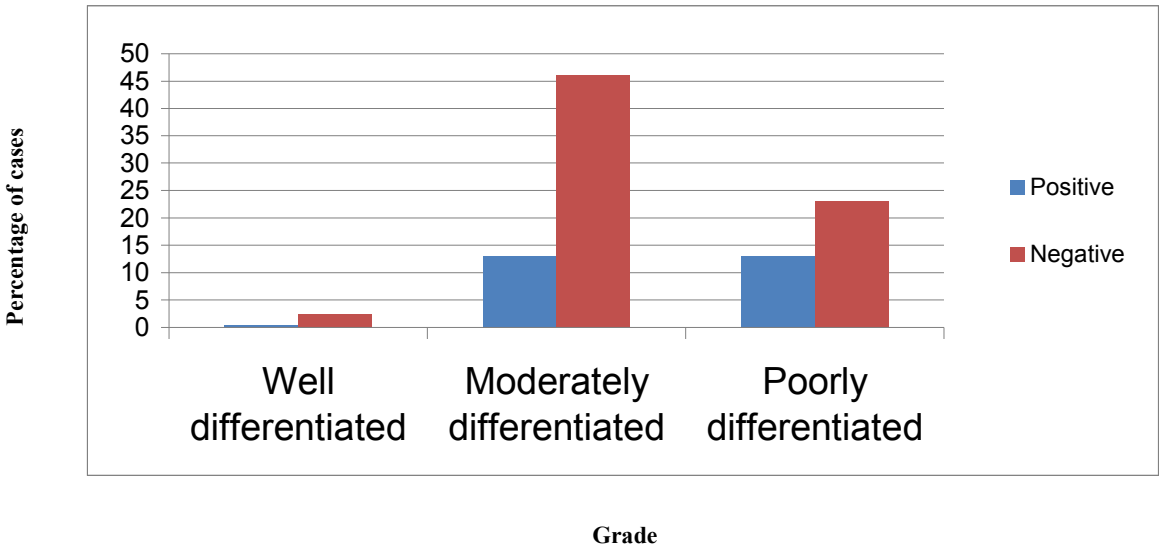

Figure (6): EBV expression in relation to tumor grade.

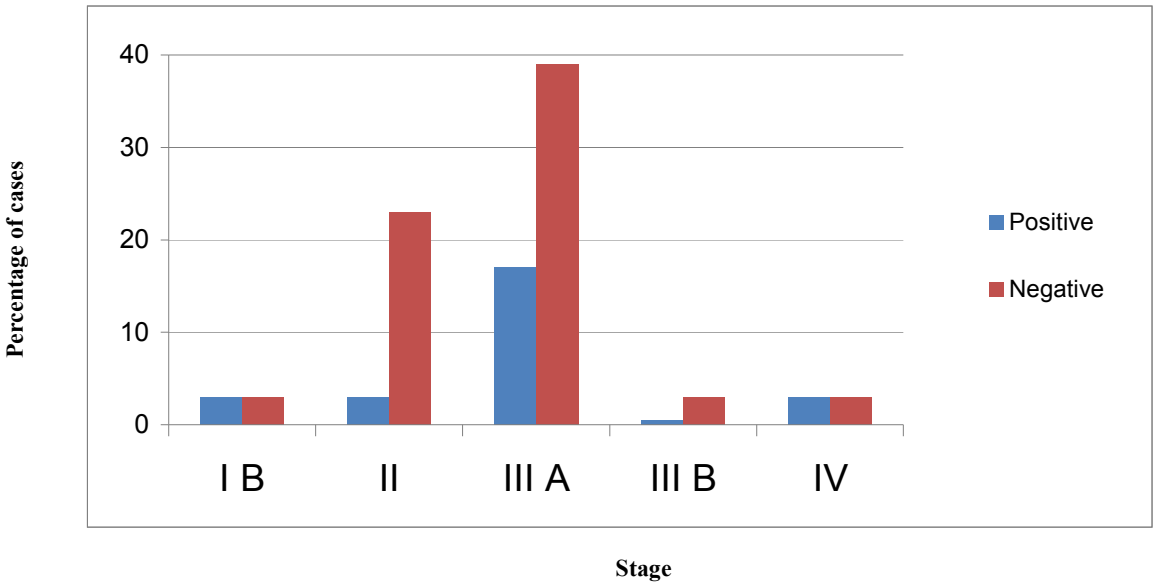

Figure (7): Distribution of patients with EBV positive & negative expression according to stage.

DISCUSSION

Stomach cancer is a global health problem. Its incidence is very significantly high in Japan, 90 per 100.000 while in western world is commoner in the low socioeconomic group [7], it reaches in the united states to 8.4 per 100.000 [8], however, in our country the incidence of gastric cancer is 2.37 per 100.000 (Iraqi cancer registry, 2001). In situ hybridization (ISH) can be considered the suitable method for analyzing viral antigen (EBV in this study) and their components in infected cells [9] as it is the most sensitive & specific tool for the detection of viral antigens in diseased tissue and tumor [10] . The role of EBV in gastric carcinogenesis has been postulated recently [3]. In this study, we investigated the association between EBV & gastric cancer by ISH. The results revealed that 26.6% of gastric cancer was EBV positive; it indicates that high percentage of gastric cancer in our collected cases was associated with EBV infection. A similar study has been done in Iraq [11] it also showed a high prevalence of EBV in gastric cancer that reached 17.1% this high EBV expression recorded in our study can be explained by:

1- The small sample size
2- High prevalence of EBV infection in lowers socioeconomic with lower standard of hygiene in developing countries including our.
3- Large geographic variations in the prevalence of infectious mononucleosis, in Iraq. It reaches 86% [12]. The incidence of EBV-GC in Japan is approximately 5-10% with regard that Japan is a country with the highest incidence of gastric cancer.
4- Other: include taking more than one section from each tumor block with multiple thickness level (to overcome error of handling) that increases diagnosis of EBV-GC.

In this study we found that EBV positive cases were equally distributed in both sexes (13.3%) for each (table 3) with no evidence of male predominance while it was observed in most of the studies [13]. EBV-GC shows no age dependence as the cases were distributed throughout the age group with high frequency in those >50 yrs but with no statistical significance (table 2) which goes with what had been found by [14] but unlike that found by [14;15]. According to our study, the primary tumors in EBV-GC, were located in the lower third of the stomach in approximately 19.9% while the proximal and middle tumor constitute only (3.3%) for each (Figure 3). The contradicts in several reports which suggest that the incidence of EBV tumors is greater in the upper stomach in comparison with the lower stomach [3;16]. This contradiction could be due to the small size in which tumor of the cardiac constitue (6.7%) one at which (3.3%) was EBV positive. Regarding the histopathological type, the proportion of EBV-GC varied. Some studies show predominance of these cases in diffuse type as in Japan and India [17; 18]. Some showed predominance in intestinal type as in Lyon/France, Netherlands and a study done for western patients [13]. Other studies showed difference in the incidence of EBV between intestinal & diffuse type as in Columbia [19]. In our study EBV expression was more in intestinal type approximately (16.6%) while only (10%) of the cases were of diffuse type (Figure 4) which could be due to high percentage at intestinal type in our cases. EBV-GC were identified more frequently in moderately & poorly differentiated adenocarcinoma when compared to other histological type with higher frequency in advanced tumor which involves the serosa [19] which is similar to what has been found in our study where EBV-GC was distributed evenly between moderate & poorly differentiated histological types (13.3% for each) (fig. 6) and was in a previous Iraqi study [13]. Regarding tumor stage EBV-GC predominated in stage III A (16.6%) which the surface was involved (Fig. 7). As a final point, the relationship between viral infection & tumor genesis remain a complex question & poorly understood.

CONCLUSION

1- There is an association between EBV and gastric cancer, however it is not significant but it sheds light upon the role of this virus in pathogenesis of gastric cancer.
2- There is neither male predominance nor age dependence in EBV-GC
3- EBV-GC slightly predominated in the distal part of the stomach.
4- The histological grade at EBV-GC shows prevalence in moderately & poorly differently type.
5- Most cases of EBV-GC were in advanced stages with serial in vision and mainly in stage III A.

RECOMMENDATION

1- Further analysis of clinicopathological features EBV GC using larger number of cases with follows up.
2- Studying the relation between EBV-GC and significant serological positive of the antibodies against EBV-VCS.
3- Studying the role of EBV in the development of cancer in other sites the body e.g. prostrated uterus.

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Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Locally Produced Soft Cheese in Baghdad Markets

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ABSTRACT

In order to investigate the presence of *Staphylococcus aureus* (*MRSA*) from locally produced soft cheese in Baghdad, a total of 75 samples were collected randomly from different markets in Baghdad during December 2011 till February 2012. The samples were analyzed and processed according to food microbiological protocols in which they enriched by peptone water and mannitol salt broth then inoculated on selective Baird-barker agar for *MRSA* type; biochemical tests including catalase and coagulase with β-hemolysis of sheep blood agar were done. Then check susceptibility of isolates to methicillin for selection of *MRSA* type *S. aureus* which is the most virulent type. Results showed that 25 (33%) samples out of the total 75 cheese samples were contaminated with *S. aureus* in which *MRSA* type were identified from 3 (4%) samples. These findings suggest presence of *MRSA* type in locally produced soft cheeses in Baghdad markets thus recommended to monitoring these products periodically to inshore public health.

***Key Words: Staphylococcus aureus*, *MRSA*, Soft Cheese**

الملخص باللغة العربية

بهدف التحري عن وجود العنقوديات الذهبية المقاومة للمضاد الحيوي الميثيسيلين في الجبن الطري المصنع محليا في بغداد اجريت هذه الدراسة على 75 أنموذج من الجبن المحلي الطري المملح والتي جمعت بصورة عشوائية من اسواق بغداد حيث تم معاملة النماذج حسب متطلبات البحث وبما يتفق مع الطرائق القياسية المتبعة في هذا المجال مع بعضالتحويرات للمدة من كانون الاول 2011 ولغاية شباط2012 .

زرعت النماذج المملحة والملائمة لطبيعة الجرثومة بعد تنشيطها باستخدام ماء الببتون ومرق المنيتول على الوسط الانتخابي (البرد بركر).

اجري للعزلات بعض الاختبارات الكيموحيوية مثل الكتليز والكوأغيوليز وتحلل الدم، كما تضمن البحث اجراء اختبار حساسية العزلات للمضاد الحيوي الميثيسلين لغرض تشخيص النوع المقاوم للمثيسيلين وقد اظهرت النتائج ان25 (33%) أنموذج من اصل 75 كانت ملوثة بجرثومة *S.aureus* كان منها 3(4%) نماذج شخصت على أنها النوع *MRSA*

نستنتج من هذه الدراسة بتلوث نماذج الجبن الطري بالعنقوديات الذهبية كان منها النوع المقاوم للمثيسيلين لذلك ينصح بمراقبة هذه المنتجات بشكل مستمر لضمان الصحة العامة.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically on the human and animal's body. Methicillin-resistant *S. aureus* (*MRSA*) includes those strains that have acquired a gene giving them resistance to methicillin and essentially all other beta-lactam antibiotics (1). This group of organisms has since emerged as a serious concern in human medicine. *MRSA* was first reported as a nosocomial pathogen in human hospitals. Although these organisms cause the same types of infections as other *S. aureus*, hospital-associated strains have become resistant to most common antibiotics, and treatment can be challenging (2). Community-associated *MRSA* first appeared in high-risk populations such as intravenous drug users, people in nursing homes, and people who were chronically ill, but they are now reported even in healthy children. *MRSA* can be transmitted between people and animals during close contact (3). Virulence factors found in *S. aureus* allow it to adhere to surfaces, damage or avoid the immune system, and produce toxic effects. All strains of *S. aureus* can cause purulent infections (4). Transmission of *S. aureus* or *MRSA* usually occurs by direct contact, often via the hands, with colonized or infected people. In hospital outbreaks, contaminated food can disseminate the organism to patients as well as to healthcare workers. Asymptomatic colonization with *MRSA*, including both nasal and rectal carriage, has been reported in animals. Carrier animals may serve as reservoirs for disease in themselves, and they may transmit *MRSA* to other animals or people (5). Environmental contamination has been reported in veterinary practices, even at times when *MRSA* patients were not detected. Food may serve as a vehicle to disseminate *MRSA*. Low degree contamination with *S. aureus* is common in retail meat, and *MRSA* has been reported in a variety of foods like raw milk and soft cheeses (6). *S. aureus* is not ordinarily invasive when eaten, except under rare and unusual circumstances. For this reason, accidental contamination with *MRSA* while handling raw food is the most important consideration. Food may also serve as a vehicle to disperse *MRSA*, if the organisms have not been destroyed by cooking (7). Acute staphylococcal gastroenteritis (food poisoning) is caused by the ingestion of preformed toxins, which are produced when *S. aureus* grows in food. The toxin, rather than the live organism, is responsible for the illness. Staphylococcal food poisoning usually develops abruptly. The symptoms may include nausea, vomiting, diarrhea, abdominal cramps, prostration and, in severe cases, headache and muscle cramps. The disease is self-limiting, and most people recover in 1 to 3 days, although some may take longer (8). *S. aureus* infections are diagnosed by culturing the affected site, while staphylococcal food poisoning is diagnosed by examination of the food for the organisms and/or toxins (9). Cheese has been made and consumed by people worldwide for thousands of years (10). Prior to modern technology and the ability to mass produce, cheese was made by hand in small batches. Cheese making was a labor intensive process, but it was a way to produce a nutritious food item with a much better shelf life than the milk from which it was made. Modern high-tech food processing methods provided a way to make cheese efficiently and produced a convenient, affordable, and consistent product that many consumers enjoyed. Cheeses are perishable products that can potentially harbor bacterial pathogens that can cause severe illness or even death. Some types of cheese, in particular soft cheeses made from raw milk have been implicated in outbreaks more often than others (11). The bacterial pathogens most often found in contaminated cheeses implicated in outbreaks of illness include *Listeria monocytogenes*, *Salmonella*, *Escherichia coli O157:H7*, and *Staphylococcus aureus* (12).

The objective of this study was to evaluate the microbiological safety, relative to standards of food regulations, of cheeses made in various places across the Baghdad markets, by testing the occurrence of *S. aureus* especially *MRSA* type in raw soft cheeses.

MATERIALS AND METHODS

Collection & Processing of Cheese Samples:

Samples of cheese were collected randomly from different part of Baghdad markets during December 2011 till February 2012. Each sample is diluted and macerated in a stomacher for 3 minutes. then it enriched in peptone water, 10gm to100ml and incubated in 35C°

for 24h then transferred to mannitol salt broth overnight then streaked on a selective media Baird-Parker agar base (Sigma-aldrich.com) 950ml with addition of 50 ml of egg yolk emulsion with potassium telleurit solution 3ml /L of agar, the proteolytic bacteria produce a clear zone around the black colonies in the media after 24-48 hrs. at 35C (13).

Isolation& Identification procedure:

S. aureus is a purple Gram positive, nonspore forming coccus, It may be found singly, in pairs, in short chains or in irregular clusters after routine staining. The colonies are circular, smooth and glistening; with black color as characteristic feature of *MRSA* type. On Sheep blood agar, they are usually beta-hemolytic. Young colonies are colorless; older colonies may be shades of white, yellow or orange. Enrichment media, as well as selective plates for *MRSA*, are available. Biochemical tests such as the catalase & coagulase tests are used to differentiate *S. aureus* from other *streptococci* & *staphylococci*.
Tube coagulase test free (extracellular) coagulase clots plasma in the absence of calcium.
The tube coagulase test with rabbit plasma and examination of tubes after incubation for 4 h and 24 h 21, 22 is the standard test for routine identification of S. aureus. Tests negative at 4 h should be re-examined at 24 h because a small proportion of strains require longer than 4 h for clot formation (13).

Methicillin Susceptibility test:

Tested the Susceptibility of isolates to antibiotics in the manner standard practice in the U.S.A. by the USDA and approved by the national committee for clinical laboratory standards (NCCLS.1990, MA-A4) and according to the instructions of the standard method of Kirby-Bauer Disc Diffusion Method (Bauer, 1966). Mueller-Hinton agar is considered to be the best for routine susceptibility testing of *S. aureus* in which it's prepared from a commercially available dehydrated base according to the manufacturer's instructions (Oxoid, UK). Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8 C). Cartridges containing commercially prepared paper methicillin disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. To standardize the inoculum density for a susceptibility test, a BaSO*R*4*R* turbidity standard, equivalent to a 0.5 McFarland standard. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The broth culture is incubated at 35C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually 2 to 8 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This result in a suspension containing approximately 3 to 5 x 10 5 cfu / ml for *S. aureus*. Then, a sterile cotton swab is dipped into the adjusted suspension, the swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level, this will remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. Then left for 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily

streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth (14).

RESULTS AND DISCUSSION

This brief study was conducted to evaluate the microbiological safety of cheeses selected from those that are produced in the Baghdad markets, Dairy products, including cheeses, are perishable products known to be potential carriers of pathogenic bacteria when care is not taken to prevent contamination with such bacteria. Results showed that 25 (33%) samples out of the total 75 cheese samples were contaminated with *S. aureus* in which *MRSA* type were identified from 3 (4%) samples as shown in table (1).

Table (1): occurrence of S. *aureus* in soft cheeses in Baghdad markets

Type of Sample	Number collected	*S.aureus* %	*MRSA* type %
Raw Soft Cheese	75	25 (33%)	3 (4%)

Colonies appeared on selective Baird-Parker agar as circular, smooth and glistening; with black color as characteristic feature of *MRSA* type, the proteolytic bacteria produce a clear zone around the black colonies in the media after 24-48 hrs. at 35C. On Sheep blood agar, they are usually beta-hemolytic. Young colonies are colorless; older colonies may be shades of white, yellow or orange. Biochemical tests such as the catalase & coagulase tests are used to differentiate *S. aureus* from other *Streptococci* & *Staphylococci* (15). Most study strains show zone of inhibition larger than 32 mm (standard sensitivity > 30 mm) in diameter in standardized disc diffusion tests with methicillin or oxacillin, with some strains resistance is seen as reduced zone sizes below 25 mm (standard resistance < 28 mm) in diameter, colonies varying in size and number within zones of inhibition. The literature on methicillin susceptibility testing is extensive, and often conflicting in recommendations regarding the most reliable method for routine use (15). This is partly because the various studies of phenotypic methods have included different strains, which may differ significantly in heterogeneity, and behave differently under particular test conditions. The farmstead dairy environment, where the animals that produce the milk used in production are raised in close proximity to the site where the dairy products are made, presents additional challenges to the prevention of contamination, compared with non-farmstead dairies, because the animals and their immediate environment are natural reservoirs of the foodborne pathogens associated with dairy products (16). Often, the same individuals who care for the animals also make the cheeses, so in the absence of adequate attention to sanitation, contamination of the cheeses could easily occur. Additionally, many of these farmstead cheeses are made from raw milk rather than from pasteurized milk, which calls for an even greater need to be certain of the microbiological safety of the milk being used. This last point can be a challenge even to the most attentive producer, because it is known that animals can be asymptomatic or subclinical carriers of *Salmonella*, *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus*.

The results of this brief and limited study indicate that the majority of the farmstead cheeses produced in the Baghdad markets and available for purchase are microbiologically safe for consumers. Staphylococcal food poisoning, caused by enterotoxins produced by some strains of *S. aureus*, is a very common and relatively mild foodborne illness, causing nausea, vomiting and diarrhea that normally subside within one to three days (17). *S. aureus* survives quite well in milk, and dairy products (including cheeses) have been implicated in cases of illness caused by enterotoxins produced by these bacteria (18). Another reason to be concerned about the presence of *S. aureus* in foods is the increasing presence of methicillin-resistant *S. aureus* (*MRSA*) in settings other than hospitals. The presence of *MRSA* in food producing animals has been demonstrated by others, with about 3-4% of *S. aureus* isolates from two studies possessing the *mecA* gene characteristic of *MRSA* (19). Because *S. aureus* can be shed in the milk of animals infected with this organism, and because *S. aureus* is destroyed by pasteurization, raw milk and cheeses made from raw milk are more likely than products made from pasteurized milk to be contaminated with *S. aureus* that could be *MRSA*. However, pasteurized products can become contaminated with *S. aureus* through post-pasteurization contamination. Because *MRSA* is resistant to most commonly used antibiotics and infections with *MRSA* can consequently be difficult to treat, cheese makers should take steps to control the presence in their products of *S. aureus*, some of which could be *MRSA*. These findings suggest presence of *MRSA* type in locally produced soft cheeses in Baghdad markets, thus recommended to monitoring these products periodically to inshore public health.

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Improvement of antibiotic production via exploiting spent culture supernatant in a bioreactor

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ABSTRACT

We report a strategy for exploiting quorum sensing molecules to elicit antibiotic production using a model bacteria *Streptomyces coelicolor*. The strategy was based on introducing an amount of spent culture supernatant from early stationary phase of *Streptomyces coelicolor* to its fermentation culture. The antibiotics studied were red-pigmented undecylprodigiosin and blue-pigmented actinorhodin. Investigations were performed in shake flasks and 2L bioreactor. Different amounts of spent culture supernatant ranging from 1 to 10% added at 0 time were used. For the test cultures, production of undecylprodigiosin was significantly increased by 2.2 -fold compared to the control cultures of *S. coelicolor*, whereas actinorhodin was increased by 1.6–fold. Furthermore, another positive outcome of this strategy was the earlier onset of antibiotics production by 24-48h compared to the control culture. Growth of *S. coelicolor* and glucose consumption of the test cultures were also studied and compared with those in the control culture. In all cases, growth and glucose consumption remained similar to those observed in the control culture. Bioreactor results confirmed our findings in the shake flasks experiments and thus, contribute to additional evidence that our strategy of using spent culture supernatant for improving antibiotic production by *S. coelicolor* is reproducible and applicable.

***Key Words*: *Streptomyces coelicolor*; Actinorhodin; Undecylprodigiosin; Spent culture supernatant; Antibiotics improvement**

الملخص باللغة العربية

Quorum sensing هي عمليه تستطيع من خلالها البكتريا تنظيم وتنسيق العديد من فعالياتها الحيويه والفسيلوجيه عن طريق انتاج جزيئات كيميائيه الى البيئه التي تتواجد فيها عند وصول عدد خلايها الى عدد محدد خلال طور الثبات Stationary phase. وجد ان انتاج المضادات الحياتيه يقع تحت تاثير انتاج جزيئات الكوروم سنسنك حيث تعمل كعوامل تحفز عميله بدء انتاج هذه المركبات. تضمنت الدراسه طريقه جديده لاستغلال جزيئات الكوروم سنسنك لغرض تطوير انتاج المضادات الحياتيه المنتجه من بكتريا *Streptomyces coelicolor* A(3)2. تعتمد الطريقه بشكل رئيسي على اضافة كميات من الوسط التخميري المستهلك لبكترايا الستربتومايسس والماخوذ خلال فترة طور الثبات الى وسطها الانتاجي بكميات تراوحت من 1–10 %. تمت دراسة نوعين من المضادات الحياتيه المنتجه من هذه البكتريا وهما الصبغه الحمراء الانديسلبروديجيوسين Undecylprodigiosinوالصبغه الزرقاء الاكتينورهودين Actinorhodin . التجارب اجريت باستخدام الدوارق الهزازه ومخمر بسعة 2 لتر. اظهرت النتائج زياده واضحه في انتاج هذين المضادين مقارنتا بانتاجهما في الحالات الطبيعيه, حيث تضاعف انتاج الانديسلبروديجيوسين بمقدار 2.2 والاكتينورهودين بمقدار 1.6 مقارنتا بمستوى انتاجهما في وسط السيطره. علاوة على ذلك وجد ان انتاج هذين المضادين قد بدأ مبكرا بحوالي 24 –48 ساعه قبل وقت انتاجهما في الحاله الطبيعه في وسط السيطره. تمت دراسة نمو بكتريا الستربتومايسس واستهلاكها للمصدر الكاربوني ووجد انهما لم يتأثرا بازدياد انتاج الانديسلبروديجيوسين والاكتينورهودين. نتائج التجارب التي اجريت باستعمال المخمر كانت مشابه لتلك التي تم الحصول عليها من تجارب الدوارق الهزازه وبذلك اعطت دليلا اضافيا على فعاليه هذه الطريقه لتحفيز انتاج المضادات الحياتيه ومدى امكانية تطبيقها بمستويات انتاجيه اعلى.

INTRODUCTION

The communication between bacterial cells is important for coordinating their behavior in many essential biological processes such as production of secondary metabolites (1). Bacteria have developed and utilized different ways to communicate such as direct cell-cell contact or via soluble secreted molecules (2).
Communications based on excreting signalling molecules are widespread among bacteria. It can be observed among the members of the same or different species (3). This kind of communication, namely quorum sensing, is normally achieved via a variety of low molecular weight molecules called autoinducers (4). During the past 30 years much more information has become available on quorum sensing in many bacteria (4-6) and in filamentous fungi (7). In general, when the signalling molecules reach a particular threshold concentration, they normally internalize into the bacterial cell and consequently, an activation in particular genes take place in all the bacterial population, for instance genes responsible for the stationary phase or secondary metabolites production for example antibiotics (8).
So far, different kinds of molecules have been discovered as quorum sensing signal molecules. For example, a wide variety of Gram-negative bacteria use acyl homoserine lactone molecules to communicate (9). In Gram-positive bacteria small linear and cyclic peptides are used as autoinducers which normally bind to the receptors on the cell membrane (10). A group of autoinducers called autoinducer-2 (AI-2) have also been discovered in both Gram-positive and negative bacteria (11). Furthermore, Gamma-butyrolactones are described as the most widely used signalling molecules by *Streptomyces* species for the regulation of antibiotic production (12). The first indication of a possible role for Gamma-butyrolactones compounds in *Streptomyces* species was obtained by Takano et al. (13), who described some molecules produced by *S. coelicolor* that can elicit the precocious production of the blue pigmented actinorhodin and red pigmented undecylprodigiosin.
Despite the important role of the quorum sensing signal molecules in the production of antibiotics, a very few attempts in the literature are found on the industrial exploitation of this molecules to improve antibiotic production particularly at the bioreactor scale. In this context, Raina et al (7) found that the addition of spent culture supernatant of *Penicillium sclerotiorum* containing butyrolactone molecules to its fermentation culture resulted in a significant increase in the production of the antibiotic sclerotiorin without causing a considerable change in the biomass production or carbon source consumption.
Streptomyces species are among the most widely used bacteria in industrial microbiology because of their ability to produce a wide range of secondary metabolites. We therefore, chose one of the best characterized members of this species, namely *Streptomyces coelicolor*, as the test microorganism in this study. It produces four known antibiotics two of which are pigmented, undecylprodigiosin (red) and actinorhodin (blue) (14). The other two antibiotics are the calcium dependent antibiotic and methylenomycin, for both of which no straightforward quantitative analytical methods exist in the literature.
Exploiting quorum sensing signaling molecules to elicit *S. coelicolor* in order to improve the productivity of antibiotics was our main objective. The present study was designed to investigate for the first time in a 2 L bioreactor, the use of spent culture supernatant from early stationary phase of *S. coelicolor* to improve the production of undecylprodigiosin and actinorhodin by this strain.

MATERIALS AND METHODS

Microorganisms and Media Composition

MT1110 strain of *Streptomyces coelicolor* A3 (2) was used in this work. This strain is $SCP1^-$, $SCP2^-$ plasmids-free and was derived from the wild type strain 1147 (14). It was kindly provided by Professor Ferda Mavituna of the School of Chemical Engineering and Analytical Science, The University of Manchester, UK.
A chemically defined liquid medium was used which contained per liter of distilled water:10 g glucose, 5 g NaCl, 5 g Na_2SO4, 4.5 g $NaNO_3$, 2 g K_2HPO_4, 1.2 g Tris base, 1 g $MgSO_4.7H_2O$, and 0.0178 g $ZnSO_4.7H_20$. The medium was supplemented with 1 ml trace salt solution containing per liter: 8.775 g $FeCl_3$, 2.040 g $ZnCl_2$, 1.015 g MnCl2.4H20, 0.425 g CuCl2.2H2O, 0.415 g NaI, 0.310 g H3BO3, 0.238 g $CaCl_2.6H_2O$ and 0.242 g $Na_2MoO_4.2H_2O$ (15).Mannitol/soya flour (MS)

agar which contained per 1liter of tap water 20 g mannitol, 20 g soya flour and 20 g agar (16) was used for maintenance and spore stocks of *S. coelicolor*.

