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IJST contact Information:
P.O. Box 2793 Amman 11953 Jordan
Tel. +96265602285
E-mails: info@icast-jo.com / info@ijst-jo.com
URL: www.ijst-jo.com

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FORWARD

It is my pleasure to welcome you back and present you this issue, Volume 5 , No. 2 (2010), of ***International Journal for Sciences and Technology (IJST)***. The members of Editorial Board, the ICAST and TSTC team work and I hope you will find this collection of research articles useful and informative.

In this issue, I am pleased to welcome the new elite members of the Editorial Board who have joined us to add another scientific dimension to the journal by wide diversity and more experiences in fields of sciences and technology.

The journal is one of the scientific contributions offered by ***the International Centre for Advancement of Sciences and Technology*** 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, Researchers and the Editorial Secretary for managing the scientific, design, technical and administration aspects of the Journal and for preparing this volume for final printing and publishing.

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A Comparative Study Between Adam-Bohart and Herkins-Jura Models for Adsorption of Pb(II) from Simulated Wastewater by Activated Carbon

Abbas H. Sulaymon, Hayder M. Abdul- Hameed

Environmental Eng. Dept. College of engineering / Baghdad University – Republic of Iraq

Email: hayder3almunshi@yahoo.com

ABSTRACT

In this research a comparison study was made between Adam-Bohart and Herkins-Jura model to determine which model give the best description for the adsorption of Pb(II) from simulated wastewater by activated carbon. Depending on the values of (R^2) and the HYBRID error function (HEF). It is found that Herkins-Jura model describe the equilibrium isotherm data better than Adam-Bohart model with (R^2) value of 0.986 and 0.737 for Herkins-Jura and Adam-Bohart model respectively and (HEF) of 0.272 and 0.484 for Herkins-Jura and Adam-Bohart respectively. Also if found the adsorption of Pb(II) from aqueous solution by activated carbon was of favourable type.

Keywords: *adsorption, activated carbon, Adam-Bohart model*

الملخص باللغة العربية

يهدف البحث الى اجراء دراسة مقارنة بين نموذج Adam-Bohart ونمـــوذج Herkins-Jura لمعرفة اي منهما يصف عملية امتزاز ايون ثنائي الرصاص من المياه الصناعية باستخدام االكربون المنشط . واعتمادا على قيم R^2 وكذلك قيم دالـــة الخطأ الهجينـــة HYBRID وجـــد ان نمـــوذج Herkins-Jura قد أوضح عملية الامتزاز ايون الرصاص الثنائي بـــشكل افـــضل مـــن نمـــوذج Adam-Bohart حيث ان قيمـــة R^2 لنمـــوذج Herkins-Jura كانت 0.986 بينما لنمـــوذج Adam-Bohart كانت 0.737 وان قيمة دالة الخطـــأ الهجينـــة كانـــت 0.272 لنمـــوذج Herkins-Jura و0.484 لنمـــوذج Adam-Bohart . وقد ثبت أافضلية عملية االمتزاز ايون الرصاص الثنائي بواسطة الكاربون المنشط .

INTRODUCTION

Lead and its salts are considered as one of the most important heavy metal elements that presented in the environment. (*1*) As this metal has a main role in different life activities and introduced in many industrial, technological, mineral and petroleum industries (*2)*

Lead salts presented in the municipal wastewater effluent especially cities and towns that located near the chemical plants and processing industries. The major risk and concern of lead salts are their presence in the waste streams or drinking water (3). The presence of lead in water will add an odd taste to the waste even when the (Pb) concentration is around 1.2 mg/l (4).

In addition to that, the accumulation of (Pb) salts in the human body causes chronic defects and diseases that lead sometimes to death. Diseases such as kidney failure, liver disease, and heart disorder are related to the presence of lead salts in the human body with relatively high concentration reaches to (3-5) mg/l. The assimilation of relatively small amounts of lead over a long period of time in the human body can lead to the malfunctioning of the organs and chronic toxicity. The toxic effect of lead ions on humans, when present above the threshold level in the human body may become lethal (5).

Lead has been shown to cause brain damage in children, arthritis, urethrities and conjunctivitis even when its existence is in an extremely low concentration (*6*).

Environmental protection Agency (EPA), the world health organization (WHO) and the Iraqi standards had been setted the limits for different heavy metals concentrations in a wastewater, potable water and the rivers. See table (1) below:

Table (1), *Permissible limits of heavy metals concentration in drinking water, rivers and wastewater

Pollutants	Iraqi Standards			WHO			USEPA		
	drinking water mg/l	river mg/l	waste-water mg/l	drinking water mg/l	river mg/l	waste-water mg/l	drinking water mg/l	river mg/l	waste-water mg/l
Pb	0.005	0.05	0.05	Nil	0.03	0.05	<0.003	0.03	0.05
Cd	0.005	0.01	0.05	Nil	0.04	0.05	<0.003	0.04	0.05
Hg	0.005	<0.05	0.05	Nil	0.03	0.05	Nil	0.03	0.05
As	0.005	0.05	0.05	Nil	0.02	0.05	Nil	0.03	0.05
Cr	0.005	0.05	0.05	Nil	0.02	0.05	Nil	0.03	0.05

** Iraqi environmental legislations, Ministry of Environment (MOE), 2007.*

widely used process for removing different pollutant species from

water and wastewater. This technique offers easy operational, economical and high efficiency for removing of these pollutants from water (7).

The use of activated carbon as an adsorbent was recorded since 500 B.C when ancient Egyptians used charcoal for water deodorizing (8).

Due to it's excellent surface properties (high surface area per unit mass), high porosity and low cost of manufacturing, these properties make activated carbon a good adsorbent in spite of the presence of many adsorbents such as silica gel, bentonite, and saw dust (9).

(Zenden, 2004) (10) found that the adsorption of Pb(II) fitted well with Langmuir isotherm for different Pb(II) equilibrium concentrations.

(*Massako, 2005*) (11) revealed that for different pH values of Pb (II), the Frendlich isotherm model was not fitted the data.

The Adam-Bohart model is valid for a single layer adsorption. It is based on the assumption that maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface, and the energy of adsorption is constant (12).

The Adam-Bohart isotherm is used to describe the adsorption data. The basic assumptions underlying Adam-Bohart model are:

- Solute molecules are adsorbed onto definite sites on the adsorbent surface.
- Each site can be a host for only one molecule (monolayer).
- The area of each site is a fixed quantity determining the geometry of the surface.
- The adsorption energy is constant for all sites.
- The adsorbed molecules can not migrate across the surface or interact with other molecules.

Adam-Bohart model was widely used to describe the adsorption process onto activated carbon and can be expressed as:

$$q_e = \frac{a}{C_o} - \frac{b}{C_e} \ln\left(\frac{C_o}{C_e} - 1\right) \qquad \textbf{(1)}$$

Where;

q_e = amount of material adsorbed per amount of adsorbent (kg/kg).

C_e = equilibrium concentration (kg/m^3). C_o = initial concentration (kg/m^3). a, b = adsorption rate constants.

And by plotting q_e versus $1/C_e$ the value of (b) will be determined from the intersection of equation (1) with y-axis and the value of (a) may be calculated.

(Haussmann, 2005) found that Adam-Bohart model gave the best fit for equilibrium data of Cd (II) adsorption by silica gel. (13)

(Nadit, 2004) investigated that Adam-Bohart model expressed a good match for representing the equilibrium data for adsorption of Hg(II), Pb(II) from wastewater by cocoanut shell. (14)

The Herkins-Jura model is generally an extended isotherm from Adam-Bohart model and corresponds to multilayer adsorption. This model assumes that numbers of layers of adsorbate accumulate at the surface, and then the Adam-Bohart model is applied to each layer. Each additional layer of adsorbate molecules is assumed to equilibrate with the layer below it and layers of different thickness are allowed to coexist (15).

Herkins-Jura model which accounts for a multilayer adsorption and for existence of heterogeneous pore distribution in the adsorption can be expressed as:

$$\frac{1}{q_e} = \frac{B}{A} - \frac{1}{A}\log C_e \qquad \textbf{(2)}$$

By plotting $1/q_e^2$ versus log C_e the constant A, B can be determined.

In addition to above mentioned models, there are a number of isotherm models describing the adsorption processes. The properties of these isotherms with characteristics are summarized in table (2)

T ***Table (2) Adsorption isotherm models characteristics (16)***

Isotherm	Equation	Advantages	Disadvantages
Langmuir	$q_e = \frac{q_m b C_e}{1 + b C_e}$	interpretable parameters	not structured, monolayer-sorption
Freundlich	$q_e = K C_e^{1/n}$	simple expression	not structured, not leveling off
combination (Langmuir and Freundlich)	$q_e = \frac{b q_m C_e^{1/n}}{1 + b C_e^{1/n}}$	combination of above	unnecessarily complicated
Radke and Prausnitz	$q_e = \frac{K_{RP} C_e}{1 + (\frac{K_{RP}}{F_{RP}}) C_e^{1-N_{RP}}}$	simple expression	empirical uses 3 parameters
Reddlich and Peterson	$q_e = \frac{A_R C_e}{1 + B_R C_e^{m_R}}$	approach Freundlich high concentration	no special advantages
Dubinin – Raduskevch	$q_e = q_{DR} \exp(-\beta \in^2)$	give a percentage filling micropors	special uses
Temkin	$q_e = \frac{RT}{b} \ln(K_T C_e)$	involving the binding energy	uses for a restricted isothermal adsorption process
Harkins - Henderson	$q_e = \frac{K^{1/n}}{C_e^{1/n}}$	multilayer adsorption and heterogeneity	not use for a general adsorption

The parameters of each model were determine from the best fitting of the equation to the measured experimental data, and the judgment on the best fitting model depended on two techniques, firstly, adopt the highest value of the correlation coefficient (R^2) resulted from the implementation of each model, secondly, conducting the lowest value of the (HYBRID) fractional error (17).

The (HYBRID) fractional error function of non-linear regression is employed, as it compensates for low concentrations by balancing absolute deviation against fractional error and is more reliable than other error function. The (HYBRID) error function is given by:

$$\mathbf{HYBRID} = \frac{100}{N-p}\sum\left[\frac{q_{e,exp}-q_{e,calc.}}{q_{e,exp}}\right] \quad \textbf{(3)}$$

Where N is the number of data points and p is the number of parameters in the isotherm model. Applying the two mentioned techniques, in this research, the model with highest (R^2) values and lowest (HYBRID) error function considered as the best fit model.

The HYBRID error function is widely used in the statistical analysis for the environmental engineering problems, since this technique provide a solid tool for assessment of any reliable process that might be used for controlling any environmental process to ensure the degree of credibility of such process to be conducted and used for environmental pollution problems (18).

MATERIALS AND METHODS

The granular activated carbon (GAC) used was brought from the local market with the following physical properties mentioned by the manufacturer and tabulated in table (3):

Table (3) physical properties of GAC

Ash content	Less than 5%
Surface area	1600-1800 m^2/gm
Bulk density	750-780 kg/m^3
porosity	0.64

Activated carbon was firstly washed with distilled water and then put in an oven at 110 ^oC for 105 minute to remove all the moist content and humidity, then a sample of (GAC) was sieved using different sieve sizes with sieve

shaker and (GAC) with 0.4mm average particle size was used. Different weights of (GAC) of (2, 4, 6, 8, 10) gms were put in a (250) ml conical flask. The Pb (II) solution was prepared using Pb $(NO_3)_2$ solution, (2.41) gms of Pb $(NO_3)_2$ were dissolved in 3.75 liters of distilled water to prepare a solution with (30) mg/l of Pb (II) (19).

A sample of (100) ml was taken from the prepared solution and put in the conical flask of different (GAC) for (24) hrs in a rotary shaker and a sample of (25) ml was withdrawn from each flask for analysis using atomic absorption device type (Perkins 91005). The equilibrium data resulting from the analysis was tabulated in table (4).

Table (4) equilibrium concentration C_e mg/l and q_e for different mass of GAC

Sample No.	Weight of GAC, gm	C_e Pb(II) equilibrium concentration	q_e Kg of adsorbed / kg of (GAC)
1	2	52.9	0.0015
2	4	11	0.0055
3	6	5.36	0.0079
4	8	4.13	0.0089
5	10	2.73	0.014

By applying equation (1) and (2) on the data tabulated in table (1) the results are show in both figures (1) and (2) respectively.

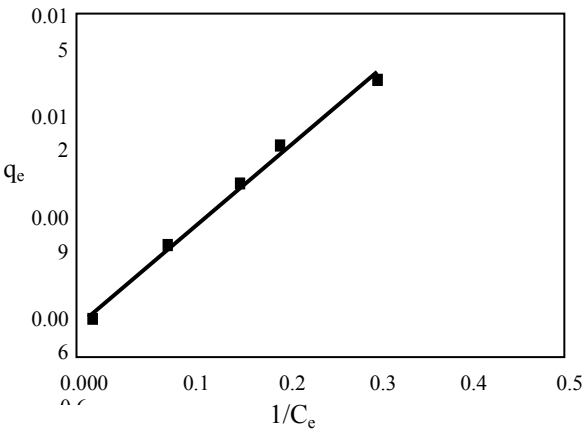

Figure (1) equilibrium isotherm applying Herkins-Jura model for Pb(II)

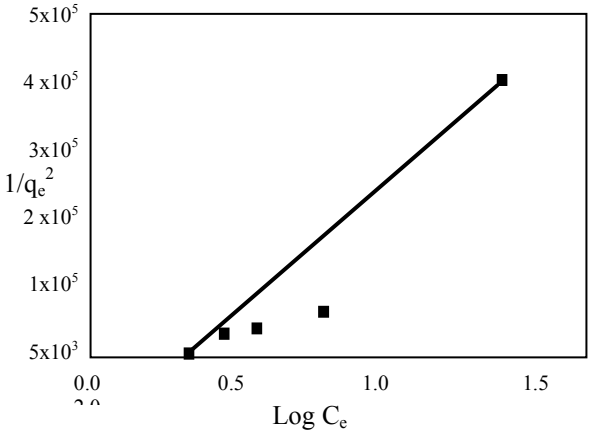

Figure (2) equilibrium isotherm applying Adam-Bohart model for Pb(II)

By applying equation (3) on the data mentioned in table (1) HYBRID error function of each model in found to be 0.272 for Adam-Bohart model and 0.484 for Herkins-Jura model.

RESULTS AND DISCUSSION

The adsorption of Pb(II) by activated carbon was of a favourable type.

An increase in the equilibrium concentration is accompanied by an increasing in the adsorption uptake (sorption) of activated carbon.

Both models can be used successfully for expressing the adsorption process of Pb(II) onto activated carbon.

Herkins-Jura model is the best model to express the data in this research in comparison with Adam-Bohart model since (R^2) values of Adam-Bohart model was (0.737) and for Herkins-Jura model was (0.986), in addition to the the (HFE) values which were (0.272) for Herkins-Jura and (0.484) for Adam-Bohart model.

Since the research appointed that Herkins-Jura model is the best model to describe the data, that may explained mass transfer of Pb (II) occurred within a multi-layers film that surrounding the (GAC) particles and the gradual concentration gradient was the main driving force of the adsorption process, rather than pore and inter-particle diffusion mechanism.

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The Adsorption of Cd (II) and Pb(II) Ions from Aqueous Solution by Sea Shell Powder

Hayder M. Abdul- Hameed

Environmental Eng. Dept. College of engineering / Baghdad University – republic of Iraq

Email: hayder3almunshi@yahoo.com

ABSTRACT

Heavy metals are considered as major environmental pollutants. Many of industrial wastewater effluents contain a wide range of these heavy metals. The adsorption of Cd^{2+} and Hg^{2+} metal ions from aqueous solution by sea shell powder was studied. The results showed that maximum adsorption capacity occurred at 471.5×10^{-3} mg/kg for Pb^{2+} ion and 531.6×10^{-3} mg/kg for Cd^{2+} ion. The adsorption in a mixture of the metal ions had an equilibrium effect on the adsorption capacity of the sea shell powder. The adsorption capacity of each metal ion was influenced by the presence of other metal ions rather than its presence individually. The study showed that the presence of other heavy metals attribute to the reduction in the sea shell powder capacity, and the adsorption process was found to obey the Freundlich isotherm for both ions.

