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EDITORIAL BOARD - 2016

FORWARD

Dear Collegues,

I used to start my message by the achievements we try always to do and by the idea that was born to put between your hands our journal – IJST. Today, I write you about how our journal is moving to the new volume as we are now in 2016, eleven years without stop, despite the challenges we faced, and despite all constraints that our beloved Arab countries have while they are looking for more development achievements. What I want to say, is that the only weapon, as well as the tool to proceed to the gate of development is science and how we can use and adopt all the ways that make our cultures, our thoughts and our talents and research efforts to be converted into practices to improve life for us and for the coming generations and let the other parts of the world listen to us very appreciately. By this year, IJST had been awarded a new scientific impact factor ***, that is (the Global Impact Factor- GIF) of a value scored 0.81.*** *I n addition,* ***IJST had awarded an increase of the value scored for SJIF to be 4.487****. By the beginning of the current year,* ***a new Editorial Board Member has joined IJST, and it is our pleasure to welcome Prof. Taha Al- Samarrai from University of Samarra*** *and wishing him the best times while in our IJST journey.*

For all what we achieved, I would like to present my deepest thanking and great recognitions for all people and institutes who faithfully gave IJST their concerns, their cares, and their patiences to keep it as one of the leading journals in Arab and international worlds.

Thanks a lot for Prof. Jamal Abbas and Dr. Abdullah Al- Shebani from University of Kufa, Dr. Atheer Al- Douri , Prof. Hazim Al- Daraji from University of Baghdad, Prof. Waleed Al- Murrani for his endless support from Plymouth University, Prof. Abdulbari Abbas Al- Faris from University of Basrah, and finally to the one who stands always behind this great effort and performs her best with no disperence, non stopping, and with full of faith, loyalty and creative footprints at IJST, the Editorial Board Secretary of IJST. With you all, IJST is now here, and will continue as long as we breath, as we believe on our goal, and as we have the power from God to be with you.

IJST was a fruitful effort issued by the International Centre for Advancement of Sciences and Technology – ICAST, which tries to take part in both globalization and revolution in information and communication technologies, because S&T development becoming not only the key elements of economic growth and industrial competitiveness, but also essential for improving the social development, the quality of life and global environment. ICAST took then a decision to establish a scientific alliance with TSTC (Tharwa for scientific Training & Consultations) and this alliance comes to support the efforts towards publishing IJST.

Today, we announce a new issue of our journal, that is the first issue from the eleven volume of IJST, March , 2016.

Finally, I hope that all significant figures of sciences whom joined the editorial board, the researchers, and the readers of our journal will keep IJST between their eyes and contribute in continuing its journey, with their remarks, valuable recommendations and their researching outcomes.

Thanks a lot for all who support IJST.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

The Referees for this Issue

** The referees and advisory group below are listed according to alphabetical order, with deep appreciation for all.*

TABLE OF CONTENTS

** Articles in this issue are listed below according to field specialties order, starting by English section and followed by Arabic section.*

(I) ENGLISH SECTION

Continue>>>> English section

VETERINARY MEDICINE

PATHOLOGY

PHYSICS

ARABIC STUDIES AND RESEARCHES SECTION – قسم الدراسات والبحوث العربية (II)

ENGLISH SECTION

Data hiding in 3D model using artificial techniques

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ABSTRACT

Hide data in three-dimensional models began to take a broad interest in recent times in spite of the difficulties faced by working with this type of media. In this study, the design and implementation of innovative algorithm based on artificial intelligence techniques were done to identify sites that were selected in order to cover-ups, depending on the values of (silhouettes). The purchasing managers' data encryption was to be hidden up to date in a manner depending on vectors triangular surface-dimensional high security to achieve. Results obtained were very good in terms of lack of observation by the eye or standards that have been adopted, which was (Housedroff Distance, RMS). Results also showed that they have good resistance to attack types of engineering such as translation, scaling, rotation and the coefficient of smoothing where a full recovery has been hidden data without any destruction, and this is what boosted scale (correlation factor) error rate.

Keywords: 3D mesh, digital watermarking, copyright protection, attack, robustness

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الملخص باللغة العربية

حظي إخفاء البيانات في النماذج الثلاثية الأبعاد باهتمام واسع في الآونة الأخيرة، بالرغم من المصاعب التي تواجه العمل مع هذا النوع من الوسائط.

في هذه الدراسة، تم تصميم وتنفيذ خوارزمية مبتكرة تعتمد على تقنيات الذكاء الصناعي في تحديد المواقع التي يتم اختيارها من أجل الإخفاء بالاعتماد على قيم (silhouettes) ، حيث تم تشفير البيانات المراد إخفاؤها بطريقة محدثة تعتمد على متجهات السطح الثلاثي الأبعاد لتحقيق درجة أمنية عالية.

إن النتائج التي تم الحصول عليها تعتبر جيدة جدا من ناحية عدم الملاحظة من قبل العين أو المقاييس التي تم اعتمادها وهي (Hausedroff Distance, RMS)، كما أظهرت الطريقة مقاومة جيدة لأنواع الهجوم الهندسي مثل التدوير، النقل، معامل التكبير والتصغير والتنعيم، حيث تم استرجاع البيانات المخفية كاملة دون أي تدمير، وهذا ما عززه مقياس معدل الخطأ (correlation factor),

INTRODUCTION

The development of digital media on the Internet, and the widespread use of a wide range of individual computer devices and multimedia applications allow users to hide information in the digital medium (such as image, audio, video and electronic documents) and its distribution channels through unsafe. On the other hand, it also means the danger that may easily be detected from the precious contents, reproduce or modified by unauthorized users.

As a way to protect confidential information hidden in digital media and to detect unauthorized tampering, information hiding is now a big draw attention in the field of information security (1).

Easy duplication properties and modification of these contents, it is necessary to develop a variety of techniques such as digital signature, 3D water plan (1), not only for the various protecting copyright and property claims, but also to find the area of content tampering (2).

Water compared with another solution such as digital signatures, encryption is less computationally cheaper costs (3).

The three-dimensional regions of interest extracted from these images using segmentation and modeling. Usually characterized by geometric objects by a group of vertices joined by line segments is determined while the neighboring surface by surface orientations rates. Drawing object can represent a model of the object extracted from the volumetric image, and can be designed using the CAD system or can be learned from an image using the shape-from-shading techniques. In the case of a graphic object one usually has to deal with the decline in the volume of data that carry geometrical information mainly (4).

Graphical 3D objects is the most difficult digital media type to design the framework of water for, as I have done many challenges, such as: 1) decrease the volume of data: the amount of data available to hide the watermark in that it is very low as a 3D model from a few thousand peaks consists Unlike the huge amount of pixels provided for in the case of images. 2) is not a unique representation: a 2D image in the form of a matrix, while the 3D model can be represented in many different ways. 3) No strong field of transformation that can be used to integrate. 4) The attacks may change engineering and network connectivity properties. and 5) High computational requirements, specifically to carry out the frequency range (5).

The idea of subdivision

The basic idea of its units can be summarized as follows:

It determines the division of smooth curve or surface as at the end of a row of successive improvements.

This description for sure is somewhat loose with lots of details as yet to be determined, but it embodies the essence. In case of a link of some of the initial number of points in the plane curves. Start with 4 points of contact through the straight line segments. Next to it is a revised version. Then, the original 4 points and plus 3 more points "between" the old points are found. By repeating this process, a piecewise linear curve looking smoother is getting. Repeating this step on the curve begins to look very nice indeed. It is easy to see that after a few steps of this procedure would be resolved output curve as well as one could hope for when using a limited decision such as that provided by a computer monitor or a laser printer.

An example of subdivision surfaces is illustrated in figure (1). In this case, each triangle is split in the original network on the left to 4 new triangles four times the number of triangles in a network. The application of the same rule partition again gives the network on the right.

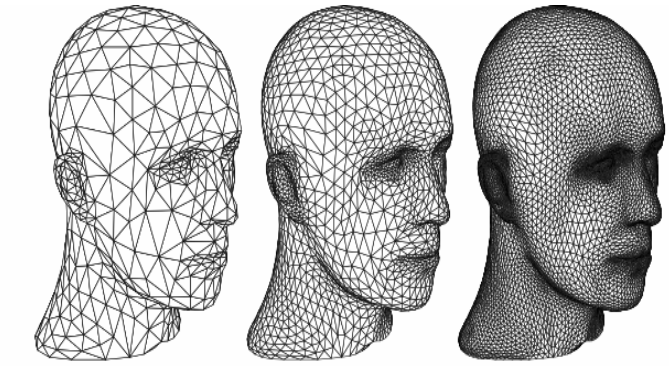

Figure (1): Example of subdivision for a surface, showing 3 successive levels of refinement. On the left an initial triangular mesh approximating the surface.

Both of these examples showed what known as interpolating subdivision. Originally points remain undisturbed while the inclusion of new points. The keys, which are generally not distortion will be shown later, and can also be generated through the subdivision (6).

Cluster analysis

Cluster analysis or clustering is the task of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar (in some sense or another) to each other than to those in other groups (clusters). It is a main task of exploratory data mining, and a common technique for statistical data analysis, used in many fields, including machine learning, pattern recognition, image analysis, information retrieval, and bioinformatics.

Cluster analysis itself is not one specific algorithm, but the general task to be solved. It can be achieved by various algorithms that differ significantly in their notion of what constitutes a cluster and how to efficiently find them. Popular notions of clusters include groups with small distances among the cluster members, dense areas of the data space, intervals or particular statistical distributions.

Clustering can therefore be formulated as a multi-objective optimization problem. The appropriate clustering algorithm and parameter settings (including values such as the distance function to use, a density threshold or the number of expected clusters) depend on the individual data set and intended use of the results. Cluster analysis as such is not an automatic task, but an iterative process of knowledge discovery or interactive multi-objective optimization that involves trial and failure. It will often be necessary to modify data preprocessing and model parameters until the result achieves**.**

Silhouette analysis

Silhouette can be used to study the distance between the masses of the resulting analysis. Fantasy plot displays a measure of how close each point in one group is a point in the neighboring communities, and thus provides a means to evaluate parameters such as the number of blocks visually. This measure has a range of [-1.1].

Imagination transactions (also referred to these values, peace be upon him) + 1 near to the sample away from the neighboring communities. A value of 0 to the sample or very close to the boundary between the two neighboring and negative values indicate that the decision of those samples have been assigned to the wrong block.

To calculate silhouette cluster, first calculate the average distance within the bloc. Each member of the cluster has an average specific distance from all the other members of the same group. This is a variation from it cluster. Members of the cluster with low variation comfortably within the bloc that is set. Average difference on the mass is a measure of the extent of the agreement it is. Note that two of the same members of the cluster may be different groups of neighboring. For points that are close to the border between two, it may be a couple of tens of difference almost equal.

The average distance to the cluster fellow members are then compared with the average distance to the neighboring members of the cluster. Figure (2) shows the process to a single point (17.27).

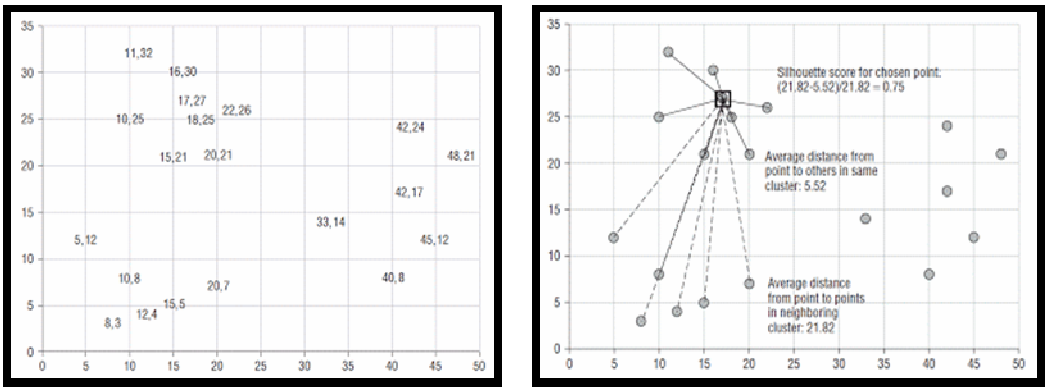

Figure (2): compute silhouettes single point (17.27).

Proposed hiding system

New algorithm for hiding data in 3d object based on clustering and silhouettes idea is proposed. The block diagram for embedding system is explained in figure (3).

1-Preprocessing step:

Subdivide the 3d object for two reasons:

A- Remove the noise.

B- Increase the capacity of the object.

2- Adaptive encryption method based on the surface of 3d object: The first step in the block diagram is performing the encryption for the embedded data to increase the security of the proposed system. The embedded data is encrypted based on XORing the data with values of normal direction computed for every vertex, so that the XOR-operation based on the surface of object, every object has a different normal direction from the others so that it can be consider as a key generated at every embedding.

In the three-dimensional case a surface normal, or simply normal, to a surface at a point P is a vector that is perpendicular to the tangent plane to that surface at P. (Figure 4).

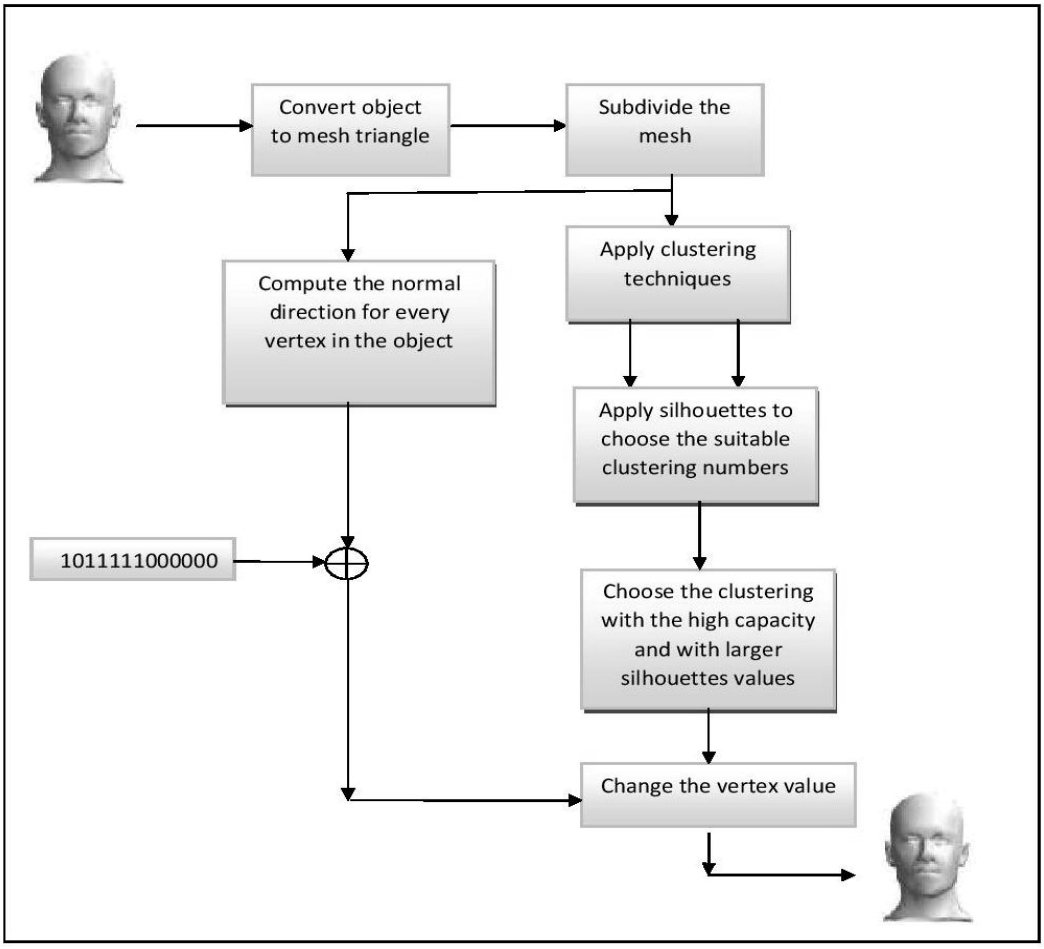

Figure (3): block diagram of the proposed system

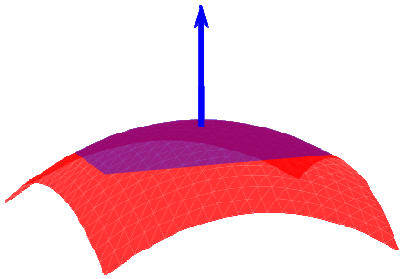

Figure (4): A normal to a surface at a point is the same, as a normal to the tangent plane to that surface at that point.

For a convex polygon (such as a triangle), a surface normal can be computed as the vector cross product of both (non-parallel) edges of the polygon. To calculate the normal, the Cross Product of these vectors is needed to calculate. This will produce us a point that is takes place perpendicular to the plane generated by the vectors. Normal is an x,y,z vector is needed. For each of x,y and z you need to make the following calculations based on the values in the Vectors.

Cross Product.x = (Vector1.y x Vector2.z) - (Vector1.z x Vector2.y) (1)
CrossProduct.y = - ((Vector2.z x Vector1.x) - (Vector2.x x Vector1.z)) (2)
CrossProduct.z = (Vector1.x x Vector2.y) - (Vector1.y x Vector2.x) (3)

To calculate the Cross Product:

Normal.x = (v1.y * v2.z) - (v1.z * v2.y); (4)
Normal.y = -((v2.z * v1.x) - (v2.x * v1.z)); (5)
Normal.z = (v1.x * v2.y) - (v1.y * v2.x); (6)

Normal of vertex at a vertex of a polyhedron is a directional vector associated with a vertex, intended as a replacement to the true geometric normal of the surface. Commonly, it is computed as the normalized average of the surface normal for the faces that include that vertex (9,10).

3- Hiding approach: After encrypted the embedded text and perform subdivision for the 3d object, k-mean clustering is applied for the cover object of n-cluster and based on silhouettes plot we decided the best number of clusters is suitable for work, and then to decide which cluster is chosen for hiding many numbers of criteria is taken.
1. the size of the cluster.
2. the cluster with the highest silhouettes values.

After choosing the suitable cluster the embedding process is start by selecting the vertex with larger silhouettes values for embedding the encrypted text.

4- Extracting watermark: The proposed system is blind watermarking so that no need for the original object to extract the watermark bit, but only knowing the position of the selection vertex that are used for embedding, so that the same steps of embedding are done, then reorder the extracted bits to collect the original watermarked bits, and last decryption the text by XORing them with normal direction of the object.

EXPERIMENTAL RESULTS

To test the proposed system, many different model of '.off' 3d graphical format used were explained in figure (5) and the mesh for each object, in table (1) the name, number of faces, and number of vertex are explained, the length of the watermark is 350 bits**.**

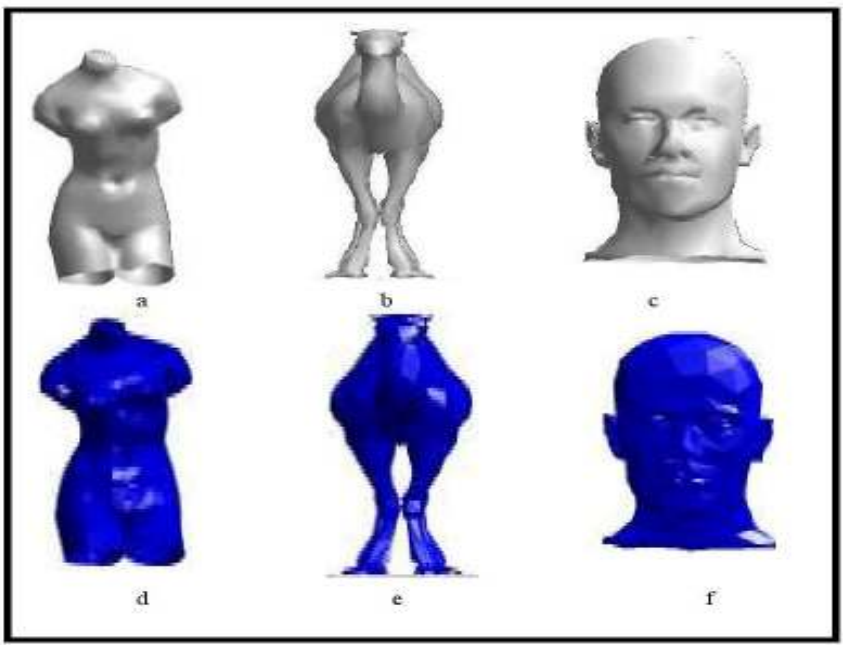

Figure (5) :(a-c) original model, (d-f) mesh for object

Table (1): Model's information

Name	No. of Faces	No. of Vertices
venus	711	1396
camel	586	1168
manuqien	428	204

The first step for the proposed system is subdivided the object, as shown in figure (6).

As described before, the watermark text is encrypted based on the normal direction for each object. Figure (7) shows the vertex normal direction for each object.

After encryption step, then selection the vertices for hiding the binary sequence is start, by using artificial intelligent clustering techniques k-means for N-times, and every time partitioning the vertices into number of clusters from 2 to N-clusters, then based on the silhouette plot the suitable numbers of clusters are selected, some number of criteria is taken to decided which cluster is chosen for hiding, the criteria are:

1. The size of the cluster.
2. The cluster with highest silhouettes values, which gives indicator that these points have good correlation between them.

Figure (8) shows the iterative k-mean based on distance for 'camel' model.

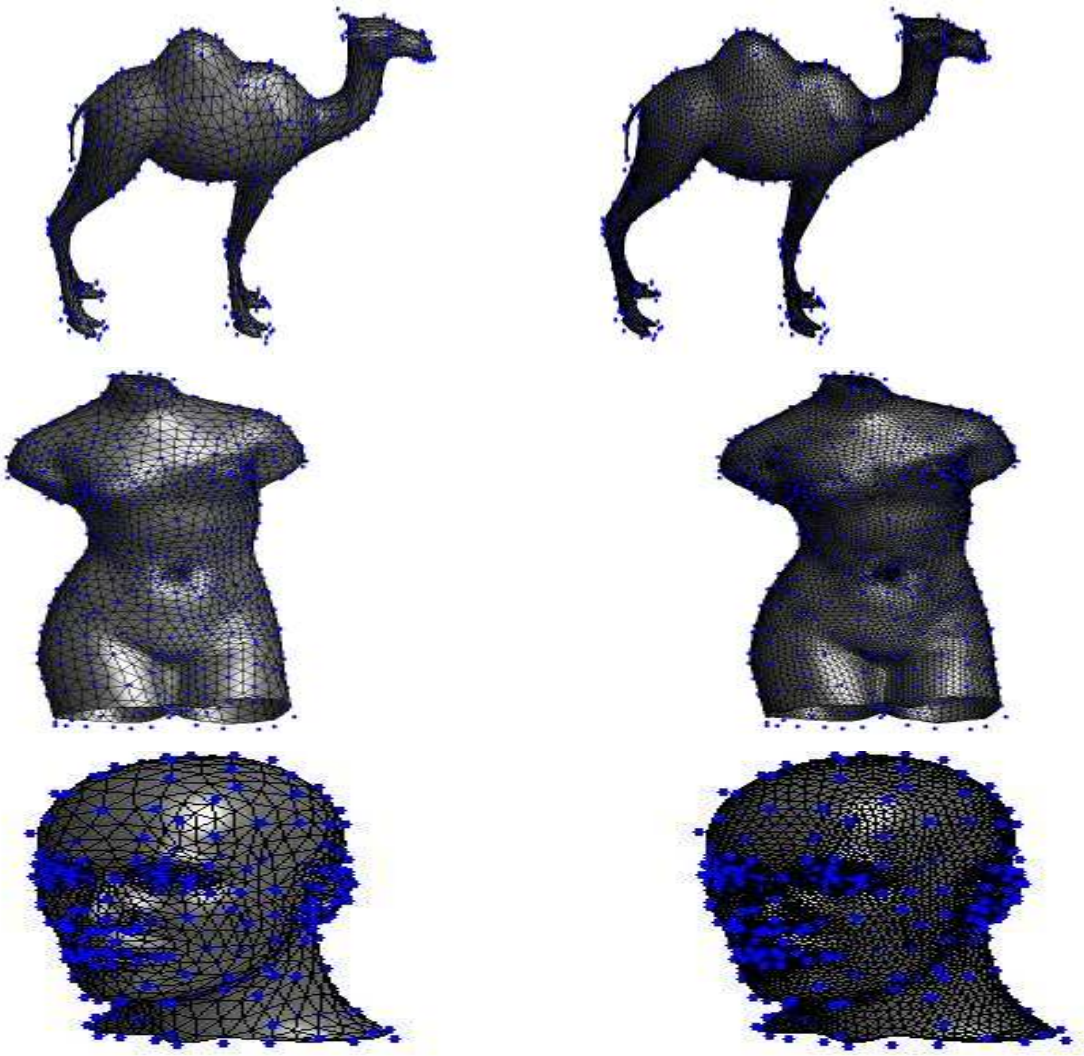

Figure (6): Two levels subdivision for 3 models

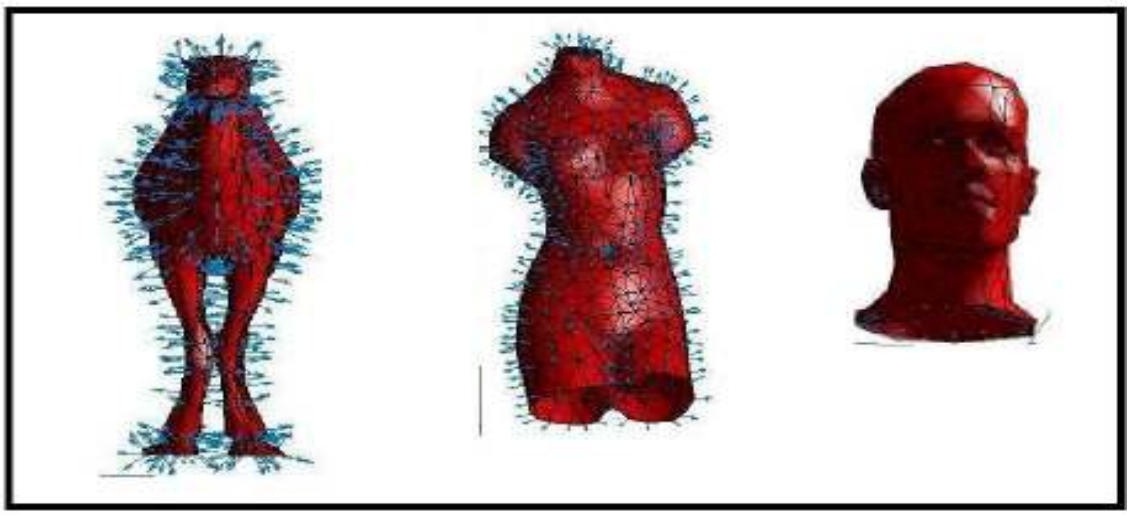

Figure (7): Vertex Normal direction for models

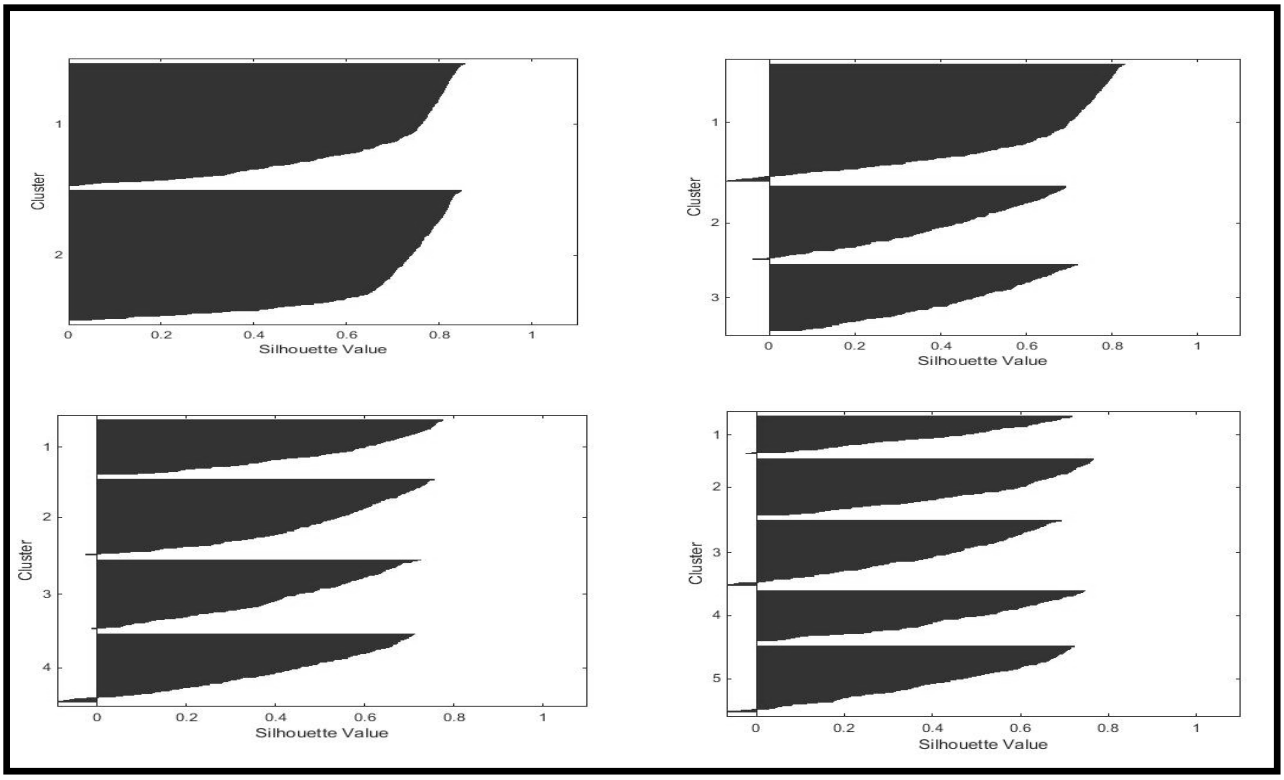

Figure (8): Iterative k-mean based on distance for 'camel'

From figure (9), we see for 'camel' the suitable number of clusters are two, because silhouettes has maximum value, then select the cluster two because it satisfied the criteria.

The objects after embedding the encryption text is explained in figure (10). The results showed that there is no difference for the eyes.

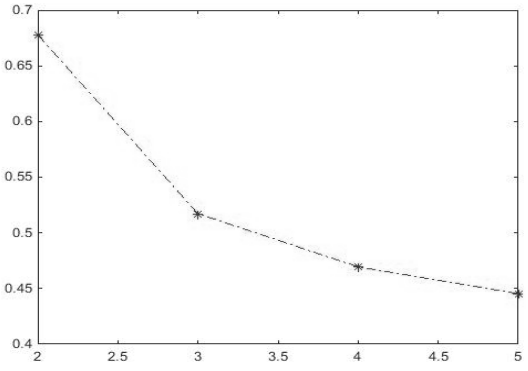

Figure (9): Silhouettes values for N-cluster

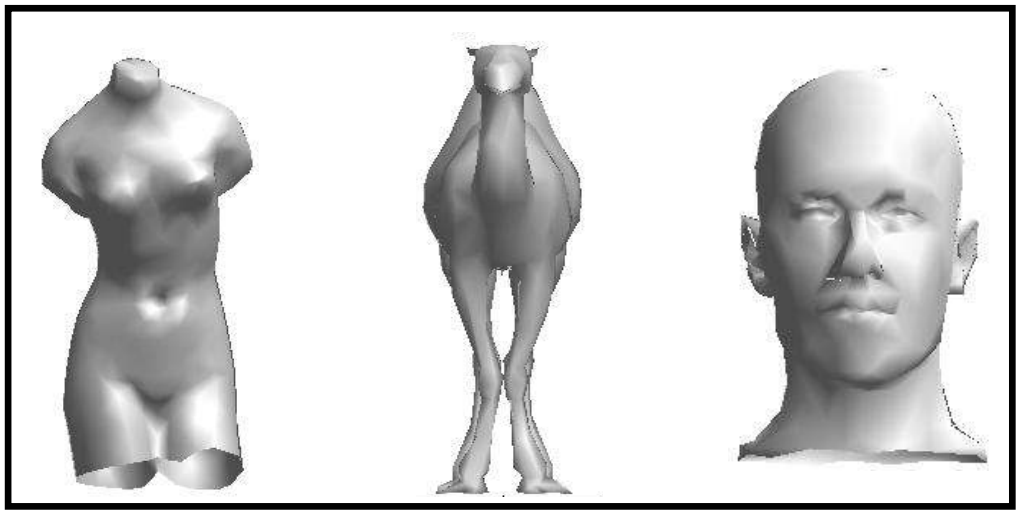

Figure (10): Watermarked 3d model

Distortion measure

To measure the effect of watermarking over the 3D model, objective measure like RMS, Hausdorff distance have been considered along with subjective assessment for visual quality of 3-D mesh.

a. The Hausdorff distance:
It is widely used to measure the distance Hausdorff similarities between the two sets of data, including comparable image and comparison 3D. Hausdorff is defined as the distance between two points and the maximum distance from one group to the nearest point in another group. Hausdorff over every point of a set of points near the object distance is estimated at some other object group, and vice versa. This distance is used to estimate the degree of similarity between two objects that are superimposed on each other. The objective is to minimize the Hausdorff distance to reduce the degree of mismatch between cover object (M_1) and watermarked object (M_2) (11).

$$H_{max}(M_1,,M_2)=\max\{\max_a€M_1 \min_b€M_2 d(a,b), \max_a€M_2 \min_b€M_1 d(a,b)\} \quad (7)$$

Originally designed to measure the similarity between the original object and simplified object. The distance Hausdorff are not the same, are evaluated two spaces where M1 and M2 are consistent and d (a, b) is the Euclidean distance between A and B in 3D space. This is usually called the scale (maximum geometric error). Hausdorff distance is the best approximate measure for assessing the similarity between the two 3D objects.

b. Root Mean Square Error (RMS):
The average error is based on the square root of correspondence between every pair of vertices of the two objects before embedding and after embedding for comparison, and therefore it is limited to the comparison between the two meshes sharing the same topology (table 2). The root mean square error as follows rating:

RMS=√(Σ (1 = 1)^1n [||v_1(1-) v_1(1)||'] [||] ^2) (8)

n is number of vertices of mesh and a vertex of object before embedding and vl' is a vertex corresponding in the watermarked object (12).

Table (2): The evaluation parameter for the watermarked models

Model name	Hough distance	RMS
man	0.0039063	0.00000094
venus	0.0019531	0.00000002
camel	0.0009766	0.00000005

Attack simulation

One of main requirements of the proposed system is robust against many type of attack, a 3-D polygon meshes has been tested against distortion attack. The correlation factor has been used by comparing the number of bits extracted. The correlation value '1' indicates 100% of bit inserted has been returned exactly. The watermark inserted has been invariant to translation, rotation and uniform scaling.
In the preprocessing step objects have been subdivision so that they are not affected by the subdivision attack. The preprocessing steps make the algorithm robust against distortion and distortion-less attack. Simplification is process of removing vertices and faces maintaining the shape of the object. Table (3) shows the robustness against simplification attack.

Table (3): the correlation robustness against attack

Name	Translation	Scaling	Rotation	Noise	Simplification	Subdivide
venus	1	1	1	0.89	0.72	1
camel	1	1	1	0.6352	0.86	1
man	1	1	1	0.904	0.82	1

CONCLUSION

In this study, a new proposed method is introduced based on clustering techniques in selecting the area for embedding.
The preprocessing step provide us good solution to increase the capacity and to robustness against smoothing attack. Encryption techniques based on the surface of the object gives good security because the normal direction change with every object.

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Physical layer security: comparison between Random Linear Network (RLNC) and Rateless Code

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ABSTRACT

Network Code is a new field in the theory of information, which is used in various network layers. One of these, RLNC,is used in the network layer to increase reliability andsecurity. But this methodhas many disadvantages, such as complexity and selecting random coefficients , rate fixing, the length of the header, and the weakness of efficiency in some types of channels. All thesedisadvantages can be overcomeby using another type of Network Code (which is called Rateless code)which is characterized by non-fixing ratethat is used in physical layer which is considered more flexible and less complex and the overcoming of the disadvantages in this way, RLNC . Rateless code is used as a method ofsecurity in physical layer. In this paper, we will make a compression between two methods of security Rateless code and RLNC according to rate ,complexity, distributed, packet header, delay, efficiency and reliability and GF (q).

Keywords: Physical layer security, Network Code (NC), Random Linear Network Code, Rateless Code

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الملخص باللغة العربية

يشكل ترميز الشبكة (Network code) حقلا جديدا في نظرية المعلومات، والذي يستخدم في مختلف طبقات الشبكة. ومن أحد تطبيقاته ترميز الشبكة الخطي العشوائي RLNC، ويستخدم في طبقة الشبكة لزيادة الموثوقية والأمان. غير أن هذا الأسلوب له عيوب كثيرة، مثل التعقيد واختيار المعاملات العشوائية، والمعدل الثابت وطول بداية الحزمة، وضعف الكفاءة في بعض أنواع القنوات. كل هذه العيوب يمكن التغلب عليها باستخدام نوع آخر من ترميز الشبكة والذي يسمى ترميز الشبكة بدون معدل(Rateless)، الذي يتميز بعدم وجود معدل ثابت ويستخدم في الطبقة المادية التي تعتبر أكثر مرونة وأقل تعقيدا، وللتغلب على عيوب هذه الطريقة RLNC يستخدم رمز Rateless كوسيلة من وسائل الأمن في الطبقة المادية. في هذا البحث، جرت مقارنة بين ترميز الشبكة الخطي العشوائي RLNC مع الترميز بدون معدل (Rateless) وفقا للمعدل والتعقيد، التوزيع ، رأس الحزمة، التأخر، الكفاءة والموثوقية وحقل GF.

INTRODUCTION

Authentication, confidentiality, and privacy are issues which are performed in the upper layers of the protocol stack in communication by using many types of private and public-key cryptosystems. Therefore, the computational security is cryptosystems which are based on mathematical operations, for example the factorization into prime factors, which is very difficult to perform by any attacker of limited power . Many types of emerging network for example mobile networks consist of a great number of devices with different capabilities, which make it difficult to perform computation a security. The results of information theory, signal processing, and cryptography suggest that we can gain a lot of security by using noise and fading which are treated as weakness in wireless communication . The result of information theory shows that they can hide messages from attackers without using secret key, hence the design of security solutions at the physical layer itself is to complement computational security (1), and physical-layer security (PLS) adds a new layer of security in communication networks (2).

Unauthorized users of interception have already overheard the wireless transmission because of the broadcast nature of radio propagation. Therefore, good vulnerability to eavesdropping attacks are accrued , thus the protection of the wireless communications from eavesdropping is needed by physical layer security in such a way as to exploit the physical characteristics of wireless channels (3). The efforts of researchers are focusing now a days, on the use of physical layer methods to improve the security of wireless links by using coding strategies (information theory) or beam forming (1).

Many network code methods are used for unicast, multicast and broadcast. For example, a network code called COPE was proposed by (4), which provides throughput gains. Another new network code called CLONE was proposed by (5), which increases reliability by sending multiple copies of the packets. A network code called FUN was proposed by (6), which is random linear coding and is more efficient than NC methods which are explained in (4) and (5). This method will achieve high throughput over wireless network. The different NC methods were described by (7) in details. Using algebraic watchdog in NC was proposed by (8) to determine malicious behaviors in network . Encryption by using RLNC (only encryption coefficients vectors) was proposed by (9). RLNC and polynomial hash were used against Byzantine attackers (10-12). Rateless codes against byzantine attack were used by (13). Thus, the present paper will explain Random Linear network code and rateless codes with examples and give comparison between RLNC and rateless. The aim of this paper was to give an idea of network coding that is used in network as security method in physical layer.

INFORMATION THEORY METHODS

Quality of Service (QoS) metrics for example Delay, Throughput and Reliability are very important to know and estimate Internet Service Providers (ISPs). Thus, this value of metrics can define the value suggested to build up incomes (14). Ahlswede (15) was the first researcher who proposed Network Coding (NC), which had emerged as a promising method used to build up the capacity of wire network. Recently, Network Coding and wireless network work together to build up the capacity of network (14).

New field or new concept in information theory has emerged what is called network coding (NC). In this field, data is relayed step by step from source to a destination without being substituted, and this field is unlike the existing store and routing schemes. The notion of mixing information is referred to as network coding information from different flows at intermediate nodes mixing in the network. To protect and recover the original data the receiver decodes these packets when he receives enough coded packets, as well as, mixing packets from different flows which are achieved by multicast capacity (15). As shown in figure (1), the function of input packets which results in output packets and some computation should be performed by each node in a network (16-18). Network coding allows intermediate nodes to mix information from different data flows and thus provides an intrinsic level of data security arguably one of the least well understood benefits of network coding (19).

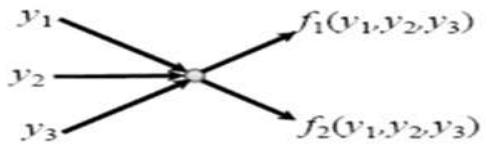

Figure (1): Basic network coding idea(16)

Network Coding (NC) is used in different layers in network for example , NC is used in network layer to achieve higher security rate if size of packets is determined appropriately and NC is performed in physical layer to achieve strong security with less computational complexity (20).

NC was classified into four classes (7), which are described in table (1).

Table (1): classifications of network coding methods

Classification	NC types	Description
Classification 1	XOR	Perform XOR operation between packets
	Binary	Chooses random coefficients over finite field, k ,and data x ,and performs :$\sum x*k$
Classification 2	Inter- session	Relays node code packets from different sources
	Intra- session	Relays node code packets from same source
Classification 3	Global	Intermediate node does not performed coding, but performs coded packet again
	Local	Intermediate node performs decoding, and performs coded packet again
Classification 4 (From the attitude security of network)	State-aware NC protocol	In this protocol , the source node mixes packets which depend on the network state information for example buffer states of neighboring for example Rateless Code (2)
	Stateless-aware NC protocols	In this protocol ,the source node mixes packets which do not depend on the network state information ,for example RLNC (2)

Benefits of NC:

1. Throughput: NC builds up network's capacity for multicast flows (in flooding case); the network bandwidth is overwhelmed by the broadcast storm. The important and effective way to deal with this great problem in both broadcasting and multicasting is NC (21)**.**

2. Reliability: The main benefits of NC involve high reliability especially in mobile network. Reliability is confirmed by encoding the packets into a single packet, which this lost single packet doesn't necessarily require in retransmissions. In the same generation, if the complete set of coded packets can be received from any node, the decoding can be successful and recovered by all the packets. In the same case, we can use partial decoding's concept (21).

3. Distributed Nature: There is no need for global information about the network with NC. We also do not care what our neighbors have received. It is greatly distributed in nature. Respectively it suites perfectly wireless networks (14, 22).

4. Low Complexity: NC works by solving the equations 'set linearly combined together in the polynomial time. Decoding works by using Gaussian elimination ways. These ways are very simple in computational power to improve the efficiency of network.

5. Mobility: In case of mobile, the network topology changes from time to time. The frequent route update and collection of new topological information are the two main difficulties for many routing protocols. The uncertainty and alleviation can be addressed by NC as the need for exchanging route updates.

6. Security: Sending the linear combination of the packets in place of uncorded packet will give a natural way to take benefit of multipath variety for security against wiretapping polynomial time (21).

Random Linear Network Coding (RLNC)

In RLNC, the given node of the output flow is obtained as a linear combining of its input flows. The coefficients (which are selected) for this linear combination are random in nature in complete way; so that they are named Random linear network coding (RLNC). The node gathers several of packets which are received into one or many of outgoing coded packets. Three operations performed by RLNC are: encoding, decoding and re-encoding (figure 2).

The encoding process of RLNC has the combination of native and original packets in linear way, with randomly selected of coefficients. Galois field (GF) is the selections of a finite field by coefficients randomly and independently. A coding vector consists of the coefficients of this combination. The implementation of encoding is shown in figure (3), re-encoding and decoding are by matrix operations. The two processes of the encoding and re-encoding is almost similar, but with the exception that the coding vector of the re-encoded packet is accounted for by the arithmetic operations between the newly generated coefficients at that node and the original coefficients of the received coded packets. The decoding operation is made at the given node by gathering the coded packets. A system of linear equations consist these packets and can be solved forming a matrix. This matrix becomes a decoding matrix (14, 23-25). The stream of packets is divided into generations of size h; the packets, which belong to the same generation addressed with a special generation number. Incoming packets within each node are divided into their base of generation numbers (2). RLNC contains three operations such as encoding, re-encoding and decoding operations. Figure (3) shows packet after encoding operation.

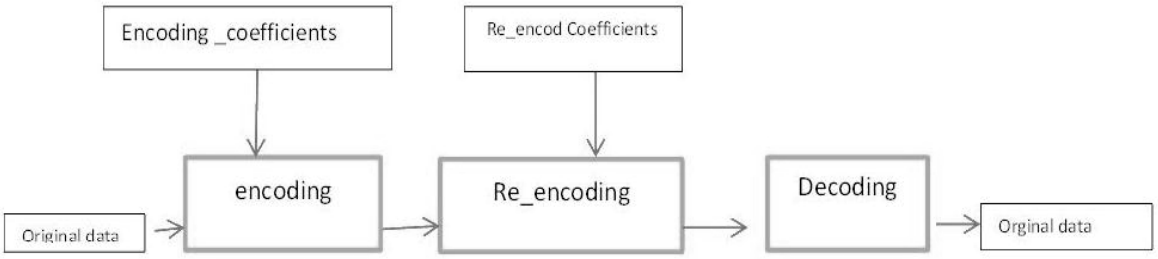

Figure (2): RLNC process(24)

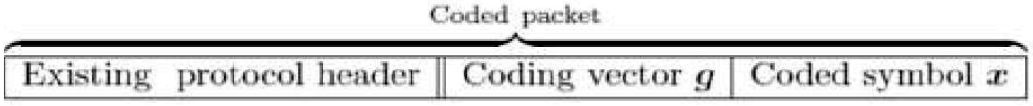

Figure (3):Coded packet

1. RLNC encoding algorithm (24):
Input: source packets.
Output: Encoding packets.
Step 1: Symbols packet generated by source node.
Step 2: Check if generation is stored in buffer in source node.
Step 3: i. if No, construct new generation with ID.
ii. Perform encode operation and produce coded vector.
iii. Create and initialize the decoding matrix and add coefficients vector to this generation , start timer T, go to step 5.
Step 4: I. if yes, search for the generation having lowest distance, if two generation have same lowdistance, the first generation must be used.
Ii: determine if such generation exists.
A: if yes encode the packet and insert in generation and performre_encodeoperation for all packets in that generation. B: if no, go to step 3.
Step5: Convert packet to another format.
Step6: Send single packet.

Example 1: RLNC encoding operation: Suppose we have 3-bytes (11, 155, 4) to be encoded, I selected 3*3=9 coefficients as 3-coded packets are to be sent for 3 –symbols.

Solution:
Vector 1: 73,221, 30.
Vector2: 7, 232, 91.
Vector3: 191, 6,200.
All operations are over GF (2^8) and encoding operation is performed as:
First encode byte: 73*11+155*221+30*4=106
Second encode byte: 7*11+155+232+4*91=49
Third encode byte:191*11+155*6+4*200=247.