Preparation of inocula

In all experiments, vegetative inocula of *S. coelicolor* were used and prepared as follows: *S. coelicolor* was cultivated on MS agar slant for 10-14 days at 30°C from the original culture on MS agar. After growth and spore formation, 5ml of sterile distilled water was added and the growth was scraped gently by a loop to release the spores into the water which were then collected in a sterile tube. This spore suspension was centrifuged at 4000 rpm for 10min to collect the spores. Next, the spores were washed and re-suspended in 1ml of sterile distilled water and counted using a haemocytometer. In all experiments, the number of spores in this inoculum was kept to approximately 1×10^9 spores/mL by adding more sterile distilled water if necessary. A vortex was used to get a homogeneous suspension of spores. 500 µL of this spore suspension was used to inoculate 100 mL defined liquid medium with 4 glass beads of 0.4 cm in 500 mL flask. These glass beads aided with mixing and dispersion of the mycelial growth. This culture was incubated for two days in an orbital shaker at 30°C and 200 rpm which was then used as vegetative inocula (17).

Cultivation methods

5% (v/v) level vegetative inoculum of *S. coelicolor* was used to inoculate 100 ml defined liquid medium with 4 glass beads of 0.4cm diameter in a 500 ml flask. An orbital shaker was used for incubation at 30°C at 200 rpm for 7 days. All experiments were accompanied with a control culture of *S. coelicolor*, and each run was conducted either in triplicate or duplicate and the results were represented as the arithmetic average.

Spent culture supernatant

S. coelicolor was grown as described earlier for 5-6 days. The culture broth was filtered using Whatman glass microfiber filters paper, GF/C, 1.2µm. The cell-free broth was further filter sterilised using a 0.2µm cellulose acetate membrane filter and added to the test cultures in volumes of 1.0, 2.0, 5.0 and 10% (v/v) at 0Time.

Bioreactor

Experiments were carried out in a 2L bioreactor constructed of jacketed Pyrex glass vessel with a working volume of 1.5 L. The internal diameter of the vessel was 11cm and the height 25cm. Its glass lid had five glass ports used for sampling, agitation, aeration, condenser and inoculation. Media (excluding glucose, phosphate and trace elements) were added to the vessel and then autoclaved at 15 psig and 121^{o}C for 45 minutes. Glucose, trace elements and phosphate were sterilized separately and then pumped into the bioreactor aseptically. The temperature was controlled at 30^{o}C. The bioreactor was operated with an aeration of 2 litres/min and agitation at 200 rpm. The pH of the medium was initially adjusted to 7.2 and then allowed to follow its natural course. Foam control was achieved by using antifoam Struktol J647.

Analyses

The growth of *S. coelicolor* was measured as the dry weight of cell material using Whatman glass microfiber filter paper (GF/C). Analox glucose analysis machine (Beckman-RIIC Ltd.) was used to determine the glucose concentration.

Concentration of actinorhodin was determined using the colorimetric method described by Doull and Vining (18). Actinorhodin was extracted from a known volume of *S. coelicolor* culture by adding an equal volume of 2 N NaOH which raised the pH above 13. Next, the pH of the mixture was adjusted to 12 using 1N HCl and mixed for at least three hours. Cell debris was removed by centrifugation at 4000 rpm for 10 min. The absorbance of the supernatant was measured at 640 nm and the concentration was calculated using the specific extinction coefficient E (1 %, 1 cm) = 355 at 640 nm as determined by Doull and Vining (18) for an alkaline solution of actinorhodin.

Using a colorimetric method described by Kang et al. (19), concentration of undecylprodigiosin was determined. Undecylprodigiosin was extracted by adding an equal volume of methanol to the cell pellets which were harvested from a known volume of the *S. coelicolor* culture by centrifugation at 4000 rpm for 10 min. The mixture was then

mixed for at least 3 hours. The absorbance at 530 nm of the supernatant was determined after removing cell debris by centrifugation at 4000 rpm for 10 min. The concentration of undecylprodigiosin was calculated by using molar extinction coefficient (E_{530} =100150 M^{-1} cm^{-1}).

RESULTS AND DISCUSSION

Biosynthesis of antibiotics is affected by various physiological and environmental factors; some of them act positively and stimulate production of antibiotics such as quorum sensing signal molecules [20, 21]. In spent bacterial culture supernatants, a wide variety of different products can be found and theoretically, some of them have the potential to act as quorum sensing signal molecules. In *S. coelicolor* culture, at least twelve extracellular Gamma- butyrolactones have been identified so far as signalling molecules that can play an important role for the onset of antibiotics production [12, 13, 22]. Our work was designed to exploit quorum sensing signal molecules to elicit antibiotics production by *S. coelicolor* based on introducing spent culture supernatant of this bacterium to its culture.

Growth and antibiotic production by *S. coelicolor* in defined liquid medium

Fig. (1) shows a typical batch culture time course indicating growth (dry weight), glucose consumption and antibiotics production in shake flasks as the average of three runs. The onset of undecylprodigiosin production occurred on the third day of incubation and reached its maximum of 0.96 mg/L on the fifth day. Whereas, the onset of actinorhodin production was observed on the fourth day of incubation and increased during the rest of the incubation reaching the maximum value of 5.3 mg/L on day seven. The maximum biomass concentration of 2.71 g/L was attained on the sixth day. Glucose concentration dropped from 9.2 g/L to 0.8 g/L during the exponential growth phase. The pH increased from the initial value of 7.2 to 8.3 with a further increase to 8.8 towards the end of the cultivation.

Accumulation of the quorum sensing signal molecules in the fermentation medium is normally concomitant with production of antibiotics. Thus, in our work, spent culture supernatant was prepared from a 5 days old culture of *S. coelicolor* (early stationary phase). The addition of spent culture supernatant to the *S. coelicolor* fermentation medium resulted in a significant enhancement in the production of undecylprodigiosin and actinorhodin as described below. For realistic comparison, we had tested the effect of addition of spent culture supernatant taken from 2-3 and 6-7 days old culture of *S. coelicolor*. The results revealed that spent culture supernatant prepared from 2-3 days old culture had no significant effect on the production of neither undecylprodigiosin nor actinorhodin (data not shown). Moreover, no further enhancement was observed when the spent culture supernatant was taken from 6-7 days old culture of *S. coelicolor* as the production of actinorhodin and undecylprodigiosin was similar to that observed in the culture supplemented with spent culture supernatant taken from 5 days old culture (data not shown).

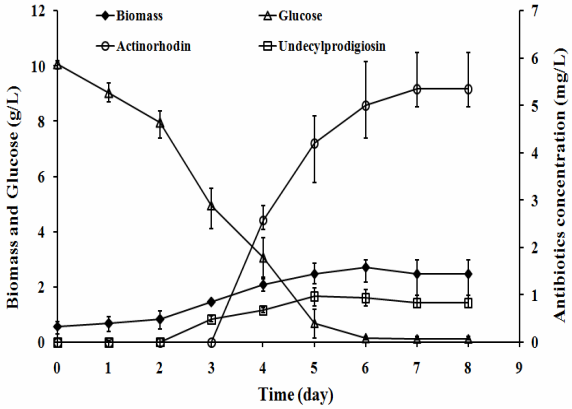

Figure (1): Growth of *S. coelicolor* in shake flasks using chemically defined synthetic medium and 5% (v/v) level of vegetative inoculums

Addition of spent culture supernatant

Fig. (2) and (3) show that the production of actinorhodin and undecylprodigiosin were significantly improved in the culture supplemented with 2.5, 5 and 10% (v/v) of the spent culture supernatant. The onset of production of these two antibiotics was earlier compared to the control. However, no significant differences in the production pattern of actinorhodin and undecylprodigiosin was observed in the culture supplemented with 1% of the spent culture supernatant as the

production was approximately similar to that in the control culture.

In the test cultures supplemented with 5 and 10% of the spent culture supernatant, production of actinorhodin started on the second day of incubation and about 48h hours before its normal production in the control culture. Concentration of actinrhodin on the second day of incubation was 0.56 and 0.69 mg/L in the test culture supplemented with 5 and 10% of the spent culture supernatant, respectively. Furthermore, in the culture supplemented with 2.5% of the spent culture supernatant, the production of actinorhodin was also started earlier on day 3 of the incubation and about 24h before its normal production in the control culture. Maximum production of actinorhodin achieved in the cultures supplemented with 2.5, 5, and 10% of the spent culture supernatant were 7.2, 9.4 and 9.7 mg/L, respectively, attained on day seven of incubation. Elicitation with spent culture supernatant therefore, corresponds to an increase of 1.2, 1.57 and 1.6-fold in the maximum actinrhodin concentration in the culture supplemented with 2.5, 5, and 10% (v/v) of the spent culture supernatant, respectively compared with the control culture.

Furthermore, production of undecylprodigiosin in the test cultures started on the second day of incubation and about 24 hours before its normal production in the control culture Fig. (3). Concentration of undecylprodigiosin on the second day of incubation was 0.03, 0.02 and 0.024 mg/L in the test culture supplemented with 2.5, 5 and 10% (v/v) of the spent culture supernatant, respectively. Maximum production of undecylprodigiosin achieved in the cultures supplemented with 2.5, 5, and 10% of the spent culture supernatant were 0.91, 1.54 and 1.85 mg/L, respectively, attained on day seven of incubation. Elicitation with spent culture supernatants therefore, corresponds to an increase of 1.1, 1.9 and 2.2-fold in the maximum undecylprodigiosin concentration in the culture supplemented with 2.5, 5, and 10% of the spent culture supernatant, respectively compared with the control culture.

The experiments in shake flasks were performed at least in triplicate for each run, and each run was then repeated twice or three times, and the results therefore, were reproducible.

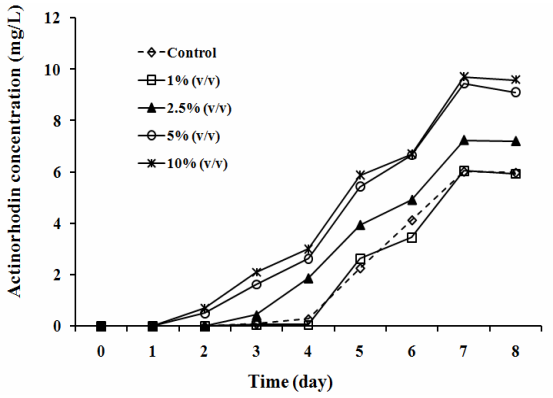

Figure (2): Production of actinorhodin by *S. coelicolor* in cultures suplemented with 1, 2.5, 5, 10 % of spent culture supernatant at zero time compared with the control

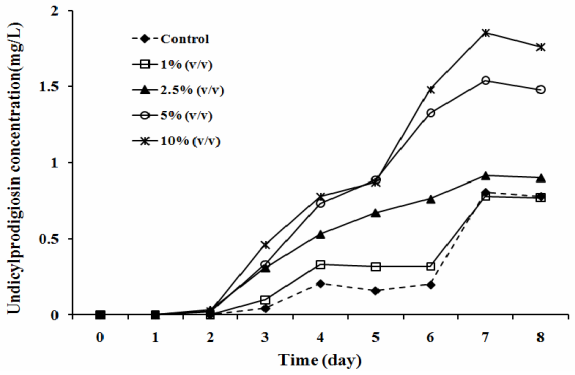

Figure (3): Production of undecylprodigiosin by *S. coelicolor* in cultures suplemented with 1, 2.5, 5, 10 % of spent culture supernatant at zero time compared with the control

Bioreactor experiments

In our work, production of antibiotics by *S. coelicolor* elicited with spent culture supernatant was further studied in a 2L bioreactor. In the light of the results obtained in shake flask experiments, 5% (v/v) of a spent culture supernatant was chosen in the bioreactor experiments. We compared growth, glucose consumption and actinorhodin production in the control and elicited bioreactor cultures.

Fig. (4), (5) and (6) show the time course of growth (dry weight), glucose consumption and

production of actinorhodin and undecylprodigiosin in the control and test cultures as the average of three runs. In the test culture, the growth pattern of *S. coelicolor* was similar to that of the control culture; the maximum biomass concentration achieved in the test culture was 2.51 g/L compared with 2.33 g/L in the control; both obtained on the seventh day of incubation Fig. (4). In both cultures, glucose consumption was concomitant with growth and reflected the changes in growth trends; however glucose consumption in the test culture was slightly high compared with the control Fig. (4). The pH values throughout the fermentation period were approximately similar in both cultures and ranged from the initial value of pH 7.2 to pH 8.8 at the end of the incubation.

Similar to the results obtained in the shake flasks, addition of spent culture supernatant had a significant effect on the production of actinorhodin compared with the control culture. In the test culture, the production of actinorhodin started about 24-35 h earlier than its normal production in the control culture. In this culture, the concentration of actinorhodin at the end of the first day of incubation was 1.12 mg/L and reached its maximum concentration of 26.5 mg/L on the sixth day. In the control culture, actinorhodin production started on day 3 of incubation and attained its maximum concentration of 21.8 mg/L on the seventh day Fig. (5). Comparing this value with the maximum concentration achieved in the test culture, the increase in the production of actinorhodin was therefore 1.2-fold.

Furthermore, addition of spent culture supernatant was also stimulated undecylprodigiosin production. As can be seen in Fig. 6, the production of undecylprodigiosin in the test culture started earlier and about 24-35 hours before its normal production in the control. At the end of the first day of incubation, the concentration of undecylprodigiosin was 0.14 mg/L and rose to the maximum of 1.25mg/L on the fifth day. The maximum production of undecylprodigiosin in the control culture was 0.44 mg/L achieved on day four of the incubation. Comparing this value with the maximum concentration achieved in the test culture, the increase in the production of undecylprodigiosin was therefore 2.8-fold.

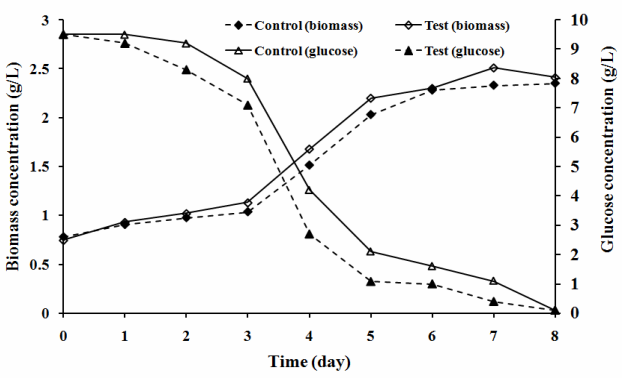

Figure (4): Growth of *S. coelicolor* and glucose consumption in 2 L bioreactor in culture supplanted with 5% of spent culture supernatant compared with control.

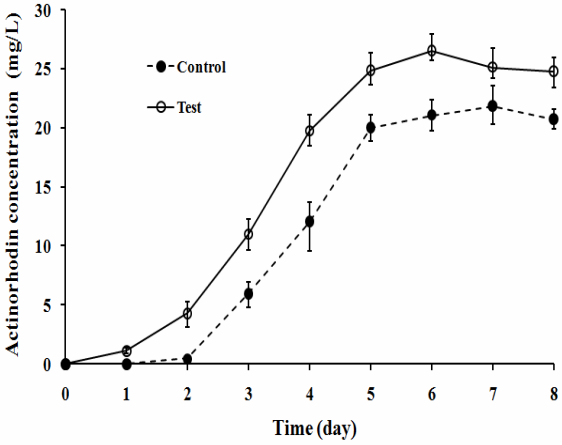

Figure (5): Production of actinorhodin by *S. coelicolor* in 2L bioreactor in culture supplemented with 5% of spent culture supernatant compared with control.

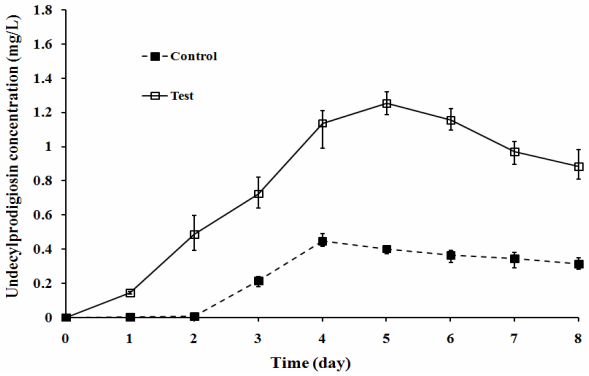

Figure (6): Production of undecylprodigiosin by *S. coelicolor* in 2L bioreactor in culture supplemented with 5% of spent culture supernatant compared with control

Some stoichiometric and kinetic parameters associated with the growth of *S. coelicolor*, glucose consumption and production of actinorhodin and undecylprodigiosin in the test and control cultures are presented in Tables 1, 2 and 3. Stoichiometric and kinetic parameters were calculated according to Mavituna and Sinclair (23) and Doran (24). The most notable differences between the two cultures were in the yield of actinorhodin and undecylprodigiosin in relation to biomass produced and glucose consumed. The enhancement in the production of antibiotics was slightly accompanied with an increase in the biomass concentration; however the maximum specific growth rate remained similar in both cultures.

Table (1): The values of some of the stoichiometric and kinetic parameters associated with the growth and glucose consumption by *S. coelicolor* in the control and culture supplemented with 5% of spent culture supernatant.

Stoichiometric and kinetic parameters	Control	Test culture
X_{max} (g L^{-1})	2.33	2.51
μ_{max} (h^{-1})	0.008	0.008
$\Delta S(\%)$	98.9	98.9
$Y'_{X/S}$ (g cells)(g Glu)$^{-1}$	0.16	0.19

X_{max} maximum biomass concentration, μ_{max} maximum specific growth rate, $\Delta S(\%)$ percentage of glucose consumed, $Y'_{X/S}$ yield factor for biomass on glucose consumed.

Table (2): Some stoichiometric and kinetic parameters associated with the production of actinorhodin by *S. coelicolor* in th control and culture suppelemented with 5% of spent culture supernatant.

Stoichiometric and kinetic parameters	Control	Test culture
P_{max} (mg/l)	21.85	26.56
q_P (mg /g cells-h)	0.055	0.073
$Y'_{P/S}$ (mg Act. / g Glu)	2.33	2.83
$Y'_{P/x}$ (mg Act. /g cells)	14.1	15.06
P_P (mg Act./l-h)	0.13	0.18

P_{max} maximum actinorhodin concentration, q_P maximum specific rate of actinorhodin production, $Y'_{P/S}$ yield factor for product on glucose, $Y'_{P/x}$ yield factor for product on biomass, P_P batch productivity for actinorhodin

Table (3): Some stoichiometric and kinetic parameters associated with the production of undecylprodigiosin by *S. coelicolor* in the control and culture supplemted with 5% of spent culture supernatnt.

Stoichiometric and kinetic parameters	Control	Test culture
P_{max} (mg/l)	0.44	1.25
q_P (mg /g cells-h)	0.001	0.004
$Y'_{P/S}$ (mg Und./ g Glu)	0.047	0.133
$Y'_{P/x}$ (mg Und./g cells)	0.283	0.709
P_P (mg Und./l -h)	0.006	0.01

P_{max} maximum undecylprodigiosin concentration, q_P maximum specific rate of undecylprodigiosin production, $Y'_{P/S}$ yield factor for product on glucose, $Y'_{P/x}$ yield factor for product on biomass, P_P batch productivity for undecylprodigiosin

CONCLUSION

When spent culture supernatant of *S. coelicolor* was added to its fermentation cultures, production of undecylprodigiosin and actinorhodin was increased as much as 2.5-fold compared to the control culture. In addition, the onset of actinorhodin and undecylprodigiosin production was earlier when there was such stimulation. The fact that spent culture supernatant is just as effective for such improvement in the antibiotic production strengthens the case for the industrial use. It is easier, quicker, and less costly to use spent culture supernatant rather than compounds (quorum sensing molecules) that need to be extracted and purified from the fermentation culture. Our work supports the exploitation of quorum sensing to improve the production of antibiotics at bioreactor scale as a preliminary step for industrial scale applications.

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Low GC and High GC bacterial DNA impact on anti DNA antibodies, IL-6 and IL-12 level in rats

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ABSTRACT

Bacterial DNA released at a site of infection triggers an immune response, differentiation, resistance to apoptosis, and release of proinflammatory cytokines. High GC content DNA of *E. coli* and Low GC content DNA of *S. aureus* succeeded in stimulating the immune system of rats to produce anti DNA antibodies, IL-6 and IL-12. However, response to *E. coli* DNA was markedly higher ($P<0.05$) than that to *S. aureus* DNA. What's more, time had a significant effect ($P<0.05$) on the levels of anti DNA antibodies, IL-6 and IL-12. In conclusion, bacterial DNA with high GC content stimulated rat immune system to produce DNA antibodies, IL-6 and IL-12 more than bacterial DNA with low GC content does.

Key words: **IL-6, IL-12, anti DNA Ab, GC, DNA**

الملخص باللغة العربية

يتحرر الدنا البكتيري عند موقع الاصابة محفزا استجابة مناعية و تمايزا و مقاومة للموت المبرمج للخلايا و اطلاق الحركيات الخلوية ما قبل الالتهابية. نجح كل من دنا بكتريا الاشيريكية القولونية عالي المحتوى من GC ودنا العنقوديات الذهبية واطيء المحتوى من GCفي تحفيز الجهاز المناعي للجرذان لانتاج اضداد الدنا و انترلوكين 6 وانترلوكين 12. في حين كانت الاستجابة لدنا الايشيريكية القولونية اعلى بشكل ملحوظ ($P<0.05$) من الاستجابة لدنا العنقوديات الذهبية. وعلاوة على ذلك كان للزمن تاثيرا معنويا ($P<0.05$) في مستويات اضداد الدنا و انترلوكين 6 وانترلوكين 12. ومن الممكن ان نستنتج ان الدنا البكتيري عالي المحتوى من GC حفز الجهاز المناعي على انتاج اضداد الدنا و انترلوكين 6 وانترلوكين 12 اكثر مما يفعل دنا البكتريا واطيء المحتوى من GC.

INTRODUCTION

Microbial DNA liberated at a site of infection converts immature antigen-presenting cells (APCs) to mature professional APCs. It triggered B cell proliferation, differentiation, resistance to apoptosis, and release of interleukien-6 (IL-6) and interleukine-12 (IL-12) (1,2). The cytokines induced by CpG motifs perform critical immunomodulatory functions. IL-12 and interferon gamma (IFN-γ) promote type 1 cytokine production and play important roles in the elimination of human pathogens (3). IL-6 is a type 2 cytokine that facilitates the growth/differentiation of T and B lymphocytes and enhances autoantibody formation in both human and mice. Furthermore, IL-6 is required for the production of anti-DNA (4).

Dalpke et al. (5) reported that the content of CG and CpG motifs also play an important role in eliciting immune responses. Bacterial species differ in their frequency of guanosine and cytosine (G +C) content; thus, differences in the frequency of CG dinucleotides (fCG) are also probable. That most often fCG correlated with fG+C, indicating that the dinucleotide [CG] showed a frequency as expected from the individual G+C content. That an increase in fCG went along with increased immunostimulation. In a survey of 15 bacterial species, the immunostimulatory capacity of bacterial DNA samples directly correlated with the frequency of CpG dinucleotides. In that study, the CpG frequency ranged from 1.44 to 12.21%. *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*, which showed the best IL-8 inducing activity, had also the highest frequency of [CG], while the lowest activity was *Staphylococcus epidermidis* was marked by a low Fcg (5).

To our knowledge, this is the first work investigating the role of low and high GC bacterial DNA effect on in vivo production of anti DNA antibodies, IL-6 and IL-12.

MATERIALS AND METHODS

Bacterial isolation and identification

E. coli and *S. aureus* were isolated from patients presented with UTI. Identification was achieved by biochemical tests according to Forbes et al. (6) and confirmed by api 20E and api 20 staph (bioMérieux, France)

Extraction of DNA

E. coli (high GC) and *S. aureus* (low GC) genomic DNA was extracted and purified from the most antibiotic (beta-lactam) sensitive isolates using Wizard genomic DNA purification kit (supplied by Promega Corporation). According to the protocol stated by the kit manufacturer, DNA was extracted from fresh cultures of the selected bacterial isolate.

DNA concentration was determined by measuring OD_{260} absorbance on a UV spectrophotometer (CECIL, France). Purity of DNA was determined by optical density at 260nm/280nm ratio ($OD_{260/280}$). All DNA used in these experiments had an $OD_{260/280}$ ratio .1.81-1.93.

In vivo study

Animals

The experiments were performed on nine conscious adult male Wister rats (*Rattus norvegicus*) weighing 242-306g. They have free access to water and food. The animals were randomly distributed into three groups of three animals (A, B and C). Groups A and B injected with *E. coli* DNA and *S. aureus* DNA, respectively; while, group C was considered as control group.

Injection protocol

Each rat was restrained using a rat restrainer and injected intravenously, by venipuncture of the tail vein, with *E. coli* or *S. aureus* DNA (1600 ng/ml) using a syringe with a 29G needle. In addition to TE buffer for control group. Thereafter the animals could move freely and have free access to water.

Blood Collection

Blood was collected from control rats as well as rats injected with bacterial DNA from the tail vein about 6, 8, 10 and 24 hr after injection. The rat was also restrained and the tail was warmed by immersing in warm water to dilate the vessels. Then the blood was collected and instantaneously transferred to a tube containing heparin (20 U/ml).

Consequently, sera were separated by centrifugation and kept at -20 °C until use.

Anti DNA, IL-6 and IL-12 level estimation

Anti DNA, IL-6 and IL-12 level were estimated in rats' sera following the manufacturer instructions of ZEUS Scientific (USA) reference, Assay Max mouse IL-6 (USA) and DRG IL P70 mouse (Germany) ELISA kits, respectively. Each assay was performed in triplicate.

Statistical analysis

Data are presented as mean ± standard deviation. ANOVA test, correlation coefficient (*r*) and $LSD_{0.05}$ were employed for data analysis using Microsoft EXCELL 2007 application.

RESULTS AND DISCUSSION

The presence of the microorganism itself or its DNA activates the immune system causing the production of proinflammatory cytokines. Cytokines have been shown to exert an important influence in the pathogenesis (7-10). Bacterial DNA succeeded in stimulating the immune system of rats to produce anti DNA antibodies (table 1), IL-6 (table 2) and IL-12 (table 3). Both cytokines and anti DNA antibodies level declined after 24 hrs from injection. However, significant differences ($P<0.05$) were observed between the impact of high GC content DNA of *E. coli* and Low GC content DNA of *S. aureus*. Furthermore, exposure period significantly affected the level of anti DNA antibodies, IL-6 and IL-12.

Takeshita et al (11) suggested that *Porphyromonas gingivalis* DNA may function as a virulence factor in periodontal disease through expression of inflammatory cytokine. The bacterial DNA markedly stimulated IL-6 production by human gingival fibroblasts. Miyazato et al. (12) observed that DNA from *C. albicans* induced the IL-12 production by murine bone marrow-derived myeloid dendritic cells.

In addition, we have found that the production of both cytokines was 1.5 times more in mice stimulated by *E. coli* DNA than *S. aureus* DNA (tables 1 and 2). Consistent with this result, Gilkeson et al. (13) found that oligonucleotides lacking CpG dinucleotide induced no cytokine (IL 6, IL 12, and IFN γ) production.

Such finding highly emphasizes the role of high GC DNA in stimulation cytokines production.

Table (1): Anti DNA (IU/ml) production stimulated by *E. coli* DNA and *S. aureus* DNA*

Time (h)	Control	*S. aureus* DNA	*E. coli* DNA
6	12 ± 1	40 ± 6	30 ± 6
8	14 ± 2	35 ± 4	27 ± 2
10	13 ± 3	24 ± 2	19 ± 3
24	12 ± 1	19 ± 2	13 ± 2

*LSD= 0.052, P= 4.42×10^{-10}

Table (2): IL- 6 production (pg/ml) stimulated by *E. coli* DNA and *S. aureus* DNA*

Time (h)	Control	*S. aureus* DNA	*E. coli* DNA
6	9.10 ± 0.26	18.33 ± 2.5	34.33 ± 4.5
8	9.36 ± 0.46	20.66 ± 3.5	31.33 ± 3.0
10	10.06 ± 1.5	17.66 ± 3.05	30.66 ± 2.08
24	9.56 ± 0.87	15 ± 3.6	24.33 ± 2.5

*LSD= 4.38, P= 3.2×10^{-12}

Table (3): IL-12 production (pg/ml) stimulated by *E. coli* DNA and *S. aureus* DNA*

Time (h)	Control	*S. aureus* DNA	*E. coli* DNA
6	3.7 ± 0.2	10.7 ± 0.26	14.9 ± 0.3
8	4.5 ± 0.1	9.5 ± 0.4	12.6 ± 0.2
10	4.1 ± 0.3	6.06 ± 0.37	9.7 ± 0.26
24	4.2 ± 0.2	4.06 ± 0.4	9.3 ± 0.36

*LSD= 0.035, P= 2.4×10^{-24}

Richards et al. (14) stated that IL-6 has been implicated both in anti-DNA antibody production and the pathogenesis of nephritis. The present work agreed with this fact as IL-6 is positively correlated with anti DNA production in mice after stimulation with both *E. coli* DNA (*r* 0.89) and *S. aureus* DNA (*r* 0.82). Obviously, it is correlated with the former more than with the latter.