Keywords *Adsorption, heavy metal ions, aqueous solution, sea shell powder.*

الملخص باللغة العربية

تعتبر العناصر الثقيلة واحدة من أهـم الملوثـات البيئية، فالكثير من المياه الصناعية تحتوي علـى عدد كبير من المعادن الثقيلة. وقد تمـت دراسـة عملية امتزاز أيوني الكادميوم والرصاص الثنائي من محاليلهما المائية باستخدام مسحوق الأصداف البحرية في البحث، وأظهرت النتـائج أن أكبـر استيعاب للامتزاز كان بمقدار 0.4715 ملغـم/ كغم بالنسبة لأيون الرصاص الثنـائي، وبمقـدار 0.5316 ملغم/ كغم بالنسبة لأيـون الكـادميوم. وأن تواجد الأيونين مع بعضهما يؤدي إلى إحداث عملية توازن في الامتزاز، وعملية الامتزاز هـذه تخضع لقانون فريندليتش.

INTRODUCTION

A great interest and concentrated studies in the research for the removal of heavy metals from industrial effluents focused on using materials such as sea shell powder that is easy handle and economical and durable materials. Adsorption process is considered as technique for the removing of heavy-metal from industrial, mineral and petroleum industry, (1). This process is mainly less cost and can be executed on site, hence, reducing the hazards of transporting the toxic materials to the de-pollution sites. The adsorption process is a good alternative for the recovery of metals contained in other media (2).

The use of sea shell powder as filters were introduced in the 1947's for the ultra purification of water in the food industry. Industry had also taken advantages of the unique ability to adsorb a variety of organic and non-organic compounds by utilizing the crushed sea shell in industrial wastewater treatment (3).

The Freundlich equation had been widely used for many years.

This equation which originally proposed as an empirical equation is used to describe the data for the heterogenicity in the adsorbent surfaces, in which the energy term (b) in the Langmuir equation varies as a function of surface coverage (q_e), that due to variations in the heat of adsorption (4).

Freundlich studied the adsorption phenomenon and showed that adsorption from solution could be expressed empirically by:

$$q_e = K C_e^{1/n} \qquad \textbf{... (1)}$$

Where, (K and n) are constants, and n > 1 (5).

As Freundlich equation is an empirical equation then it is useful as a means for data description. Data are usually fitted to the logarithmic equations as follows:

$$\mathrm{Log}\, q_e = \mathrm{Log}\, K + \frac{1}{n}\mathrm{Log}\, C_e \qquad \textbf{... (2)}$$

This equation gives a straight line with a slope of (1/n) and an intercept equal to the value (Log K), for C = 1. The intercept is roughly indicated of adsorption capacity.

Freundlich equation generally agrees quite well for the experimental data of a wide range

of concentration (6)

Zenedy and Murphy (2007) found that the equilibrium data for adsorption of mercury onto activated carbon were correlated well with Langmuir and Freundlich equations. (7)

Prasert and Parasaut (2006) found that the pistachios shell can be examined for removal of copper and lead from aqueous solution and the Freundlich isotherm models well fitted the data. (8)

Badmus and Anyate (2007) showed that the adsorption isotherm of zinc onto reed bed seemed generally to approach Freundlich models. (9)

Diarati and Taliki (2003) showed that the Freundlich isotherm fitted well with the data of adsorption of chromium by powdered activated carbon at 24^{o}C. (10)

Qader and Akhtars (2005) found that the adsorption kinetic study of lead and cadmium individually, and the resulted data fitted well with Freundlich isotherm. (11)

The mechanism of adsorption may be particle diffusion controlled (12) or a film diffusion controlled depends on many parameters that will dominate which mechanism of the above of favorable depends on the slowest step of the adsorption process that will consider the rate step. Here the result of the amount adsorbed against time for a mixture of the metal ions.

Since adsorption is a particle diffusion controlled (12) and this could be affected by the following processes:

(i) diffusion of the solute from the solution to the film surrounding the particle;
(ii) diffusion from the film to the particle surface (external diffusion);
(iii) diffusion from the surface to the surface to the internal sites (surface diffusion or pore diffusion), and
(iv) Uptake which can involve several mechanisms: physicochemical sorption, ion exchange, precipitation or complexation (13). The first process is bulk diffusion, the second is the external mass transfer resistance and the third is intraparticle mass transfer resistance.

When the adsorption is particle diffusion controlled, it means that intraparticle mass transfer resistance is rate limiting. Therefore, in the presence of a mixture of the metal ions, the metal ions (i.e. compete) for the adsorption sites on the adsorbent. This competition affects the diffusion properties of the metal ions, hence decreases the adsorption capacity of the metal ions. Thus, the metal ion that successfully reaches the adsorption site faster depends on the above factors and also on the ionic radii of the metal ions. Competition among the metal ions for adsorption sites deviously affected the adsorption capacity (12).

It seems that surface attachment might also take place on the functional groups on the surface of the adsorbent. More of what happens is volumetric filling of the micro-pores found in the adsorbents. Since adsorption takes place in these micro-pores (14), these results decrease in the amount of metal adsorbed with time, by inspection of the plots, the application of the lagergen equation (equation 3) shows a zero order reaction. This is true since amount adsorbed remains fairly constant with increased time. The lagergen equation is given by:

$$\textbf{Log (qe-q) = log qe – } K_{ad}\textbf{ t/2.303. (3)}$$

Where q is amount adsorbed (mg/g) at time t, q_e is amount adsorbed (mg/g) at equilibrium time and K_{ad} is the rate constant of adsorption (min^{-1}) (12)

Scope of Study;

In this work, sea shell powder is being used as adsorbent for the removal of Cd (II) and Pb (II) ions and the mechanism of sorption was investigated respectively. The effect of having two metal ions in the wastewater had been studied. Since the pollution of the environment with heavy metals is important and as result of many human industrial, agricultural and petroleum activities, it means that these effluents would carry heavy metal ions in solution.

MATERIALS & METHODS

All materials used are from the Iraqi market, a solution of $Cd(NO_3)_2.H_2O$ and $Pb(NO_3)_2.2H_2O$ were prepared with initial concentration of 1000 mg/l by the dissolving 1.35 gm of $Pb(NO_3)_2.2H_2O$ in 4.3 l of distilled water and 1.21 gm of $Cd(NO_3)_2.H_2O$ in 4.6 l distilled water. The sea shell was first collected from Al-Razzazza artificial lake (107 km south west of Baghdad),the sea shell then washed with distilled water and dried in the oven at 80 C for 24 hour, then the sea shell were crushed and grinded by a ball mill with an average particle size used is 600 μm, the process of screening was held in the petroleum research and development center lab / Ministry of Oil using screen shaker of type (BAUS). 40 ml of each of two metal ions were mixed and put in a conical flask containing 50 g of sea shell powder. The flask was uniformly agitated at a temperature of 25^{o}C and optimum pH of 7 using a rotary shaker. Figure (1.a) shows the agitation process using rotary shaker at constant agitation speed.

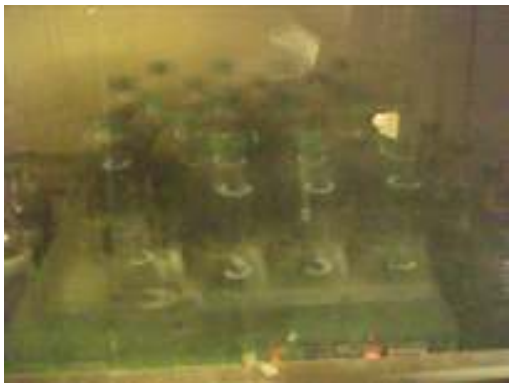


Fig. (1.a): Agitation process for different flasks

The experimental set-up was repeated for time intervals of 20, 30, 40, 50, and 60 min after the end of every (10min) a sample was taken from the flask and the concentration of (Pb, Cd) in the filtrate water was measured using atomic absorption device in Baghdad environmental directory / Ministry of Environment. Figure (1.b) shows the samples after treatment with sea shell powder.

Fig. (1.b): The samples after treatment with sea shell powder

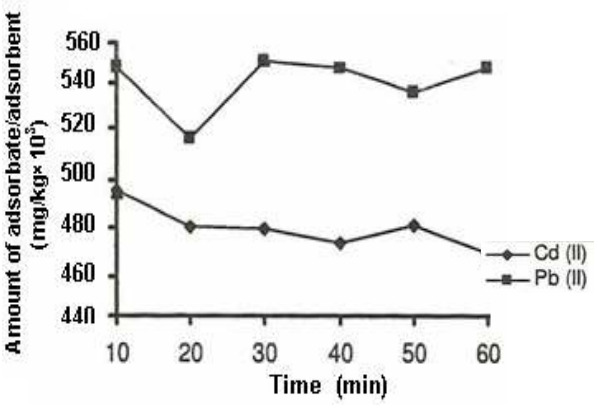
RESULTS & DISCUSSION

The amount of the adsorbate adsorbed at different time interval for both Cd, Pb ions were measured and table (1) refers the values of these amounts.

Table (1) Amount of adsorbate adsorbed by sea shell powder

Time (min)	Amount Pb adsorbed (mg/kg)×10^3	Amount Cd adsorbed (mg/kg)×10^3
10	531.60	471.50
20	515.20	470.11
30	547.31	473.20
40	542.01	471.75
50	530.57	475.54
60	545.71	436.71

Figure (2) shows the amount of metal adsorbed against time for sea shell powder, 1000 mg/l initial metal ions concentration and 600 μm average particle size.



From Figure (2), its clear that the adsorption was unsteady for Pb(II) but varied a little for Cd(II) on sea shell powder. The adsorption will expected to become less varied in the curve behaviour as preceding the time interval.

By applying (equation 1) the equilibrium concentration for each ions C_e at different q_e values were calculated and tabulated in Table (2).

Table (2) Equilibrium concentration C_e (mg/l) for Cd and Pb

Cd	Pb
862.60	878.20
871.20	880.70
863.17	881.70
864.49	882.06
866.07	881.11
863.57	890.89

By applying the linearized form of Freundlich isotherm model, Equation (2) to the data in Tables (1) and (2) and plotting the equation, a straight line will be resulted according to the model isotherm as in Figure (2) which is refered to a good fitting of the data with Freundlich isotherm for both Cd and Pb.

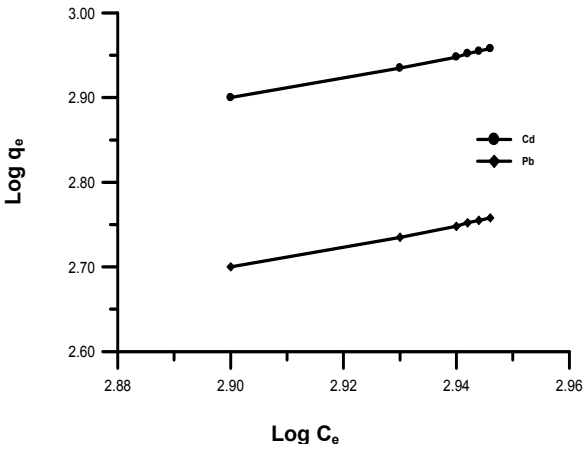


Fig. (3): The linearized form of the equilibrium concentration for both Cd and Pb

CONCLUSION

The amount of Cd (II), Pb (II) metal ions in aqueous solutions adsorbed did not increase as time increased as expected. Rather, the amount adsorbed remained fairly constant with time during the competitive sorption. This was attributed to the fact that all the metal ions, will have to be struggling for the same number of adsorption sites at the same time. Therefore, this study significantly reveals that the presence of other heavy metals and chemicals are influential factors and should be design parameters in the treatment and management of heavy metal pollutants using sea shell powder.

Furthermore, the fluctuation in the amount of Cd adsorbed with respect to time rather than Pb may attribute to the high affinity of the Pb rather than Cd for adsorption onto sea shell powder due to the variation in the electro-charges properties of both ions. As Pb is more attracted (attached) than Cd on the vacant sites of the sea shell powder due to is less positivistic than Cd. Both Cd and Pb obeys well Freundlich isotherm.

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The Evaluation of Antitumor Activity of S-layer Proteins in Comparison with Cell Free Filtrate of *Lactobacillus spp. in Vitro*

Maysaa Ch. AL-Yas,(1) Amina N. AL-Thwani(2), Raghad A. Hassan(3)

Department of Biotechnology, College of Science, Al-Nahrain University / Republic of Iraq. (1), (3)

Genetic Engineering and Biotechnology Institute for Postgraduate Studies, Baghdad University / Republic of Iraq (2)

E-mail: dr.maysaa78@yahoo.com

ABSTRACT

This project was conducted to evaluate the activity of S-layer proteins isolated from *Lactobacillus* in comparison with the activity of concentrated filtrate of *Lactobacillus* against tumor cell lines *in vitro*.

Twelve isolates of *Lactobacillus spp.* obtained from, vinegar, human milk, cow milk, yoghurt and vagina, were used to detect the S-layer protein (Slp) by Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) then extracted it by excised the Slp pand and treated with 6M guanidin hydrochloride (G-HCl) to eluted the protein from gel. The Molecular weights (MW) of Slps were estimated between (37-63 kDa) depending on the *Lactobacillus* species. The concentrations of Slp were estimated by using a Kit based on the Biuret method. One isolate of each of *Lactobacillus acidophilus* and *Lactobacillus casei*, were selected depending on the MW and concentrations of S-layer proteins.

When the cytotoxic activity of S-layer proteins and *Lactobacillus* concentrated filtrate by using different concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) was applied against two tumor cell lines (RD and L20B) and incubated

for 48 hr., results confirmed that all extracts (S- layer proteins and filtrate) have cytotoxic effects. Moreover, *Lactobacillus* concentrated filtrate had the highest significant cytotoxic effect on growth of both tumor cell lines when compared with S-layer proteins. We concluded that S-layer proteins and concetrated filterate of *L.acidophilus* and *L.casei* had cytotoxic effect on growth of both tumor cell lines RD and L20B depending on concentrations. The concentrated filtrate gave highest cytotoxic effect than S-layer protein.

الملخص باللغة العربية

اجريت هذه الدراسة لتقييم فعالية البروتينات السطحية المعزولة من بكتريا حامض اللاكتيك العصوية بالمقارنة مع فعالية رواشح البكتريا الخام المركز باستخدام تراكيز مختلفة: 31.25,62.5,250,500,1000 ضد نوعين من خلال فترة حضن 48 ساعة. اذ استخدمت (12) عزلة من بكتريا حامض اللاكتيك المعزولة من الخل وحليب البشرو حليب البقروالبن والمهبل (L20B, RD) خطوط الخلايا السرطانية للكشف عن وجود البروتينات السطحية ثم عزلت Poly acrylmide gel بطريقة electrophoresis sodium Dodecyl Sulfate (SDS-PAGE) (G-(Hcl البروتينات guanidine hydrochloride بواسطة قطع حزمة البروتين السطحي ومعاملتها مع تركيز 6 عياري لاسترجاع البروتين من الهلام. قدرت الاوزان الجزيئية للبروتينات وكانت تتراوح مابين (37 – 63) كيلو دالتون حسب اختلاف انواع البكتريا حامض اللاكتيك, كذلك حسبت تراكيز البروتينات السطحية باستخدام عدة تعتمد في اليه عملها على طريقة البيوريت. وتم اختيار *Lactobacillus* و *Lactobacillus acidophilus* اعتمادا على الوزن الجزيئي وتركيز البروتين*casei* للعزلتين.