After that form 3-packets, each packets contains coefficients and encoded byte, as shown in table (2).

Table (2): Packets coding

NO.	Coefficients Coding vector	Coded
Packet 1	73,221,30	106
Packet 2	7,232,91	49
Packet 3	191,6,200	247

2. Re-encoding:
Consider Anode that has received and stored a set (g1, X1), (gm, XM) of encoded Coding vector Coded packets of 12 bytes of each packet. New encoded packet (g′, X′) can be generated by node A by picking a set of coefficients of K= [K1, KM] εGF (2s) and performing the linear combination.

2.1 Re-encoding algorithm (24):
Input: Set (g1, X1), (gm, XM) of encoded.
Output: New encoded packet (g′, X′).
Step 1: While node receives the encode packet.
Step 2: Check if generation-id is stored in buffer.
1: if no, new generation is creating with given ID, create decoding matrix.
2: Add packet's coding vector to this generation and start timer T.
3: check if timer T has expired:
I: if yes, calculate not (transmissions for its generation), convert packets to required format.
Ii: Do not send packets, if Not< 1, send with probability NT.
Iii: if no, wait for more innovative packets, go to step 1.
Step 3: Check if packets are innovative
I: if no, discard the packets, go to step 1.
Ii: if yes, the received sequence numbers match the IP addresses of added coefficients with the existing coefficients in the generation.
Iii: move conflicting packet's coding coefficient to the free space or increase the generation size to add this coefficients.
Step 4: Check if packet is rebroadcasted after T expired, if no go to step 1.

i. If yes, calculate not
ii. If Not>1, rebroadcast only one packet.
iii. If Not< 1 rebroadcast with probability NT.
Step 5: End while.

Node S1 has a packet n4 to send. The packet is encoded with random coefficient, x3 = k5 * n4, where k5 is taken from GF (2^8). A new generation is created if no generation is stored in node and x3 is inserted in the new generation and is sent. It is assumed that the node has two coded packets in the same generation in the buffer, k1 * n1+ K2 * n2 and k3 * n1 +k4 *n3. The two coded packets contain symbols from 3 original packets, n1, n2 and n3.The source node put its packet in this generation. The node packet is encoded and x3 is added to this decoding matrix. Now the generation has symbols from 4original packets. The source re-encodes all these packets into a single re-encoded packet and broadcasts, the source will generate 3 random coefficients; these are k6, k7 and k8. It will combine the coded packets and create a single re-encoded packet as follows:
k6 (k1n1+k2n2) + k7 (k3n1+k4n3) + k8 (k5n4)
= (k6k1+k7k3) n1+ (k6k2) n2+ (k7k4) n3+ (k8k5) n4 , as shown in figure (4), which describes the new packet.

Generation identifier (Gen ID)	[n1 n2 n3 n4]	Generation distance (GD)	[(k6k1+k7k3) k6k2 k7k4 k8k5]	Payload

Figure (4): Packets after re-encoding operation

3. RLNC decoding operation:
In decoding operation it is very important to solve a group of linear equations. Encoded packets which are received can be stored by a node; according to the original packets which are stored also row by row. At first the matrix is either empty or consists its own non- encoded packets with decoding matrix. Initially this matrix is empty or it contains its own non-encoded packets which are similar to the vectors of encoding. The encoded packets arrive; they are stored in the final row of decoding matrix. Decoding operation can use Gaussian elimination in which only innovative packets are inserted. If a packet is non-innovative it is discarded (24).

3.1 RLNC decoding algorithm:
Input: Encode packets
Output: Source packets.
Step1: Received innovative packet.
Step2: Store innovative packet as last row in decoding matrix.
Step3: Check rank is full.
A: if yes, all packets which are stored in matrix are decoded and given to upper layer in matrix which is not decoded
B: if no, check if sub matrix rank is full.
1: if no, do nothing, go to step 4.
2:i.If no, decode the matrix partially, and store the decoded packets in memory to avoid duplicate decoding.
ii: send the decoded packets to upper layer which is not already decoded for generation.
Step 4: End.

Fountain codes

The first practical class of Rateless codes was developed by Lucy (2002), which was called Lucy Transform (LT) codes. Then the LT codes to Raptor codes is extended by Shokrollahi (2006), in which a type of powerful Rateless codes needs very small overhead to recover completely the source data with linear encoding and decoding time (25,26). Initially Rateless code is developed to achieve efficient transmission in erasure channels. The LT encoded symbols are generated by using at once a specific degree distribution and then there are potentially an unlimited number of encoded symbols. Until an acknowledgment (ACK) response of successful decoding is received at the transmitter, these symbols are generated on the fly and broadcasted to the receiver (27). The initial efforts of working on Rateless Codes have been limited to erasure channels with the primary applications in multimedia video streaming. The researchers recently used the design of Rateless Codes for wireless channels, such as binary symmetric channels (BSC), additive white Gaussian noise (AWGN) channels. Rateless Codes are used in a very wide spectrum of applications in various modern wireless communication networks such as to control the peak-to-average power ratio in OFDM systems and to enhance the transmission efficiency in IEEE ad-hoc 802.11b wireless networks and cooperative relay networks. In contrast to traditional fixed-rate coding schemes, Rateless Code is very efficient in wireless transmissions because the transmitter does not need to know the channel state information before sending its encoded symbols, and a resilient decoding performance can be retained by receiver .In the encoding operation, firstly the encoder draws a value of (d) from a distribution node called the check node degree distribution. To generate an encoded symbol, the encoder selects randomly (d) systematic message symbols, and performs binary summation (i.e. XOR) on these selected symbols. The relationship of systematic message symbols and encoded symbols can be represented by a bipartite Tanner graph. Figure (5) shows the Tanner graph of the conventional LDGM-like rate less codes in the Tanner graph, the systematic message symbol is denoted by ○ and the encoded symbol by □.The vertices ■ check node represents the parity-check node. The overhead represents the number of collected binary symbols away from the optima of a decoder (27-29).

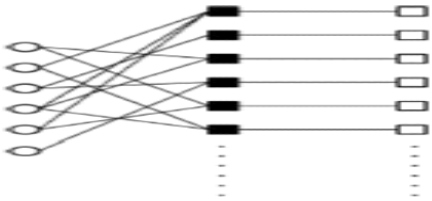

Figure (5): The tanner graph of the conventional LDGM- like rate less codes (17).

1. LT coding operation (29,30):

LT coding algorithm can be described as below :
Step 1:While no feedback message is received from the decoder do
Step 2: Determine degree k by sampling the degree distribution Ω(x).
Step 3: According to value of k, select set of bit from thej-information bits.
Step 4: Perform XOR operation on the selected k information bits to produce coded bits.
Step 5: Send the coded bit over the channel.
Step 6: End while.

Example (31):
If choose degree distributed randomly d=2, encode operation in LT encode performed as; the first step is shown in figure (6) and final step is shown in figure (7). If d=2

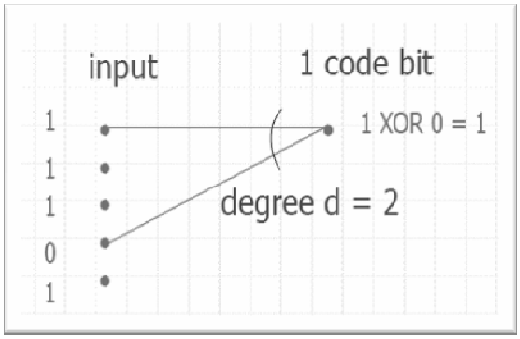

Figure (6): First step

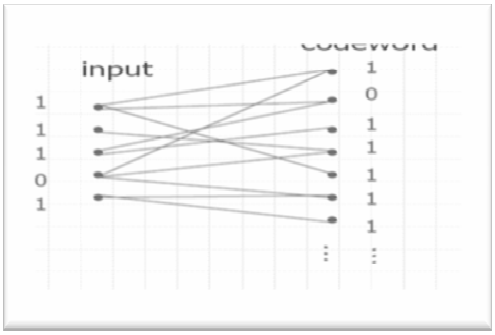

Figure (7): Final step

2. LTDecoding operation (31):

Belief propagation decoding algorithm steps are:
Step 1: Search for any output node that has degree one. If not found, decode will fail
and in this state more output packets areneeded to restart decoding.
Step 2: Send the selected output node value**k**to the input node which is connect bydividing k by the weight of the connected edge.
Step 3: Output node should be removed from the graph of decoding as well as itsedge.
Step 4: The value of input node should spread to recover all output nodes which areconnected; by adding the value of input node to each output value.
Step 5:The edges should be removed from the graph of decoding .The end of decoding will succeed if all input nodes are found. If this does not exists we should go to step 1.
Step 6: End.

Comparison between RLNC and Rateless code

1. Rate: encoding operation of Rateless codes has feature "**on the fly**" which mean each source node continues producing encode words until it receives feedback from destination node after decoding operation is completed, stop encoding operation and send another output packet but in RLNC source send fix rate of encode packets (32).
2. Distributed: The ability to construct a distributed encoding of the information is begin an important property of Network Coding. The information is encoded at the source and also all the nodes of the network support the encoding of the packets, therefore gaining a real distributed encoding scheme. This is not possible completely in rateless codes. Many researchers faced this problem, so different solutions are proposed for various network configurations, but they have didn't solved the problem, the encoding of rateless codes is centralized. The main problem is still which is the possibility of encoding information by using rateless codes in a distributed way (14, 22, 32).
3. Delay problem: The delay of the transmission is very common in network coding and rateless codes according to the decoding of the packets. Classically the systems of distributed coding is ready for use conversely, when the information is received; and in order to retrieve the plain information, the encoding schemes add a further delay. In addition, in order to address this issue the

decoding algorithms which are able to decode the packets early must be studied in order to decrease the decoding delay, incremental decoding and partial decoding are two methods which are created for that. Hence, Rateless code is faster than RLNC (32).

4. GF (q) nature: In RNC, a linear collection has made new packets in the node that has the information about its input symbols in GF (q),where q is great enough integer numbers. Now encoded packets are created by other nodes in the network, this will be by a linear collection in GF (q) of the packets which is received previously; q must be greater than numeral of peers in the network. This problem (which makes this planning) is difficult to achieve in the real application according to its computational complication. The RNC achievement is powerless. The computational complication of encoding and decoding in GF(q) rises with the rising of (q) and it is still very costly because all the decoding and encoding operations are made in a GF(q) , the RNC is not appropriate in the real application, but in real-time application, the rateless code is used because rateless uses GF(2) (32,33).

5. Packet header: In RLNC, set bits must be added to the header of each data packet to inform the decoder of the encoding vector for the packet. The encoding vector determines the generation identity and coefficients that were mixed with data packet. For fountain coding, it is very important to store sequence number in the header for the data packet only, because the sender and receiver know the mapping between the encoding vectors for the fountain code, hence fountain code is simple (18).

6. Complexity: RLNC has computational complexity, for any integer k, decoding operation can be performed by Gaussian elimination on the packets they receive to invert the linear coding operations which require O (k^3) operation and encoding operation need so (k^2) operations (32, 34, 35). For any integer k and any real € >0, Raptor codes in this class produce an infinite stream of symbols so that any subset of symbols of size (k (1+€)) is sufficient to recover the original symbols with high probability. Each encoding process uses (O (log (1/€)) operations, and decoding process (O (k log (1/€)) operations (36).

7. Efficiency and Reliability: Rateless coding has many advantages as compared to the RLNC. One of them, since RLNC does not rely on channel realization we can choose either efficiency or reliability when it works in time-varying channels. While, in rateless code the code word length is limited by the channel realization, so that it achieves higher reliability and efficiency. For multicast and broadcast transmission, the excessive RLNC, which caused by feedback messages decreases the throughput. While rateless codes are a solution for applications where Channel State Information (CSI) at the transmitter is not available. In addition, rateless codes have increased throughput over relay networks (29). At the end, LT or Raptor codes are not systematic which makes them one of the disadvantages; that mean the input symbols are not always reproduced among the output symbols, this is called error floor (27). Comparison between Rateless and RLNC can be described in table (3).

Table (3): Comparison between Rateless and RLNC

	RLNC	Rateless code
1.Rate	Fix rate	On fly rate ,not fixed rate
6.Complexity	high complexity	Low complexity
2.Distributed	Yes	No
5.Packet header	Add set bits to header	Does not add bits to header
3.Delay	More delay	Less delay
7.Efficiency and Reliability	Low	High
4.GF(Q)	GF (q), q integer, ex; 4, 8,	FG(2)

CONCLUSION

A network code is considered as a new concept of information theory which is used for increasing through put, reliability and security in wireless network. RLNC combines input data from different flows after execution of XOR operation for each data with random coefficients, RLNC has many problems which can be solved by using Rateless method in which it has limitless rate for code and generates codes on fly. At the end rateless suffers from error floors that input cannot reproduce from output. Many researchers are dealing with this problem to solve it.

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Gender determination by using dimorphism in permanent maxillary and mandibular canines in Iraqi adults

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ABSTRACT

Odontometric measurement of teeth crowns are easy to perform in living and in fossil forms, simple, inexpensive, more accurate and less time consuming.
The aim of study was to evaluate the sexual dimorphism in permanent (maxillary andmandibular canines , among Iraqi population . The study was conducted on 60 dental students ,selected from Institute of Medical Technology / Baghdad , aged between (18-28) years.
The mesiodistal and buccolingual diameters of the permanent maxillary and mandibular canines were measured in dental cast using electronic digital caliper, which has an accuracy ± 0.01 mm. The descriptive statistics and t-test were calculated.
The results showed that in Iraqi population mesiodistal and buccolingual diameter of permanent maxillary and mandibular canines were larger in males than in females and the difference was statically significant ($p<0.0001$). The sexual dimorphism in mandibular canines was 3.25 % by using buccolingual dimension, while sexual dimorphism in mandibular canines was 2.55% by using mesiodistal dimension , while in maxillary canine, the sexual dimorphism was 1.22% by using mesiodistal dimension and 1.02% by using buccolingual dimension. The study showed mandibular canines exhibiting significant sexual dimorphism.

Keywords: Canine, sexual dimorphism, Inter canine distance

الملخص باللغة العربية

إن القيام بأخذ القياسات السنية لتيجان الأسنان من النماذج الحية والأحفورية أمر سهل، وبسيط وغير مكلف وأكثر دقة وأقل وقتا. وكان الهدف من الدراسة هو تقييم ازدواج الشكل الجنسي في الأسنان الدائمة (الأنياب العلوية والسفلية). أجريت الدراسة على 60 طالب عراقي تم اختيارهم من المعهد الطبي التقني في بغداد، حيث تراوحت أعمارهم بين (18−28) سنة، حيث تم قياس أبعاد السن باستخدام الفلجار الرقمي الإلكتروني بدقة ± 0.01 ملم، كما تم إجراء اختبار الإحصاء الوصفي واختبار (ت).

أظهرت نتائج الدراسة أن قياسات عرض السن وقطره لأنياب الفك العلوي والفك السفلي الدائمة لدى العينة كانت أكبر في الذكور من الإناث، وكان الفارق كبيرا عند مستوى دلالة ($p <0.0001$) ، كان ازدواج الشكل الجنسي في أنياب الفك السفلي 3.25٪ باستخدام القطر، بينما كان ازدواج الشكل الجنسي في أنياب الفك السفلي 2.55٪ باستخدام العرض بالنسبة لناب الفك العلوي، وكان ازدواج الشكل الجنسي 1.22٪ باستخدام العرض و 1.02٪ باستخدام القطر. وأظهرت الدراسة أن أنياب الفك السفلي تمتلك الازدواج الشكلي الجنسي بشكل كبير.

INTRODUCTION

Odontometric measurements seem to be the most reliable method since teeth represent the most durable and resilient part of the skeleton (1). Teeth of various species are known to exhibit asexual dimorphism (2), which refers to the systemic difference between individual of different gender in the same species. Tooth size standards based on odontometricsInvestigation can be used in age and sex determination (3). Mesiodistal (MD) and buccolingual (BL) diameter of the permanent tooth crowns are the most two commonly used in determining sex on the bases of dental measurement .The mesiodistal crowns diameter of the teeth are reduced by inter proximal wear and the buccolingual measurement may prove more useful for sex identification. The sexual dimorphism is more pronounced in permanent dentition than in deciduous teeth (4). One study was by (5) as a odontometric study in permanent maxillary canine among Kosovo-Albanian population, and in similar, (6) conducted a study on maxillary canine teeth in a sample of adults Indian population.
Studying permanent maxillary and mandibular canines teeth offer certain advantages in that they are least affected by periodontal disease, are exposed to less plaque, calculus, abrasion from brushing, and are the least teeth to be extracted with respected to age (7).

The Aim of the Study

This study aimed to investigate the accuracy of the method with which sex can be differentiated by odontometric analysis of maxillary and mandibular canine teeth in a sample of Iraqi adults**.**

MATERIALS AND METHODS

Subjects

A sample consisted of 60 individuals (30 males and 30 females) were included from institute of medical technology in Baghdad aged between (18-28 years), and were selected based on following criteria:
1- Complete set of fully erupted teeth.
2- Non carious and periodontal healthy teeth.
3- Non attired and intact.
4- Aligned teeth (without any crowding).
5- No history of trauma and any orthodontic treatment.

Materials

The following tools and instruments were used in the experiment:
1- Dental mirrors.
2- Dental probes.
3- Dental tweezers.
4- Rubber base, impression material.
5- Dental stone.
6- Electron digital vernier calipers.

Experimental method

Dental stone casts were prepared from alginate impression taken in selected study casts and labeled with sex and the age of subjected labio–lingual dimensions of maxillary and mandibular canine. The inter canine widths were measured with digital vernier caliper boss. Mandibular canine index and sexual dimorphism were calculated according to special formula**.** All the measurements were achieved by a single examiner to eliminate inter examiner errors. Data were subjected to statistical analysis using t-test.
Measurement of mesiodistal (MD) and buccolingual (BL) crown diameter of right and left permanent maxillary and mandibular canines (MC) were taken in dental casts using electronic digital caliper boss, which has an accuracy degree ± 0.01 mm. The mesodistal crown diameter was defined as the greatest mesiodistal dimension taken parallel to the occlusal and facial surface. The buccolingual crown diameter was defined as the greatest distance between the buccal (or labial)and lingual or (palatal)surface perpendicular to the mesiodistal diameter (8). Each measurement was taken three times and the average of three values was obtained to minimize the intra observer error. The descriptive statistics were calculated (mean, range, and standard deviation) for maxillary and mandibular canines. Statistically significant sexual dimorphism in male and female odontometric features were tested by students' t-test. The level of statically significance was set up at $p<0.05$. The percentages of sexual dimorphism were calculated using the formula given by (9), as follows:

1- sexual dimorphism = $[X_m / X_f] -1 \times 100$
Where,
X_m =mean value of males.
X_f= mean value of females.

2- canine index:

$$\frac{\text{mesio-distal diameter of the canine}}{\text{Intercanine distance}} \times 100$$

RESULTS

Table (1) shows mean value of inter canine distance and canine index for upper jaw, values of S.D, t test and p values for both males and females . The values for inter canine distance and canines index in males and females showed significant differences.
Table (2) shows inter canine distance and canine index, t test and p value in mandibular canines. The value for inter canine distance and canine index in males and females showed significant difference.
Table (3) Showed mean value of mesiodistal and buccolingual measurement and maxillary canines, value S.D, t test and P values in both male and female groups. The difference between male and female for both MD and BL dimensions of maxillary canines was significant.

Table (1): Inter canine distance and canine index for upper jaw (maxilla)

Value	Female (n=30)	Male (n=30)	t- test	P-Value	C.S
	Mean ±SD	Mean ±SD			
Inter Canine distance upper	35.99 ± 6.96	37.03 ±2.17	8.402	P<0.005	(S)
Canine index upper	0.352± 2.20	0.326± 1.28	1.322	P<0.005	(S)

Table (2): Inter canine distance and canine index for lower jaw (mandible)

Value	Female (n=30)	Male (n=30)	t- test	P-Value	C.S
	Mean ±SD	Mean ±SD			
Inter Canine distance lower	30.56± 4.35	27.38± 1.922	9.218	P<0.005	(S)
Canine index lower	0.252± 2.20	0.245± 2.20	4.154	P<0.005	(S)

Table (3): Mesiodistal and buccolingual measurements, mean, S.D, t- test and p value of maxillary canines

Value	Female (n=30)	Male (n=30)	t- test	P-Value	C.S
	Mean ±SD	Mean ±SD			
B.L.distance upper	7.66± 0.56	6.53±0.67	3.18	P<0.002	(S)
M.D.distance upper	7.14± 0.36	6.75± 0.57	2.12	P<0.001	(S)

Table (4) showed mean values of MD and BL dimensions of mandibular canines, values of S.D, t test and P values in both male and female groups. The differences between the male mean and female mean for both MD and BL dimension of mandibular canines were also significant**.**

Tables (5) and (6) showed sexual dimorphism in maxillary and mandibular canines respectively. It was found that the sexual dimorphism in maxillary canine was 1.22% using MD dimension and 1.02% using BL dimensions. Where as, in the mandibular canines sexual dimorphism was 2.55% using M.D dimension and 3.25% using BL dimensions**.**

When values for both males and females were measured and compared, males showed greater mean dimensions of teeth than females. Results were statistically significant. The most sensitive predictors of greater and gender determinator from canine were the mandibular inter canine distance and canine index**.**

Table (4): Mesiodistal and buccolingual measurements, Mean, S.D.t Test and p value of mandibular canines

Value	Female (n=30)	Male (n=30)	t- test	P-Value	C.S
	Mean ±SD	Mean ±SD			
B.L.distance lower	5.80± 0.42	5.88±0.45	2.14	P<0.003	(S)
M.D.distance lower	6.23± 0.43	6.05± 0.45	3.22	P<0.002	(S)

Table (5): Sexual dimorphism in permanent maxillary canines

Dimensions of canines (mm)	Mean (male) Xm	Mean (female) Xf	Sexual dimorphism
Mesiodistal dimension of maxillary canine.	8.366	6.661	1.22%
Buccolingual dimension of maxillaycanine .	6.452	6.133	1.02%

Table (6): Sexual dimorphism in permanent mandibular canines

Dimensions of canines (mm)	Mean (male) Xm	Mean (female) Xf	Sexual dimorphism
Mesiodistal dimension of maxillary canine.	6.452	6.133	1.02%
Buccolingual dimension of maxillaycanine .	6.557	6.455	3.25%

DISCUSSION

Metric methods of gender determination consider objective method because they rely on standard land mark and result in lower incidence of errors for this reason; the present study was performed to check the relevance of odontometric measurement of maxillary and mandibular canines**.**

Canines demonstrated higher degree of sexual dimorphism among the population (10, 11), and are considerate the most resistant teeth in dentition, remaining intact in several post mortem sceneries (12). In the present study, there was no significant difference between measurement at the permanent maxillary caning in right and left sides (asymmetry) for both sexes. A non-significant difference of the measurement of permanent teeth between right and left sides were reported in studies of samples from three population from Egypt, Mexico and USA (13-15). In Saudi Arabia, even there was a study with contra dictory findings, where there were statistically significant differences between right and left sides of the maxillary canine (15). This difference can be attributed to several factors namely, environmental, ethnic and nutritional factors.

The present study showed that both maxillary and mandibular canines in males were greater in mesiodistal and buccolingual dimensions than females. These differences were found to be statistically significant. In accordance with present study, (1) showed statistically significant differences between males and females, while measuring the mesiodistal and labiolingual diameters of both maxillary and mandibular canines. Richardson *et. al.* (16) found that teeth of males tend to be larger than female for each type of tooth in both the maxillary and mandibular arches. Howe *et. al.* (17) found that combined mesiodistal width for males was more compared to females. Partabha Rani etal (2009) studied that males showed greater bucclo_lingual dimensions of teeth in comparison of female (18). The present study revealed that inter canine distances of maxillary canine was greater in males than in females and it was statistically significant. However, the differences in mandibular inter canine distance between both sexes were statistically significant.

As in study conducted by (1), maxillary inter canine distance showed statistically significant differences between both sexes.

The study conducted by (18) showed that inter canine distance of the upper and lower dental arches were significant greeter in males than in female in Saudi population group study. Contrary to results of present study, Kaddah (19)stated that no statistically significant differences were obtained between males and females while measuring the inter – canine distance. In the present study, mandibular canines were more sensitive predictor of gender because they showed greater sexual dimorphism than maxillary canine . These results are in agreement to the results obtained by (9), who found that mandibular canines exhibit the greatest sexual dimorphism amongst all teeth . a study conducted by (20), revealed that the mandibular canine showed a greater degree of sexual dimorphism than the maxillary canine. A study reported that in most living human population , lower canines showed that the greatest dimorphism followed by upper canines where as the premolars were the least dimorphism (21).

The sexual dimorphism of canines is attributed to the many theories : The first theory states that according to Moss, there is a greater thickness of enamel in male due to long period of amelogenesis and slower rate of maturation of teeth than in female. Second theory said the sexual dimorphism is due to more dentine in the crowns of male teeth (22).

The third theory said that Y chromosome control the thickness of dentine , whereas X-chromosome seems to be responsible for modulating the thickness of enamel (19).

There can be a complex interaction between a variety of genetic and environment factor that is responsible for the variation in the magnitude of dimorphism . Different human populations may show different expression of sexual dimorphism . In some populations, this dimorphism may be greater developed than others. Sexual dimorphisms in tooth size are population specific (10) and varied among different ethnic groups (9).

CONCLUSION

The study revealed that males showed larger mean dimension of teeth than females in the study groups inter canine distance and mandibular canine index are more sensitive predicators for gender determination . Mandibular canine teeth show significant and consistent results for sexual dimorphism and can be used as an adjunct along with other procedures .for sex determination in mass disasters**.**

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Identification of salivary protein biomarkers (IL-1RA and SLPI); matrix metalloproteinase (MMP-2 and MMP-9) and IL-6 levels in type 1 diabetic patients in relation to HbA1c in Iraqi population

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ABSTRACT

Type I diabetes mellitus (type-I DM) is a complex polygenic autoimmune disease resulting in the targeted destroy of beta-cell in pancreas that result in deficiency or absence of insulin production. Several studies have been reported the salivary biochemical and biomarkers alterationsin diabetic patients.

The aims of the study were to evaluate the salivary Interleukin-6 (IL-6), Interleukin-1 Receptor Antagonist (IL-1RA) and Secretory Leukocyte Protease Inhibitor (SLPI), matrix metalloproteinase (MMP-2 and MMP-9) in uncontrolled type-I DM and investigating the association of salivary biomarker to the HbA1c%.

The total sample composed of 90 adult aged 18-35 years. Divided into 60 uncontrolled diabetes (HbA1c >7%) and 30 appeared healthy control group. The BMI, HbA1c and duration of disease were recorded through the visits. Unstimulated whole saliva was collected from each patient under standardized condition. The salivary flow rate and PH were measured; the salivary IL-6, IL-1RA, SLPI, MMP-2 and MMP-9 levels were measured by using multiple immunoassay analysis.

The salivary MMP-2,MMP-9 and IL-6 were elevated in individuals with type I diabetes mellitus in comparison to the healthy controls, but the statistical difference was non-significant between the study groups except the IL-6 was higher than control among type-I diabetes mellitus group, which was statistically significant ($p=0.033$). IL-1RA was highly significant lower among uncontrolled diabetic group than control group ($p<0.001$). On other hand, SLPI of uncontrolled diabetic group was lower than control group with no statistically significant difference between the two groups. Among uncontrolled diabetic group the salivary MMP-2 was highly significant correlated with MMP-9 ($r=0.468$, $p<0.001$) in positive direction.The SLPI was positively highly significant correlated with salivary IL-1RA($r=0.579$, $p<0.001$) and positive correlation with salivary MMP-2 and MMP-9 ($r=0.394$, $p=0.002$; $r=0.328$, $p=0.01$, respectively). HbA1c% was correlated significantly with salivary SLPI in positive direction. The salivary flow rate among uncontrolled type-I DM was highly significantly lower than control group ($p<0.001$), while the salivary PH among uncontrolled diabetic group was non-significantly different in comparison to the control group.

In conclusion, the diabetic and poor metabolic control has a significant influence on salivary biomarker, matrix metalloproteinase and salivary flow rate.The salivary biomarkersmay represent a useful method in predictive of the progression and control of DM type-I.

Keywords: Diabetes mellitus, saliva, IL-6, IL-1RA and SLPI, matrix metalloproteinase.

الملخص باللغة العربية

يعتبر النوع الأول من مرض السكري مرضا مناعيا ذا جينات متعددة ومعقدة، يؤدي إلى تدمير مستهدف من خلايا بيتا في البنكرياس والتي تنتج نقصا أو غيابا في إنتاج الأنسولين، وقد وجد العديد من الدراسات الخاصة بالتعديلات البيوكيميائية والمؤشرات الحيوية اللعابية لدى مرضى السكري.

الهدف من الدراسة هو تحديد مستوى الانترلوكين 6(IL-6)،(IL-1RA) وإفراز خلايا الدم البيضاء المثبطة للبروتياز(SLPI)، وإنزيمي (MMP-9 , MMP-2) لدى مرضى السكري النوع الأول غير المنضبط وتحقيق رابطة العلامات البيولوجية اللعابية بالنسبة إلى (HbA1C%).

شملت عينة الدراسة 90 شخصا بالغا تتراوح أعمارهم بين 18−35 سنة، مقسمة إلى 60 مريض مصاب بداء السكري غير المنضبط بنسبة (HbA1C%>7) و 30 شخص سليم غير مصاب بداء السكري كمجموعة ضابطة. وتم تحديد مؤشر كتلة الجسم، اختبار الكلايكيتيد هيموغلوبين، ومدة الإصابة بمرض السكري من خلال الزيارات السريرية . وقد تم جمع عينات اللعاب غير المحفز من كل مريض تحت ظروف قياسية. وتم قياس معدل تدفق اللعاب وقيمة الأس الهيدروجيني ومستويات(IL-1RA , SLPI , MMP-9 , MMP-2 , IL-6) في اللعاب باستخدام طرق التحليل المناعية المتعددة.

أظهرت النتائج أن مستويات (IL-6 , MMP-2 , MMP-9) اللعابية مرتفعة لدى مرضى السكري النوع الأول مقارنة بالمجموعة الضابطة، لكن لا توجد فروق ذات دلالة إحصائية مهمة بين مجموعات الدراسة باستثناء مستوى (IL-6) في اللعاب لدى مرضى السكري الذي كان مرتفعا بدلالة إحصائية معنوية ($P = 0.033$) . وكان مستوى (IL-1RA) أقل أهمية لدى مرضى السكري غير المنضبط مقارنة بالمجموعة الضابطة ($P<0.001$) . ومن ناحية أخرى، كان مستوى (SLPI) لدى مجموعة مرض السكري غير المنضبط أقل من المجموعة الضابطة مع عدم وجود فروق ذات دلالة إحصائية بين المجموعتين.

وكشفت النتائج أن إنزيم (MMP-2) قد ارتبط بعلاقة إيجابية مع إنزيم (MMP-9) وارتبط بروتين (SLPI) بعلاقة إحصائية معنوية مهمة للغاية مع بروتين (IL-1RA) وباتجاه إيجابي، كما ارتبط مع إنزيمي (MMP-9 , MMP-2) بعلاقة إيجابية أيضا. وقد ارتبطت نسبة (HbA1C%) بشكل كبير مع بروتين (SLPI) اللعابي باتجاه إيجابي، وكان معدل تدفق اللعاب لدى النوع الأول من مرضى السكري غير المنضبط أقل بكثير من المجموعة الضابطة، في حين كان قياس مستوى الأس الهيدروجيني لدى مجموعة مرضى السكري غير المنضبط غير ملحوظ بالمقارنة مع المجموعة الضابطة.

وقد خلصت الدراسة إلى إن سيطرة التمثيل الغذائي والفقيرة لمرضى السكري لديها تأثير كبير على العلامات البيولوجية اللعابية، الماتركس ميتالوبروتينيس ومعدل تدفق اللعاب. وتمثل المؤشرات الحيوية اللعابية طريقة مفيدة في التنبؤ من التقدم والسيطرة عند النوع الأول من السكري.

INTRODUCTION

The abnormally high blood glucose level is the characteristic feature of Diabetes mellitus. Type 1 diabetes (T1D) is a highly complex polygenic autoimmune disease resulting in the loss of pancreatic β-cells and absence of insulin production (1), the diagnosis of itmay be occur at any age and the majority of the patients with type 1 diabetes were diagnosed before the age of twenty which means that it usually manifests in childhood, adolescence or early adulthood. At birth the individuals with a genetic susceptibility have normal beta cell mass but start to lose beta cells secondary to autoimmune destruction that occurs over months to years (2).

Saliva consists of two main components that are secreted by independent mechanisms; the first component includes ions, which is produced mainly by parasympathetic stimulation and second protein component, which is released mainly in response to the sympathetic stimulation (3). In saliva the inflammatory molecules are derived from the oral mucosa and from the periodontium through influx of gingival crevicular fluid (4).

Saliva is carrying a spectrum of immunologic and non-immunologic proteins that characterized by having the antibacterial properties (5). Secretory Leukocyte Peptidase Inhibitor (SLPI) possesses antimicrobial defenses and cytotoxic properties in addition to the antifungal and antiviral activity (6).Interleukin-6 (IL-6) has a chief regulator of acute-phase inflammatory response (7). However, it plays a critical role in the transformation from acute to chronic inflammation (8). The concentration of IL-6 has been shown to be normal or higher in DM1 people in comparison to healthy people (9), on other hand the interleukin-1 receptor antagonist (IL-1Ra) plays important role in protecting the periodontium from the inflammatory effect of interleukin-1 (IL-1). The IL-1 is proinflammatory protein that arrests the function beta cells and develops apoptosis. Naturally the body secretes IL-1Ra which is anti-inflammatory protein, it has the inhibiting function of both types of IL-1 signaling and protecting the beta cells so it's used to counter the effect of IL-1 (10, 11).

Matrix metalloproteinases (MMPs) are family of metal dependent proteolytic enzymes that mediate the degeneration of extra cellular matrix and basement membranes and play a role in collagen degradation of osseous and connective tissue (12). Recent studies suggest that the elevations in blood sugar may abnormally affect MMP enzyme activity (13). The MMP-2 and MMP-9 are called gelatinase A and gelatinase B, respectively, because gelatin was identified as one of the key substrates for these two enzymes. Gelatinases can also break down other ECM proteins such as type IV collagen (14).

The present study was designed to investigate the salivary biomarkers and matrix metalloproteinase and may represent a useful method in predictive of the progression and glycemic control in patients with Type 1 DM.

PATIENTS AND METHODS

Ninety adult patients (90) were recruited from Diabetic - Endocrinology Center in AL- Kindy Teaching Hospital in Baghdad city during the period from (November 2014 to April 2015). Inclusion criteria were: Aged 18-35 years of both genders have type 1 Diabetes mellitus for more than 3 years duration. Patients with any other systemic diseases, taking any medications like antihypertensive, anti-lipid and aspirin and subjects less than 18 years of age were excluded from this study. The samples were divided into two groups: 60 patients with uncontrolled type-I diabetes mellitus (HbA1c >7%) and non-diabetic subjects as a control group were included 30 healthy subjects who did not suffer from any systemic disease and matching with the study group. Approximately 1.5 to 2 ml of saliva were collected by spitting method for 10 mints at the same day of blood sample aspiration for HbA1c measurements and after informed consent was obtained from all individuals. The salivary samples were centrifuged at (4000 rpm for 10 minutes) to remove any unwanted particles; then the supernatant has been taken by micropipette, aliquot into Eppendorf tubes (500µl) and stored immediately at - 20°C for matrix metalloproteinase while the sample used for protein and interleukin analysis stored at -70°C until analysis. The immunoassay analyses of salivary sample were doing to measure the concentration of biomarkers by using an enzyme-linked immunosorbent assay (ELISA technique) according to the manufacturer's instructions,IL-6 (Abcam Human ELISA Kit, U.S.A), IL-1RA, SLPI (BioAssay ELISA Kit, U.S.A.) MMP-2, MMP-9 (MyBioSource, ELISA Kit). The saliva sample that used to measure the IL-1RA and SLPI was diluted by using phosphate buffer 150 fold; the concentration read from the standard curve must be multiplied by dilution factor. Statistical analyses were done using SPSS version 21 computer software (Statistical Package for Social Sciences) in association with Excel version 5. The statistical significance of differences in mean between 2 groups weres assessed using the independent samples t-test. P value less than 0.05 was considered statistically significant and highly significance when $P< 0.01$.

RESULTS

The characteristics of the study subjects (age, body mass index, salivary flow rate and PH) are presented in table (1). The (mean ± SD) of age in patients with diabetes was (24.8±5.4) years, while the (mean ± SD) of age in healthy controls subjects was (23.8±5). The (mean ± SD) of BMI in diabetic patients was (24.3 ± 4.3) kg/m², while the (mean ± SD) of BMI in healthy (24.4 ± 4.4) kg/m².Statistically no significant difference in mean age and mean BMI between groups (p=0.44, p=0.91) respectively. The mean salivary PH was 7.6 (±0.5) in healthy group and 7.5(±0.5) in diabetic group which was statistically non-significant (p=0.3), whereas the salivary flow rate mean was higher in healthy control (0.56 ±0.19) compared to

diabetic group (0.35±0.29) with statistical highly significant difference (P< 0.001).

Table (1): The DM type 1- controls difference in mean age, BMI, salivary PH and flow rate (ml/min) among study group

Parameters	Healthy controls mean	Diabetic type-I mean	P-value
Age	23.8(±5)	24.8(±5.4)	0.44[NS]**
BMI kg/m²	24.4(±4.4)	24.3(±4.3)	0.91[NS]***
Salivary pH	7.6 (±0.5)	7.5 (±0.5)	0.3[NS]***
Salivary flow rate (ml/min)	0.56 (±0.19)	0.35 (±0.29)	<0.001*

(p<0.01) highly significant, ** (p<0.05) significant, * (p>0.05) non-significant*

In table (2), the females 34 (56.7%) more than males 26 (43.3%). The age of those patients was <20 years 10(16.7%); 20-24 years was 25(41.7%); 25-29 years was 14(23.3%) and 30+ years was 11(±18.3). The frequency distribution of the sample according to the BMI was divided into three categories, the acceptable categories (<25kg/m²) were 31(51.7%), over weight categories (25-29.9 kg/m²) were 23(38.3%) and obese categories (30+ kg/m²) were 6(10%) and according to the smoking habit into smoker were 10(16.7%) and non-smoker were 50 (83.3%).

The salivary parameters were shown in table (3) (mean/median and p-value) among diabetic and healthy controls groups. The highest salivary MMP-2, MMP-9 and IL-6 values was represented in diabetic group (30.4, 51.4, 4.6) in comparison with control group (28.5, 49, 2.6), respectively. Result revealed that salivary MMP-2 and MMP-9 had no statistically significant difference (p=0.67, p=0.11, respectively), whereas the salivary IL-6 had statistically significant difference (p=0.033). While the lowest salivary IL-1RA and SLPI values were found in saliva among diabetic group (15.9, 31.5) compared to control group (24.7, 36.8) respectively. The mean salivary IL-1RA had highly significant difference between study groups (p<0.001), in contrast no statistically significant difference in SLPI was found between two groups (p=0.09).

Table (2): Frequency distribution of diabetic type-I (study sample) by selected variables (gender, age, body mass index BMI and smoking habit)

Variables	No.	%
Female	34	56.7
Male	26	43.3
Age groups (years)		
<20	10	16.7
20-24	25	41.7
25-29	14	23.3
30+	11	18.3
BMI (kg/m²)-categories		
Acceptable(<25)	31	51.7
Overweight(25-29.9)	23	38.3
Obese(30+)	6	10.0
Smoking habit		
Non- smoker	50	83.3
Smoker	10	16.7

Table (3): Frequency distribution of the DM type 1- and control in relation to selected salivary parameter among study group

Salivary parameter	Healthy controls mean/median	Diabetic type-I mean/median	P- value
MMP-2 (pg/ml)	28.5	30.4	0.67[NS]***
MMP-9 (pg/ml)	49	51.4	0.11[NS]
IL-6 (pg/ml)	2.6	4.6	0.033**
IL-1RA (ng/ml)	24.7	15.9	<0.001*
SLPI (ng/ml)	36.8	31.5	0.09[NS]

(p<0.01) highly significant, ** (p<0.05) significant, * (p>0.05) non-significant*

Table (4) illustrates that the correlation of SLPI with HbA1c% was significant in positive direction among uncontrolled diabetic group (r= 0.276, p= 0.033). On other hand, table (5) showed the correlation between the salivary parameters among type-I DM group, salivary MMP-2 was highly significant correlated with MMP-9 in positive direction (r=0.468, p<0.001), SLPI had highly significant positive correlation with salivary IL-1RA (r=0.579, p<0.001) and significant positive correlation with MMP-2 and MMP-9 (r= 0.394, p= 0.002, r= 0.328, p= 0.01 respectively). No significant correlation in negative direction between SLPI and IL-6 (r=-0.054, p= 0.68).

Table (4): Correlation coefficients between the HbA1c% and salivary SLPI among type-I DM group

Glycated Hemoglobin	Uncontrolled type-I DM Salivary SLPI	
	r	p
HbA1c%	0.276	0.033

Table (5): Correlation coefficients between the salivary parameters among type-I DM group

	Uncontrolled type-I DM Salivary parameters			
	MMP-2 (pg/ml)	MMP-9 (pg/ml)	IL-6 (pg/ml)	IL-1RA (ng/ml)
MMP-9 (pg/ml)	r=0.468 P<0.001*			
IL-6 (pg/ml)	r=0.155 P=0.24[NS]	r=0.243 P=0.06[NS]		
IL-1RA (ng/ml)	t=0.196 P=0.13[NS]	r=0.098 P=0.46[NS]	r=-0.02 P=0.88[NS]	
SLPI (ng/ml)	r=0.394 P=0.002**	r=0.328 P=0.01**	r=-0.054 P=0.68[NS]	r=0.579 P<0.001*

**(p<0.01) highly significant, ** (p<0.05) significant*

The salivary flow rate and salivary IL-1RA were the two parameters associated with highest ROC area (0.796, 0.770 respectively) (p<0.001) as shown in table (6), and therefore were the most affected by type I DM disease process, (in other words it showed the largest difference between type I DM and controls); followed by IL-6 (0.638, p= 0.063), whereas the salivary MMP-9, SLPI and MMP-2 were less affected by type I DM disease under ROC (0.605, 0.601, 0.515) (p= 0.11, p= 0.12, p= 0.81) respectively, in addition to salivary PH (0.515, p=0.37).

Table (6): ROC area for selected parameters when used as test to differentiate between cases with type-I DM and healthy control

	ROC	P – value
Salivary flow rate	0.796	<0.001*
Salivary IL-1RA (ng/ml)	0.770	<0.001
Salivary IL-6 (pg/ml)	0.638	0.033**
Salivary MMP-9 (pg/ml)	0.605	0.11[NS]***
SLPI (ng/ml)	0.601	0.12[NS]
Salivary pH	0.558	0.37[NS]
Salivary MMP-2 (pg/ml)	0.515	0.81[NS]

(p<0.01) highly significant, ** (p<0.05) significant, * (p>0.05) non-significant*

DISCUSSION AND CONCLUSION

Salivary diagnostics is a potential method for numerous biological and technical studies and saliva is carrying a spectrum of immunologic and non-immunologic proteins that characterized by having the antibacterial properties (5). The present study was carried out to elucidate the effect of hyperglycemia on salivary parameter like MMP-2, MMP-9, IL-6, IL-1RA and SLPI in uncontrolled diabetes mellitus type-I and may also be an indication of glycemic status in type 1DM. The salivary flow rate was significantly lowered in uncontrolled type – I diabetics, a reduction in the flow rate of saliva may give an indication that diabetic has an influence on salivary flow rate. This result comes in agreement with some studies (15, 16) and disagreement with other studies (17, 18). No significant difference in salivary PH between diabetic patient and healthy control. This result is in disagreement with other studies (19, 20).

In the present study, individuals with type 1 diabetes mellitus and poor glycemic control have highest salivary MMP-2 and MMP-9 levels in comparison to the healthy controls, but the difference observed failed to reach the level of statistically significant may be due to small sample size, level of metabolic control and duration of disease. This result is in agreement with other studies (21, 22). Positive correlation between salivary MMP-2 and MMP-9; this may be due to the similarity in the structure and mechanism of these two enzymes in degrade type IV collagen (14).

Interleukin-6 is a pleiotropic cytokine with a wide range of biological activities in hematopoiesis, immune regulation inflammation and oncogenesis (23). The level of salivary IL-6 was higher in DM type I patients than healthy controls however, there is a significant differences in mean of IL-6 between two groups. A possible explanation for increased salivary Il-6 is the chronic hyperglycemia that affects the migration and phagocytic activity of mononuclear and polymorph nuclear phagocytic cells resulting in establishment of more pathogenic sub-gingival flora, this triggers an infection-mediated pathway and dis-regulates cytokine production and expression (24). This result is in agreement with study by (25).

Data of the present study revealed the level of salivary IL-1RA and SLPI was significant lower among uncontrolled diabetes group than control group, the lower level of IL-1RA may be related to xerostomia, the IL-1RA limits the immune and/or inflammatory response in the mouth, so the patients with lower level of salivary IL-1RA or reduction of IL-1RA production precedes the first symptom of dry mouth by several years that lead to formation of dental caries and early tooth loss (26).The correlation of SLPI with IL-1RA and HbA1c% was in positive direction, both SLPI and IL-1RA has inhibitory activity against the bacteria and inflammatory process that may give an indication about the correlation between them.

Jindal (27) found the subject with poor glycemic control has more severed gingival inflammation by the higher score of gingival index. The positive correlation between HbA1c% and SLPI may be attributed to gingivitis. In patients with type 1 DM (especially poor metabolic control), the incidence of gingivitis can be explained by the fact that the excessive blood glucose which enters the oral cavity through saliva and gingival crevicular fluid, soaks the biofilm and causes an increase in total biofilm accumulation. This result is in agreed with other studies that reported the levels of SLPI are significantly increased during gingivitis and periodontitis (28, 29) and dis agreement with studies by (30, 31) who found the SLPI level become decreased.

In the present study, the SLPI have a positive correlation with salivary (MMP-2, MMP-9), the data of the present study suggest the correlation between them are explained by the important role of SLPI in the control of tissue destruction by inhibiting the elastase activity which is related to connective tissue destruction that associated with inflammatory process (4, 32). Moreover, the MMP-2 & MMP-9 are subgroup of enzymes within the MMP family that are highly similar in their substrate specificity and structure, but differ in their cellular origin. Both enzymes are associated with degradation type IV collagen and regulate basement membrane remodeling (14), so the SLPI, MMP-2 and MMP-9 are related to the periodontal diseases monitoring. This result is in agreement with other studies (33, 34).

The results of this study showed the salivary IL-1RA is a parameter associated with highest ROC area, therefore considers the most parameter affected by type-I DM disease process, also this protein can be used as a biomarker that may be prone to periodontitis in diabetes mellitus. This result is in agreement with study by (35) who reported this biomarker may be affected by diabetic patients with periodontitis by its protection action of periodontium from the IL-1 inflammatory effect.