IL-12 plays a crucial role in a variety of immune responses that are involved in immuno-senesence and autoimmune diseases. IL-12 treated mice had significant elevated levels of anti-DNA antibodies. Administration of IL-12 to mice reversed their Th1/Th2 cytokine profile and thus rendered them vulnerable to the induction of experimental systemic lupus erythematosus (15).

Since high GC bacterial DNA was able to stimulate rat immune system to increase production level of IL-6 and IL-12 as well as anti DNA antibodies, we believed it may has an important role in evoke an autoimmune reaction. Much work is needed to elucidate the mechanism lay behind such stimulation.

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Simple Kit for Detection of Rotavirus

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ABSTRACT

In this pilot study, we developed diagnostic kit depend upon reverse passive heamagglutination (PHT) as rapid and sensitive test to determine the presence of Rotavirus in children stool. The kit depend upon sensitize formalized RBC with antibodies prepared from human rotavirus using the Nebraska calf diarrhea virus (NCDV), which is antigenically related to human strains. Forty stool samples collected from children with diarrhea. The kit depends upon passive heamagglutination test compared with latex agglutination test. Drop of water mixed with the stool and drop of prepared kit and from latex, agglutination was observed in case of presence of Rotavirus in stool. The results shows that 38 (95%) out of 40 stool samples were positive, when we used the locally prepared kit compared with 35(87.5%) positive case in case of slide latex agglutination test used. The positive cases showed the highest normality which was (1/64) by using passive heamagglutination test (PHT) ,while the intensity of clumping ranged from 2-3 minutes when tested by latex agglutination on the slide compared to positive and negative control. This simple kit very useful for diagnostic laboratory, because it's simple, not expensive and accurate.

الملخص باللغة العربية

فى هذه الدراسة التجريبية وضعنا عدة لتشخيص الروتا فيروس فى براز الاطفال تعتمد على طريقة التفاعل الدموى المنفعل. يعتمد الفحص على تحسيس كريات الدم بواسطة مضادات محضرة ضد فايروس الروتا العزول من العجول فى نبراسكا والمسمى فايروس نبراسكا والذى يرتبط مستضديا الى السلاسلات البشرية.

وفي بحثنا هذا استعملنا طريقة التفاعل الدموي المنفعل المحضرة محليا والتي تعتبر من الطرق الاكثر حساسية مقارنة بطريقة تلازن اللاتكس على الشريحة و بينت النتائج وجود فرق معنوي عند فحص (40) نموذج براز لأطفال يعانون من اسهال شديد بطريقة التلازن الدموي المنفعل واعطت 38 (95%) نتيجة موجبة في حين بينت النتائج عند فحصها بطريقة تلازن اللاتكس على الشريحة واعطت 35 (87.5 %) نتيجة موجبة مقارنة بالسيطرة الموجبة والسيطرة السالبة، وبينت النماذج الموجبة اعلى عيارية وكانت 64/1 عند استعمال طريقة التفاعل الدموي المنفعل في حين تراوحت شدة التلازن من 2-3 دقيقة عند فحصها بطريقة تلازن اللاتكس على الشريحة مقارنة بالسيطرة الموجبة والسالبة .

INTRODUCTION

Rotavirus infects human and animal causing inflammatory bowel disease and severe diarrhea in babies, which ranges in age from less than a month to two years. As a result of this virus, children become dry and salt imbalance in their body.

The virus can be diagnosed by passive interaction, which is the most sensitive ways of interacting with antibodies to rotavirus associated with red blood cells, particularly with the virus made up clumping, appears on the form of a network during the examination period (1,2).

The virus had been diagnosed with the disease by immunofluorescence dye used by Bridnojamaath and by Julkn *et al*. (3).

Rotaviruses replicate mainly in the gut, and infect enterocytes of the villi of the small intestine, leading to structural and functional changes of the epithelium. The triple protein coats make them resistant to the acidic pH of the stomach and the digestive enzymes in the gut (4,5). Rotavirus gastroenteritis is a mild to severe disease characterized by vomiting, watery diarrhoea, and low-grade fever. Once the virus infects a child, there is an incubation period of about two days before symptoms appear. Symptoms often start with vomiting followed by four to eight days of profuse diarrhoea. Dehydration is more common in rotavirus infection than in most of those caused by bacterial pathogens, and is the most common cause of death related to rotavirus infection (6,7).

Rotavirus particles in faces with gastroenteritis can be detected by electron
microscopy (EM) (8),counter - immunoelectro - osmophoresis
(CEP) (9), or radioimmunoassay(RIA) (9). However, EM requires time and expensive equipment, while the CEP method is slightly less sensitive than EM. Although the sensitivity of RIA seems the best of all, it also requires elaborate procedures. We have developed a passive haemagglutination (PHA) method for the detection of human rotavirus using the Nebraska calf diarrhoea virus (NCDV), which is antigenically related to human strains (10,11)

MATERIALS AND METHODS

Sample collection:

Stool sample was collected from babies aged (2-24) month and from calf Rotavirus diarrhoea during (1-4) days of infection. Rotavirus was diagnosed by immunological method described below**.**

Viral purification:

The virus was collected from calf stool with acute gastroenteritis. We had taken (10%) of stool suspension prepared by Tris Buffer Saline (TBS, PH 7.5-Tris) . Centrifugation was made by 10,000r/min. for 10 minutes in 4ºC. The sediment was suspended in 2ml TBS.

Antibody preparation:

Two ml of viral suspension was mixed with complete Freund's adjuvant. About 0.3ml of suspension was injected intramuscularly in rabbit every four days for one month ,after week of the last injection, a five ml of blood sample was taken and centrifuged .The serum was treated by Ammonium Sulphate according to Campbell :1964 (12), with some modification in immune serum precipitation. Absolute protein quantification was made by bioret and Bradford method on 545nm wave length (3).

Sephadex G200 preparation:

Red blood cells were placed in 500 ml flask with alkaline tris buffer pH 7.8 for 24 hour till the RBCs were swelled.

The RBCs were gently transferred to Sephadex Colum (20× 2.5) by using 10 ml pipette gently to prevent bubble formation in Sephadex RBCs. The suspension was washed by Tris buffer with pH 8.7 for several times and carefully to made tric buffer cover the gel to prevent dryness. The IgG Antibodies were passed throw the prepared Sephadex tube, then Antibodies were collected, transferred to small bottles (1ml in each). Optical density was performed on 280nm. Curve was made then samples were condensed by PEG 6000 and protein quantification as explained.

Agarose gel Immunodiffution and precipitation test:

Ouchterlony procedure was used in this test as described by Kwapiniski :1972 (13).

Formalized red blood cell treated with tannic acid:

A twenty ml of sheep blood was obtained and added to seffer solution, washed for three time with phosphate buffer solution with pH 7.2 (3000 RPM for 10 min.) to remove the seffer solution**,** the precipitate was re -suspended in 10% phosphate buffer ,the suspension was occupied in dialyzer, and double volume of formalin was added , placed on magnetic stirrer in 4ºC overnight.
Then formalized RBCs suspension was washed by phosphate buffer for seven times (10000 RPM)to remove the formalin. The precipitate was re-suspended to reach 8% concentration, then formalized RBCs was a added to a homologue amount of Tannic acid ,stored in 37ºC for half hour, the RBCs washed for twenty min.(1500 RPM). Again and by the same buffer the formalized RBCs was washed twice (3000 r/cell) for 10min.
Sensitization of formalized red blood cell by Antibodies: One mlg /ml of Antibodies was added to the formalized RBCs as (one volume of RBCs with duplicated Antibody volume) incubated at 37ºC for 30 min. centrifuged at 3000 RPM for 10 min. to remove any unbounded antibodies ,then washed twice by the same buffer at 3000 RPM for 10 min.

Rotavirus Antigen Detection:

A 10% suspension of each stool sample was prepared in TSB and homogenized thoroughly in a vortex mixer. The homogenized suspension was clarified by centrifugation, the supernatant of buffer solution was separated, and the PHT and LA test was performed. The PHT was performed by added the formalized sensitize RBC with Rotavirus antibodies, while the LA was performed by using commercial kit (Bioret/ Spanish). The PHT considered positive for Rotavirus if distinct haemaggultination was observed.

RESULTS AND DISCUSSION

Rotavirus isolation

Rotavirus was isolated successfully from calf feces detected by latex agglutination test which visualized during 2-3 min and confirmed by Agarose gel Immunodiffution and precipitation test. The virus purified by ultracentrifugation (5000 RPM) at 4ºC. The Antigen protein was condensed by bioret method to obtain a 6g/ml. The antibody concentration prepared against rotavirus Ag was made (3.8 mg/ml).

The agarose gel Immunodiffution and precipitation test :

Agarose gel Immunodiffution and precipitation prepared for immune serum with rotavirus Ag showed precipitation line after 24 h. in 37ºC.

Passive heamagglutination test (PHT):

Table (1) reveals the results of detection of Rotavirus antigen in stool samples collecte for 40 children suffer from gasteroenteritis diarrhea. Out of 40 stool samples,38 samples were positive (95%) by using passive heamagglutination test (PHT) which was prepared locally in comparison with slide latex agglutination test (LA) (Bioret/ Spanish) 35 samples were positive (87.5%).

Table (1): comparison between passive heamagglutination (PHT) and Latex heamagglutination (LA)

Stool sample (40)	Passive heamagglutination test		slide latex agglutination test	
	No.	%	No.	%
Positive	38	95%	35	87.5%
Negative	2	5%	5	12.5%
Total	40	100%	40	100%

To confirm the results, eight stool sample were taken (four stool sample which were found to be positive for rotavirus and four cases which were negative for the virus) were diluted and positive and negative control of the test were added. Different stool sample diluents had been examined by passive heamagglutination

test (PHT) and found that the highest normality was in 1/64 & 1/32 diluents while 1/128 found to be negative for the test. (Table 2).

Table (2): Detection of highest normality for diluted suspension of stool sample tested by passive heamagglutination test (PHT)

Antibody titer							
positive Rotavirus(4)	1/2	1/4	1/8	1/16	1/32	1/64	1/120
+ve	4	4	4	4	2	1	0
-ve	0	0	0	0	2	3	4
Negative Rotavirus(4)							
+ve	0	0	0	0	0	0	0
-ve	4	4	4	4	4	4	4
Positive control	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Negative control	-ve	-ve	-ve	- ve	- ve	- ve	- ve
***Test cells**	-ve	-ve	-ve	- ve	- ve	- ve	- ve
Control **cells	-ve	-ve	-ve	- ve	- ve	- ve	- ve

**Sensitized RBCs with antibodies.*

This study confirmed that the kit prepared locally, which depend upon the properties posses by rotavirus to induce passive haemagglutination when formalized sheep RBC sensitized with Rotavirus antibodies and treated with Rotavirus antigen. The current test was very sample, not expensive and accurate. The methods of choice for the detection of rotavirus in stool samples should have high degrees of sensitivity and specificity, high predictive values, and reproducibility, which ensure consistency of performance in the laboratory.

The results show the importance of using the current kit in diagnosis of Rotavirus in children stool. Rotavirus infects mostly the children and causes acute gastroenteritis. Some reports mentioned that this virus causes CNS disorder in children (1). There are increasing reports of cases in which patients who have seizures after an episode of rotavirus diarrhea have evidence of rotavirus in their CSF.

More extensive studies are necessary to determine the prevalence of Rotavirus in Iraq in order to design effective control measures to protect our children against this pathogen. The Rotavirus vaccine was scheduled by ministry of health through immunization programmers for children.

The significant of current kit that there were no false negative results obtained by passive heamagglutination test (PHT) in comparison with slide latex agglutination test, although latex agglutination test is a highly specific and rapid method and it may be useful in certain situations, such as in outbreaks, its low sensitivity can make it unsuitable for use in routine clinical practice.(14,15)

Rotavirus used in this test was obtained from human and bovine types because of their similarity in viral antigens. Also we have diagnosed rotavirus which infect human by IgG antibodies absorbed on sensitized RBCs. In this study we found that the best volume of immunoglobulin's used in binding in order to prevent the non specific agglutination was made 1mlg/ml of immunized globulins.

The rapid diagnosis of rotavirus infection in patients admitted to the hospital with symptoms of gastroenteritis would enable better treatment of the patient, such as isolation or discharge, as in many cases effective rehydration can be achieved at home, and most rotavirus infections are selflimiting. An accurate diagnosis of rotavirus is essential since it obviates the unnecessary use of antibiotic therapy.

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Study the immunological effect of human choriogonadotropin like hormone isolated from local isolate *Staphylococcus epidermidis* bacteria

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ABSTRACT

Twenty *Staphylococcus spp* were collected from blood of cancer patients .The results of re characterization methods showed there were sixteen isolates classified as *S.epidermidis* according to the several biochemical tests, and all sixteenth isolates were coagulase and manitol negative, and the results of sugar fermentation showed there were several changes. .The extract was prepared, the protein and carbohydrate concentration were measured, which were 300 mg/ml and 96 mg/ml respectively. The result of purification procedure showed there were three main peaks which occurred at fractions (8-17),(18-32),(33-43) respectively ,the protein concentration in samples which were (32 , 50 ,33.4) mg/ml respectively , while the carbohydrates concentration (12 , 42 ,25) mg/ml respectively . The first peak fractions was chosen. The HPLC result for this peak showed three main peaks with the retention time (1.868 , 2.642 , 2.874) respectively , the last peak retention time showed closely related with that peak of standard formulated hCG hormone that occurred at 2.836,and when more purification was done, the first peak (7-16) fractions, the protein concentration for this peak was (87.6 mg/ml), and was gave a positive result with pregnancy test kit, and when used it as immune stimulator for lab. mice showed , there were crossly internal organs enlargement(Liver, Spleen) The whole Liver microscopically shows normal structure appearance with kupffer cell hyperplasia with mono nucleus infiltration . While spleen tissue showed follicular hyperplasia of the white pulp ,with megakaryocytic cells infiltrate , focal infiltration of mono nucleus in the tissue and diffuse hyperplasia of lymphoid tissue.

الملخص باللغة العربية

جمعت عشرون عينه بكتيريه من دم مرضى مصابين بمرض السرطان شخصت انها تعود لجنس *Staphyloccocus spp.* . وكانت نتيجه عمليه اعاده التشخيص هو وجود 16 عزله صنفت على انها بكتيريا *S. epidermidis* اعتمادا على عدد من الفحوصات الكيمياحيويه فجميعها اعطت نتيجه سالبه لفحص انزيم coagulase وسكر المانيتول . وكانت نتائج عمليه تخمر عدد من السكريات الى حدوث عدد من التغيرات لبعض العزلات . حضر المستخلص ،وكان تركيز البروتين والكاربوهييدرات هي 300ملغم /ملليتر و 96 ملغم /ملليتر على التوالي . وكانت نتيجه عمليه التنقيه ظهور ثلاث قمم رئيسيه وكانت ضمن الاجزاء (8−17)،(18−32)،(33−43)على التوالي . وان تركيز البروتين لهذه الاجزاء هي (32 , 50 ,33.4) ملغم/ مل على التوالي بينما تركيز الكاربوهيدرات كان (12، 42، 25) ملغم/مل على التوالي . اختيرت اجزاء القمه الاولى ، وكانت نتائج الفحص بتقنية الـــ HPLC ظهور ثلاث قمم رئيسيه وان زمن الاحتجاز لها كان (1.868 , 2.642 , 2.874) على التوالي . زمن الاحتجاز للقمه الاخيره اعطى نتيجه مقاربه جدا لزمن احتجاز قمه الانموذج القياسي لهرمون hCG والتي كانت. 2.836 وعند اجراء عمليه تنقيه اضافيه ، اختيرت القمه الاولى للاجزاء (7−16) وكان تركيز البروتين 87.6 ملغم /مل , حيث اعطى نتيجه ايجابيه لاختبار عده فحص الحمل , وعند اختبار الانموذج كمحفز مناعي لفئران المختبر , اظهر حصول تضخم عياني واضح لعضوي الكبد والطحال ، ومجهريا حيث اظهر الكبد تركيبا طبيعيا مع زياده في خلايا كوبفر وارتشاح خلايا احاديه النواه , بينما نسيج الطحال اظهر زياده حويصليه في اللب الابيض مع ارتشاح خلايا عملاقه وخلايا احاده النواه وزيادات منتشره للنسيج اللمفاوي.

INTRODUCTION

Human chorionic gonadotropin is a pregnancy hormone , composed of 237 amino acids with a molecular mass of 36.7 kDa. It is one kind of glycoprotein hormone normally produced in the trophoblast of placenta during pregnancy, and it is important for gonad and reproductive system physiology (1). It is heterodimeric, with an α (alpha) subunit identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and β (beta) subunit that is unique to hCG (2).The α (alpha) subunit is 92 amino acids long. The β-subunit of hCG gonadotropin contains 145 amino acids ,these subunits are joined non covalently. Active synthesis of material with immunological, physicochemical, and *in vitro* and *in vivo* hormonal activities similar to those of human choriogonadotropin (hCG) by several strains of different bacterial species has been demonstrated by (3). Most of these bacteria were isolated from fluids or tissues of patients with clinically manifested cancer. Moreover, the synthesis of the hCG-like material occurs in bacterial species belonging to the indigenous flora of humans (4,5). The limited number of bacteria studied, several expressing the hCG-like antigen are coagulase-negative *staphylococci*. Because of the biological and physiological implications of these findings, a systematic investigation for the presence of the hCG-like material in bacteria was undertaken with the aim of determining, if possible, to what extent bacteria express the hCG-like material are capable of expressing the hCG-like material and those that are not(6). These bacteria often showed characteristics of cell wall-deficient bacteria (7). Since only a few bacterial strains were investigated, it is not known how ubiquitous is the expression of these hormone like substances by prokaryotes. As in the case of the numerous bacteria expressing hCG-like material, the biological role of these peptides in bacteria is unknown (8).

As part of this effort, the results of our investigations of 16 strains of coagulase-negative *Staphylococcus* species isolated from locally patients with clinical manifested cancer.

MATERIALS AND METHODS

This procedure was done according to the method described by Robinson (9) and modified by the researcher

Identification and re-characterization methods :

The identification of 20 selected strains from different *Staphylococcus* genus .16 strains are re characterized as *S. epidermidis* by checking on mannitol agar and coagulase test, and several biochemical tests All of these strains were isolated from blood of the cancer suspected patients were obtained from hospital (leukemia & cancer dept.) who had positive blood cultures for *S .epidermidis* .

Culturing the strains:

S. epidermidis (previously re characterized), 16 strains of coagulase – negative, all strains were incubated in modified media contain lysine -trypticase agar (17.9g), yeast extract (10g), galactose (5g), k2HPO4(2.5g) for five days .

Sugar Fermentation test :

This test was done according to the method described by(10). Sugar stock solution for each sugar (Mannitol, Sucrose, Lactose, Inline, Fructose) was prepared .

Extraction of HCG like material:

After culturing for five days .the growth are scraped from modified media with Tris-HCL buffer (1M) pH (8) (prepared by dissolving 60.5 g of Tris -base in 400ml dist. Water ,then regulating the pH 8 ,by using Hcl solution (1M), and completed the buffer in to 500ml) and autoclaved .Then centrifugation was done with cooling centrifuge for 15 min (8000 rpm),the supernatant is denied & the pellets were washed with dist. water for two-fold .then the pellets were re suspend with EDTA buffer (0.5M) pH (8) (prepared by dissolving 93.6 g of EDTA in 400ml dist. Water ,then regulating the pH 8 ,by using NaOH solution (10M), and completed the buffer in to 500ml) and autoclaved .

A- Sonication:

The bacterial cells in the previous suspension were ruptured by sonication (8min. with 1min. interval)

B-Centrifugation:

The sonication suspension was centrifuged for 20 min. (10,000rpm).the supernatant were collected & the pellet was denied .

C-Partial purification:

The partial purification was done by using Ion –exchange (DEAE cellulose) .After the activation of the resin by using NaOH buffer(0.25M) pH 8(prepared by dissolving 5 g of NaOH in 400ml dist. Water and regulated the pH 8 , then completed the buffer in to 500ml), and HCL buffer (0.25M)(prepared by diluted 10.4 ml of conc. HCL with 400ml dist. Water, then completed the buffer in to 500ml) with washing the resin interval by dist .water .finally washed the resin with Tris-HCL pH (8).

D-Fractions collection :

The fraction were collected according to the main peaks which were generated from ion exchange.

Protein analysis:
The protein concentration was measured according to the Bradford method (11).

Protein standard curve preparation:

Standard curve for protein was prepared by using different concentration of bovine serum albumin(20,40,60,80,100)µg/ml.

Carbohydrates analysis:

The carbohydrates concentration was measured according to the Dubois method (12).

Carbohydrate standard curve preparation:

standard curve for carbohydrate was prepared by using different concentration of glucose (20,40,60,80,100) µg/ml.

Standard hormone :

Formulated hCG hormone IVC-C inj. Manufactured by LG PhD company (Korea) was used as standard hormone which contain 5000IU.

Collected and concentrated the fractions :

The fractions for three peaks were collected and dialyzed by dialysis sac. to concentrated the fractions against sucrose .

High performance liquid chromatography analysis :

The samples and standard of HCG were analyzed by HPLC separation with column Luna 5u C_{18} (250 × 4.6) mm internal diameter. The mobile phase was acetonitrile (ACN) 100% with a flow rate of o.5 ml/min. Injection volume for sample and standard solution was 10 µl. The pH was adjusted to 3.5. The detection occurred at UV light at 254 nm.

Immunization procedure:

immunization schedule was done by immunized the mice according to(Hernan*,et.al*.1985)(13)

Pregnancy test kit:

Spinreact,S.A.-Ctra. Santa Coloma,7
E-17176 SANT ESTEVE DE BAS – (Girona) SPAIN . hCG- LATEX . ISO 9001

RESULTS AND DISCUSSION

The Results of re characterization methods for twenty *Staphylococcus spp*. Showed there were sixteen isolates classified as *S.epidermidis* according to the several biochemical tests as shown in table (1) , and all sixteenth isolates were coagulase negative.

Table(1) Sugar fermentation by *S.epidermidis* isolates

Isolate number	Fructose	Lactose	Mannitol	Inulin	Maltose
1S	+	-	-	+	+
2S	+	+	-	+	+
3S	-	-	-	+	+
4S	+	-	-	+	+
5S	+	+	-	+	+
6S	+	+	-	+	+
7S	+	+	-	+	+
8S	+	+	-	+	+
9S	-	+	-	-	+
10S	+	+	-	+	+
11S	+	+	-	-	-
12S	+	+	-	+	+
13S	+	+	-	+	+
14S	+	+	-	+	+
15S	+	+	-	+	-
16S	+	+	-	+	+

From above results showed, there were several changes occurred in fermentation of some sugars such as 1S, 3S ,and 4S isolates which were not fermented lactose ,3S and 9S showed negative result for fructose ,while 9S and 11S showed negative for inulin ,and 11S , 15S showed negative for maltose , the reason for this variation may be due to some isolates were become mutant so they could not ferment these sugars. (14).

The 3S isolate was chosen as a sample because of the variation that occurred in its ability of fermentation .The protein and carbohydrate concentration were measured by the Bradford method with Bovine serum albumin as a reference and by a phenol –sulfuric acid method with glucose as a reference respectively. The results of the crude extraction the method showed the concentration of protein was 300 mg/ml, while the concentration of carbohydrates was 96 mg/ml . That mean ,the extract was mainly composed of protein and contained a significant amounts of carbohydrate substances . The purification procedure was done by ion exchange (DEAE) to increase the purity of hCG- like material in the solution . The result of the fractions showed there were three main peaks (8-17) ,(18-32),(33-43) respectively (Figure 1) , every peak was collected , dialyzed against distilled water and Bradford method were detected the protein materials concentration in samples which were (32 , 50 ,33.4) mg/ml respectively .

While the carbohydrates concentration were measured by Dubios the results showed the separation of three main peaks (Figure 1), and detected the carbohydrates materials concentration in samples which were (12 , 42 ,25) mg/ml respectively .

The first peak (8-17) fractions was chosen as a result of the compatibility between protein and carbohydrate contants (figure 1) , because the top of the first peaks for both of them were occurred at fraction eleven .

The second peak is tested also to find any substance is related with the hCG like hormone. The HPLC was used to measure the approximate amount of hCG like hormone hence because this instrument is not sensitive for the amount less than (0.2 mg/ml) (6), and in the same time to measure the degree of the hCG like hormone purity , the results showed the three main peaks were separated with the retention time (1.868 , 2.642 , 2.874) respectively for the first peak fractions (figure 2), , and when compared with the retention time for the main peak of the standard formulated hCG hormone which occur at 2.836 (figure 3), and when compare with the second peak (figure 4).

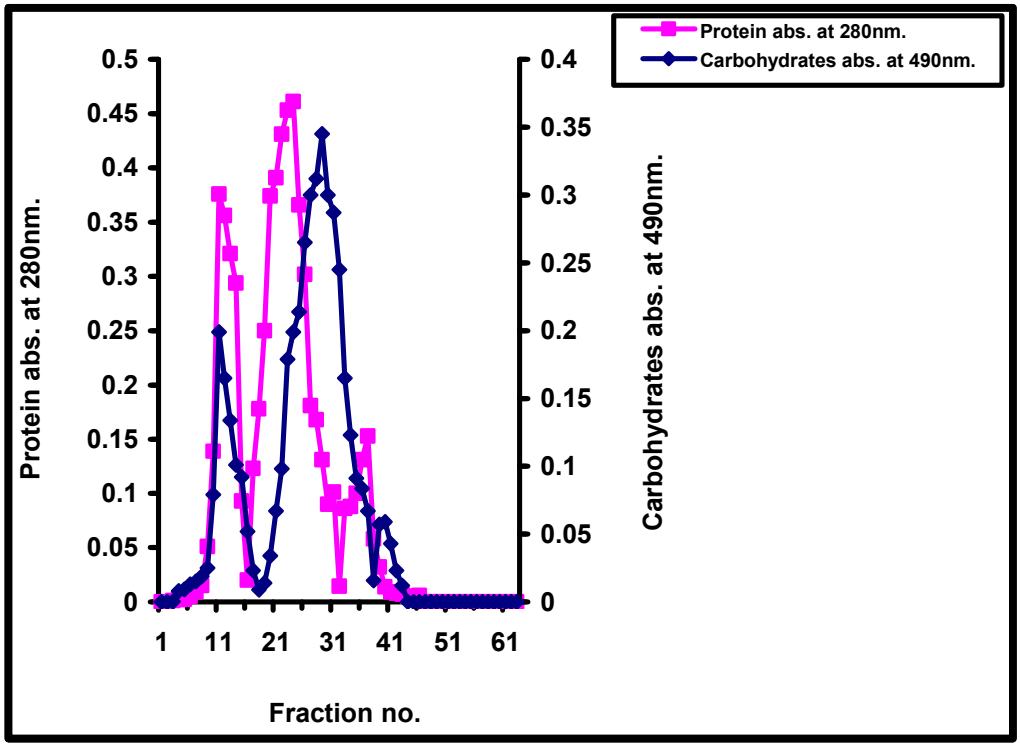


Figure (1): Ion exchange diagram for protein fractions by usage column (DEAE) in dimension 50 × 2.5 c m .washed with Tris-HCL pH (8.4) buffer in flow rate 3 ml / fraction with flow rate speed 36 ml/hr.