INTRODUCTION

The beneficial role of probiotic bacterium in the intestinal lumen were described and many clinical benefits to these specific non pathogenic organism were studied like diarrhea treatment , antimicrobial activity , anticarcenogenic activity , immune modulation , reduction of cholesterol level and other (1)

The consumption of probiotic cultures may decrease cancer risk. The mechanisms by which lactic acid bacteria inhibit colon cancer may include alteration of the

metabolic activities of intestinal microflora, (2) alteration of physicochemical conditions in the colon, binding and degradation of potential carcinogens, (3) quantitative and qualitative alterations in the intestinal microflora incriminated in the production of carcinogens, production of antitumorigenic or antimutagenic compounds, enhancing the host's immune response and effects on the physiology of the host (4,5)

An important property proposed for a probiotic bacterium is the ability to adhere and colonize host tissues, which enhances multiplication and survival of bacteria in the host and prevents colonization by pathogenic bacteria (6)

The role of proteinaceous surface molecules in adhesion has been proposed in several studies (7). Like many other bacteria, several species of *Lactobacillus* have a surface (S-) layer as the outermost component of the cell (8). The function of *Lactobacillus* S-layers characterized so far is involved in mediating adhesion to different host tissues. In addition to surface layer proteins (Slps) adhesive properties, the very large number of S-layer subunits present on the cell surface has prompted research aiming at the use of S-layers as a vehicle for the delivery of biologically active compounds, such as drug molecules, antibodies, enzymes and vaccine antigens (9). This study aimed to extraction S-layer proteins from *Lactobacillus spp.* of different sources, evaluating antitumor activity of S-layer proteins in comparison with concentrated filtrate of *Lactobacillus in vitro*.

MATERIALS AND METHODS

Bacterial Isolates:

Bacterial isolates used in this study were obtained from different sources: two isolates of *Lactobacillus acidophilus* isolated from chicken intestine/ College of Veterinary Medicine/ Baghdad University, *Lactobacillus acidophilus* and *Lactobacillus casei* were isolated from faeces of children / Biotechnology Research Centre /AL-Nahrain University.

Isolation of *Lactobacillus* from different sources

Two samples of vinegar, five (3ml) of human milk (taken from healthy women), three of cow milk, and four of yoghurt were collected in order to isolate *Lactobacillus*, also *Lactobacillus* isolates were isolated from the vagina of healthy premenopausal women by the gynecologist doctor in Kamal AL-Samarai hospital, Baghdad. *Lactobacillus* isolated according to the method was performed by (10).

One ml of each sample was transferred to a test tube containing 9 ml of sterilized MRS broth and incubated anaerobically overnight at 37 ^{o}C, and this was repeated for three times to increase bacterial numbers, Serial dilutions were made from peptone water, and 1 ml of the last one was cultured on MRS-CaCO3 agar and incubated for 24 hr. at 37 ^{o}C, under anaerobic conditions using anaerobic jar. After incubation, colonies surrounded by clear zones (due to the production of acid hydrolyzing CaCO3) were picked and grown on MRS broth.

Local isolates of Lactobacillus sp. were first identified the morphologically under light microscope to notice the cells shape, Gram reaction, grouping and *spore forming* and cultural characteristics (shape, color, size, edges and height) of colony on MRS agar plates according to (11). Biochemical tests: catalase, oxidase, gelatinase, acid production in litmus milk media and growth at 15-45 ^{o}C were also performed (12).

Detection of S-layer proteins

Lactobacillus cells grown in MRS broth were collected by centrifugation at 10,000 rpm for 10 min at 4ºC and washed once with 0.5M Tris-HCl, pH 7.5. The pellet, equivalent to 1 ml of culture, was dissolved directly in 200 µl of Laemmli sample buffer and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis 10% (SDS-PAGE) (13).

Extraction of the S-layer protein:

The bands which located in the range between Transferrin and Trypsine was excised and cut into

pieces.(14) The protein was eluted from the gel pieces in 1.5 ml of 6 M guanidine hydrochloride-0.5 M Tris-HCl-2 mM EDTA, pH 7.5, by incubating in an end-over mixer at room temperature for 10 h. The elute was dialyzed against 0.1M Tris-HCl, pH 8.5, at +4°C for 10 h. also analyzed by (SDS-PAGE), In order to ensure the purity of protein.

Determination of Total Protein:

Protein concentration was estimated by using the specific kit which depended on Biuret method. The principle of this assay was protein form a blue/violet complex when mixed with copper ions in alkaline solution (Biuret reagent) each copper ion binding with 5 or 6 peptide bonds. Tartarate was added as a stabilizer and iodide was used to prevent auto-reduction of the alkaline copper complex.

Procedure:

- Blank: by mixing 0.02 ml of D.W with 1ml of reagent.
- Standered: by mixing 0.02 ml of the standard with 1ml of reagent.
- Sample: by mixing 0.02 ml of sample with 1 ml of reagent.

The mixture was incubated at room temperature for 5 minute. Absorbance of sample (As) and standard (Astd) against reagent blank at wavelength of 546 nm, was measured.

Calculation: Total protein g/dl = As /Astd × concentration of standard (Indicated in the kit).

Preparation of S-layer protein dilutions:

To prepare stock solution 1 ml of S-layer proteins of each *Lactobacillus spp.* were transferred to containers contained 1 ml of serum free medium, then sterilized by filtration using Millipore 0.22µm filter paper. From this stock solution two fold dilutions were made starting from concentration 1000µg/ml ending with the concentration 31.25 µg/ml. The dilutions were done in the wells of microtiter plate using serum free medium.

Preparation of *Lactobacillus* concentrated filtrate dilutions:

A portion of 100mg from each of *Lactobacillus spp.* concentrated filtrate was dissolved in 10 ml to of serum free medium, and then

sterilized by Millipore 0.22µm filter. From these stock solutions two fold dilutions were made starting from concentration 1000µg/ml to 31.25 µg/ml. The dilutions were dumped in the wells of microtiter plate using serum free medium.

Cytotoxic Effect of *Lactobacillus* Concentrated Filtrate and S-layer Proteins isolated from *Lactobacillus* on Tumor Cell Lines:

The growth inhibition was carried out according to a method that was adopted by [15].

The method included the following steps:

The cells (L20B and RD) were supplemented as a monolayer attached cells in falcon culture flasks (25) cm^2 containing RPMI-1640 medium. The cells were washed with sterile PBS under aseptic conditions after decanted off the old medium, and then a 2-3 ml of trypsin-versene solution was added with gentle shaking until the cells were detached from the flask surface. A quantity of 20 ml of the new growth medium was added to falcon containing a suspension of single cells with well stirring followed by transferring the contents of each falcon into another in a way that each falcon contained equal volume of both culture medium and cells (subculture). These falcons were incubated at 37°C for 24 hr. for L20B and RD cell lines.

Counting of viable cells was carried out using trypan blue dye; dead cells usually take up the dye within a few seconds making them easily distinguished from viable cells. For this, one part of cell suspension was mixed with equal volume of diluted trypan blue stain (0.2 ml trypan blue in 1.6 ml PBS).

Cell suspension was prepared by treating the container of (25) cm^2 with trypsin-versene solution followed by the addition of 20 ml of growth medium supplemented with 10% fetal calf serum. The cells were seeded in the wells of 96 well tissue culture plate, which carried out by transferring 200 µl of cell suspension in to each well, in somehow that each well had contained ($1x\ 10^5$) cell/well, and the plate was incubated overnight at 37°C for L20B and RD cell lines.

The day after, the wells examined to inspect the formation of cell monolayer, and then 200 µl/well from each concentration (1000, 500, 250, 125, 62.5 and 31.5 µg/ml) that were previously prepared for each extract as much as three replicates, also 12 replicates were made for the control which contained only the cells with 200 µl/well of serum free medium (SFM), then the plates were wrapped with para-film and incubated at 37°C for 48 hr. in an incubator supplemented with (5%) CO_2.

After elapsing the incubation period the media was decanted off, 50 µl/well of neutral red dye were added and incubated again for 2 h. after incubation, The contents of the plate were removed by washing the cells with PBS to remove the excess dye followed by the addition of 20 µl/well of extraction dye solution that draw out the dye from the viable cells that had stained. The results were read using ELISA reader at wave length 492 nm. The percentage of growth inhibition was calculated according to the following equation:

Growth inhibition % =

$$\frac{\text{Control} - \text{Treatment cell}}{\text{Control}} \times 100$$

Statistical Analysis:

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using SPSS computer program version 7.5.

Differences in results were considered significant at probability value equal or less than 0.05.

RESULTS AND DISCUSSION

A) Identification Lactobacillus Isolates:

Lactic acid bacteria were primarily identified depending on the formation of clear zone around their growing colonies on MRS agar containing calcium carbonate (1%) as a result of acid production and CaCO3. After that, lactic acid bacteria isolates were identified by biochemical tests, results shown that they were negative to oxidase,

catalase, and to the production of gelatinase after grow on gelatin medium, while they were positive to litmus milk test. When *Lactobacillus* isolates grew in MRS broth at 15 °C and 45 °C under anaerobic conditions, results showed that most of them grew at 45 °C but few of them could grew at 15 °C especially *Lactobacillus casei* and *L. plantarum* which are considered as mesophillic group of *Lactobacillus* bacteria. These results were agreed with those mentioned by (16).

Cultural and Morphological Characteristics:

All colonies of *Lactobacillus* isolates on MRS agar appeared; white to pale in color, round shape, convex, soft, mucoid and having smooth edges. Microscopic examination showed that cells were gram positive, short or long bacilli, found mainly in chain containing 3-8 cells and non-spore forming. These results coincide with those mentioned by (17) . Depending on the results of the cultural and microscopic examination, thirteen isolates were belonging to the genus *Lactobacillus*

B) Concentrations of Lactobacillus S-layer proteins:

The concentrations of extracted S-proteins from *Lactobacillus* were determined by using Kit which depended on Biuret method.

Results of the concentrations of S-proteins showed that were ranged from 1.87 mg/ml for *L.acidophilus* (isolated from chicken intestine) to 0.13 mg/ml for *L. curvatus* (from cow milk). Under laboratory cultivation conditions, yield of the S-layer glycoprotein ranges between 0.5 and 2.0 g wet weight per liter of growth medium (18).

Two S-layer proteins extracted from *L. acidophilus*1 and *L.casei* which their molecular weight were (47 and 44 kDa) and their concentrations were (1.87 and 1.39 mg/ml), respectively, were used in this study to evaluate the biological role of S-layer proteins.

C) Cytotoxicity effect of S-layer proteins of L. acidophilus and L. casei and Lactobacillus concentrated filtrates on Tumor Cell Lines**:**

Two tumor cell lines (RD and L20B) were used in this study for one time of exposure (48 hr). Cell lines were subjected to six concentrations of S-layer protein and of *Lactobacillus* filtrates (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) that were prepared.

Results obtained may be discussed as follows:

1- Effect of S-layer proteins and *Lactobacillus* filtrates on RD cell line:

After cancer cell line (RD) was treated with S-layer proteins and concentrated filtrates of *Lactobacillus acidophilus* and *Lactobacillus casei*, results showed significant cytotoxic effect started at the certain concentration and continued to the higher concentrations reaching the last concentration when compared with the control.

Surface protein from *Lactobacillus acidophilus* had significant cytotoxic effect ($P \leq 0.01$) on growth of RD cell line at the concentrations of 500 and 1000 µg/ml with growth inhibition percentage 26.61% and 49.81%, respectively, as shown in figure (1).

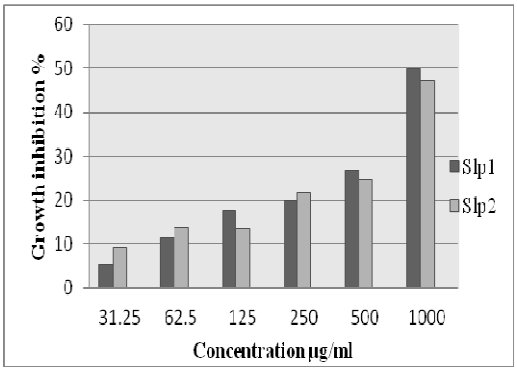

Fig (1): Histogram of growth inhibition percentages (GI %) for different concentrations of S-protein of *L.acidophilus* (Slp1) and S-protein of *L. casei* (Slp2) on RD cell line.

S-layer protein from *Lactobacillus casei* showed significant toxicity ($P \leq 0.01$) on growth of RD cell line at the concentration 1000 µg/ml with growth inhibition percentage 47.19%, as shown in figure (1).

As the concentration decreased, there was a decline in the inhibitory effect with no significant cytotoxic effect when compared with the control.

Figure (2) revealed the percentage of growth inhibition of concentrated filtrates *Lactobacillus acidophilus* and *Lactobacillus casei* on growth of RD cell line and. There was a high significant cytotoxic effect ($P \leq 0.0001$) for the concentrated filtrate of *Lactobacillus acidophilus* which appeared at concentrations of 62.5

and 125 µg/ml with growth inhibition percentages 73.74% and 54.32%, respectively, but cytotoxic inhibition rate was decreased with increasing concentrations. The significant toxicity ($P \leq 0.001$) was appeared at the concentrations of 250, 500 and 1000 µg/ml to give growth inhibition percentages of 40.13%, 40.46% and 40.78%, respectively.

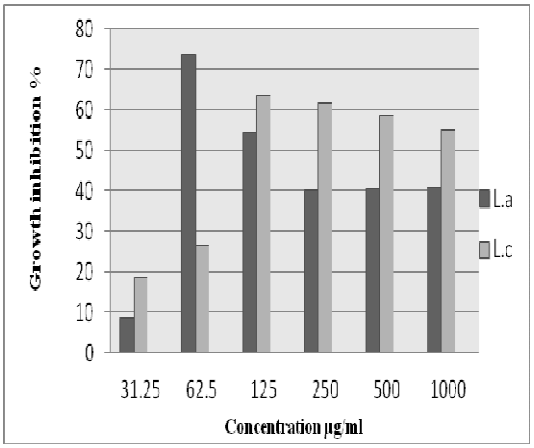

Fig (2): Histogram of growth inhibition percentage (GI %) for different concentrations of concentrated filtrate of *L.acidophilus* (*L.a*) and *L.casei* (*L.c*)on RD cell line.

An explanation for this behavior is that in the design of cell culture experiment it was important to be aware of the growth state of the culture, as well as the quantitative characteristics of cell strain or cell line. Cultures will vary significantly in many of their properties between exponential growth and stationary phase (19).

The concentrated filtrate of *Lactobacillus casei* has significant effect ($P \leq 0.001$) when compared with the control at concentration of 1000 µg/ml with growth inhibition rate of 54.97%, the cytotoxic inhibition rate was increased with the concentration decreased. There was a high significant effect ($P \leq 0.0001$) at the concentrations of (125, 250, 500) µg/ml to give growth inhibition rates of (63.78%, 61.66% and 58.40%), respectively.

2- Effect of S-layer proteins and *Lactobacillus* concentrated filtrate on L20B cell line:

Effect of S-layer proteins of *L.acidophilus* and *L.casei* on growth of L20B cell line at significant difference ($P \leq 0.05$) level, and growth inhibition percentages are shown in figure (3).

S-layer protein from *L.acidophilus* showed significant differences started from concentration 500 µg/ml ($P \leq 0.01$) to 1000 µg/ml when compared with the control. S-layer

protein from *L.casei* had significant cytotoxic effect on growth of L20B cell line at the concentration 1000 µg/ml, when compared with control, while no significant cytotoxic effect was appeared when the concentration decreased below that.

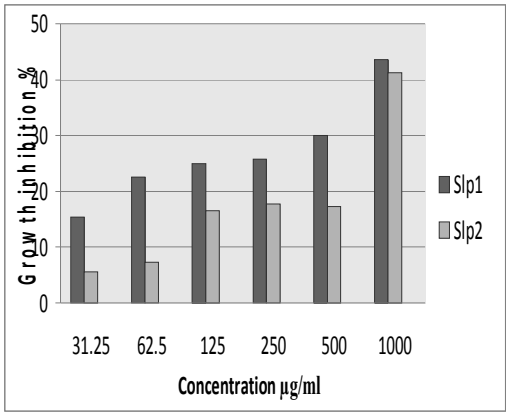

Fig (3): Histogram of growth inhibition (GI %) for different concentrations of S-layer protein of *L.acidophilus* (Slp1) and S-layer protein of *L. casei* (Slp2) on L20B cell line.