The salivary MMP-9, MMP-2 and SLPI in this study show less affected by type-I DM, but the increase in the salivary concentration of MMPs may result from gingival and periodontium inflammation that associated with uncontrolled diabetic patients due to impair neutrophil function and chemotaxis become decreased, however, advanced periodontitis in type I DM seems to be linked with elevated MMP-9 level and lowered level of SLPI that might be useful in monitoring the periodontal disease with diabetes patients. This result is in agreed with other

studies (21, 34, and 22). In contrast, (36) concluded that the concentration of MMP-2 and MMP-9 were lower in subjects with diabetic than in non-diabetic controls.

In conclusion the poor metabolic control in diabetic type-I patients has a significant influence on salivary biomarker, matrix metalloproteinase and salivary flow rate. The regular oral screening programs are considered as a standard of care for young patients with type-I diabetes to decrease the incidence of oral disease.

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Prevalence of intestinal protozoa in some asymptomatic children aged five years old who attend private day care centers

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ABSTRACT

Intestinal protozoa parasites infections have a public health importance mainly in symptomatic children, because they consider to be a source of infections. *Ciardia lamblia , Entamoeba histolytica* and *Cryptosporidium parvium* are the most common infectious parasites, which transmit by contaminated food and water. The prevalence of intestinal protozoa in asymptomatic pre-school aged children was the objective of the study. Gender and social levels were in account. Direct microscopic examination, staining ,flotation methods were used for the intestinal protozoa diagnosis in 105 stool samples collected from 4 privet day care centers during April, 2015. The results revealed that 19.04% was the total prevalence rate with a highly significant differences P<0.01 between male 23.33% and female 13.33% , high 12.72% and poor 26% social levels. In addition, results indicated that only two types of intestinal protozoa were detected with a higher prevalence rate for *G.lamblia* 80% in comparison with *E.histolytica* 20%. The study concluded that children aged 5 years old are most frequently infected with intestinal protozoa parasites which was associated with the lack of major contributor factors.

Keywords: Intestinal parasites, protozoa, *Giardia lamblia, Entamoeba histolytica*

الملخص باللغة العربية

تحظى الإصابة بطفيليات الأوالي المعوية باهتمام بالغ في الصحة العامة خصوصا حين يصاب بها الأطفال عديمي لذين لا تظهر عليهم الأعراض المرضية، باعتبارهم مصدرا للإصابة. ومن أكثر الإصابات الطفيلية شيوعا هي الإصابة بالجيارديا الإثني عشرية الأميبة والكربتوسبوريديم، حيث تنتقل بالطعام والماء الملوث. هدفت الدراسة إلى التحري عن انتشار الأوالي المعوية بين الأطفال عديمي الأعراض المرضية بعمر ما قبل المدرسة، وقد شملت دراسة الجنس والمستوى الاجتماعي، تم استخدام الفحص المجهري المباشر، التصبيغ والتطويف لتشخيص الأوالي المعوية في عينات البراز التي جمعت من (105) أطفال من 4 حضانات أهلية خلال شهر نيسان عام 2015 . أظهرت النتائج أن نسبة الانتشار الكلية 19.4% مع وجود فروقات معنوية كبيرة P<0.01 بين الذكور 23.33% والإناث 13.33% وبين المستوى الاجتماعي الجيد 12.72% والفقر 26%. كما أشارت النتائج إلى وجود نوعين فقط من الأوالي المعوية التي تم تشخيصها مع نسبة انتشار أعلى للجيارديا 80% مقارنة مع الأميبا 20% .استنتجت الدراسة إلى أن الأطفال بعمر 5 سنوات هم في أكثر الأحيان عرضة للإصابة بطفيليات الأوالي المعوية والتي لها علاقة بفقدان العوامل المساهمة الرئيسة.

INTRODUCTION

People of all ages can get parasitic infections, which are considered to be as a serious problem in public health throughout the world, especially in developing countries (1). The main causative of these infections are protozoa**,** helminthes**,** and ectoparasites (2). A parasite is an organism that depends on another organism (host) and gets its food from its host (2). WHO "estimated that 3.5 billion people are infected by intestinal parasites (3). *Entamoeba histolytica* and *Giardia lamblia* are also estimated to infect about 60 million and 200 million people worldwide, respectively" (4). Protozoa parasites have only one cell and can multiply inside the human body and cause serious infections. *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium* are the most common intestinal protozoa parasites, which enter the body through contaminated food and or water.

G. lamblia is a pear-shaped, flagellated protozoan that lives in small intestine and causes giardiasis (a wide variety of gastrointestinal complaints) (5). Life cycle includes two stages: trophozoite and cyst, ingestion of at least 10 to 25 cysts (infective stage) can cause infection in humans (6). *E. histolytica*, is about 10 to 60 µm in length , moves through the extension of finger-like pseudo-pods and caused amoebiasis. *Cryptosporidium parvum* about 3-6 µm in size, primarily localized to the distal small intestines and causes cryptosporidiosis (7). Although infections transmission mainly occurred through contaminated food and water but person to person and contaminated soil were documented (8).

Diarrhea is the main clinical sign for infections in symptomatic patients which is passing loose, watery stools 3 or more times a day, while asymptomatic patients show no clinical signs but have an importance in spread the infections (9). Most of the previous studies had focused on the distribution and prevalence of intestinal parasitic infections, mainly among school children (12 – 16 years). Only few studies have reported on pre- school children (under-five years old) (10).

PATIENTS AND METHODS

Samples collection

One hundred and five stool samples were collected from asymptomatic children aged 5 years old, who attended 4 private day care centers (1,2 day care centers as high social class and 3,4 as poor social class) in sterile plastic cups during April, 2015, including 60 males and 45 females.

Microscopic examination:

All samples were examined using saline and iodine wet mount smears of fresh stool method for parasitic detection (11).

Small amount of feces was taken by wooden stick and mixed with a droop of normal saline and a droop of Lugol's iodine on glass slid that covered with cover slip and examined under microscope (10x,40x) , for crypto sporidialoocysts were recovered by modified acid-fast stain as described by Bronsdon (12). Briefly; the fecal smears were fixed with methanol for 10 minutes and 5 drops of carbol fuchsin was added. Smears were decolorized by 5% sulfuric acid and then stained with methylene blue. To confirm the results another zinc sulphate solution flotation method was used (13). A data sheet was used to record information such as age, antibiotic consumption, medical history and personal hygiene habits also A questionnaire was also used to assess water consumption habits, eating and living places.

Statistical analysis

Statistical analysis was done by SPSS, the inertial statistic use chi-square-test with-p-value if $p<0.05$ significant, $p>0.05$ Non significant, $p<0.01$ High significant (14).

RESULTS AND DISCUSSION

Out of 105 stool samples, only 20 (19.04%) samples were detected to be infected with intestinal protozoa. Significant differences $P<0.05$ in the prevalence rate were recorded between various day care centers, the lowest infestation was shown in number one 10%, while the higher prevalence rate was demonstrated in number four (31.81%). *G. lamblia* and *E. histolytica* were the most common intestinal protozoa recovered. No *cryptosporidium* species were detected (table 1).

Table (1): Prevalence and types of protozoa infection

Types of protozoa	Prevalence rate %	Positive No. for parasites	No. examined	Day care center
1	30	3	10	*Giardia lamblia, Entamoeba histolytica*
2	25	4	16	*Giardia lamblia*
3	28	6	21.42	*Giardia lamblia Entameba histolytica*
4	22	7	31.81	*Giardia lamblia, Entameba histolytica*
Total	105	20	19.04	

**Chi-square =3.628 P<0.05 significant*

Few studies were focus on the prevalence of intestinal protozoa parasites in asymptomatic children aged 5 years old ,the overall conclusion of these studies was "The prevalence of intestinal parasites in children varies according to different regions, Younger children are predisposed to heavy infections with intestinal parasites since their immune systems are not yet fully developed" (15).

In general, the total recorded prevalence rate 19.04% may be attributed to both poor personal hygiene and poor environmental conditions. According to the data from questionnaire of this study an inadequate supply of drinking water, a waste disposal system which does not correspond to approved standards, the activities of children in contaminated recreational water ,not washing hands after using the toilet and owning animals in homes play a role as source of parasites infections.

The total prevalence rate was less than that reported in other studies, this may be due to the size of samples and the periods of study. In Thailand the findings of a study showed that 66 asymptomatic children aged 5 years old were infected with intestinal parasites from the total 236 examined 27.9% (16). Also Marry (17) indicated that at least one pathogenic parasite identified in children with prevalence rate 27%.

According to gender, the results indicated a highly significant differences P<0.01 in the prevalence rat between males and females, 14 male from the total examined number 60 were positive for intestinal parasite with a higher prevalence rate 23.33% in compared with the prevalence rate for females 13.33% as 6 females showed positive results from the total number examined 45 (table 2).

Table (2): Prevalence of intestinal protozoa according to gender

Day care center	Total examined number		Positive parasitic number		Prevalence %	
	M	F	M	F	M	F
1	20	10	2	1	6.66	10
2	13	12	3	1	23.07	8.33
3	9	9	4	2	21.05	22.22
4	8	14	5	2	62.5	14.28
Total	60	45	14	6	23.33	13.33

**Chi-square =15.522 P<0.01 High significant / M: male, F: female*

The higher prevalence rate was observed in males, this was a result of the different behaviors of the males delegates for females in terms of playing and dealing with the environment. Aly and Mostafs (18) noted that the differences in recreational activities play a role in the rate of infection between male and female. Similarity in results was observed with a study done by Ahmed (19), and another study, which revealed that prevalence was significantly higher in males than females with rates of 51.8% (604/1162) and 30.8% (381/1238) respectively (20). Disagreement was recorded with Cláudia's study (21) in that prevalence was similar among genders 6.9% in male and 6.5% in female.

In table (3), data demonstrated that the most common intestinal protozoa parasite identified was *G.lamblia*, the prevalence rate in males and females were 78.5%, 83.33% respectively. For *E.histolytica* the prevalence rate in males and females recorded 21.42% and 16.66% respectively. On the other hand, *G.lamblia* was more common among females 5/6(83.33%) while, males showed the higher prevalence rate for *E.hitolytica* 3/14(21.42%). The total prevalence rate for *G.lamblia* was 80%, while *E.histolytica* recorded 20%, A highly significant differences P<0.01 were recorded.

Table (3): Types of protozoa in examined stool samples associated with gender

Day care center	Total examined number		Positive parasitic number		Prevalence %	
	M	F	M	F	M	F
1	2	1	1 (50%)	1 (100%)	1 (50%)	-
2	3	1	3 (100%)	1 (100%)	-	-
3	4	2	3 (75%)	1 (50%)	1 (50%)	1 (50%)
4	5	2	4 (80%)	2 (100%)	1 (20%)	
Total	14	6	11 (78.57%)	5 (83.33%)	3 (21.42%)	1 (16.66%)
Prevalence rate %	---		80		20	

**Chi-square =14.238 P<0.01 High significant / M: male, F: female*

Agreement in results was observed with a study that reported 5.4% and 8.9% prevalence of *Entaemoeba histolytica* and *Giardia lamblia*, respectively (22). Similar results was conducted by Boonchal (16) *as G.lamblia* was the higher prevalence 23.3% among 236 asymptomatic children. In 2011, a study done in Baghdad reported that from a total 131 diagnosis number with intestinal parasites in different age group, (30.4%) were infected with *G.lamblia* at age 2-5 while *E.histolytica* recorded (24.1%) also male were the higher infected than females 33.5%, 28.6% for *G.lamblia* and 19.4%, 29% for *E.histolytica* respectively (23). *G.lamblia* was the most prevalent, seen in 19% of children while *Entamoeba histolytica* 1% (17). All samples were collected from privet day care centers which classified according to social levels in to 1 and 2 as a high class while 3 and 4 as a poor class depending on geographic regions, education level for parents and hygienic concern, results analysis showed highly significant differences P<0.01 between the two categories. Seven children were recognized with intestinal protozoa parasites from the total of 55 stool samples examined from 1 and 2 day care centers with a prevalence rate 12.72%. For the 50 examined samples collected from poor day care centers 3 and 4, twenty children showed positive for infection with a prevalence rate recorded 26% (table 4).

Table (4): prevalence of protozoa infection according to social level

Day care center	Social level	Total examined number	Positive number for infection	Prevalence %
1 and 2	High	55	7	12.72
3 and 4	Poor	50	13	26
		105	20	19.04

**Chi-square =15.846 P<0.01 High significant*

Multiple features of host social behavior, exposure to child and institutional care centers, maternal education and weakened immune system influence parasitic infections in children. The poorest people are at highest risk of parasitic infection and other conditions of ill-health (24). The most common outcome of *Giardia* infection is often asymptomatic, especially for children, it has been estimated that as many as 50% to 75% of *Giardia*-infected persons may be asymptomatic (25). As shown in results for the higher prevalence rate was demonstrated in poor day care centers this finding was in line with similar studies conducted (15, 26, 27). A study in Turkey did not show any significant association between intestinal parasitic infection and some factors such as maternal education (28).

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Immunohistochemical localization of over-expressed P53 gene and CD56-tumor infiltrating lymphocytes in tissues with papillary thyroid cancer

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ABSTRACT

The aim of the present study was to evaluate of P53 expression tumor suppresser gene in papillary thyroid cancer and the role of immune defense by CD56 in papillary thyroid tissue by Immunohistochemistry technique.
The study was designed as a retrospective study, where (60) samples from papillary thyroid cancer patients were collected from patients wo attended Medical City Hospital in Baghdad and Al-Yarmouk Teaching Hospital during the period May, 2013 to May 2014.

Keywords: P53 gene, CD56, Immunohistochemistry, papillary thyroid cancer

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الملخص باللغة العربية

هدفت الدراسة إلى التحري عن انتشار البروتين المطفر والمشفر من هذا الجين المثبط للأورام نمط (P53) في العينات النسيجية للغدد الدرقيه بأستخدام الطريقة الكيميائية النسيجية المناعية (IHC)، بالإضافة إلى التحري عن CD56 في العينات النسيجية في الغدد الدرقيه السرطانيه ومقارنتها مع الغدد الغير السرطانيه بأستخدام الطريقة الكيميائية النسيجية المناعية (IHC).
شملت عينة الدراسة (60) خزعة نسيجية من الغدة السرطانية الحليمية المحفوظة بالفورمالين والمطمورة بشمع البرافين، وقد جمعت النماذج من أرشيفات الأنسجة المرضية في المختبرات التابعة لمستشفى مدينة الطب ومستشفى اليرموك التعليمي في بغداد، بالإضافة إلى مجموعة من مختبرات الانسجة المرضية الخاصة الموجودة في بغداد، وذلك خلال الفترة من مايو / أيار 2013 إلى مايو / ايار 2014، وقد قسمت العينة إلى مجموعتين، المجموعة الأولى شملت (30) عينة نسيجية، أخذت من مرضى مصابين بسرطان الغدة الحليمية ، في حين كانت المجموعة الثانية مكونة من (30) قطعة نسيجية من عينات الغدد الطبيعية الحليمية والتي تعتبر نماذج سيطرة للمقارنة، حيث خضعت للفحص التأكيدي الدقيق للتشخيص النهائي للعينات النسيجية من قبل استشاريي البحث باختصاص التشخيص النسيجي المرضي وباستخدام صبغتي الأيوسين والهيماتوكسلين.
سجلت النتائج نسبة انتشار البروتين المطفر والمشفر P53 بطريقة الكيميائية المناعية في 28 حالة (70%) موجبة من مجموع الحالات، و 12 حالة سالبة بنسبة (30%)، كما وجدت نسبة موجبة من CD56-IHC متساوية لكل من الأنسجة السرطانية الحليمية وأنسجة تضخم الغدة الدرقية البسيط (2 حالة موجبة بنسبة 6.7%).

INTRODUCTION

Papillary thyroid cancer is the most common types of thyroid cancer (1), representing 75% -85% of all thyroid cancer cases (2). It occurs more frequently in women and presents in the 20–55 years age group. It is also the predominant cancer type in children with thyroid cancer, and in patients with thyroid cancer, who have had previous radiation to the head and neck (3). Previous studies had indicated that history of radiation and viruses, particularly during childhood, might be the main established and associated risk factors for thyroid cancer. Thyroid cancer is the most common type of endocrine-related cancer in 2013, as indicated by National Cancer Institute, with an estimated (60,220) new cases in the United States. Recent statistics have found that thyroid cancer constituted 3.6% of all new cancer cases (4). The CD56-immune profile is a newly reported and promising good negative -marker in diagnosing, as well as differentiating the PTC, as a thyroid cancers, from the benign nature of thyroid tumors of uncertain malignant potential (5,6). Human p53 gene has a location on the chromosome 17p and consists of 11 exons and 10 introns. It is "guardian of the genome", and the first tumor suppressor gene to be identified in 1979. It eliminates and inhibits proliferation of the abnormal cells, and ultimately preventing tumor development (7)**.** Therefore, the loss of p53 functions may have severe consequences. In an estimated 50% of human cancers the p53 function is lost by mutations or deletions in p53 gene (7). The EBV LMP1 protein can repress p53-mediated DNA repair likely through the NF-κB pathway and, as such, it can inhibit p53-dependent transcriptional activity. Based on these results, LMP1 disrupts the activities of p53 in trans activation and DNA repair, there by contributing to the oncogenesis of LMP1 in human epithelial cells (8).

MATERIALS AND METHODS

This study was designed as retrospective study. Sixty (60) selected formalin fixed, paraffin embedded thyroid tissue blocks were enrolled and were distributed on the following groups: I- Thirty tissue blocks from patients with papillary thyroid cancer, II- Thirty tissue blocks with benign thyroid lesions as control thyroid tissues group. The age of these patients were ranged from 10 to 75 years. Malignant and normal thyroid tumors tissue blocks were collected from the archives of histopathology laboratories of different general hospital including Al- Yarmouk Teaching Hospital (Baghdad), Baghdad Medical City Teaching Hospital as well as many private laboratories in Baghdad.

Immunohistochemistry

CD56 and P53: The samples were rehydrated and treated with protein blocking agent to reduce non-specific binding of antibodies. The tissues were incubated with Primary AB to binds to specific Ag, Biotinylated secondary Ab to binds to the primary Abs, Streptavidin peroxidase reagent to binds to secondary Ab. The streptavidin binds to biotin on the secondary Abs: then peroxidase serves as the indicator enzyme.The last step addition of peroxidase substrate (hydrogen peroxide) and colored chromogen resulted in the formation of colored in the tissue Ag.

1- Slides' preparation: Serial thin sectioning of (4 m) thickness was done for each paraffin-embedded tissue block and sticking the sections on charged slides. Paraffin sections were deparaffinized (Dewax) in oven at 60Co overnight.

2- Deparafin and rehydration were done by serial steps in glass staining jars containing the following:

- Xylene (100%) for 15 minutes (two times).
- Ethanol (100%) for 5 minutes (two times).
- Ethanol (95%) for 5 minutes (one time).
- Ethanol (70%) for 5 minutes (one time).
- Distilled water for 5 minutes. (one time).
- Dry the slides for 5 min at 37C°.
- Wash the slides 3 times by 1xPBS 5 min.

3- Rehydration, which was done according to the following steps:

- Add Xylene: 100% for 15 minutes 2 times.
- Add Ethanol: 100 % for 5minutes 2 times.
- Add Ethanol: 95% for 5 minutes.
- Add Ethanol: 70% for 5min.
- Wash in distilled water for 5min.
- Air dry section for 5 min. at 37C°.

4- Add enough drops of hydrogen peroxide Block to cover the section incubate 10 min .Wash 2 times in buffer and Air dry section.

5- Retrieval: Unraveling antigenic epitopes by retrieval methods is important for successful immune histochemically staining and detection of protein. Slides were placed in bath containing retrieval solution. 1 ml of citric acid + 100ml D.W was added and boiled in 95C°/pH=6 for 20 min., and then washed with Buffer for 5 min.

6- Power block: enough drops of protein block were added to cover the sections for10min in 25C ° and slides were put in humidity chamber. Then slides were drained for 5 min. Slides were air –dried.

7- Primary antibody: the slides were covered with enough drops of ready to use primary (Anti-P53,CD56 antibody) after that incubated for overnight in a humidity chamber at RT. After that, all the slides were rinsed with PBPs for 5 minutes. Primary Anti-P53 antibody [PAb240] ab26 was prepared by mixing (1ul Anti-P53 antibody {PAb240}ab26 to 25 ul of diluted antibody) for each section , this protocol for detection P53 in thyroid tissue. CD56 antibody preparation [1ul of Anti-NCAM antibody (RNL-1) was added to the 25 ul of diluted antibody].

8- Apply secondary antibody: Adding enough drops of complement to the section, incubating for three hours and then placing the slides in wash buffer for five minutes , lastly drying the slides.
9- HRP conjugate reagent: Adding enough drops of HRP conjugate to the section and incubating for 15 minutes at room temperature, then washing the slides by wash buffer for 5 min.
10- Chromogen :Enough drops of DAB chromogen were applied in dark room (30µlDAB chromogen +1.5mlDAB substrates) for 10 min and buffer for 5min and slides were dried.
11- Hematoxylin counter stain :Enough drops of haematoxylin were added for 2minutes and Washed by tap water.
12- Dehydration:-dehydrated the sections by using series concentration of alcohol:(100%,95%,70%%), for one time and 100% (two times), two minutes for each solution ; finally incubation in xylene 100% for 2 min.
13- Mounting: Enough drops of mounting media were placed to cover the section and let dry over night at RT.

Statistical analysis

Data were translated into a computerized database structure. The database was examined for errors using range and logical data cleaning methods, and inconsistencies were remedied. An expert statistical advice was sought for. Statistical analyses were done using IBMSPSS version 21 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2013.

RESULTS

At high power field examination, positive- P53 immuno-staining reactions were detected by IHC test in a total 60 tissues with either papillary thyroid cases, simple nodular goiter (Table 1). It was found that the highest positive results of P53- IHC reaction were showed in papillary thyroid tissues (21 cases; 70 %) then those in normal thyroid tissues (6 cases each; 20.0%), while P53- IHC reactions were revealed in only two cases (6.7 %) with simple nodular goiter. Statistically**,** there are high significant differences among these results ($p<0.01$). Mutated P53- protein was detected by IHC at high power field examination as a brownish discoloration at nuclear and cytoplasmic localization (figure 1a-b). Mutated P53- protein was detected in 28 cases (70%) of the studied cases. Twelve cases (30.0 %) showed negative IHC reactions. It was found that the highest positive results of P53 - IHC reaction were showing moderate score (14 cases; 35.0 %) then those showing strong and weak scores (7 cases each; 17.5%). In the control groups (SNG), positive-IHC test reactions were shown in (6.7%) of the studied tissues, (table 2).

Table (1): The total percentage of immunohistochemical results of over- expressed protein of P53- tumor suppressor gene among studied groups

Immunohistochemical results of P53		Studied groups		Pearson Chi-Square (P-value)
		SNG	PTC	
Negative	N	28	9	**P = 0.00**
	%	93.3	30.0	
Positive	N	2	21	HS*
	%	6.7	70.0	
Total	N	30	30	(P<0.01)
	%	100.0	100.0	

**HS = Highly Significant difference (P<0.01)*

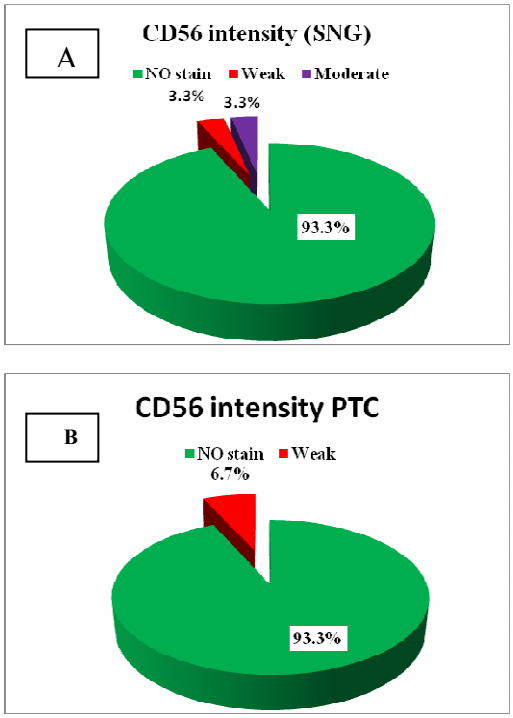

Figure (1-a, b): Pie-Histographic representation of CD56- IHC results of the studied groups according to their signal intensities

The percentages of the examined cells, which were evaluated for the intensity of P53- IHC reaction signals in the examined tissues at a high power fields, are shown in table (3). In PTC group, the moderate intensities of P53 -IHC reactions were observed in (63.3%) while weak and strong intensities in (3.3%, each. In SNG group, moderate intensity of P53- IHC reactions was observed in (6.7%) while none of weak and strong intensities were observed. Statistically, there are highly significant differences among the studied groups ($p<0.05$) (table 3). The CD56- markers were observed by immuno histochemistry as brownish discoloration in the examined thyroid tissues . percentages of CD56-IHCreactions in either thyroid papillary or simple nodular goiter (2 positive cases; 6.7%, each). Statistical analysis showed highly

significant differences among studied groups, regarding CD56 marker (p<0.01) (table 4). among studied groups: The percentage of PTC with low (1+) signal score for CD56 - IHC test was (6.7%) while PTC with high (3+) and moderate (2+) scoring were not found in the examined PTC tissues. Negative CD56- IHC reactions constituted (93.3%) of the PTC group. In the SNG, the percentage of tissues that showed low (1+) signal score for CD56- IHC test was (6.7%) while no high (3+) or moderate (2+) scoring were detected. Negative CD56- IHC reactions constituted (93.3%) of the SNG group. The percentages of the examined cells, which were evaluated for the intensity of CD 56- IHC reaction signals in the examined tissues at a high power fields, are shown in table (5). In PTC group, the weak intensity of CD 56- IHC reaction was observed in (6.7%) while moderate and strong intensities were not observed. In SNG group, weak and moderate intensities of CD 56- IHC reactions were observed in (3.3%) while none of strong intensity was observed. Statistically, there are highly significant differences among the studied groups (P<0.01) (table 5). Table (6) shows a strong negative correlation (with non - significant difference) between CD56 with P53 scoring results were observed {r = -0.58,P value=0.586(P>0.01)}.

Table (2): Results of P53- IHC signal scoring among studied groups

Studied groups	P53 Score		Frequency
SNG	Negative	N	28
		%	93.3%
	Positive	N	2
		%	6.7%
	Total	N	30
		%	100.0%
PTC	Negative	N	9
		%	30.0%
	Positive	N	21
		%	70.0%
	Total	N	30
		%	100.0%

Table (3): Distribution of IHC - intensity results of P53- marker in papillary thyroid cancer , simple nodular goiter

P53 intensity		Studied groups		Pearson Chi-Square (P-value)
		SNG	PTC	
NO stain	N	28	9	P = 0.00
	%	93.3%	30.0%	
Weak	N	0	1	HS*
	%	0%	3.3%	
Moderate	N	2	19	(P< 0.01)
	%	6.7%	63.3%	
Strong	N	0	1	
	%	0%	3.3%	
Total	N	30	30	
	%	100.0%	100.0%	

Table (4): Distribution of CD56 positive immune cells among studied groups

CD56 test		Studied groups		Pearson Chi-Square (P-value)
		SNG	PTC	
Negative	N	28	28	P = 0.00
	%	93.3%	93.3%	
Positive	N	2	2	HS*
	%	6.7%	6.7%	
Total	N	30	30	(P<0.01)
	%	100.0%	100.0%	

**HS = Highly Significant difference (P<0.01)*

Table (5): Evaluation of CD56- markers in the studied groups

Studied groups	CD56 Scores		Frequency
SNG	Negative	N	28
		%	93.3%
	Positive	N	2
		%	6.7%
	Total	N	30
		%	100.0%
PTC	Negative	N	28
		%	93.3%
	Positive	N	2
		%	6.7%
	Total	N	30
		%	100.0%

Table (6): The relation between scoring test results of CD56 with P53 scores

Spearman's rho		P53 Scores
CD56 Scores	r.	-.058
	P-value	.586

DISCUSSION

In natural situations, the protein product of P53-tumor suppressor gene has many major regulatory functions, among them the cell- cycle progression, DNA- repairing and its role in programmed cell death (9). Over expression of somatically-mutated P53 gene is still believed to play an important role in the initiation and / or progression steps of tumorigenesis ,and also is among the critical determinants of the phenotype of many forms of human cancers (10). Inactivating- p53 mutations have been found in about 50% and 15% of human cancers and malignant thyroid tumors, respectively (9,10). The present results are compatible with the results obtained by (11), who found over-expression of mutated p53 in 78% (32 out of 41) of their studied papillary thyroid cancer cases.

These results were also supported by the results of a recent study done by (9), who had noticed that P53 expression was statistically more frequently than in benign lesions, too. Furthermore, (9) found, and by using a quantitative IHC analysis of a large number

of patients, that p53 exerted an important physiological role in well-differentiated thyroid tumor containment, especially those with papillary thyroid carcinomas. The results of present study were compatible with the results of (9), where among the 83 examined cases with papillary thyroid cancers (79.0%) have positive IHC reactions for p53- mutated protein ,while less IHC reactions positivity for p53- mutated protein in benign lesions was observed, given that only (25.7%) out of 105 benign lesions presented with some positivity for this mutated protein.

Results of the present study are also consistent with results of (12), who found that 52% (9 out of 17) of their examined thyroid carcinomatous tissues have an intense p53 -cytoplasmic staining in front of only two out of 17 (11.8 %) goiters and benign lesions have exhibited a strong cytoplasmic p53-expression. The results of (13) were also consistent with our results, too, where they found that 43% of papillary thyroid cancer cases(29 out of from 68) have over-expression of p53- mutated protein. The present results should also receive a considerable attention and relative importance (along and supporting the findings of many other studies) regarding the p53 testing in the present study on the cases with PTC to be ,(and among all investigated other markers) as a promising predictive and prognostic biomarker in thyroid cancers (14). In addition, recently and confirming such speculations and in turn to the results of the present study, (15, 16) have found that p53 was significantly augmented in PTC patients when compared with benign thyroid disorders as well as they found that p53 was more frequently expressed in tumors with more aggressive features. In this study, the results showed a significant reduction in the CD56 expression in the group of papillary thyroid cancer cases, where there were only two cases of PTC showed CD56 expression . In consistency with our results, Park *et. al.* (5) had observed that 92.5% (62 of 67) of the examined tissues with papillary thyroid cancer have negative - CD56 expression , and that the tumor cells of one case in this PTC-studied group has showed a strong as well as a diffused pattern of CD56- positivity . In this respect, CD56-IHC testing has ranking 92.5% sensitivity and 86.4% specificity in differentiating papillary thyroid cancer. These results were definitely compatible with the results obtained in the present study. Previous studies by (17,18) have found that CD56 is a marker whose expression is reduced or absent in papillary thyroid cancer , these results were also consistent with the current results. It was found that CD56 has revealed a malignant profile when the expression of CD56 absent in more than 90% of the tumor cells of papillary thyroid cancer, and these results were also consistent with our results (6). Same authors (6) also found that CD56 was a highly sensitive marker of PTC, and showed a malignant profile (no expression) in the majority of cases (84.8 %), and this result was well-suited with our results. While in benign thyroid cases CD56 has revealed a positive immune staining in 7 from 11, benign cases (63.6%). Mokhtari *et. al.* (19) showed in their results that CD56 expression was seen in 70 samples of non-papillary carcinoma lesion (95.8%) versus one case of papillary thyroid cancer (1.3%). Therefore, CD56 was 98.6% sensitive and 95.8% specific in distinguishing PTC from other follicular thyroid lesions. In addition, CD56 positive expression was found in the 10 samples of normal thyroid tissue 10/10 (100%), which showed strong membranous CD56 expression in >50% of the cells (score 3). Among the solitary follicular patterned thyroid nodules; positive CD56 expression was observed in 42 out of 47 cases (89.4%). On the other hand, assessment of CD56 staining in the 29 PTC cases showed negative CD56 expression in 24 out of the 29 cases (82.8%). These cases included 11 out of 13 cases of classic PTC (84.6%) and 13 out of 16 cases of FVPC (81.3%).

In contrast, immune regulatory cells showed a higher infiltration in PTC than goiter tissues, pointing to the fact that an immune regulatory pattern of NK cells is required for thyroid carcinogenesis.

CONCLUSION

The present study revealed the following conclusions:

1- The importance of CD56 marker has been found to play a role or it may be better to be used as a negative- diagnostic biomarker for papillary thyroid cancer in differentiating it from other malignancies as well as benign lesions of the thyroid gland, individually as well as in combination with other markers for clinical evaluation of those patients.

2- The evident high mutated p53- over expression, as reflected by abnormal gene product, among papillary thyroid cancer patients indicates for a pivotal role of such genetic mutation in their carcinogenesis as well as could be useful in the clinical evaluation of patients with papillary thyroid cancer, too.

3- The high coexistence of p53-mutation with EBV in PTC could point for participation of EBV – transformation genes in the p53 activation in high proportion of PTC.

RECOMMENDATIONS

1- Study Screening of p53- gene mutation prior to clinical diagnosis of papillary thyroid cancer via serological testing of anti-p53 antibodies against mutated p53 protein, especially in individuals who are at high risk of such cancer, to afford a simple and rapid screening test.

2- study each subtype of T-helper cells and their role in the viral as well as tumor immunity against different thyroid patients.

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Immunomodulatory effect of aqueous extract of *Piper nigrum* L. in mice model

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ABSTRACT

The study was carried out to evaluate the effects of water extracts of *Piper nigrum* L. fruitsat two doses (1 and 5) mg/ kg body weight daily for 30 days on the immune response of BALB/c mice by estimating of serum concentration of IL-2, IL-4, IL-10 andINF-γ using ELISA test.

Oral administration of the extract at the two doses did not produce signs of toxicity, behavioral changes or animal death.

The highest serum concentration values of IL-2 and INF-γ were founded in mice group treated with 5 mg/kg of the aqueous extract (182.40 pg/ml and 1547.00 pg/ml ,respectively), while the lowest concentration were founded in the group treated at dose 1 mg/kg of the aqueous extract (97.60 pg/ml and 945.20 pg/ml) respectively. On the other hand the highest concentrations of IL-4 and IL-10 (78.30 pg/ml and 51.90 pg/m respectively) were founded in mice group treated with 1 mg/kg of *P.nigrum* aqueous extract while the lowest concentration of IL-4 and IL-10 (55.70 pg/ml and 37.50 pg/ml respectively) were founded in group treatedcat dose 5 mg/kg.

Keywords: aqueous, black pepper, extract, *Pipernigrum* L., cytokine

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الملخص باللغة العربية

أجريت هذه الدراسة لتحديد تأثير تركيزين (1و5 ملغ/ كغم من وزن الجسم) للمستخلص المائي لثمرة نبات الفلفل الأسود (*Piper nigrum* L) المعطى يوميا لمدة 30 يوما على الاستجابة المناعية للفئران المختبرية نوع BALB/c من خلال تحديد تراكيز IL-2، IL-4، IL-10، INF-y، في المصل باستخدام اختبار ELISA.

أظهرت النتائج أن التجريع الفموي للمستخلص المائي بالتركيزين لم يحدث أية أعراض للتسمم أو تغييرا في السلوك أو موت الفئران المختبرية خلال مدة التجربة، وقد اعطت المجموعة المعاملة بالمستخلص المائي لنبات الفلفل الأسود بتركيز 5 ملغ/كغم من وزن الجسم أعلى تراكيز IL-2، INF-y (182.40 pg/ml ، 15.47 pg/ml) على التوالي، بينما كانت أقل النتائج في المجموعة المعاملة بتركيز 1 ملغ/ كغم من وزن الجسم (97.60 pg/ml و 945.20 pg/ml) على التوالي.

من ناحية أخرى، وجدت أعلى تراكيز IL-4 و IL-10 في مجموعة الفئران المعاملة بالمستخلص المائي بتركيز 1 ملغ/كغم من وزن الجسم (78.30 pg/ml و 51.90 pg/ml) على التوالي، فيما وجدت التراكيز الأقل في المجموعة المعاملة بالمستخلص المائي بتركيز 5 ملغ/كغم من وزن الجسم (55.70 pg/ml و 37.50 pg/ml) على التوالي.

INTRODUCTION

The black pepper, *Piper nigrum* (Piperaceae) has traditionally been used as both spice and medicine. It contains small quantities of chemopreventive compounds such as β-carotene, piperine, tannic acid and capsaicin (1). It stimulates the digestive enzymes of pancreas, protects against oxidative damage, lowers lipid peroxidation, and enhances the bioavailability of a number of therapeutic drugs (2). In addition, its anti-inflammatory activities have been demonstrated in rat models of carrageenan-induced rat paw edema, cotton pellet-induced granuloma, and a croton oil-induced granuloma pouch (3).Black pepper and cardamom extracts act as potent modulators of the macrophages (4). Macrophages function as antigen-presenting cells (APCs) and participate in the activation of the adaptive arm of the immune response (5). These inflammatory cells produce large amounts of tumor necrosis factor (TNF), interleukin (IL)-12 and interleukin (IL)-23 and therefore are important drivers of antigen specific type I helper T cell responses (6). T cell activation is therefore the hallmark of the initiation of the adaptive immune response (7). Indeed it is now well known that APC maturation via CD40 ligation (8) and notch stimulation in T cells is the connecting link between innate and adaptive immunity (9).

T helper (Th) lymphocytes differentiate into Th1, Th2 and regulatory T (Treg) cells, and play an important role in the serial adaptive immune response to various infectious agents through the production of specific cytokines. Th1 cells secrete interferon gamma (IFN-γ) and protect their host against intracellular pathogens and viruses (10). Th2 cells produce interleukin 4 (IL-4), IL-5 and IL-13, and support the role of B cells in removing parasites (11). Additionally, Treg cells play a critical role in the regulation of immune cellhomeostasis by producing IL-10 and transforming growth factor-beta (TGF-β) (12, 13).

The aims of the present study were to evaluate the effect of the aqueous extract of *Piper nigrum* L. on adaptive immune response in BALB/c mice.

MATERIALS AND METHODS

Preparation of aqueous extract of *Piper nigrum*

Water extraction was prepared by boiling 100 gram of *Piper nigrum* in 1000 ml distilled water for 15 minutes. The flask was plugged and removed from the heat and allowed to cool at room temperature.

After cooling, the content of the flask was filtered and dried to prepare the required concentrations (14).

Experimental animals

Thirty BALB/c mice 4-5 weeks old weighting 15-28 grams were obtained from the animals unit, college of medicine, university of Baghdad, Iraq. The animals were divided into three groups, each group consists of 10 mice, and the animals were bred in standard mice cages and fed with a suitable quantity of water and complete diet.

The first and second groups were given 0.1 ml as an oral dose of 1 mg/kg b.w. and 5 mg/kg b.w. respectively of *Piper nigrum* aqueous extract daily for 4 weeks. While third mice group were given 0.1 normal saline daily for the same period.

The animals were monitored for apparent signs of toxicity for 30 days. On the 31st day after treatment, the animals were scarified and the serum was separated after the blood to measure the levels of IL-2, IL-4, IL-10 and INF-γ.

Estimation of IL-2, IL-4, IL-10 and INF-γ value in serum

Immunomodulatory effect of the aqueous extract *P. nigrum* were evaluated by estimation of serum IL-2, IL-4, IL-10 and INF-γ.

These interleukins were measured in serum by using ELISA according to the instructions of eBioscience company, USA.

Briefly, microtiter plate was coated with 100 µl/well of capture antibody (pre-titrate purified anti- IL-2, IL-4, IL-10 or INF-γ antibody). The plate was sealed and incubated overnight at 4°C. Cover film was removed and the plate was washed with 250 µl/well washing solution (1xPBS, 0.05 Tween-20) this procedure was repeated five times. Wells were blocked with 200 µl/well of 1x Assay Diluent and incubated at room temperature for 1 hour. Washing step was as mentioned above. 1x Assay Diluent was used to perform 2-fold serial dilutions of standards to make the standard curve. 100 µl/well of 1x Assay Diluent was added to the blank well. One hundred µl/well of standards and serum samples were loaded to appropriate wells and the wells were covered and incubated at room temperature for 2 hours. Plate was washed as mentioned above. 100 µl/well of detection antibody (pre-titrated biotin-conjugated antibody) was added to each well. The plate was sealed and incubated at room temperature for 1 hour. Cover film was removed and the plate was washed as described previously. 100 µl/well of Avidin-HRP was added to each well and the plate was sealed and incubated for 30 minutes at room temperature. Plate was washed as in step 2 and repeated for total seven washes. 100 µl/well of substrate solution, tetramethylbenzidine (TMB), to each well and incubated for 15 minutes at room temperature. The reaction was stopped by adding 50 µl of stop solution to each well. The absorbance of each well was read at 450 nm using microplate reader. The sample concentrations were determined depending on a standard curve**.**

Statistical analysis

Data are expressed as the mean values ± standard deviation (SD) of samples. The statistical significance of the differences between various groups was determined by PostHoc test (LSD alpha 0.05) and one-way analysis of variance (ANOVA) using SPSS version 18.0 software. Differences were considered statistically significant for $p<0.05$.

RESULTS

Enzyme linked immune-sorbent assay test were done to estimate immune responses after oral inoculation of *Piper nigrum* to determine the titers of IL-2, INF-γ, IL-4 and IL-10 in mice sera.
Tables (1-4) show the mean and standard deviation values of serum concentration of IL-2, INF-γ, IL-4 and IL-10 respectively in mice sera.

Table (1): The ELISA results of IL-2 concentration in serum expressed as pg/ml

Mice groups	No.	Mean	S.D	S.E
1 mg/Kg of *Piper*	10	97.60	±9.070	2.868
5 mg/Kg of *Piper*	10	182.40	±4.648	1.470
Control	10	21.30	±6.533	2.066

Table (2): The ELISA results of INF-γ concentration in serum expressed as pg/ml

Mice groups	No.	Mean	S.D	S.E
1 mg/Kg of *Piper*	10	945.20	±11.282	3.568
5 mg/Kg of *Piper*	10	1547.0	±5.538	1.751
Control	10	321.30	±7.660	2.422

Table (3): The ELISA results of IL-4 concentration in serum expressed as pg/ml

Mice groups	No.	Mean	S.D	S.E
1 mg/Kg of *Piper*	10	78.30	±11.624	3.676
5 mg/Kg of *Piper*	10	55.70	±7.379	2.334
Control	10	22.20	±6.374	2.015

Table (4): The ELISA results of serum IL-10 concentration expressed as pg/ml

Mice groups	No.	Mean	S.D	S.E
1 mg/Kg of *Piper*	10	51.90	±4.954	1.567
5 mg/Kg of *Piper*	10	37.50	±13.770	4.354
Control	10	21.20	±6.070	1.919

Tables (1-4) showed serum concentration values of IL-2, INF-γ, IL-4 and IL-10, respectively. Serum concentration values of IL-2 and INF-γ were 97.60 pg/ml and 945.20 pg/ml , respectively at dose 1 mg/kg and 182.40 pg/ml and 1547.00 pg/ml ,respectively at dose 5 mg/kg. On the other hand the concentrations of IL-4 were 78.30 pg/ml and 55.70 pg/ml at dose 1 and 5 mg/kg respectively, while the concentrations of IL-10 were 51.90 pg/ml and 37.50 pg/ml at dose 1 and 5 mg/kg respectively.
There was significant difference ($p<0.05$) between treated and control groups (figures 1-4) of serum interleukins concentration IL-2, INF-γ, IL-4 and IL-10, respectively. On other hand Figures 1 and 2 show that serum concentrations of IL-2 and INF-γ in group 2 were highest than the concentration of group 1, while the highest values of IL-4 and IL-10 were in serum concentration of group 1 rather than group 2.

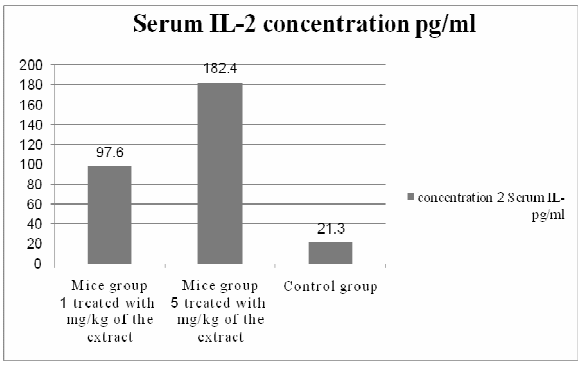

Figure (1): The ELISA results of IL-2 concentration in serum expressed as pg/ml

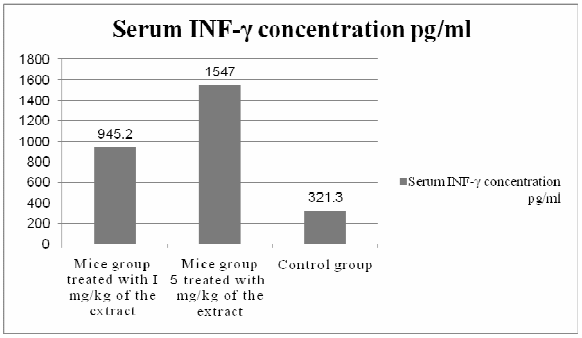

Figure (2): The ELISA results of INF-γ concentration in serum expressed as pg/ml

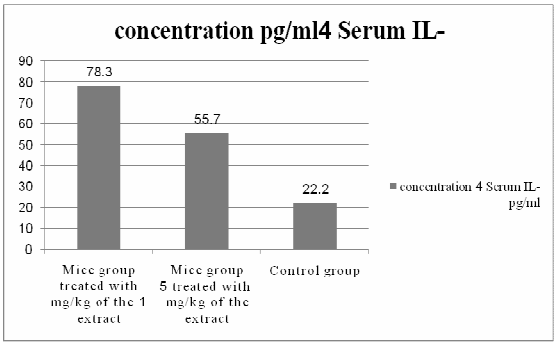

Figure (3): The ELISA results of IL-4 concentration in serum expressed as pg/ml

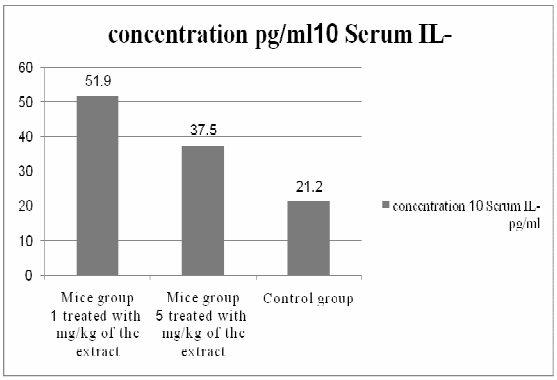

Figure (4): The ELISA results of IL-10 concentration in serum expressed as pg/ml

DISCUSSION

After the mice were orally given daily doses 1, 5mg/kg of the water extract from the dried fruits of *P. nigrum*, neither signs of toxicity nor death of mice were observed during the 30 days experimental period, similar results were also obtained by studying the same doses on mice in which the pepper did not cause toxicity at the acute toxicity study (15**).**

In this study the concentration of IL-2 and INF-γ in mice sera was significantly increased ($p>0.5$) by the increase of the concentration of aqueous extract (5 mg/kg) as shown in figures 1 and 2. IL-2 and INF-γ concentration in mice sera were estimated to reflect TH1 response. These result are in agreement with Vaidya and Rathod, (4), who found that aqueous extracts of black pepper is potentially capable of modulating the function of macrophages. Exposure of P388D1 cells to high concentrations of black pepper extract led to enhanced proliferation of these cells, whereas when exposed to low concentrations of extract of black pepper, the cells displayed greatly reduced proliferative activity (4).