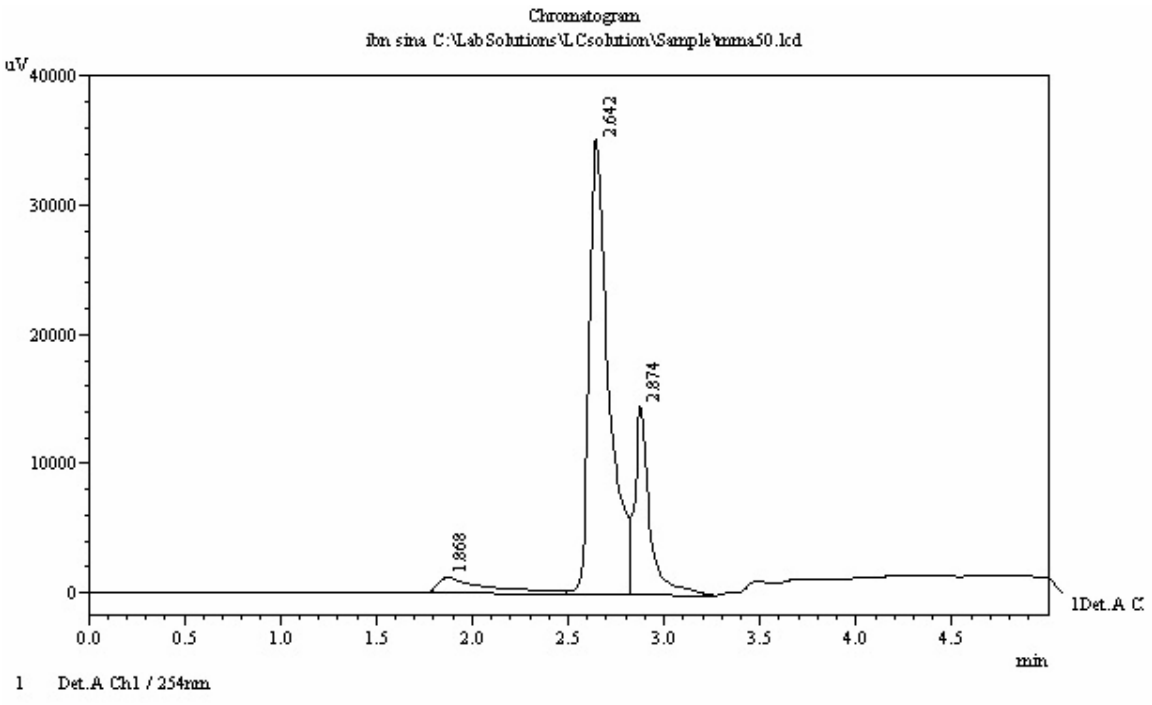


Figure (2): HPLC analysis for first peak

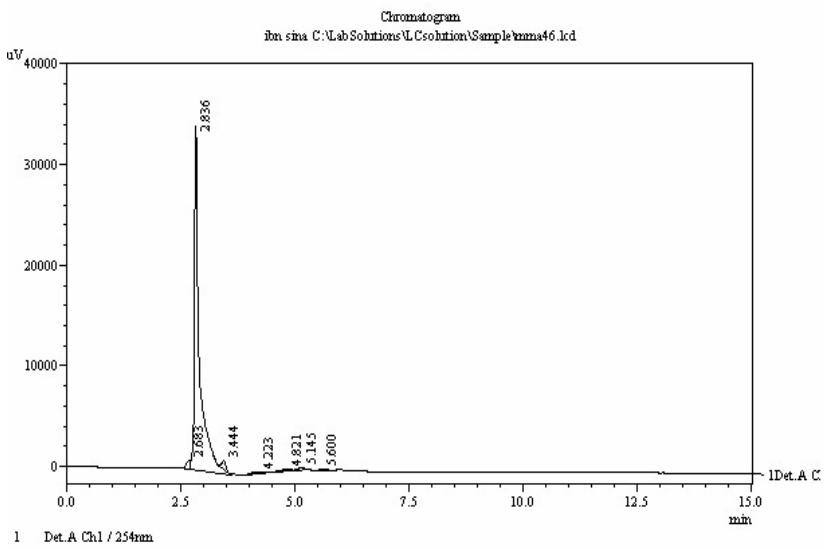
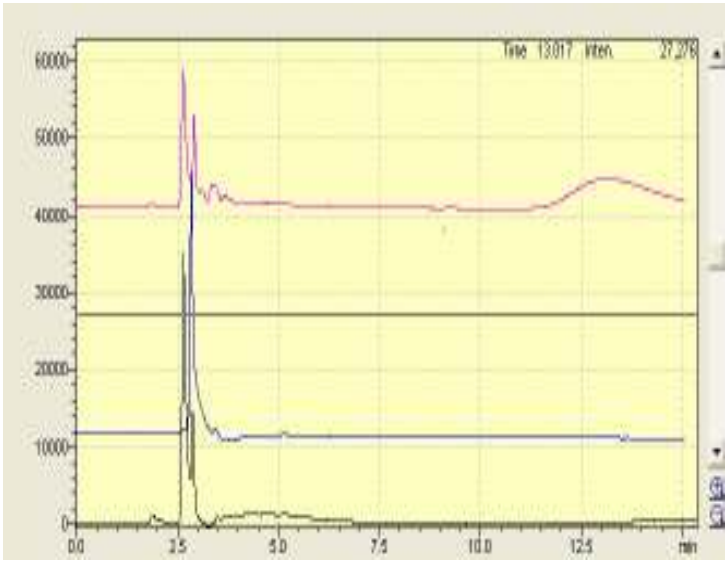


Figure (3): HPLC stander curve for formulated hCG hormone



1-Second peak (red)
2- Standard (blue)
3- First peak (black)

Figure (4): HPLC analysis for second, standard ,first peak

so the third peak is represented the hCG like hormone (figure 4) because of closely related with that(main peak) of standard curve as shown in(figure 4) .

This result showed there were several impurities in the sample, and when more purification was done for the tested sample (first peak) by the same previously column the fractions (7-16) were collected and concentrated, the concentration of protein for the first peak was (87.6 mg/ml) (figure 5).

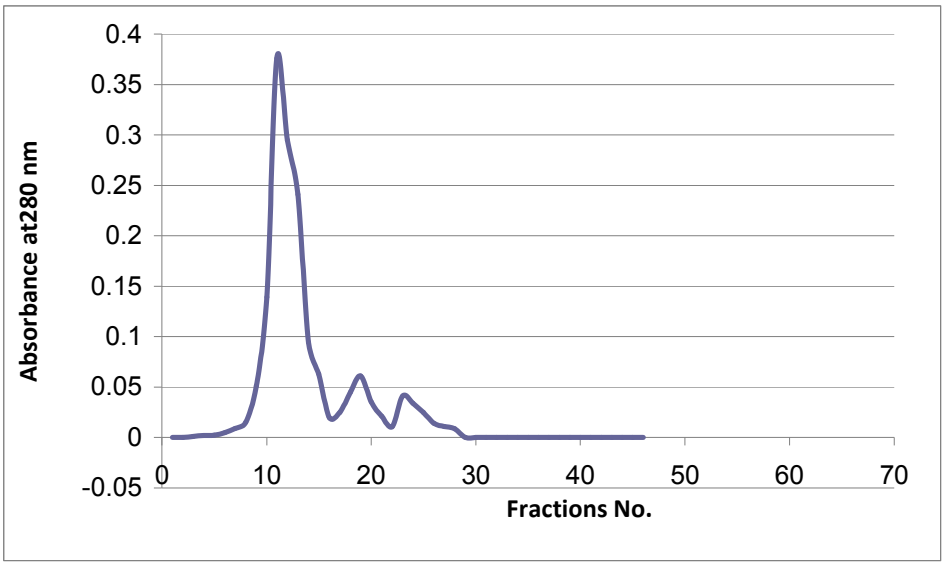
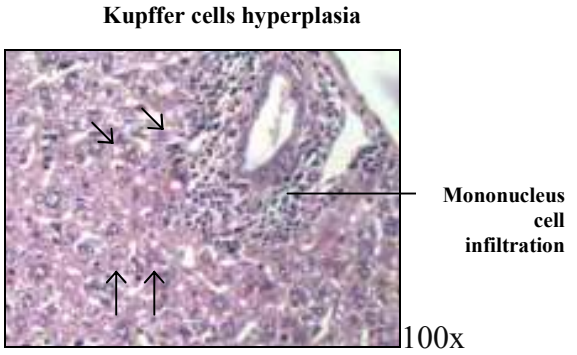
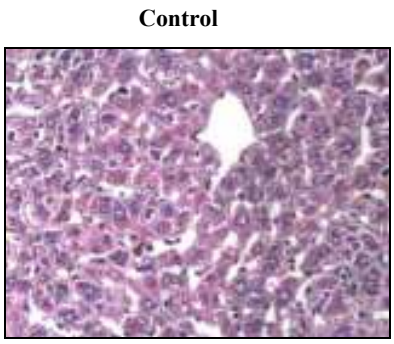


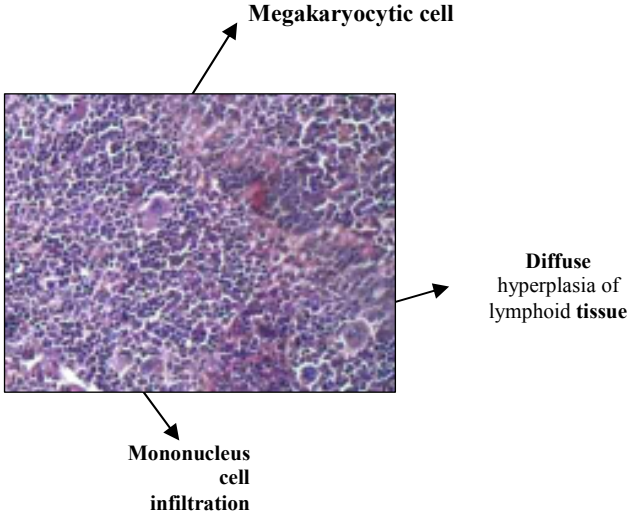
Figure (5): Ion exchange diagram for protein fractions by usage column (DEAE) in dimension 50 × 2.5 c m .washed with Tris-HCL pH (8.4) buffer in flow rate 3 ml / fraction with flow rate speed 36 ml/hr.

The conclusion of HPLC results showed , there is hCG like hormone in the cellular membrane . of *S. epidermidis* bacteria .And when tested the sample (after concentration) with pregnancy test kit ,it gave a positive result for the sample (12), that mean hCG like material extracted from *Staph. epedermidis* like hCG extracted from human.

On the other hand, The fractions (7-16) (figure 6) after concentration ,it was used as immune stimulator for lab. mice .The immunological study showed , there were crossly internal organs enlargement(Liver, Spleen) in the immunized mice after sixteen days from first injection, that mean , hCG like material has activity on these organs. The whole Liver shows normal structure appearance with kupffer cell hyperplasia with mono nucleus infiltration . While spleen tissue showed follicular hyperplasia of the white pulp ,with megakaryocytic cells infiltrate , focal infiltration of mono nucleus in the spleen tissue and diffuse hyperplasia of lymphoid tissue.







From this study we can concluded ,the extraction method was efficient in extraction of hCG like material from *S. epidermidis* , and there was a significant result in the yield of hCG like material when compared with global research . The *invivo* study showed, the first peak fractions have positive immunologically effects in animals,.

Acknowledgements:

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Fast Disintegrating Tablets of Meloxicam : Preparation Characterization and Evaluation

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ABSTRACT

Tablets containing 15 mg of meloxicam were prepared using direct compression method. Meloxicam with other excipient (mannitol, talc, Mg stearate, mint, and aspartame) and superdisintegrant mixed together and compressed into tablets. Two Different percentages (5.4% and 8%) of three different superdisintegrants (croscarmellose sodium, crospovidone and sodium starch glycolate) were used to evaluate their effect on the properties of the prepared tablets. The prepared tablets were characterized by weight variation, friability, hardness, wetting time, disintegration time and content uniformity. In vitro dissolution was measured for the prepared formulae using USP dissolution test apparatus. Formula containing 8% crospovidone was chosen as the best formula and evaluated for long term stability studies. The chosen formula left on the shelf at room temperature for 12 months and evaluated for friability, hardness, wetting time, disintegration time and content uniformity. In vitro dissolution test shows no significant difference and suggest that the formula is stable at the shelf.

الملخص باللغة العربية

تم تحضير حبوب تحتوي على 15 ملغم من الميلوكسيكام بأستخدام طريقة الكبس المباشر للحبوب. الميلوكسيكام تم مزجه مع المواد الاخرى في التركيبه وهي مانيتول , تالك , مغنيسيوم ستياريت , نكهة النعناع , اسبارتام و مفتت الحبوب سريع المفعول وكبست بشكل حبه. تم استخدام مفتت الحبوب سريع المفعول بنسب مختلفه وهي 5.4% و 8% وكذلك تم استخدام ثلاثة انواع مختلفه من مفتت الحبوب سريع المفعول وهي كروسكارميلوز صوديوم , كروسبوفيدون و صوديوم ستارج كلايكوليت لدراسة تأثير كل منهم على خواص الحبوب المحضره. تم دراسة الخواص التاليه للحبوب المحضره وهي اختلاف الوزن , نسبة التفتت , الصلابه , وقت امتصاص الحبه للماء , وقت تفتت الحبه و تجانس كمية الدواء في كل حبه . كذلك تمت دراسة وقت ذوبانية وامتصاص الحبوب المحضره لكل تركيبه . تم اختيار التركيبه التي تحتوي على 8% كروسبوفيدون كأحسن تركيبه لتحضير هذا النوع من الحبوب وتم اختيارها لدراسة مدى ثبوتية الحبه خلال فترة الخزن الطويله. تم وضع الحبوب المحضره على الرف لمدة 12 شهر وبعدها تم تقييم الحبوب لغرض معرفة تأثير وقت الخزن عليها. تم دراسة نسبة التفتت , الصلابه , وقت امتصاص الحبه للماء , وقت تفتت الحبه و تجانس كمية الدواء في كل حبه للحبوب المحضره. دراسة وقت ذوبانية وامتصاص الحبوب المحضره بعد الخزن لم تظهر اي اختلاف ملحوظ في النتائج مقارنة بالحبه الاصليه مما يدل على ثبوتية الحبه خلال الخزن.

INTRODUCTION

Efforts made over the years for the development of new delivery systems or improve the existent ones (1-4). Oral route of administration is the most popular rout because of the ease of administration and free of pain and solid oral dosage forms are the most popular ones. One of the disadvantages of tablets is the difficulty in swallowing in some patients such as children, elderly and psychiatric patients (5,6).

Orally disintegrating tablets ODTs are a new generation of tablets that allow the swallowing of the tablet as liquid dosage form without the use of water in less than one minute. It disintegrates and dissolves rapidly once placed on the tongue. ODTs offer several advantages over traditional solid dosage forms and improve patient compliance. ODTs offer tablets with rapid onset and higher bioavailability because some of the drug will be absorbed in the oral cavity and avoid first pass metabolism (7,8). Several techniques used for the preparation of ODTs such as direct compression, spray drying, sublimation, freeze drying, lyophilization and solid dispersion method (9-12). One of the advantages of ODTs is rapid relief of pain because of its rapid onset and for that reason several attempts for the preparation of meloxicam as ODT were observed (13).

Meloxicam is a non-steroidal anti-inflammatory drug used to relief paine especially for patients with rheumatoid arthritis, osteoarthritis, fever and dysmenorrheal. Meloxicam inhibits cyclooxygenase synthesis which is responsible for converting arachidonic acid into prostaglandin H2 (14).

The aim of our work is to prepare orally disintegrating tablets of meloxicam by direct compression using different super-disintegrants which are croscarmellose sodium, crospovidone and sodium starch glycolate and investigate the effect of the concentration of each type of super-disintegrants on the properties of the prepared tablets.

MATERIALS AND METHODS

Materials

Meloxicam, croscarmellose sodium, crospovidone, sodium starch glycolate were a gift from the college of pharmacy /Baghdad University. Mannitol, mint, aspartame, talc, Magnesium stearate purchased from commercial sources.

Methods

Preparation of orally disintegrating tablets of meloxicam by direct compression:-

The active ingredient (15 mg) and other excipients (croscarmellose sodium, crospovidone, sodium starch glycolate, mannitol, mint, aspartame and talc) were accurately weighed using electronic balance and thoroughly mixed together according to the formulations in table (1) The tablets compressed using TDP-single punch tablet machine (shanghai tainHe pharmaceutical machinery Co.itd) by direct compression method. All the prepared tablets were stored in airtight containers at room temperature.

Evaluation of the prepared tablets

All the prepared tablets were evaluated for weight variation, friability, hardness, wetting time, disintegration time and content uniformity according to the official methods (15,16) and the results shown in table (2)

Weight variation

The mean weight of 10 tablets was calculated and the deviation from $\pm$ 7.5% according to USP was calculated.

Friability and hardness test

The tablets weighed and placed in DBK Friability test apparatus (LABLIN, India). The device was set at 25 rpm for four minutes. The tablet then removed from the fribilator, cleaned and weighed again. The percentage of friability calculated according to the following equation

$$\text{Friability \%} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}} \times 100$$

The hardness of the prepared tablets was measured using Monsanto hardness tester.

Wetting time and Disintegration time

For the calculation of wetting time a piece of tissue paper 10cm in diameter folded four times and placed in Petri dish containing 5 ml of distilled water. A tablet placed in the middle of Petri dish and observed. The time calculated for complete wetting of the tablet. The disintegration time for each tablet was determined using USP disintegration apparatus

using phosphate buffer PH 6.8, 900 ml at 37^0 C as the disintegrating medium.

Drug content

Ten tablets were weighed and powdered. An amount of the powder equivalent to one tablet (15 mg of meloxicam) was weighed and dissolved in 800 ml of phosphate buffer PH 7.4 and samples analyzed using UV for their drug content.

Dissolution time

In vitro dissolution studies of selected formulas was performed using USP type II paddle apparatus operated at 50 rpm (900 ml phosphate buffer PH 6.8)at $37^0C \pm 0.5^0C$. Samples withdrawn periodically and analyzed for drug content using UV-165-OPC spectrophotometer (Shimadzu, Japan) at 360 nm and cumulative percentage of drug release was calculated.

Long term stability studies

The best formula that meets our goals was chosen for long term stability studies (F5). The prepared tablet placed in an amber container with a dissicator and left on the shelf at room temperature for 12 moths. After that the tablet evaluated for their wetting time, disintegration time, drug content and dissolution time.

Table (1): Composition of different formulae for the preparation of orally disintegrating tablets.

Ingredients of the formula in mg	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Meloxicam	15	15	15	15	15	15
Mannitol	150	150	150	1	150	150
Croscarmellose sod.	10	__	__	15	__	__
Crospovidone	__	10	__	__	15	__
Sod. starch glycolate	__	__	10	__	__	15
Aspartam	5	5	5	5	5	5
Talc	2	2	2	2	2	2
Mint	0.1	0.1	0.1	0.1	0.1	0.1
Mg stearate	0.1	0.1	0.1	0.1	0.1	0.1

RESULTS AND DISCUSSION

The present work used to formulate and evaluate an oral disintegrating tablet of meloxicam using direct compression method. Different tablet formulae were prepared as shown in table 1. Different superdisintegrants (croscarmellose sodium, crospovidone, sodium starch glycolate) used in different concentrations (5.4% for formula F1 to F3 and 8% for formulae F4 to F6) to show their effect on tablet parameters. All the prepared formulae were evaluated for weight variation, friability, hardness, wetting time, disintegration time and content uniformity and the results shown in table 2. All the prepared formulae passed the weight variation test according to USP since none of the prepared tablets exceed $\pm$ 7.5%.

Friability test was performed for all the prepared formulae. The friability % of all the prepared formulae was below 1% which indicates good mechanical strength. The hardness of all the prepared formulae was between (2.5-4 kg/cm^2). Formulae F1 to F3 shows low hardness compared to Formulae F4 to F6. The wetting time for all the prepared formulae was between 20 to 6o seconds which is in the acceptable limits of ODTs (15). The disintegration time of the prepared formulae was between 18 to 27 seconds which indicates that the prepared tablets have the ideal characteristics of ODTs. The Formula containing 8% of crospovidone shows the lowest disintegration time (18 sec.) compared to croscarmellose sodium F4 and sodium starch glycolate F6 which is consistent with other results for crospovidone preparations (17). The drug content of all the prepared formulae was between 94% to 101% .Which is within the acceptable limits of USP.

Dissolution of all the prepared formulae was performed and the results shown in figure 1. The best dissolution rate observed with F5 (8% of crospovidone). The best formula that meets our goals was F5 which has the best wetting time and disintegration time. F5 shows good mechanical strength compared to other formulae.

Long term stability studies were conducted for F5 and the results shown in table 3 and figure 3. No significant difference observed on wetting time, disintegration time and in-vitro dissolution time. The drug content was 92%$\pm$ 0.31 which is acceptable according to USP.

CONCLUSION

Different formulae for orally disintegrating tablets of meloxicam were prepared. The formula with the best properties was F5 (8% of crospovidone). F5 shows sufficient mechanical strength, wetting time and disintegration time. Dissolution test revealed that 78% of the drug released in 10 minutes and complete release achieved in 50 minutes. Stability studies revealed no significant difference in disintegration time, wetting time, drug content and dissolution test. These formulae provide a promising way to prepare fast disintegrating tablets by simple direct compression method for meloxicam.

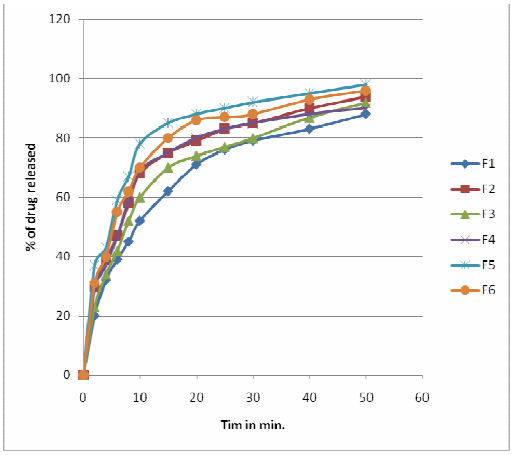

Figure (1): In vitro dissolution profile of the prepared formulae

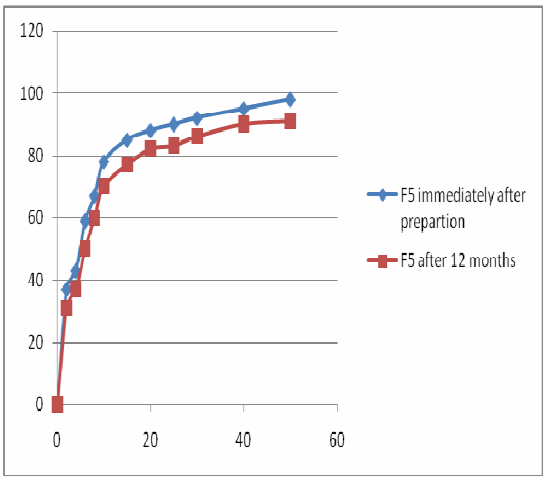

Figure (2):Copmarision of in-vitro release of F5 immediately after preparation and after 12 months

Table (2): Different parameters of the prepared tablets of Meloxicam.
(The results is ±SD, n=4 except for weight variation and content uniformity)

Formula no.	Weight variation	Friability %	Hardness (Kg/cm2)	Wetting time sec.	Disintegrating time sec.	Content uniformity %
F1	Pass	0.72±0.23	2.5±0.32	30±0.41	25±0.28	100.5%±0.23
F2	Pass	0.61±0.24	2.5±0.26	20±0.22	18±0.27	101%±0.27
F3	Pass	0.53±0.32	3.5±0.14	60±0.28	27±0.2	98%±0.21
F4	Pass	0.51±0.3	3.3±0.21	46±0.3	23±0.24	99%±0.26
F5	Pass	0.48±0.19	4±0.2	50±0.21	20±0.18	96%±0.19
F6	Pass	0.56±0.21	3.7±0.23	40±0.27	22±0.2	94%±0.24

Table (3): Different parameters of the prepared tablets of Meloxicam after long term stability studies ±SD, n=4.

Formula no.	Wetting time sec.	Disintegrating time sec.	Content uniformity %
F1	60±0.21	25±0.25	92%±0.31

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قسم الدراسات العربية

ARABIC SECTION

استحثاث الكالس من البذور الناضجة لمحصول الباذنجان *Solanum melongena* L خارج الجسم الحي

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الملخص باللغة العربية

استخدم الوسطان الغذائيان MS و SH مضاف اليهما تراكيز وتوليفات مختلفة من الاوكسين نفثالين حامض الخليك NAA والسايتوكاينين بنزيل امينوبيورين BAP لاستحثاث الكالس من بذور الباذنجان الناضجة للهجين ريما، كما اختبر تاثير تركيز السكروز ايضا في استحثاث الكالس و زيادة معدل وزنه الطري والجاف اذ اضيف الى الوسط الغذائي SH بثلاث تراكيز 30 ، 40 ، 50 غم/لتر. اختلفت النتائج باختلاف الوسط المستخدم وتوليفة منظمات النمو النباتية المضافة اليه، فقد تفوق الوسط الغذائي SH على الوسط الغذائي MS معنويا في استحثاث الكالس وزيادة وزنه الطري والجاف اذ بلغ معدل الوزن الطري والجاف للكالس المستحث على الوسط SH 0.413 و 0.033 غم على الترتيب، في حين كان اعلى معدل وزن طري وجاف للتوليفة 1 ملغ/لتر BAP، 1 ملغ/لتر NAA مع الوسط الغذائي SH اذ تفوقت على جميع التوليفات الاخرى في كلا الوسطين وبشكل معنوي اذ بلغ 0.887 و0.069 غم على الترتيب. اما افضل تركيز للسكروز في استحثاث الكالس ووزنه الطري والجاف فكان للتركيز 40 غم/ لتر اذ تفوق وبشكل غير معنوي على التركيز 50 غم/ لتر وبشكل معنوي على التركيز 30 غم/لتر فقد بلغ معدل الوزن الطري والجاف للكالس المستحث 0.700 غم ، 0.051 غم على الترتيب.

الكلمات المفتاحية: *زراعة انسجة نباتية، بنزيل امينو بيورين، نفثالين حامض الخليك، سكروز، مركب الحديد.*

ABSTRACT

Different concentration and combination of NAA and BAP were used in MS and SH media to induce callus from seeds of hybrid Reema of eggplant. Three concentration of sucrose (30, 40, 50) g/l were added to SH medium to test for callus induction and its increase in fresh and dry weight. Callus induction and weight increase varied in different media and combination of NAA and BAP.The rate of fresh and dry weight of callus induced on SH medium (0.413 and 0.033) g respectively. The highest fresh and dry weight of callus obtained was recorded 0.887 and 0.069 g respectively was induce on SH medium containing 1 mg/l NAA and 1 mg/l BAP which was significantly higher than all other combinations. The highest fresh and dry weight of callus produced on SH medium containing 40 g/l sucrose was recorded (0.700 and 0.051) g respectively and was significantly higher than the 30 g/l and not significantly with 50 g/l.

المقدمـــة

الباذنجان *Solanum melongena* L (2n=24) محصول ذو اهمية غذائية واقتصادية باعتباره احد محاصيل الخضر الصيفية من العائلة الباذنجانية. ينمو غالبا في المناطق الاستوائية وشبه الاستوائية من العالم (1-4). يستهلك الباذنجان طازج (غير مطبوخ) او مطبوخا او محشوا او يستعمل في المخللات الخ (5)، كما لا يخلو من الفوائد الطبية اذ يستعمل علاجا لداء السكري والتهاب القصبات والربو والام الجهاز البولي ويستعمل ايضا لخفض نسبة الكولسترول في الدم وللتخلص من السمنة ، وهو مصدر جيد للفيتامينات والمعادن وله محتوى مرتفع من البوتاسيوم الا انه منخفض السعرات الحرارية(6،7) كما تساعد صبغة الانثوسيانين في قشور الباذنجان على التخلص من الجذور الحرة باعتبارها مضاد اكسدة(8) وكذلك حماية الفئران ضد التطفير mutagenesis الناشيء عن cyclophosphamide في الكائن الحي *in vivo* (9).

يعتمد استحثاث الكالس في الباذنجان على عمر البادرة والمرحلة التطورية لها وكذلك على الجزء النباتي الماخوذ منها مثل الاوراق الفلقية والسويقة الجنينية السفلى والعرق الوسطي للورقة midrib والساق والورقة والجذر (3،4،10). تستخدم انواع وتراكيز مختلفة من منظمات النمو النباتية لغرض استحثاث الكالس اذ جهز وسط MS بـــ 2 ملغ/لتر BAP و 0.5 ملغ/ لتر NAA، لاستخدام الساق والورقة والجذر كاجزاء نباتية (10)، في حين جهز باحث اخر وسط MS بـــ 2 ملغ/ لتر NAA و 0.5 ملغ/ لتر BAP لزراعة الاوراق الفلقية والسويقة الجنينية السفلى (4).

يعد نسيج الكالس مصدرا لدراسة انواع الخلايا وعزل البروتوبلاست والانتخاب الخلوي وتكوين الاجنة الجسمية والاعضاء وانتاج مركبات الايض الثانوي(11)، وكذلك انتاج نباتات خالية من الامراض (10)، كما ويعد اخلاف نبيتات عن طريق الكالس احد الخطوات المهمة لاي نوع من انواع التحول الوراثي (12).

يهدف البحث الى اختزال مرحلة انبات البذور والحصول على البادرة باعتبارها مصدر الاجزاء النباتية لاستحثاث الكالس، واستخدام البذور الناضجة للباذنجان مباشرة للحصول على نوعية كالس جيدة وذلك بتحوير وسط SH والتوصل الى التركيز والتوليفة الافضل من منظمي النمو NAA و BAP لهذا الغرض.