The significant cytotoxic effect ($P \leq 0.001$) of the concentrated filtrate of *Lactobacillus acidophilus* was started at concentration of 31.25 µg/ml with growth inhibition percentage of 40.36%. The significant cytotoxic effect was increased toward the higher concentrations ($P \leq 0.0001$) when compared with the control. Higher growth inhibition percentages 85.06%, 75.28% were observed at the concentrations of 125 and 250 µg/ml as shown in figure (4).

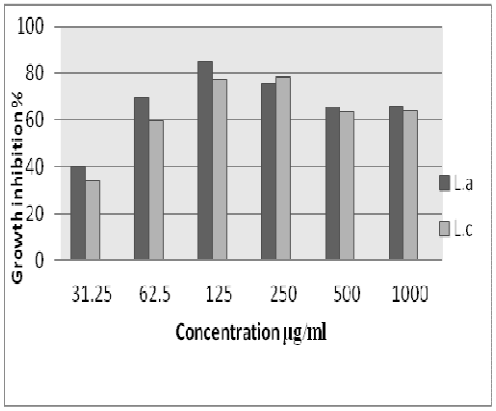

Fig (4): Histogram of Growth inhibition percentage (GI %) for different concentrations of concentrated filtrate of *L.acidophilus* (*L.a*) and *L.casei* (*L.c*) on L20B cell line.

Concentrated filtrate of *Lactobacillus casei* has high significant cytotoxic effect ($P \leq 0.001$) on growth of L20B started at concentration of 31.25 µg/ml with growth inhibition percentage of 33.67% when compared with the control. Significant effect was increased toward the higher concentrations, but concentrations of 125, 250 µg/ml have higher growth inhibition percentages (76.92%, 78.21%), respectively, compared to the concentrations of 500, 1000 µg/ml which gave growth

inhibition percentages of 63.33%, 64.10%, respectively, as shown in figure (4). Statistical analysis comparison between the effect of S-layer protein and concentrated filtrate of *L.acidophilus* and *L. casei* on RD cell line at the incubation period 48hr. clarified that S-layer protein of *L.acidophilus* had more effect than S-layer protein of *L.casei* because at the concentration 500 µg/ml it had significant difference of ($P \leq 0.01$) when compared with Slp of *L.c* which showed the same signifigant difference at the concentration 1000 µg/ml. On the other hand *L. acidophilus* filtrate had more effective than *L. casei* filtrate because at the concentration of 62.5 µg/ml had high significant difference ($P \leq 0.0001$), when compared with *L. casei* filtrate that showed high significant difference ($P \leq 0.0001$) at 125 µg/ml.

Lactic acid bacteria can inhibit genotoxicity of dietary carcinogens *in vitro* considering that the degree of inhibition was strongly species dependent (20), (21) when examined the cytotoxicity of four lactic acid producing bacteria (LAB) strains, (*Bifidobacterium animalis*, *Lactobacillus delbreukii, Lactobacillus plantarum* and *Lactobacillus acidophilus*) on four human cancer cell-lines (HEPG-2 (Liver carcinoma); MCF7 (Breast carcinoma); HELA (Cervix carcinoma) and HCTI16 (colon carcinoma), they found that maximum inhibition was exerted by the *B.animalis* on the four cell lines which were totally unaffected by the *L. acidophilus*.

Statistical analysis comparison between the effect of S-layer protein and concentrated filtrate of *L.acidophilus* and *L. casei* on L20B cell line at incubation period of 48 hr. showed that the Slp of *L.acidophilus* more effect than Slp of *L.casei* because at the concentration 500 µg/ml it had significant difference ($P \leq 0.01$) when compared with Slp of *L.casei* that gave same significant difference at 1000 µg/ml when compared with control. In case of filtrates, the output revealed that *L. acidophilus* filtrate had more cytotoxic effect than *L. casei* filtrate, because it had high significant effect ($P \leq 0.0001$) at

concentration of 62.5 µg/ml, while *L. casei* filtrate had high significant difference ($P \leq 0.0001$) at concentration of 125 µg/ml. Depending on statistical analysis, the crude filtrate of LAB gave better cytotoxic effect on tumor cell line than S-layer protein, and concentrated filtrate of *L. acidophilus* was more effect than that of concentrated filtrate of *L. casei*.

Extracellular polysaccharides (EPS), which are metabolites of some LAB strains, were also reported to exhibit antitumor activity, macrophage activation, mitogenic activity, and induction of cytokines. However, most of these studies were performed in vitro and little information is available for in vivo experiments involving oral administration (22). The EPS are taken up by Peyer's patches in the intestine and stimulate antigenpresenting cells, such as dendritic cells, through tolllike receptors. This would result in selective enhancement of T-helper 1 (Th1) cell proliferation, and the subsequent production of IL-2 and IFN-γ, which are cytokines that are vital for cell mediated immune responses (23).

Regarding the cytotoxicity of S-layer proteins on tumor cells, this effect may be because S-layer proteins are mostly composed of glycoproteins and involved in the adherence *Lactobacillus* to host tissues (9). (24) reported that binding of LAB may give a good effect on colon cancer by balancing the Th1/Th2 cytokine profile, but it is critically dependent on binding capacity of LAB to tumor/cancer cells and thus the pattern of cytokine production is that due to the LAB adhesion may be bacterial strain- specific.

Regarding susceptibility of the tumor cell lines to these extracts, it was noticed that L20B cell line was more sensitive than RD cell line, this may be due to the presence of several compounds that posses effective ability against this type of cell line, or due to the increased activity of Glutathione-S-transferase (GSTs) that acts as an anti-oxidation agent especially in cancer cells. The (GSTs) acted as an anti oxidant causing cellular detoxification by inhancing their

combination with reduced glutathione leading the cancer cell toward programmed cell death, apoptosis (25).

We concluded that S-layer proteins and concetrated filterate of *L.acidophilus* and *L.casei* had cytotoxic effect on growth of both tumor cell lines RD and L20B depending on concentrations. The concentrated filtrate gave highest cytotoxic effect than S-layer protein.

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Effect of 2, 4- D and Kin combinations on callus induction on *Nigella sativa* explants and production of Thymol

Nabeel K. Al- Ani (1), Zaynab Saad (2)

Biotechnology Department / College of science/ Al-Nahrain university / Republic of Iraq , (1) Iraqi center for cancer & Medical genetics research / Al-Mustansiriya University / Republic of Iraq (2)

E-mail: nkalani54@yahoo.com

ABSTRACT

Callus of *Nigella sativa* was induced from leaves, hypocotyls, and roots using different concentrations of 2, 4-D and Kinetin on MS media. The best calluses from these explants were subculture until we get 1400 mg fresh weight within 75 days. The production of Thymol was determined in a leaf callus. Thymol was maximum at (75 days) for leaf callus as detected by HPLC. The amount of Thymol in leaf was higher than that in the hypocotyl and root callus.

Key words: *Nigella sativa*, callus culture, Thymol production *in vitro.*

الملخص باللغة العربية:

استخدم الوسط الغذائي المسمى MS بتراكيز مختلفة من 2,4-D والكاينتين لاستحداث الكالس من اوراق ، السويقة الجنينة ، والجذور لنبات حبة البركة ***Nigella sativa***. واجريت اعادة الزراعة لاحسن النموات لغاية حصولنا على 1400 ملغم وزن طري وكان ذلك من الاوراق بعمر 75 يوم من الزراعة . قدر الثايمول لهذا الكالس بعمر 75 يوم باستخدام HPLC . تفوقت نماذج كالس الاوراق على بقية النماذج من سويقة فلقية وجذور .

الكلمات المفتاحية نبات حبة البركة ، انتاج الكالس ، انتاج الثايمول خارج الجسم الحي

INTRODUCTION

Nigella sativa, a genus from Ranunculaceae family which includes some annual herbs. In Arabic known as black seed coequal names of its seed in Arabic

countries are Al-Habbah Al-Sawda, Kamoun Aswad, Scuniz and Kodria. The main inspiration of black seed comes from the famous saying (Hadith) of our Prophet Mohammed; (GPUH), that "Habbat Al-soda is remedy for all diseases except death."(1). The black seed has several biological activities (2), like antiinflammation (3), antitumor (4), and the action on relaxing of central nervous system (5), also its activity against strok (6), and decreasing sugar and cholesterol level in the blood (7). Thymoquinone and dithymoquinone, active principles of *Nigella sativa*, had cytotoxic effect against parental and multi-drug resistant human tumour cell lines which were over 10-fold more resistant to doxorubicin and etoposide (8). Thymol, thymoquinone, carvacol, anthole and terpinol are the active compounds of black seed oil, which acts as an antioxidant compounds in *Nigella sativa* (9, 10). This experiment was carried out to find an alternative method for Thymol production using *in vitro* culture.

The history of Plant Tissue Culture or sometimes known as Micropropagation or *in vitro* culture go back to Haberlandt experiments (1902). The basic of plant tissue culture technique depends on is the definition Totipotency which means the ability of each cell in plant to divide and grown into complete plant if suitable conditions found like temperature, light and nutrients (11). The production of secondary metabolites by tissue culture describes, as use instead of conventional culturing method, which provides industrial source of different necessary metabolic compounds, including alkaloid, phenol, terpenoid, vitamins and other of compounds which are necessary in medical uses (12). This is may be the first report in Baghdad for isolating Thymol from tissue culture of *Nigella sativa*.

MATERIALS AND METHODS

***In vitro* culture:** the mature seeds of *Nigella sativa* were obtained in 2006 from (Agriculture Research and Food Technology/ Ministry of Science and Technology/ Baghdad/ Iraq). Seeds were sterilized by

soaking for 5 minutes in 3% sodium hypochlorite (NaOCl), and rinsed three times in sterile distilled water. Seeds were then germinated on petri dishes lined with filter paper soaked with sterile distilled water in light condition. Leaves, hypocotyls, and roots were isolated from seedlings after 10 days of germination and cultured on MS medium (13) contained both kinetin at concentrations (0, 1, 1.5, 2, 2.5, 3 and 5 mg /l) and 2, 4-D at concentrations (0, 1, 2, 3 and 4 mg/l), 30% sucrose, 1% casein hydrolysate and solidified with agar. The pH of the media was adjusted to 5.7 prior autoclaving. The explants were incubated at 25 ± 1 ºC with a photoperiod of 16 hours light/8 hours' dark. Subcultures were carried out every 3 weeks on the same media and growth conditions.

Induced callus: The callus was established from 3 weeks old parts cultured *in vitro*. The best callus was maintained on MS medium supplemented with 1 mg/l of 2,4-D and 1.5 mg/l of Kin. and subcultured every 3 weeks. The production of Thymol was estimated after the last subculture left 75 day.

Extraction and quantification of Thymol: Thymol from leaf callus (75 day old) was extracted according to the method described by (14). Thymol was identified and quantified by comparing it with standard Thymol. It was quantified using HPLC technique as described by the separation of Thymol occurred on a reversed phase de base Colum (MDB) 250 x 4.6 mm internal diameter (I.D). The mobile phase that is used Buffer phosphate: methanol (30:70), the pH was adjusted to 3.5. The detection occurred at UV light at 254 nm wave length, with flow rate 1 ml / min, the temperature was 30 ºc.

RESULTS AND DISCUSSION

Production of callus: Leaves, hypocotyls, and roots were isolated from germinated seeds. In fig 1 fresh weight of leaf callus were determined in MS medium supplemented with Kinetin and 2,4-D in different concentrations. It seems that the best fresh callus

production (1400mg/l) was produced on medium contained 1.5 mg/l Kinetin plus 1 mg/l 2,4-D. It is noticed as the plant growth regulators going higher the fresh weight for the callus reduced in both regulators (2,4-D and Kinetin).

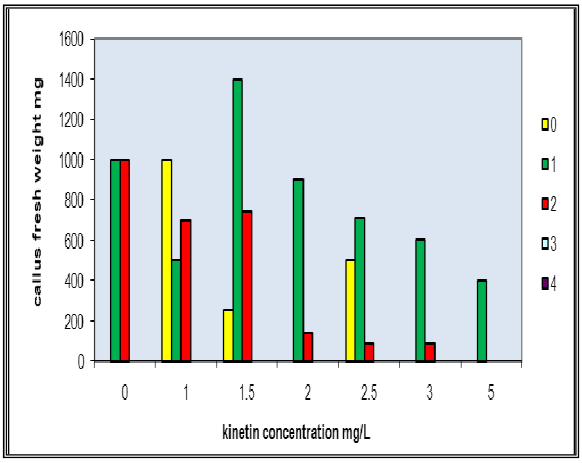

Fig (1): Callus fresh weight induced from leaf explants with 2,4- D and kinetin

Moreover, it was also noticed that the control treatment (without regulator) produced a good callus, and this may be due to endogenous hormones inside the leaf tissue. (15)

Similar results were found in root culture fig 2 beside .

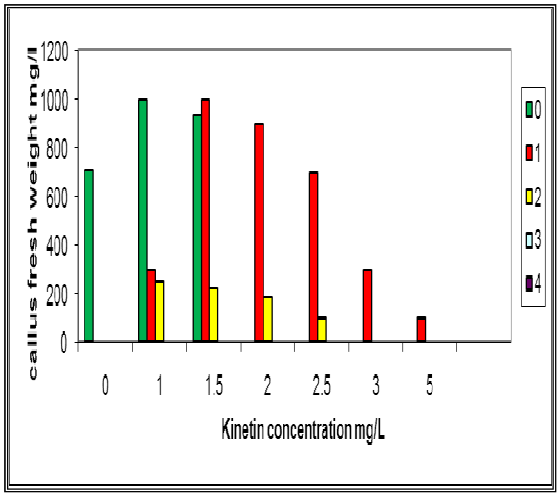

Fig (2): Caluus fresh weight induced from root explants with 2,4- D and kinetin

The best callus production was on medium contained 1.5mg/l Kinetin and 1 mg/l 2,4-D but the fresh weight was less 1000 mg per sample.

In the hypocotyl experiment the results were different .Best callus production was on control treatment 1500 mg fresh weight per sample. Then the weight of callus was reduced as we go up with the concentration of both regulators. Fig 3.

We can conclude from above results that the leaves and roots exhusted their endogeous regulators as results for their activities, while the hypocotyl still

have higher levels of these regulators that why they do not need exogenous regulators for their growth.

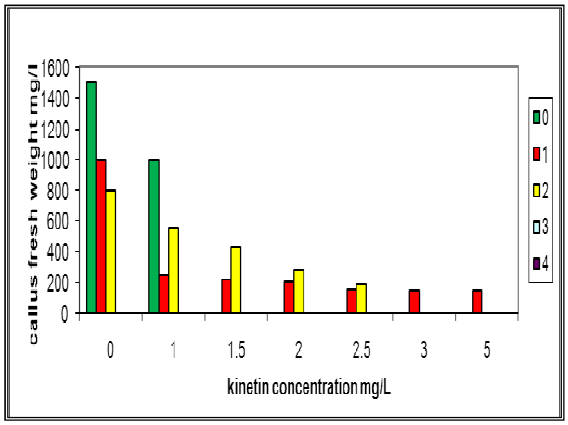

Fig (3): Callus fresh weight from hypocotyl explants with 2,4- D and Kinetin

Thymol production from callus: growth of callus was measured by determination the fresh weight in a period 75 day old. Generally, the Thymol content increase as the callus growth and it appears to remain high even after the growth of callus has stopped and began to decline. The maximum Thymol in leaf callus was after 75 days of culture. Controversially the hypocotyls, and root callus become old at 75 days which leads to decrease content and concentration of Thymol.

The similarity between standard and thymol isolated from leaf callus (75 day old) in the retention time between both samples (5.358) and (5.517) minutes, respectively as shown in (fig 4 and fig 5), concentration of isolated thymol is equalls to 30.5 mg/ml in the leaves callus.