On the other hand, IL-4 and IL-10 concentration in mice sera were estimated to reflect TH2 response. From the presented results (figures 3 and 4) IL-4 and IL-10 concentration in mice sera were significantly ($p>0.5$) decreased with the increase of the aqueous extract of *P. nigrim*. This finding were in line of Kim and Lee, (16), who founded that piperine from *P.nigrum* has been observed to exert a suppressive effect on OVA-induced asthma in mice (16).

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Hematological changes of duck (*Anser anser)* infected with *Cyclocolum* sp. Digenea: cyclocoelid and *Fimbriaria* sp. (Cestoda: Hymenolepidae) from Basra province, Iraq

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ABSTRACT

The present study aimed to observe hematological parameters of some naturally infected ducks that are either infected with single infection with cyclocoelid trematode or double infection with Digenea cyclocoelid trematode and Cestode *Fimbriaria* sp. Parasites. Samples were collected during April month of 2014. A total of 30 male ducks were examined,, four of them were infected with single infection with Digenea parasite cyclocoelid trematode and five of them were infected with double infection with Digenea *Cyclocolum sp* and Cestode *Fimbriaria* sp. The result of the study showed that the ducks were infected with two types of parasites four of them were single infected with Digenea cyclocoelid trematode with prevalence of 13% and mean intensity of 9.25. Five were infected with the cestode parasite *Fimbriaria* sp. with prevalence of 16% and mean intensity of 4.6 and five of them with mixed infection of two type of parasite with a prevalence of 16% and mean intensity of 4.4. The study chick the blood parameters of infected ducks that's are RBC , WBC; and count of RBC, HB, WBC, MCV and other and compared with normal one (uninfected). The hematological parameters of the infected ducks were different from bird to bird.

Keywords: Hematological, Observations, *Anser anser*, Infected birds cyclocoelid trematode and *Fimbriaria* sp. Cestoda: Hymenolepidae

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الملخص باللغة العربية

هدفت الدراسة الحالية إلى إجراء الصورة الدموية لعينة قوامها (30) من ذكور البط نوع (*Anser anser*) المصابة بشكل طبيعي بنوع واحد من الطفيليات الثنائية المنشأ وهو *Cyclocolum sp* واصابة مزدوجة مع طفيلي شريطي هو *Fimbriaria* sp. Cestode. تمت الدراسة خلال شهر نيسان /أبريل من عام 2014.

أظهرت النتائج أن من بين عينة طيور البط، وجد أربعة منها مصابة باصابة مفردة بالطفيلي الثنائي المنشأ، وخمسة منها كانت مصابة إصابة مزدوجة. وأظهرت النتائج أيضا أن ذكور البط المصابة بالطفيلي الثنائي المنشأ بلغت نسبتها 13% وبشدة إصابة متوسطة بكثافة 9.25 ، وخمسة منها مصابة بالطفيلي الشريطي *Fimbriaria* sp. ، حيث كانت نسبة الإصابة 16%، وشدة الأصابة 4.6 ، خمسة منها كانت إصابتها مختلطة بنوعين من الطفيليات، حيث كانت نسبة الإصابة 16% وشدة إصابة بلغت 4.4.

INTRODUCTION

Hematological studies are important for diagnosing the structural and functional status of the body.
In the last few years many authors worked on hematological parameters of vertebrates in relation to toxicology but not much work done on hematological aspect of vertebrates related to parasitic infections. Tapeworm infection is a major health problem in birds, because it affects the normal blood parameters and produces anemia, lymphocytosis etc. (1). Therefore the study of hematological parameters is very important.
Only little information is available on the hematological parameters of birds. Various workers studied the hematological parameters or changes of some birds due to parasitic infection. For example, on pigeon (2) , on *Great tit* (3), and on local ducks (4).
Only few investigations of their parasitic fauna have been under taken in Iraq. On the other hand many investigations concerning the pathogenicity of the bird's parasites have been carried out in other parts of the world by (5-7). Some of the literatures are available on aquatic bird helminthes in Iraq, of the last ninth years, and made them available for other investigators who are interested in this field. The first one was introduced by (8,9) which consists of the helminthes fauna recorded in the period from 1977 to 1991 in Basra.
Avian helminthes have been studied by workers in many parts of the world, and published works are too extensive to review here. On the other hand, helminthes fauna of birds in center and north of Iraq is still incompletely knows, and such investigations are limited in Iraq.
The aim of this study was to determine the hematological parameters of ducks due to parasitic infection.

MATERIALS AND METHODS

The (30) male ducks (*Anser anser)* were collected from local markets of Basra city. Blood sample were collected aseptically with sterile syringe and needle either from the heart or from the wing vein of ducks . After blood collection, duck were killed for dissection and examined for helminthes infection. The methods and techniques used for collection, relaxation, fixation, staining and mounting of helminthes are basically those described (10). Ducks were examined only for internal and external parasite parasites, the abdominal cavity of each duck was opened and the intestine was separated from the other visceral organs and placed in a Petri-dish containing physiological saline and examined for parasites. The parasites were washed in a 0.6% saline solution and fixed in 70% ethanol. They were stained with alum carmine, dehydrated and then cleaned in xylene and mounted in Canada balsam, the specimens were deposited in the Department of Marine vertebrate , Marine Science Center, University of Basrah, Iraq. Parasites identification was done with the aid of (11) ,(12). Immediately after collection the blood was transferred into sterile glass bottles containing Ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Estimation of Hb, PCV, MCV and determination of WBC, and RBC using the Hematology Analyzer (Mind ray BC-3000). The ducks were identified according to (13).
The Prevalence and intensity of infection were achieved by the adoption of the method described by (14).

Prevalence (%) = Number of infected / Number of examined ×100

Mean intensity = Number of parasite/ Number of infected ducks

RESULTS

The results of the study showed that nine of the (30) male duck were infected with two types of parasite. Four ducks were infected with digenea cyclocoelid trematode, with prevalence of (13%) and mean intensity of (9.25), five of them were infected with the Cestode parasite *Fimbriaria* sp. with prevalence of (16%) and mean intensity of (4.6), and five of them were of mixed infections with the two types of parasite with a prevalence of (16%) and mean intensity of (4.4) (table 1).

Table (1): The prevalence and intensity of infections

Parasite	Number of infected ducks /total	Number of parasites	Prevalence	Mean intensity
Cyclocolum elongates	4/30	37	13%	9.25
Fimbriaria spp.	5/30	23	16%	4.6
Mixed infections	5/30	22	16%	4.4

Cyclocolum sp.

Description was based on two specimens of parasite.
Site of infection: Intestine (Rectum) (figure 1-A) .
The parasite of cyclocoelid trematode (figure 1- A) ,has an oval body, which measures 25-32(28.5) mm in length and 8.47-12.33 (10.4) mm in width. Oral sucker are terminal and measured 1.1-1.7 (1.4) mm, pre-pharynx is absent or short depending on the state of contraction the pharynx is barrel shaped and measures 0.89-1.9 (1.395) mm. The esophagus is small and bifurcates into two simple wide caeca, which terminate blindly a little in front of the posterior extremity. The Ovary situated in the last trimester of the body. The vitellaria are composed of small irregular follicles extending along the caeca from the level of the intestinal bifurcation to the end of the body.

***Fimbriaria* sp.**

Description was based on two specimens of the parasite.
Site of infection: Intestine (Rectum) (figure 1-B).
The Cestodes measures 25.9-36(30.95) mm in length and the maximum width is 5.2-5.9(5.55) mm. Pseudo scolex poorly developed and the neck are absent. The Proglottides measures 0.95- 1.6 (1.27) mm in length and 11.7 – 13.5 (12.6) mm in width.

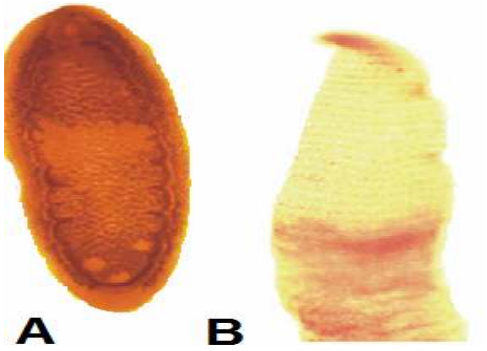

Figure (1): A*:* cyclocoelid trematode and B: *Fimbriaria* sp.

Hematological values

The present study indicated a very interesting feature that accounts for the restlessness of infected birds and the different types to helminthes produce different types of changes in hematological parameters in birds.
Data of the hematological values of the ducks (*Anser anser)* , of both uninfected and infected with cyclocoelid trematode and Cestode Fimbriaria sp. were presented in table (1). The blood parameters of HGB, PCV, WBC, MCV, MCH, neutrophil and lymphocytes' values of the infected duck were significantly different at ($p<0.05$) from the non-infected counterparts. Table (2) showed the different hematological changes in male ducks infected with the two types of parasites. The WBC counts in infected group was higher than that of controls, 3.20 , 86.52, 87.66, 88.7 in single infection and 71.41, 80.72, 81.55, 80.67, 43.65and 75.65 in double infection were lower than that of controls 69.44 ($p<0.05$). whereas RBC values of infected group in single infection are 0.14,20.7, 1.99, 0.0,and the double infection are 1.00, 1.26, 1.24, 1.24,0.0, and the control are 1.45,1.40, and hemoglobin values are 217, 218,217 in single infection and 118,129, 129,128 and 79 in double infections, whereas 152, 145 in the control . The results of this study indicated that cestode parasite affect the blood parameter of duck (*Anser anser*) a significant reduction in the level of fact that PCV, RBC count Haemoglobin concentration show significant changes when compare with the normal, also the Increase in WBC count, MCV while decrease in RBC count from normal.

DISCUSSION

The Cyclocoelinae is a subfamily of parasitic flatworms of the order *Echinostomida*. Their definite hosts are waterfowl and other (mostly aquatic) birds such as waders, where the worms are mainly found in the airways. Three subfamilies are recognized of Cyclocoelidae according to (15).
The genus *Fimbriaria,* Fuhrmann (1932), was based on personal observations and on the detailed work of (16) accepted this species as valid, because his specimens were mature and ovigerous, without any sign of maceration, and because the genera *Diorchis and Fimbriaria* are not closely related. With the generic diagnosis of (17), the differences in some measurements of the body that probably influenced by the state of the development of the specimens.
Intestinal parasites are widely spread, and affecting various types of poultry such as chickens and turkeys (18). It is one of the important problems of Helminthiasis in chickens. The tapeworms infection in birds is one of the causes of the decrease in production of chicken and the chicken does not cause weight loss, but also cause many problems and affect the preparation of chicken diseases also caused conditions such as intestinal blood loss and production loss and neurological signs loss (19).
The present study indicated a very interesting feature that accounts for the restlessness of infected birds and the different types to helminthes produce different types of changes in haematological parameters in birds. These results are in agreement with those of (20), which were quite comparable to those observed in mammals including man.
The similar results of the decrease in RBC counts and increase in WBC counts in infected host as compared with normal host were also reported by (21) in albino rats infected with *Plasmodium* parasites. Similar findings also were recorded in blood parameters from *Capra hircus* infected with nematodes (22). The Increase in WBC count, MCV and the decrease in RBC were counted from normal. These results were in agreement with (23, 24), which showed in infected dove *Columba livia* and chickens . In the infected birds, the clinical disease is associated with fever, depression, anorexia, loss of body weight, dyspnea, hepatomegaly, splenomegaly, ocular haemorrhage, haemolytic anaemia, haemoglobinuria, leukocytosis, lymphocytosis, hypoalbuminaemia, nephritis, fatty liver, oedema of the lungs, hydropericardium and occlusion of capillaries of the brain (25).

Table (2): The values of blood parameters of infection with *Cyclocolum elongates* and *Fimbriaria* spp. parasites

Parameters	Single infection*	Single infection*	Single infection**	Single infection**	Double infection	Double infection	Double infection	Double infection	Double infection	Control	Control
WBC $10^3/\mu L$	3.20	86.52	87.66	88.7	71.41	80.72	81.55	80.67	43.65	75.65	69.44
NEU $10^3/\mu L$	--	--	--	--	16.71	--	20.31	21.33	0.00	17.25	0.0
LYM $10^3/\mu L$	--	--	--	--	48.27	--	58.31	59.9	43.65	55.60	0.0
MON $10^3/\mu L$	0.0	0.0	0.0	0.0	5.28	0.0	2.36	2.42	0.0	1.74	0.0
EO $10^3/\mu L$	0.0	0.0	0.0	0.0	1.07	0.0	0.57	0.59	0.0	1.06	0.0
BAS $10^3/\mu L$	0.0	0.0	0.0	0.0	0.07	0.0	0.00	0.08	0.0	0.0	0.0
NEU%	--	--	--	--	23.4	0.0	24.9	23.9	0.0	22.8	0.0
LYM%	--	--	--	--	67.6	0.0	71.7	72.8	100.00	73.5	0.0
MON%	0.0	0.0	0.0	0.0	7.4	0.0	2.9	2.8	0.0	2.3	0.0
EO%	0.0	0.0	0.0	0.0	1.5	0.0	0.7	0.9	0.0	1.4	0.0
BAS%	0.0	0.0	0.0	0.0	0.1	--	--	0.12	--	--	--
RBC 106/µL	0.14	20.7	1.99	0.0	1.00	1.26	1.24	1.24	0.0	1.45	1.40
HGB g/L		217	218	217	118	129	129	128	79	152	145
HCT %	0.0	31.4	30.3	32.7	15.3	19.0	18.8	18.6	0.0	21.3	20.2
MCV fL	0.0	151.5	152.1	150.8	153.4	150.7	151.0	150	0.0	146.6	144.4
MCH pg	0.0	104.6	109.8	108.9	118.3	102.0	103	102.6	0.0	104.5	103.6
MCHC g/L	0.0	691	722	690	771	677	687	685	0.0	713	717
RDWsdfL	0.0	59.8	55.3	56.9	62.8	74.6	73.9	73.7	0.0	64.9	67.0
RDWcv %	0.0	8.6	7.9	8.2	9.0	11.4	11.0	11.3	0.0	10.2	10.0
PLT 103/µl	5	27	36	32	30	21	24	22	3	21	89
PCT %	0.0	0.02	0.02	0.02	0.02	0.01	0.02	0.03	0.0	0.01	0.05
MPV fL	0.0	5.8	6.9	5.9	7.4	5.9	8.0	7.9	0.0	5.3	5.9
PDWsd fL	0.0	18	19.5	19.1	18.5	19.0	21.4	21.5	0.0	19.6	16.6
PDWcv %	0.0	39	39.6	39.3	39.3	39.6	39.6	39.6	0.0	39.6	39.6

The implications of the reduction of the parameter lead to anaemia, which may be functionally defined as a decreased oxygen- carrying capacity of the blood. a very interesting feature that accounts for infected birds show restlessness and different types to helminthes produce different types of changes in haematological parameters in birds, which were quite comparable to those in mammals including man (23). Hemoglobin level indicated the oxygen-carrying capacity of the blood, and it was mentioned that in the case of nutritional deficiency or exposure to parasites, hemoglobin levels might be reduced (24).

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Prevalence of some intestinal helminthes eggs in some rivers of south Iraq

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ABSTRACT

A total of 30 water samples were collected from different site of the Shatt- Al-Arab, Ashar and Alkhora rivers to assess its contamination level with intestinal helminthes, all of the studied areas were found to be positive for helminthes ova. All samples grown in this area were found positive for *Ascaris lumbricoides*, *Enterobius sp, Trichuris trichiura* and hookworms eggs. *A. lumbricoides* and *Hymenolepis nana* were the most predominant species observed in all samples. The numbers of eggs were more than 2000 eggs/liter. The results indicated that the waste water effluent of the Shatt Arab, Ashar and Alkhora Rivers cannot be considered as a safe source of water for drinking and agriculture uses.

Keywords: intestinal helminthes egg, Shatt Al-Arab River, South of Iraq

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الملخص باللغة العربية

جمعت 30 عينة من المياه من مناطق مختلفة من شط العرب ونهر العشار ونهر الخورة لتقييم مستوى التلوث ببيوض الديدان الطفيلية المعوية. أظهرت نتائج الدراسة فيما يتعلق بتواجد وإعداد بيوض الديدان *Ascaris lumbricoides, Trichuris trichiura* . والديـــدان الخطافيـــة *A. lumbricoides and Hymenolepis*بأنها الأكثر في جميع العينات، حيث وصل أعداد بيوضهما إلى أكثر من 2000 بيضه باللتر. وبهذا، فإن النتائج أعطت مؤشرا على أن النفايات السائلة في مياه نهر شط العرب تجعله مصدرا غير آمن للمياه لاستخدامات الشرب والزراعة

INTRODUCTION

Passing of clean water acts in developing countries in 1970 created a big distance of quality standard that wastewater must meet through pretreatment process. The concentration of people in large towns has favored the production of the sludge on the site where no agricultural use can really be found. Sludge contains pollutants as well as height concentration of pathogenic viruses, bacteria and parasites (1).

Wastewater or natural water supplies into which wastewater has been discharged, are likely to contain pathogenic organisms similar to those in the original human excreta, disease prevention programmers have centered upon four groups of pathogens that potentially present in such wastes: bacteria, viruses, protozoa and helminthes. There have been extensive reviews published on the range of these pathogenic organisms normally found in human excreta and in wastewater (4).

The following short information is extracted from those reviews and is presented to establish a basic understanding of the pathogens and their abundance.

"The degree of contamination of the environments with the products of the intestinal parasite is enormous and depend largely on the inadequate excretes disposal; there are risks that the use of water polluted with wastewater , irrigation or drinking may facilitate the transmission of excreta-related diseases" (5).

The World Health Organization (WHO) established that the major risks are the transmission of intestinal nematodes infection both to those working in the wastewater irrigated field and those consuming vegetable grown in these field. These infections are due to *Ascaries lumbricoides*, *Trichuries trichiur* and, *Ancylostoma duodenale* and *Necator americanus* (1).

The eggs removal efficiency and quality of treated wastewater should meet the current WHO guideline, which state that only treated wastewater containing no more than 1 human intestinal nematode egg per liter should be used for irrigation (2).

The aim of this study was to determine the degree of contamination with the eggs.

MATERIALS AND METHODS

Three monthly samples were collected from February to March 2007 from three stations. Ten of samples were collected from Al-Aashar River, Al-Rebat River that drain in Shatt –Al Arab River, and Shatt Arab, south of Iraq.

The parasitological analysis of concentrated water samples was conducted at the laboratory, following the modified Bailenger method, as recommended by WHO (2).

The Laboratory of Parasitology had applied several adaptations to the modified Bailenger analytical methods as to improve the efficiency of the helminthes eggs recovery process. The main objective of those adaptations was to increase the absolute eggs recovery rate, up to 80-90%, as compared to the normal eggs recovery rate of 30-74% that can be achieved by the modified Bailenger method. One liter of each sample were collected, samples were allowed to sedimentation for 1-2 hrs., and thwn siphon was used to remove 90% of the supernatant. The sample sediments were transferred for macro centrifugation at1000g for 15 min. The supernatants were discarded, and the sediments were collected into one tube and re-centrifuged at 1000g for 15 min. The pellets were suspended in acetoacetic buffer pH 4.5, then two volumes of ethyle acetat ether were added and shacked by hand, and the sample were centrifuged at 1000g for 15min. The samples were separated into three distinct phases, recovered the volume of the pellet containing the eggs, and then pour off the rest of the supernatant in one smooth action.

Reuspend the pellet in zinc sulfate solution (ZNSO4)1:5 final products were removed with pipette and transferd to the McMaster chamber slide for final test .The slides were left to stand on flat surface for 5 mints before testing to allow all eggs to float to the surface.

The numbers of eggs per litter were calculated from the following equation:

$$N=AX/PV$$

Where:

N=number of egg per liter of sample

A=number of egg counted in Macmaster slide chamber mean numbers of counts from two or three slides

X=volume of final product

P=volume of Macmaster slide (0.3ml)

V=original sample volume (liter)

The identification of helminthes egg based on (1) as follows:

Wastewater frequently contains the eggs of parasites of animals, e.g. rats, domestic animals such as pigs and dogs, and birds. Although it is not necessary to identify these positively, it is important to recognize that they are not of human origin. To determine whether eggs are of human or animal origin, e.g. the eggs of *Ascaris suum* (from pigs) and *A. lumbricoides* (from humans) are morphologically indistinguishable. Similarly, the eggs of *Trichuris* spp. are all of similar colour and shape. Eggs of the human whipworm, *T. trichiura,* can only be differentiated from those of animal species by careful measurement. Human parasitic helminthes egg can be accurately identified using an eyepiece micrometer in a calibrated microscope.

RESULTS AND DISCUSSION

Microscopic examination of the water samples was used to identify the eggs of two groups of helminthes parasite nematodes and cestodes.
The quantification of helminthes egg in water samples from three stations revealed different groups of helminthes nematode *Ascaris lumbricoids*, *Trichuris* sp., *and* hookworms *Ancylostoma sp., Cestode* unclouded *Hymenolepis nana* and unidentified different species of protozoa and unidentified larval stages of parasite.
There was a difference between the total counts of eggs/L in the stations, and the counts of the eggs were equal to more than 2000 egg/L.
Table (1) shows that the concentration of *Ascaris lumbricoids* eggs detected in influent water samples varied from 2000to 8000 eggs/L, *Trichuris* sp eggs detected in influent water samples varied from 1000to 4000 eggs/L, *Ankylostoma sp* eggs detected from 2000to 4000 eggs/L, and the *Enterobius* sp 0to 5000 eggs/L and *Hymenolepis nana* 1000to 4000 eggs/L .
The concentration of parasitic helminth eggs, as indicator of the sanitary risk associated to reuse of reclaimed water for unrestricted irrigation, is one of the water quality parameters included in the third edition of the Guidelines published by the World Health Organization (WHO) in 2006 and untitled "Guidelines for the safe use of wastewater, excreta and greywater". WHO recommends a water quality limit equal or lower than 1 parasitic helminth egg per liter for the safe reuse of reclaimed water for unrestricted irrigation, and a more restrictive limit equal or below 0.1 parasitic helminth egg per liter.

Table (1): The prevalence of helminthes eggs in the water of the three stations

The parasite	Al-Ashar river	% infection	Al-Rebat river	% infection	Shatt –Al Arab river	% infection
Ascaris lumbricoids	+++++*	27%	+++++	23%	++	50%
Trichuris sp	++++	21%	++++	19%	+	25%
Ankylostoma sp	++	10%	++++	19%	++	50%
Enterobius sp	+++++	21%	++++	19%		0%
Hymenolepis nana	++	10%	++++	19%	+	50%

** (+) equal to 1000 eggs/L*

The results showed that there are varieties of parasite eggs in these stations, as well as high values of mean number of egg/L. this was probably due to the migration of sewage or sludge into these rivers (figure 1).

Figure (1): Optical microscopy views of 1: *Enterobius* sp. 2: *Hymenolepis nana* 3: Ascaris lumbricoids in the sample seed used in the demonstration project

The wastewater parasitological highlight frequency of positive sample was very high. These data indicate a strong pollution by the raw wastewater .Raw wastewater samples of this station contained different helminthes eggs this result in agreement with other studies which have shown the frequent presence and high numbers of *Ascaries* eggs in raw wastewater worldwide (3, 4).
The results also showed the high numbers of *Ascaris lumbricoids* eggs, the resistance of these egg to external condition allows the egg to remain viable for longer period than the other helminthes egg (5-8). The concentration of parasitic helminth eggs, as indicator of the sanitary risk associated to reuse of reclaimed water for unrestricted irrigation, is one of the water quality parameters included in the third edition of the Guidelines published by the World Health Organization (WHO) in 2006 and untitled "Guidelines for the safe use of wastewater, excreta and greywater". WHO recommends a water quality limit equal or lower than 1 parasitic helminth egg per liter for the safe reuse of reclaimed water for unrestricted irrigation, and a more restrictive limit equal or below 0.1 parasitic helminth egg per litter when children younger than 15 years of age can be exposed to contact with reclaimed water.
The helminthes egg indicator refers to a group of intestinal nematodes and is not specific to one, thus it covers a range of potential parasitic diseases. However, parasitic helminthes are not endemic in all areas, therefore, constant monitoring in some areas may not be needed and some of the most important parasites are *Ascaris*, *Trichuris* and a number of hookworms (2,6).
The helminthes egg water quality indicator appears to have gained wide international acceptance (2,6) and now it forms a basis for guidelines on wastewater treatment efficiency. National acceptance has not been fully established as reported in the recent WHO Guidelines (2), but only now being evaluated and considered for adoption as national health standards in developing countries. Although gaining recognition and acceptance, there is little experience in applying helminthes egg

organisms as a water quality indicator or as a measurement of the efficiency of treatment.

"The helminthes *egg guideline* value is intended as a design goal for wastewater treatment systems, and not as a standard requiring routine testing of effluent quality. The most sensitive techniques currently available for the detection of helminthes eggs in wastewater are able to detect a minimum of the order of one egg per liter. However, these are not practicable for field monitoring purposes (1).

The results shown in the present study indicated that the wastewater effluent of the Shatt Al-Arab River cannot be considered safe sources of water for drinking and agriculture uses.

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Valuation of activity of purified flavonoid compounds obtained from *Curcuma longa* planta on glucose and lipid profile of Alloxan induced diabetes in female rats

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ABSTRACT

Flavonoid compounds extracted from Turmeric planta (*Curcuma longa*) that was identified by employing high performance liquid chromatography technique were injected intraperitoneally to normal and to diabetics alloxan induced female Rats. The effect on serum; glucose level, lipid profile, GOT and GPT were studied. The results showed that, there were significant reductions in the serum level of; glucose, total cholesterol and triglyceride in both normal and diabetic rats after 10 days of treatment. No significant reduction was observed for serum level of high density lipoprotein. A significant change was observed in the serum level of; low density lipoprotein, GOT and GPT.

Keywords: Diabetes mellitus, flavonoids extract, HPLC, Alloxan**,** *Curcuma longa*

الملخص باللغة العربية

تم حقن مركبات الفلافونويد المستخلصة من الكركم التي تم تحديدها باستخدام التحليل الكروماتوغرافي السائل عالي الكثافة، في السائل البريتوني لعينة من إناث الجرذان المحفزة للإصابة بمرض السكري بالألوكسان، لدراسة تأثيرها على المصل، ومستوى السكر والدهون. وقد أظهرت النتائج وجود انخفاض معنوي على تلك المقاييس بالإضافة إلى انخفاض معنوي في مستوى الجليسيرايدات الثلاثية والكولسترول في كل من المجموعة الطبيعية والمجموعة المصابة بالسكر من عينة الدراسة، وذلك بعد 10 أيام من المعالجة. فيما لم تظهر النتائج أي انخفاض معنوي في مستوى الليبوبروتينات عالية الكثافة. بينما وجد تغير معنوي في مستوى الليبوبروتينات منخفضة الكثافة.

INTRODUCTION

Curing with medicinal plants is as old as mankind itself. The linking between man and his search for drugs in nature dates from the far past, of which there is sufficient evidence from numerous sources: written documents, preserved monuments, and even original plant medicines (1). Turmeric (*Curcuma longa.*) is one of the most widely used herbs. Its nutraceutical property has been of interest in both; food processing and pharmaceutical industries. Many patients that do not response to conventional therapies often seek help in complementary alternative medicine treatment (2). The genus *Curcuma* (family: *Zingiberaceae)* includes more than 80 species of rhizomatous herbs. The herb is widely distributed throughout the tropics of Asia, Africa and Australia. The most common species is *C. longa* (or turmeric), which is used as a natural food colorants and as one of the ingredient in various medicinal formulations (3). The medicinal properties of *C. longa* have been attributed to the presence of curcumin, an essential oils and phenolics (3). Curcuma plants have a camphoraceous aroma and contain many functional compounds such as phenolics, flavonoids and different antioxidant substances (4). *C. longa* (turmeric) is a tropical plant, perennial herb, belongs to member of the ginger family. It can grow up to 100 cm high, and has oblong, tufted leaves. The yellow spice is made from the rhizomes (5). The active component in turmeric is curcumin that may constitute 2 to 8% of the spice. Curcumin is a water non-soluble polyphenol that can be obtained from *C. longa* by ethanol extraction (6). A long-term study on healthy subject revealed no changes in fasting plasma glucose or lipid levels (7). Given curcumin to diabetic rats showed a significant reduction of renal dysfunction and oxidative stress (8). This may indicate that curcumin has a protective role against diabetic nephropathy (9).
Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in; insulin secretion, and action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (10). Association diagnosis and classification of diabetes mellitus (10). It is classified on the bases of pathogenic process that leads to hyperglycemia. The two broad categories of DM are designated as type one DMI and type two DMII. Other forms of DM are also categorized separately from these two types, which include, gestational diabetes, congenital diabetes (due to genetic defects of insulin secretion), cystic fibrosis-related diabetes, steroid diabetes (induced by high doses of glucocorticoids), as well as several forms of monogenic diabetes (11). This study was carried out to find the effect of flavonoid components of turmeric on alloxan monohydrate induced diabetes of rats.

MATERIALS AND METHODS

Preparation of flavonoid extract

Turmeric Plant bought from the local markets (Samarra city, Iraq), had been classified by the department of biology, college of education, The University of Samarra. Then, the plant was well cleaned up from impurities and grinded to fine powder using a blender. After that, the powder was extracted with ethanol (70%) using Soxhlet device. Then ethanol extracts were collected and evaporated by rotary evaporator apparatus at room temperature. The gummy residue was dissolved using 50 ml of hot methanol (45-50^{o} C) followed by vigorous mixing. This step resulted in production of brown precipitated products. Then, the precipitated products were filtered and the solid material was collected. This product termed flavonoid compounds, were used for further fractionation and identification of flavonoids (12).

High performance liquid chromatographic purification, identification and quantification of flavonoid compounds in planta extract

The solid material was dissolved in 1 ml MQ water containing 0.05% acetic acid. Samples of flavonoid compounds in 0.05% acetic acid, were separated on fast liquid chromatographic, using propyl cyanide column (50×2.0 mm) on Shimadzu high-performance liquid chromatography system (HPLC, 6AVP, Koyoto, Japan). Separation was carried out according to the manufacturer's operating specifications using solvent A (0.05% acetic acid) and B (methanol in 0.05% acetic acid) employing a stepwise gradient from 0-95 % (v/v) methanol containing 0.05% (v/v) acetic acid. Samples (20 µl) were injected onto the column at a flow rate of 1 ml/min and washed with the same solvent at 1 ml/min for a further 10 min. linear gradient (B=0% to 100%) from t=0 to 10 min, were also employed on standard containing 25 µg\ml flavonoid compounds. The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the sample (13).

Animals and induction of diabetes

Healthy female albino rats were chosen. They were housed in well ventilated cages under normal environmental conditions (temperature and humidity). Animals were fed on commercial balanced diet and tap water. The rats were divided into 3 groups of 5 rats; group I, (control), group ll, diabetic rats (induced by alloxan) and group lll, diabetic rats given flavonoid extract (20 mg/Kg body weight) daily using an intraperitoneally tube for 10 days. Rats of group (ll + lll) injected intraperitoneally with alloxan monohydrate

dissolved in normal saline at a dose of 50 mg/Kg body weight (14). Rats with moderate diabetes having hyperglycemia (blood glucose level>200 mg/dl) have been used for the experiment. At the end of the experiment (10 days), the animals in all three group were fasted for 12 hrs. and blood samples were collected.

Biochemical analysis

At the end of 10 days, the rats fasted overnight. Then, blood was collected by heart puncture. Clear serum samples were separated by centrifugation at 3000 rpm for 20 min. Fasting blood glucose level was evaluated (control, diabetic and treated groups). Lipid profiles, serum glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) were evaluated at day 10 for all groups. All the above biochemical parameters were carried out according to the manufacturer's instruction, while low density lipoprotein cholesterol (LDL-C) concentration is calculated by using the Friedwald formula which was based on the assumption that very low density lipoprotein cholesterol (VLDL-C) is present in serum at a concentration equal to one fifth of the TG concentration (15).

Statistical analyses

Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS) (2012).

RESULTS AND DISCUSSION

Turmeric Plant grinded to fine powder was extracted with ethanol (70%) using Soxhlet device. The gummy residue was dissolved in hot methanol (45-50 ^{o}C). This product termed flavonoid compounds, were used for fractionation and identification of flavonoids. Fractionation and identification was carried employing HPLC. Figure (1) presents the HPLC separation of the standard flavonoids compound. The sequences of appearance of the compounds were; curcumin, quercetin, galangin, demethoxy curcumin, bisemethoxy curcumin and germacrone respectively. Fractionation and identification of flavonoids extracted from Curcuma planta were compared with the consequences of appearance of flavonoids of the standard (figure 1). Similar pattern for the appearance of flavonoid was obtained (figure 1).

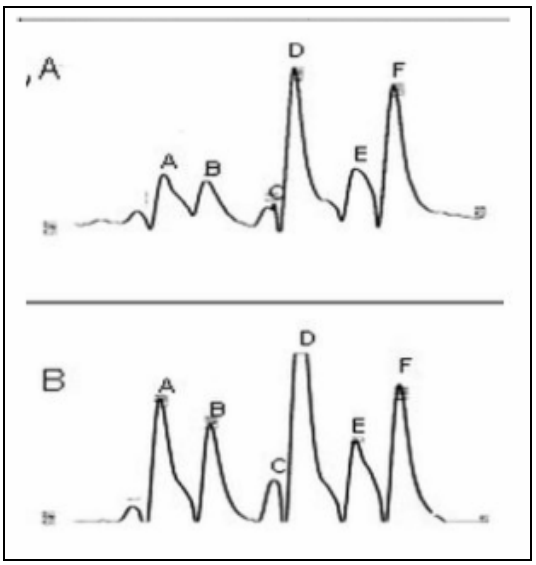

Figure (1): HPLC chromatogram of: A. Curcuma planta flavonoids; (a), Curcumin. (b), Quercetin. (c), Galangin. (d), Demethoxycurcumin (e), Bisemethoxycurcumin. (f), Germacrone. B. standard Curcuma planta flavonoids; (a), Curcumin. (b), Quercetin. (c), Galangin. (d), Demethoxycurcumin (e), Bisemethoxycurcumin. (f), Germacrone

The retention time of flavonoids extracted from Curcuma planta and standard were presented in table (1). The retention times of flavonoids extract are in agreement with those observed for standard (reference) sample. In addition, this result coincided with the findings obtained by (16). Flavonoids obtained by HPLC from Curcuma plant are presented in table (2). The concentration of germacrone,desmethoxycurcumin,bisemethoxycurcumin, quercetin, galangin and curcumin were 236.667 µg/ml, 164.089 µg/ml, 152.499 µg/ml, 120.491 µg/ml, 112.847 µg/ml and 96.170, µg/ml respectively. The effect of flavonoid compounds purified from Curcuma planta on serum level of glucose in alloxan-induce diabetic rats are presented in table (3). The serum level of blood glucose for control (group I), Alloxan treated (group II) and Alloxan with flavonoid extracts (group III) were 62.35 mg/dl, 265.33 mg/dl and 83.57 mg/dl respectively. Treatment with flavonoids extract resulted in dropping of blood glucose level to 83.57 mg/dl after 10 days of the treatment in comparison with diabetic rats. The treatment of the diabetic rat for one weeks resulted in lowering the level of blood glucose to the normal value in comparison with normal control. Alloxan causes β-cell necrosis and induces experimental diabetes in various animal models (17). The destruction of β-cells during diabetes ultimately causes degradation or loss of structural proteins due to the unavailability of carbohydrates for energy production (18). Insulin deficiency resulted from β-cells destruction ultimately results in increased production of glucose

by the liver, and decreased utilization of glucose in peripheral tissues (19). The elevated blood glucose level observed in the diabetic rats was significantly reduced in treated group with flavonoids extract. The decline may be due that, flavonoids extract working to increase and improve insulin secretion from β- cells or may work on the renewal of β- cells (20). It is well known that in cases of uncontrolled diabetes mellitus, there is an increase in total cholesterol, Triglycerides, VLDL-C and LDL-C and a decrease in HDL-C. These changes in the levels are contributes to coronary artery disease. Although abnormalities in cellular cholesterol level in diabetes occur, the precise mechanism underlying these enzymatic changes have not been elucidated. Such a significant increase in TG may be due to the lack of insulin under diabetic condition, while insulin activates the enzyme lipoprotein lipase to hydrolyze TG under normal condition.

Table (1): Retention time of flavonoids extracted from Curcuma plant and standard

Number of fractions	Flavonoid compounds	Retention time (min)	
		Standard	Sample
1	Curcumin	1.58	1.65
2	Quercetin	2.48	2.44
3	Galangin	3.64	3.61
4	Demethoxy curcumin	4.07	4.06
5	Bismethoxy curcumin	5.12	5.20
6	Germacrone	5.88	5.87

Table (2): Concentrations of flavonoids extracted from Curcuma plant by HPLC chromatogram

Flavonoids	Concentration (µg/ml)
Curcumin	96.170
Quercetin	120.491
Galangin	112.847
Demethoxy curcumin	164.089
Bismethoxy curcumin	152.499
Germacrone	236.667

Table (3): The effects of flavonoids compounds in extract of curcuma planta on serum biochemical analysis in alloxan-induced diabetic rats

Parameters	Group I* control	Group II* (Alloxan treated)	Group III *
Glucose (mg/dl)	62.3 ± 8.5	265 ± 1.99	83.6 ± 10.6
Cholesterol (mg/dl)	155 ± 52.2	165.1 ± 2.7	122.3 ± 8.5
Triglycerides (mg/dl)	51 ± 56.8	56.8 ± 48.9	48.9 ± 3.8
HDL-C (mg/dl)	92.7 ± 3.2	49,1 ± 12.96	82.7 ± 15.6
VLDL-C (mg/dl)	10.8 ± 3.2	11.4 ± 7.1	9.8 ± 7.6
LDL-C (mg/dl)	51.5 ±4.13	104 ± 3.7	29.8 ± 2.9
GOT (I.U/ml)	38.7 ± 4.5	52.3 ± 4.5	19.7 ± 6.4
GPT (I.U/ml)	29.7 ± 2.3	41.3 ± 5.5	51.3 ± 1.5.3

**Values are expressed as mean ±SD*

It was found that, the total cholesterol, triglycerides and LDL-C level were elevated in diabetic group II and it was decreased after 10 days of treatment with the extract, while the level of HDL-C was significantly elevated. It was reported that, the levels of serum GOT and GPT are elevated as a consequence of metabolic changes in the liver, as in cases of administration of; toxin, cirrhosis of the liver, hepatitis, liver cancer and inflammatory conditions including diabetes. It was reported that the levels of serum GOT and GPT in alloxan induced diabetic rats were elevated (21). These findings were coinciding with our results. This might be due to adverse effect of alloxan that lead to leaks of the enzymes from the tissues followed by migration of the enzymes to the circulation. The transaminase enzymes have been used as markers to assess the extent of liver damage in streptozotocin in induced diabetic mice (21). These results were in agreement with the findings of (22), who found that hepatic damage was restored and the elevated transaminase activities was significantly reduced by hypoglycemic plant (22). The diabetes complication such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities (22). The results we have had achieved in this study indicated that administration of flavonoids extract for 10 days in diabetic rats, significantly reduced glucose, cholesterol, triglyceride, LDL-C, VLDL-C, GOT and GPT levels as in comparison to diabetes.

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Anatomical, histological and histochemical developmental study of sclera ossicle in broiler chicken

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ABSTRACT

The sclera in some vertebrate (birds and turtles) was consist of scleral ossicles protect the eye during deformation. This study involved collection of broiler chicken embryos at 7,8,9,10,and 20 days of incubation , and the eye ball were removed and the anterior portion of the eye ball were radiography and stained with alizarin red . The histological and histochemical study were carried out on the anterior portion of the eye, this study showed that the sclera papilla appeared firstly at eighth day of incubation ,while the first intramembranous ossification of sclera ossicles noticed at tenth day of incubation. The fourteen square sclera ossicles appeared overlapping forming complete ring at twenty day of incubation. This result did not notice any differences in the shape and number of sclera ossicles in the right and left eye. The Histochemical study showed that alkaline phosphatase activity, mitochondrial size and Golgi elements was increased in the osteobalstic stage compared to the preosteoblastic stage.

***Keywords*:** ossicles, ossification, alizarin, eyeball, skeleton

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الملخص باللغة العربية

تمتلك صلبة العين في بعض الفقريات مثل الطيور والسلاحف والأسماك عظيمات لحماية العين. في هذه الدراسة تم جمع أجنة لعينة من دجاج اللحم بأعمار (7، 8، 9، 10، 20) يوم حضانة، ورفعت كرة العين وأخذ الجزء الأمامي من كرة العين للدراسة التشريحية والنسيجية وتم صبغه بصبغة الإيزارين الحمراء، إضافة إلى فحص بعض النماذج بجهاز الأشعة.

أوضحت الدراسة التشريحية والنسجية للجزء الأمامي من كرة العين ظهور بعض الحليمات الصلبة لدى الدجاج في عمر 8 أيام حضانة، بينما ظهر التعظم الغشائي أولا عند عمر 10 أيام حضانة في أربعة عشر عظيمة، وهذه العظيمات الأربعة عشر المربعة الشكل أخذت تنمو واحدة فوق الأخرى مكونة حلقة عظمية متكاملة عند عمر 20 يوما حضانة، كما أوضحت الدراسة عدم وجود اختلافات في شكل وعدد العظيمات بين العين اليمنى واليسرى.

وبهذا، يمكن الاستنتاج بأن الدراسة الكيميائية النسجية أظهرت بأن فعالية إنزيم الفوسفاتيز القاعدي وحجم المايتوكوندريا وعناصر أجسام جولجي ازدادت في مرحلة بانيات العظم مقارنة بمرحلة قبل التعظم.

INTRODUCTION

The vertebrate eyes are located in the orbit, and consisted from internally nervous tunica, tunica vasculosa and tunica fibrosa, which involved the cornea and sclera (1,2). The sclera is the external layer covering the eye and consists of the hyaline cartilage and fibrous layer, but some animals have sclera ossicles (3). The sclera ossicles are found in ring embedded in the sclera surrounding the cornea beneath the conjuntival zone and cover the anterior region of eye of non mammalian vertebra (4). The scleral ossicles are common among many vertebrates such birds, turtles and lizard, but are absent in snake, crocodilian, mammal and amphibian (5). The number of sclera ossicles as well as the pattern in which these ossicles overlap are differed among the diffefent groups of animals (6). The sclera ossicles play role in protecting and supporting the eye ball function of the cilliary muscles, especially in the anterior part of the cornea, suggesting a role of visual accommodation (7). It was reported that the sclera ossicles may support bin ocular vision, enabling the animal to adjust the shape of cornea to modify its focusing power (8). Most of birds can not move their eyes as well as have the best visual capacity, because their eyes are very large compared to the size of the body, and have special structures to enhance the ability of vision that are not found in other creatures, and change the focal length, while squinting to get a clear vision (1,9). Embryologically, the optic cup is covered by loose mesenchymal tissue, which is derived from the neural crest, these cells form the white, densely collagenous sclera (10). The neural crest–derived cell only form scleral ossicles after interacting with specialized papilla in the sclera epithelium, these papilla induce the formation of the scleral ossicles (11,12). This study aimed to investigate the gross appearance with alizarin red stain, radiological with micro X-ray , some cytological(mitochondria, Golgi bodies) and Histochemical alkaline phosphatase enzyme developmental study of sclera ossicles in the broiler chicken during different days of gestation.

MATERIALS AND METHODS

Thirty (30) hatching eggs of broiler chick (*Gallus domestica*) were obtained from a commercial hatchery,(the fertilization appearance was determinate via incubation, then checking by lamp at the first fourth day of incubation showing the capillaries network). The eggs were continues incubated in an automatic incubator at (37ºC) with humidity (50%-70%) for different days in the histological laboratory. The embryos were removed by open shell of eggs and then washing with normal saline using five embryo for each age (7,8, 10, and 20 days of incubation). For the anatomical study the anterior ring portion of eye bull surrounding the cornea were stained with alizarin red for 10 min according to (6). Briefly this portion of right and left eye were dissected at embryo age (7,8, 10 and 20 days of incubation), fixed in 10% formaldehyde solution over night at a room temperature and then stained with 1% Alizarin Red stain(1 gm of alizarin red stain dissolved in 99 ml of distilled water) via immersion of the embryo in the staining solution for 10 minute, the stained eye were de stained with distilled water for three hours and photographed under dissecting microscope. Several samples of the ossicles were radiography using dental x-ray (Trydent system). For histological study the anterior portion of eye were processed according to (13), and stained with haematoxyline eosin stain. For mitochondria study the separated sclera ossicles at age 10 days of incubation were fixed in Helly's fluid then stained with authors silver-reduction method (14). The alkaline phosphatase activity was investigated by the calcium phosphate method (14). For Golgi elements the separated ossicle at age of 10 day of incubation were processing according to methods of (14). Briefly the ossicles fixated by Helly's fluid and the section stained with silver–reduction method. Finally, the tissue sections were examined using light microscope connected with camera.

RESULTS

The anatomical observation revealed that On the seventh day of incubation the eye was very conspicuous, pigmented and the eye lids covered the eye as well as no any markers for ossification (figures 1-A and 1-B). During the tenth day of incubation the ossification of the sclera ossicle was first appeared around the ciliary body as single ossification centers located in the center of the area surround the cornea and cilliary body (figures 1- C and 1-D). On the twenty day of incubation the oosicle ossification increased in size toward the outer edge and full overlapping forming complete ossicle ring consist of fourteen square scleral ossicle(figures 2- A and 2-B). The results did not show any difference in the number of the scleral ossicles in the right and left eye (figures 2 –C and 2-D).

The histological observation noticed that on the seventh day of incubation the section surrounded the cornea showed mesenchymal cells (figure 3-A). On the eighth day of incubation there were mesenchymal condensations which forming sclera papilla (figure 3-B). The ossified scleral ossicles was appeared at the tenth day of incubation, this ossification showed spicules, bone cells and vascularization by membranous ossification (figure 3-C). The histochemical study appeared that alkaline phosphatase activity, the size of Golgi elements and mitochondria number were increased in the ossification stage compared to the pre osteoblastic stage (figures 4-A,B,C,D,E and F).