المواد وطرائق العمل

المادة النباتية: استخدم في هذا البحث بذور الباذنجان الهجين ريما هولندي المنشأ ويصلح للزراعة المحمية الشتوية فقط، المجموع الخضري جيد جداً، شكل الثمرة بيضوي متطاول ذات لون اسود وتتلون باللون الارجواني عند ارتفاع درجة الحرارة، متوسط التحمل للامراض الفطرية وغير مقاوم للفايروسات، النبات متوسط الانتاج ووزن الثمرة 300 غم ويصلح للزراعة في البيوت المحمية العالية، مصدر بذوره شركة .S.G (Sluis Growth).

تحضير الاوساط الغذائية وتعقيمها:

استخدم الوسطان الغذائيان MS (13) بجميع مكوناته والوسط SH (14) بجميع مكوناته ايضا باستثناء المايو اينوستول اضيف بتركيز 100 ملغ/ لتر بدل 1000 ملغ/ لتر، كذلك جرب تركيز 100 مايكرومولر من مركب الحديد بدل تركيز 54 مايكرومولر مع الوسط الغذائي SH، كما اضيف الى الوسطين نفثالين حامض الخليك NAA بتراكيز0.0، 0.1، 0.5، 1.0 ملغ/ لتر بمفرده او مؤلفا مع BAP كما في الجدول 1، وكذلك اضيف 30 غم/ لترسكروز للوسط MS و30 ، 40 ، 50 غم/ لتر الى الوسط SH و8 غم/ لتر اكار لكلا الوسطين، وعدل الرقم الهيدروجيني الى 5.8+0.1 قبل التعقيم بجهاز الموصدة (autoclave) على درجة حرارة 121 درجة مئوية و ضغط 1.04 كغم/سم^2 ولمدة 20 دقيقة ووزع في انابيب الزراعة (80 X 25) ملم بواقع 5 مل مرة، ودوارق مخروطية 50 مل بواقع 15 مل مرة اخرى وبعشر مكررات لكل معاملة، وتركت لتبرد لحين الاستخدام. جدول (1).

الجدول (1): توليفات منظمات النمو المضافة الى الوسطين SH و MS لاستحثاث الكالس من بذور الباذنجان الناضجة

الاوكسين NAA ملغ/لتر	السايتوكاينين BAP ملغ/ لتر			
0.0	0.0	0.1	0.5	1.0
0.1	0.0	0.1	0.5	1.0
0.5	0.0	0.1	0.5	1.0
1.0	0.0	0.1	0.5	1.0

تعقيم البذور وزراعتها: غسلت البذور بماء الحنفية الجاري لمدة 30 دقيقة ثم غمرت في محلول التعقيم 50% القاصـــر التجاري الحاوي 5-6% هايبوكلوات الـــصوديوم واضـــيف اليه قطرتين من مادة tween 20 ولمدة 15 دقيقة ثم غسلت بالماء المقطر المعقم ثلاث مرات للتخلص من بقايا محلـــول التعقيم لمدة 5 دقائق لكل مرة، ثم زرعت في الانابيب بواقع بذرة لكل انبوبة وبذرتين لكل دورق فـــي كابينـــة الهـــواء الطبقي، ثم حضنت الزروعات في غرفة النمو في الظـــلام بدرجة حرارة 25+2 مْ يوميا لمدة 4 اسابيع ثم سجل الوزن الطري والجاف للكالس المستحث ولونه ونوعيته.

التحليل الاحصائي: استخدام التـــصميم العـــشوائي الكامـــل Completely Randomized Design (CRD) لتحليـــل البيانات وتم مقارنة المعدلات حسب اختبار اقل فرق معنوي (LSD) على مستوى احتمال 0.05.

النتائج والمناقشة

1.تاثير الوسط الغذائي في النسبة المئوية للانبات واستحثاث الكالس:

اظهرت النتائج ان لنوع الوسط الغذائي تاثير في النسبة المئوية للانبات اذ بلغت 34.38% عند زراعة البذور على الوسط SH بعد 5 ايام من الزراعة في حين بلغت 52.5 % عند زراعة البذور على الوسط MS كما في الجدول 2، وارتفعت بعد 15 يوما من الزراعة وبنسبة 97.51 % في الوسط SH في حين كانت الزيادة في الوسط MS بنسبة 92.5 % وبنفس المدة وعند قضاء مدة 30 يوم من عمر المزرعتين بلغت النسبة المئوية الكلية 98.15% في الوسط SH في حين كانت النسبة المئوية الكلية للانبات في الوسط MS 96.25% الجدول (2) وبفرق بسيط وغير معنوي وهو ربما يعود الى النسبة المئوية لحيوية البذور المزروعة. اما بشأن النسبة المئوية لاستحثاث الكالس بعد 30 يوما فبلغت 100% في حالة الوسط SH في حين انخفضت في حالة الوسط MS الى 94.17% الجدول (2) وهو فرق غير معنوي ايضا ويعود الى الاختلاف في تراكيب الوسطين الغذائيين.

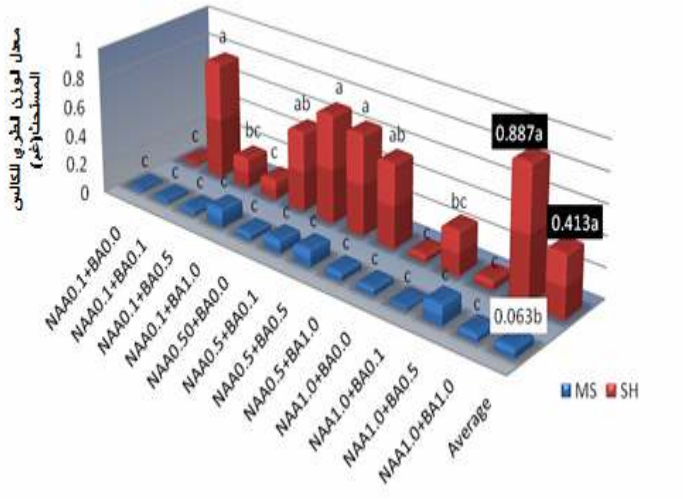
جدول (2): النسبة المئوية لانبات البذور واستحثاث الكالس في الوسطين SH و MS

الوسط الغذائي	% للانبات		% لاستحثاث الكالس
SH	بعد 5 ايام من الزراعة	34.38	100%
	بعد 15 يوم من الزراعة	63.12	
	بعد 30 يوم من الزراعة	0.63	
	% الكلية	98.13	
MS	بعد 5 ايام من الزراعة	52.50	94.17%
	بعد 15 يوم من الزراعة	40.00	
	بعد 30 يوم من الزراعة	3.75	
	% الكلية	96.25	

2. تأثير الوسط الغذائي ومنظمات النمو في الوزن الطري والجاف للكالس المستحث:

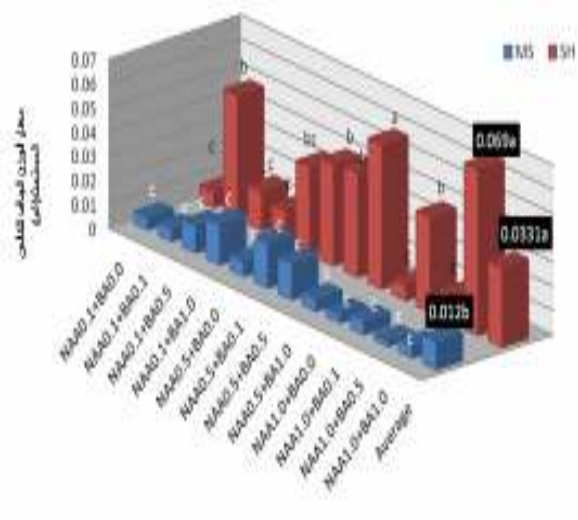
اظهرت النتائج تفوق الوسط الغذائي SH على الوسط الغذائي MS معنويا في استحثاث الكالس اذ بلغ معدل الوزن الطري والجاف الكلي للكالس المستحث في الوسط SH 0.413 غم ،0.033 غم على الترتيب في حين انخفض في الوسط الغذائي MS اذ بلغ 0.063 غم ، 0.012 غم على الترتيب كما في الشكلين 1 و2. اما بشأن تأثير منظمات النمو فلم يستحث الكالس في حالة اضافة السايتوكاينين BAP الى الوسط الغذائي بمفرده في كلا الوسطين وتحفز تكوين البراعم العرضية في الوسط MS في التركيز 1 ملغ/ لتر في حين لم يتحفز تكوين البراعم العرضية في الوسط الغذائي SH لذلك استبعد من التحليل الاحصائي لاستحثاث الكالس.

اما فيما يتعلق بتوليفات منظمي النمو NAA و BAP فقد تفوقت التوليفات المتساوية التراكيز من كلا NAA و BAP (0.1 + 0.1، 0.5 + 0.5، 1.0 + 1.0) ملغ/ لتر معنويا في معدل الوزن الطري والجاف مقارنة بالتوليفات التي ارتفع فيها تركيز BAP على NAA ولم تختلف عن بعضها معنويا اذ بلغ اعلى معدل وزن طري وجاف للكالس المستحث في التوليفة NAA و BAP 1 ملغ/ لتر لكل منهما 0.887، 0.069 غم على الترتيب في حالة الوسط الغذائي SH. في حين تفوق التركيز المرتفع من NAA على BAP فقط في التوليفة 1+ 0.5 ملغ/ لتر على الترتيب في حالة الوسط الغذائي MS الشكلين (1، 2).



الشكل (1): تأثير الوسطين SH و MS وتوليفات BAP+NAA في معدل الوزن الطري للكالس المستحث (غم).

- *المعدلات التي تحمل احرف متشابهة لا تختلف عن بعضها معنويا حسب اختبار LSD وعلى مستوى احتمال 0.05.*



الشكل (2): تأثير الوسطين SH و MS وتوليفات BAP+NAA في معدل الوزن الجاف للكالس المستحث (غم).

- *المعدلات التي تحمل احرف متشابهة لا تختلف عن بعضها معنويا حسب اختبار LSD وعلى مستوى احتمال 0.05.*

ان التفوق الحاصل في كمية الكالس المستحث عند استخدام التوليفات الحاوية تراكيز متساوية من **BAP و NAA** وفي كلا الوسطين في البحث الحالي والذي اختلف فيه عن نتائج دراسات اخرى سابقة (4، 10، 15، 16) والتي ازدادت فيها كمية الكالس الستحث عند استخدام التوليفات الحاوية تراكيز مرتفعة من الاوكسين NAA على مايبدو يعود الى التركيب الوراثي للهجين ريما المستخدم في البحث الحالي اذ حصل بعض الباحثين على نفس النتيجة باستخدامه الوسط MS مضافا اليه 2 ملغ/ لتر BAP و0.5 ملغ/ لتر NAA (3).

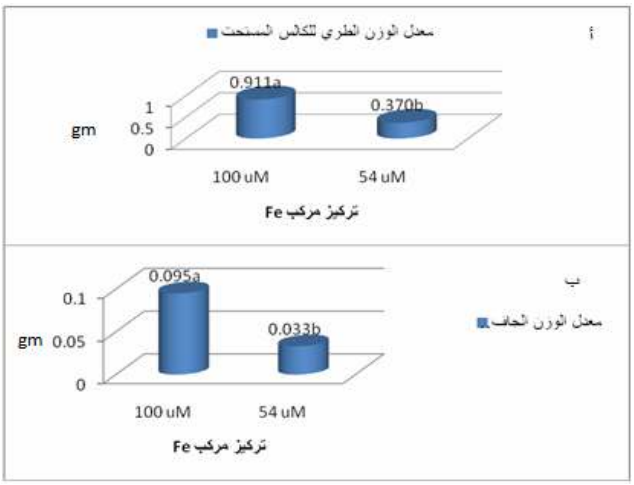
يتفوق الوسط الغذائي SH على الوسط MS في تركيز بعض المكونات الغذائية مثل KI وبعض العناصر النادرة مثل Cu, Mn, B, Mo والتي تعد محفزة للنمو (14). كما ويتفوق في تركيز فيتامين Thiamine- HCl، فيتامين Nicotinic acid وهي ايضا محفزة للنمو لكن مايو اينوسيتول محفز ضعيف للنمو (14)، لذلك خفض الى التركيز 100 ملغ/ لتر. كذلك تعتمد استجابة الانسجة النباتية في اوساط مزارع الانسجة على التفاعل بين مكونات الاوساط الغذائية المختلفة ونوع منظمات النمو وتراكيزها وتوليفاتها في الوسط الغذائي، وتتطلب وجود املاح مختلفة في الوسط الغذائي في مراحل مختلفة من عمر المزرعة النسيجية وهذه المتطلبات من الاملاح تختلف من نوع نباتي الى اخر (4، 15، 17).

3. تاثير الوسط الغذائي ومنظمات النمو في نوعية الكالس المستحث:

اظهرت النتائج ان الكالس المستحث على الوسط MS قوي متماسك النسجة وبلون اصفر باهت مائل الى البني او ابيض مائل الى الكريمي في حين كان الكالس المستحث على الوسط SH قوي متماسك النسجة في الغالب وهش سهل التفتت friable في بعض التوليفات وخصوصا (0.1 + 0.1، 1.0 + 0.1، 1.0 +0.5) من NAA و BAP على الترتيب وفيما يتعلق بلون الكالس المستحث فقد كان لونه كريمي، او اصفر باهت او اصفر مائل الى الاخضر.
ان نوعية الكالس المستحث من ناحية قوة تماسكه او هشاشته تعود الى تركيبة الوسط النامية فيه البذور المستحث منها الكالس اولا وكذلك الى ارتفاع تركيز الاوكسين NAA على تركيز السايتوكاينين BAP وهذه النتائج مؤكدة بدراسات اخرى سابقة (10، 14، 17، 18).

4. تاثير تركيز الحديد في الوزن الطري والجاف للكالس المستحث:

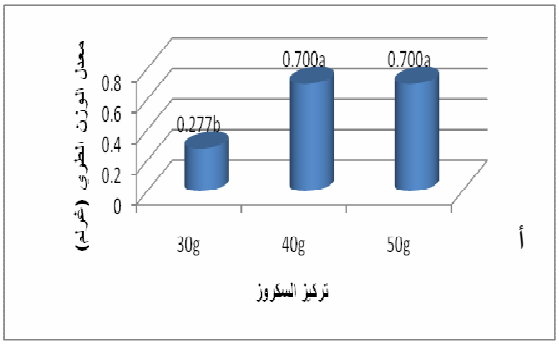
بينت النتائج ان لزيادة تركيز الحديد في الوسط الغذائي SH باستخدام مركب الحديد للوسط الغذائي MS ، 100 مايكرومولر تاثير في زيادة معنوية في معدل الوزن الطري والجاف للكالس المستحث على الوسط نفسه وبتركيز مركب الحديد الاصلي 54 مايكرومولر اذ بلغ 0.911 غم وبلون كريمي مخضر مقارنة مع 0.370 غم بلون اصفر مائل الى البني الشكل (3: أ).
اما فيما يتعلق بالوزن الجاف فقد تفوق ايضا معدل الوزن الجاف للكالس المستحث على الوسط الغذائي SH المضاف اليه مركب الحديد بتركيز 100 مايكرومولر الخاص بالوسط MS معنويا على معدل الوزن الجاف للكالس المستحث على الوسط نفسه بتركيز مركب الحديد الاصلي للوسط SH، 54 مايكرومولر اذ بلغ 0.095 غم مقارنة بـــ 0.033 الشكل (3: ب).
يعد تركيز مركب الحديد 100 مايكرومولر المضاف الى الوسط SH بدل تركيز 54 مايكرومولر مثالي لنمو الانسجة كما في دراسات سابقة على نباتات مختلفة (13،19،20،21)، اذ يعمل على زيادة جاهزية كاتيونات cations العناصر الغذائية الثنائية الصغرى مثل Co,Mn,Zn الخ في الوسط الغذائي حيث تكون معتمدة على ثباتية خلبها مع EDTA (22)، وخصوصا ان تراكيزها مرتفعة في الوسط SH بالنسبة لتراكيزها في وسط MS، كما ان انخفاض تركيز مركب الحديد في الوسط الغذائي يعمل على انخفاض نمو الانسجة النباتية (23).

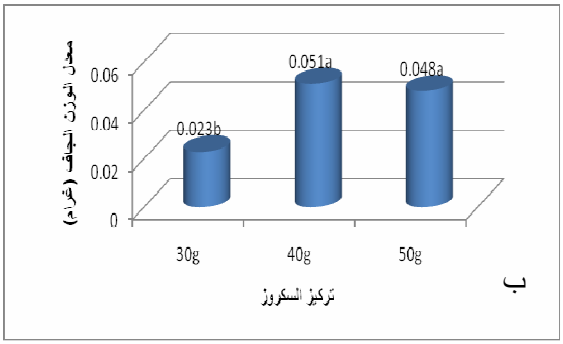


الشكل (3): تاثير تركيز الحديد في معدل الوزن الطري في أ، والجاف في ب، للكالس المستحث (غم).

5. تاثير تركيز السكروز في معدل الوزن الطري والجاف ونوعية الكالس المستحث:

بينت النتائج ان لزيادة تركيز السكروز في الوسط الغذائي SH تاثير في زيادة الوزن الطري والجاف للكالس المستحث من البذور الناضجة للباذنجان اذ تفوق معدل الوزن الطري للكالس المستحث معنويا عند تركيز 40 و50 غم/ لتر سكروز في الوسط الغذائي وبلغ 0.700 غم لكل منهما وبلون اصفر باهت مائل الى الاخضرار مقارنة مع 0.277 غم بلون ابيض الى كريمي عند تركيز 30 غم/ لتر سكروز الشكل (4: أ)، كذلك تفوق معدل الوزن الجاف للكالس المستحث معنويا في حالة تركيز سكروز 40 و50 غم/ لتر في الوسط الغذائي اذ بلغ 0.051 و 0.048 غم على الترتيب مقارنة مع 0.023 غم عند تركيز 30غم/ لتر في الوسط الغذائي الشكل (4: ب).





الشكل (4): تاثير تركيز السكروز في الوزن الطري في أ، والجاف في ب، للكالس المستحث.
* المعدلات التي تحمل احرف متشابهة لا تختلف عن بعضها معنويا حسب اختبار LSD وعلى مستوى احتمال 0.05.

بالرغم من ان كثير من البحوث تشير الى ان تركيز 30 غم/ لتر سكروز هو التركيز الشائع الاستخدام (10،13،14، 24) ولكن بالامكان تجربة تراكيز اخرى تصل الى 50 غم/ لتر لمعرفة التركيز الامثل (11،13،25) وحسب النوع النباتي لكونه يعد مصدرا للطاقة في مزارع الانسجة النباتية (11، 25) ويؤثر في الوزن الجاف لمزارع الخلايا المعلقة (26) وكذلك في زيادة النسبة المئوية للكالس المستحث من المتوك (27) بسبب الاختلاف في الضغط الازموزي بين الانسجة النباتية المزروعة والوسط الغذائي المستخدم (28). وفيما يتعلق بنوعية الكالس فقد تميز الكالس المستحث على الاوساط الحاوية 40 و 50 غم/ لتر سكروز بقوة تماسكه وبلون اصفر مائل الى الاخضرار في حين كان الكالس المستحث على الوسط الغذائي الحاوي 30 غم/ لتر سكروز هش وسهل التفتت friable وبلون اصفر باهت.
ان زيادة تركيز السكروز في الوسط الغذائي والذي يستخدم كمصدر طاقة للخلايا المزروعة يجعل الكالس المستحث قوي بدل من كونه هش سهل التفتت وهذا ما اكده بعض الباحثين (10).

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تأثير استخدام بذور الحلبة خلال مرحلة النمو في الأداء الإنتاجي والفسلجي وقطعيات الذبيحة لذكور أمهات فروج اللحم (فاوبرو)

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الملخص باللغة العربية

أجريت هذه التجربة في حقل الدواجن التابع لقسم الثروة الحيوانية في كلية الزراعة / جامعة بغداد لدراسة تأثير مستويات مختلفة من بذور الحلبة في عليقة النمو للفترة من 29 – 56 يوماً في بعض الصفات الإنتاجية والفسلجية وقطعيات الذبيحة لذكور أمهات فروج اللحم (فاوبرو) .

استخدم 225 فرخاً من ذكور أمهات فروج اللحم (فاوبرو) بعمر يوم واحد وزعت عشوائياً على خمس معاملات بثلاث مكررات للمعاملة (15 فرخ / مكرر) غذيت الطيور بعليقة البادئ (عليقة قياسية) للفترة من 1 – 28 يوما لجميع معاملات التجربة بدون أي إضافة ، وأضيفت بذور الحلبة بالمستويات 0.0 ، 0.5 ، 1.0 ، 1.5 ، 2.0 % إلى عليقة النمو للفترة من 29 – 56 يوم. أظهرت نتائج التجربة حصول زيادة عالية المعنوية على مستوى ($P<0.01$) في معدلات وزن الجسم والزيادة الوزنية تحسن معنوي على مستوى ($P<0.05$) في معامل التحويل الغذائي لمعاملات استخدام بذور الحلبة في عليقة النمو بالمقارنة مع معاملة السيطرة ، في حين لم تظهر فروق معنوية في كمية العلف المستهلك ونسبة التصافي وأوزان الأجزاء الداخلية وأوزان قطعيات الذبيحة (الفخذ ، الظهر والأجنحة) بين معاملات التجربة المختلفة بينما زادت معنوياً ($P<0.05$) أوزان قطعة الصدر وانخفضت معنوياً ($P<0.01$) أوزان قطعة الرقبة لمعاملات استخدام بذور الحلبة في عليقة النمو بالمقارنة مع معاملة السيطرة . وأنخفض معنوياً تركيزي الكلوكوز والكولسترول في بلازما دم الطيور المغذاة على نسب مختلفة من بذور الحلبة عن معاملة السيطرة .

نستنتج من هذه الدراسة إمكانية استخدام مستويات مختلفة من بذور الحلبة في عليقة النمو لذكور أمهات فروج اللحم لتحسين الأداء الإنتاجي وبدون ظهور أي تأثيرات سلبية على الطيور .

الكلمات المفتاحية: ذكورامهات فروج اللحم، بذورالحلبة، الصفات الانتاجية، الصفات الفسلجية، قطعيات الذبيحة

ABSTRACT

The experiment was conducted in the field of Poultry of Animal Resources Department in the University of Baghdad / College of Agriculture to study the effect of different levels of fenugreek seeds in diet during period of 29- 56 days on some physiological and productivity traits and carcass cuts to male broiler breeder (fawbro) by using 225 male broiler breeder chicks one day old distributed randomly to five treatments three replicate for the treatment (15 chicks /replicate). Chicks fed starter ration (standard diet) for all treatment (without any addition) from period 1-28 days of age , and to grower ration from 29-56 days of age supplementing to the diet fenugreek seeds at levels 0 , 0.5 , 1.0 , 1.5, 2.0% .

The results of experiment revealed increase highly significant ($P< 0.01$) in the rates of body weight and weight gain and significantly improvement ($P<0.05$) in feed conversion ratio by used fenugreek seeds in all treatment compared with control group , while no significant differences in feed consumption , dressing percentage , weight of internal parts and weight of carcass cuts (thigh and drumstick , back , wings) between experimental treatments , the weight of breast cut increased significantly while the weight of neck cut decreased significantly for all treatments used fenugreek seeds in growing ration compared with control group.

Significantly decreased in concentration of glucose and cholesterol in blood plasma of birds fed different levels of fenugreek seeds.

Concluded from this study the possibility of using different levels of fenugreek seeds in diet of male broiler breeder to improving performance of production without any negative effects for the birds.

المقدمة

اتجه الاهتمام الى العديد من النباتات الطبية كاضافات غذائية طبيعية لتحسين اداء الطيور الداجنة لاهميتها كمضادات بكتيريه وفطريه وانعكاس ذلك على مناعة الطيروبالتالي زيادة حيويته (1)، كما استخدمت كبدائل عن المضادات الحيويه ومحفزات للنمو(2) وتبين ان المستخلصات الزيتيه لبعض النباتات الطبيه لها تأثيرامحفزا للجهاز الهضمي من خلال زيادتها لافرازالانزيمات الهاضمه وتحسن الاستفاده من المادة الغذائيةعن طريق التحسن في الوظيفة الحيويه للكبد (3) .

ومن النباتات الطبية المستخدمة قديما بذور نبات الحلبة والتي تستعمل كمادة مشهية ومعالجة لاضطرابات الجهاز الهضمي وطارده للغازات في الانسان (4) وذلك لاحتوائها على مركبات فلافونيدية منها Apigenin و Luteolin و Orientin (5) وهذا يمنحها القدرة على كبح نشاط الجذور الحره واكساب البذوردورا فعالامضادا للاكسده (6),وصابونينات اهمها Diosgenin و Gitogenin (7) والتي لها دورا مهما في تقليل مستوى كولسترول بلازما الدم من خلال تكوينها مركب معقد مع الكولسترول في القناة الهضمية وبذلك تثبط امتصاص الكولسترول في الامعاء الدقيقة (8) .

كما تحتوي على قلويدات Gentianine, Trigonelline , Carpaine (9) ومعادن الحديد والكالسيوم والفسفور وبعض الفيتامينات مثل Vit.D (10).

واشارت Chadha (11) الى احتواء زيت بذور الحلبة على الاحماض الدهنية Linolenic و Linoleic و Oleic بالنسب 13.8 و33.7 و35.1 %على التوالي كنسبة مئوية من الزيت .

اختلفت نتائج الدراسات في تأثير الحلبة في الاداء الانتاجي للدجاج البياض وفروج اللحمحيث زاد وزن الجسم وتحسن معامل التحويل الغذائي معنويا عند اعطاء 100, 250 ,500 ملغم من مغلي بذور الحلبة في علائق فروج اللحم (12) في حين التجربة التي اجرتها (13) على الدجاج البياض من نوع نيوهمبشايربعمر28 اسبوع ولمدة ستة اسابيع خفضت معنويا من معدل وزن الجسم واستهلاك العلف عند استخدام المستويات 2و4 و6 % من بذور الحلبة بالمقارنة مع معاملة السيطرة ,كذلك عندما غذت نفس الطيور بعمر 42 اسبوعا لمدة 4 اسابيع وبالمستويات 6 و10 % من بذور الحلبة حصل ايضا انخفاض معنوي في معدل وزن الجسم بينما لم تكن هناك فروق معنويه في استهلاك العلف ومعامل التحويل الغذائي , عليه اجريت هذه الدراسه بتغذية فروج اللحم بمستويات من بذور الحلبة للفتره من 29 –56 يوما لمعرفة تأثيرها في الاداء الانتاجي والفسلجي , علما ان هناك جزء اخر من هذه الدراسه غذيت فيها بذور الحلبة من عمر يوم ولغاية 56 يوما .

المواد وطرق العمل

شملت الدراسة 225 فرخاً من ذكور أمهات فروج اللحم (فاوبرو) بعمر يوم واحد ، وزعت على خمس معاملات بثلاث مكررات (15 فرخ / مكرر) قدمت عليقة البادئ (عليقة موحدة) لجميع المعاملات للفترة من 1 – 28 يوماً وعند عمر 29 يوماً أضيفت المستويات 0.0 ، 0.5 ، 1.0 ، 1.5 ، 2.0 % من بذور الحلبة إلى العلائق وحسب الجدول (1) وأجري التحليل الكيمياوي لبذور الحلبة من العناصر الغذائية الجدول (2) . قدم الماء بشكل حر ومع توفير كافة الظروف الملائمة التي تحتاجها الطيور خلال فترة التربية .

أتبع البرنامج الوقائي لتحصين الطيور ضد الإصابة بالأمراض بتلقيحها بلقاحي النيوكاسل والكمبورو في الأعمار 9 ، 20 ، 30 يوماً عن طريق ماء الشرب .

وزنت الطيور فردياً نهاية كل أسبوع وحسبت معدلات أوزان الجسم (غم) وكمية العلف المستهلك (غم) والزيادة الوزنية (غم) وكفاءة التحويل الغذائي (غم علف / غم زيادة وزنية) أسبوعياً خلال فترة النمو وهي الفترة الذي غذيت فيها الطيور على مستويات مختلفة من بذور الحلبة ، كما حسبت نسبة الهلاكات % لنفس الفترة وقيست في نهاية التجربة عند عمر 56 يوماً نسبة التصافي وأوزان قطيعات الذبيحة وأوزان الأجزاء الداخلية المأكولة وكذلك مستويات الكلوكوز والكولسترول في بلازما دم الطيور (ملغم / 100 مل بلازما) حسب طريقتي (14) للأولى و (15) للثانية وذلك عند نهاية فترة التجربة بعمر 56 يوماً .

تم تحليل البيانات باستخدام البرنامج الإحصائي الجاهز (16) للتصميم العشوائي الكامل (CRD) وقورنت الفروق المعنوية بين المتوسطات باستخدام أختبار دنكن متعدد الحدود (11) .