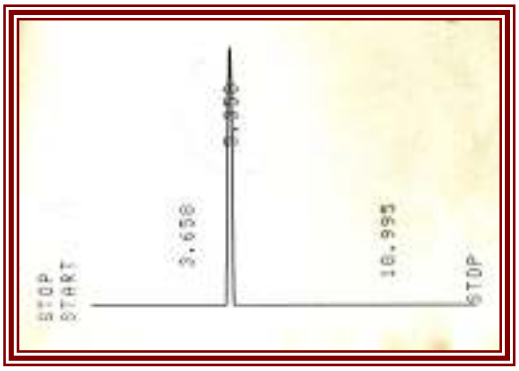

Figure (4): HPLC of the standard Thymol

The presence of the second peak as shown in (Fig 5) of the isolated thymol may be due to several reasons. The presence of isomeric thymol and these may be other phenolic compounds which has hydroxyl group in the sample or interacted with the solvent groups (14), the second reason may be separating another active compound from tissue culture or separating some pigments from the

callus, beside the high sensitivity of the method used to lowest concentrations and finally some factors may also affect like temperature and pH (16, 17).

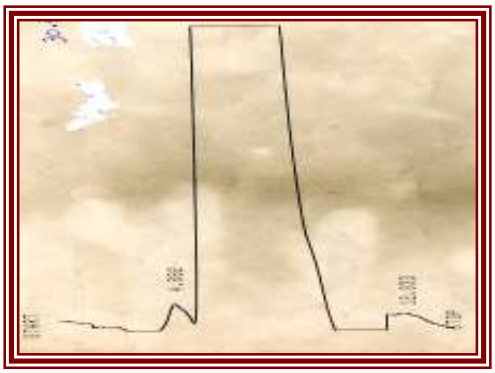

Figure (5): HPLC of isolated Thymol from leaf callus

From these results it was concluded that the *in vitro* production of Thymol varies with different plant parts. Thymol content in callus were found to be much lower than in *in vivo* plants, therefore, further studies should be done to increase the *in vitro* production of Thymol by manipulating the nutrient and environment conditions and the growth regulators.

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مرجع باللغة العربية:

14. التميمي، ازهار عادل 2004. دراسة بعض المتغيرات البايولوجية بفعل مركب الثايمول المعزول من المزارع النسيجية لنبات الحبة السوداء *Nigella sativa* العراق. أطروحة دكتورا، كلية العلوم، جامعة الموصل .

Antibiotics Susciptibility Patterns of Some Pathogenic Bacteria Isolated from Patients with Osteomyelitis

Shrooq Rayes (1), Suhad hatim (2), Ramina Mekhael (3)

College of pharmacy-Al –Mustansiriyah university/ Republic of Iraq, (1) , (2)
College of Science /Baghdad university / Republic of Iraq. (3)
E-mail: shrooq7@yahoo.com

ABSTRACT

Seventy two clinical swabs were collected from male bone infections to study the antibiotic susceptibility patterns of some bacterial isolates from osteomyelitis and their plasmid profile. 63 isolates were identified (87.5%)by bacteriological & biochemical tests,54 isolates were Gram-negative bacteria; *Pseudomonas aeruginosa* was the predominant pathogen (23.8%),followed by *Klebsiella pneumoniae* (20.6%),*Escherichia coli* and *Enterobacter* (14.2%) and *Proteus* (12.6%).Nine isolates of Gram positive bacteria were detected as *Staphylococcus aureus* (14.2%). 12 kinds of antibiotics were used :amikacin , ofloxacillin , tetracycline ,ciprofloxacin,cefotaxim,pipracillin,trimethoprime-sulfamethaxazole ,penicillin,ampicillin,gentamicin,augmentin and imipenem to test the susceptibility of 63 bacterial isolates on Mueller-Hinton agar by disk diffusion method . All the isolates were sensitive to imipenem (100%),& different susceptibility to most of the antimicrobial agents so imipenem was the most effective drug among the other kinds of drugs for treatment .

Most of the isolates exhibiting resistant to Amikacin (81.2%) & for ciprofloxacin it was (78.4%),while the result was (68.8%) for ofloxacin, & (51.2%) for gentamicin.The isolates exhibit low susceptibility to penicillin (12.8%),augmentin (16%),trimethoprime (17.6%),co-

trimethaxazole (28.8%) & reduced susceptibility (30.4%) to both pipracillin & tetracycline.

Key words:*Osteomyelitis ,bacteria ,antibiotic.*

الملخص باللغة العربية:

جمعت اثنان و سبعون مسحة سريرية من جروح اخماج العظام من الرجال لدراسة انماط الحساسية لبعض العزلات البكتيرية تجاه بعض المضادات الميكروبية المستخدمة محليا .

شخصت 63 عزلة بكتيرية بالأختبارات البكتريولوجية و البيوكيمياوية, 54 عزلة منها كانت سالية لصبغة كرام وكانت *Pseudomonas aeruginos* هي السائدة بنسبة (23.8%) تلتها بكتريا *Klebsiella pneumoniae* بنسبة (20.6%) و *Escherichia coli* و *Enterobacter* بنسبة (14.2%) و بكتريا ال *Proteus* بنسبة (12.6%) وكذلك شخصت 9 عزلات لبكتريا *Staphylococcus aureus* بنسبة (14.2%) .

استخدم 12 نوع من اقراص المضادات الحيوية (amikacin و ofloxacillin و ciprofloxacin و tetracycline و cefotaxim و pipracillin و rimethoprime-sulfamethaxazole و penicillin و ampicillin و gentamicin و augmentin و imipenem) للتحري عن حساسية العزلات للمضادات الحيوية على وسط المولار هنتون بطريقة الأنتشار بالأكار . ابدت كل العزلات حساسية للمضاد الحيوي imipenem بنسبة (100%) ولذا يعد هذا المضاد الأفضل للأستخدام في العلاج .

كانت معظم العزلات مقاومة لل Amikacin بنسبة (81.2%) و ciprofloxacin (87.4%) و ofloxacin (68.8%) و gentamicin (51.2%) .بينما اظهرت العزلات حساسية واطئة لل penicillin وبنسبة ,(12.8%) وال augmentin16% و trimethoprime (17.6%) وكذلك co-trimethaxazole (28.8%) , اما الحساسية تجاه المضادين pipracillin وtetracycline فكانت بنسبة (30.4%) .

INTRODUCTION

Osteomyelitis is the medical term for an infection in a bone. Infections can reach a bone by traveling through bloodstream or spreading from nearby tissue. Infections can also begin in the bone itself if trauma exposes bone to germs. Bone infections commonly affect the long bones of body, such as leg bones and upper arm bone, as well as spine and pelvis. Bone infections can occur after trauma (post-traumatic osteomyelitis), such as broken bones that break the skin (compound fracture) or open

wounds to the surrounding skin and muscles.Post-traumatic osteomyelitis can also occur after surgery if metal pins, screws or plates are used to secure the broken bones. Open wounds offer easy access for bacteria to enter the bone or nearby tissue directly (1) .

The most common organisms seen in osteomyelitis from areas of contagious infections, *S. aureus* & *S.epidermidis* are the most causes of hip & knee infections (2), gram negative bacteria including *Pseudomonas aeruginosa* ,*Escherichia coli* & other enteric bacteria (3).

osteomyelitis is also seen in patients with distant foci of infection such as those with infected urinary catheters.Infection occurs where there is direct contact of infected tissue with bone as may occur during a surgical procedure or following trauma. Clinical signs tend to be more localized and there are often multiple organisms involved.

Treatment of osteomyelitis remains a real challenge in medicine necessitating the use of broad-spectrum antibiotics, because of the variety of the pathogens causing the infection and the fact that the infected bone may become necrotic and vascular, preventing systemic antibiotics from adequately penetrating to the infection site (4). Osteomyelitis is also a disease in transition, with ongoing changes in predisposing factors, causative organisms and treatment . The advancing age of the general population has contributed to the increase in the incidence of diabetes and peripheral vascular disease (PVD), which are predisposing and complicating factors of osteomyelitis. There have also been dramatic changes in therapy, which include new antibiotics, new surgical techniques and outpatient parenteral antimicrobial therapy (5).

The aim of this study is to determine the bacteriological profile of chronic wound infections & know the antibiotic susceptibility pattern of the specimens.

MATERIALS AND METHODS

Specimens: 72 clinical specimens of chronic bone wound infections (osteomyelitis) from men (victims of explosions).The specimens were collected from Baghdad hospital during January –November 2008. The specimens were directly cultured on Blood agar, MacConkey agar (oxoid),and by EPI 20 in the hospital laboratory as described by (6).

Antibiotic susceptibility test:

12 kind of antibiotic disks (amikacin , ofloxacillin , tetracycline ,ciprofloxacin,cefotaxim,pipracillin,trimethoprime-sulfamethaxazole ,penicillin,ampicillin,gentamicin,augmentin and imipenem) were used to test the susceptibility of 63 bacterial isolates by disk diffusion method on Mueller-Hinton agar (Oxoid) (6).

Standard strains: *E.coli* ATCC (25922), *P.aeruginosa* ATCC (27853) and *S.aureus* ATCC (25923).

RESULTS AND DISCUSSION

There were 73 clinical swabs examined, 63 isolates (87%) were identified by bacteriological & biochemical tests as in (table 1) & ten swabs failed to yield any bacterial growth. *Pseudomonas aeruginosa* was the predominant isolate (15:23.8%), followed by *Klebsiella* (13:20.6 %), while isolates of Escherichia *coli, Enterobacter*, & *Staphylococcus aureus* were (14.2) and *Proteus* (12.6%).

Table (1): Bacterial isolates of osteomyelitis

Bacteria	No. of Isolates	%
E. coli	9	14.2
Enterobacter	9	14.2
K.pneumoniae	13	20.6
Proteus	8	12.6
P.aeruginosa	15	12.6
S.aureus	9	14.2
Total	63	

Many studies on the incidence of pathogenic bacteria from osteomyelitis , mentioned that *S.aureus* was (9%) & *P.aeruginosa*

was (4.4%) as in (7,8) but in another study *Proteus* isolates were predominant (50%),followed by *S.aureus* (42%) then *P.aeruginosa* (14%),& *E.coli* (11%) according to types of locations & hospitals.The most common pathogens of osteomyelitis are *S.aureus* ,*P.aeruginosa* & *E.coli* (9,10).Management & treatment of bone fractures should be early systemic, wide spectrum antibiotic & cover gram negative & positive bacteria(11).

The differences in numbers and species of isolates in our study and the other studies are refer to total number of specimens ,hospitals ,environment and kinds of antibiotics which are used in the countries that definitely affect on the species of dominant bacteria in hospitals and patient wounds .

All the isolates were sensitive to imipenem (100%),as showed in (table 2) & different susceptibility to most of the antimicrobial agents,so imipenem was the most effective drug among the other kinds of drugs (12) .

More than the half of the isolates exhibiting resistant to Amikacin (81.2%) & for ciprofloxacin it was (78.4%),while the result was (68.8%) for ofloxacin, & (51.2%) for gentamicin.The isolates exhibit low susceptibility to penicillin (12.8%),augmentin(16%),trimethoprime (17.6%),co-trimethaxazole (28.8%) & reduced susceptibility (30.4%) to both pipracillin & tetracycline.

Fluoroquinolones are as effective as beta-lactams for the treatment of osteomyelitis and can be considered as a useful alternative in the physician's armamentarium. The value of fluoroquinolones for the treatment of osteomyelitis lies in the fact that they can be administered in an outpatient setting (4).

Multiple antimicrobial resistance was also observed in this study , as in (table 3) ,one isolate of *Enterobacter* was resistant to (9,5) kinds of antibiotics subsequently .Two isolates were resistant to 8 & 7 kinds ,Two isolates of *E.coli* were resistant to 10 & 7 kinds of antibiotics,one isolate to 9 kinds & 4 isolates to 6 kinds. Six isolates of *Klebsiella* were resistant to 8 kinds of antibiotics, 3 isolates to 7 & 6

kinds .Three isolates of *proteus* were resistant to 7 kinds of antibiotics & 2 isolates to 6 kinds.One isolate of *Pseudomonas* were resistant to 9 & 8 kinds of antibiotics ,5 isolates to 7 kinds, & 4 isolates to 6 kinds.One isolate was resistant to 9 & 6 kinds .A new study on *E.coli* revealed a plasmid which refers resistance to gentamycin,tetracycline,ciprofloxacin & trimethoprime (13*).*

Table (2): Antibiotic susceptibility of osteomyelitis isolates

Antibiotics	R or S	*E.coli*	*Enterobacter*	*K.pneumoniae*	*Proteus*	*P.aeruginosa*	*S.aureus*	Total
Ak	R	-	4	-	-	-	8	12
	S	9	5	13	8	15	1	51
OFX	R	4	3	2	3	5	3	20
	S	5	6	11	5	10	6	43
TE	R	6	4	9	8	14	3	44
	S	3	5	4	-	1	6	19
CF	R	4	2	1	1	2	4	14
	S	5	7	12	7	13	5	49
CTX	R	6	7	9	4	13	6	45
	S	3	2	4	4	2	3	18
PIP	R	7	7	12	4	11	3	44
	S	2	2	1	4	4	6	19
Tr	R	6	8	11	7	15	5	52
	S	3	1	2	1	-	4	11
P	R	9	8	11	7	15	5	55
	S	-	1	2	1	-	4	8
AM	R	9	8	12	6	15	5	55
	S	-	1	1	2	-	4	8
GM	R	6	4	5	2	9	15	49
	S	3	5	8	6	6	4	32
AC	R	8	8	12	7	12	6	53
	S	1	1	1	1	3	3	10
IMP	R	-	-	-	-	-	-	-
	S	9	9	13	8	15	9	63

P. aeruginosa exhibits remarkable ability to acquire resistance to these agents. Acquired resistance arises by mutation or acquisition of exogenous resistance determinants and can be mediated by several mechanisms degrading enzymes, reduced permeability, active efflux and target modification.These enzymes confer high level resistance to antipseudomonal cephalosporins (14).

Table (3):Multiple antimicrobial resistance of osteomyelitis isolates

Bacteria	No. of resistant isolates	No. of antibiotics
E.coli	2	10
	1	9
	2	7
	4	6
Enterobacter	1	11
	1	9
	2	8
	2	7
	1	6
Klebsiella	6	8
	3	7
	3	6
Proteus	3	7
	2	6
Pseudomonas	1	9
	1	8
	5	7
	4	6
S.aureus	1	9
	2	8
	2	7
	1	6

CONCLUSION

Pseudomonas aeruginosa was the predominant isolate (15:23.8%), followed by *Klebsiella* (13:20.6 %), while isolates of Escherichia *coli, Enterobacter*, & *Staphylococcus aureus* were (14.2) and *Proteus* (12.6%). The differences in numbers and species of isolates in our study and the other studies are refer to total number of specimens ,hospitals ,environment and kinds of antibiotics which are used in the countries that definitely affect on the species of dominant bacteria in hospitals and patient wounds . All the isolates were sensitive to imipenem (100%).

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Cytotoxicity and antitumor activity of *Nigella sativa* Seed

Sundus H. Ahmed

Food technology center/ Ministry of science and technology/ Republic of Iraq

E-mail: hams_63@yahoo.com

ABSTRACT

The cytotoxicity study with cancer cell line A-549 indicated that the methanol insoluble fraction of methanol extract *Nigella sativa* is toxic (CTC50 165,110 µg/ml) in microculture tetrazolium assay and sulphorhodamine B assays. However, an antitumor screening by the short-term toxicity study with DLA cells showed that the methanol insoluble fraction showed the highest toxicity in compare with the other extract .

Key words: *Nigella sativa*, cytotoxicity, antitumor, medicinal plants.

الملخص باللغة العربية

اظهرت نتائج دراسة السمية في خط الخلايـــا A-549 ل*Nigella sativa* الجزء الغير ذائب لمستخلص الكحول المثيلي ل *Nigella sativa* ذو سمية (CTC50 165, 110 µg /ml) عنـــد اســـتخدام طريقـــة microculture tetrazoliumassay و sulphorhodamine B assay وعنداجراء الدراسة المسحية للفعاليـــة المـــضادة للسرطان باختبار السمية القصيرة الامد باستخدام DLA cells بينت ان المستخلص نسبيا عـــالي السمية.