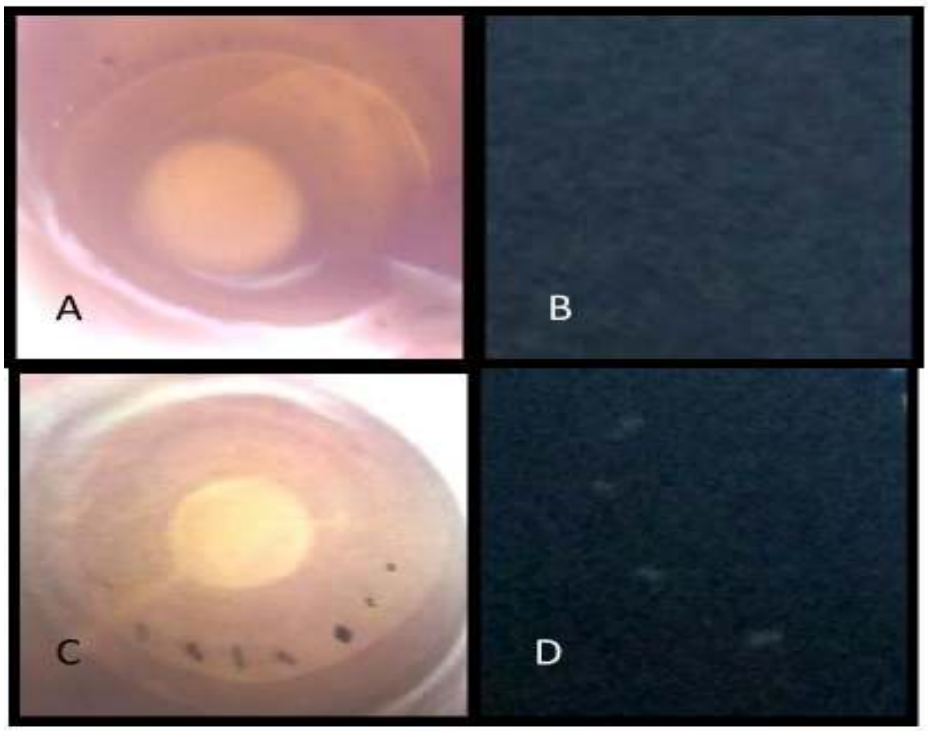

Figure (1): (A) Sclera ossicle show no ossification(alizarin red stain). (B) Sclera ossicle show no ossification (radiography). (C) Sclera ossicle show ossification (alizarin red stain). (D) Sclera ossicle show ossification (radiograpghy. 4X).

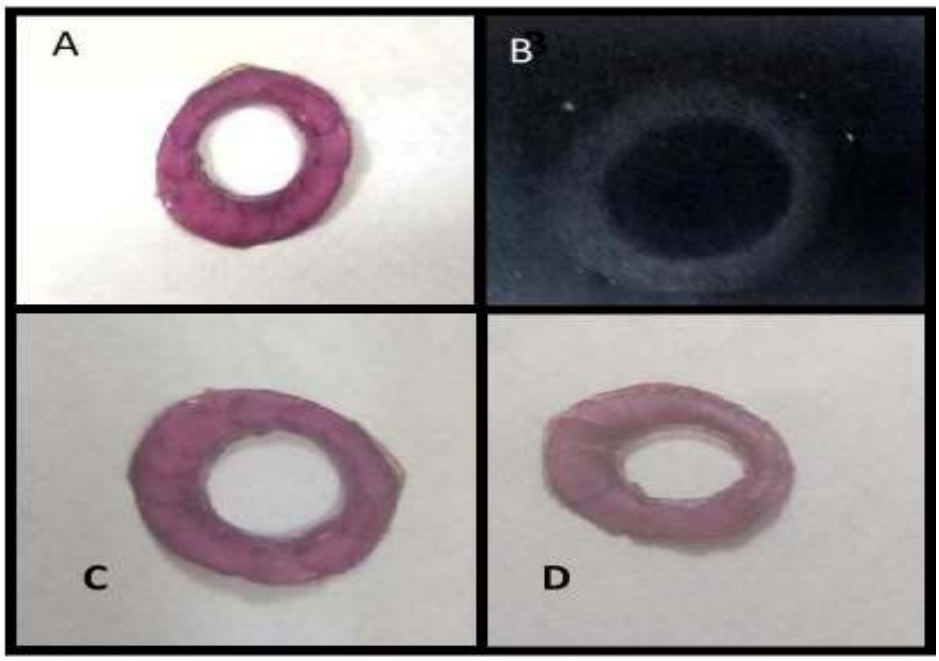

Figure (2): (A) Scleral ossicles showed overlapping (alizarin red stain), (B) Scleral ossicles showed overlapping (radiography), (C) Scleral ossicles of right eye (alizarin red stain), (D) Scleral ossicles of left eye (alizarin red stain .4X).

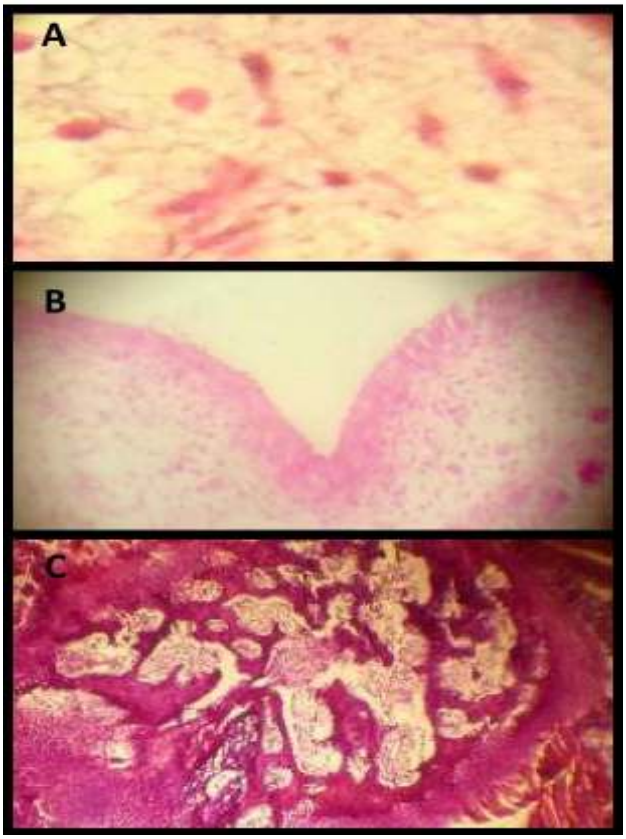

Figure (3): (A) Section of ossicles at 10 day of incubation show Scleral ossification (H&E 40X) ,(B) Section of ossicles at the seventh day of incubation show mesenchymal cells (H&E 40X), (C) Scleral Section of ossicles at 8 days of incubation shows Scleral papillae (mesenchymal cell condensation) (H&E 40X)

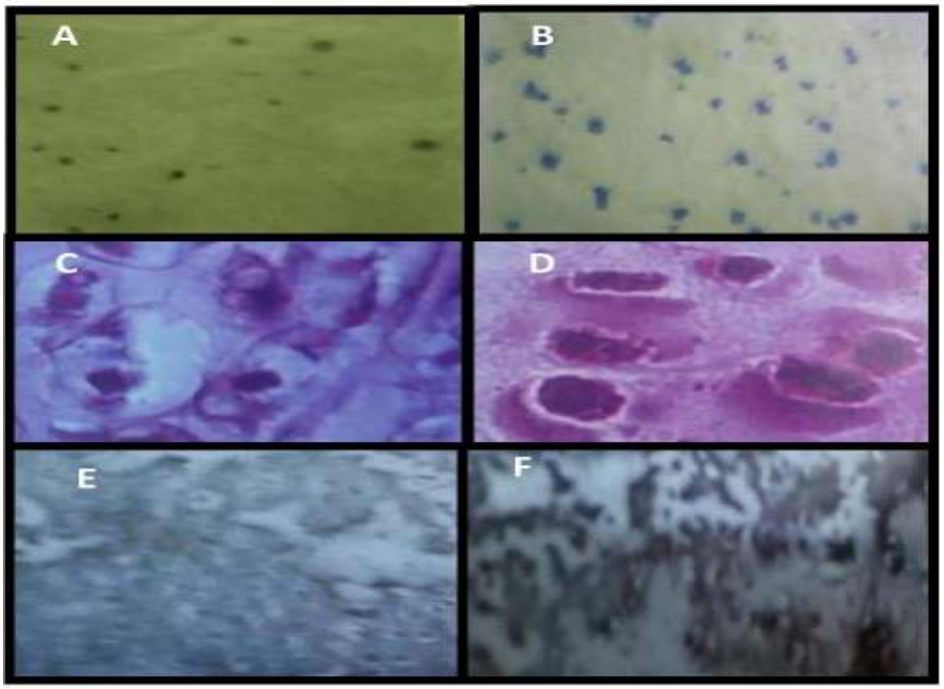

Figure (4): (A) Golgi elements in pre osteoblastic stage of ossicles(40X),(B)Golgi elements in osteoblastic stage of ossicle (40X), (C) Mitochondrial size in pre osteoblastic stage of ossicle (400X), (D) Mitochondrial size in osteoblastic stage of ossicles (200X), (F) Alkaline phosphatase activity in preosteoblastic stage of ossicles (200X).

DISCUSSION

The results of the present study revealed that the eye at the seventh day of incubation appeared conspicous, pigmented and the eye was covered by eye lids. These findings are in agreement with those revealed by (15), who noticed that on seventh day of incubation the eye of chick broiler embryo was very conspicuous with dark color. The results of the histological study showed that the sclera ossicles at the seventh day of incubation consisted of mesenchymal cells. This was similar to results obtained by (16) on chick broiler embryo. The present results also showed sclera papilla at the eighth day of incubation in the form of mesenchymal condensation, which was not appeared grossly. This result may be contributed to the difficulty of observations due to fact that they do not protrude far above the conjunctival epithelium (5), as well as difficulty of obtaining the correct age of embryo, and the papilla had short life cycle. It was noticed that the appearance of scleral papilla induced the scleral ossicles, and then this papilla degenerated lately so these papilla were transient (17). These results were in agreement with results of (18), who noticed fourteen papilla at seventh day of incubation in chick, while (19) did not observe any sclera papilla in turtles species, but (20-23) observed transient sclera papilla in *apalane spinifera, chrysemy picta, pelodiscus sinensus and tseripta* (turtles species). The present study showed that sclera ossicles first appeared by intramembranous ossification at tenth day of incubation. These results are in agreement with (24), while he noticed chondral ossification in sclera ossicles in turtles as similar to *jeleost* bony fish. Our results showed that the ossified ossicles appeared centrally, while (5) observed that ossicles firstly appeared in the posterior–most ossicles in turtles. The sclera ossicles in this study at twenty day of incubation appeared as overlapping forming complete ossicles ring, this result agree with present of complete ring in *ornithiscahia* bird and disagrees with incomplete ring in *saurischian,* which were observed by (25). The present study noticed fourteen scleral ossicles. These results are in agreement with the result of (26) in *archaeopteryx*, and results of (27), who noticed that most species had 14-15 ossicles, but in *osteichthyan* fish there were 0-4 scleral ossicles (28). 6-13 ossicles in *tostudines* were reported by (29). The shape of sclera ossicles in the present study in chicken was square, this shape agrees with the result of (6) in brazillian bird, as well as the result of (30) in *falconi formis* species and *psittaciformis* species, but in *piciformis* species were irregular while in reptile were flat and some time it was slightly curved. The present study did not show any difference in number of sclera ossicles in the right and left eye balls, while (6) noticed that differences may be found in this number in some species bird such as *spheniscus magellanicus*, which showed thirteen ossicle in right eyes and fourteen ossicles in the left eyes, while in *Elanus leusleucurus* fifteen ossicles in right eyes and sixteen in left eyes. Small peripheral bone in the left eye of *leptadon* species was noticed by (6) as named sclera sesamoid bone in *Bubobuo* genus (7). The histochemical study showed that alkaline phosphatase activity, mitochdrial hypertrophy and size of Golgi elements had increased in osteoblast stage compared to pre osteoblast stage, which were in agreement with those obtained by (14). These findings of Histochemical study may be attributed to the presence of relationship between cell proliferation and these histochemical changes. The Alkaline phosphatase is an important component in the hard mineral formation. The mechanism with which this enzyme carries out its function is not completely understood, but it appears to act both to increase the local concentration of inorganic phosphate, a mineralization promoter, and to decrease the concentration of extracellular pyrophosphate, an inhibitor of mineral formation (31). The high activity of mitochondria and Golgi bodies in the osteoblast was due to the energy to production of the mineral and support the thesis that the osteoblast is metabolically very active.

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Detection of sheep mange in different regions of Basrah province

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ABSTRACT

This study was conducted to detect mange mites of sheep in different regions of Basrah province, and to study the effects of the sex and age on infestation from the period of October 2014 to May 2015. The results showed the highest percentage infestation (51.96%) of *Sarcoptes ovis,* followed by *Psoroptes ovis* (35.24%), while the *Chorioptes* ovis recorded lowest appearance (11.76%) , also the mixed infestation with *Sarcoptes ovis* and *Psoroptes ovis* was 0.99%.

The results also indicated that percentage of occurrence of mange mites in female was (22.70%), while in male was (22.24%), and the percentage of infestation in *Sarcoptes ovis* in age (0-1 ,1-2 and >2) were (28.30%, 33.96% and 37.73%), *Psoroptes ovis* was (33.33% ,22.22% and 44.44%) and *Chorioptes ovis* (30% ,36.66% and 33.33%). The mixed infestation with *Sarcoptes ovis* and *Psoroptes ovis* were (0%. 0% and 100%) respectively.

Results of samples survey from different regions of Basrah province revealed that Al-Zabair region was highest prevalence (36.27%) followed by Al-Qurna region and Al- Madaina (24.90% , 11.17 %) respectively, while, Basrah center , Safwan and Abu Al-Khaseeb recorded (9.60% , 7.84% ,6.27%) respectively. The lowest rates were detected in Qarmat Ali (3.92%). The analysis revealed that the differences in the infestation rates among the regions of the study were significant ($p \leq 0.05$), and recorded seasonal prevalence of mites, which was gradually rose in February in male and females (30.07% , 32.64%) respectively, while during May there was no infestation with any cases of mange mites.

Keywords: *Sarcoptes ovis*, *Chorioptes ovis,* sheep

الملخص باللغة العربية

أجريت هذه الدراسة للكشف عن جرب الأغنام في مناطق مختلفة من محافظة البصرة، وتأثير الجنس والعمر وأشهر السنة على الإصابة، وذلك خلال الفترة من شهر أكتوبر / تشرين أول 2014 إلى شهر مايو / أيار 2015 . تم فحص 2264 رأس غنم (872 ذكور و 1392 إناث). أظهرت الدراسة إصابة 510 رأس أغنام بالجرب، حيث كانت أعلى نسبة إصابة بـــالنوع *Sarcoptes ovis* 51.96% و *Psoroptes ovis* *K % 36.24* بينما *Chorioptic ovis* 76.11% . كما سجلت الدراسة إصابة مشتركة بالنوع *Sarcoptes ovis* مع *Psoroptes* ovis بنسبة إصابة 0.99% . كما أظهرت الدراسة أن نسبة إصابة الإناث كانت 22.70% وفي الذكور كانت 22.24% ونسبة الإصابة في *Sarcoptes ovis* في عمر (0–1، 1–2 و> 2) كانت (28.30٪، 33.96٪ و 37.73٪) ، وللنوع *Psoroptes ovis* (33.33٪، 22.22٪ و 44.44٪). كما كانت *Chorioptic ovis* (30٪، 36.66٪ و 33.33٪) والإصابة مختلطة *Sarcoptes ovis* مع *Psoroptes* ovis (0٪، 0٪ و 100٪) على التوالي .

كشفت نتائج عينات مسحية من مناطق مختلفة من محافظة البصرة أن منطقة الزبير سجلت أعلى نسبة إصابة 36.27٪ ، تليها منطقة القرنة والمدينة 24.90٪ و11.17 ٪، في حين سجلت الإصابات في كل من مركز البصرة وصفوان وأبو الخصيب 9.60٪، 7.84٪، 6.27٪ على التوالي، وأدنى نسبة إصابة كانت في كرمة علي 3.92٪ . أظهر التحليل الإحصائي أن هناك فروقا معنوية في معدلات الإصابة بين مناطق الدراسة تحت مستوى ($p \leq 0.05$) .

وسجلت نسبة الانتشار الموسمي ارتفاعا في نسبة الجرب تدريجيا في شهر فبراير/ شباط في الذكور والإناث 30.07٪، 32.64٪ على التوالي، بينما خلال شهر مايو / أيار لا توجد إصابات.

INTRODUCTION

Skin diseases caused by ectoparasites are among the major diseases of small ruminants causing serious economic losses to small holder farmers, and the tanning industries and, skin diseases cause mortality, decrease production ,reproduction , and according to tanneries report, skin diseases due to external parasites causes 35% sheep skin and 56% goat skin, respectively (1).

The mites of mammals and birds inhabit their skin, where they feed on blood lymph, skin debris or sebaceous secretions, which they ingest by puncturing the skin, scavenge from the skin surface or imbibe from epidermal lesions, most mites spend their entire lives in intimate contact with their host (2), s o that transmission from host to primarily by physical contact, the generalized veterinary term for an infestation by mites in an animal is called acariasis and can result in severe dermatitis, known as mange or scabies, which may cause significant welfare problems, economic losses and outright deaths (3).

Mange causes itching, which results in loss of hair or wool and skin damage, loss of body condition and death (4), the mites cause intense itching and discomfort which are associated with decreased feed intake and production and the scratching and rubbing caused by mites result in extensive damage to hides and skin (5) .

The great economic losses due to damaged skin and wool , anemia, physical condition ,decreased milk and meat production and meat production and suboptimal lambing and growth rates (6) .

About 50 mites species in 16 families and 26 genera may cause mange where all the major mange mite species are within the orders Astigmata and Prostigmata, the Astigmata include the medical or veterinary important families Sarcoptidae and Psoroptidae which include Sarcoptes mite that causes Sarcoptic mange *(scabies*) in humans and other mammals as *a* zoonotic disease*,* while Prostigmata include the Cheyletiellidae**,** Demodecida*e* and Psorergatidae (7), mange mites are mainly of three types: *Sarcoptic* (barn itch**),** *Psoroptic* (sheep scab, body Mange, ear Mange), *Chorioptes* (tail mange, leg mange, scrotal mange).

MATERIALS AND METHODS

Study areas

Sampling was done from different region of Basra Province (Basrah center ,Shatt Al-Arab ,Al- Qurna , Al-Midaina , Abu Al-Khaseeb, Zubair, Safwan, and Qarmat Ali) which represented same locations. A random selection of sheep flocks included 872 males and 1392 females. samples were collected from October 2014 to May 2015, where the samples were divided into three groups: group (0-1) years old, group (1-2) years old and group (more than 2 years).

Skin scarping

Samples were collected weekly from the animals which showed clinical signs of the mange infestation such as hair loss, severe itch and crusty or scaly skin lesions, the wool was clipped out with scissors and then a drops of glycerin were added on the edge of lesion to moisten the area, skin scrapings were taken only from animals suspected for having clinical signs of mange by scraping 2.5 cm area of the affected lesions in black plastic containers according to (8,9).

Laboratory examination

A complete history of each animal and date of examination were recorded and all samples were processed within 12 hrs. after collection. Briefly, 20 ml of 10% KOH solution was added to each sample container and boiled in water bath for 5-10 min, after that, samples were centrifuged at 1500 rpm for 4-5 min, then some drops are drawn from the sediment with a pipette and placed on a glass slide and covered with a cover slide ,it is examined under microscope under microscope with power (10x ,40x and 100x) to confirm the presence of parasite and diagnose the species depend on morphology and features of mites as described by (10) in diagnosis of the samples of the study.

Statistical analyses

All values were expressed as the mean (M) ± standard error (SE), Chi-square was used to analysis the significance of the difference between the groups (11).

RESULTS

Characteristics and types of mange mites that appeared in skin scraping

A. *Sarcoptes Scabiei var Ovis* De Geer 1778: Adults *S. ovis* are rounded in sheep with four pairs of legs (two pairs in front and two pairs in behind). They were recognized by the ventrally surface flattened and which has the epimeres which form Y shape and in addition noticed the mouth parts in the body of the mites (figure 1), while the dorsally surface is convex –like body and multiple cuticular spine and setae like tortoise. Adults *S.ovis* are very small 0.3 to 0.5 mm in length.

The males were smaller than females and have suckers on the all pairs of legs except the third pair have hairs (bristles). The body has hair in ventral surface (figure 2). The suckers have bell like shape, while in third and fourth pairs of legs three were long hair like setaes (figure 3). The larvated eggs contained six legged larvae (figure 4).

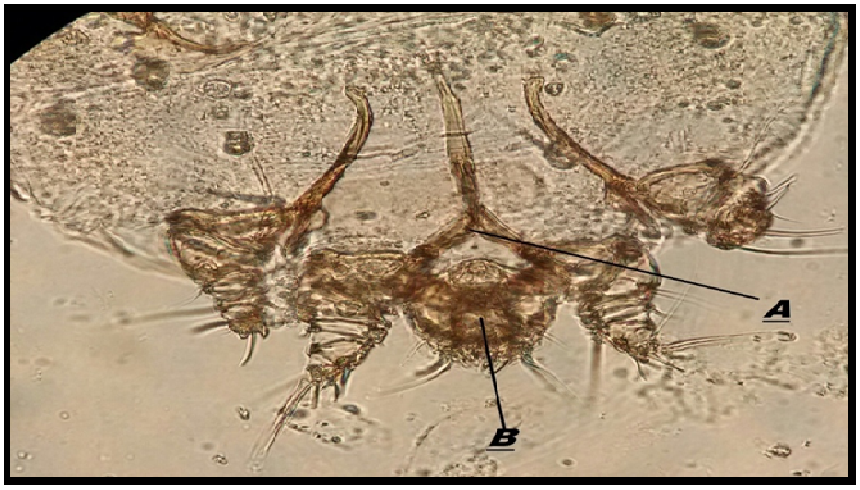

Figure (1): *Sarcoptes ovis* A. Y shape epimeres B. mouth part. (100 X)

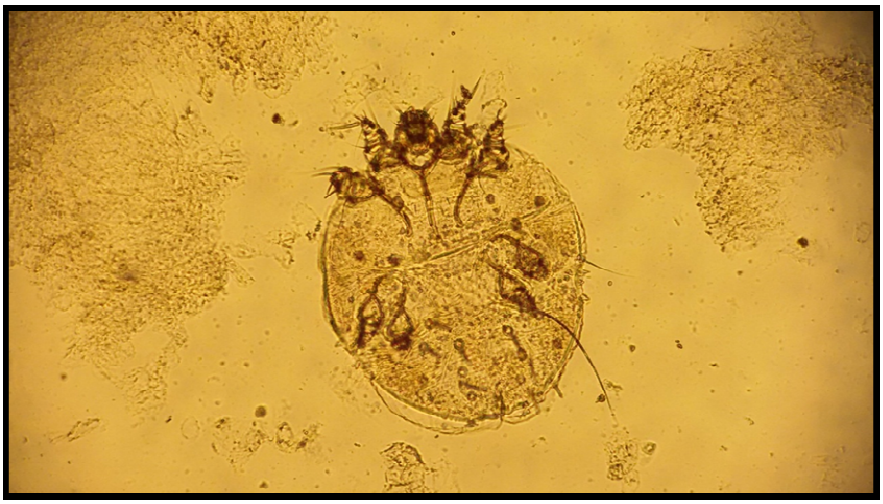

Figure (2): ***Sarcoptes ovis*** **female with four pairs legs (40 X.)**

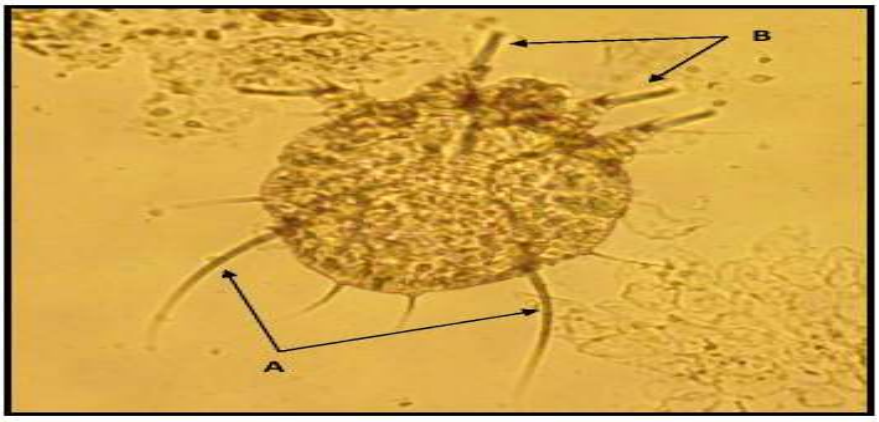

Figure (3); legs of A. bell shape suckers on first two pairs of legs 40x
B. Hair like setae on 3rd and 4th pairs of legs 40 X.

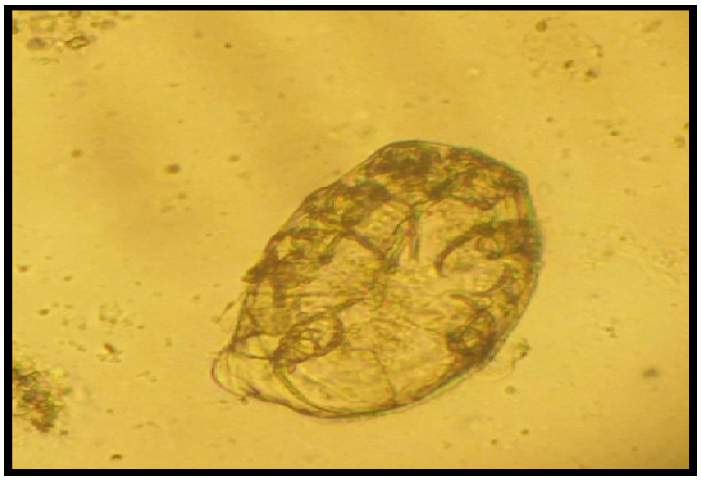

Figure (4): Larvae of *Sarcoptes scabiei ovis*(six legs larvae) (40 x.)

B. *Psoroptes ovis* Gervais,1841: This type of mange mites appeared as an oval shape. The adult mites have four pairs of long legs, which extended out of the body (figure 5). Adult *P.ovis* was 0.75-0.90 mm in length , the male is smaller than female in size and 1st ,2nd and 3rd pairs of legs end with suckers and noticed adanal suckers pairs and two posterior lobes in the end of ventral surface and some legs has hair (figure 6). The *psoroptes ovis* brown –yellow in color and six legged larva which appear in side of eggs (figure 7).

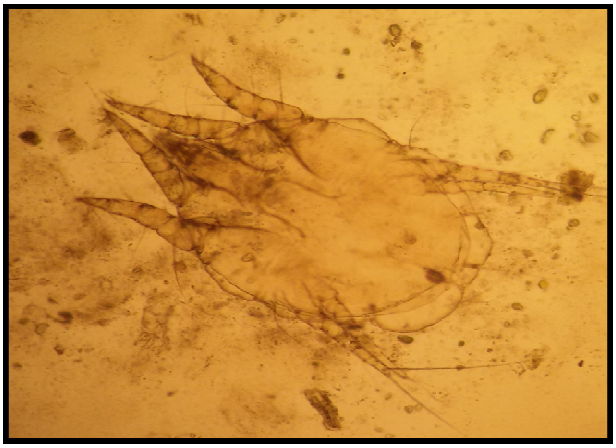

Figure (5): Female Ventral surface of *Psoroptes ovis* with four pairs legs (40x.)

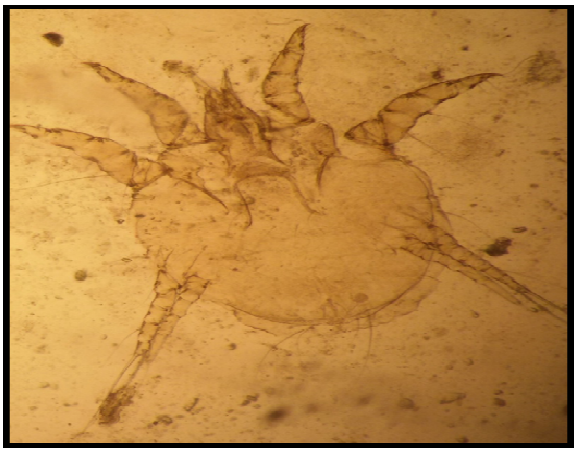

Figure (6): Male of *Psoroptes ovis* , showed end of dorsal surface (40X.)

Figure (7): Six legged larva in egg of *Psoroptes ovis* (40 x.)

C. *Chorioptes ovis* Railliet ,1893: Adults of both sexes have anterior and posterior cuticular shields and a variety of mostly short, hair-like setae ,they are also very small (0.4 to 0.6 mm).
Ventrally, the female ovipore is a transverse slit with a pair of trailing apodemes , the mouthparts are unremarkable, and the legs are moderately long and robust, except the fourth pair in the male are very short, and in female the third and fourth pairs are more slender.
All legs in male and female are terminate distally in empodial suckers with short, unjointed stalks, but in third pair of female, which end in two long whip like setae each. The male also has a long, whip-like seta on each third leg and a pair of adanal suckers (figure 8).

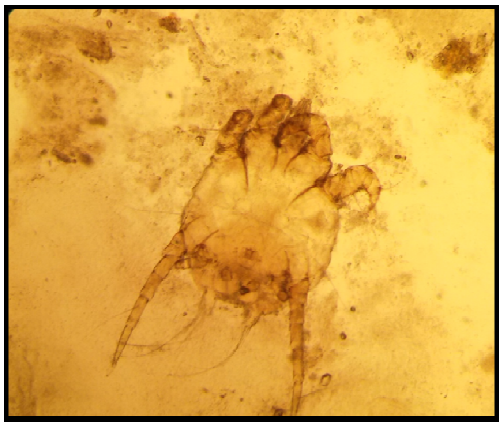

Figure (8): *Chorioptes ovis*

Prevalence of mange mite species

According to the results obtained from the present study , three types of sheep mange mites (table 1) *Sarcoptes ovis* recorded the highest percentage (51.96%) ,followed by *Psoroptes ovis* (35.29%), while *Chorioptes ovis* recorded lowest appearance (11.76%). Also the mixed infestation with *Sarcoptes ovis* and *Psoroptes ovis* (0.99%) were noted (diagram 1).

Table (1): Prevalence of sheep mange mites species

Type of mange	No. of infested animals	%
Sarcoptes ovis	265	51.96
Psoroptes ovis	180	35.29
Chorioptes ovis	60	11.76
Sarcoptes ovis+ *Psoroptes ovis*	5	0.99
Total	510	--

Diagram (1): Relationship between the percentage infestation with types sheep of mange mites

According to sheep age , the study indicated that the prevalence of infested with *Psoroptes ovis* > 2 age was highest and infestation, while the infested by *Sarcoptes ovis* was low infestation than the *Chorioptes ovis* recorded was lowest percentage, so that in case mixed infested with *Sarcoptes ovis* and *Psoroptes ovis* were high (table 2). Significance differences between the age and sex were indicated in percentages of infestation mange age of animals high in significant ($P \leq 0.05$).

Table (2): Relationship between the sheep age and infestation with species mange mites

Species	Age	No. of examined animals	No. of infested animals	%
S. ovis	0-1	231	75	28.30b
	1-2	383	90	33.96b
	>2	470	100	37.7a
X^2=14.647	---	1084	265	---
P .ovis	0-1	191	60	33.33b
	1-2	295	40	22.22b
	>2	302	80	44.44a
X^2=24.665	---	788	180	---
C.ovis	0-1	30	18	30b
	1-2	164	22	36.6a
	>2	189	20	33.33b
X^2=64.256	---	382	60	---
S. ovis + *P .ovis*	0-1	0	0	0
	1-2	0	0	0
	>2	10	5	100a
X^2=13.333	---	10	5	---

The relationship of sex and age of sheep with mange infestation

The laboratory results showed that 510 samples from a total of 2264 samples collected from sheep were infested with mange. The prevalence rate was (22.52%) recorded during the study period from (October 2014 to May 2015). In relationship to sex,

the abundance of mange species recorded 22.24 % in male and 22.70% in female (table 3). Significant differences (P≤0.05) were indicated between males and females.

Table (3): percentage of infestation and relationship sex and age

Sex	No. examined Animals	No. Suspected Animals	No. infested Animals	%
Male	872	286	194	22.24a
Female	1392	661	316	22.70a

Male X^2 =77.174 Female X^2=77.738

Geographic distribution of mange mites infestation

Results of samples survey from different regions of Basrah province revealed that Al-Zubair region was highest prevalence (36.27%) followed by Al-Qurna region and AL- Madaina (24.90% , 11.17 %) respectively while, Basrah center , Safwan and Abu AL-Khaseeb recorded (9.60% , 7.84% ,6.27%) respectively. The lowest rates were detected in Qarmat Ali (3.92)% , the analysis revealed that the differences in the infestation rates among the regions of the study were significant (p≤ 0.05) (table 4, diagram 2).

Table (4): Geographical distribution of sheep mange infestation mange in Basrah region

Region of study	No. of examined animals	No. of infested animals	%
Al-Zubair	542	185	36.27 a
Al-Qurna	449	127	24.90 c
Al-Madaina	341	57	11.17 b
Basrah center	175	49	9.66 b
Safwan	319	40	7.84 b
Abu Al-Khaseeb	233	32	6.27 b
Qarmat Ali	205	20	3.92 b
Total	2264	510	

Different letters=Significant different (p≤0.05) .
Similar litter= non Significant different (p>0.05)

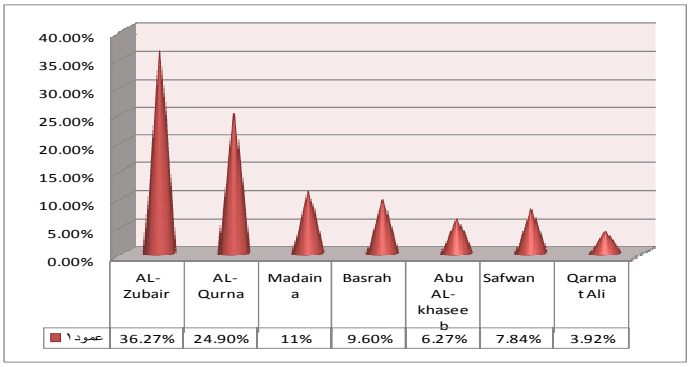

Diagram (2): Relationship between the percentage of infestation in regarded study region

Effect of a year months on mange mite infestation

The results of study showed the infestation and percentage with mange mite in female and male were high in February 30.07%, 32.64% respectively, while the infestation in female and male disappear in May (table 5, diagram 3). The significant differences (P ≤0.05) were of infestation rates among months in female and male.

Table (5): Distribution of infestation among study period

Month	No. female examined	No. infested	%	No. male examined	No. infested	%
October	78	17	21.79b	81	11	13.58a
November	96	19	19.79b	99	19	19.19a
December	278	35	12.58a	115	8	6.95
January	302	88	29.13b	210	44	20.95a
February	409	123	30.07b	291	95	32.64b
March	83	19	22.89b	45	13	28.88b
April	76	15	19.73a	31	4	12.90a
May	70	0	0	0	0	0
Total	1392	316	--	872	194	--

Female X^2=111.268, Male X^2 =41.16
Different letters=Significant different (p≤0.05) .
Similar litter= non Significant different (p>0.05

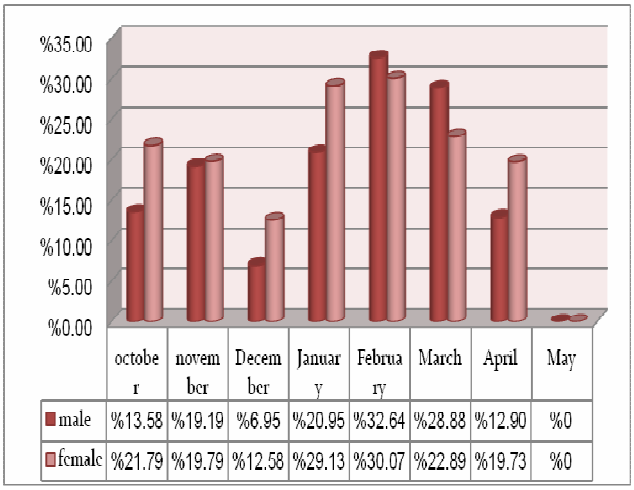

Diagram (3): Relationship between percentage infestation with months

DISCUSSION

Mange is a highly contagious and debilitating skin diseases of sheep which badly affect the health and productive capacity of these animals ,the species of the three main genera i.e *S. ovis* , *P. ovis* and *C.ovis* are particular clinical important (12). *S. ovis* recorded the highest percentage 51.96% ,followed by *P. ovis* 35.29% , while *C. ovis* recorded lowest appearance 11.76% ,also the mixed infestation with *S. ovis* and *P. ovis* 0.99% , due to Basrah commercial site and transfers of sheep between the southern provinces on the one hand and the gulf states on the other hand, in addition to the green open spaces and the spread of grazing herds of sheep in Basrah lead to the spread and in addition to illegal smuggling of the sheep between provinces to Basrah because of its commercial site.

The main reason for the difference percentage of infestation with mange due to the geographical location of the affected areas, mixed farming system, low grade nutrition, poor manage mental condition. In addition the difference of infestation due to the lack of control and treatment programs during the seasons of infestation or non –use treatment ivermectin and cypermethrin periodically.

Our study was higher than the percentage recorded by (13) in Najaf province, which was 22.16% with *S. ovis* . This study was in disagreement with (10), who recoded over percentage was 3.65% and *S. ovis* 31.18% , *P. ovis* 52.15% and *C. ovis* 8.06%. It was showed that percentage of infestation was 11.37% , *P. ovis* 6.62% , *S.ovis* 4.75% (14), while by (15) percentages were 28.1% , *P. ovis* 19.5% and *S.ovis* 8.4%**.**

The current study demonstrated to higher the percentage of infestation with *S. ovis* 51.96% than other species ,due to *S. .ovis* burrowing mites that live in tunnels of stratum corneum in the skin and softer skin and tissue enabling easy penetration of the burrowing mites into the body of the host . These mites complete their entire life cycle on the host, and survive for long periods in the environment and the transmission of *S. ovis* between animals is probably by direct contact or in contaminated bedding and similar fomites in addition the *Psoroptes* mites do not dig tunnels and *Psoroptes* mites infests the superficial layers of the skin ,this result agreed with (16), which record highest ratio of *S.ovis*, while disagreed with (17, 18) whose had registered higher percentage of infestation with *P.ovis* than *S. ovis* and *C. ovis* due to Irritation of the outer skin by the mite's mouthparts and saliva results in a complex form of cutaneous hypersensitivity and inflammatory exudation of serum and fresh cells, the mites feed on this moist exudates .

This study showed contrast in mange mite infestation percentage and numbers depending on animals age ;the animals age was >2 years that may depend on the nutritional status, where well-fed animals can better withstand parasites infestation than animals on an inadequate diet, which can influence the level of immunity.

Alternatively, mange might be a cause for poor body condition; hence high prevalence was computed in this group of animals , these results have agreed with (10, 19) , and our finding incompatible with each of (20, 21) those registered the plausible motivation of higher infestation in younger sheep might be ascribed to an immature immune system that has yet to be challenged by introduction of mite infestation and softer skin and tissue enabling easy penetration of the burrowing mites.

Also our study recorded 36.27% in Al-Zubair region compared with the other regions, this return to animals density in these areas ,do not use regular prevention and control the diseases , the absence of cultural awareness to owner ,in addition poor management and lack of nutrition and the decreased rate of infestation in this regions ,due to the presence of that most of the animals are individually so they treat their owners and the prevention of mange mites and that the use of common compounds Ivermectin may be a reason for the decreased rate of infested. This study was in agreement with (22, 23), who mentioned that animals in poor conditions appear to be more susceptible to infestation.

According to the time of the infection the highest infection was 30.64% ,30.07% male and female in February, while the lowest rate was 0% in May, this return to the variance in environmental stress factors temperatures and relative humidity and levels of rainfall between seasons and months of the year which lead to direct effect on activity and survive and addition to the competing animals with each may help to increase the friction and then spread infested among the flock , this results agreed with results of (24), who found the highest infection rate in February, while it was the lowest in June. Stock density of animals and least exposure to sunlight in closed housing were recorded by (23, 25), which made animals more prone to arachnids.

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Effect of vaginal sponge and drug delivery system to estrous synchronization in local goats

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ABSTRACT

The aim of the present study was to compare the effect of vaginal sponge and drug delivery to synchronize estrus in local goats, through induction of estrus pregnancy diagnosis by ultrasound to confirm the presence of fetus.
Twenty does aged, (2-3) years old , weighted (25-35)kg were used in this study, with three buck age about (2-3) years. They were divided randomly into two equal groups, each group contained ten does : group (A) I/M injected with progesterone hormone as a drug delivery with chitosan ,while group (B) received intra vaginal sponges, which was impregnated with 20 mg (MAP) medroxy progesteron acetate. All does after 14 days were injected by I/M of 500 IU (PMSG) pregnant mare serum gonadotropin. All females were examined by abdominal palpation , ultrasonography to confirm presence of pregnancy before beginning the study.
Results showed that animal response to estrus in group (A) was 80% with duration of response 18.6± 11.80 hrs., while estrus response in group(B) is high 90% with duration of response 21.6±12.50 hrs. The pregnancy rate recorded in group (B) was 90%, which was higher than group (A) (80%), and nature of parturition recorded high of normal parturition (70%) and low dystocia (29.35%) . Type of parturition single was high 82.35%, while twines 17.6% . The presence of living new born was high 95% and the dead was 5%. Pregnancy diagnosis by ultrasonography was done in 30, and 100 days after natural insemination by buck.
The conclusions of this study demonstrated the vaginal sponge is the best in estrus synchronization after 14 days injection by (PMSG) of 500 IU as compared with progesterone hormone injection as a drug delivery with chitosan.

Keyword: Estrous synchronization, goats

الملخص باللغة العربية

سعت هذه الدراسة إلى إجراء مقارنة ما بين تأثير استخدام الإسفنجات المهبلية ونظام تحميل الأدوية لتوحيد الشبق في الماعز المحلي. وقد تم استحداث الشبق وفحص الحمل بالسونار بالموجات فوق الصوتية لتحديد وجود الجنين.
استخدم في هذه الدراسة عشرون من إناث الماعز، التي تراوحت أعمارها (2–3) سنة وأوزانها (25–35) كيلوغراما، مع ثلاثة ذكور بأعمار (2–3)سنة، وتم فحص الإناث للكشف عن الحمل بواسطة الجس عن طريق البطن والموجات فوق الصوتية قبل بدء التجربة. وخلال فترة العلاج الهرموني (هرمون البروجستيرون) لم تظهر أي علامات للشبق على جميع حيوانات الدراسة. وقد جرى تقسيم الحيوانات بشكل عشوائي إلى مجموعتين (A,B)، فأما المجموعة A ، فقد شملت (عشرة حيوانات) تم حقنها في العضلة بهرمون البروجستيرون، المحمل على الكيتوسان، في حين شملت المجموعة B (عشرة حيوانات) وضعت لها إسفنجات مهبلية مشبعة بمقدار 20 ملي غرام من (ميدروكسي بروجستيرون أستيت) لمدة 14 يوما، وفي اليوم الرابع عشر تم حقن الحيوانات في كلا المجموعتين (A,B) بمقدار 500 وحدة دولية من مصل الفرس الحامل في العضلة.
أظهرت نتائج الدراسة أن نسبة حصول الشبق في المجموعة (A) بلغت 80% ، ومدى وقت ظهور الشبق بالساعة (18.6±11.80) ، وفي المجموعة (B) 90%، مع مدى ظهور الشبق 21.6±12.50 ساعة، وكانت نسبة الحمل في المجموعة (B) 90% وهي أكبر من المجموعة (A) 80%، وكانت طبيعة الولادة 70.5 %ولادة طبيعية، و29.4% ولادة عسرة، أما نوع الولادة، فقد سجلت 82.35% ولادة مفردة و17.6 ولادة توائم. وكانت نسبة الأجنة الحية عالية (95%) مقارنة بالأجنة الميتة (5%). وق تم فحص الحمل بواسطة الموجات فوق الصوتية بعد 100، 30 يوما من التلقيح الطبيعي، وكانت نسبة الحمل في المجموعة (B) 90% أعلى من نسبة الحمل المسجلة في المجموعة (A) وهي 80 %.
خلصت هذه الدراسة إلى أن الإسفنجات المهبلية هي الأفضل في استحداث الشبق بعد حقنها بهرمون مصل الفرس الحامل (PMSG) 500 IU بالمقارنة مع هرمون البروجستيرون المحمل بالكيتوسان.

INTRODUCTION

Goats are the first to be domestic for milk, meat, and hair fibers. They are seasonally polyestrous. This seasonality is governed by photoperiodicity with oestrus activity. They are spontaneously ovulate. The onset and length of the breeding season depend on various factors such as latitude, climate, breed, light, physiological stage, presence of the male, breeding system and the specific photoperiod (1-3).

Oestrus cycle control in goats serves the purpose of synchronizing oestrus in groups of animals to be bred or inseminated at a specific time or inducing out-of- oestrus season (4). The stimulation of oestrus can be achieved with hormonal treatments, manipulation of the photoperiod or by the male effect (3-5). For the control of the oestrus cycle, progesterone or one of its synthetic analogues is preferred. The most widely used procedures for synchronization and /or the induction of estrous are 12 to 21 days of fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) impregnated intra vaginal sponge treatment (4, 6-8) .and an intramuscular injection of pregnant mare serum gonadotrophin (PMSG) at progestagen withdrawal (9,10).

A drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product and the subsequent transport of the active ingredients across the biological membranes to the site of action (11). However, much attention has been focused on the natural and synthetic polymers. Chitosan is a popular type of drug carrier. It is a very important, naturally occurring polysaccharide derived from the deacetylation of chitin and has been used extensively in pharmaceutics because of its excellent biocompatibility and biodegradability (12). Among the variety of polymers that were used for drug-loaded nanoparticles, chitosan has received great attention in both the medical and pharmaceutical fields (13).

Recently much attention has been given to the use of chitosan in veterinary applications ,as a wound healing agent ,bandage material, skin grafting template, hemostatic agent and drug delivery vehicle (14).

The aim of the present study was to investigate the chitosan effect on reproduction of goat and to compare between vaginal sponge and chitosan drug delivery.

MATERIALS AND METHODS

This study was carried out on 20 healthy goats (local breed) range in age from (2-3) years old and weight (25-35) kg. And 3 fertile buck about 2 years old in age and (35-45) kg in body weight. The animals were housed semi opened, in animal place at Surgical and obstetric department of the college veterinary medicine Basrah university. Water and green food were offered to all goats, in addition to a half kg of barley for each goat daily along the period of experiment. All the goat were examined carefully to ensure that they are healthy.

All experimental animals were undergone to a program of vaccination as following against enterotoxaemia at a dose of 1ml S/C, also deformed with cur fluke (Ireland) via oral route against liver fluke and gastrointestinal parasites twice a week and Ivermectin dose 2mg was administered S/C against external and some internal parasite.