جدول (2): التحليل الكيمياوي لبذور الحلبة

العنصر الغذائي	النسبة المئوية %
البروتين الخام	26.4
الطاقة الممثلة المحسوبة* kg / kcal	1538
الرطوبة	6.7
الألياف الخام	11.4
الدهن الخام	2.2
الكربوهيدرات الذائبة	49.4
الفسفور	0.30
الكالسيوم	0.10
الرماد	3.5

* تم حسابها بالاعتماد على معادلة حساب الطاقة الممثلة المحسوبة والمذكورة في NRC (15) .

جدول (1): النسب المئوية والتركيب لعلائق التجربة خلال مرحلتي البادئ والنمو من 1 – 56 يوماً

المادة العلفية %	عليقة البادئ للفترة من 1 – 28 يوماً	عليقة النمو من 29 – 56 يوماً مستوى بذور الحلبة في العليقة %				
		السيطرة 0.0	% 0.5	% 1.0	%1.5	% 2.0
ذرة صفراء	59	59	59	59	59	59
كسبة فول الصويا 44% بروتين	37	28	28	28	28	28
* بريمكس	3	3	3	3	3	3
نخالة	-	5	4.5	4	3.5	3
حجر كلس	0.7	1.2	1.2	1.2	1.2	1.2
حلبة	-	0.0	0.5	1.0	1.5	2.0
ملح طعام	0.3	0.3	0.3	0.3	0.3	0.3
دهن نباتي	-	3.5	3.5	3.5	3.5	3.5
المجموع	100	100	100	100	100	100
التركيب الكيمياوي المحسوب						
الطاقة الممثلة kg/Kcal	2818.1	2998	2991	2985	2878	2972
بروتين خام%	21.5	18.3	18.2	18.1	18.0	18.0
نسبة الطاقة إلى البروتين C/P Ratio	131.6	164.3	164.6	165.0	165.3	165.7
الألياف الخام %	3.911	3.814	3.816	3.818	3.847	3.849
% Ca	1.234	1.310	1.520	1.512	1.630	1.540
% Lysine	1.44	1.378	1.366	1.395	1.404	1.413
Meth.+Cystine	0.698	0.607	0.710	0.613	0.717	0.719

C.P ، % 4 = M.E 550 = kg/kcal ، Ca ، % 16 = P ، % 10.6 = Na ، % 4 = Mn 2750 = kg/mg ، Fe 1670 = kg/mg ، Zn 2670 = kg/mg ، Cu 335 = kg/mg ، Co 8.35 = kg/mg، kg/mg So = I ،Se 6.7 = kg/mg ، Methin 27 = kg/g ، Cys + Methin 27.6 = kg/g ، Niacin 1.350 = kg/g ، vitA 400000 = kg/IU ، vitD3 85000 = kg/IU ، vitE 1400 = kg/mg ، vitK3 100 = kg/mg ، VitB1 85 = kg/mg ، vitB2 200 = kg/mg ، vitB6 400 = kg/mg ، vitB12 = 0.680kg/mg.

النتائج والمناقشة

أظهرت نتائج الجدول 3 تأثير إضافة بذور الحلبة للفترة من 29 – 56 يوماً في معدلات أوزان الجسم خلال فترة التجربة حيث زادت معنوياً أوزان الجسم لجميع معاملات إضافة بذور الحلبة لعليقة فروج اللحم وكانت 2565.17 ، 2489.00 ، 2562.00 ، 2563.70 غم للمستويات 0.5 ، 1.0 ، 1.5 ، 2.0 % من بذور الحلبة ، وبالمقارنة مع معاملة السيطرة 2252.00 غم عند عمر 8 أسابيع وسارت بنفس المنحى معدلات الزيادة الوزنية للجسم لنفس الفترة (جدول 4) بزيادة معنوية لمعاملات إضافة مستويات مختلفة من بذور الحلبة بالمقارنة مع معاملة السيطرة التي سجلت أدنى زيادة وزنية 1421.56 غم ولم تلاحظ فروق معنوية في معدلات استهلاك العلف (غم / طير) في الأسبوع الثامن من العمر وكذلك للفترة الكلية التي غذيت بها الطيور بمستويات مختلفة من بذور الحلبة من 5 – 8 أسبوع (الجدول 5) .

وكنتيجة لحصول زيادة معنوية في معدلات أوزان الجسم والزيادة الوزنية أنعكس ذلك على تحسن معنوي في كفاءة التحويل الغذائي للأسابيع 6 ، 7 ، 8 وللفترة الكلية من 5 – 8 أسبوع في جميع معاملات استخدام بذور الحلبة وكانت للفترة الكلية 1.75 ، 1.85 ، 1.75 ، 1.80 غم علف / غم زيادة وزنية للمعاملات 0.5 ، 1.0 ، 1.5 ، 2.0 % بذور الحلبة مقارنة مع معاملة السيطرة 2.20 غم علف / غم زيادة وزنية (جدول 6) . وربما تعود الزيادة المعنوية في أوزان الجسم بإضافة مستويات مختلفة من بذور الحلبة إلى

احتواء بذور الحلبة على بروتين غني بالحامضين الأمينين اللايسين والتربتوفان (9،17).
كما أن كل 100 غم من بذور الحلبة يحتوي على 395 ملغم Ca و 51 ملغم فوسفات و 16.5 ملغم حديد و 52 ملغم فيتامين E كذلك تحتوي على تراكيز قليلة من مجموعة فيتامين B وحامض النيكوتنيك (18)، والتحليل الكيميائي لبذور الحلبة يشير إلى وجود تراكيز مختلفة من الكاروتين والثيامين والرايبوفلافين علاوة على وجود كميات قليلة من فيتامين C والبايوتين (11) ولوجود جميع هذه العناصر الغذائية في بذور الحلبة أظهرت هذه الدراسة زيادة معنوية في أوزان الجسم والزيادة الوزنية وتحسن معنوي في كفاءة تحويل الغذاء على الرغم من عدم حصول زيادة معنوية في استهلاك العلف .
ولم تظهر الدراسة فروق معنوية في نسب التصافي ونسب أوزان الأجزاء الداخلية (القلب والقانصة والكبد) نسبة إلى وزن الذبيحة كذلك في نسبة الهلاكات عند استخدام مستويات مختلفة من بذور الحلبة في عليقة فروج اللحم (الجدول 7) .
ويشير جدول (8) عدم وجود فروق معنوية في النسب المئوية لقطعيات الظهر والأفخاذ والأجنحة في حين انخفضت معنوياً النسب المئوية لأوزان الرقبة وزادت معنوياً النسب المئوية لأوزان الصدر باستخدام مستويات مختلفة من بذور الحلبة بالمقارنة مع معاملة السيطرة .
يوضح الجدول (9) تركيز كل من الكلوكوز والكولسترول في فروج اللحم المضاف إلى علائقه مستويات مختلفة من بذور الحلبة وأظهرت الدراسة انخفاضا معنوياً في تركيزي الكلوكوز والكولسترول بزيادة مستويات بذور الحلبة في العليقة .
وربما يعود السبب في انخفاض سكر الكلوكوز في الدم بوجود بذور الحلبة في العليقة إلى احتواء بروتينات بذور الحلبة على الحامض الأميني 4 – hydroxyisoleucine الذي له دور في تحفيز البنكرياس على إفراز هرمون الأنسولين الذي يعمل على خفض سكر الدم (19) ، أو وجود المركبات الفعالة في بذور الحلبة على سبيل المثال الميوسيلج Mucilage وهو مركب كلايكو بروتين قطبي لزج من السكريات المتعددة الطرفية – Polar glycoprotein ولزوجة هذا المركب تخفض من سكر وكولسترول بلازما الدم عند الإصابة بالسكري (20) .
وقد يعود خفض كولسترول الدم في الفروج المغذى على بذور الحلبة ربما يعود إلى احتواء ألياف بذور الحلبة على glatactomannan الذي يزيد من لزوجة الكتلة الغذائية ومن ثم تخفيض مستوى الكولسترول بتثبيط امتصاصه من الأمعاء الدقيقة وتثبيط أعادة امتصاص أحماض الصفراء من قبل الجزء العلوي للفائفي (21) أو وجود الصابونينات في بذور الحلبة والتي تشكل 1.5 – 2.2 % (25) ومن أهمها Hecogenin , Gitogenin , Diosgenin , Yamogenin ودور الصابونينات مهم في تقليل مستوى كولسترول بلازما الدم من خلال تكوينها مركب معقد مع الكولسترول في القناة الهضمية وبذلك تثبط امتصاص الكولسترول في الأمعاء الدقيقة (8) أو تمنع امتصاص أحماض الصفراء (22) أو قد تؤخر الصابونينات امتصاص الأمعاء لدهون الغذاء عن طريق تثبيط فعالية أنزيم اللايبيز (23).
وربما دور بذور الحلبة المضاد للبكتريا الضارة والفطريات (24) أنعكاس على تقليل نسبة الهلاكات وتحسين الأداء الإنتاجي للفروج عند إضافة بذور الحلبة إلى علائق فروج اللحم بكافة مستوياتها للفترة من 29 – 56 يوماً من عمر الطيور . عليه يمكن التوصل من هذه التجربة إلى إمكانية استخدام بذور الحلبة في العلائق بالمستويات 0.5 ، 1.0 ، 1.5 ، 2 % بدون ظهور أي تأثير سلبي على الأداء الإنتاجي والفسلجي للفروج وإنما على العكس تحسنت الصفات الإنتاجية وانخفضت نسبة الهلاكات باستخدام بذور هذا النبات الطبي في العليقة .

جدول (3): تأثير مستويات مختلفة من بذور الحلبة في علائق فروج اللحم خلال مرحلة النمو 29 – 56 يوماً في معدل وزن الجسم

المعاملات		معدل وزن الجسم غم / طير للفترات أسبوعيا				
رقم المعاملة	نسبة الحلبة %	4 (28 يوم)	5 (35 يوم)	6 (42 يوم)	7 (49 يوم)	8 (56 يوم)
Control 1	0.0	803.38±6.5	c 1226.53±11.3	b 1625.67±10.5	c 1887.65±7.6	b 2252.00±8.3
2	0.5	794.68±9.0	b 1252.67±10.4	b 1647.33±9.4	b 1942.17±8.9	a 2565.17±9.1
3	1.0	828.81±11.1	b 1254.83±12.3	ab 1658.50±8.6	b 1961.00±7.8	a 2489.00±9.5
4	1.5	844.12±8.8	a 1283.79±8.7	a 1688.50±11.3	b 1971.47±9.5	a 2562.00±10.4
5	2.0	845.31±10.7	ab 1261.67±9.6	a 1682.17±12.1	a 2029.00±6.9	a 2563.70±11.2
مستوى المعنوية		NS	*	**	**	**

الحروف المختلفة ضمن العمود الواحد تشير إلى وجود فروق معنوية بين متوسطات المعاملات .
** = وجود فرق معنوي عند مستوى احتمال P<0.01 .
- = وجود فرق معنوي عند مستوى احتمال P<0.05 .

جدول (4): تأثير مستويات مختلفة من بذور الحلبة في علائق فروج اللحم على معدل الزيادة الوزنية للجسم للمدة من 29 – 56 يوماً من العمر

المعاملات		معدل الزيادة الوزنية للجسم غم / طير للفترات أسبوعياً				
رقم المعاملة	نسبة الحلبة %	5 29 – 35 يوم	6 36 – 42 يوم	7 43 – 49 يوم	8 50 – 56 يوم	8 – 5 29 – 56 يوم
Control 1	0.0	423.15±7.8	399.14±9.2	b 262.01±12.2	b 337.33±9.7	b 1421.56±10.1
2	0.5	430.99±10.3	394.66±8.1	b 294.84±11.4	a 623.00±8.9	a 1743.50±9.9
3	1.0	426.02±9.8	4.3.69±8.6	b 302.47±10.5	a 528.15±10.0	a 1660.40±11.2
4	1.5	439.67±12.0	4.4.67±9.0	b 283.01±11.3	a 590.52±11.2	a 1717.80±10.5
5	2.0	416.36±11.1	420.49±10.1	a 346.85±10.7	a 553.00±10.8	a 1736.56±12.1
مستوى المعنوية		NS	NS	*	**	**

الحروف المختلفة ضمن العمود الواحد تشير إلى وجود فروق معنوية بين متوسطات المعاملات .
** = وجود فرق معنوي عند مستوى احتمال P<0.01 .
* = وجود فرق معنوي عند مستوى احتمال P<0.05 .
N.S = عدم وجود فرق معنوي .

جدول (5): تأثير نسب مختلفة من بذور الحلبة في علائق النمو لفروج اللحم على معدل استهلاك العلف للفترة من 29 – 56 يوم من العمر

المعاملات		معدل استهلاك العلف غم / طير للفترات أسبوعياً				
رقم المعاملة	نسبة الحلبة %	5 29 – 35 يوم	6 36 – 42 يوم	7 43 – 49 يوم	8 50 – 56 يوم	8 – 5 29 – 56 يوم
Control 1	0.0	605.5±8.6	ab 790.0±10.2	b 775.0±7.4	940.5±11.1	3111.0±9.3
2	0.5	586.0±9.2	b 733.5±9.8	a 844.3±8.1	955.0±10.3	3089.5±10.5
3	1.0	570.0±7.9	a 800.0±9.2	a 828.0±9.1	905.5±9.5	3103.5±11.2
4	1.5	577.5±10.1	b 755.0±8.8	b 762.5±8.9	954.5±10.1	3099.5±9.7
5	2.0	591.4±8.9	ab 795.0±8.5	a 872.5±7.8	980.0±9.6	3239.0±8.7
مستوى المعنوية		NS	*	**	NS	NS

الحروف المختلفة ضمن العمود الواحد تشير إلى وجود فروق معنوية بين متوسطات المعاملات .
* = وجود فرق معنوي عند مستوى احتمال P<0.05 .
N.S = عدم وجود فرق معنوي .

جدول (6): تأثير مستويات مختلفة من بذور الحلبة في علائق النمو لفروج اللحم على معامل التحويل الغذائي للفترة من 29 – 56 يوماً

المعاملات		معامل التحويل الغذائي غم علف / غم زيادة وزنية للفترات أسبوعياً				
رقم المعاملة	نسبة الحلبة %	5 29 – 35 يوم	6 36 – 42 يوم	7 43 – 49 يوم	8 50 – 56 يوم	8 – 5 29 – 56 يوم
Control 1	0.0	1.45±0.01	a 1.98±0.02	a 2.93±0.03	a 2.81±0.00	a 2.20±0.02
2	0.5	1.40±0.00	ab 1.86±0.03	b 2.89±0.02	c 1.55±0.01	b 1.75±0.01
3	1.0	1.35±0.02	a 2.07±0.01	c 2.75±0.01	b 1.80±0.02	b 1.85±0.03
4	1.5	1.35±0.05	b 1.85±0.02	c 2.67±0.01	b 1.70±0.01	b 1.75±0.02
5	2.0	1.50±0.02	ab 1.95±0.00	d 2.52±0.02	b 1.75±0.03	b 1.80±0.01
مستوى المعنوية		NS	*	**	**	**

** = وجود فرق معنوي عند مستوى احتمال P<0.01 .

*= وجود فرق معنوي عند مستوى احتمال P<0.05 .

جدول (7): تاثير نسب مختلفة من بذور الحلبة في علائق النمو لفروج الححم في وزن الذبيحة المنظفة ونسبة التصافي ونسبة الهلاكات عند عمر 8 اسابيع.

المعاملات		الصفات المدروسة				
رقم المعاملة	نسبة الحلبة%	نسبة التصافي *	القلب %	القانصة%	الكبد%	الهلاكات%
1	0.0	75.64± 9.30	0.802±0.02	2.859±0.16	3.640±0.20	3.4±0.0
2	0.5	76.20±8.80	0.813±0.03	2.841±0.32	3.500±0.21	2.0±0.3
3	1.0	74.55±6.90	0.771±0.05	2.416±0.18	3.440±0.15	2.1±0.0
4	1.5	76.33±7.85	0.785±0.06	2.660±0.19	3.610±0.28	2.2±0.0
5	2.0	75.17±8.70	0.731±0.02	2.750±0.20	3.760±0.19	2.5± 1.3
مستوى المعنوية		N.S	N.S	N.S	N.S	N.S

* = حسبت على اساس وزن الذبيحة المنظفة الفارغة مع الأجزاء الداخلية المأكولة القلب ، الكبد ، القانصة ، (الفياض وناجي،1989)

N.S = عدم وجود فرق معنوي.

جدول (8): تاثير نسب مختلفة من بذور الحلبة في علائق النمو لفروج اللحم في النسب المئوية لاجزاء الذبيحة عند عمر 8 اسابيع.

المعاملات		النسبة المئوية للقطعيات %				
رقم المعاملة	نسبة الحلبة %	الظهر	الافخاذ	الاجنحة	الرقبة	الصدر
1	0.0	29.97±2.4	30.74±3.1	12.20±1.5	4.91±1.1	b 22.59±2.6
2	0.5	29.37±2.3	30.60±1.5	11.11±1.0	c 4.39±1.1	ab 23.75±3.5
3	1.0	29.95±2.1	30.62±2.4	11.66±1.6	b 4.61±0.9	ab 22.77±2.8
4	1.5	28.19±2.6	30.16±2.2	12.12±1.3	c 4.29±0.7	ab 23.02±3.1
5	2.0	27.66±3.2	32.38±1.8	11.36±1.7	ab 4.82±0.8	a 24.90±2.7
مستوى المعنوية		N.S	N.S	N.S	**	*

N.S = عدم وجود فرق معنوي . * *= وجود فرق معنوي عند مستوى احتمال P<0.01. * = وجود فرق معنوي عند مستوى احتمال P<0.05.

جدول (9): تاثير مستويات مختلفة من بذور الحلبة في علائق فروج اللحم في تركيز الكلوكوز والكولسترول في بلازما الدم عند نهاية فترة التجربة عند عمر 8 اسابيع.

المعاملات		مستوى الكلوكوز والكولسترول في بلازما الدم ملغم/100 مل بلازما	
رقم المعاملة	نسبة الحلبة %	تركيز الكلوكوز	تركيز الكولسترول
1	0.0	a 249.0±4.90	a 119.5±5.62
2	0.5	a 236.0±4.42	b 96.0±1.64
3	1.0	b 202.0±5.16	c 79.3±2.85
4	1.5	b 203.2±2.86	d 69.5±3.40
5	2.0	c 162.5±3.92	c 75.3±3.74
مستوى المعنوية		**	**

** = وجود فرق معنوي عند مستوى احتمال P<0.01.

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التحري وتنمية طفيلي بلانتيديم كولاي *Balantidium coli* من الأغذية

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الملخص باللغة العربية

يعد طفيلي *Balantidium coli* من الابتدائيات المرضية المهدبة التي تصيب الإنسان ويسبب الطفيلي مرض الديزنتريا البلانتيدية وهو من الأمراض المشتركة بين الإنسان والحيوان.
تم فحص اربعين نموذج من الخضار الورقية (كرفس ، رشاد ، بقدونس ، نعناع ، سبانغ ، سلق ، خيار) جمعت بصورة عشوائية من الأسواق المحلية لمدينة بغداد.
تم تنمية طفيلي *Balantidium coli* في المختبر على وسطي Sabouraud Glucose Agar و Sabouraud Glucose Broth واظهرت اربعة نماذج من نباتي السلق والكرفس احتوائها على طفيلي *Balantidium coli* باستخدام الفحص المجهري المباشر وتمت تنمية الطفيلي على وسطي Sabouraud Glucose Agar و Sabouraud Glucose Broth بدرجة حرارة 25 م° لمدة (24–48) ساعة حيث تم ملاحظة الطور المتكيس والمتغذي بسهولة في وسط Sabouraud Glucose Broth
يهدف البحث ولأول مرة في العراق التحري عن طفيلي *Balantidium coli* في الأغذية وتنميته في المختبر.

ABSTRACT

Balantidium coli is the pathogenic ciliated protozoan parasite infect human responsible for the zoonotic disease balantidiosis .
Fourty samples from leafy vegetables (Celery,Cress,Parsley,Spearmint, Spinach, chard ,Cucumber)collected randomly from local markets around Baghdad capital were examined.
Culture medium include Sabouraud Glucose Agar and Sabouraud Glucose Broth used for cultivation and growing of *balantidium coli* in laboratory.
The results show four samples (celery and chard) contain the parasite *balantidium coli* by using direct microscopic examination.
We success in cultivation of *balantidium coli* on both Sabouraud Glucose Agar and Sabouraud Glucose Broth at 25 C^o for 24 – 48 hour , on the culture medium we detected the cyst and trophozoite phase easily.
The aim of this study at the first time detection of *balantidium coli* in food and cultivation in laboratory.

المقدمة

يعتبر طفيلي *Balantidium coli* من الابتدائيات المرضية المهدبة الوحيدة التي تصيب الإنسان ويسبب مرض يعرف بالديزنتريا البلانتيدية (Balantidiasis) وهو من الأمراض المشتركة بين الإنسان والحيوان (1) ، تعد الكثير من الحيوانات الأليفة والمتوحشة خازنة وناقلة للطفيلي لذلك تصبح مصدرا لنقل الإصابة للإنسان مثل الأبقار والجاموس والجمال والكلاب والقطط والجرذان والخنازير والقرود وان أكثر الحيوانات الخازنة للطفيلي والتي تكون مصدر إصابة للإنسان هي الخنازير (2، 3، 4) . بعض الحشرات يمكن أن تكون عامل ناقل لهذا الطفيلي الى الإنسان مثل الصراصير (5).

تحدث الإصابة بهذا الطفيلي نتيجة تناول المياه أو الأغذية الملوثة بالبراز الحاوي على الطفيلي والإصابة من شخص إلى آخر ممكنة الحدوث أيضا ، ويسبب المرض مشاكل صحية عند تلوث مصادر المياه ببراز الإنسان والحيوان (6، 7).

يتواجد الطفيلي في الأمعاء الغليظة للإنسان (القولون والأعور) ويمتاز بدورة حياة بسيطة لاتحتاج إلى مضيف وسطي ويمر بطورين ، الطور المتغذي Trophozoite الذي يتواجد في الأمعاء الغليظة ويتراوح طوله (50–200) ملي مايكرون وعرضه (40–70) ملي مايكرون ويغطي جسم الطفيلي الأهداب Cilia التي تجعل الطفيلي ذو حركة سريعة ونشطة دائرية مميزة ويحتوي الطفيلي على نواتين مميزتين واضحتين الأولى كبيرة الحجم ذات شكل كلوي والأخرى صغيرة دائرية الشكل،عند خروج الطفيلي مع الغائط يتحول إلى الطور المتكيس Cyst الذي يتميز بكونه اصغر من الطور المتغذي وبقطر (40×60) ملي مايكرون ويتصف بشكله الدائري الخشن ويحاط بغلاف مكون من طبقتين ويعتبر هذا الطور هو الطور المرضي الفعال للطفيلي (8).

يتسبب الطفيلي في إحداث حالات مرضية تختلف في شدتها من العلامات غير الظاهرة والتي يصبح المريض حامل للطفيلي ويتم طرحه بشكل مستمر مع الغائط ، أن مثل هؤلاء الأشخاص وخاصة العاملين في مجال تحضير الأغذية ممكن أن يشكلو خطورة في نقل المرض (1) ، أما الحالات الشديدة للمرض فتتميز بوجود المخاط والدم في الغائط ومن الممكن أن يؤدي النزيف الشديد إلى الصدمة والموت (9) وفي الحالات المزمنة يلاحظ فقدان في الوزن والغثيان والم في البطن والتهاب وتقرح القولون (10) وإصابة الرئتين وصعوبة في التنفس حيث تحدث الاصابة بهذا الطفيلي عند استنشاق الهواء الحاوي على الطور المتكيس للطفيلي من قبل العاملين في حضائر الحيوانات وخاصة اولئك الذين يستخدمون براز الحيوانات في تسميد مزارعهم (11) ، وهناك عدة عوامل تزيد من شدة الأعراض مثل الأشخاص اللذين يعانون من سوء التغذية ومدمني الكحول والأشخاص المصابين بالأمراض المثبطة للجهاز المناعي مثل مرضى الايدز (11) ، ويمكن للطورالمتغذي أن يخترق الغشاء المخاطي للأمعاء الغليظة مسبباً تقرحات في القولون (12، 13، 14).

نظراً للاحتياج اليومي من الغذاء والماء لديمومة الحياة بات من الضروري أن يكون هذا الغذاء والماء خالين من الملوثات البيولوجية المرضية لذا يهدف البحث ولأول مرة في العراق التحري عن طفيلي *Balantidium coli* في الأغذية واختيار الطريقة المثلى لتنميته في المختبر.

المواد وطرائق العمل

النمذجة :

تم فحص (40) نموذج من الخضار الورقية (كرفس ، رشاد ، بقدونس ، نعناع ، سبانخ ، سلق ، خيار) جمعت بصورة عشوائية من الأسواق المحلية لمدينة بغداد للفترة من شباط/2011 ولغاية تشرين الثاني/2011 .

طريقة الفحص المباشر :

تم التحري عن طفيلي *Balantidium coli* بعد غسل ونقع كمية من الخضار (كل نموذج على حدة) في وعاء زجاجي نظيف ومعقم يحتوي على محلول معقم من المحلول الملحي المحضر من اذابة 8,5 غرام من كلوريد الصوديوم في لتر من الماء المقطر .ترك النموذج لمدة نصف ساعة مع التحريك في درجة حرارة الغرفة ، اخذ المحلول الفسيولوجي ونبذ في جهاز الطرد المركزي بسرعة 1500 دورة/دقيقة لمدة ثلاث دقائق وتم التخلص من الرائق وأعيد تعليق الراسب وأخذت قطرة من العالق ووضعت على شريحة زجاجية ثم وضع غطاء الشريحة وفحص مباشرة بالمجهر الضوئي بقوة تكبير (40 X) .

طريقة تصبيغ الطفيلي :

استخدم محلول اليود الذي حضر من إذابة 50 غرام من اليود و 100 غرام يوديد البوتاسيوم في 1000 مليلتر من الماء المقطر اللايوني وقبل استعماله مباشرة يخفف محلول اليود بنسبة 1:5 بالماء المقطر اللاأيوني ، يحضر هذا المحلول قبل تصبيغ الطفيلي مباشرة وذلك بوضع قطرتين صغيرتين من العينة المركزة على شريحة زجاجية ويضاف لإحدى القطرات قطرة من المحلول الملحي المعقم والى القطرة الثانية تضاف قطرة من محلول اليود المخفف وتغطى كل قطرة بغطاء الشريحة وتفحص تحت المجهر للتحري عن الطفيلي بطوريه (المتغذي والمتكيس) حيث تم فحص النموذج المركز المعلق بالمحلول الملحي أولا ثم يتم تأكيد النتيجة بفحص القطرة المصبوغة بصبغة اليود.

التحري عن طفيلي *Balantidium coli* في براز الخنازير :

تم فحص براز الخنازير (منطقة البوعثة/بغداد) للتحري عن طفيلي *Balantidium coli* حيث أخذت كمية صغيرة من الغائط وتم تعليقها بالمحلول الملحي المعقم ويرج بلطف وتم عمل شريحة زجاجية من هذا المحلول الحاوي على البراز وصبغه بمحلول اليود وشخص الطفيلي بوضوح بالفحص المجهري المباشر ودراسة شكله ومكوناته الداخلية وحركته اللولبية المميزة.

تنمية طفيلي *Balantidium coli* في المختبر :

تم تنمية طفيلي *Balantidium coli* على الأوساط الزرعية التي شملت وسط Sabaorouad Glucose Agar (SGA) و Sabaorouad Glucose Broth (SGB)

مكونات وسط السبوريد الصلب Sabaorouad Glucose Agar (SGA)

Mycological peptone	10 gm
Glucose	40 gm
Agar	15gm
Distilled water	1000ml

تذاب المواد ويعدل الأس الهيدروجيني إلى (5،6±0،2) ويعقم الوسط بجهاز التعقيم بالحرارة الرطبة بدرجة حرارة 121 م° وضغط 15 باوند/انج^2 لمدة 15 دقيقة.
اما بخصوص وسط Sabaorouad Glucose Broth (SGB) تم تحضره بنفس الطريقة السابقة بدون إضافة مادة الاكار ويعقم الوسط بنفس الظروف.

تنمية الطفيلي على وسط (SGA) ووسط (SGB) :

بعد التأكد من وجود طفيلي *Balantidium coli* في النماذج المفحوصة مجهرياً يتم نشر (0.1) , (0.5) و (1) مليلتر من العالق المركز على سطح وسط (SGA) ويترك لفترة حتى يمتص العالق على الوسط وفي نفس الوقت تضاف نفس الحجوم من العالق المركز في أنابيب تحتوي على وسط(SGB) وترج بلطف ، حضنت الأطباق والأنابيب بدرجة حرارة 25 م° و 37 م° لمدة (24-48) ساعة.