INTRODUCTION

Many studies have been conducted, particularly during the last two decades, on the effect of *N. sativa* L. seed extracts on various body systems *in vitro* or *in vivo*(1,2). The pharmacological

investigations of the seed extracts reveal a broad spectrum of activities including immunopotentiation (3,4) and antihistaminic (5, 6), antidiabetic (7), anti hypertensive (8), anti-inflammatory (9), and antimicrobial activities (10). Many of these activities have been attributed to the quinone constituents of the seed (11,12). Furthermore, black seed preparations may have a cancer chemopreventive potential and may reduce the toxicity of standard antineoplastic drugs (13). In fact, topical application of a blackcumin extract inhibited the two stage initiation-promotion of skin carcinogenesis in mice by dimethylbenz [a]anthracene (croton oil) (13). In addition, others have reported an antitumor activity of some crude and purified components of *N. sativa.* In fact, Salomi et al. (14) have shown that a crude methanol extract of blackseed exhibited a strong cytotoxic action on Erlich ascites carcinoma, Dalton's ascites lymphoma and sarcoma 180 cells, with minimal cytotoxicity to normal lymphocytes. These investigators have also described the cytotoxic property of extracts of the seeds *in vivo*, as shown by inhibition of the growth of Erlich ascites carcinoma in mice receiving 2 mg of the extract per mouse per day for 10 days (14). Furthermore, a new triterpene saponin (-hederin) recently isolated from blackseed showed significant dose-dependent tumor inhibition when given intraperitoneally for 7 days at 5 and 10 mg/kg to mice with formed tumors (15). Similarly, Worthen and colleagues (4) have tested *in vitro* a crude gum, a fixed oil and two purified components of the seed (thymoquinone (TQ) and dithymoquinone (DTQ)) for cytotoxicity to some parental and multidrug-resistant human tumor cell lines. Protein extract of the seeds possess antitumor activity against Yeshida sarcoma in rats and mice (16). thymoqinoun was effective in reducing solid tumour mass stored in desiccators for pharmacological experiments. This investigation evaluates the cytotoxicity and antitumor properties of *N sativa*.

MATERIALS AND METHODS

Preparation of extract

Dried seed powder of Black cumin was extracted with 50% aqueous ethanol in cold maceration method (17) at room temperature. After filtration, extracted twice in the same conditions. Ethanol was removed under vacuum and the aqueous residue was left to dryness(crude 50% ethanolic extract) to take exact weight from this crude material and then were fractioned in petroleum ether, chloroform and methanol. The crude (50% ethanolic extract and methanol soluble and insoluble fractions of crude were stored in desiccators for pharmacological experiments.

Determination of mitochondrial synthesis by microculture tetrazolium (MTT) assay

Human cell line A – 549 from small cell lung carcinoma (morphology: epithelial) was used in the experiment. The cell line was obtained from UFSP Medical College, Sao paolo, Brazil. The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitrochondrial activity per cell (5). The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells is proportional to the extent of formazan production by the cells used.The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x105 cells/ ml using the medium containing 10%new born calf serum. Each well of the 96 well microtitre plate, 0.1 ml of the cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 □l of different drug dilutions concentrations was

added to the cells in microtitre plates. The plates were then incubated at 37C for 72 h in 5% CO2 atmosphere. Microscopical examination was carried out and observations were recorded every 24 h. After 72 h, the extraction of black cumin solutions in the wells were discarded and 50 Ml of MTT in MEM-PR was added to each well. Then plates were gently shaken and incubated for 3 h at 37□C in 5% CO2 atmosphere. The supernatant was removed and 50 □l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. (18). the percentage growth inhibition was calculated using the formula:

Growth inhibition (%) =

$$100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

The plates were then incubated at 37□C for 72 h in 5% CO2 atmosphere. Microscopical examination was carried out and observations are recorded every 24 h. After 72 h, 25 ml of 50% trichloro acetic acid was gently added to the wells such that it forms a thin layer over the drug dilutions to form a over all concentrations of 10%. The plates were incubated at 4C for 1 h. The plates were flicked and washed five times with tap water to remove traces of medium, extracts and serum, and were then air-dried. The air-dried plates were stained with sulphorhodamine B for 30 min. The unbound dye was then removed by rapidly washing four times with1% acetic acid. The plates were then air-dried. 100 ml of 10 mM tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 min. The absorbance was measured using a microplate reader at a wavelength of 540 nm (18). The percentage growth inhibition was calculated using the formula shown beside.

Determination of total cell protein content by sulphorhodamine B (SRB) assay

Sulphorhodamine B (SRB) is a bright pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, sulphorhodamine B binds to the proteins basic amino acid residues in TCA (trichloro acetic acid) fixed cells to provide a sensitive index and cellular protein content that is linear over a cell density range of at least two orders of magnitude.Colour development in sulphorhodamine B assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. When TCA-fixed and sulphorhodamine B stained samples are air-dried, they can be stored indefinitely without deterioration. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x105 cells /ml with medium containing 10% new born calf serum. Each of the 96 wells of microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed once with medium and 100 ml of different drug concentrations were added to the cells in microtitre (19).

Anti-tumor screening

Cells were obtained from Brazil Cancer Institute, and was propagated and maintained in the peritoneal cavity of mice at, UFSM, Santa Maria / Brazil. The tests rely on a break down in membrane integrity determined by the uptake of a dye such as (Tryphan blue, erythrorisin and nigrosin) to which the cell is normally impermeable. DLA cells were cultured in peritoneal cavity of mice by injecting a suspension of DLA cells (1.0 x105 cells/ml) intraperitoneally. The DLA cells were withdrawn from the peritoneal cavity of the mice between 15 - 20 days with the help of a sterile syringe. The cells were washed with HBSS and centrifuged for 10 - 15 min at 10,000 rpm. The procedure was repeated thrice. The cells were suspended in known quantity of HBSS and the cell count was adjusted to 2 x 106 cells /ml. The cell suspension was distributed

into Eppendorf tubes (0.1 ml containing 2 lakhs cells). The cells were exposed to drug dilutions and incubated at 37□C for 3 h. After 3 h dye exclusion test, that is, equal quality of the drug treated cells were mixed with tryphan blue (0.4%) and left for 1 min. It was then loaded in a haemocytometer and viable and non-viable count was recorded within 2 min. Viable cells do not take up colour, whereas dead cells take up colour. However, if kept longer, live cells also generate and take up colour (20). The percentage of growth inhibition was calculated using the following

Formula:

Growth inhibition (%) =

$$100 - \frac{\text{Total cells- dead cells}}{\text{Total cells}} \times 100$$

Statistical analysis

Data from all experiments were statistically evaluated using analysis of variance (ANOVA) followed by Duncan's test; $p > 0.05$ were considered significant (21).

RESULTS & DISCUSSION

The present study was under taken to provide comparative data on the *in vitro* cytotoxic activity of different fractions of *N. sativa* by using different assays. The cytotoxicity study indicated that the methanol insoluble fraction of crude extracts forms toxic (CTC50 , 165, 110 µg/ml) to the cell in both asoluble (cancer cell line A- 549 - small cell lung carcinoma; whereas all the other fractions soluable and insoluable showed toxicity at a higher concentration only (Table 1) shows that total cellular protein mitochondrial synthesis by microculture tetrazolium (MTT) assay in A-549 cell line in different fraction of *N. Sativa* where highest in insoluable fraction at the concentration 31.25 µg/ ml , the major constituents of the essential oil of *N. sativa*, were cytotoxic to all cell lines (4). Furthermore, the presence of terpenes as major components in the *N. sativa* may explain its high cytotoxic activity. In fact, the anti-cancer potential of these products has been recently reported (23-24) Percentage of

growth inhibition in determination of total cellular protein mitochondrial synthesis by micro culture tetrazolium assay (MTT) in A-549 cell line in different extract of *N. sativa* were shown in table (2) .

Table (1): Determination of total cellular protein mitochondrial synthesis by microculture tetrazolium (MTT) assay in A-549 cell line in different extract of *N. Sativa*

Methanol fraction	**Mean absorbance**						
	Control	1000	500	250	125	62.5	31.25
Control	0.213± 0.004	0.036± 0.001	0.095± 0.003	0.122± 0.005	0.136± 0.012	0.155± 0.001	0.201± 0.002
Methanol soluble Fraction	0.721± 0.006	0.045± 0.001	0.058± 0.014	0.075± 0.001	0.096± 0.003	0.136± 0.001	0.168± 0.014
Methanol insoluble fractiopn	0.721± 0.012	0.025± 0.002	0.038± 0.006	0.068± 0.001	0.088± 0.007	0.012± 0.016	0.155± 0.003

Table (2): Percentage of growth inhibition in determination of total cellular protein mitochondrial synthesis by micro culture tetrazolium assay (MTT) in A-549 cell line in different extract of *N. sativa* seeds.

Methanol fraction	Percentage of growth inhibition						CTC50
	1000	500	250	125	62.5	31.25	g/ml
Crud	58.78	35.15	20.00	14.84	3.75	0.00	450
Methanol soluble fraction	65.33	35.54	12.35	4.20	0.0	0.0	375
Methanol In soluble fraction	87.22	73.61	64.33	55.21	41.15	33.53	165

Table (3): Percentage of growth inhibition in the determination of total cellular protein by Sulphorhodamine B (SRB) assay in A- 549 cell line in different extract of *N. sativa* seeds.

Methanol fraction	Percentage of growth inhibition					CTC50
	1000	500	250	125	62.5	g/ml
Crud	53.61	33.22	13.14	10.19	07.45	>500
Methanol soluble fraction	60.32	34.69	20.49	17.42	17.42	400
Methanol Insoluble fraction	73.79	57.18	49.47	36.98	28.41	110

Table (4): The antitumor screening (short term toxicity studies) used by DLA cells in different extract of *N.sativa* seeds.

Drug	Percentage of growth inhibition					CTC50
	1000	500	250	125	62.5	mg/ml
Crud	59.14	42.62	29.12	16.16	10.03	740
Methanol soluble fraction	64.46	49.78	31.26	23.26	14.64	510
Methanol Insoluble fraction	81.41	70.16	63.16	47.01	32.33	145

We found that the highest percentage of growth inhibition by insoluble fraction was 87.22% in the highest concentration 1000 µg / ml while the inhibition by soluble fraction was 65.33% in comparison with control. In antitumor screening by the short term toxicity studies used by Dalton's lymphoma ascities (DLA) cells all the extracts showed less toxic (CTC50 740, 510 except Methanol Insoluble fraction, 145, mg/ml) (Table 4). *N. sativa* are more toxtic to tumour cells than normal cells (6). *N. sativa* is effective in reducing solid tumour mass development induced by Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells. DLA cell line was more sensitive to *Nigella* than EAC (20). *Nigella* has a highly toxic protein (LD50, 0.018 mg/kg body wt of mice) present to extent of 0.22% in the seed. It has been studied intensively for its antitumour activity (24).

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أثر التحفيز الكهربائي وحقن المحاليل الملحية على محتوى بروتينات المايوفيبرل و الأحماض الأمينية الحرة في تحسين طراوة اللحوم المنتجة من إناث الماعز المسنة

اميرة محمد صالح الربيعي (1) ، مهدي ضمد القيسي (2)، . زهير فخري الجليلي(3)

قسم الثروة الحيوانية، كلية الزراعة،ابو غريب/ بغداد، العراق (1) ، (3) ،
وزارة الزراعة، بغداد، العراق(2)

البريد الإليكتروني: alrubeii@yahoo.co.uk

ABSTRACT

The objective of present study was to investigate the effect of electrical stimulation and salt infusion on myofibril proteins and free amino acids content from meat of carcasses aged female goats. Thirty female goats with age 7-8 years and 40.50 kg in weight were managed, fed and slaughtered thereafter. The carcasses were divided into 7 groups and each group were subjected to one of the following treatments : T1 : Control , T2 : electrical stimulation (80 volts , 25 Hz for 2 min), T3 : electrical stimulation (200 volts , 25 Hz for 2 min) , T4 : sodium chloride infusion (0.6 M) , T5 : calcium chloride infusion (0.3 M) ,T6 : T2 + T4 and T7 : T2 + T5.

The results obtained were summarized as follows:There were significant differences in the release of free amino acids among treatments T7 surpassed the other treatments in this respect. Gel electrophoresis pattern of myofibril protein extraction from the three muscles in T2-T7 demonstrate distinct hydrolysis of most meat proteins . The results also indicate that among the six treatments (T2-T7) . T7 revealed higher ability in analyzing and breaking down the meat proteins.It can be concluded from this study that electrical stimulation and infusion of NaCl or

CaCl2 improve tenderness of aged female goat meat.

الملخص باللغة العربية

استهدفت التجربة دراسة تأثير معاملتي التحفيز الكهربائي والحقن بالأملاح والتأثير المشترك لهما في بروتينات المايوفيبرل ومحتوى اللحم من الاحماض الامينية الحرة لثلاث عضلات في ذبائح إناث الماعز المسنة. استخدم (30) أنثى ماعز مسنة تتراوحت أعمارها بين 7–8 سنوات بمتوسط وزن حي 40.50 كغم. وزعت عشوائياً على سبعة معاملات هي عينة المقارنة (T1)، التحفيز الكهربائي (80 فولت بتردد 25 هيرتز لمدة دقيقتين) (T2)، التحفيز الكهربائي (200 فولت بتردد 25 هيرتز لمدة دقيقتين) (T3)، الحقن بمحلول كلوريد الصوديوم تركيز (0.6 مولار) (T4)، الحقن بمحلول كلوريد الكالسيوم تركيز (0.3 مولار) (T5)، التحفيز الكهربائي (80 فولت) + الحقن بمحلول كلوريد الصوديوم تركيز (0.6 مولار) (T6) والتحفيز الكهربائي (80 فولت) + الحقن بمحلول كلوريد الكالسيوم بتركيز (0.3 مولار) (T7). ثم أجريت مجموعة من القياسات الكيميائية. وقد بينت النتائج مايلي :لوحظت زيادة معنوية ($p<0.05$) في الاحماض الامينية الحرة في معاملات التجربة وللعضلات LD , SM وBF عند المقارنة مع معاملة السيطرة . كما ظهرت اعلى نسبة مئوية في الزيادة المتحققة في كمية الاحماض الامينية الحرة الكلية في المعاملة T7 وللعضلات المذكورة انفا مقارنة مع المعاملة T1 .اوضحت نتائج الترحيل الكهربائي الهلامي لمستخلص بروتينات المايوفيبرل في العضلات LD , SM وBF حصول تحلل بروتيني واضح في اغلب بروتينات اللحم بتاثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما مقارنة مع معاملة السيطرة . كذلك يتضح من النتائج ان مقدار تحلل وتكسير بروتينات اللحم في المعاملة T7 يفوق ما حدث في بقية المعاملات الاخرى.يستنتج من نتائج الدراسة بان استعمال التحفيز الكهربائي والحقن باملاح كلوريد الصوديوم وكلوريد الكالسيوم في ذبائح اناث الماعز المسنة يؤدي الى تحسين طراوة اللحوم المنتجة منها .

مفتاح الكلمات :التحفيز الكهربائي ,المحاليل الملحية ,بروتينات اللحم ,

- البحث مستل من اطروحة دكتوراه للباحث الاول .