The animals were exposed to the same environment conditions, including climate management and feeding for one month (before starting experiment) to acclimatize and adopt them to the place. The goats were divided randomly to two groups: A and B. Each group includes ten goats. They were submitted to trans-abdominal ultrasonography to ensure that goats were not pregnant, free from any infection, and abnormality.

The first group included (ten goats) were I/M injected by progesterone hormone mix with chitosan powder that is dissolved in ethanol (0,2mg), then after 14 days, at the time of sponge removal, they were injected I/M by 500 I U PMSG.

The second group included (ten goats) Had received intra vaginal sponge which impregnated with 20 mg of (MAP), and coated with an antiseptic cream, a sterilized glass applicator and a speculum were used to insert the sponge in the vagina of the animals , Insertion of the sponge was performed on 10 November/2014 and 24 November/2014 the sponge removes, Sponge was left on for 14 days. On day 14, all does were injected I/M by 500 I.U PMSG. Three fertile males were used to estrus detection and natural insemination. (The age of these males was 2 years old).

Ultrasonographic examinations were conducted using a convex 7.5 MH2 trans-abdominal (4.0 cm length). 7.5 transducer was well lubricated with (carboxy methyl-cellulose contact gel) and applied to the test side area of 150-200 cm2 on the right flank above and under after removing the hairs over it. Then, the transducer was placed at the right side of the goats 5.0cm in front of the rear leg and 2.5 cm above the teat.

Pregnant and non-pregnant goats were determined using real- time monitor by detection of fetal-heart, spinal cord, head, limbs, fetal organ and others. Ultrasonography examination (abdominal) was done in a special room, using special gel (coupling) for the probe and the examination (ventro-lateral area) area was shaved and disinfected carefully. The interval period to examination is done on 30, and 100 days. The animal was put in a setting position on its tail with the hind limbs extended on ground while the four limbs left up and controlled by assistant.

Pregnant and non-pregnant goats were determined using real- time monitor by detection of fetal-heart, spinal cord, head, limbs, fetal organ and others. Ultrasonography examination (abdominal) was done in a special room, using special gel (coupling) for the probe and the examination (ventro-lateral area) area was shaved and disinfected carefully. The interval period to examination is done on 30, and 100 days. The animal was put in a setting position on its tail with the hind limbs extended on ground while the four limbs left up and controlled by assistant.

Animals were under supervision after treatment for detecting the estrus behavior and natural mating to record the ratio of response to hormonal treatment.

The statistical procedures were performed by using the computerized software STATGRAPHICS (15). The Results are expressed as means ± standard deviation; means calculated by one-way ANOVA, F test, Q square test, and significant difference for comparison. The Difference between the means was considered significant at ($p<0.05$).

RESULTS

The results in table (1) described the type of treatment , animal response ,duration of response and pregnancy rate in goats ,goat response to estrus in group (A) that received (chitosan +progesterone +PMSG) is 80% with duration of response 18.6±11.80 ,while group (B) that received (vaginal sponge +MAP+PMSG). The response to estrus was 90% and duration of response was 21.6±12.50. Thus, the response of group (B)is higher than group (A) significantly ($P<0.01$) with typical estrus sign included plenty of clear viscous mucous vaginal secretion, hyperemia of vaginal mucosa, mild edema of valve, tail shaking, restlessness, homosexual behavior (riding a goat another in common). Poly urea, does in estrous always seek male finally accept the ride by the male.

Table (1): The type of treatment ,animals response, duration of response, and pregnancy rateined goats.

Groups	No. of goats	Type of treatment	Animals response (estrus show)		Duration of response M ±SE hrs.	Pregnancy rate	
			No.	%		No.	%
A	10	Chitosan+progesteron+ PMSG 500 I.U	8	80	18.6±11.80 b*	8 b*	80
B	10	Vaginal sponge+ PMSG 500 I.U	9	90	21.6±12.50 a*	9 a*	90

** Different small letters mean sig. differences ($p<0.01$)*

The results in table (2) explain the nature of parturition, which was 70.5% for normal and 29.4% represented dystocia parturition. The single type of parturition was recorded to be 82.35%, while the twining parturition was recorded to be 17.35% in goats. In group (A), single parturition was 6 from 10 goats and twin is 2 from 10 goats. In group (B), single type of parturition was 8 from 10 goats and twin is one from 10 goats. Twin in group (A) is higher than group (B). The viability of off spring recorded 95% alive and 5% dead. The availability of group (A) was 8 from 10 goats higher than group (B), which was 9 from 10 goats. The sex of lambs recorded 55.7% of male compared with 54.25% of female. The results of table (2) showed pregnancy rate of group (B) which was 90% and higher than pregnancy rate of group (A) that was 80%. Traditional methods for pregnancy diagnosis in small ruminants were applied, such as abdominal palpation , ballottement and noting udder enlargement. However , these methods are applicable only in late pregnancy. Currently trans-abdominal ultrasonography was used with great deal of accuracy as a means for pregnancy diagnosis.

The results of uterus of goats (group B) on day 30 of pregnancy presented the aminion sac surrounded the embryo as a thin hyprechoic line (figure 1). Image of placentomes was detected on 100th day of gestation (group A) and presented fetus heart and spinal cord (figure 2). Observations on images of fetal and related structures were carried out using probes (trans abdominal probes) on percentage of occurrence of fetus of (group B) and related structures for stages of pregnancy were observed.

Table(2): The nature and type of parturation in goats, sex and viability of newborns

Groups	No. of goats	Pregnancy rate		Nature of parturition		Type of parturition		Sex of goats		Viability	
		No.	%	N	D	S	T	M	F	A	D
A	10	8 b*	80	6	2	6	2	7	3	10	--
B	10	9 a*	90	6	3	8	1	4	6	9	1
Total	20	85%		12/17 70.5 a*	5/17 29.4 b*	14/17 82.35 a*	3/17 17.64 b*	11/20 55.7 a*	9/20 54.25 b*	19/20 95 a*	1/20 5 b*

N=normal, D=dystocia, S=single, T=twin, M=male, F=female, A= alive,D=dead

** Different small letters mean sig. differences ($p<0.01$)*

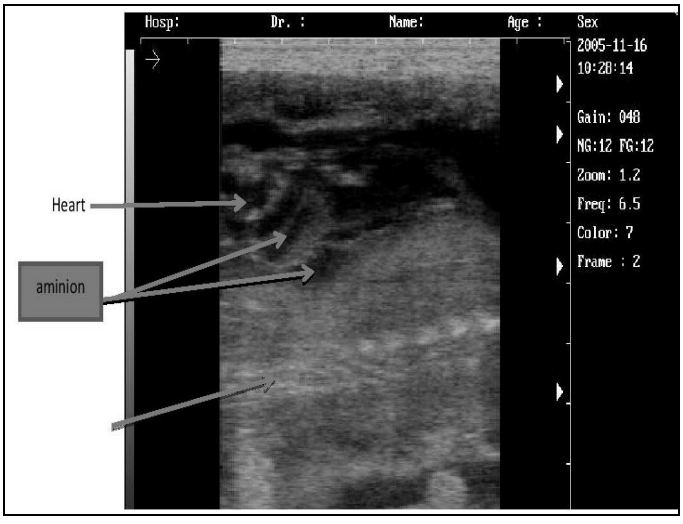

Figure (1): late stage image of placentomes on day 100 of gestation using trans abdominal probe, Group A.

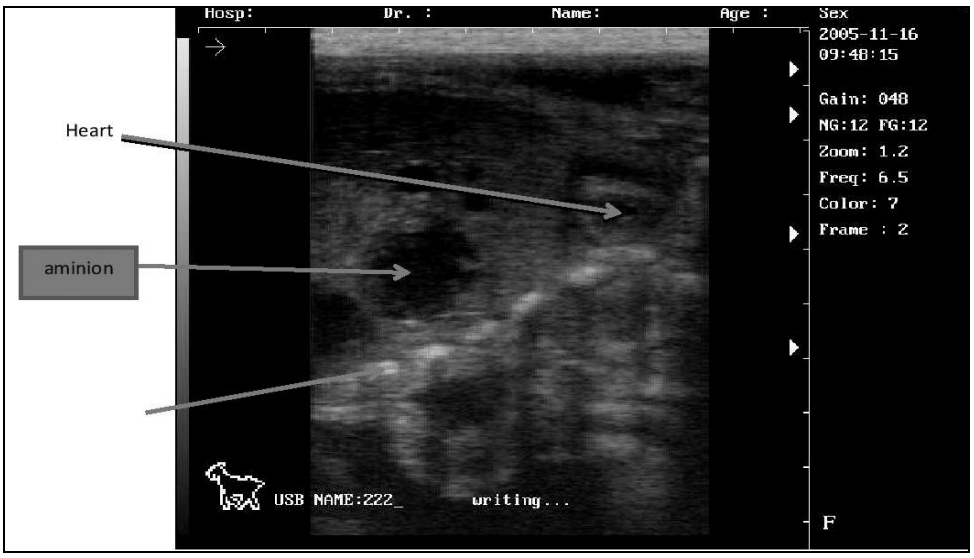

Figure (3): Image of fetus (a) heart (b) spinal cord on 100th day of gestation using transabdominal probe, group B

DISCUSSION

Estrus synchronization plays a major role in fixed time breeding, artificial insemination(AI) and embryo transfer(ET((16). There are a number of synchronizing methods for goats, the most common administration of progestagen application in goats is via intravaginal sponge (17). The most widely used procedure for synchronizing of estrus are 12-21days of fluorogestone acetate (FGA) or medroxy progesterone acetate (MAP) impregnated intravaginal sponge treatment (4, 8), and intramuscular injection of pregnant mare serum gonadotrophin (PMSG) at progestagen with (9,10). In this study, results showed no signs of estrous during progesterone treatment till 14 days ,when the sponge withdrawal and I/M injection of (PMSG)500 IU.

The estrus induction/ synchronization program has been highlighted as a helpful biotechnology to be used during the breeding or non-breeding season to increase the productivity of ovine (18). Several estrous induction protocols are currently available with varying doses, duration, type and route of a demonstration. The most commonly protocols used are progesterone slow release intra-vaginal sponges contain Medroxy progesterone acetate (MAP) by (19,20).

The results of no estrous during progesterone treatment were in agreement with that reported by (21-23), who suggested that the insertion of the sponge containing progesterone has the ability to stop the estrous as long as they exist inside the vagina. Impregnated sponges are considered as artificial source of progesterone insertion. The 20 mg of MAP, which is used in this study was enough to suppress the production of gonadotropin, while removing the blockage of progesterone leads to release gonadotropin and sequent estrous and ovulation in female treated with progesterone. In Iraq, it was recorded that 100% estrous after using 40mg MAP impregnated sponge in goat (22). While by (23), 40 mg progesterone in dose breeding season was used to synchronize estrus in goat by locally prepared progesterone impregnated sponges (60 mg MAP) and all goat exhibited estrus within 21-100 h of the sponge removal, While (25) synchronized estrus with(MAP 60mg) for 11 day and at 48 hrs. period of sponge removal ,I/M injection of 400IU of ecG equine chorionic Gonadotropin and cloprostenol (0,075) was given 94.5% of Dose showed estrus between 12- 24 hrs. after sponge removal and pregnancy rate recorded is 29.4%.

The results in table (1) showed that the time of estrous emergence from withdrawal of 20mg sponges was 21.6±12.6 hrs, in group (B), while the results of group (A) were 18.6±11.80 hrs. Good response of animal to treatment indicated the correct dose of 20 mg and 500 IU. PMSG used in the present study. This result is in agreement with (26), who synchronized estrus via impregnated (MAP 20 mg) for 13 day and I/M injection of 500 IU (PMSG) 24 hrs before removing sponge. All the dose showed (100%) estrus in 24-60 hrs. after sponge withdrawal. It was described by (27), that the ovulation in the female goat usually occurs 30-36 hrs. after onset of estrous. In addition, the same results were described when the PMSG hormone given 24 hrs. before the removal of sponge outside the breeding season and the dose come in heat during the 12-36 hrs. by removing the sponge. The insemination will be done at 48 hrs. of estrous (10.27).

In another study conducted by (28), animals were applied intra vaginal sponges (40 mg FGA) for 12 days in (October) and administered with 500 IU of (PMSG) on a day of sponge removal. The time of

estrus was 32.0 ±3.4 hrs. Furthermore, (29) reported that application of intravaginal sponges impregnated with (30mg of FGA) during breeding season for 13 day resulted in estrus in 32.9±9.7 hrs. after sponge removal On the other hand, (30) reported the mean time of estrus at 18.0±1.9 hrs. This difference may have arisen from geographical region, type of feeding animal breed and season. A study conducted by (31) had synchronized estrus by applying progestagen-impregated intra vaginal sponges for either 7or 12 day to lactating goat during breeding season and IM injection of 400 IU (PMSG) and recorded pregnancy rate 55%. Estrus response to intra vaginal sponges varies greatly and depends on breed, co treatment, management and mating system. However, the data is almost consistent to the heat induction with vaginal sponges in temperate goat breeds (8, 32). Ultrasonography is a non-invasive and it plays valuable roles in diagnosis of various physiological and pathological conditions of the reproductive organs of ruminants (33,34). Early diagnosis of pregnancy and fetal sexing using ultrasonography enhances reproductive management on farms and improves the commerce of pregnant animals (20, 35-37).

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Hormonal improvement of pregnancy rate in cows suffered from repeat breeders

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ABSTRACT

The present study was conducted in many farms in Basra province, from November 2014 to April 2015. The study sample included 50 cows suffered from repeat breeders syndrome. The sample were treated by two hormonal regime treatments of repeat breeder cows like human chorionic gonadotropin (hCG) and progesterone. The results showed that the injection of these hormones improved the pregnancy rate (the injection of hCG recorded 50% as pregnancy rate), while the injection of hCG and progesterone recorded 70% as pregnancy rate.

Keywords: repeat breeding, hCG, progesterone

الملخص باللغة العربية

أجريت هذه الدراسة في عدد من الحقول في محافظة البصرة، في الفترة من شهر تشرين الثاني/ نوفمبر عام 2014 لغاية شهر نيسان/ إبريل عام 2015. شملت عينة الدراسة 50 بقرة كانت تعاني من تكرار الصراف، وقد تمت معالجتها بنظامين من الهرمونات، النظام الأول اعتمد على الهرمون الغذائي المشيمي وقد سجل نسبة حمل 50% ، أما النظام الثاني اعتمد تطبيق هرمون البروجستيرون مع الهرمون الغذائي المشيمي وكانت نسبة الحمل 70%.

INTRODUCTION

Repeat breeder cows syndrome is a condition in which the cows have normal estrus cycle , no abnormality in the vaginal discharge, no palpable abnormality in the reproductive tract, but they have failed to conceive after three or more numbers of services by a fertile bull or inseminated by good semen quality (1-3). Repeat breeder is a substantial problem in cattle breeding leading to large economic loss for the dairy producer due to more inseminations. Repeat breeder has multifactorial etiology such as infectious or inflammatory processes, nutritional deficiencies, management practices, hormonal disturbances that lead to ovulatory disturbances. Ovulatory disturbances are one of the main causes of it. An ovulation and delayed ovulation characterized by fertilization failure and / or embryonic death are two other major causes of repeat breeder in high yielding crossbred dairy cows (1). These conditions remain undiagnosed unless repeated per rectal examination of the reproductive tract is undertaken. In dairy cows, luteal insufficient and lower progesterone concentrations are known as a cause of embryonic mortality and reduce the pregnancy rates during early embryonic development (4). During the pre-implantation phase of embryonic development , direct progesterone supplementations and GnRH / hCG injections are the approaches to improve embryonic survival in repeat breeder cows (5) . It has been hypothesized that increasing peripheral progesterone concentrations during the diestrus after insemination may improve embryonic development and may suppress luteolysis resulting in reduced embryonic loss (6).

Hormonal disturbances

The hormones have the majestic role in the control of the reproduction. All of the reproductive functions and developments are done under the hormonal control, from ovulation, to implantation, and growth of embryo until the parturition. The defects in any hormone may change or affect the establishment of pregnancy (7). Progesterone represents one of the most important hormones, which interacts with the pregnancy. During the first stage of pregnancy, the progesterone concentration is essential for both uterine secretions and the survival of the embryo or fetus (8). In dairy cattles, the luteal insufficiency and low level of progesterone concentrations represent the major causes of embryonic mortality and reduce conception rates during early embryonic development . (9) found about 50% of embryonic losses in buffaloes due to low level of progesterone. The aim of this study was to improvement of pregnancy rates and efficacy of reproduction performance in cows suffered from repeat breeders by using different protocols of hormonal treatment

MATERIALS AND METHODS

1. Expermental animals:

This study was conducted on 50 cows in different farms in Basra province, from November 2014 to April 2015. This study depended on the case-history of these cows from owners, 50 cows suffered from repeat breeder problem. All cows were healthy and the clinical examination to the reproductive system was normal from any defect, and their parity range from 0 to 5.

Cows belonging to different breeds (cross-breed Holstein) were considered for hormonal treatment. The cow body weights ranged between 200-350 kg BW . Body weights of the animals below 200 kg were not considered for the study. Animals suffering from clinical reproductive problems were also excluded. A total of 50 repeat breeder cows were considered for application of three different protocols. For the first protocol (figure 1), 10 cows were selected. They were subjected to rectal palpation. On palpation of functional CL, 1500 IU of Chorulon (Human chorionic gonadotropin) was injected IM at day 15th of estrous and 2nd Chorulon on a day of estrous and were inseminated at fixed time twice.

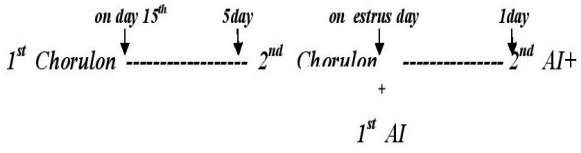

Figure (1): Human chorionic gonadotropin. Chorulon based protocol 1st and 2nd LH , A.I. –Artificial Insemination, 1st AI 120 hrs. post 1st LH (on estrous day), 2nd AI 24 hrs. post 1st AI.

For the second protocol (figure 2) 10 cows were selected. Chorulon was injected on estrous day with 1st AI and then 2nd AI and progesterone was injected 24 hrs. post injection of Chorulon and after 24 hrs. progesterone (5 ml) injected daily for 5 days.

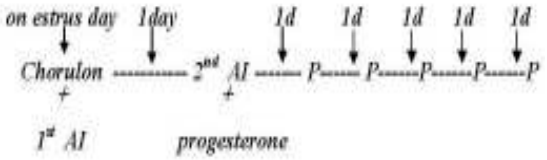

Figure (2): Chorulon LH and based protocol progesterone on estrous day with 1st AI and 2nd AI and progesterone was injected 24 hrs. post Chorulon was done 24 hrs progesterone (5 ml) injected daily for 5 days.

For the third protocol, 10 cows were selected. The protocol is similar to the second protocol without injection of Chorulon (figure 3).

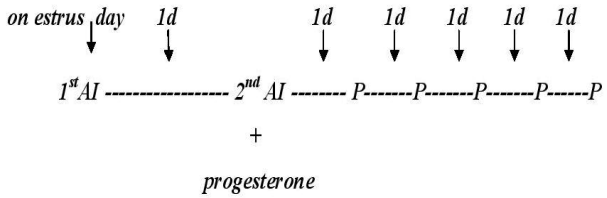

Figure (3) : Progesterone , based protocol progesterone A.I. – Artificial Insemination 1st AI on estrous day post 24 hrs. the 2nd AI and progesterone, then 5 ml progesterone injected daily for five days.

For the forth protocol (figure 4) 10 cows were selected. On palpation of functional CL, 1st Chorulon was injected on day 15th of estrous and (1500 IU) of 2nd Chorulon was injected on estrous day post 1st Chorulon injection with 1st A.I then 2nd A.I was done after 24 hrs. post 1st A.I then progesterone (5 ml) injected with 2nd A.I then 5 ml progesterone injected daily for five days.

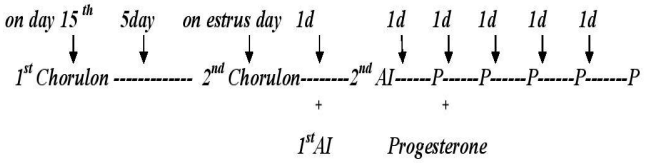

Figure (4): Chorulon LH and based protocol progesterone. Chorulon was injected on day 15th of estrous and 2nd Chorulon injection with 1st A.I at estrous day then 2nd A.I with progesterone was injected 24 hrs post 2nd Chorulon, then progesterone (5 ml) was injected daily for 5 days.

For the fifth protocol (figure 5), 10 cows were selected. Chorulon (1500IU) was injected on estrous day with 1st AI and 2nd AI at 24 hrs post Chorulon injection.

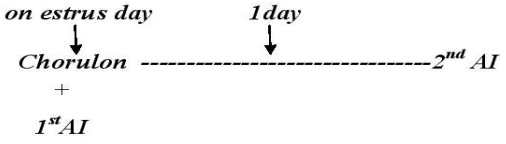

Figure (5): Chorulon LH and based protocol Chorulon A.I. –Artificial Insemination and chorulon with injection at estrous day then 2nd AI at 24 hrs post 1st AI.

These cows were suffering from repeat breeding , and the cows that suffering from reproductive problems were neglected. By rectal palpation, the status of C.L. and uterine tone could be judged. Lysis of C. L. About 70% of the cows were confirmed in estrous observed by swollen vulva and uterine tone on rectal palpation. Cows were diagnosed for pregnancy via rectal palpation on day 72 post A.I Pregnancies resulting from A.I. were validated by rectal palpation by a second technician and by calving dates results for number of pregnant animals**.**

2. Statistical analysis

The Results are expressed as; means calculated by Qi square test, and significant difference for comparison. The Difference between the means were considered significant at ($p<0.05$).

RESULTS AND DISCUSSION

Effect of progesterone on repeat breeders

The percentage of progesterone hormonal treatment system (protocol) was 20% as in table (1), it agreed with (11,12), Where progesterone is very important hormone to success the pregnancy, because of its role in preparing the uterus to receive and nourish the embryo (10,11). The defect in the progesterone may lead to early embryonic death and lead to repeat breeders cow syndrome (12). While were disagree with (13). It appears that inducing accessory CL, thereby increasing progesterone, may improve fertility in repeat breeder dairy cows.

Administration of hCG following AI

Several studies have investigated that the effects of human chorionic gonadotropin (hCG) on fertility with little or no effects realized. However, few studies have utilized large number of cows to assess the effectiveness of hCG on conception rates and pregnancy loss of high-yielding dairy cows under field conditions (14).

The effects of hCG administered on day 5 after AI on CL number, conception rate, and pregnancy loss in high-producing dairy cows were evaluated by (15). A total of 40 cows were injected with either hCG after AI. Treatment with hCG on day 5 resulted in 70% of the cows with more than one CL as in table (1). However, there were differences between groups for number of pregnancy losses. Therefore, The benefit of hCG to increase pregnancy rate. while were disagree with A recent study with embryo transfer recipients detected an increase in pregnancy rate of recipients treated with hCG (16).

Pregnancy rate in cows receiving hCG on day 5 was higher (70%) or cows receiving hCG on day 1 (42.5%) after estrus. This reinforces that induction of an accessory CL and increased progesterone concentrations reduce early embryonic mortality in cattle. In another study utilizing repeat breeder cows, hCG was given at day 5 post AI (17). There was a significant ($P < 0.05$).

The results revealed that there is high percentage of conception in fourth group where it was (70%) then the 2nd group followed it where was (60%), the 5th group the percentages of conception was (50%) ,while in the 1st group was (40%). For the 3rd group, the percentages was the lowest one, where it was (20 %) among the five groups of study (table 1).

These differences are probable or may be caused by environmental conditions or the breeds or to different site all over the world or this difference may be due to resistance of some cows to the hormonal treatment or hormonal programs or may be due to causes not hormonal but may be uterine inflammation such as sub-clinical endometritis or due to unknown and undiagnosed reasons.

Table (1): percentages of conception rate

Treatment group		Cow not return to estrus after 21 days
T1	D	10/4 (40%)
T2	B	10/6 (60%)
T3	E	10/2 (20%)
T4	A	10/7 (70%)
T5	C	10/5 (50%)

CONCLUSION

Throughout this study, the following points were concluded:

1- The study described LH decline as one of the causes of repeat breeders cow.

2- Double artificial inseminations is impartment for decrease cases of repeat breeders cows.

3- Administration of LH and progesterone is impartment for increase pregnancy rate and decrease RBC syndrome.

RECOMMENDATIONS

According to the results and conclusions, the following recommendations are suggested:

1- Apply new protocols in cases of RBC syndrome.

2- Apply new protocols such as hormonal treatment with antibiotics.

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Morphological and histochemical study of harderian gland in local buffalo *Bubalus bubalis*

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ABSTRACT

The aim of the present study was to investigate the morphological and histochemical structures of the harderian gland in local buffalo (*Bubalus bubalis*) . Head of ten healthy adults of both sexes (male and female) were obtained and processed routinely to observe the morphology and histochemical structures of the gland that was located ventromedially around the posterior part of the eyeball in the base of third eyelid, supported with T-shape hyaline cartilage that extend into the gland. H.G was lobulated and elongated in shape , light yellow color with irregular out line as well as the gland enveloped by a membrane which penetrated the gland .The mean length of H.G. was 40.8± 0.30 mm ,the mean width was 17.25± 1.93 mm, while the mean of thickness was 4.88±0.58 mm. In general, histological of H.G. showed lobulated appearance that surrounded by connective tissue capsule, which send septa into the gland divided it into several lobules. H.G. consists of acinar serous and mucous units lined by simple columnar epithelium tissue. Histochemially, H.G. was positive to periodic acid Schiff, as well as the masson trichrome stained connective tissue septa surrounded the acinus .H. G. strongly stained with Alcian blue pH 2.5, especially the deep region of gland that near the hyaline cartilage while the peripheral gland was less reaction, whereas the gland was weakly stained with vanGison stain.

Keywords: *H.G., Bubalus bubalis*

الملخص باللغة العربية

هدفت هذه الدراسة إلى تحديد الصفات الشكلية والكيمونسيجية لغدة هاردر في الجاموس المحلي. تم أخذ عشر نماذج من رؤوس الحيوانات البالغة والخالية من الأمراض من كلا الجنسين، وخضعت لسلسلة من التحضيرات لملاحظة شكل الغدة والتحضير للفحص المجهري لها.

تقع غدة هاردر حول الجزء الخلفي لكرة العين في قاعدة الجفن الثالث مدعومة بغضروف زجاجي على شكل T يمتد وسط الغدة . تغلف غدة هاردر بغشاء يمتد داخل الغدة وهي تبدو مفصصة ذات لون أصفر باهت مع شكل خارجي غير منتظم . متوسط طول غدة هاردر في الجاموس المحلي كان 40.8±0.30 mm ، بينما معدل العرض 17.25±1.93 mm ، وكان معدل السمك 4.88±o.58 mm . بشكل عام، نسيجيا تتكون الغدة من عدة فصوص محاطة بمحفظة من نسيج رابط يخترق الغدة ويقسمها إلى عدة فصوص وفصيصات، وهي تتكون من وحدات إفرازية عنبية الشكل مصلية ومخاطية الإفراز مبطنة بنسيج طلائي عمودي بسيط.

أما كيميائيا، فقد أبدت الغدة تفاعلا إيجابيا لحمض البيريودك شيف (PAS) والماسون ثلاثية الكرومات، إذ صبغت الحواجز المحيطة بالوحدات الفارزة . وكانت الغدة شديدة التفاعل مع الاليشين بلو (الأس الهيدروجيني 2.5) ، خاصة المناطق العميقة القريبة للغضروف، أما المنطقة البعيدة عن الغضروف كانت اقل اصطباغا. كما كانت الغدة ضعيفة التفاعل مع صبغة فان كيزن.

INTRODUCTION

The Harderian gland is a gland found within the eye's orbit, which occurs in tetrapods the possess a nictitating membrane (1) . In mammals, the gland is well developed, especially in rodents such as rats and hamsters (2). Externally, the harderian gland is enveloped by a membrane, which penetrates the gland and divides it into several lobules (3,4). Harderian gland in mammals excretes an oily substance used to preen the fur (5). Histologicaly in mammals, the secretory tubules of the harderian glands are usually formed by a simple epithelium composed of cuboidal cells (6). The gland can be compound tubular or tubuloalveolar and the fluid it secretes (mucous, serous or lipid) varies between different groups of animals, as well as the gland has several function including that photo protective organ, a location of immune response, a source of thermoregulatory lipids, a source of pheromones and site of osmoregulation (1). In the present study, the morphological, histological and histochemical structure of this gland in local buffalo were investigated.

MATERIALS AND METHODS

Heads of ten healthy adult of local buffalo of both sexes were obtained from slaughter house. The harderian glands were dissected out and then these glands were fixed in 10 % buffered formalin solution and embedded in paraffin blocks, sections 5µm were made and stained with haematoxylin and eosin for general histological examination by light microscope. Several sections were made for special stain to obtain the collagen fibers with Masson-strichrom. Periodic acid-Schifft (PAS) reaction was employed to determine neutral mucosubstance and Alician blue (pH 2.5) was used for determining acidic muco substances (6).

RESULTS

Results of the study revealed that the harderian gland of local buffalo was lobulated tubuloalveolar, elongated in shape, light yellow color with irregular outline organ , located ventromedially around the posterior part of the eyeball in the base of third eyelid supported with T-shaped cartilage and this cartilage extends into the gland (figures 1-A, 1-B) . Externally, the haderian gland enveloped by a membrane which penetrated the gland divided it into several lobules .The mean of morphometrical parameters of the harderian gland was 4o.8±0.30 mm in length , width 17.25 ± 1.93 mm , and 4.88± o.58 mm (table 1).

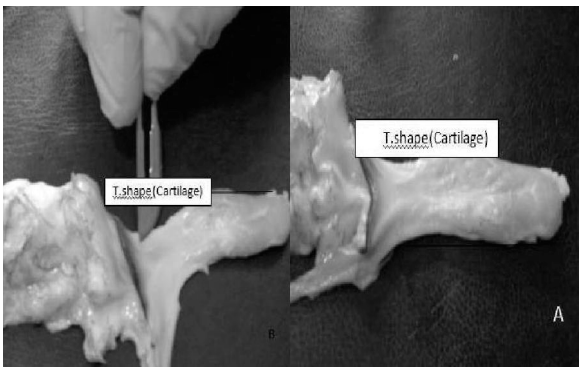

Figure (1): A:Morpholoical Shape of Harderian Gland explain thickness of first part of H.G. B:A:Morpholoical Shape of Harivan Gland explain thickness of second part of H.G

Table (1): Mean of length and width and thickness of haderian gland. significant with (p<0.05).

Length	M+_S.D 40.8 + _0.30 mm	S.R (0.95)
Width	17.25+_1.93mm	(0.611)
Thickness	4.88+_0.58mm	(0.18)

Histologically in general stain (figure 2-A), the Harderian gland H.G. showed lobulated appearance and it surrounded by connective tissue capsule which sends septa into the gland divided it into several different lobes and lobules as well as the connective tissue contain the normal components at connective tissue such as collagen fiber ,nerve and blood vessels. H.G. consists of alveal small lumen acini lined by simple columnar epithelium cells of varying height .the acini was composed of two types of neighboring cells ,the first mucus cells was dark stain and the second was light with circular nucleus (figures 2-D, 2-E). There are such duct in this gland the interlobular duct was lined by simple columnar epithelial tissue and the duct bifurcated the lobule into intralobular duct lined by simple columnar epitheilial tissue (figure 2-D).

The harderian gland was strong reaction and the glandular cells stained with dark pink during Periodic acid Schifft (figures 2-C, 2-F) (PAS), while in case of the Alcian blue (figure 2-G) (pH 2.5). The apical part of glandular cells was strong, whereas in the rest of the cytoplasm weak stain in other hand the peripheral of gland stained dark in compare with central of it (figure 2-G). The Masson-strichrome stained connective tissue septae surround acinus in harderian gland with blue color (figure 2-B), while the stoma gland stained with pink color. The gland was weakly reaction for Vangison stain (figure 2-F).

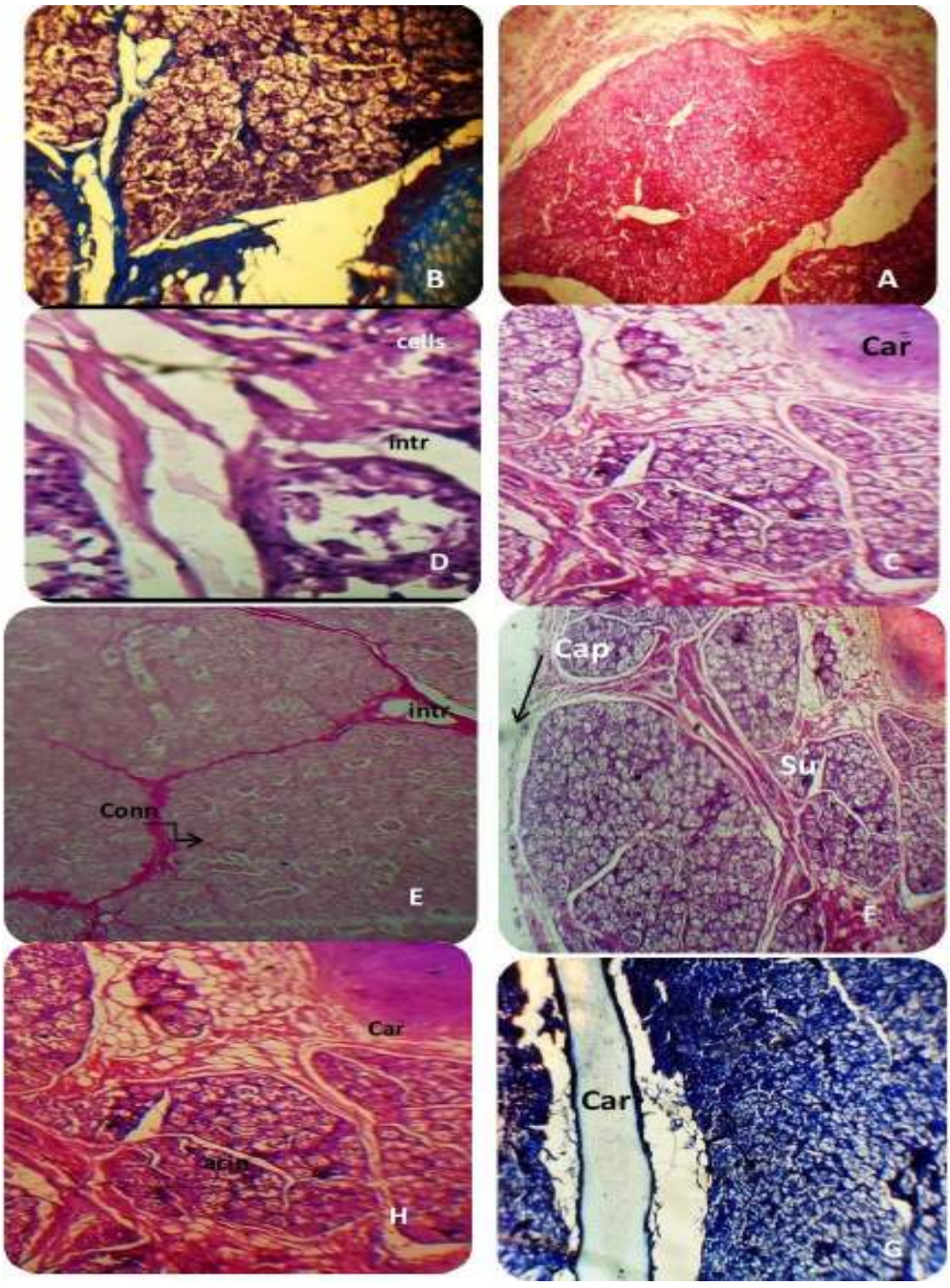

Figure (2): A)histoloicalsecation through harderian gland showed stain H&E ,10X ,B):section though, C)section though gland PAS stain 40X , D) section though GLAND stain H&E ,40X E) section though SECRTORY UNIT , 40X F) section though secretary unit stain Van Ggison 40X G)section though CARILAGE AND SECRTORY UNIT Alacin blue stain 10X , H)) section though GLAND stain H&E (40X)

DISCUSSION

Results obtained by this study found that the Harderian gland(H.G.) in local buffalo was lobulated and elongated with irregular outline. In a study conducted by (9), the superior gland of the third eyelid in camel was observed to be oval shaped and composed of tubuloacinar units. AS well as the study showed that H.G. supported with T-shaped hyaline cartilage that extended into the gland. this result was in agreement with (7-9) in ruminant the third eyelid gland is supported by a T-shaped piece of hyaline cartilage, whereas the type of cartilage in cat , horse and pig was elastic (10) . Means parameters of H.G. in buffalo it was 40.8mmleught ,width was 17.4 THICHNESS 4.88 $\pm$ 0.58 mm histological this study thickness while in sheep (9)showed the superior gland thired eyelid measures 22mm in height ,14 mm width and its s 4.5 mm . In Camel the mean lengh 28.7 mm and mean width was 17.4 mm(7) .Histoloically, this study showed the H."G composed of several different lobes and lobules surrounded by connective tissue capsule this gland consist of small lumen acinar lined by simple columnar epithelial cells of varying height where as American bison and cattle (11). The Harderian gland was revealed tubuloalveolar combined with large lumens gland. This finding is supported by (7,12), who showed the H.G well developed composed tubulaoalveolar capsulated and secretory unit showed a narrow lumen irregular lined with two types of secretary cells columnar cells and another lined with cuboidal cells.

In rabbit the superficial gland was composed of alveolar arrangement (13). This finding indicated the H.G. in local buffalo was supported by hyaline cartilage in the central of gland that similar to the hyaline cartilage shaft of third eyelid gland in camel (7) and sheep (9) In opposite side the H.G. was supported by elastic cartilage in horse , pig and cat (10 , 14). In this current study H.G. has inter and interalobular duct lined by simple columnar epithelial tissue . According (15) in Wister rat there is no ducts were observed within the H.G. there was specialized excretory duct which originated in the hilus this duct lined by a stratified , cuboidal epithelium tissue . Histochimicaly we are observed H.G. was positive for PAS , Alician blue pH 2.5 and it strongly stained this agreement with (11) in American Bison and cattle , Lizard podarcis(16) and three Balaenopterid species(17), they indicated the H.G. characterized by the presence of both acid and neutral mucosubstance therefore it was positive for PAS ,Alician blue 2.5. Masson trichrom reaction was appeared connective tissue present in septa between acinae was blue in color that is the same in (7)who showed connective tissue septa which surrounded individual acinus and tubules in camel superior gland stained well with Masson-strichromstain.H.G. in buffalo was weakly stained with Vangison that reveled the gland has few fiber.

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Study the effect of proanthocyanidin and ranitidine on fertility efficiency in adult female rabbits with gastric ulceration induced by indomethacin

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ABSTRACT

This study was done to induce gastric ulceration in female rabbits by using indomethacin in dose 75mg/kg for two days, and to study the effect of that on fertility and pregnancy efficacy. In addition, this study was an attempt to investigate the curing effect of proanthocyanidin extracted from grape seeds (*Vitis vinifera*) on female reproductive dysfunction caused by giving indomethacin. Thirty adult female rabbits weight ranged between (1500-2000.0 mg) were used in this study, divided into five equal groups (6 rabbits/group) as the following: Group1:- called negative control group, drenched 3 ml of normal saline for 10 days; Group 2:- (positive control group) was drenched indomethacin drug (75mg/kg B.W.) to induce gastric ulceration for two days; Group3:- at first drenched indomethacin (75mg/kg B.W.) for two days, followed by giving proanthocyanidin extract (100mg\kg B.W.) for 10 day; Group 4:- initially drenched indomethacin (75mg/ kg)for two days, followed by giving proanthocyanidin extract (200mg/ kg) for 10 days; Group 5:- was given indomethacin (75mg/ kg) for two days, followed by giving ranitidine (50mg/ kg) for 10 days. The obtained results revealed that a significant decrease ($P \leq 0.05$) in serum concentrations of FSH, LH, E_2, P_4 have been shown in serum positive control group and ranitidine group compared with negative control group and proanthocyanidin at a dose (100mg/kg and 200 mg/kg). In addition to, the rate of fertility was16.66% in female rabbits that treated with indomethacin and 50% in female rabbits that treated with ranitidine and 100%in groupstreated with proanthocyanidin at a dose (100mg/kg and 200 mg/kg) compared with negative control group, in which fertility rate 83.33%. There is reduction in number and weight of newborns with occurrence of several mortality and malformation during pregnancy in positive control group. Our conclusion of this study is that GSE may be promising as a natural therapeutic agent, can be used as get rid of indomethacin side effect on female reproductive functions.

Keywords: Proanthocyanidin, ranitidine, Fertility, Gastric Ulcer, Rabbits, Indomethacin

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الملخص باللغة العربية

سعت هذه الدراسة إلى إحداث قرحة معدية في إناث الأرانب بواسطة عقار الأندوميثاسين بجرعة 75ملغم/كغم لمدة يومين، بهدف دراسة تأثيرها على الكفاءة التناسلية والحمل لدى الأرانب، بالإضافة إلى دراسة التأثير العلاجي لبروانثوسياندين المستخلص من بذور العنب (كرمة العنب الأوربي) على الاختلال الوظيفي للجهاز التناسلي الأنثوي الناجم عن إعطاء عقار الأندوميثاسين. استخدمت الدراسة ثلاثين أنثى أرنب بالغة تراوحت أوزانها بين (1500− **2000** ملغم) وقسمت إلى خمس مجموعات متساوية، كل مجموعة تكونت من ستة أرانب بالشكل التالي: المجموعة الأولى أعطيت جرعة مقدارها (3 مل) من المحلول الملحي الفسلجي الطبيعي، لمدة عشرة أيام واعتبرت مجموعة السيطرة السالبة. المجموعة الثانية أعطيت الاندوميثاسين بجرعة مقدارها 75ملغم /كغم لمدة يومين لإحداث القرحة واعتبرت مجموعة السيطرة الموجبة. المجموعة الثالثة أعطيت ً الأندوميثاسين أولا بجرعة مقدارها 75ملغم /كغم لمدة يومين، ثم جُرعت البروانثوسياندين بجرعة مقدارها 100ملغم /كغم لمده عشرة أيام. المجموعة الرابعة أعطيت الأندوميثاسين بجرعة مقدارها 75ملغم/كغم لمدة يومين ثم جُرعت البروانثوسياندين بجرعة مقدارها 200 ملغم /كغم لمدة عشرة ايام. المجموعة الخامسة أعطيت الاندوميثاسين بجرعة مقدارها 75ملغم /كغم لمدة يومين، ثم أعطيت الرانتدين بجرعة مقدارها 50ملغم/ كغم لمدة عشرة أيام.

أظهرت نتائج الدراسة أن هناك انخفاضا معنويا عند مستوى ($P \leq 0.05$) في التركيز المصلي لبروجستيرون(P4)، الأستروجين(E2)، الهرمون المنبه للجريب(FSH) ، والهرمون المنشط للجسم الأصفر(LH) في مصل مجموعة السيطرة الموجبة ومجموعة الرانيتيدين، مقارنة مع مجموعة السيطرة السالبة والبروانثوسياندين بجرعة (100ملغم/ كغم و 200 ملغم / كلغم). وكان معدل الخصوبة 16.66٪ في إناث الأرانب التي أعطيت الإندوميتاسين و 50٪ في إناث الأرانب التي أعطيت الرانيتيدين و 100٪ في المجموعات التي أعطيت البروانثوسياندين بجرعة (100ملغم/ كغم و 200 ملغم / كلغم) مقارنة مع مجموعة السيطرة السالبة التي كان معدل الخصوبة فيها 83.33٪. وايضا كان هناك انخفاض في عدد وأوزن الحيوانات حديثي الولادة، مع حدوث عدة وفيات وتشوهات أثناء فترة الحمل في مجموعة السيطرة الموجبة. وبذلك خلصت الدراسة إلى أن البروانثوسياندين المستخلص من بذور العنب يمكن أن يكون علاجا طبيعيا يستخدم للتخلص من الآثار الجانبية للأندوميثاسين على وظائف الجهاز التناسلي الأنثوي

INTRODUCTION

In the gastric mucosa, Prostaglandins (PGs) E and F2α have been shown to be synthesized. PGE2-like material was also shown to be present in gastric Juice. Prostaglandins protect the gastric mucosa against injuries. One rate-limiting step in prostaglandin synthesis is mediated by prostaglandin endoperoxide synthase (PGHS), the target enzyme of non-steroidal anti-inflammatory drugs (NSAIDs).Two isoforms of PGHS exist: a constitutive (PGHS-1) and an inducible (PGHS-2) enzyme. Non-steroidal anti-inflammatory drugs (NSAIDs) are common medications used for the curing inflammatory diseases due to their abilities to decrease swelling, pain of inflammation, fever and headache(1). Enzyme in the NSAIDs acts by inhibition of cyclooxygenase (COX) that is the rate limit prostaglandins (PG) synthesis (2). COX-1 and COX-2 have important role in ovulation, fertilization and implantation of ovum, in addition to angiogenesis for setting up of placenta (3-4). NSAIDs inhibit ovulation in all mammalian species (5-7). Inhibition of COX-2 and prostaglandin (PG) synthesis by NSAIDs is the main cause of anti-ovulatory properties of these drugs (8-11). Prostaglandins are necessary mediators of ovulation, they have the ability to prod mobilization of granulosa and theca interna cells within the ovaries. COX-2 dependent PGs may have the ability to generate proteolytic enzymes that cause follicles rapture (12). The relationship between prostaglandin and ovulation is depended on many guides as the following:

1- preovulatory follicles produce prostaglandins (PGs) in response to the preovulatory LH surge, prostaglandins (PGs) reach highest concentrations around the time of ovulation(13-15).

2- Prostaglandin synthesis and ovulationare inhibited by indomethacin, which is one of non-steroidal anti-inflammatory (5-7,16).

3-ovulation failure was observed in mice which have genetic deficiency incyclooxygenase-2 (COX-2) or PGE_2 receptors (17-18).

Rats treated with indomethacin, most newly formed corpora lutea showed abnormal follicle rupture at the basolateral sides. Moreover, granulosa cells and follicular fluid invaded ovarian stroma and blood and lymphatic vessels was observed. $PGE_{1\alpha}$, inhibited abnormal follicle rupture and restored ovulation while $PGF_{2\alpha}$ was only partially effective in inhibiting abnormal follicle rupture and restoring ovulation (19). Indomethacin inhibit the tissue changes that occur at apex of follicle during ovulation that lead to prevent follicular rapture (20-22). Antiovulatory action of indomethacin is mediated by COX-2 inhibition because treatment with prostaglandin restored ovulation in indomethacin-treated rats(19). Rats treated with indomethacin show series alteration in ovarian, this due to the release of the cumulus-oocyte complex (COC), granulosa cells, and follicular fluid to the ovarian interstitium. Also Cycling rats treated with indomethacin during the preovulatory period show abnormal ovulation (23). However, high doses of indomethacin inhibited ovulation significantly in in rats (24-25). While the use of low-dose indomethacin can abolish ovarian PGE2 synthesis but low-dose indomethacin failed to affect ovulation (25).