النتائج والمناقشة

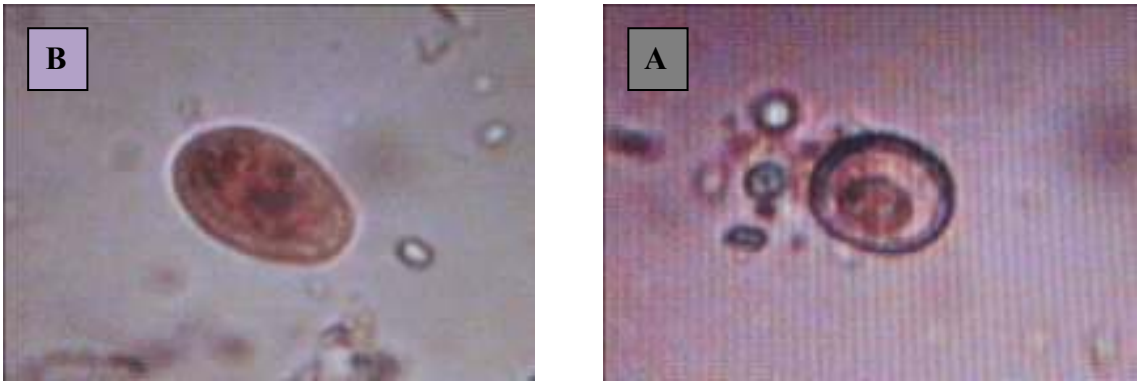
أظهرت أربعة نماذج من الخضار الورقية (السلق والكرفس) احتوائها على طفيلي *Balantidium coli*.
إن تواجد هذا الطفيلي في الأغذية يعني إمكانية إصابته للإنسان بسهولة مع العلم أن الطور المتكيس لهذا الطفيلي يكون مقاوم للمعقمات المستخدمة في معاملة الخضار الورقية ، تتلوث الخضار الورقية عادة بطفيلي *Balantidium coli* من براز الإنسان والحيوان التي تستعمل عادة في تسميد مزارع الخضار الورقية ويمكن أن تحدث الإصابة للإنسان نتيجة تلوث مصادر المياه والخضار ويمكن أن تحدث الإصابة من شخص إلى آخر (15).
كما أظهرت نماذج براز الخنازير احتوائها على الطفيلي بطوريه المتغذي والمتكيس صورة (1)، تعتبر الخنازير من اكثر الحيوانات الخازنة لهذا الطفيلي ومن الممكن ان تنتقل الاصابة الى الانسان عند تناوله الاغذية ومياه الشرب الملوثة ببراز الخنازير الحاوي على الطفيلي (16).
إن الأعراض المرضية الحادة لمرض الديزنتريا البلانتيدية والمتمثلة بوجود المخاط والدم غالبا ما يخطأ في تشخيصها من قبل الأطباء حيث يشتبه بها بقرحة القولون أو يمكن أن يشتبه بها كحالات الزحار الأميبي وان إجراء الفحص المجهري المباشر لغائط الإنسان المصاب يمكن أن يشخص الطفيلي بسهولة سواء كان الطور المتغذي الذي يتميز بكبر حجمه وحركته الدورانية المميزة والتجويف الفمي او الطور المتكيس الذي يتميز بجداره السميك المكون من طبقتين واحتوائه على النواة ، كذلك يمكن أن تتلوث مزارع الخضار من براز الحيوانات مثل الكلاب السائبة والقطط والجرذان والمجترات بصورة عامة (8).
لقد تم تنمية طفيلي *Balantidium coli* على وسطي (SGA) و (SGB) فعلى وسط (SGA) نمى الطفيلي على شكل مستعمرات تشبه مستعمرات البكتريا من حيث قوامها وحجمها ولونها وقد تم تعين وجود الطور المتكيس عند استخدام حجم 0.5 مليلتر فقط صورة (2) ، أما وسط (SGB) فقد نمى الطفيلي بشكل أفضل وتم ملاحظة الطور المتغذي بشكل واضح ومميز بحجمه الكبير واحتوائه على نواتين احدهما كبيرة كلوية الشكل مع وضوح التجويف الفمي والأهداب التي تساعده على الحركة اللولبية السريعة وقد تم تعين الطفيلي في الأنابيب التي لقحت بحجوم (0.5) و (1) مليلتر صورة (3).
ان تنمية هذا الطفيلي على الأوساط الزرعية في المختبر تم لأول مرة في العراق وسوف يفتح آفاق مستقبلية جديدة في إجراء دراسات أخرى على هذا الطفيلي حيث يمكن من خلال تنميته زيادة اعداد الطفيلي ومن ثم سهولة تشخيصه إذ أن الكثير من الدراسات التي أجريت في تعيين الطفيلي باستخدام الفحص المجهري المباشر للغائط والتي بينت نتائج سالبة أظهرت نفس العينات نتائج موجبة عند تنمية الطفيلي لنفس العينات في المختبر (8).

الاستنتاجات

1. تواجد طفيلي *Balantidium coli* في الخضار الورقية واحتمالية الاصابة بهذا الطفيلي عند عدم التنظيف والتعقيم الجيدين لهذه الخضار ويمكن استخدام هايبوكلورايت الصوديوم بتركيز 1% حيث تكون فعالة في القضاء على الطور المتكيس لهذا الطفيلي.
2. تنمية طفيلي *Balantidium coli* علي وسطي (SGA) و (SGB) التي يمكن استخدامها في تشخيص الطفيلي المنقول بالاغذية

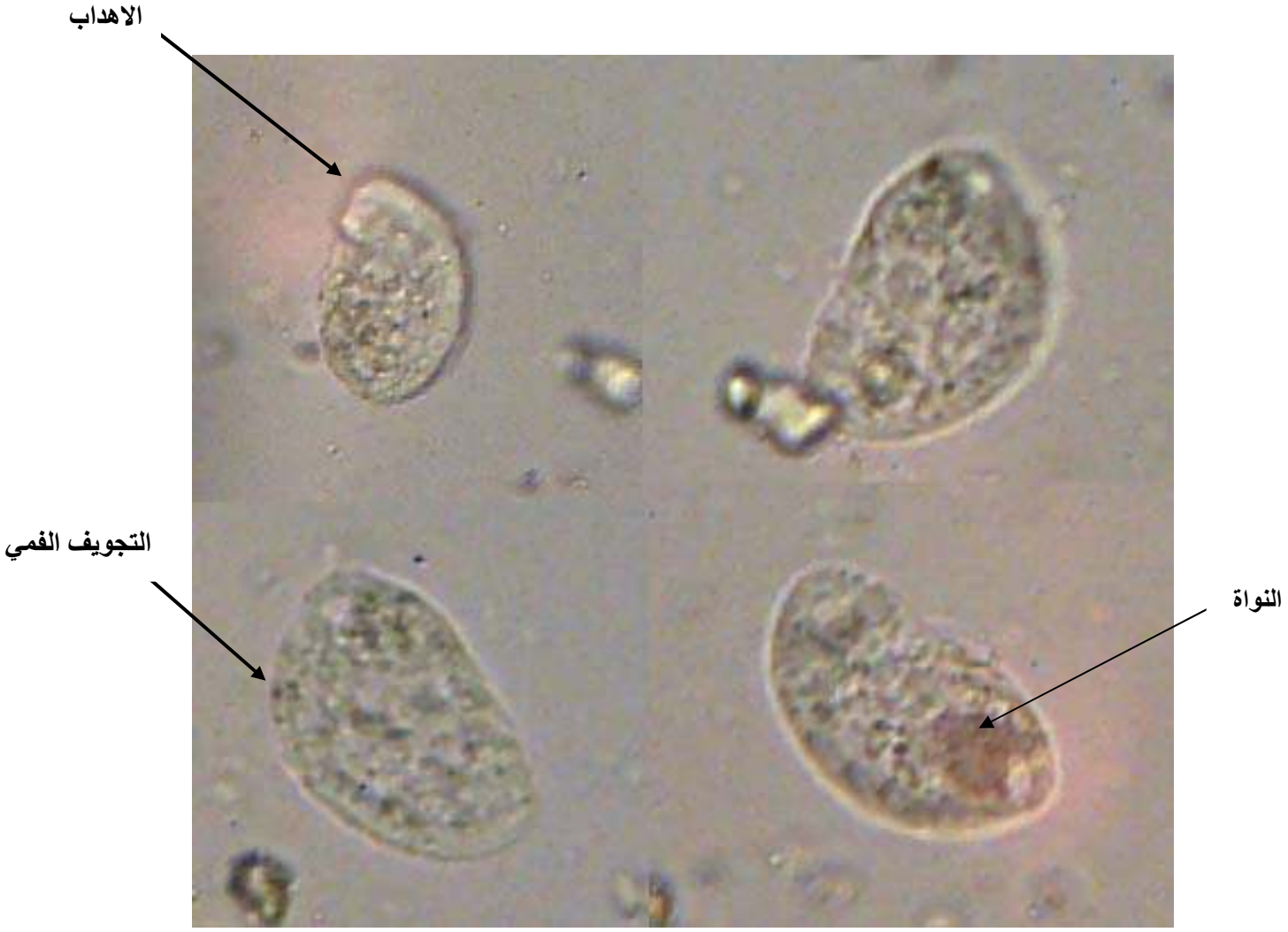
التوصيات

1. للوقاية من مرض الديزنتريا البلانتيدية يجب عدم استخدام براز الإنسان كسماد في الزراعة ، عدم شرب المياه الملوثة ,غسل وتعقيم الفواكه والخضار جيداً والطبخ الجيد للحوم إضافة إلى زيادة الوعي الصحي للمواطنين بالاعتناء بالنظافة الشخصية وغسل اليدين جيداً بعد ملامسة الحيوانات والخروج من المرافق الصحية وخاصة العاملين في مجال إعداد الأغذية في معامل صناعة الأغذية والمطاعم.
2. بناء قاعدة معلوماتية حول تواجد هذا الطفيلي في براز الاطفال اللذين يعانون من حالات الاسهال
3. اجراء دراسات حول تواجد الطفيلي في براز الحيوانات المجترة والكلاب والقطط لمعرفة دور هذه الحيوانات في نقل الاصابة الى الانسان



صورة (1) طفيلي *Balantidium coli* في براز الخنازير (A) الطور المتغذي (B) الطور المتكيس

صورة (2) الطور المتكيس لطفيلي *Balantidium coli* على وسط SGA



صورة (3) الطور المتغذي لطفيلي *Balantidium coli* على وسط SGB

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الرش باندول حامض الخليك(IAA) وحامض الجبرليك (GA_3) وأثرهما في صفات النمو لنبات القرنفل *Dianthus caryophyllus* L.

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الملخص باللغة العربية

نفذ البحث في الظلة الخشبية التابعة لقسم البستنة وهندسة الحدائق / كلية الزراعة – جامعة الكوفة في الفترة من 2009/9/15 لغاية 2010/6/1 لدراسة تأثير الرش بأندول حامض الخليك(IAA) وحامض الجبرليك(GA_3) في صفات النمو لنبات القرنفل المعمر *Dianthus caryophyllus* L. var. chabaud ، إذ تضمنت التجربة ست عشرة معاملة عامليه عبارة عن التداخل بين أربعة تراكيز من اندول حامض الخليك (IAA) هي (0 ، 25 ، 50 ، 75) ملغم.لتر$^{-1}$ وأربعة تراكيز من حامض الجبرليك (GA_3) هي (0 ، 100 ، 200 ، 300) ملغم.لتر$^{-1}$ وبواقع ثلاث رشات بتاريخ 2010/3/1 و 2010/3/15 و 2010/4/1 ، نفذت التجربة العامليه Factorial experiment بتصميم القطاعات العشوائية الكاملة Randomized Complete Block Design (R.C.B.D.) بثلاث مكررات وقورنت المتوسطات باستعمال اختبار دنكن متعدد الحدود وعند مستوى احتمال 0.05 .

أظهرت النتائج أن رش النباتات باندول حامض الخليك بتركيز 50 ملغم.لتر$^{-1}$ أو الرش بحامض الجبرليك بتركيز 300 ملغم.لتر$^{-1}$ أدى إلى زيادة معنوية في جميع الصفات المدروسة ، آذ تفوقت النباتات المعاملة بإعطاء أعلى معدل لارتفاع النبات ، المساحة الورقية، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي ، عدد الجذور الرئيسة ، الوزن الجاف للمجموع الجذري، محتوى الأوراق من اندول حامض الخليك، محتوى الأوراق من حامض الجبرليك , محتوى الأوراق من الكاربوهيدرات الذائبة الكلية ، طول الساق الزهرية ، قطر الزهرة , عدد البتلات، الوزن الجاف للزهرة وعمر الأزهار في المزهرية مقارنة بأدنى القيم التي نتجت من معاملة المقارنة(الرش بالماء المقطر فقط) .

أظهرت نتائج التداخل بين منظمي النمو أن رش النباتات باندول حامض الخليك بتركيز 50 ملغم.لتر$^{-1}$ وحامض الجبرليك بتركيز 300 ملغم.لتر$^{-1}$ تفوقت معنوياً في جميع الصفات المدروسة مقارنة بالنباتات التي لم ترش باي من المنظمين (المقارنة) .

الكلمات الدالة: هرمونات النمو,نبات القرنفل,اندول حامض الخليك(IAA),حامض الجبرليك(GA_3)

** البحث مستل من رسالة ماجستير للباحث الثاني*

ABSTRACT

This research was conducted in Lath house of botanical department in The technical institute AL-Kufa from 15/9/2009 to 1/6/2010 to study the effect of spraying of Indol -3-acetic acid(IAA) and Gibberellic acid(GA3)on growth characteristics of Carnation plant *Dianthus caryophyllus* L.Var chabaud ,The experiment contains sixteen Factorial treatments i.e. interactions between four concentrations of Indol -3-acetic acid (0,25,50 and 75)$Mg.L^{-1}$ and four concentrations of Gibberellic acid (0,100,200 and 300)$Mg.L^{-1}$ in three sprayers 1/3/2010, 15/3/2010, 1/4/2010,Complete randomized design was used with three replicates, using Duncan's multiple range test at probability of 0.05 was adopted to compare means.

Results showed that spraying plant with Indol acetic acid at concentration 50 $Mg.L^{-1}$ or Gibberellic acid at concentration 300 $Mg.L^{-1}$was increased significantly in all the studied characteristics which gave the higher plant height, leaf area, shoot dry weight, leaf contents of total chlorophyll, numbers of mean roots, root dry weight, leaf content of Indol acetic acid, leaf content of Gibberellic acid, leaf content of total soluble carbohydrates, flower length stem, flower diameter , number of petals, flower dry weight, and vessel life compared with lower values which were produced from the plants control (spraying with distillate water only) .

Result revealed that interaction between two plant hormones the treated plants with Indol acetic acid in concentration 50 $mg.L^{-1}$ and Gibberellic acid in concentration 300 $mg.L^{-1}$ was increased significantly in all the studies characteristics compared with non- spraying with two hormones (control) .

المقدمة

ينتمي القرنفل *Dianthus caryophyllus* L. إلى العائلة القرنفلية Caryophyllaceae. وهو من النباتات العشبية المعمرة ذات التربية الخاصة، إذ يحتاج إلى خبرة فنية عالية بغية أنتاج أزهاره بنوعية تجارية جيدة. ويُعد القرنفل من أزهار القطف المهمة عالمياً ذات القيمة التنسيقية والجمالية العالية ، إذ احتلت زراعته وإنتاج أزهاره الصالحة للقطف موقع متقدم في دول العالم المنتجة لهذه الأزهار، آذ بلغ أنتاجها في مدينة فيكتوريا والتي تعد اكبر مركز لإنتاج الزهور في الولايات المتحدة الأمريكية بحدود (140) مليون زهرة صالحة للقطف للمساحات المزروعة والتي تقدر بـ (100)هكتار(1). لذا نجد أن أنتاج ازهار القطف يمكن أن يحل مشكلة العائدات الاقتصادية في بلدان مختلفة ومنها ازهار القرنفل المعدة لغرض التصدير وذلك من خلال الاعتناء بالعمليات الزراعية إثناء موسم النمو لغرض تحسين نمو النباتات وزيادة أنتاج إزهاره ذات المواصفات التجارية والتي نصت عليها مقاييس الجودة للاتحاد الاقتصادي الأوربي (European Economic Community) ، ومن هذه العمليات استخدام منظمات النمو , التي تشكل احد الاتجاهات الأساسية للأبحاث العلمية الأكاديمية والتطبيقية على حدٍ سواء ، والتي تلعب دوراً أساسيا في تحسين وإنتاج إزهار النباتات(2). ومن هذه المنظمات النمو اندول حامض الخليك(IAA) وحامض الجبرليك(GA_3)، التي تؤدي دوراً مهماً في تنشيط وتشجيع استطالة الخلايا وبدء تكوين الإزهار وتطورها من خلال توجيه نواتج التمثيل الغذائي نحو الإزهار النامية(3)وبالتالي فإنها تعمل بالنهاية على زيادة حجم الإزهار وعدد البتلات النامية فيها وطول الساق الزهرية.فقد ذكرت (4) أن رش نباتات الداليا *Dahlia variabilis* L بحامض الاندول بيوتريك(IBA) بتركيز 200 ملغم.لتر$^{-1}$ أدى إلى زيادة قطر الساق والوزن الطري والجاف للنباتات . كذلك أوضحت الدراسات إن حامض الجبرليك يشجع استطالة السيقان عن طريق تحفيز استطالة الخلايا والتغلب على التقزم الوراثي وتشجيع انقسام وتوسيع الخلايا (2). فقد وجد (5) أن رش نباتات المنثور (الشبوي) *Matthiola incana* L. بحامض الجبرليك بالتراكيز 100 , 200 و 300 ملغم.لتر$^{-1}$ أدى إلى زيادة معنوية في ارتفاع النبات وعدد الأوراق.وذكر (6) إن رش نباتات القرنفل *Dianthus caryophyllus* L. بحامض الجبرليك بتركيز 300 ملغم.لتر$^{-1}$ أدى إلى زيادة قطر الزهرة وزيادة عدد البتلات فيها معنويا .

ونظراً للإقبال المتزايد على اقتناء مختلف أنواع الإزهار المقطوفة كنتيجة للتطور الاقتصادي والاجتماعي الحاصل في بلادنا العزيز وتداولها في كثير من المناسبات والأعياد الوطنية والاجتماعية. و الأهمية الاقتصادية الكبيرة لنباتات القرنفل ، فقد اتجهت النية إلى أجراء هذه الدراسة والتي تضمنت رش أربعة تراكيز مختلفة من منظمي النمو اندول حامض الخليك (IAA) وحامض الجبرليك (GA_3) على نبات القرنفل لدراسة تأثيرها على مؤشرات النمو وإمكانية تحسين الصفات النوعية لإزهار القطف ذات المواصفات التجارية لهذا النبات.

المواد وطرائق العمل

نفذ هذا البحث في الظلة الخشبية التابعة لقسم البستنة وهندسة الحدائق / كلية الزراعة – جامعة الكوفة في الفترة من 2009/9/15 لغاية 2010/6/1 ، لدراسة تأثير كل من اندول حامض الخليك (IAA) وحامض الجبرليك في صفات النمو لنبات القرنفل المعمر *Dianthus caryophyllus* L. صنف Chabaud ذو الإزهار القرمزية. أنتاج شركة Semillas-fito الاسبانية.

زرعت البذور بتاريخ 2009/9/15 في إطباق بلاستيكية (Plastic trays) ذات 72 جوره مملوءة بالبيت موس (Peat moss) وضعت داخل الظلة الخشبية ،تم نقل الشتلات بتاريخ 2009/11/15 ذات الزوجين من الأوراق الحقيقة وارتفاع 5–8سم في أصص بلاستيكية مملوءة بوسط زراعي معقم بمبيد فطري البنتانول 50% بتركيز (50 سم^3. 100 لتر$^{-1}$ ماء) للوقاية من الإصابة الفطرية(7) مكون من تربة مأخوذة من النهر والبيت موس (Peat moss) بنسبة 2 : 1 على التوالي وبمعدل 1.5 كغم وسط لكل أصيص. وقد تم اخذ عينة من تربة الدراسة قبل مزجها مع البيت موس واجري لها تحليلات كيميائية وفيزيائية في مختبرات كلية الزراعة/جامعة الكوفة وان الجدول (1) يوضح نتائج هذا التحليل. إما البيت موس فكانت صفاته مثبتة على الأكياس والجدول (2) يوضح الصفات الكيمائية للبيت موس إنتاج شركة Sab-Germany الألمانية. كذلك فقد أخذت المعدلات الشهرية لدرجات الحرارة العظمى والصغرى والرطوبة النسبية لمدنية النجف من محطة الأنواء الجوية في محافظة النجف (جدول 3)

جدول(1): الصفات الفيزيائية والكيميائية للتربة المستخدمة في الدراسة

نوع التحليل	القيمة/وحدة القياس
نسجه التربة	رملية غرينية
الرمل	700.8 غم.كغم$^{-1}$
الغرين	200.2 غم.كغم$^{-1}$
الطين	90.0 غم.كغم$^{-1}$
pH	7.71
EC	2.25 ديسي سيمنز.م$^{-1}$
مادة عضوية	1.70 غم.كغم$^{-1}$
Mg^{++}	8.0 ملي مول.لتر$^{-1}$
Ca^{++}	14 ملي مول.لتر$^{-1}$
Na^{+}	3.0 ملي مول.لتر$^{-1}$
K^{+}	1.0 ملي مول.لتر$^{-1}$
Cl^{-}	2.5 ملي مول.لتر$^{-1}$
$SO4^{=}$	20.0 ملي مول.لتر$^{-1}$
HCO_3^{-}	0.5 ملي مول.لتر$^{-1}$

جدول (2) الصفات الكيميائية للبتموس أنتاج شركة Sab-Germany الألمانية

Salt content $g.L^{-1}$	Ph	N $mg.L^{-1}$	P_2O_5 $mg.L^{-1}$	K_2O $mg.L^{-1}$
0.9–0.7	5.7–6.5	160–70	180–70	190–80

جدول (3) المعدلات الشهرية لدرجات الحرارة العظمى والصغرى والرطوبة النسبية لمدينة النجف لعام 2009–2010*

المعدلات / الشهر	درجة الحرارة العظمى (م°)	درجة الحرارة الصغرى (م°)	الرطوبة النسبية (%)
أيلول	40.2	25.1	35.1
تشرين الأول	35.8	20.6	47.7
تشرين الثاني	24.6	12.1	57.7
كانون الأول	21.4	9.4	69.5
كانون الثاني	21.6	8.1	62.8
شباط	23.2	10.3	56.5
آذار	28.8	14.6	49.0
نيسان	32.1	18.5	50.2
آيار	40.0	24.5	21.2
حزيران	45.0	29.0	16.2

أجريت كافة العمليات الزراعية المتبعة في تربية هذا النبات من قبل منتجي ازهار القطف التجارية بشكل كامل لكل الوحدات التجريبية وكلما دعت الحاجة لذلك وتضمنت الري والتسميد بالسماد الكيميائي المركب (N.P.K)20:20:20 والذي رش على النباتات بتركيز 600 ملغم.لتر$^{-1}$ بواقع ثلاث رشات وبفارق شهر بين رشة وأخرى وقرط للقمم النامية بعد تكوين النبات لسبعة أزواج من الأوراق الحقيقة بعدها تم انتخاب أربعة أفرع بعد وصول التفرعات الجانبية إلى أطوال (5–7)سم وإزالة البراعم الجانبية بشكل مستمر عند ظهور البراعم الزهرية مع ترك برعم طرفي واحد لكل فرع لغرض الوصول إلى الحجم الجيد للازهار , وقد تمت إزالة البراعم الجانبية حينما إلى حجم يسمح مسكها باليد وبحجم ألحمصه (8) . تم تحضير محاليل اندول حامض الخليك(IAA) بإذابة 25 ملغم من المسحوق النقي منIAA في 20 مل من الكحول الاثيلي بتركيز 95% وأكمل الحجم إلى لتر بالماء المقطر للحصول على التركيز 25 ملغم.لتر$^{-1}$ وأضيف مع كل تركيز 1 سم^3 من مادة الزاهي كمادة ناشرة وتم تحضير التراكيز الأخرى بنفس الطريقة إي بوزن(50 ، 75) ملغم منIAA و (100 ، 200 ، 300) ملغم من GA_3 للحصول(50 ، 75) ملغم.لتر$^{-1}$ من IAA و (100 ، 200 ، 300) ملغم.لتر$^{-1}$ من GA_3. واستعملت المرشة اليدوية(سعة 2 لتر) في إجراء عمليات الرش للمعاملات ، إذ تم الرش عند الصباح الباكر بالنسبة إلى اندول حامض الخليك(IAA) حتى حصول البلل التام للنبات إما حامض الجبرليك(GA_3) فقد كان الرش قبل غروب الشمس بسبب ارتفاع درجات الحرارة ولإعطاء الوقت الكافي لإزالة اثر محلول المعاملة السابقة من سطح النباتات ، كما رشت معاملة المقارنة بالماء المقطر فقط ورعي أثناء عملية الرش غسل المرشة اليدوية بالماء المقطر بعد كل معاملة لإزالة إي اثر لمحلول المعاملة السابقة حتى لا يؤثر في تركيز المعاملة التي تليها وفصل المعاملات باستعمال حاجز لتجنب الرذاذ المتطاير بين المعاملات المتجاورة وتمت عملية الرش بعد إن أجريت عملية السقي ولكلا الموقعين قبل يوم واحد من عملية الرش لزيادة كفاءة النباتات في امتصاص المادة المرشوشة (9). رشت النباتات بمنظمي النمو أعلاه وبواقع ثلاث رشات الأولى بتاريخ 2010/3/1 والثانية بتاريخ 2010/3/15 و والثالثة 2010/4/1 .

نفذت تجربة عامليه(4×4) بعاملين الأول أربعة تراكيز من IAA هي (75,50,25,0) ملغم.لتر$^{-1}$ الثاني أربعة تراكيز من GA3 هي (300,200,100,0) ملغم.لتر$^{-1}$ وفق تصميم القطاعات العشوائية الكاملة Completely Randomized Block Design (R.C.B.D.) وبثلاثة مكررات والمكرر عبارة عن (16) وحدة تجريبية وبواقع خمسة أصص لكل وحدة تجريبية. وتمت مقارنة المتوسطات وفق اختبار دنكن متعدد الحدود Duncan's Multiple Range Test وتحت مستوى احتمال 0.05 (10).

وفي نهاية التجربة تم دراسة الصفات الآتية :-

1.ارتفاع النبات (سم)

2.المساحة الورقية(سم^2)

تم حساب المساحة الورقية وفقا للمعادلة الآتية الواردة في (11) وهي :

المساحة الورقية (LA): LW × 0.91

L = طول الورقة (سم) W = عرض الورقة (سم)

3.الوزن الجاف للمجموع الخضري(غم)

4.محتوى الأوراق من الكلوروفيل الكلي(ملغم.100غم$^{-1}$ وزن طري)

تم تقدير صبغة الكلوروفيل حسب طريقة (12). بأخذ عينة 1غم من الزوج الثالث للأوراق وأضيف له 10 مل من الأسيتون 85% وسحق النسيج بالهاون حتى ابيِّض النسيج ثم رشحت باستعمال ورق الترشيح وأكمل حجم الراشح إلى 100 مل بالأسيتون،وبواسطة جهاز UV-visible spectrophotometer المنتج من قبل شركة Shimadzu الألمانية تم قياس الامتصاص الضوئي للصبغة وعلى طوليين موجيين هما (645 و 663) نانوميتر ثم حسبت كمية الصبغة (ملغم.100 غم$^{-1}$ نسيج ورقي طازج) بتطبيق المعادلة الآتية:

$$\text{Total chlorophyll} = 20.0 \times D_{(645)} + 8.02 \times D_{(663)} \quad (v/w \times 1000)$$

5.عدد الجذور الرئيسة (جذر. نبات$^{-1}$) : بعد فصل المجموع الخضري عن الجذري وضع المجموع الجذري في وعاء كبير مملوء بالماء لحين فصل جميع الأتربة من حول الجذور جميع الأتربة بعدها غسل وتم قياس عدد الجذور .

6. الوزن الجاف للجذور(غم)

7. محتوى الأوراق من اندول حامض الخليك وحامض الجبرليك(ملغم.كغم$^{-1}$ وزن طري)

تمت عملية التقدير الكمي لهرموني النمو(IAAو GA3)وفق الطريقة التي اتبعها(13) وبواسطة جهاز

UV-visible spectrophotometer المنتج من قبل شركة Shimadzu الألمانية وعلى طول موجي 280 نانوميتر لهرمون IAA و254 نانوميتر لهرمون GA3 في مختبر الدراسات العليا–كلية الزراعة /جامعة الكوفة.