المقدمة

تعد الطراوة من أكثر خواص الاستساغة أهمية للمستهلك وهي أولى الخواص التي يشعر بها الفرد عند أكل اللحوم وتقطيعها في الفم إلى قطع صغيرة (الطائي ,1986).وتتأثر طراوة اللحوم بصلابة الليفات العضلية الناتجة من تداخل بروتينات التقلص (الاكتين والمايوسين) مع بعضهما في العضلة وتعتمد صلابة الألياف العضلية على عاملين هما : الوقت ودرجة الحرارة أثناء تطور التيبس الرمي وأيضا فعل الإنزيمات المحللة خلال مدة الإنضاج (Hertzman واخرون ، 1993). لكن ضعف الارتباط او التداخل بين الاكتين والمايوسين المقرون مع تجزئة البناء الخويطي واستطالة الساركومير يسهم في زيادة الطراوة بعد التيبس الرمي (Taylor واخرون ، 1995) . و أشار Davey وGraafhuis (1976) الى ان فقدان صفة التخطيط من الليفات العضلية خلال مدة الإنضاج وحدوث تغيرات بنائية في التركيب النسيجي (كتلة الألياف العضلية) وبتعبير أدق في بروتينات الليفات العضلية في الساركومير وفي اتجاهين طولي وجانبي يعتمد على مدى التحلل البروتيني (Proteolytic) مما يؤدي الى فقدان تماسك وارتباط الألياف العضلية مع بعضها البعض (Tornberg ، 1996) . وفي الاتجاه نفسه وجد Ouali (1990) ان التمزق في منطقة N2-line (منطقة ارتباط خويطات الاكتين مع Z– line) له دور رئيسي في عملية تطرية اللحوم . وافاد Robson واخرون (1997) ان اكثر البروتينات التي تعاني تحلل وتكسر هي سبعة أنواع وتشمل : التتين ، الدسمين ، النيبولين البارانمين (Paranemin) ، السينمين (Synemin) ، الفلمين (Filamin) والتروبونين – T وتصنف هذه البروتينات بأنها كلايكوبروتين ولها دور كبير في تركيب الساركومير و لاحظ Huff – Lonergan واخرون (1996) ان كلا من بروتين التتين والنبولين يعانيان تكسرا وتحللا في وقت مبكر بعد الذبح ويكتمل تحللهما بعد ثلاثة ايام من الذبح مما يسبب ضعفا في قوة الليفات العضلية و يعد المفتاح للخطوة الأولى في طراوة اللحوم بعد مرحلة التيبس الرمي . لذلك اتجهت صناعة اللحوم الى إيجاد تقنيات سريعة وبديلة للتعتيق في الإسراع من عملية التطرية في اللحوم ومنها استعمال تقنية التحفيز الكهربائي (Hertzman واخرون ، 1993 و Devine واخرون ، 2001) ، وتقنية الحقن أو التنقيع بالأملاح ($CaCl_2$ NaCl) (Geesnik واخرون ، 1994) .ومن المؤشرات المعتمدة لقياس طراوة اللحوم تقنية الهجرة الكهربائية بهلام متعدد الاكريلامايد (SDS-PAGE) لفصل وتقدير الوزن الجزيئي للببتيدات والبروتينات المختلفة الناتجة من تكسير وتحلل بروتينات الساركوبلازما والليفات العضلية (Claeys وآخرون، 1995). وكذلك تقدير الأحماض الأمينية الحرة فقد أشارت الدراسات إلى زيادة تركيز الأحماض الأمينية الحرة خلال مدة الخزن بعد الذبح بسبب التحطيم والتكسير في الببتيدات المتعددة وبروتينات الساركوبلازما والليفات العضلية بفعل البروتيزات (الكالبينات والكاثبسينات)، لذلك فان تقدير الأحماض الأمينية الحرة هو مؤشر على التحسن الذي يحصل في طراوة اللحوم اذ يعطي تقييما

غير مباشرا (Feidt وآخرون، 1996). لذلك استهدفت الدراسة الحالية بيان تأثير كلا" من التحفيز الكهربائي والحقن بالاملاح والتأثير المشترك لهما في بروتينات المايوفيبرل ومحتوى اللحم من الاحماض الامينية الحرة في ذبائح إناث الماعز المسنة.

المواد و طريقة العمل

استعمل في التجربة (30) أنثى ماعز محلي مسنة تتراوحت أعمارها من 7–8 سنوات وبمتوسط وزن حي 40.50 كغم . خضعت لبرنامج تغذوي واحد طوال مدة التجربة (70 يوم) في محطة تجارب الإنتاج الحيواني التابعة لمركز إباء للأبحاث الزراعية بالعراق. تم ذبح الإناث كافة وسلاخة الذباح وتجويفها وتوزيعها عشوائياً إلى سبع معاملات هي :1.السيطرة (T1)، 2.التحفيز الكهربائي بمقدار 80 فولت (T2)، 3.التحفيز الكهربائي بمقدار 200 فولت (T3)، 4. الحقن بكلوريد الصوديوم تركيز 0.6 مولار (T4)، 5. الحقن بكلوريد الكالسيوم تركيز 0.3 مولار (T5)، 6. التحفيز الكهربائي 80 فولت +الحقن بكلوريد الصوديوم 0.6 مولار (T6)، 7. التحفيز الكهربائي 80 فولت + الحقن بكلوريد الكالسيوم 0.3 مولار (T7).

وتم تطبيق تقنية التحفيز الكهربائي خلال مدة 20 دقيقة بعد الذبح باستعمل جهاز التحفيز الكهربائي (المصمم والمصنع من قبل الدائرة الهندسية / منظمة الطاقة الذرية العراقية Electrical Stimulation (ES)لتوليد سلسلة من النبضات عددها 3000 نبضة تشمل (1500 نبضة on) و (1500 نبضة off) وبتياركهربائي حوالي 3 أمبير بتردد ثابت مقداره (25 هيرتز) على مدى دقيقتين وبفولتيتين (80 أو 200 فولت) و يصل التيارالكهربائي عن طريق سلكين في نهاية كل منهما الالكترود أحداهما وضع في تماس للمنطقة الواقعة بين الفقرة الخامسة والسادسة من فقرات الرقبة أما الالكترود الآخر فقد وضع في تماس مع العضلات الموجودة قرب منطقة الوتر العرقوبي. أما عملية الحقن بالأملاح فقد اتبعت الطريقة الموصوفة من قبل (1989).et al Koohmaraie في الحقن بأملاح كلوريد الصوديوم (0.6 مولار) وكلوريد الكالسيوم (0.3 مولار) في الوريد الوداجي في منطقة الرقبة وبنسبة حقن 1% من الوزن الحي لكل أنثى اي مايعادل مل /100 غرام من وزن الحيوان الحي بواسطة حقنة نبيذة syringe سعة (50 مل) وخلال مدة 20 دقيقة من بعد الذبح. أما في حالة التأثير المشترك لكلا التقنيتين ، فقد تم أولا تطبيق التحفيز الكهربائي ثم عملية الحقن بالأملاح ، ثم تركت الذبائح في غرفة درجة حرارتها (16 + 2 ْم) لمدة (3 ساعات) بعدها نقلت إلى غرفة التبريد عند درجة حرارة (2 ْم) لمدة 24 ساعة.بعد ذلك وبطريقة تشريحية فصلت ثلاث عضلات رئيسية هي العضلة الطويلة الظهرية (LD) Longissimus Dorsi والعضلة نصف الغشائية Semimembranosus (SM) والعضلة الفخذية ثنائية الرأس Biceps (BF) femoris استناداً إلى طريقة (1983) .et al Butterfield ثم إزالة الدهن المترسب حولها وتغليفها بأكياس من البولي اثيلين لحين إجراء القياسات عليها. تم تقدير الاحماض الامينية الحرة

باستعمال جهاز الكروماتوكرافي السائل عالي الاداء (HPLC) وحضرت العينات استنادا" الى طريقة Mikami واخرون (1994) و استعملت الطريقة الموصوفة من قبل Heinrikson و Meredith (1984) في تحضير المشتقة و اتبعت في الفصل الظروف التي ذكرها McClung و Frankenberger (1988) وباستعمال عمود الطور المعكوس (C–18) Ultra Sphere- ODS ذو الابعاد (4.6 × 250 ملم) وبحجم جزيئات (5 مايكروميتر) اما بخصوص الهجرة الكهربائية لهلام الاكريلامايد المتعدد – SDS .

Poly Acrylamide Gel Electophoresis

SDS-PA استعملت طريقة Laemmli (1970) و Greaser واخرون (1981) في دراسة الهجرة الكهربائية لبروتينات مستخلص المايوفيبرل ومتابعة تاثير المعاملات المختلفة في هذه البروتينات بطريقة SDS-PAGE باستعمال جهاز الهجرة الكهربائية المجهز من قبل شركة Pharmacia السويدية . واستعمل البرنامج الإحصائي الجاهز(2001) SAS في تحليل البيانات كما استعمل اختبار دنكن لتحديد الفروقات المعنوية بين المتوسطات للمعاملات وبين العضلات ضمن المعاملة الواحدة.

النتائج والمناقشة

توضح الجداول (3,2,1) التحليل الكمي للاحماض الامينية الحرة (ملغم / 100 غم لحم) في معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما في العضلات SM , LD و BF. ويلاحظ من الجداول ان كمية الاحماض الامينية الحرة ازدادت بتاثير المعاملات المذكورة انفا مقارنة مع معاملة السيطرة مما يدل على ان استعمال هذه المعاملات ادى الى زيادة انفراد الاحماض الامينية الحرة الكلية وكمية كل حامض اميني على حدة .اذ يلاحظ ان اعلى زيادة في انفراد الاحماض الامينية الحرة للعضلات SM , LD و BF كانت في المعاملة T7 . في حين كانت اقل زيادة في انفراد الاحماض الامينية الحرة للعضلات SM , LD و BF في المعاملة T1 .كذلك سجلت المعاملة T3 أعلى زيادة في انفراد الحامض الاميني الهيدروكسي برولين وبلغت 4.79 ، 4.45 و 4.19 ملغم/100غم لحم في العضلات SM , LD و BF على التوالي وكذلك في الحامض الاميني الهستدين وبلغت 6.18 ، 6.05 و 5.95 ملغم/100غم لحم في العضلات المذكورة انفا على التوالي . كما تفوقت المعاملة T5 في زيادة انفراد الحامض الاميني الارجنين وبلغت 5.29 ، 5.01 و 4.90 ملغم/100غم لحم في العضلات المذكورة انفا على التوالي مقارنة مع معاملة السيطرة . اما بخصوص زيادة انفراد الاحماض الامينية الحرة في بقية المعاملات فقد اختلفت معنويا ($p<0.o5$) بين معاملة وأخرى وكان تسلسلها وحسب الاهمية من الاعلى زيادة في انفراد الاحماض الامينية الحرة الى الاقل هي T6, T5, T3ثم T4واخيرا T1 على التوالي .

الجدول (1): تاثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما في محتوى اللحم من الاحماض الامينية الحرة (ملغم/100 غم/لحم) في العضلة الطويلةالظهرية (LD) لذبائح اناث الماعز المسنة .

T7	T6	T5	T4	T3	T2	T1	الحامض الاميني
A 2.03	A 1.96	AB1.90	AB1.80	AB1.89	BA1.85	B 1.65	الاسبارتك
A31.25	A29.51	B 27.66	CB26.10	CB26.90	C 25.63	D15.32	الكلوتاميك
A 4.73	AB 4.67	BC 4.46	BC 4.31	AB 4.64	AB4.52	C 4.23	السيرين
A 4.63	B 3.15	CB 2.90	C 2.70	A 4.79	B 3.08	D 1.50	هيدروكسي برولين
A 8.03	BA 7.83	BA 7.75	BA 7.80	B 7.51	B 7.48	B 7.67	الكلايسين
A 6.11	BA 5.96	BA 5.81	BC 5.60	A 6.18	BA 5.92	C 5.42	الهستدين
A 3.90	BA 6.76	BA 3.66	BA 3.63	BA 3.67	BA 3.61	B 3.52	الثريونين
A 37.10	A 36.91	BC 36.33	D 35.61	AB 36.59	DC 35.94	E34.39	الالنين
AB 5.21	AB 5.18	A 5.37	A 5.29	C 4.86	BC 4.98	AB 5.22	الارجنين
A 3.38	A 3.30	A 3.40	A 3.34	A 3.22	A 3.11	A 3.29	البرولين
A 2.66	A 2.60	A 2.53	A 2.42	A 2.40	A 2.25	A 2.21	التايروسين
A 4.21	A 4.14	AB 4.01	AB 3.94	AB 3.97	AB 3.91	B 3.76	الفالين
A 1.79	AB 1.72	ABC 1.50	BC 1.43	ABC 1.63	AB 1.51	C 1.34	الميثايونين
A 1.52	A 1.41	A 1.24	A 0.90	A 0.95	A 0.91	A 1.55	السستين
A 3.12	AB 3.01	CD 2.71	CD 2.61	ABC 2.85	BCD 2.75	D 2.47	الايزوليوسين
A 5.09	AB 4.85	BC 4.60	CD 4.39	B 4.70	BC 4.64	D 4.30	اليوسين
A 3.35	AB 3.26	ABC 2.93	BC 2.80	ABC 3.16	ABC 3.13	C 2.70	الفنيل النين
A 4.81	AB 4.65	CDE 4.40	ED 4.33	AB 4.60	BCD 4.46	E 4.19	اللايبسين

تشير الحروف المختلفة ضمن الصف الواحد الى وجود اختلافات معنوية بين المعاملات بمستوى (P<0.05)

الجدول (2) : تاثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما في محتوى اللحم من الاحماض الامينية الحرة (ملغم/100 غم/لحم) في العضلة نصف الغشائية (SM) لذبائح اناث الماعز المسنة .

T7	T6	T5	T4	T3	T2	T1	الحامض الاميني
A 2.00	AB 1.85	AB 1.82	BC 1.66	AB1.80	AB 1.79	C 1.53	الاسبارتك
A 30.90	B 28.93	C 27.04	D 25.70	D 25.95	D 25.35	E 14.82	الكلوتاميك
A 4.51	AB 4.42	AB 4.30	AB 4.18	AB 4.37	AB 4.25	B 4.11	السيرين
A 4.20	CB 2.93	CD 2.72	D 2.57	A 4.45	B 3.05	E 1.40	هيدروكسي برولين
A 7.82	A 7.77	AB 7.60	AB 7.68	BC7.40	BC 7.35	AB 7.52	الكلايسين
AB 5.94	BC 5.88	BCD 5.62	DC 5.51	A 6.05	AB 5.76	D 5.35	الهستدين
A 3.68	A 3.61	A 3.52	A 3.47	A 3.51	A 3.44	A 3.34	الثريونين
A 36.77	B 36.29	C 35.89	D 35.31	BC 36.09	D 35.54	E 33.93	الالنين
AB 4.93	AB 4.91	A 5.17	A 5.01	B 4.55	AB 4.80	AB 4.96	الارجنين
A 3.30	A 3.26	A 3.31	A 3.28	A 3.17	A 3.09	A 3.21	البرولين

التايروسين	A 2.03	A 2.12	A 2.15	A 2.13	A 2.07	A 2.20	A 2.26
الفالين	B 3.50	AB 3.71	AB 3.75	AB 3.73	AB 3.79	A 3.84	A 3.92
الميثايونين	B 1.28	AB 1.43	AB 1.52	AB 1.40	AB 1.46	A 1.65	A 1.70
السستين	A 1.40	A 077.	A 0.85	A 0.81	A 1.07	A 1.17	A 1.24
الايزوليوسين	C 2.35	ABC 2.61	AB 2.78	CD 2.54	CD 2.58	AB 2.86	A 2.99
اليوسين	D 4.21	ABC 4.60	AB 4.65	CD 4.30	BCD 4.54	AB 4.77	A 4.94
الفنيل النين	C 2.37	AB 2.88	AB 2.90	BC 2.55	BC 2.64	AB 2.85	A 3.00
اللايبسين	A 4.10	A 4.22	A 4.42	A 4.17	A 4.26	A 4.38	A 4.56

تشير الحروف المختلفة ضمن الصف الواحد الى وجود اختلافات معنوية بين المعاملات بمستوى (P<0.05)

الجدول (3) : تاثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما في محتوى اللحم من الاحماض الامينية الحرة (ملغم/100 غم/لحم) في العضلة الفخذية ثنائية الرأس (BF) لذبائح اناث الماعز المسنة .