Abnormal follicle rupture is responsible for the antiovulatory action of indomethacin (19). A previous study in monkeys and women treated with COX inhibitors denoted that delayed ovulation (26) or failure follicle rupture (4) were the main causes of NSAID-induced ovulatory dysfunction. Inhibition of follicular rapture by indomethacin is due to inhibition of proteolytic enzyme needed for ovulation (5-7). The inhibitory action of indomethacin was not due to the inhibition of follicle rupture, but rather lead to the induction of abnormal spatial target of follicle rupture (27-29), but follicles rapture occur at any site of follicles wall(27-28). However this could explain existence of some ovulated oocytes till in use higher of indomethacin doses (7). The ovulation inhibited significantly in high doses of indomethacin in rats (24-25). While the use of low- dose indomethacin can abolish ovarian PGE2 synthesis but low-dose indomethacin failed to affect ovulation (25).

MATERIALS AND METHODS

Drugs and chemicals

Indomethacin was obtained from Safa company Diyala-Iraq, and ranitidine was provided from Glaxo Smith Kline, S.A. Aranda de Duero, Spainwere suspend in 2 ml of normal saline. Serum concentration of FSH, LH, estrogen and progesterone were determined by using commercial ELISA kits Mono bind Inc. lake forest CA 92630, USA.

Plant Material

Proanthocyanidin was extracted from black grape seeds that were used in this study. The black grape was hand-picked from local market with full skin intact. It was washed with tap water, the skin and fleshes were removed and the seeds are dried .The seeds of the grape were turned to powder with the help of an electric grinder and kept in dark container at 25C°.

Preparation of proanthocyanidin extract from grape seeds

Fifty grams of dried grape seeds powder were defatted with (500 ml) of n-hexane for 2 hrs. by soxhlete. The combined n-hexane extract was concentrated below 50°C under reduced pressure in a rotary evaporator to get 7ml of yellow oily mass. This mass was dried at room temperature and further (40 gm.) was refluxed in (500ml) methanol

(80%) in water with 3% hydrochloric acid for one hour then filtered by Buchner funnel and filter paper (Wattman No.185) .The filtrate was extracted with an equal volume of chloroform to remove pigments. The alcoholic layer was extracted with an equal volume of ethyl acetate treated with 2% of hydrochloric acid ,the ethyl acetate layer was concentrated by rotary evaporator at 45˚C and dried at room temperature (30-31). The resultant extract (2.5 gm) was pink color and dry. The extract was kept in dark glass container at 4°C (figure 1).

Figure (1): steps for preparation of proanthocyanidin

Experimental animals

Thirty adult female rabbits weight ranged between (1500-2000.0mg) were kept for an adaptation period for one month at the animal house of Veterinary Medicine College / Basrah University. The experimental animals were kept in individual cages, provided with ration composed fodder in addition to green alfalfa (Medicago *sativa*) and tap water *ad libitum* and given a prophylaxis drug against coccidiosis (Amprollium 1g/L of drinking water).

Experimental design

The rabbit divided into five group comprising of 5 animals in each as the following:
Group1:- healthy (-ve control group) oral administration 3ml of normal saline (0.9 of NaCL) for 10 days.
Group 2:- oral administration with indomethacin 75mg\kg B.W. for dissolve with 3ml of normal saline two days(+ve control) group and remain without treated for10 days.
Group 3:- treated with indomethacin 75mg\kg B.W. dissolve with 3ml of normal saline for two days, then treated with proanthocyanidin 100mg\kg B.W. dissolve with 3ml of normal saline for 10 days.
Group 4:- treated with indomethacin 75mg\kg B.W. dissolve with 3ml of normal saline for two days, then treated with proanthocyanidin 200mg\kg B.W. dissolve with 3ml of normal saline for 10 days.
Group 5:- treated with indomethacin 75mg\kg B.W. dissolve with 3ml of normal saline for two days, then treated with ranitidine 50mg\kg B.W. dissolve with 3ml of normal saline for 10 days.

Induction of gastric ulcer

Gastric ulcers were induced in twenty four non starved rabbits by giving indomethacin (Safa company Diyala-Iraq) orally by one ml size syringe and in dose 75mg/kg for two days (figure 2).

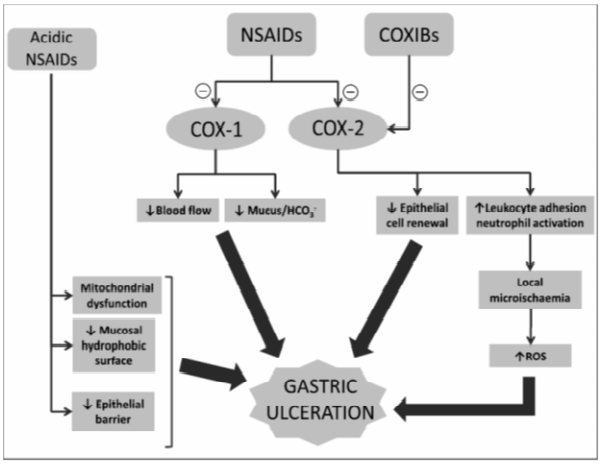

Figure (2): pathways for induction of ulcer by indomethacin

Reproductive efficiency

1- Fertility activity:
After 10 days of treating female rabbits caged overnight with males (2♀:1♂) of proven fertility. Vaginal smears were examined on the following morning for the presence of spermatozoa in the vaginal smears, this was considered as indication of pregnancy, and this day was counted as day 0 of pregnancy.

2- Measurement of the length of gestation period:
In this experiment, the treated pregnant rabbits were carefully noticed until normal delivery occurred. At day 2 or 3 before delivery, the rabbits were caged individually. The time and day of delivery was recorded. In addition, weight of the litters was recorded.

3- Implantation Site:
In this experiment, after delivery of pregnant rabbits were sacrificed immediately for watching the effect of treatments on implantation site of blastocysts, as described by (32). The animals were sacrificed, their uteri were exposed and opened by cutting longitudinally to expose the bluish implantation sites, to be counted. Weight of uterus and the left and right ovaries were taken. The ovaries were removed from each animal, placed on a Petri–dish and the number of corpa lutea were counted. The percentage of success of implantation was calculated according the following equation:

$$\text{Percentage of success (Ps)} = \frac{\text{No. of implants}}{\text{No. of corpa lutea}} \times 100$$

4- Collection of Blood Samples:
Blood samples (15ml) were collected from each animals at end of experiment by the heart (cardiac puncture). The blood was deposited into tube without anticoagulant and then the blood samples were centrifuged at (3000 rpm) for 15 minutes and serum samples stored in polyethylene eppendorff tubes at (-20ºC), which used for studied hormonal analysis (FSH, LH, estrogen and progesterone).

5- Histological techniques:
The animals were sacrificed from all groups at the end of the experiment (after42 days from the beginning of the experiment) stomach, ovary and uterus were fixed in 10% buffered formalin, dehydrated progressively in increased ethanol concentrations, treated with xylene and embedded in paraffin. Five microns thickness sections of paraffin-embedded tissue were mounted on glass slides and stained with Hematoxyline and Eosin stain (H and E stain) (33-34).

Statistical analysis

The results of the present study were analyzed by using two-way covariance (ANOVA) test in all study. All statistical calculations were carried out by the aid of the statistical package SPSS V. 11 (SPSS Inc.). The data were expressed as means ± standard deviation (X ± SD). Least significant different test (LSD) was calculated to test difference between means of groups and subgroups (Stat soft, 2006).

RESULTS

The mean values of FSH, LH, Estradiol (E_2), Progesterone (P_4) concentrations as presented in the table (1). The results indicated a significant ($P \leq 0.05$) decrease in serum FSH concentration in female rabbits with gastric ulceration (+ve control group) and ranitidine group compared with (-ve control group) and another treated groups (PA at a dose 100mg/kg and PA at a dose 200 mg/kg).
LH concentration was a significantly ($P \leq 0.05$) decreased in serum of female rabbits with gastric ulceration (+ve control group) and ranitidine group compared with (–ve control) group and another treated groups (PA at a dose 100mg/kg and PA at a dose 200 mg/kg) respectively.
LH concentration was a significantly($P \leq 0.05$) increased in groups treated with(PA at a dose 100mg/kg and PA at a dose 200 mg/kg) respectively compared with (–ve control) group.
E_2 concentration was significantly ($P \leq 0.05$) decreased in serum female rabbits with gastric ulceration (+ve control group) and ranitidine group compared with (-ve control group) and another treated groups (PA at a dose 100mg/kg and PA at a dose 200 mg/kg) respectively.
P_4 concentration was significantly ($P \leq 0.05$) decreased in serum female rabbits with gastric ulceration (+ve control group) and ranitidine group compared with (-ve control group) and another treated groups (PA at a dose 100mg/kg and PA at a dose 200 mg/kg) respectively.
The results presented in (table 2 and figure 3) revealed affected fertility by treatment with indomethacin (+ve control) group and ranitidine group. Not all female rabbits were mated with male and showed positive spermatozoa became pregnant. The results revealed some female rabbits treatment with indomethacin (+ve control) group and ranitidine inability pregnancies compared with (–ve control) group, 16.66% fertility rate in female rabbits treated with indomethacin (+ve control) group and 50% fertility rates in gastric ulceration of femalerabbit treated with ranitidine and 100% fertility rate in gastric ulceration female rabbits treated with(PA at a dose 100mg/kg and PA at a dose 200 mg/kg) respectively compared with (–ve control) group fertility rate 83.33% (table 3, figures 4-17). The rabbits treated with indomethacin (+ve control group) revealed a significant ($P \leq 0.05$) decrease newborns weights and revealed significant ($P \leq 0.05$) increase gestation of period and presence malformation in some newborns, the gestation period affect, and in this study found that those rabbits that had treated with indomethacin (+ve control) group suffered from partial reproductive failure. These effects were seen in the smaller size of their litter, the number of stillbirth and early fetal deaths. Moreover the rabbits treated with ranitidine revealed non-development of fertile ova after mating but treated with (PA at a dose 100mg/kg and PA at a dose 200 mg/kg) respectively lead to significant ($P \leq 0.05$) increase in fertility rates, weight of new born, number of newborn and no effect of gestation period compared with (–ve control group).

Table (1): Effect of Proanthocyanidin and Ranitidine on Serum Concentrations of FSH, LH, Estrogen and Progesterone in Gastric Ulceration Female Rabbits induced by Indomethacin (Mean ±SD) (n=6)

Parameters / Treatment	FSH (µlU/ml)	LH (ng/ml)	Estrogen (µg/dl)	Progesterone (ng/ml)
Control (-ve) Normal Saline(0.9%NaCl)	6.55±2.53 A	5.63±0.11 B	45.00±7.61 A	9.66±5.86 A
Control (+ve) Indomethacine(75mg/kg)	3.51±1.94 B	2.78±0.01 6 C	33.5±9.93 B	5.10±3.68 B
Indometh+Proantho (100mg/kg)	6.03±1.70 A	8.16±0.83 A	47.00±6.16 A	9.12±4.06 A
Indometh+Proantho (200mg/kg)	6.63±2.14 A	7.66±0.61 A	44.33±6.15 A	7.37±4.81 A
Indometh+Ranitidine (50mg/kg)	4.03±1.02 B	3.16±0.23 C	31.00±5.40 B	3.46±1.90 B

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control

Table (2): Effect of proanthocyanidin and ranitidine on reproductive efficiency in gastric ulceration female rabbits induced by indomethacin (Mean ± SD) (n=6)

Parameters / Treatment	N0. of female pregnancy	Gestation period	No. of New born	No. of New born Total and range	Weight of New born	Fertility Rate %	Malformation%	Malformation	Aborted rabbits %	Dead newborn	Mortality Rate Of newborn
Control(-ve) Normal Saline (0.9%NaCl)	5	30 ± 1.02 B	4±1.5	20 4	21.33 ± 10.53 B	83.33 %	0%	0	0%	0	0%
Control(+ve) Indomethacine (75mg/kg)	1	34 ± 1.05 A	0.66 ± 0.02	5 0-5	16.33 ± 3.14 C	16.66 %	20%	1	0%	0	100%
Indometh+PA (100mg/kg)	6	29 ± 1.56 B	8.66 ± 1.03 A	51.6 7-10	33.25 ± 1.43 A	100%	0%	0	0%	0	0 %
Indometh+PA (200mg/kg)	6	28 ± 4.64 B	8.33 ± 1.03 A	49.8 7-10	33.56 ± 1.94 A	100%	0%	0	0%	0	0%
Indometh + Ranitidine (50mg/kg)	3	0	0	0	0	50%	0%	0	0%	0	0%

N=number of animals., Capital letters denote differences between groups ,P≤0.05 vs. control

Table (3): Effect of Proanthocyanidin and Ranitidine on Site of Implantation, Number of Corpora Luteum and Successful Implantation % in Sacrificed Gastric Ulceration Female Rabbits Induced by Indomethacin (Mean±SD) (n=6)

Treatment	Parameters			
	Site of Implantation	Number of Corpora Luteum	Successful Implantation %	Resorption of fetuses
Control (-ve) NormalSaline(0.9%NaCl)	4.00 ± 2.09 B	4.33 ± 2.25 B	92.37%	0.0±0.0 B
Control (+ve) Indomethacine(75mg/kg)	0.83 ± 0.04 C	2.83 ±0.63 C	29.32%	2.83±1.16 A
Indometh +Proantho (100mg/kg)	7.83 ± 1.47 A	8.33 ± 1.03 A	93.99%	0.0±0.0 B
Indometh +Proantho (200mg/kg)	7.50 ±1.87 A	7.83±1.47 A	95.78%	0.0±0.0 B
Indometh +Ranitidine (50mg/kg)	2.83± 0.75 B	6.16 ±0.75 AB	45.94%	3.16±0.75 A

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control

Figure (3): Newborns of mother rabbits controls negative during pregnancy, showing newborns healthy at birth.

Figure (4): Newborns of mother rabbits undergoes gastric ulceration during pregnancy. Showing malformation (M) of one of newborn and weakness another newborn.

Figure (5): Newborns of mother rabbits undergoes gastric ulceration and treated with (PA at 100 mg/kg). Showing newborns healthy at birth.

Figure (6):Newborns of mother rabbits undergoes gastric ulceration and treated with(PA at 200 mg/kg), showing newborns healthy at birth.

Figure (7): Fetus of mother rabbits undergoes gastric ulceration and treated with ranitidine at 50mg/kg. showing absorbed embryo and non-development, only present site of implantation (SIT)in the uterus.

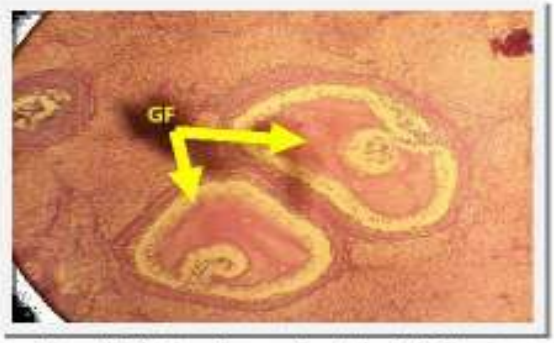

Figure (8): Section of ovary of control rabbit. Showing normal ovarian cellular tissue with normal Graafine follicles (GF), normal primary follicles (PF) and secondary follicles(SF), stain (H&E) 400X.

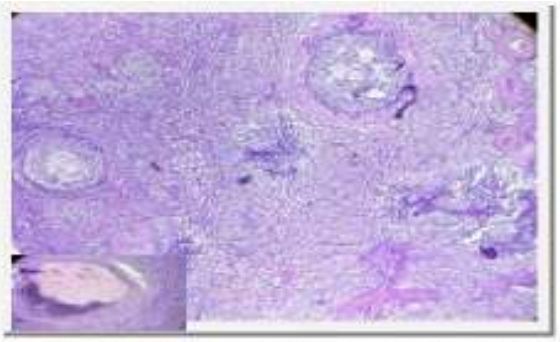

Figure (9): Section of ovary of gastric ulceration female rabbit induced by indomethacin (control positive), showing. primary follicles (PF) and secondary follicles(SF), stain (H&E) 400X.

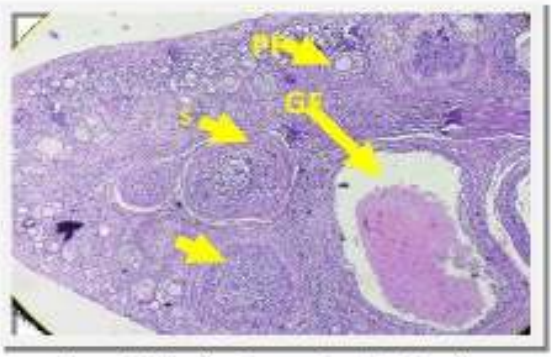

Figure (10): Section of ovary of gastric ulceration female rabbit treated with (PA at dose 100 mg/ kg. Showing normal architecture, normal ovarian cellular tissue with normal Graafine follicles (GF), normal primary follicles (PF) and secondary follicles (SF), stain (H&E) 400X.

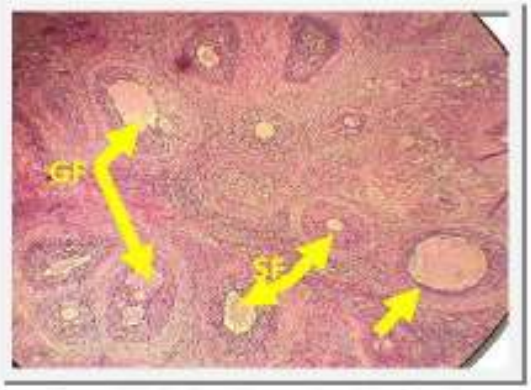

Figure (11): Section of ovary of gastric ulceration female rabbit treated with (PA at dose 200 mg/ kg. Showing normal architecture, normal ovarian cellular tissue with normal Graafine follicles (GF), normal primary follicles (PF) and secondary follicles(SF), stain (H&E) 400X.

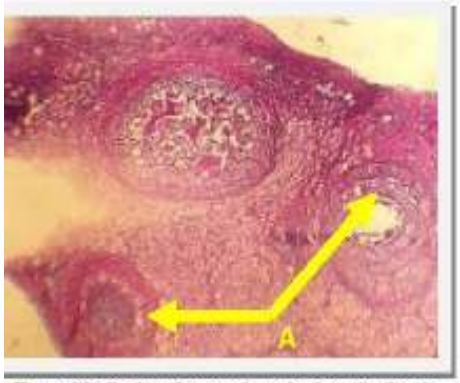

Figure (12): Section of ovary of gastric ulceration female rabbit treated with ranitidine at dose 50 mg/ kg, showing atretic follicles

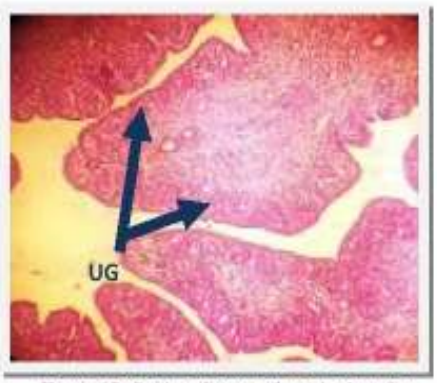

Figure (15): Section of uterus of gastric ulceration female rabbit treated with (PA at dose 100 mg/ kg. Showing normal architecture, normal uterine glands (UG), dilated lumen and papillary epithelium, stain (H&E) 400X.

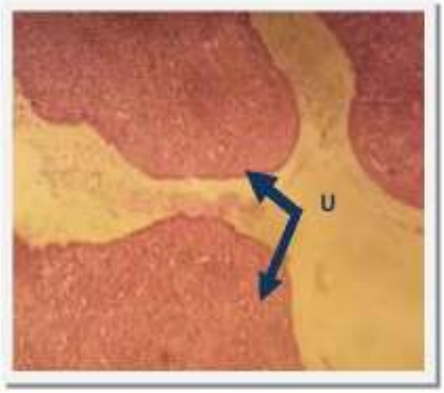

Figure (13): Section of uterus of female rabbit (control negative). Showing normal architecture, normal endounterium, (proliferative phase), normal uterine gland (UG), normal uterine lumen, stain (H&E) 400X.

Figure (16): Section of uterus of gastric ulceration female rabbit treated with (PA at dose 200 mg/ kg. Showing normal architecture, normal uterine glands (UG), dilated lumen and papillary epithelium, stain (H&E) 400X.

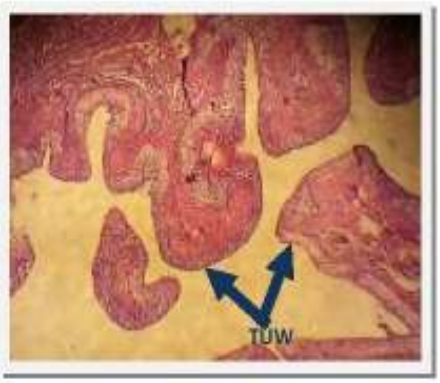

Figure (14): Section of uterus of gastric ulceration female rabbit induced by indomethacin (control positive). Showing thickened uterine wall(TUW) with prominent myofibrosis stroma with endometrial mucosa glands, also part of the uterus lined by papillary vacuolated proliferating epithelium, stain (H&E) 400X.

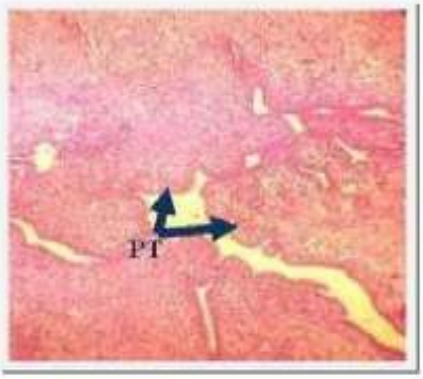

Figure (17): Section of uterus of gastric ulceration female rabbit treated with ranitidine at dose 50mg/kg. Showing rich in endometrial glands (EG) and rich myofibrosis stroma and dilated paplapian tubules(PT) stain (H&E) 400X.

DISCUSSION

Peptic ulcer disease (PUD) is common disorder of the gastrointestinal system .The causes of PUD are increased aggressive factors and / or reduction of gastric protection factors. Peptic ulcer diseases occur mainly due to consumption of NSAIDs, infection by *Helicobacter pylori*, stress or due to pathological conditions such as Zollinger-Ellision syndrome (35). Cause of PUD due to NSAIDs include factors that increase acid secretion, reduction of gastric mucosal blood flow, inhibition of prostaglandin synthesis, disruption of mucosalbarrier, inhibition of mucus and bicarbonate secretion in the gastro-intestinal mucosa (36-37). Indomethacin caused gastric damage in all animals administered it, this was further confirmed by the gross section and histopathologic lesions produced.The results indicate that proanthocyanidin has anti-ulcer effect, this related to its antioxidant activity , cause lowering in the gastric secretion by acting on the gastric mucosa and inhibiting the generation of reactive oxygen species that initiate the oxidative stress in the gastric lumen (38). In addition to, grape Seed Extract has anti-histamine properties (it stabilizes the release of histamine from mast cells) (39). From the study, proanthocyanidin showed more antiulcer activity at the dose of 100mg/kg and 200mg/kg when compared with standard drug ranitidine.

PGF2α is important factor in terminating inflammatory changes linked with ovulation process (40). Gonadotropins (FSH/LH) (increase during this period) are responsible for stimulation of PGF2α(41).Inhibition of PGF2α by NSAIDs administration may block or prevent the termination of the inflammatory process and allowing it to extend longer than normal and the slight difference observed between the different NSAIDs may reflect the difference in potency ofPGF2α inhibition and anti-inflammatory action between the different NSAIDs according to the difference in their selectivity towards COX enzyme selectivity and in their pharmacokinetic properties (1) .

In this study, significant decrease in gonadotropin hormone (FSH and LH) were due to suppression of PGE2 by indomethacin centrally in hypothalamus. A previous study indicated that PGs have little direct effect on gonadotropin secreted from the pituitary, while NSAIDs seems to suppress these hormones at the hypothalamic level by inhibition on GnRH release (42).This finding agree with (43) who indicate very high dose of aspirin administration caused a complete cessation of ovulation with a significant decrease in both FSH and LH , this decrease was thought to be due to suppression of PGE2 by aspirin centrally in hypothalamus and locally on the ovarian level PGs. While disagree with(42) who indicate thatthe low doses of aspirin cause unsuccessful in inducing any significant changes in the serum level of both gonadotropins (FSH/LH), as well aslow doses of NSAIDs (2.5 mg/kg B.W. of aspirin , 8.33mg/Kg mefenamic acid , 10mg/Kg ibuprofen and 0.0033 mg/kg B.W. of meloxicam) were unsuccessful in inducing any significant changes in the serum level of (FSH , LH, progesterone andEstrediol)(44). This failure to induce significant change may due to several causes : NSAIDs doses used were too low to induce suppression of hypothalamic PGE2(45). The other possibility is that NSAIDs acted locally at ovarian level without any effect on gonadotropins (FSH/LH) (46).Several reports have shown that, ovulation can be inhibited by NSAIDs despite of undetectable changes in several key hormones of ovulation (FSH, LH,E2 and P4), suggesting that local ovarian factors are the predominant driving force in ovulation(47).Indomethacin cause inhibition of ovulation due to inhibition of PGs and proteolytic enzymes such as collagenase and plasmin which increase in response to LH surge preovulatory , that lead to inhibition of collagenolytic activity (6, 48) and prevent follicular rupture(21-22). Regarding reduction in ovulation rate (number of flushed ova) which was reduced significantly in treated animals compared to control group , the underlying cause could be due to incomplete central inhibition of gonadotropin release (FSH and LH) which are known to be a key hormones for ovulation (49). Indeed, the incidence of pregnancy (1 of 6) in this study because some follicles showed severalrupture sites and release of oocyte (27-28). this finding are in accordance with(7) who indicated that presence of some ovulated oocytes even with the higher possible indomethacin doses.In this study, oral administration of proanthocyanidin cause significant increase in FSH, LH, progesterone and estrogen. This result may be due to antioxidant and scavenger of free radical of proanthocyanidin (50). The protective effect of GSE treatment agreed with (51) who reported that oral intake of GSE reduced the oxidative stress. In addition to, GSE treatment considerably increased the formation of antioxidant products which may be regarded to the phenolic constituents of GSE and its antioxidant activity. Also, proanthocyanidin may increase level of PGs centrally in hypothalamus that lead to increase production of FSH and LH, which cause increase of PGs and proteolytic enzymes such as collagenase and plasmin at ovarian, then produce follicular rupture. This result is supported by histological findings of uterus and ovary in the present study which indicated markedly increased in site of implantation and number of corpora luteum. This result is agreed with (52) who indicate grape seed extract alleviate reproductive toxicity caused by aluminum chloride in male rats, Moreover, rats orally administered GSE alone showed highly significant increase in sperm count, GSE increased the process of steroidogenesis and hence testosterone production and improved sperm production and the process of fertility.

Oral administration of ranitidin cause significant decrease in FSH, LH,progesterone and estrogen. This reduction is due to inability of ranitidine in

restoration adequate level of PGs centrally that lead to decrease level of FSH and LH, then decrease PGs and proteolytic enzymes at ovarian.This result is supported by histological findings of uterus and ovary in the present study which indicated markedly suppressed in site of implantation and number of corpora luteum.

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Histopathological changes induced by sodium dichromate at the liver and kidney of male and female rats

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ABSTRACT

140 female and 70 male rats were divided into six different treated groups according to the exposure developmental period during pregnancy and the dose of SDC used in accordance with registered design of FDA **(1):**T1P1 group mean animals dosed orally SDC at (3mg/kg B.W)during 1st trimester of pregnancy, T1P2 group mean: animals dosed orally SDC at (3mg/kg B.W)during 2nd trimester of pregnancy, T1P3 group mean: animals dosed orally SDC at (3mg/kg B.W) during 3rd trimester of pregnancy, T2P1 group mean: animals dosed orally SDC at (9mg/kg B.W) during 1st trimester of pregnancy**,** T2P2 group mean: animals dosed orally SDC at (9mg/kg B.W) during 2nd trimester of pregnancy and T2P3 group mean: animals dosed orally SDC at (9mg/kg B.W) during 3rd trimester of pregnancy.

The kidney showed significant changes such as necrotic, vacculation changes, shrinkage of Bowman's capsules, while the hepatic histologically changed were revealed as congestion of hepatic central vein and sinusoids, necrotic hepatocytes also noted congestion of portal vein, degenerated hepatocytes, which appeared eosinophilic.

Keywords: Histopathology, sodium dichromate, liver, kidney

الملخص باللغة العربية

شملت عينة الدراسة 210 من الجرذان، 140 منها كانت إناثا، 70 منها ذكورا، وقسمت إلى ست مجموعات مع مجموعة السيطرة، وذلك اعتمادا على مراحل تطور الجنين خلال فترة الحمل . وقسمت اعتمادا على جرعة ثنائي كرومات الصوديوم المستخدمة إلى (T2P3 ,T1P1,T1P2,T1P3,T2P1,T2P2) ومجموعة السيطرة التي جرعت بالماء المقطر طيلة فترة التجربة.

أظهرت نتائج الدراسة مجموعة من التغيرات النسيجية في الكلية: علامات النخر ، التنكس الفجوي، تقلص في محفظة بومان ، .بينما التغيـــرات الملاحظة في الكبد كانت احتقان الأوعية الدموية الكبدية، والجيبانيات، والنخر في الخلايا الكبدية، واحتقان الوريد الكبدي البابي، إضافة إلى وجود تنكس في الخلايا الكبدية.

INTRODUCTION

Sodium dichromate is an inorganic chemical compound with the formula $Na_2Cr_2O_7$, salt, red to bright in color and it can be considered as main source for hexavalent chromium Cr (VI) . Usually, this salt is handled as its 104 ehydrate $Na_2Cr_2O_7{\cdot}2H_2O$. Almost all chromium ore is processed via conversion to sodium dichromate (SDC) and all compounds and materials based on chromium are prepared from this salt (2). Chromium (VI) is known to cause problems, kidney and liver damage, alteration of genetic material. The absorbed hexavalent chromium is distributed through out the body ,blood,, bone, testis, brain ,breast milk and uterus (3,4). Hexavalent chromium has been detected in fly ash from power plants (5). Both hexavalent and trivalent of chromium enter water resources by leaching from soil or from industrial contamination. The results of a study conducted by (6) showed that human and animal chromium are widely distributed in the body after exposure to Cr (VI), with spleen, liver, bone, and kidney having higher concentrations than other tissues such as in bone, kidney, liver and testis (7,8) showed that chromium can cross the placenta. The administered Cr(VI) in the drinking water to different kind of laboratory (rats ,mice, and guinea pigs) was administered in a study conducte by (9), chromium in blood and kidney increased with exposure concentration. In another study done by (10), exposed male and female rats and mice to sodium dichromate 104ehydrate in drinking water for 3 months resulted in reduction of body weights, and increased bile acid concentrations in exposed rats due to altered hepatic function.

Histopathology of major organs was similar among all groups. In a study of (11), urinary volume was increased with increasing renal effects experientially in rats administered Cr (VI) (for 20 days) by gavage included increased accumulation of lipid, triglycerides, and phospholipids in different regions of the kidney than controls and inhibited kidney membrane enzymes (alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, and lipase)(12). In addition, Oliguria and proteinuria were observed in rats exposed to Cr (VI) for 28 days in drinking water reported by (13).

It was reported by (14) that cancers, damage to the liver and kidneys, infertility in both males and females ,defects in embryo and developmental problems in young children also were signs of health effected by Cr (VI) and this agreed with (15-17). The study conducted by (18), showed that the hepatocellular apoptosis in rat liver after exposed to Cr(VI) contaminated water also there were increase in serum glucose and alanine aminotransferase levels. Renal lesions in animals are confine to the proximal convoluted tubules reported by (19,20). It was reported by (21) that ingestion of hexavalent chromium caused gastric lesions in rats, hepato- and nephrotoxicity as well as hematological changes were seen in rats.

At the higher doses administration of Cr (VI), the results of (22) revealed oxidative stress in liver and kidney that were reflected by changes histopathologically, with degenerative changes and dilatation of sinusoids in liver. Kidney sections showed degeneration of tubular epithelial cells, cystic dilatation of tubules, hyaline casts, congestion of blood vessels and dilatation of bowmans space. Severe histological changes in the liver and kidney of Cr treated rats were earlier reported by (23).

MATERIALS AND METHODS

Experimental animals

Two-hundred and ten (210) male and female albino rats with ages about three months and body weight ranged between (150-200g) were used with their pups to perform this experiment .The animals were raised and bred in the animal house of College of veterinary Medicine/ University of Baghdad where the research was done. The animals were kept in cages of (20*30*50) cm^3 dimensions in average of three rats in each cage one month before study for acclimatization in optimum conditions of breeding at (22±3) ºC with a (14/10) hrs. (Light/Dark) cycle. Commercial feed pellets and drinking water were given all the time of experiment.

The dose of experimental depend on oral LD_{50} of male rat that reported by (24) represented 0.1ml of LD_{50} of SDC that reported at (24). Therefore, 3mg/kg B.W was prepared by dissolving 30mg of SDC in 10 ml of distilled water. The concentration was 3mg/ml, while the dose was 0.1ml/100g B.W.

Experimental design

Two-hundred and ten (210) male and female albino rats were divided randomly, each sex alone and subgroups to 6 groups with control group giving Sodium dichromate (SDC) orally, 3 and 9 mg/kg B.W. daily.

Insurance of pregnancy

The pregnant female rats were examined daily after mating for five days. Vaginal smears were prepared by using vaginal swabs and methyline blue stain to detect proestrus phase. Pregnancy was detected by observation of pale mucous membrane of vagina and sperms in the third day after conception (25).

Histopathological study

Animals were anesthetized by intrapretonial injection ketamin 10mg/ 100gm.B.W. The animals were sacrificed in closed chamber saturated with chloroform to achieve a good muscular relaxation, then, the organs involved in the study (Liver and Kidney) were obtained and preserved in 10%

formalin, then sent to laboratory of histopathology. Slides were prepared and stained with Hematoxyline and Eosin stain (26).

RESULTS

T1P1 group showed the following changes at the:

Liver: thickening of portal areas seen due to infiltration of mononuclear cells**,** congested portal blood vessels and severe dilation of congested sinusoidal capillaries, there were also focal areas of necrosis and diffused infiltration of inflammatory cells mainly MNCs (lymphocytes, macrophages) within the hepatic lobule (figure 1).
Kidney: There were degenerated epithelial lining cells of renal tubules in cortex and medulla. Vacuolar degenerative changes of endothelial cells of glomerular tufts and increased spaces of bowman´s capsule (atrophid glomerulus), some tubules contained proteintious material (hyaline casts), RBCs and inflammatory cells (figure 2).

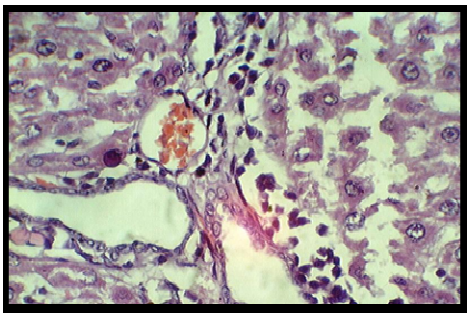

Figure (1): section of liver from (T1P1) group showed the portal area thickened and congestion of B.V. and infiltration of inflammatory cells MNCs. (↗) 40X H&E stain

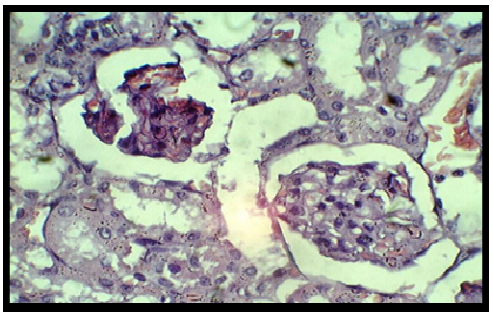

Figure (2): Kidney (T1P1) showed atrophied tuft and degenerative changes of the renal tubules. (40X H&E stain)

T1P2 group showed the following changes at the:

Liver: necrotic hepatocytes characterized by losing nuclei, infiltration of MNCsmainly and congestion of blood vessels, hypertrophy of portal blood vessel (figure 3).
Kidney: atrophy of glomerular tufts and cystic dilation of renal tubules, congestion and dilated blood vessels (figure 4).

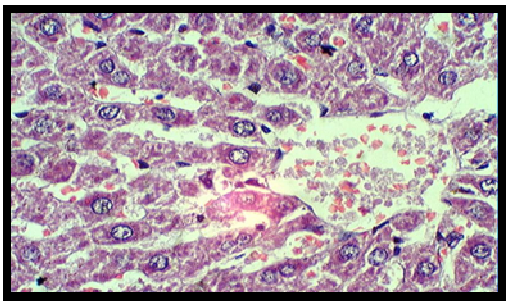

Figure (3): liver (T1P2) showed hyper atrophy of portal blood vessels, of hepatocytes infiltration of inflammatory cells mainly MNCs .H&E stain 40X

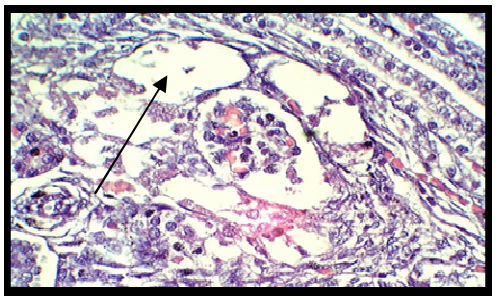

Figure (4): kidney (T1P2) showed degenerative changes of renal tubes, atrophy of tuft, dilated of Bowman's capsule & aggregation of MNCs around .(→)H&E stain 40X

T1P3 group showed the following changes at the:

Liver: Infiltration of inflammatory cells mainly mononuclear cell thickening in portal area are congestion of blood vessels, degenerated hepatocytes. (figure 5).
Kidney: Congestion of glomerular tufts (dilated capillaries and filled with blood) with destination of bowman's spaces, flattened their walls (figure 6).

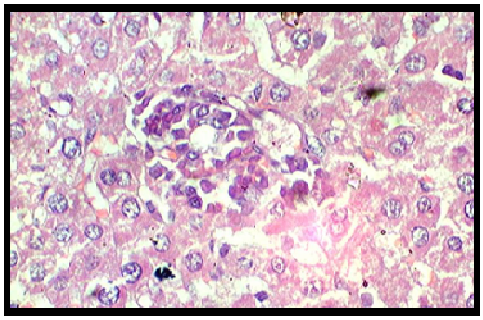

Figure (5): liver (T1P3) group showed infiltration of MNC's around B.V. with necrosis of hepatocyte. H&E stain 40X

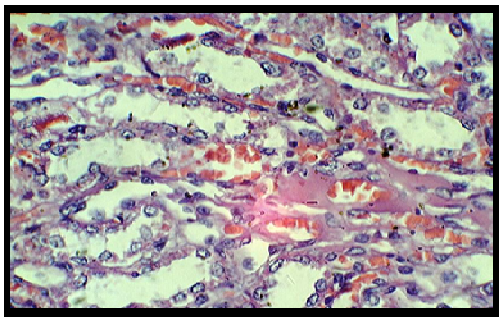

Figure (6): kidney (T1P3) group show congested B.V.& vaculation degeneration of collected tubules with aggregation of inflammatory cells. H&E stain 40X

T2P1 group showed the following changes at the:

Liver: Congestion of sinusoidal capillaries with focal diffused infiltration of MNCs, congestion of hepatic and portal blood vessels appeared dilated, filled with blood and few inflammatory cells (figure 7).
Kidney: Vacuolar degeneration of endothelial cells lined glomerular capillaries, severe congestion of renal blood vessels, in addition to proteintious fluid in dilated collecting tubules, necrosis of epithelial lining tube (figure 8).

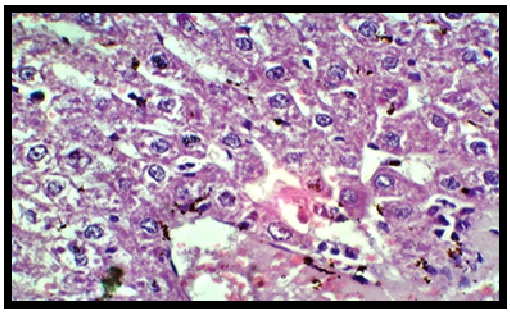

Figure (7): liver T2P1 showed sinusoidal congestion of blood capillaries with focal diffused in filtration of MNCs ,congestion of b.v. filled with blood and few inflammatory cells.40X H&E stain

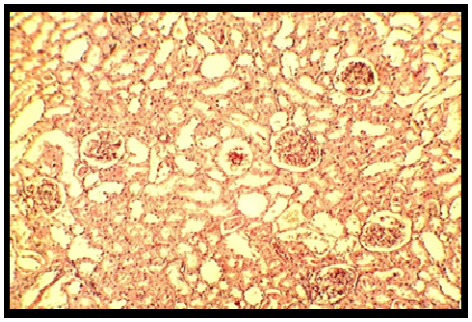

Figure (8): kidney (T2P1) vacuolar degeneration of endothelial cells of glomerular tufts ,sever congestion of renal b.v. 10X H&E stain

T2P2 group showed the following changes at the:

Liver: Sever hepatic blood vessels congestion, central veins and sinusoids. Vacuolar degenerative changes of hepatocytes , in addition to necrotic cells which lost their nuclei. Lymphocytes also infiltrated in sinusoidal wall (figure 9).
Kidney: Great distention of bowman's space due to congestion of glomerular capillary tuft. Degeneration of epithelial lining cells of proximal convoluted tubules, infiltration of MNCs also noted. In other section hyalinization and thickening of bowman's capsule presence of hyaline casts in renal tubules (figure 10).

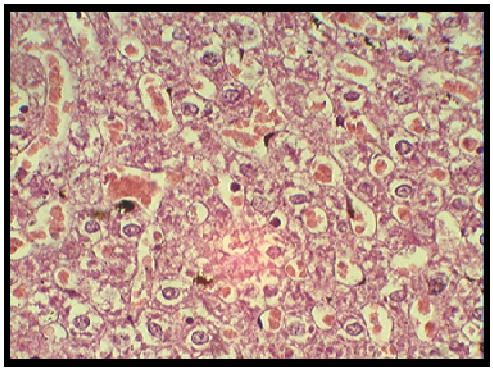

Figure (9): histological section of liverT2P2show sever congestion of sinusoidal capillaries appeared dilated and filled with blood (H&E stain,40x).

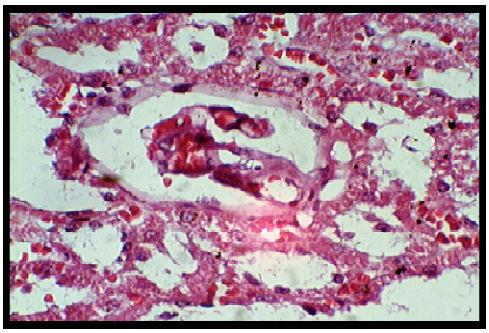

Figure (10): histological section of kidney T2p2 showed Hyalinized and thickened wall of atrophic glomerulous in (H&E stain,40X).

T2P3 group showed the following changes at the:

Liver: Severe congestion of hepatic blood vessel and sinusoids, necrotic hepatocytes also noted congestion of portal vein, degenerated hepatocytes, which appeared eosinophilic (figure 11).
Kidney: Nephritis characterized by infiltration of inflammatory cells in interstitial tissue mainly mononuclear cells and necrotic renal epithelial cells also noted (figure 12).

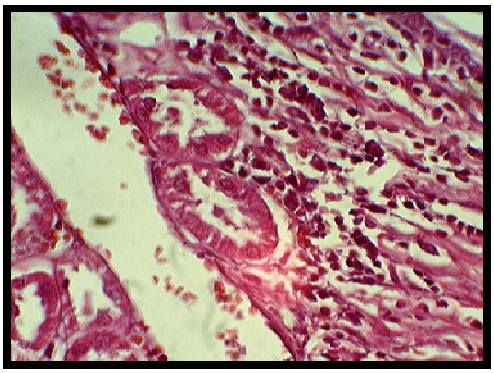

Figure (11): liver showed congestion of portal vein, degenerated hepatocytes which appeared eosinophilic 40X H&E stain

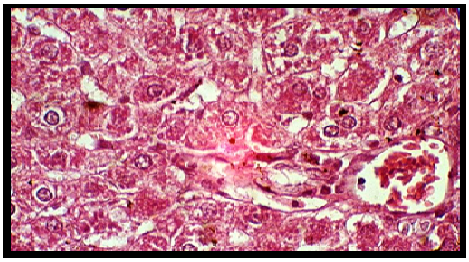

Figure (12): kidney T2P3 showed nephritis characterized by infiltration of inflammatory cells in interstitial tissue mainly mononuclear cells and necrotic renal epithelial cells also noted 40X H&E stain

DISCUSSION

The present study explained the serious pathogenic effects of The kidney showed significant changes such as necrotic, vacuolation changes, atrophy of glomerulus tuft, which were in agreement with (27,28). In addition, there were degeneration of epithelial lining with cystic dilatation of the tubules, congestion of B.V., hyaline casts ,with mild swelling of glomerular tufts, also dilatation of bowman's space that reported by (29).
Cr (VI) administrated to female rats during late pregnancy and early postnatal periods showed sever kidney damages in dams and their off springs (30). It was found that Cr (VI) induced liver damages as evidenced by the elevation of plasma aminotransferases, lactate dehydrogenase activities, and bilirubin levels.
Impairment of the hepatic function corresponded histological. Results also revealed hemorrhage, leukocytes infiltration cells, and necrosis, which were more pronounced in the hepatocytes of mothers than in those of their suckling pups that due to mothers dose more than the pups and also the immunity of the pups is higher than mothers (31), liver changes were as following vacuolar degeneration of the hepatocytes, dilation of sinusoids, congested B.V. of the liver and activated macrophages liver changes were as following vacuolar degeneration of the hepatocytes, dilation of sinusoids, congested B.V. of the liver and activated macrophages, which agree with (32). Also (33) reported that Cr(VI) is carcinogenic and mutagenic effected the liver by produced reversible hepatic damage. A study conducted by (18) reported an apoptosis hepatocellular of rats liver exposed to Cr (VI) orally for 10 weeks.
According to (31), the histological and functional disturbance were due to Cr(VI)administration to female rats, the histopathological changes which indicated by infiltration of inflammatory cells, necrosis, hemorrhage, were more sever of mothers than in pups. There were degenerative changes ,vascular congestion, sinusoids dilatation (31) ,while at the portal area showed significant increase infiltration of inflammatory cells (MNCs).

CONCLUSION

In conclusion, sodium dichromate could cause changes for the liver and kidney of rats male and female histologically, as well as changes in their biological functions.