8 – محتوى الأوراق من الكاربوهيدرات الذائبة الكلية (ملغم.100غم$^{-1}$ وزن جاف): تم تقدير الكاربوهيدرات وفق الطريقة التي اتبعها (14) وبواسطة جهاز UV-visible spectrophotometer المنتج من قبل شركة Shimadzu الألمانية وعلى طول موجي 490 نانوميتر .

9– **طول الساق الزهرية (سم):** قيست أطوال السيقان الزهرية لكل الإزهار المتكونة بعد تفتحها تفتحا كاملاً ابتداءً من منطقة اتصال الساق الزهرية بالساق الرئيس وحتى القاعدة السفلية للتخت باستعمال شريط القياس.

10– قطر الازهار(سم): قيس قطر الازهار عند التفتح التام لها بين ابعد نقطتين في القطر وبشكل متقاطع بواسطة القدمة Veriner caliper وسجل معدلها .

11– عدد البتلات (زهرة .نبات$^{-1}$).

12 – الوزن الجاف للزهرة(غم).

13– العمر المزهري للازهار (يوم) : قطفت أربع ازهار من كل معاملة في المرحلة التي كان فيها البتلات متوازية مع سيقانها الزهرية من منطقة اتصالها بالساق الرئيس للنبات ، وقد تمت عملية القطف في الصباح الباكر (15) ثم وحدت أطوال السيقان الزهرية إلى 30 سم (16) ووضعت الازهار مباشرة في أواني نظيفة ومعقمة ومتساوية في الحجم كانت قد ملئت بالماء المقطر وأضيف اليها 1% سكر و $\frac{1}{2}$ قرص أسبرين لكل أناء الذي كان يحوي على المادة الفعالة (حامض السليسليك) (17) ومبيد فطري غير سام للازهار لمنع نمو الفطريات وكان قد ضبط pH المحلول على 4.5 (18) , وكان الماء يستبدل كل يوم ، وحسب عدد الأيام التي بقيت فيها الزهرة بحالة نضرة في جو الغرفة الاعتيادي مع الاحتفاظ بحيويتها بحيث تصلح للاستعمال في التنسيق .

النتائج والمناقشة

يلاحظ من الجدول(4) أن الرش بمنظم النمو أثر ايجابياً في صفات المجموع الخضري ,إذ تفوقت معاملة الرش بانـدول حامض الخليك تركيز (50 ملغم.لتر$^{-1}$) بإعطاء أعلى معدل لارتفاع النبات ,المساحة الورقية ,الوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي بلغ(71.5) سم ,(12.1) سم^2 ,(7.1) غم و(52.9) ملغم.100غم$^{-1}$ وزن طري مقارنة بأقل معدل (58.1) سم ,(8.0) سم^2 ,(5.7) غم و(46.6) ملغم.100غم$^{-1}$ وزن طري وعلى التوالي والذي نتج من معاملة المقارنة. وقد يعزى ذلك إلى إن الاوكسين يعمل على زيادة انقسام الخلايا واستطالتها (19 و 20) مما يؤدي بالنهاية إلى زيادة ارتفاع النبات. كما إن الاوكسين يؤثر على ليونة جدار الخلية إذ يؤثر على المطاطية Elasticity واللدونة للجدار الخلوي Plasticity (21) إضافة الى انه يساعد على تنشيط نوع معين من الجينات لبناء RNA المهم لبناء البروتينات (2) وهذا ينعكس بالنهاية على زيادة المساحة الورقية للنبات والوزن الجاف للمجموع الخضري للنبات .ومن جهة أخرى ذكر (22) إن الاوكسينات هي من هرمونات الصبا التي تقلل من هرمونات الشيخوخة التي تحلل الكلوروفيل.وهذا بدورة ينعكس على زيادة محتوى الأوراق من الكلوروفيل.

كذلك يلاحظ من الجدول (4) وجود فروقات معنوية في عدد الجذور الرئيسة والوزن الجاف للمجموع الجذري للنبات نتيجة المعاملة بمنظم النمو ، إذ تفوقت النباتات المعاملة باندول حامض الخليك بتركيز (50 ملغم.لتر$^{-1}$) بإعطاء اكبر عدد للجذور الرئيسة والوزن الجاف للجذور بلغ (16.8) جذر و(0.87) غم مقارنة بأقل عدد للجذور الرئيسة ووزن جاف للجذور بلغ (12.8) جذر و(0.74) غم وعلى التوالي والذي نتج من معاملة المقارنة.ويعزى ذلك لدور منظمات النمو ومنها الاوكسين في زيادة نشاط عملية البناء الضوئي نتيجة زيادة المساحة الورقية مما يضمن وجود كميات جيدة من نواتج البناء الضوئي لأجراء الفعاليات الحيوية في النبات وبالتالي زيادة نمو الجذور (23) ، وهذا ما قد ينعكس بالنهاية على زيادة عدد الجذور الرئيسة والوزن الجاف للمجموع الجذري.

أدى رش النباتات بحامض الجبرليك الى زيادة معنوية في ارتفاع النبات والمساحة الورقية والوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي مقارنة بمعاملة المقارنة ، اذ تفوق التركيز (300 ملغم.لتر$^{-1}$) بإعطاء أعلى معدل لارتفاع النبات,المساحة الورقية , الوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل بلغ (76.1) سم , (11.0) سم ، (6.7) غم و(50.7) ملغم.100غم$^{-1}$ وزن طري قياسا بمعاملة المقارنة والتي أعطت اقل معدل لارتفاع للنبات ,المساحة الورقية,الوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل إذ بلغت (54.7) سم ,(6.8) سم^2 ,(5.1) غم و(45.7) ملغم.100غم$^{-1}$ وزن طري وعلى التوالي (جدول7). وقد تفسر نتائج ذلك الى ان الرش بالجبرلين يسبب رفع مستوى الجبرلينات الداخلية في النبات (جدول7) ، والتي تعمل على زيادة نفاذية جدران الخلايا وجعلها مركز استقطاب Sink للمواد الغذائية مما يزيد من قابليتها على الانقسام والاستطالة (27) وهذا بدوره قد يؤدي الى زيادة ارتفاع النبات.فضلا عن ان الجبرلين يساعد على انتقال المواد الغذائية من الجذور وتوجيهها نحو النمو الخضري والأوراق مما يزيد من نمو الخلايا واتساعها (28). ويعمل بالنهاية على زيادة المساحة الورقية للنبات والتي تعني زيادة البلاستيدات الخضراء وبالتالي زيادة محتوى الأوراق من الكلوروفيل وهذا بدوره قد ينعكس على زيادة الوزن الجاف للمجموع الخضري . وتتفق هذه النتيجة مع ما وجدته (29) على نباتات Lisianthus و (30) على نبات القرنفل من ان الرش بحامض الجبرليك زاد من المساحة الورقية للنبات والوزن الجاف للمجموع الخضري .

كما يلاحظ من الجدول (4) تفوق النباتات التي رشت بحامض الجبرليك معنوياً عن تلك التي لم ترش في صفة عدد الجذور الرئيسة والوزن الجاف للجذور ، اذ أعطت النباتات المعاملة بحامض الجبرليك تركيز (300 ملغم.لتر$^{-1}$) اكبر معدل لعدد الجذور الرئيسة والوزن الجاف للجذور بلغ (16.1) جذر و(0.84) غم مقارنة بمعاملة المقارنة التي أعطت اقل معدل بلغ (12.2) جذر و(0.70) غم وعلى التوالي.وقد يعزى ذلك للأسباب نفسها التي ذكرت في زيادة الصفات أعلاه عند معاملة النباتات باندول حامض الخليك وهذا يتفق مع ما توصل إليه (5) على نبات المنثور و (31) على نبات الفوجير *Nephrolepis exaltata* L. و (30) على نبات القرنفل.

جدول(4) تأثير الرش باندول حامض الخليك وحامض الجبرليك والتداخل بينهما في صفات النمو الخضري والجذري لنبات القرنفل

تراكيز اندول حامض الخليك			ارتفاع النبات(سم)	المساحة الورقية(سم^2)	الوزن الجاف للمجموع الخضري (غم/نبات)	محتوى الأوراق من الكلوروفيل الكلي (ملغم. 100 غم$^{-1}$ وزن طري)	عدد الجذور الرئيسة (جذر/نبات)	الوزن الجاف للمجموع الجذري (غم/نبات)
(IAA) (ملغم.لتر$^{-1}$)	0		58.1 c	8.0 d	5.7 c	46.6 c	12.8 c	0.74 d
	25		66.4 b	9.7 b	6.1 b	48.5 b	14.6 b	0.78 c
	50		71.4 a	12.1 a	7.1 a	52.9 a	16.8 a	0.87 a
	75		62.6 b	9.3 c	6.1 b	48.7 b	14.0 b	0.80 b
(GA_3) (ملغم.لتر$^{-1}$)	0		54.7 c	6.8 c	5.1 b	45.7 b	12.2 c	0.70 c
	100		65.1 b	10.7 b	6.3 a	50.3 a	14.9 b	0.80 b
	200		66.1 ab	10.6 b	6.5 a	50.0 a	15.0 b	0.84 a
	300		76.1 a	11.0 a	6.7 a	50.7 a	16.1 a	0.84 a
× IAA GA_3	0	0	48.1 h	5.8 i	4.6 c	43.1 c	9.9 d	0.63 b
		100	55.7 e	6.8 h	5.2 bc	46.1 b	12.0cd	0.65 d
		200	63.1 e	8.9 e	6.0 b	48.1 b	14.4 bc	0.82 a
		300	65.3 de	10.5 c	6.6 ab	48,9 b	14.8 bc	0.83 a
	25	0	54.1 g	6.1 h	4.7 c	45.7 c	12.6 cd	0.63 d
		100	61.9 f	9.7 d	6.0 b	47.8 b	14.3 bc	0.81 a
		200	67.5 d	11.1 b	6.7 ab	49.4 b	15.2 b	0.84 a
		300	70.2 c	11.6 b	6.9 a	51.1 ab	16.0 b	0.84 a
	50	0	56.6 fg	7.2 g	5.5 b	46.5 b	13.0 c	0.76 ab
		100	72.2 b	13.4 a	7.2 a	54.5 a	16.6 b	0.89 a
		200	73.1 b	13.7 a	7.5 a	54.8 a	16.7 b	0.91 a
		300	78,8 a	13.9 a	7.9 a	55.7 a	20.8 a	0.92 a
	75	0	59.8 f	8.0 f	5.7 b	47.5 b	13.3 c	0.79 ab
		100	70.7 b	12.6 ab	7.0 a	52.8 ab	16.3 b	0.85 a
		200	60.5 f	8.7 e	5.8 b	47.6 b	13.3 c	0.79 ab
		300	67.1 f	7.7 g	5.6 b	49.9 b	13.0 c	0.76 ab

*المعدلات التي تحمل أحرفا متشابهة ضمن العمود الواحد لا تختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود وعلى مستوى احتمال 0.05

يتضح من نتائج جدول (4) ان رش النباتات باندول حامض الخليك مع حامض الجبرليك ادى إلى حدوث زيادة معنوية في ارتفاع النبات ,المساحة الورقية ,الوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي ، فقد أعطت النباتات التي رشت باندول حامض الخليك بتركيز(50 ملغم.لتر$^{-1}$) مع حامض الجبرليك بتركيز (300 ملغم.لتر$^{-1}$) أعلى معدل لهذه الصفات آذ بلغت (78.8) سم ,(13.9) سم^2 ,(7.9) غم و(55.7) ملغم.100غم$^{-1}$ وزن طري مقارنة بأقل معدل بلغ (48.1) سم,(5.8) سم^2 , (4.6) غم و(43.1) ملغم.100غم$^{-1}$ وزن طري وعلى التوالي والذي نتج من رش النباتات بالماء المقطر فقط .

كذلك ادى تداخل الرش بالمنظمين الى تأثير معنوي في صفة عدد الجذور الرئيسة والوزن الجاف للجذور ، اذ أعطى تداخل رش النباتات باندول حامض الخليك بتركيز(50 ملغم.لتر$^{-1}$) مع حامض الجبرليك بتركيز (300 ملغم.لتر$^{-1}$) اكبر معدل لهذه الصفتين بلغت (20.8) جذر و(0.92)غم مقارنة بأقل معدل لها بلغ (9.9) جذر و(0.63) غم وعلى التوالي والذي نتج من عدم الرش بأي من المنظمين (جدول 4).

جدول (5) تأثير الرش باندول حامض الخليك وحامض الجبرليك والتداخل بينهما في محتوى الأوراق من اندول حامض الخليك وحامض الجبرليك والكاربوهيدرات الذائبة الكلية لنبات القرنفل

تراكيز اندول حامض الخليك (IAA) (ملغم.لتر$^{-1}$)			محتوى الأوراق من اندول حامض الخليك (IAA) (ملغم.كغم$^{-1}$ وزن طري)	محتوى الأوراق من حامض الجبرليك (GA3) (ملغم.كغم$^{-1}$ وزن طري)	محتوى الأوراق من الكاربوهيدرات الذائبة الكلية (ملغم.100 غم$^{-1}$وزن جاف)
(IAA) (ملغم.لتر$^{-1}$)	0		67.81 c	16.50 c	35.71 d
	25		67.82 b	16.54 b	45.56 b
	50		67.84 a	16.61 a	56.57 a
	75		67.82 b	16.54 b	43.50 c
(GA_3) (ملغم.لتر$^{-1}$)	0		67.80 b	16.46 d	39.5 c
	100		67.83 a	16.59 b	42.2 b
	200		67.83 a	16.58 c	41.6 b
	300		67.83 a	16.59 a	58.0 a
IAA × GA_3	0	0	67.78 i	16.38 n	40.66 g
		100	67.80 hi	16.47 m	48.61 f
		200	67.82 e	16.56 g	54.87 cd
		300	67.83 d	16.57 f	56.78 c
		25	67.79 h	16.45 k	46.66 g
		100	67.82 fg	16.43 m	53.44 d
		200	67.83 d	16.57 f	58.33 c
		300	67.84 cd	16.59 e	61.00 bc
		50	67.81 f	16.48 j	47.22 f
		100	67.84 cd	16.63 c	63.11 b
		200	67.85 b	16.66 b	64.50 b
		300	67.86 a	16.68 a	70.55 a
		75	67.81 g	16.51 I	61.00 b
		100	67.847 c	16.62 d	61.67 b
		200	67.82 e	16.52 h	51.44 d
		300	67.81 g	16.51 i	50.17 e

*المعدلات التي تحمل احرفا متشابهة ضمن العمود الواحد لاتختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود وعلى مستوى احتمال 0.05

جدول(6) تأثير الرش باندول حامض الخليك وحامض الجبرليك والتداخل بينهما في صفات النمو الزهري لنبات القرنفل

تراكيز اندول حامض الخليك (IAA) (ملغم.لتر$^{-1}$)			طول الساق الزهرية(سم)	قطر الزهرة (سم)	عدد البتلات (بتلة.زهرة$^{-1}$)	الوزن الجاف للزهرة(غم)	العمر المزهري (يوم)
(IAA) (ملغم.لتر$^{-1}$)	0		50.2 c	4.2 d	27.1 c	0.38 c	3.3 c
	25		54.9 b	4.5 b	30.8 b	0.40 b	4.1 b
	50		62.6 a	5.1 a	34.3 a	0.44 a	5.5 a
	75		56.1 b	4.3 c	29.5 b	0.40 a	3.9 b
(GA_3) (ملغم.لتر$^{-1}$)	0		48.9 c	3.8 c	28.7 b	0.37 b	3.5 c
	100		56.7 b	4.6 b	28.8 b	0.41 a	3.9 b
	200		57.2 b	4.8 a	29.2 b	0.42 a	3.7 b
	300		60.8 a	4.8 a	34.9 a	0.42 a	5.6 a
IAA × GA_3	0	0	40.6 g	3.5 h	24.5 e	0.36 b	2.5 f
	100		48.6 f	3.8 f	25.5 e	0.37 b	2.8 f
	200		54.8 cd	4.6 d	25.1 e	0.40 ab	3.0 d
	300		56.8 c	4.7 cd	32.9 c	0.41 a	5.0 b
	0	25	46.6 e	3.7 g	28.2 d	0.37 b	3.0 d
	100		53.4 d	4.6 cd	31.5 cd	0.40 a	4.3 c
	200		58.3 c	4.7 cd	28.6 de	0.41 a	3.0 d
	300		61.0 bc	4.8 c	35.0 b	0.41 a	6.0 a
	0	50	47.2 f	3.9 f	32.7 c	0.38 b	4.6 c
	100		63.1 b	5.1 b	33.2 c	0.45 a	5.6 b
	200		64.5 b	5.3 b	31.7 cd	0.45 a	5.0 b
	300		70.5 a	5.9 a	39.2 a	0.47 a	6.6 a
	0	75	61.0 bc	4.1 e	29.1 d	0.39 b	4.0 c
	100		61.6 b	4.8 c	26.5 e	0.42 a	3.0 d
	200		51.4 d	4.4 d	30.1 d	0.40 ab	3.8 cd
	300		50.1 e	4.0 e	32.2 c	0.38 b	4.8 c

*المعدلات التي تحمل احرفا متشابهة ضمن العمود الواحد لاتختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود وعلى مستوى احتمال 0.05

يتضح من نتائج جدول (4) ان رش النباتات باندول حامض الخليك مع حامض الجبرليك ادى إلى حدوث زيادة معنوية في ارتفاع النبات ,المساحة الورقية ,الوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي ، فقد أعطت النباتات التي رشت باندول حامض الخليك بتركيز(50 ملغم.لتر$^{-1}$) مع حامض الجبرليك بتركيز (300 ملغم.لتر$^{-1}$) أعلى معدل لهذه الصفات آذ بلغت (78.8) سم ,(13.9) سم^2 ,(7.9) غم و(55.7) ملغم.100غم$^{-1}$ وزن طري مقارنة بأقل معدل بلغ (48.1) سم,(5.8) سم^2 , (4.6) غم و(43.1) ملغم.100غم$^{-1}$ وزن طري وعلى التوالي والذي نتج من رش النباتات بالماء المقطر فقط .

كذلك ادى تداخل الرش بالمنظمين الى تأثير معنوي في صفة عدد الجذور الرئيسة والوزن الجاف للجذور ، اذ أعطى تداخل رش النباتات باندول حامض الخليك بتركيز(50 ملغم.لتر$^{-1}$) مع حامض الجبرليك بتركيز (300 ملغم.لتر$^{-1}$) اكبر معدل لهذه الصفتين بلغت (20.8) جذر و(0.92)غم مقارنة بأقل معدل لها بلغ (9.9) جذر و(0.63) غم وعلى التوالي والذي نتج من عدم الرش بأي من المنظمين (جدول 4).

ومن نتائج الجدول (5) يتبين إن رش النباتات باندول حامض الخليك قد اثر معنوياً في زيادة محتوى الأوراق من اندول حامض الخليك وحامض الجبرليك والكاربوهيدرات الذائبة الكلية ، إذ تفوق التركيز 50 ملغم.لتر$^{-1}$ بإعطاء أعلى معدل لمحتواها من اندول حامض الخليك وحامض الجبرليك بلغ (67.84) ملغم.كغم$^{-1}$ وزن طري و(16.61) ملغم.كغم$^{-1}$ وزن طري و(56.57) ملغم.100 غم$^{-1}$وزن جاف قياساً بأقل معدل بلغ (67.81) ملغم.كغم$^{-1}$ وزن طري و(16.50) ملغم.كغم$^{-1}$ وزن طري و(35.71) ملغم.100 غم$^{-1}$وزن جاف وعلى التوالي والذي نتج من معاملة المقارنة ، وقد يعزى ذلك الى إن عملية الرش بهذا المنظم تؤدي الى زيادة نسبته في الأوراق عن طريق المحافظة على الاوكسينات الداخلية من التحطم, كما إن للاوكسينات و الجبرلينات دوراً تعاضدياً في زيادة احدهما للاخر (24). كذلك فقد تعزى الزيادة في محتوى الارواق من الكاربوهيدرات الذائبة الكلية نتيجة رش النباتات باندول حامض الخليك بتركيز 50 ملغم.لتر$^{-1}$ إلى الزيادة في المساحة الورقية والوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي (الجدول 3) ، وهذا بدوره ينعكس على كفاءة عملية البناء الضوئي وبالتالي زيادة المواد الغذائية المصنعة في الأوراق وزيادة كمية الكاربوهيدرات المصنعة ، حيث ذكر (25) إن زيادة المساحة الورقية ومحتوى الأوراق من الكلوروفيل سيؤدي إلى تصنيع المواد الغذائية في الأوراق وبالتالي زيادة كمية الكاربوهيدرات فيها .

يشير الجدول (5) إلى ان كل تراكيز الجبرلين تفوقت على معاملة المقارنة إلا إن أعلى معدل لمحتوى الأوراق من اندول حامض الخليك كان عند التركيز 300 ملغم.لتر$^{-1}$ بلغ 67.83 ملغم.كغم$^{-1}$ وزن طري مقارنة بأقل معدل 67.80ملغم.كغم$^{-1}$ وزن طري نتج من معاملة المقارنة, إما بالنسبة لمحتوى الأوراق من حامض الجبرليك فقد بلغ أعلى معدل 16.59 ملغم.كغم$^{-1}$ وزن طري عند التركيز 300 ملغم.لتر$^{-1}$ مقارنة بمعاملة المقارنة التي أعطت اقل معدل بلغ 16.46ملغم.كغم$^{-1}$ وزن طري كما ازداد محتوى الأوراق من الكاربوهيدرات الذائبة الكلية عند رش الجبرلين بتركيز 300 ملغم.لتر$^{-1}$ إذ بلغ 58.0 ملغم.100 غم$^{-1}$ وزن جاف مقارنة بمعاملة المقارنة والتي أعطت اقل قيمة بلغت 39.5 ملغم.100 غم$^{-1}$ وزن جاف وعلى التوالي . وقد تفسر زيادة محتوى الأوراق من اندول حامض الخليك وحامض والجبرليك إلى إن ارتفاع مستوى الاوكسين يكون بواسطة فعالية الجبرلينات ، حيث تؤدي الجبرلينات الى خفض الاوكسينات غير الحرة وزيادة تكوين وإنتاج الاوكسينات الحرة ، وان للجبرلينات اثراً تعاضدياً في زيادة الاوكسينات عن طريق المحافظة عليها من التحطم (24) , كما إن الزيادة في محتوى الأوراق من الكاربوهيدرات قد تعود إلى نفس الأسباب التي ذكرت في زيادة محتوى من الكاربوهيدرات نتيجة الرش باندول حامض الخليك .

يُلاحظ من الجدول (5) التأثير المعنوي للتداخل بين الرش باندول حامض الخليك بتركيز (50 ملغم.لتر$^{-1}$) مع الرش بحامض الجبرليك بتركيز (300 ملغم.لتر$^{-1}$) في زيادة محتوى الأوراق من اندول حامض الخليك وحامض الجبرليك والكاربوهيدرات الذائبة الكلية ، اذ أعطى هذا التداخل أعلى المعدلات بلغت (67.78) ملغم.كغم$^{-1}$ وزن طري و (16.38)ملغم.كغم$^{-1}$ وزن طري و(70.55) ملغم.100 غم$^{-1}$وزن جاف مقارنة بأقل المعدلات بلغت (67.81) ملغم.كغم$^{-1}$ وزن طري و(16.59)ملغم.كغم$^{-1}$ وزن طري و(40.66) ملغم.100 غم$^{-1}$وزن جاف وعلى التوالي والذي نتج من معاملة المقارنة (الرش بالماء المقطر فقط).

يُظهر الجدول (6) التأثير الايجابي للرش بمنظم النمو في طول الساق الزهرية للنبات,عدد البتلات,الوزن الجاف للزهرة والعمر المزهري للازهار ، إذ تفوقت النباتات التي رشت باندول حامض الخليك معنوياً في هذه الصفات مقارنة مع تلك التي لم ترش وتميزت النباتات التي رشت بالتركيز (50 ملغم.لتر$^{-1}$) بإعطاء أعلى معدل لطول الساق الزهرية, قطر الازهار , عدد البتلات,الوزن الجاف للزهرة والعمر مزهري اذ بلغت (62.6) سم, (5.1) سم , (34.3) بتله ,(0.44)غم و(5.5)يوما قياساً بمعاملة المقارنة التي أعطت اقل القيم اذ بلغت (50.2) سم , (4.2) سم , (27.1) بتله ,(0.39) غم و(3.3) يوماً وعلى التوالي. وقد يعود السبب إلى إن لمنظمات النمو تؤثر في انقسام الخلايا (21) فضلاً عن دورها في نقل المغذيات (26) مما سبب بالنهاية زيادة طول الساق الزهرية وعدد البتلات وقطر الزهرة و الوزن الجاف للازهار.كما ان لدور الاوكسين في زيادة المساحة الورقية ومحتوى الأوراق من الكلوروفيل (جدول 3) مما عمل على زيادة المحتوى الكاربوهيدراتي للنبات نتيجة تحسين كفاءة عملية التركيب الضوئي مما انعكس بالنهاية على اطالة العمر المزهري للازهار في المزهرية.

لقد كان لمعاملة النباتات بحامض الجبرليك تأثير معنوي في صفة طول الساق الزهرية وعدد البتلات والوزن الجاف للزهرة والعمر المزهري للازهار ، اذ أعطى الرش بتركيز (300 ملغم.لتر$^{-1}$) أعلى معدل لطول الساق الزهرية, قطر الازهار , عدد البتلات ,الوزن الجاف للزهرة والعمر المزهري إذ بلغت (60.8) سم , (4.8) سم , (34.9) بتله ,(0.42) غم و(5.6) يوما مقارنة بأقل معدل لهذه الصفات بلغ (48.9) سم, (3.8) سم , (28.7) بتله ,(0.37) غم و(3.5) يوماً وعلى التوالي والذي نتج من معاملة المقارنة, على التوالي (جدول 9). وقد يعزى ذلك للأسباب نفسها التي ذكرت سابقاً في زيادة معدل الصفات اعلاه عند الرش باندول حامض الخليك.وهذا يتفق مع ما وجدته (32) على نبات الاقحوان ومع ما وجده (5) على نبات المنثور من ان رش النباتات بحامض الجبرليك أدى إلى زيادة الوزن الجاف والعمر المزهري للازهار.

ويلاحظ من الجدول(6) تأثير التداخل بين انـدول حـامض الخليك وحامض الجبرليك اذ تفوقت النباتات التـي رشـت بتركيز (50 ملغم.لتر$^{-1}$) من اندول حـامض الخليـك مـع (300 ملغم. لتر$^{-1}$) من حامض الجبرليك معنوياً بإعطـاء أعلى معدل لطول الساق الزهريـة,قطـر الزهـرة , عـدد البتلات,الوزن الجاف للزهرة والعمر المزهري للازهـار اذ بلغت (70.5) سم, (5.9) سم , (39.2) بتله , (0.47) غم و(6.6) يوماً مقارنة بأقل المعدلات اذ بلغت (40.6) سـم, (3.5)سم , (24.5) بتله , (0.36) غم و(2.5) يوماً والـذي نتجت من معاملة المقارنة التي رشت بالماء المقطر فقـط. وقد يعزى ذلك إلى الدور الايجابي لكل من اندول حـامض الخليك وحامض الجبرليك في زيادة قيم هذه الصفات أو قـد يعزى إلى التركيز الملائم من اندول حامض الخليك الـذي ادى إلى زيادة تأثير حامض الجبرليك في النبات حيث ذكـر (33) و (34) ان معاملة النباتات بحامض الجبرليك مرافقـاً لاندول حامض الخليك بتراكيز قليلة منه تؤدي الـى زيـادة تأثير الجبرلين في حين تعمل التراكيز العالية مـن انـدول حامض الخليك على تقليل الاسـتجابة للمعاملـة بـالجبرلين وإبطال تأثيره.

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The title page should include a concise and informative title, author names in full, and affiliations. The name of the corresponding author as well as his/her mailing address, telephone and fax numbers, and e-mail address should be provided in a footnote.

Abstract

The abstract should be one paragraph, no longer than 250 words. No references should be cited in the abstract. Abbreviations should be avoided, but if they have to be used, they must be defined the first 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.

General Arrangement of Text

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.

1. Introduction

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.

McCarthy, AJ. 1989. Thermomonospora. In: Bergey's Manual of Systematic Bacteriology (ed. Williams ST, Sharpe ME, Holt JG), Vol. 4, pp. 2552-2572. Williams and Wilkins, Baltimore, MD.

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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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