الحامض الاميني	T1	T2	T3	T4	T5	T6	T7
الاسبارتك	C 1.44	B 1.64	B 1.70	C 1.45	AB 1.76	AB 1.80	A 1.90
الكلوتاميك	E 14.22	D 24.75	D 25.25	D 25.20	C 26.55	B 28.60	A 30.25
السيرين	C 3.92	ABC 4.02	AB 4.20	BC 3.96	BC 3.99	AB 4.22	A 4.26
هيدروكسي برولين	E 1.35	B 2.95	A 4.19	D 2.49	CD 2.56	BC 2.80	A 3.91
الكلايسين	CD 7.22	D 7.10	CD 7.20	BC 7.43	BC 7.40	AB 7.50	A 7.71
الهستدين	D 5.25	ABC 5.70	A 5.95	CD 5.44	BCD 5.50	AB 5.78	A 5.90
الثريونين	B 3.41	AB 3.56	AB 3.65	AB 3.59	AB 3.63	AB 3.71	A 3.80
الالنين	E 33.35	CD 34.98	B 35.79	D 34.66	BC 35.50	B 35.90	A 36.51
الارجنين	A 4.81	AB 4.71	B 4.35	A 4.90	A 4.97	AB 4.74	AB 4.78
البرولين	A 3.18	A 2.98	A 3.10	A 3.24	A 3.26	A 3.21	A 3.23
التايروسين	A 2.10	A 2.18	A 2.20	A 2.23	A 2.32	A 2.35	A 2.40
الفالين	A 3.66	A 3.82	A 3.93	A 3.81	A 3.89	A 3.98	A 4.05
الميثايونين	C 1.20	C 1.30	BC 1.35	C 1.25	C 1.28	AB 1.60	A 1.62
السستين	A 1.16	A 0.70	A 0.74	A 0.72	A 0.89	A 0.92	A 1.05
الايزوليوسين	C 2.15	BC 2.39	AB 2.60	C 2.19	BC 2.29	AB 2.64	A 2.79
اليوسين	D 3.95	BC 4.25	BC 4.27	CD 4.13	CD 4.20	AB 4.50	A 4.63
الفنيل النين	C 2.55	AB 3.03	AB 3.10	BC 2.67	ABC 2.82	AB 3.08	A 3.15
اللايبسين	A 3.87	A 3.96	A 4.15	A 3.91	A 3.95	A 4.17	A 4.29

تشير الحروف المختلفة ضمن الصف الواحد الى وجود اختلافات معنوية بين المعاملات بمستوى (P<0.05)

واشارت النتائج في (جدول 4) الى وجود زيادة في انفراد كمية الاحماض الامينية الحرة الكلية بتأثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتأثير المشترك لهما وكانت النسبة المئوية للزيادة المتحققة في انفراد كمية الاحماض الامينية الحرة الكلية في المعاملات المذكورة اعلاه عن معاملة السيطرة (T1) وللعضلات LD , SM و BF 12.49و13.14و12.88% في المعاملة T2و 15.88و15.74 و16.06 % في المعاملة T3 و11.99و 12.56و12.97% في المعاملة T4 و 14.96و5.06و15.37% في المعاملة T5 في العضلات المذكورة انفا على الترتيب وحققت المعاملتان T6 و T7 اعلى % في الزيادة المتحققة في انفراد كمية الاحماض الامينية الحرة الكلية مقارنة مع معاملة السيطرة اذ بلغت 18.09و7.93و18.67% في المعاملة T6و21.20و21.17و21.72% في المعاملة T7 في العضلات LD , SM وBFعلى التوالي , الامر الذي يعطي ارجحية متفوقة للمعاملتين T7 ,T6 ويؤشر الاهمية النسبية المتفوقة لمعاملتي التحفيز الكهربائي والحقن بالاملاح في زيادة طراوة لحوم اناث الماعز المسنة ، تلتها بالمرتبة الثانية معاملة الحقن بمحلول كلوريد الكالسيوم ومن ثم معاملة التحفيز الكهربائي (200 فولت) وبالمرتبة الثالثة معاملة الحقن بمحلول كلوريد الصوديوم ومعاملة التحفيز الكهربائي (80 فولت) واخيرا في المرتبة الرابعة معاملة السيطرة T1. وقد يعزى تفوق المعاملتين T7و T6 الى فعل التحفيز الكهربائي في تمزيق الالياف العضلية وشبكة الياف الكولاجين وزيادة تكسر بروتينات الليفات العضلية (2ool, Thompson , Hopkins) , فضلا عن ذلك فأن الانخفاض السريع في الاس الهيدروجيني وارتفاع درجة الحرارة يؤدي الى تحرر انزيمات الكاثبسينات التي تعمل على تحلل بروتينات الليفات العضلية من جهة , وزيادة فعالية انزيم الكالبين الذي يعمل على زيادة التحلل البروتيني لبروتينات الليفات العضلية من جهة اخرى (Mikami واخرون ، 1994). فضلا عن ذلك ان التحفيز الكهربائي يعمل على زيادة فعالية انزيمات Transaminase بمقدار 20% مما يؤدي الى زيادة انفراد الاحماض الامينية الحرة وعلى وجه الخصوص حامض الكلوتاميك (Sekikaw واخرون 1999).كذلك الى زيادة القوة الايونية التي تؤدي الى اضعاف وتحطيم تركيب الليفات العضلية وزيادة ذائبية بروتيناتها مما يؤدي الى زيادة انفراد الاحماض الامينية الحرة (Dransfield ,1999) , فضلا عن ذلك زيادة فعالية انزيمات الكالبينات التي تسهم في تكسير وتجزئة بروتينات Z-line وزيادة ذائبيتها (Takahashi , 1999) . وان كمية الاحماض الامينية الحرة في هذه الدراسة كانت مقاربة للكميات التي وجدها Mikami واخرون (1994) في لحوم الابقار المحفزة كهربائيا فقد بلغت كمية الاحماض الامينية الحرة 112.77 ملغم /100غم لحم في لحوم الذبائح المحفزة كهربائيا في حين كانت 105.99 ملغم/100غم لحم في لحوم الذبائح غير المحفزة .

ومن الجدير بالاشارة ان محتوى حامض الهيدروكسي برولين قد ارتفع في الدراسة الحالية بتاثير معاملات التجربة وبشكل خاص في المعاملة T3 , الامر الذي يؤشر حصول زيادة في ذائبية الكولاجين بتأثير تلك المعاملات مقارنة بمعاملات

السيطرة . ففي دراسة Feidt واخرون (1996) وجد ان النسبة المئوية للهيدروكسي برولين المتحرر في اليوم العاشر بعد الذبح في الابقار كانت 4.6% في عضلة LD في حين كانت النسبة المذكورة في الدراسة الحالية , 3.5% 3.84 , 3.69 في العضلات BF, SM , LD على التوالي بعد يومين من الذبح في المعاملة T3 , الامر الذي يؤشر اهمية استعمال التحفيز الكهربائي ودورة في تجزئة شبكة الياف الكولاجين ومن ثم زيادة الطراوة للحوم الحيوانات المسنة .

اما بشأن تاثير نوع العضلة ضمن المعاملة الواحدة في التحليل الكمي للاحماض الامينية الحرة (ملغم/100غم لحم) فقد اشارت النتائج بشكل عام الى عدم وجود اختلافات معنوية ($p> 0.05$) بين العضلات BF, SM , LD في زيادة انفراد الاحماض الامينية الحرة الكلية في حين لوحظ وجود اختلافات معنوية ($p<0.05$)بين العضلات BF, SM , LD في الاحماض الامينية التالية : السيرين والهستدين والكلايسين والالنين في المعاملات. الامر الذي يؤشر وجود تباين في تأثير معاملات التجربة في زيادة انفراد الاحماض الامينية في العضلات . وقد يعزى الاختلاف بين العضلات في كمية الاحماض الامينية الحرة المنفردة الى اختلاف التركيب والصفات الفيزوكيميائية بين العضلات مما تؤدي الى اختلاف في حساسية بروتينات الليفات العضلية لانزيمات التحلل البروتيني (Feidt واخرون , 1996) . وكذلك التباين في فعالية انزيمات الكاثبسينات والكالبينات بين العضلات (Feidt واخرون , 1998).

الجدول (4) : تاثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما في مجموع الاحماض الامينية الحرة (ملغم/100 غم/لحم) في العضلات : الطويلةالظهرية (LD) و العضلة نصف الغشائية (SM) و العضلة الفخذية ثنائية الرأس (BF) لذبائح اناث الماعز المسنة .

المعاملة العضلة	T1	T2	T3	T4	T5	T6	T7	المتوسط العام ± SE
LD	104.73^{C}_{a}	119.68^{B}_{a}	$^{BA}_{a}124.51$	119.00^{B}_{a}	$^{BA}_{a}123.16$	127.87^{AB}_{a}	$^{a}132.92^{A}$	2.42 ± 121.695
SM	101.41^{B}_{a}	$^{BA}_{a}116.76$	120.36^{A}_{a}	$^{BA}_{a}115.98$	119.40^{A}_{a}	$^{a}123.57^{A}$	$^{a}128.66^{A}$	2.51 ± 118.022
BF	$^{a}98.81^{D}$	113.42^{C}_{a}	$^{BC}_{a}117.72$	113.54^{C}_{a}	$^{BC}_{a}116.76$	$^{a}121.50^{BA}$	$^{a}126.23^{A}$	2.26 ± 115.425
المتوسط العام ± SE	101.650 1.48 ±	116.620 1.60 ±	120.863 2.23 ±	116.173 2.62 ±	119.773 1.90 ±	± 124.313 1.96	± 129.270 1.47	

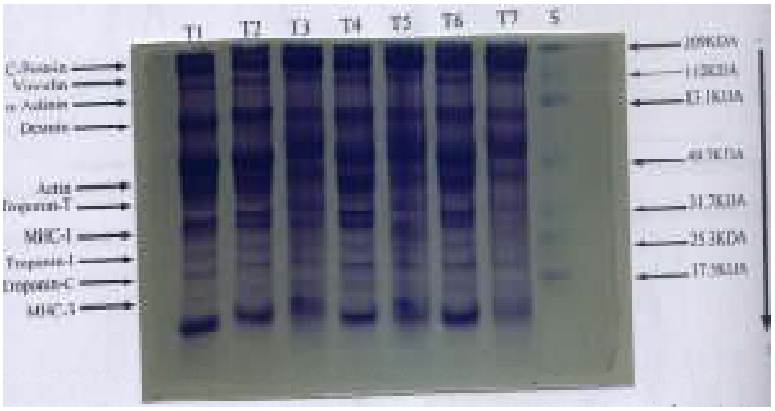
- **تشير الحروف المختلفة الكبيرة ضمن الصف الواحد الى وجود اختلافات معنوية بين المتوسطات بمستوى ($p<0.05$).**
- **تشير الحروف الصغيرة المتشابهة ضمن العمود الواحد الى عدم وجود اختلافات معنوية بين المتوسطات بمستوى ($p>0.05$).**

الترحيل الكهربائي الهلامي

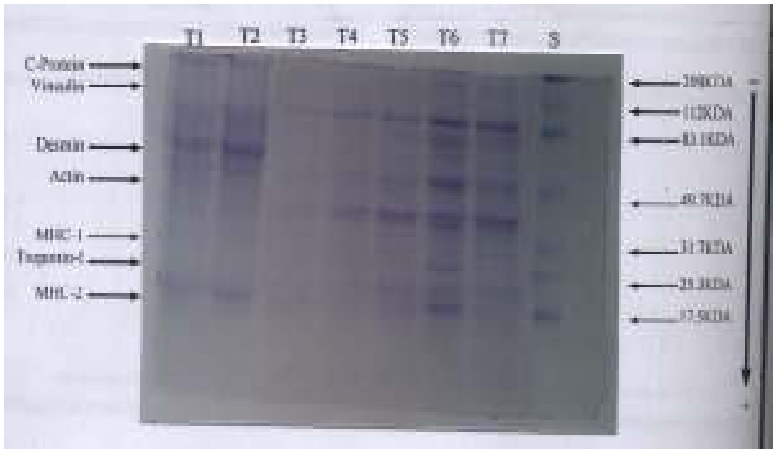
اتبعت طريقة الترحيل الكهربائي باستعمال هلام متعدد الاكريل أمايد للتعرف على مقدار الزيادة الحاصلة في عدد الحزم البروتينية ومدى التغير الذي حدث في بروتينات المايوفيبرل بتاثير معاملتي التحفيز الكهربائي والحقن بالاملاح والتاثير المشترك لهما في العضلات SM , LD وBF . ويتضح من الاشكال (1و2و3) عند النظر بالعين المجردة ان المعاملة بالتحفيز الكهربائي بالفولتيتين (80 و 200) والحقن بالاملاح ($CaCl_2$, NaCl) والتاثير المشترك لهما في العضلات LD وSM وBF سبب حدوث تغيرات واضحة في بروتينات المايوفيبرل المرحلة كهربائيا على الهلام والمحددة بجهاز المفراس (Densitometer).فقد اظهرت الاشكال (1و2و3) حدوث تجزئة وتحلل للبروتينات ذات الوزن الجزيئي العالي مما يؤدي الى ظهور حزم بروتينية جديدة بفعل معاملات التجربة المذكورة انفا مقارنة مع معاملة السيطرة . مما يدل على زيادة ذوبان بعض البروتينات وانفصالها عن البروتينات الاخرى وظهورها بشكل حزم جديدة منفصلة بفعل معاملات التجربة المذكورة انفا مقارنة مع معاملة السيطرة،.ونلاحظ ان الارتفاع في عدد الحزم البروتينية يعود الى حدوث تكسر في بعض الحزم البروتينية الى حزمتين او اكثر نتيجة للتحلل البروتيني مما يؤدي الى ظهور زيادة في عدد الحزم البروتينية بتاثير معاملات التجربة كذلك قد يحصل اندماج وتكون المحصلة النهائية نقصان عدد الحزم .

ومن خلال النتائج يمكن التوصل إلى أن معاملتي التحفيز الكهربائي والحقن بالاملاح كانت ذات فعل تحللي واضح في بروتينات المايوفيبرل والذي انعكس على انخفاض الامتصاص وزيادة عدد الحزم البروتينية وتكسرها وتجزئتها كنتيجة لفعل عملية التحفيز الكهربائي في تكسير وتمزيق الالياف العضلية (Savell واخرون ، 1978) كما ان الانخفاض السريع في الاس الهيدروجيني وارتفاع الحرارة بفعل التحفيز الكهربائي يؤدي الى تحرر انزيمات الكاثبسينات وزيادة نشاطها التحللي لبروتينات المايوفيبرل (Dutson واخرون ، 1980)، فضلا عن ذلك يعمل التحفيز الكهربائي على زيادة نشاط انزيم الكالبين وعلى وجه الخصوص انزيم CDp-I بوقت مبكر (Lee واخرون ، 2000). اما الزيادة المتحققة في عدد الحزم البروتينية في بروتينات المايوفيبرل لمعاملتي الحقن بالاملاح فيعزى الى زيادة القوة الايونية التي تسهم في زيادة ذائبية وانفصال بروتينات الليفات العضلية (Geesink واخرون ، 1994) والى زيادة نشاط انزيم الكالبين والاذابة المعتمدة على الكالسيوم (الضعف غير الانزيمي لليف العضلي) بتاثير الحقن بكلوريد الكالسيوم (Whipple و Koohmaraie ، 1993). واسهمت هذه الاسباب جميعها الى تحسين طراوة لحوم ذبائح اناث الماعز المسنة من خلال انخفاض صلابة الليفات وتجزئة البناء الخويطي لليف العضلي . وجاءت هذه النتائج متفقة مع ما توصل اليه كل من Wu و Smith (1987) و Koohmaraie و Shackelford (1991) و Ho واخرون (1997) و Geesink واخرون (2001) الذين اشاروا الى حصول تكسر وتجزئة في بروتينات المايوفيبرل عند استعمال التحفيز الكهربائي والحقن بالاملاح في الذبائح.

الشكل 1: تأثير المعاملة بالتحفيز الكهربائي والحقن بالأملاح والتأثير المشترك لهما في فصل بروتينات المايوفيبرل في العضلة الطويلة الظهرية (LD) باستخدام الترحيل الكهربائي SDS – PAGE.



الشكل 2: تأثير المعاملة بالتحفيز الكهربائي والحقن بالأملاح والتأثير المشترك لهما في فصل بروتينات المايوفيبرل في العضلة نصف الغشائية (SM) باستخدام الترحيل الكهربائي SDS – PAGE.



الشكل 3: تأثير المعاملة بالتحفيز الكهربائي والحقن بالأملاح والتأثير المشترك لهما في فصل بروتينات المايوفيبرل في الفخذية ثنائية الرأس (BF) باستخدام الترحيل الكهربائي SDS – PAGE.

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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

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

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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