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Study the pathological effects of aqueous (AE) and ethanolic (EE) extracts of *Artemisia herbaalba* on the growth of transplanting tumor in white mice in vivo

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ABSTRACT

This study represents the first attempt to use the aqueous extracts of *Artemisia herbaalba* AE of the plant as anticancer agent when the tumor-bearing mice treated with different doses of the AE. Two parameters were used to evaluate the anticancer activity of the AE, these are growth inhibition percentage (GI %) and relative tumor volume (RTV %). The preliminary step to detect the therapeutic doses that used in the treatment of transplanted murine mammary adenocarcinoma in mice was determination of LD50 in mice. They were (0.5, 0.25 and 0.125 g/kg), administered by two different routes, intraperitoneal and per os. The results of *in vivo* study indicate high effectiveness of AE in reducing the tumor volume in a dose- and time-dependant manner. The best effective dose was 0.5 g/kg when administered intraperitoneally or orally. The comparison of relative tumor volumes of different groups revealed high significant differences between all treated groups and those of untreated (control) groups. Coincidently, the histopathological changes in treated and untreated tumor masses showed that necrosis and fibrosis were the predominant features occurring with the advanced time of treatment proportional to the reduction in tumor volume. In advanced time of treatment, there were only few islands of tumor tissue sequestered by massive mature fibrous tissue.In conclusion, the results of this study revealed the high anticancer effect of AE when used in treatment of transplanted tumor in mice.

Keywords: *Artemisia herba alba,* tumor, mice, adenocarcinoma

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الملخص باللغة العربية

تمثل هذه الدراسة المحاولة الأولى لاستخدام المستخلص المائي لعشب الشيح كمادة مضادة للسرطان بعد إعطاء جرع مختلفة من المستخلص لفئران تم غرسها بخلايا سرطان الظهارة للغدة اللبنية الفأري. وقد كانت الخطوة الأولى في اختيار الجرعة العلاجية هو تحديد الجرعة المميتة الوسطية للفئران حيث كانت تساوي 5.5 غم/كغم من وزن الجسم لذا كانت الجرعة العلاجية (0.5 ، 0.25 ، 0.125 غم/ كغم من وزن الجسم) وقد أعطيت عن طريقين (عن طريق الخلب وعن طريق الفم). لقد استخدم اثنان من المعايير لتقييم الفعالية المضادة للسرطان وهما النسبة المئوية لتثبيط نمو الورم وحجم الورم النسبي (%).

لقد دلت نتائج البرنامج العلاجي للفئران الحاملة للورم على وجود فعالية عالية للمستخلص المائي في تقليل حجم الورم معتمداً على الجرعة ومدة العلاج حيث كانت أفضل النتائج بعد إعطاء الجرعة0.5 غم/كغم عن طريق الخلب أو عن طريق الفم. كما بينت المقارنة بين حجم الورم النسبي للمجاميع المعالجة وحجم الورم النسبي لمجاميع السيطرة وجود فروقات مهمة احصائيا وطيلة فترة العلاج.

لقد كان التنخر والتليف هما السمتان البارزتان في المجاميع المعالجة بعد إجراء الفحص النسجي المرضي حيث زاد ظهورهما مع تقدم العلاج وبشكل مرتبط مع صغر حجم الورم لذا نلاحظ أن المراحل الأخيرة من العلاج تُظهر إن الخلايا السرطانية موجودة بشكل جزر صغيرة محصورة في نسيج ليفي كثيف.

وبذلك، خلصت الدراسة إلى أن المستخلص المائي لعشب الشيح له فعالية مضادة للسرطان بعد استعماله في علاج السرطان المغروس في الفئران.

INTRODUCTION

The use of wild herbs in folk medicine is old as man himself. Our ancestors started to learn from nature by testing and using what was available. It is well known that old civilians have flourished in the middle east and used the natural plants for various daily needs, such as food, shelter, clothes and medicine. Traditionally, such habits have been inherited by successive generations, and, thus, some of the plants became well known for their uses especially by herbalists. The use of plants in medicine was best known among Greeks, Arabs, the Chinese in the old world, and the red Indian in the new world (1). However, the use of medicinal plants was highly practiced by Arabs during the middle ages and through them it was transferred to Europe (2). Herbal medicines are culturally accepted and widely used in many countries for treatment of disorders and hence are of great importance as a mechanism to increase access to health care services.

However, only few countries have some forms of policy/mechanism on traditional/complementary and alternative medicine (TCAM). Other countries need to develop their policy on TCAM to provide a sound basis in defining the role of TCAM in national health care delivery, ensuring that necessary, regulatory and legal mechanisms are created for promoting and maintaining good practices, that access is equitable, affordable and that authenticity, safety and efficacy of therapies are ensured (3). Recently synthesized drugs started to replace natural ones due to many well-known reasons. But after the increase of drug industry and modern technology, man began to test plant products due to some harmful side effects or symptoms caused by some synthesized drugs (4). However, as for cancer, the disease is complicated and heterogeneous, which makes it difficult to be well diagnosed, especially by traditional healers. The ethnomedical information obtained for a plant extract that is used to treat cancer might therefore not be reliable (5).

This study aimed to Study the effect of aqueous extract of *Artemisia herba alba* on the growth of transplanting tumor in mice *in vivo*.

MATERIALS AND METHODS

Extraction of test plant

1. Plant collection: *Artemisia herba alba* was collected from Al-Najaf province, south of Baghdad in December 2003, and was shed and dried at room temperature (figure 1). A voucher specimen of the plant was deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib (Certificate no. 3522 in 23/12/2003). The dried plant then was separated into: roots and aerial parts, then the aerial (leaves and barks) parts were ground into powder by coffee electrical grinder (mesh no.50), and the powdered parts were kept in a plastic bags in deep freeze (-20˚C) until the time of use (6).

Figure (1): ***Artemisia herba alba***

2. Preparation of aqueous and ethanolic extracts of *A. herba alba:* according to (6), aqueous extract of plant was prepared as follows:

1- aliquots of 50 g of the powdered plant were suspended in 200 ml of distilled water (D.W.) in Erlyn Myer flask and stirred on a magnetic stirrer over night at 45˚C.

2- After 24 hrs, the sediments were filtered by gauze and then by filter papers.

3- Steps (1) and (2) were repeated 4-5 times.

4- The pooled extract was evaporated to dryness (45˚C) under reduced pressure in a rotary evaporator.

5- The weight of crude extract resulted from that amount of powdered plant was measured.

6- The crude extract then was kept at -20˚C until the time of use.

7- The ethanolic extract of *A. herba alba* was prepared in the same manner as that of the aqueous extract except using of 70% ethyl alcohol instead of D.W.

For following experiments, 1 g of powdered plant extract was dissolved into 100 ml PBS (as solvent), the suspension then filtered and sterilized by using 0.4 µm sterile millipore filter and kept in deep freeze (-20˚C) until use.

Determination of LD50

Graded doses of aqueous extract of aerial parts of A. *herbaalba* in 0.2 ml PBS were administered intraperitoneally to eight groups (each of six mice). The ranges of single intraperitoneal doses which were used in the determination of LD50 of aqueous extract were (2-9 g/kg). Mortality was recorded after 24 hrs. The LD50 was determined according to the formula employed by (7), which is described as follows:

LD50=Highest dose -Σ(a x b)/n

Where: a=difference between two successive doses, b =the mean of dead animals of two successive groups, n=the number of animals in the group.

Tumor Growth Inhibition (*in vivo* study)

1. Transplantation of tumor cells in the mice: Single tumor (mammary adenocarcinoma) – bearing mouse was supplied from ICCMGR. This mouse was used to obtain tumorcells which later transplanted into adult female albino mice. The following protocol was followed to perform the transplantation process (8), which occurred under highly sterile conditions:

a) The tumor mass region was well disinfected with 70% ethyl alcohol.

b) By using 10 ml disposable syringes, the contents of tumor mass tissue were withdrawn into sterile flask and suspended into 50 ml of sterile PBS.

c) The solid contents were allowed to settle down while thesupernatant discarded.

d) The sediments washed 2-3 times with sterile PBS. By final wash, appropriate amount of PBS was stayed. This amount was comparable with the number of animals which prepared for transplantation. Generally, the withdrawn contents from tumor mass of single mouse were adequate for transplantation of average 20-25 mice.

e) Homogenized suspension of cells made through mechanicad disaggregation of cells in the withdrawing materials. This made via vigorous pipetting (withdraw and return of contents several times).

f) Each adult female albino mice (2-2½ months old) become ready to tumor transplantation, 1 ml of tumor cell suspension was transplanted through insertion of a needle (gague no.18) subcutaneously from thigh region toward the shoulder region where the injection was performed.

g) Tumor growth, tumor measurement was observed and recorded by using vernia caliber:

Tumor volume (mm^3) =aXb^2 / 2 (9)

Where, a = length of tumor mass(mm), b= width of tumor mass(mm).

2. **Treatment of tumor by using plant extracts:** Once tumor reached at least 6.5 mm in any dimension, mice were randomized into eight treatment groups (each contain of 5 adult female albino balb/C mice), three experimental groups were intraperitoneally (I.P) administered with (0.5, 0.25, 0.125) g/Kg. of aqueous extract of *A. herba alba*. Other three groups were orally administered with the same above doses by using oral gavage. Another two groups only administered PBS I.P and orally and they were used as control groups. The administration of the aqueous extract of the plant was performed at one day intervals, for 25 days from the beginning of the treatment, and the tumor volume was estimated and recorded at days (0, 5, 10, 15, 20 and 25) from the starting of treatment.

Measurements

Relative tumor volumes (R.T.V.) were calculated using the following formula**:**

R.T.V.(day X)= {tumor volume (day X) / tumor volume (day 0)} X100 (10)

Percent tumor growth inhibition (GI %) was calculated as described by (11).

GI %={(tumor volume of untreated group) - (tumor volume of treated group) / (tumor volume of untreated group)} X 100

Histopathological study

Immediately at the time of death or at the end of experiments, the animals were sacrificed. The macroscopic appearance were studied to detect any abnormal gross changes in internal organs and to describe the tumor mass. Specimens were taken from the tumor mass, kidneys, liver, lung, spleen and sometimes stomach. The tissues were preserved in 10 % formaldehyde immediately after removal. After fixation, and processing with a set of increasing alcohol concentrations, tissues were embedded in paraffin blocks, and sectioned by microtome at 5 µm for all tissues. All sections were stained with hematoxylin and eosin stain and the histopathological changes were observed (12).

RESULTS

Determination of LD50:

The median lethal dose (LD**50**) of aqueous extract was performed for 24 hrs through intraperitoneal injection. It was 5.5 g/kg (table 1). The animals showed rapid shallow respiration within the first hours which became more deep and wheezy with general lassitude. Loss of appetite, staggery gate, and soft stool was the signs observed later.

Table (1): LD50 determination parameters in albino female mice, after intraperitoneal injection with different concentrations of aqueous extract of *Artemisia herba alba*

Dose g/kg	No. of animals	No. of dead animals	a	b	a x b	∑(a x b)
2.0	6	0	--	--	--	---
3.0	6	0	1.0	0.0	0.0	---
4.0	6	1	1.0	0.5	0.5	---
5.0	6	2	1.0	1.5	1.5	---
6.0	6	4	1.0	3.0	3.0	---
7.0	6	5	1.0	4.5	4.5	---
8.0	6	6	1.0	5.5	5.5	---
9.0	6	6	1.0	6.0	6.0	21.0

LD50=9.0 - 21 / 6 =5.5 g/kg
a=difference between two successive doses.
b=the mean of dead animals of two successive groups

Growth inhibition (GI %) and relative tumor volume (RTV%):

Table (2) illustrated the values of tumor volume in control groups treated with PBS in two routes (intraperitoneal and per os). The results indicated the rapid growth rate of tumor mass, particularly after day (5) from the beginning of treatment. This supported by statistically different values of tumor volumes as well as relative tumor volumes (table 3), which were showed the double growth rate and more with each time of measurement and record. The values of control groups were important in calculation of tumor growth inhibition percent. The response to treatment revealed considerable variability in dose and time-dependant manner, as well as the route of administration. The best therapeutic dose was (0.5 g/kg), and less effective dose was (0.125 g/kg), while (0.25 g/kg) administered group showed moderate inhibitory effect. Table (4) revealed high significant decrement effect (P< 0.0001) at day 10 and later when animals treated with 0.5 g/kg. The cessation of tumor growth after treatment was noticed with (0.25 g/kg). This indicated by no statistical significant differences in tumor volume until the end of experiment in comparison with the onset of treatment. Whereas the lowest dose (0.125g/kg) showed lowest effect on tumor volume which increased with advance of therapeutic period even there was a significant increment in tumor volume at days 20 and 25. The relative tumor volume (%) was used to follow-up the development (progression or regression) of tumor growth with increased time of treatment in comparison to day (0) of treatment. The relative tumor volumes of I.P. administered groups (table 5) were indicated that there was high significant decrease in RTV arriving to only 22.6% of original volume after 25 days of treatment with 0.5 g/kg.

However, 0.125 g/kg treated group showed increasing RTV which appeared statistically significant at day 20 and later. The group treated with middle dose (0.25 g/kg) was also showed decreasing RTV but as not significant values where compared with day 0 of treatment. The comparison of RTV of each time of I.P. treatment in all groups with corresponding RTV of the same time in control group showed high significant decrease (P<0.0001) in RTV of all treatment groups. This indicates the high effectiveness of all doses (0.5, 0.25 and 0.125 g/kg) of AE where administered intraperitoneally to treat the transplanted tumor in mice. The oral administration of AE showed slight difference in the response to treatment from that of I.P. administration. The results of (0.5 g/kg) treated group per os revealed no significant effect at day 5 of treatment, but became effective at subsequent records (table 6). In other hand, the group treated 0.125g/kg showed the increment of tumor growth became more faster and the statistical significant differences appeared earlier (day 10) in comparison to that of I.P. route (day 20). The RTV of oral injected groups revealed slightly less decline in tumor development between 0.5 and 0.25 g/kg administered groups of both routes while the third group showed slightly larger increase arriving to six times in comparison to that of I.P. route, which only reached to doubled size at the end of experiment (table 7). Similar comparison between RTV of control group and treated group where orally administered also carried out. The results revealed high significant effectiveness of used doses at each time of measurement and record. The most clear evaluation to tumor growth inhibition were obtained after calculation of growth inhibition index (GI %). The inhibition increased in a time and dose-dependent manner. The values represented in. Both figures showed great inhibition in tumor growth after five days of treatment with different doses of AE and in both routes of administration. The great growth inhibition values occurred after treatment with 0.5 g/kg intraperitoneally and 0.25 g/kg orally (82.63% and 72.15% respectively).The final GI % at the end of the experiment showed the greatest values after treatment with 0.5 g/kg in both routes of administration (more than 99%, while the least GI% (91.95 and 84.94%) were noticed in groups treated intraperitoneally and orally with 0.125 g/kg respectively.

Table (2): Tumor volume (mm^3) in control groups when intraperitoneally and orally treated with phosphate buffer saline

Day	I.P (Mean±SE)	P-value	Oral (Mean±SE)	P-value
0	261.40±40.97	--	248.00±65.44	--
5	714.00±266.01	N.S	776.75±143.06	N.S
10	1940.0±453.89	N.S	2240.2±315.18 *	0.001
15	3695.7±755.07*	0.001	4788.0±490.16 *	0.001
20	4951.0±2169.0*	0.001	8132.5±1120.50*	0.0001
25	5571.5±2140.5*	0.0001	8918.0± 0.0 *	0.0001

**The mean difference is significant in comparison with (day 0) group of the same route of administration*

Table (3): Relative tumor volume (%) in control groups when intraperitoneally and orally treated with phosphate buffer saline

Day	I.P (Mean±SE)	P-value	Oral (Mean±SE)	P-value
0	100.00±00.00	--	100.00±00.00	--
5	275.60±86.33	N.S	313.00±57.63	N.S
10	743.00±173.7	N.S	902.75±126.9*	0.001
15	1415.7±289.0*	0.001	1930.3±197.7*	0.001
20	1897.0±831.0*	0.001	3279.0±452.0*	0.001
25	2134.5±820.5*	0.001	3595.0±0.0 *	0.001

**The mean difference is significant in comparison with (day 0) group of the same route of administration*

Table (4): Tumor volume (mm³) in the groups when intraperitoneally injected with different concentrations of aqueous extract of *Artemisia herba alba*

Day	0.5 g/kg (Mean±SE)	P-value	0.25 g/kg (Mean±SE)	P-value	0.125 g/kg (Mean±SE)	P-value
0	176.5±18.7	--	209.2±25.5	--	188.2±30.4	--
5	124.0±12.5*	0.005	196.2±29.6	N.S	218.2±36.8	N.S
10	92.5±5.5*	0.0001	185.2±35.7	N.S	275.5±43.0	N.S
15	68.0±6.3*	0.0001	169.0±43.9	N.S	319.2±35.1	N.S
20	46.2±8.2*	0.0001	165.7±46.0	N.S	419.5±68.0*	0.01
25	40.0±9.6*	0.0001	162.7±54.2	N.S	448.0±76.8*	0.005

*****The mean difference is significant in comparison with (day 0) group*

Table (5): Relative tumor volume (%) in the groups when intraperitoneally injected with different concentrations of aqueous extract of *Artemisia herba alba*

Day	0.5 g/kg (Mean±SE)	P-value	0.25 g/kg (Mean±SE)	P-value	0.125 g/kg (Mean±SE)	P-value
0	100.0±00.0	--	100.0±00.0	--	100.00±00.0	--
5	70.25±7.1*	0.0001	93.75±14.0	N.S	116.25±19.5	N.S
10	52.25±3.1*	0.0001	88.25±17.1	N.S	146.50±22.8	N.S
15	38.50±3.6*	0.0001	81.00±21.3	N.S	169.75±18.6	N.S
20	26.25±4.5*	0.0001	79.50±22.0	N.S	221.25±36.2*	0.005
25	22.50±5.4*	0.0001	77.75±25.9	N.S	238.25±40.8*	0.005

**The mean difference is significant in comparison with (day 0) group*

Table (6): Tumor volume (mm³) in the groups when orally administered with different concentrations of aqueous extract of *Artemisia herba alba*

Day	0.5 g/kg (Mean±SE)	P-value	0.25 g/kg (Mean±SE)	P-value	0.125 g/kg (Mean±SE)	P-value
0	270.0±15.7	--	216.5±41.6	--	201.0±31.3	--
5	253.7±17.5	N.S	216.2±35.0	N.S	336.7±68.3	N.S
10	217.5±27.4*	0.05	217.7±35.3	N.S	535.5±80.7 *	0.01
15	148.6±13.5*	0.0001	206.7±43.0	N.S	917.0±144.7*	0.0001
20	74.6±4.4 *	0.0001	192.7±51.3	N.S	1263.0±43.5*	0.0001
25	64.6±3.8 *	0.0001	191.7±59.9	N.S	1342.3±48.8*	0.0001

**The mean difference is significant in comparison with (day 0) group*

Table (7): Relative tumor volume (%) in the groups when injected orally with different concentrations of aqueous extract of *Artemisia herba alba*

Day	0.5 g/kg (Mean±SE)	P-value	0.25 g/kg (Mean±SE)	P-value	0.125 g/kg (Mean±SE)	P-value
0	100.0±00.0	--	100.0±00.0	--	100.00±00.0	--
5	93.96±06.4	N.S	100.0±16.1	N.S	167.50±34.1	N.S
10	80.50±10.0*	0.05	100.7±16.4	N.S	266.25±40.2*	0.01
15	55.00±5.03*	0.0001	96.50±20.7	N.S	456.25±72.0*	0.0001
20	27.66±1.76*	0.0001	88.75±23.7	N.S	628.33±21.8*	0.0001
25	24.00±1.52*	0.0001	88.50±27.8	N.S	667.66±24.1*	0.0001

**The mean difference is significant in comparison with (day 0) group*

Tumor growth inhibition:

Macroscopic study:

The predicted time for the formation of suitable and ready tumor mass for starting the treatment was between (10-20) days after transplantation. At that time the tumor was macroscopically described as hard round expanding mass . Dissection of the tumor mass revealed lobulation of the tumor tissue and marked vascularization. In control groups with advanced growth of tumor, the mass was enlarged with cystic changes showing fluid accumulation, and erosion of the overlying involved skin as a result of necrotic process. Some of animals showed escaping of necrotic materials from the eroded skin. On dissection, the mass revealed lobulation with softness of the tissue texture with marked vascularization . In response to treatment, the tumor mass showed great decrease in size with marked increase in the hardness, with peripheral replacement of tumor tissue by fibrous tissue.

Histopathological Study:

The tumor mass of murine mammary adenocarcinoma showed all histological features of malignancy when examined after 10-20 days of transplantation. These changes included glandular configuration, cellular pleomorphism, hyperchromatism, loss of differentiation as well as the neovascularization (figure 2). The tumor mass was already surrounded by loose fibrous connective tissue (figure 3). These features were clear in specimens of control group in all times of treatment. In response to treatment with different doses of AE and in both routes of administration, the histological examination revealed fibroblast proliferation, and formation of immature fibrous tissue (figure .4), and marked infiltration of inflammatory cells within the tumor tissue (figure 5). with progression of treatment, the fibrous tissue become thick and mature and there was decrease in tumor tissue which gradually replaced by mature fibrous tissue. Other characteristic feature with time of treatment was the occurrence of necrosis (figures 6, 7). In advanced time of treatment, extensive areas of necrosis (figure 8) and islands of tumor cells within the thick, mature fibrous tissue could be seen (figure .9). Sections from lungs, liver, kidney, spleen and stomach have taken to identify any metastatic effect of the primary tumor as well as to assess the side effects of treatment on these organs. All sections showed no histological changes in these internal organs of control and treated groups.

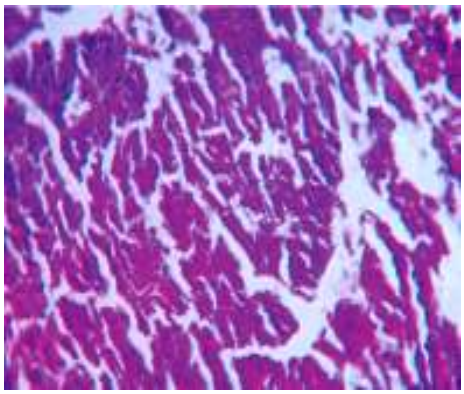

Figure (2): Part of the tumor mass showing hyperchromatic,leomorphic, poor differentiated malignant cells. (400X, H&E stain).

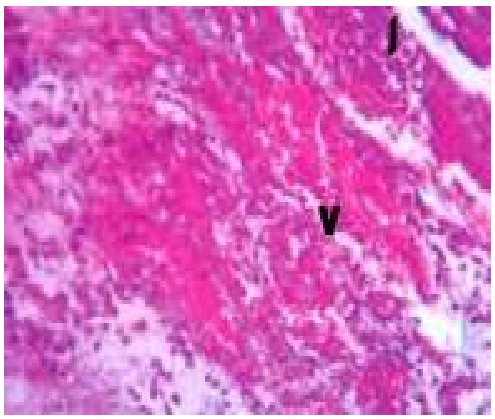

Figure (5):Marked vascular proliferation and congestion (V) with marked infiltration of inflammatory cells (I) at periphery of the tumor. (400X, H&E stain)

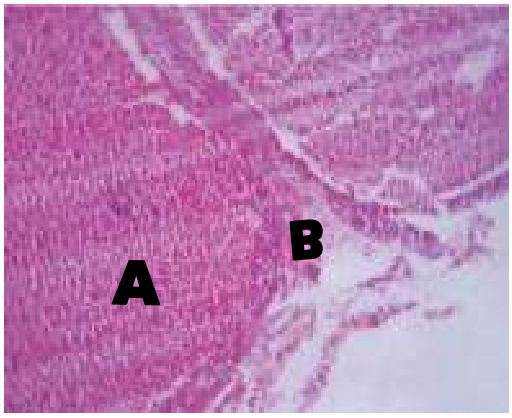

Figure (3): Mass of adenocarcinoma (A) surrounded and penetrated by fibrous band (B). (100X, H&E stain).

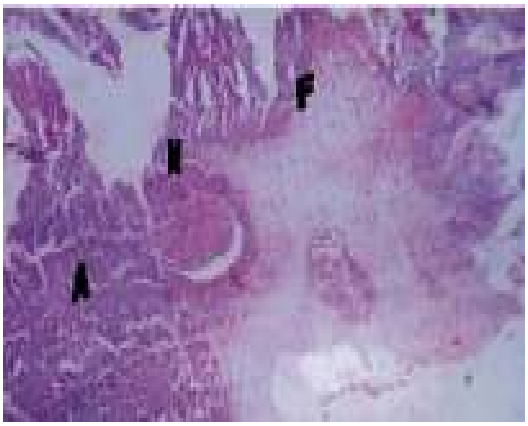

Figure (6): Abundant mature fibrous tissue (F) surrounding the tumor mass (A) that showed marked necrosis (N). (100X, H&E stain)

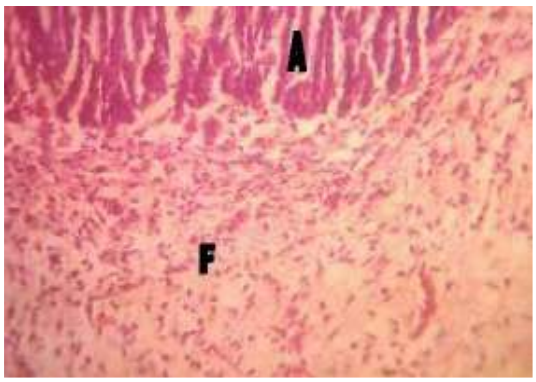

Figure (4):Immature fibrous tissue (F) surrounding the tumor mass (A) showing fibroblast proliferation. (200X,H&E stain)

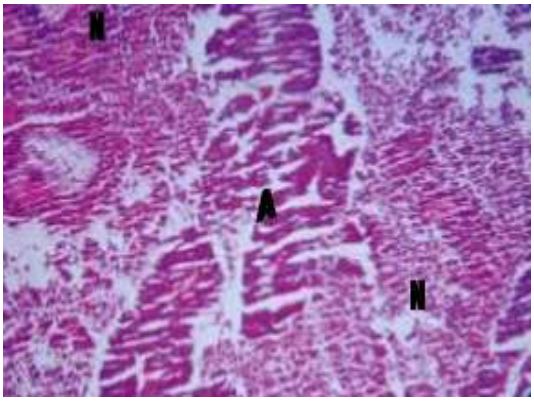

Figure (7): Extensive necrosis (N) of the tumor mass (A) (200X, H&E stain)

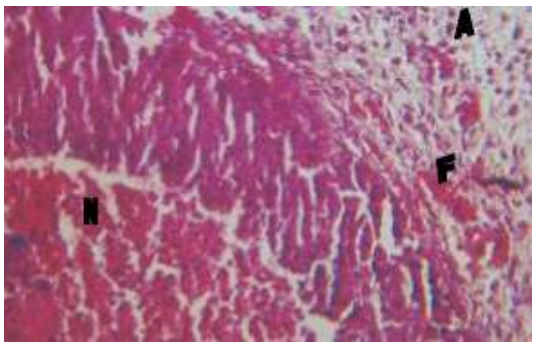

Figure (8):Tumor mass (A) showing extensive necrosis (N) and marked fibroblastic proliferation (F). (200X, H&E stain)

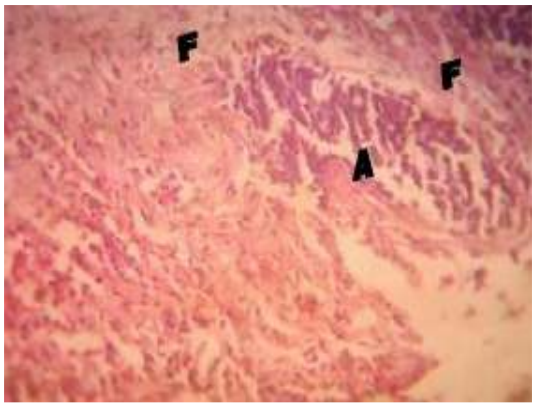

Figure (9): Island of malignant tissue (A) completely surrounded by fibrous tissue (F). (200X. H&E stain)

DISCUSSION

The resulted LD50 (= 5.5 g/kg) reflects a broad margin of safety of this extract when used *in vivo*. This result was consistent with that obtained by (1), where she had obtained LD50 equal to 4.498 g/kg. While (13) found the chronic treatment of mice with ethanolic extract of aerial parts of *Artemisia abyssinica* and *A. inculta* showed significant mortality with LD50 up to 3 g/kg per os. The main leading cause for this low toxic effect of *Artemisia herba alba* is probably the presence of an essential oil, thujone in small amounts (1).The difference in the values of LD50 could be attributed to the variation in collection time of the plant, parts of the plant used for extraction, method of extraction as well as the animal species and route of administration used in the research experiments.

Tumor growth inhibition (*In vivo* study):

The transplantation of murine adenocarcinoma was successfully established in a high percent of injected healthy mice. This was consistent with that mentioned by (10). The high percentage of successful transplantation in this study could be explained that the transplanted tumor cells have species-specific adaptation property as a result of recurrent transplantation in same species of mice, and the inability of host immune system to recognize these transplanted cells as foreign cells. This will result in earlier and most successful chances for transplantation, even without use of any immunosuppressive agents. The failure of transplantation in some animals could be attributed to non-specific defense mechanisms. Natural killer (NK) and lymphokine-activated killer (LAK) cells inhibit growth of animal tumors and are found within human tumors. These non-specific immune defense mechanisms may be of value in rejection the transplantation, particularly if the tumor cell numbers were small (14). The gross and dissecting features of the tumor mass showed an increase the volume in untreated groups and vice versa, there was decline in tumor volume with increased doses of the AE. The erosion of overlying involved skin, was characteristic feature particularly in the over expanding tumor mass of the untreated (control) groups. This could be interpreted that the rapid proliferation of tumor cells may outstrip the capacity of the new vessels to supply adequate oxygen and nutrient. The resulting patchy necrosis is characteristic of rapidly growing malignant tumors (15). The gross appearance of necrosis varies considerably and is, to some extent, dependent on the balance which is struck between the processes of denaturation and the autolytic changes (14). There are two parameters used in the evaluation of tumor growth after oral and intraperitoneal administration of therapeutic doses of AE as well as in the untreated control groups. Growth inhibition (GI %) and relative tumor volume (RTV%)were beneficial to observe the development of tumor volume according to duration of treatment and dose of extract. The comparisons were carried out firstly between the untreated groups (control groups) and treated groups at each record time of volume (5, 10, 15, 20, 25 days). Another comparison was done to detect the relative tumor volume in each day of record in account to the day 0 of each group. The results showed considerable inhibitory effect of AE on the growth of tumor mass in a dose- and time-dependant manner. The results showed that the greatest values were obtained after treatment with dose of (0.5 g/kg) in both routes of administration (more than 99%), while the lower doses (0.25, 0.125 g/kg) revealed less but still high percentage of growth inhibition. The literature survey was showed lack in the references that concerned with the use of plant extracts in a scientific regimen for treatment of tumors in animals as well as human.

The interpretation for the growth of tumor in the mice with and without treatment were consistent with the suggestions of several studies that carried out to explore the mechanism of anticancer activity of several compounds isolated from some of medicinal plants. (16) showed that the tricin, a flavone found in rice bran was inhibited the growth of human derived malignant MDA-MB-468 breast

tumor cells at submicromolar concentrations. The volume of tumors in animals bearing cells pre-exposed to 11 micro M of tricin was less a third of that in mice with control cells, while tumors from cells incubated with 0.1 or 1.1 micro Moftricin were indistinguishable from controls. These results suggest that potent breast tumor cells growth-inhibitory activity of tricin*in vitro* does not directly translate into activity in the nude mouse bearing the MDA-MB-468 breast tumor cells. Various properties were suggested to account for therapeutic findings, including ability to scavenge reactive oxygen species, inhibition of tyrosine kinases and potentiation of prostaglandin generation (17). Both the nutritive and non-nutritive components of the diet play a significant role in the inhibition of carcinogenic process. The non-nutritive constituents exert their anticarcinogenic effect by various mechanisms**:**

(I) by virtue of their antioxidant property, (II) de-activating the carcinogens, or (III) enhancing the tissue levels of protective enzymes in the body. Toxic metabolites of harmful drugs and chemicals are detoxified by the body's defense system. Phytochemicals in spices like, tumeric, mustard and allium vegetables may act in more than one way to confer beneficial effect (18). This approach to explain the mechanisms by which the transplanted tumor would be reduced in volume may be in controversy with the following studies. In one study, Artemisinin, an *Artemisia annua* derivative was showed selective immunosuppressive activity, didn't exhibit immunostimulatory activity when studied its effect in normal mice (19). And the *Artemisia* derivatives enhance the reactive oxygen response of neutrophils but depress their phagocytic ability at therapeutic blood levels (20)**.**

In another study, scoparone, the active principle from *Artemisia scoparia* may be useful as a new template for the development of better immunosuppressive agents with vasorelaxant actions for use against transplantation rejection and autoimmune disease (21). The essential oils of *Artemisia afra Jacq*. exhibited hydroxyl radical scavenging agents when assessed in the deoxyribose degradation assay (22). On the other hand, the antioxidant action of *Artemisia campestris* was examined *in vitro* and *in vivo* by (23). They were showed that the water extract of *A. campestris* has a strong scavenging action for diphenyl-2-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radical in CCl4-derived lipid peroxidation in the liver. Other indications for the role of antioxidation as a mechanism for action of plant components as anticancer agents were supporting our approach, like; The extract of *Artemisia asiatica* (DA-9601) may reduce chemically induced liver injury by complex mechanisms which involve prevention of lipid peroxidation and preservation of hepatic glutathione (24).The scoparone was a simple coumarine isolated as natural product from different species of *Artemisia* including desert wormwood. This has potent inhibitory effect of lipid peroxidation and act as scavenger of superoxide anion radicals and of aqueous alkylperoxyl radicals. This coumarine also inhibits the proinflammatory-5-lipoxygenase enzyme at micromolar concentrations (25; 17).Generation of intracellular reactive oxygen intermediates is a mechanism shared by many anticancer drugs. Resistance to such drugs can be acquired through increased expression of genes that code for oxygen-scavenging systems such as glutathione, or the enzyme glutathione-S-transferase (14).The susquiterpene lactones, a large group of useful compounds in the treatment of various disorders, are readily form adducts with glutathione, or free thiols and can thereby affect the metabolism, activity and toxicology of a wide array of pharmacological agents (26). The histological examination of the sections prepared from tumor masses of treated and untreated groups showed several variable changes. In untreated groups, the histological features of the tumor were typical to the malignant murine adenocarcinoma. The fibrous connective tissue response that occurred in the treated groups was almost identical to that described for healing process by fibrosis following acute inflammation, i.e. organization of fibrin with formation of granulation tissue which subsequently matures into fibrous tissue replacing the progressively necrotic tumor tissue (14). Coincident with fibroblast proliferation, there was proliferation of small blood vessels, a process called angiogenesis or neovascularization (15). Tumor cells, and perhaps also the macrophages which infiltrate among them, release tumor angiogenic factors, notably growth factors of the b-FGF family and a molecule with RNAse-like structure called angiogenin. As a result a network of thin-walled vessels with actively proliferating endothelium spreads within the growing tumor (27). This pattern of tumor vasculature has important implications for tumor therapy. The success of new modes of cancer therapy (and some more established ones like hyperthermia) may depend upon critically damaging the endothelial cells (28). The necrosis is a characteristic histological feature which subsequently replaced by mature fibrous connective tissue as well as marked reduction in the tumor tissue.

Our interpretations for the growth inhibition efeect against ransplanted tumor were consistent with suggestions of (29) and (30). The firsts suggest that the tumor growth inhibition could be through modulation of the expression of Bcl-2/Bax family of apoptotic regulatory factors. The later were mentioned that the attempt to influence the natural phenomenon of programmed cell death stems from the fact that it is reduced in cancer in several clinical situations. Thus; chemicals that can modify programmed cell death are likely to bepotentially useful drug. In contrast, (31) mentioned that some of phytotherapeutic agents affect prostate cancer cell growth without inducing apoptosis or cell cycle arrest but interfere with 5-alpha-reductase activity.

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Effect of Sr Substitution on Structural and electrical Properties of the Ba2-xSrxCa2Cu3O10+δ

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ABSTRACT

In this study, the superconducting compounds $Ba_{2-x}Sr_xCa_2O_{10+\delta}$ had been prepared for values of ($0 \le x \le 0.3$) by SSR method and the effects of substitution Ba by (Sr) were studied. For the purpose of the formation and stability of the high phase of the high Tc (0223) superconductor. The results of XRD analysis showed that the composite phases have: the high phase superconducting (LTP) with the presence of impurities developed and that all samples have one structure (orthorhombic), also this study had been done to compare the effects of injection with Ba and (Sr) on the analysis of the crystal structure where he found that the grafted samples with Sr have an increase in the length of the c-axis.

The percentage of the high phase (0223) had been calculated using XRD analysis, and by comparing between them. it was found that a partial compensation of Ba by Sr element lead to increase in the proportion of the high super conduction phase; also the results show a decreasing in dielectric constant with an increasing in the frequency of the applied field in addition to decreasing of the dielectric constant with an increasing of the dapping ratio by Sr.

Keywords: Structural and electrical properties, $Ba_{2-x}Sr_xCa_2Cu_3O_{10+\delta}$, superconducting

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الملخص باللغة العربية

تم تحضير المركبات $Ba_{2-x}Sr_xCa_2O_{10+\delta}$ الفائقة التوصيل ولقيم ($0 \le x \le 0.3$) بطريقة تفاعل الحالة الصلبة (SSR). وتمت دراسة تأثيرات التعويض الجزئي لعنصر Ba بـــعنصر (Sr) لغرض تكوين واستقرار الطور الفائق التوصيل ذو الدرجة الحرجة العالية (0223). أوضحت نتائج تحليلات الأشعة السينية (XRD) بأن المركب يمتلك الطور الفائق التوصيل العالي (HTP) والطور الفائق التوصيل المنخفض (LTP)، مع وجود طور الشوائب وأن جميع العينات لها تركيب (معيني متعامد المحاور). وقد أجريت دراسة لمقارنة تأثيرات التطعم بـــعنصر (Sr) على تحليلات التركيب البلوري، حيث وجد أن النماذج المطعمة بـــعنصر Sr تظهر زيادة في طول محور c .

ومن تحليلات الأشعة السينية أيضا، تم حساب كل من النسبة المئوية للطور العالي (0223) للنماذج المحضرة كثافتها، وبإجراء المقارنـــة بينهـــا، وجد أن التعويض الجزئي لعنصر Ba بالعنصرSr قد عمل على زيادة نسبة الطور الفائق التوصيل العالي. كذلك أظهرت النتائج تناقصا في ثابت العزل الكهربائي مع زيادة تردد المجال المسلط، والذي يعزى إلى ميكانيكيات الاستقطاب، كما لوحظ تناقص ثابت العزل مع زيادة نسبة التشويب بعنصر (Sr).

INTRODUCTION

High T_c superconductivity has been found now in many materials .An example of an interesting discover is superconductivity at 39K in MgB_2. Improvement of T_c was frequently reported during the first two years by the discovery of $YBaCu_3O_{7-\delta}$ T_c=90K (1), Bi-based materials with Tc (90-110K)" (2,3), "Tl-based materials with T_c equal 110K' (4), and" Hg-1223 with Tc=135K' (5). "All cuprite high temperature superconductors have a perovskite-like layer structure. In spite of the large number of compounds, the structure can be represented by the generic formula $A_mE_2R_{n-1}Cu_nO_{2n+m+1,}$ where A, E and R are various cations, often with A=Tl,Bi,Hg, E=Ba,Sr and R=Ca or a rare-earth element. It can be designated simply by Am 2 (n-1)n or, by O2(n-1)n" (5), when A is displaced in this search study the effect of partial substitution of either Sr instead of Ba on the structural and dielectrically properties of Ba-Ca-Cu-O system were studied.

METHODS AND EXPERIMENT

The samples of $Ba_{2-x}Sr_x$ $Ca_2Cu_3O_{2n+4+\delta}$ for x=0-0.3 were prepared by a SSR methods .Each sample is formed by mixing their materials in vortex mixer for 1.5 hrs, then drying using electrical oven at 100 ˚C. Mixtures were placed in a steel die (of 1.5 cm in diameter and about 0.2 cm in thickness) and compacted at 5 MPa for one minute, using hydraulic press. Sintering process was carried out by using electric Furnace, samples were fired at heating rate of 5 ºC/min up to the firing temperature of about (850) ºC and held at that temperature for 60 hrs, when time is completed, turn off the furnace and leave the samples into the furnace to cool down to room temperature. The phases structure of these minerals are characterized by XRD measurement by using (X-Ray Diffraction unit) "A computer program was used to calculate the lattice parameters a, b and c. This program is based on Full prof Suite toolbar" (6). "The volume fractions of different phases were calculated based on the relation" (7):

$$V_{ph} = \frac{\sum I_0}{\sum I_0 + \sum I_1 + \sum I_2 + \sum I_{other\ (peaks)}} \times 100\ \%$$

The samples density (d_m) were calculated by the following equation (8):

$$\mathbf{d_m = W_m / N_A V}$$

where, N_A = Avogadro's number in unit (particles/g.mol), V =volume of unit cell and W_m = molecular weigh in unit (amu) (8).

By using the following equation:

$$\varepsilon'_r = \frac{c_p}{c_o} \Rightarrow \frac{c_p d}{\varepsilon_o A}$$

the value of the dielectric constant (ε'_r) can be evaluated (9,10).

RESULTS AND DISCUSSION

The present study had investigated that the effect of Sr doping in the Ba -0223 superconductor by preparing of specimens with complete stoichiometry Ba_{2-x} Sr_x $Ca_2Cu_3O_{10+\delta}$ with x ranging from 0 to 0.3. The superconducting properties of the specimens have been examined by structurally measurements and DC dielectrically measurements. Figures (1-3) showed the patterns of x-ray diffraction for samples dopped by Sr with x=0.0,0.1and 0.3. respectively. All the major peaks were corresponded to the (Sr / Ba)-0223 phase (11). The minor impurity phases detected include Ca_2CuO_3 (11). Also we have seen an improvement in the structure properties and the higher rate of phase (Sr / Ba)-0223 that appears with increasing Sr content up to x=0.3. The increase of Sr content (x>0.3) leads to the substitution of Sr cause more cuprate vacancies that the HTSc need, to a high scattering effect of supper electrons in crystalline structure. The lattice parameters of Sr doped Ba-0223 are reported in table (1), which showed that as the (a) lattice parameter changes little, but the (c) lattice parameter slightly increases with increasing Sr concentration (table 1).

Figure (4) shows the dialectic constant dependence of the frequency electrical for a $Ba_{2-x}Sr_x$ $Ca_2Cu_3O_{10+\delta}$ sample with $0 \le x \le 0.3$. This figure shows the dielectric constant values start to be stable and became approximately constant for many phases at frequencies ($F \ge 1*10^4$Hz).The decreases of the dielectric constant values , which are illustrated in figure (5) with increase (Sr)doping. The (έ) gives the level of energy stored in the matter at electric field applied. The most likely place at which this energy could be stored is the material is within the grain acts like termination ends for the crystal (12,13).

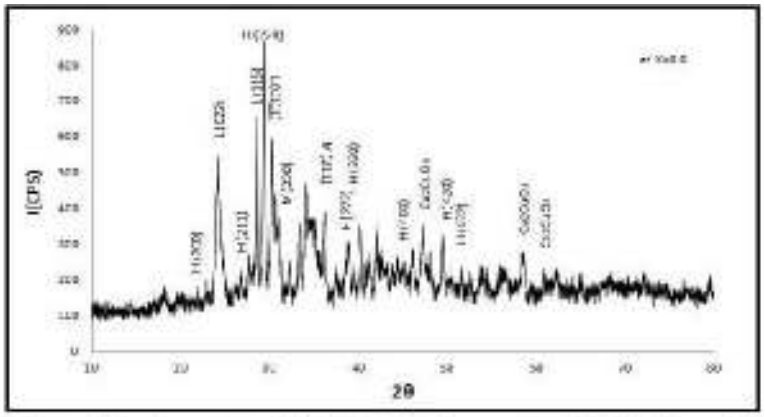

Figure (1): X-ray diffraction patterns for the $Ba_2Ca_2Cu_3O_{10-\delta}$ samples. H-HighT$_c$ phase, L-low T$_c$ phase, impurity phase Ca_2CuO_3

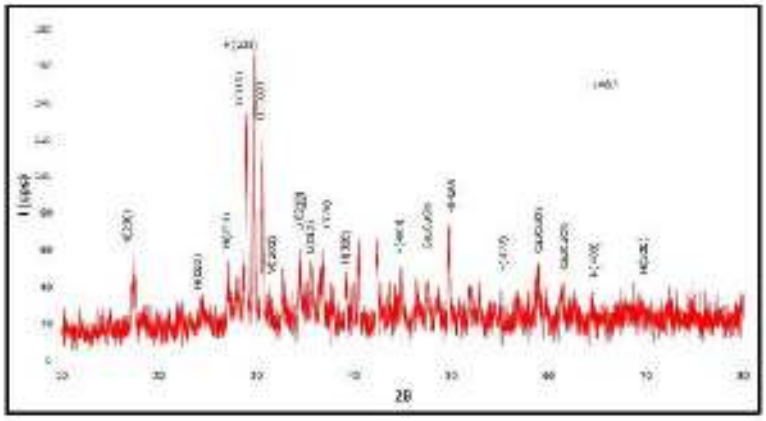

Figure (2): X-ray diffraction patterns for the $Ba_{1.8}Sr_{0.1}Ca_2Cu_3O_{10-\delta}$ samples. H-High T$_c$ phase, L-low T$_c$ phase, impurity phase Ca_2CuO_3

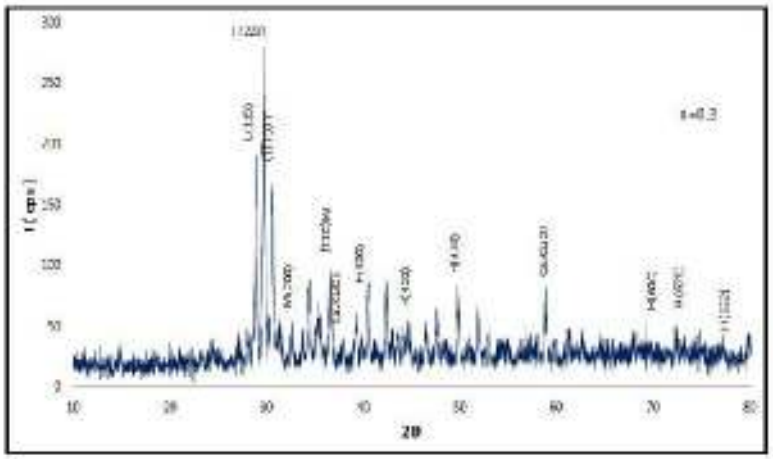

Figure (3): X-ray diffraction patterns for the $Ba_{1.7}Sr_{0.3}Ca_2Cu_3O_{10-\delta}$ samples. H-High T$_c$ phase, L-low T$_c$ phase, impurity phase Ca_2CuO_3

Table(1): Lattice parameters(Å) of the superconducting system $Ba_{2-x}Sr_x Ca_2Cu_3O_{10+\delta}$

Sample	a(Å)	b(Å)	c(Å)	c/a	dm(gm/cm^3)	High (Vph) %	Medium (Vph) %	Low (Vph) %	$CaCuO_3$ (Vph) %	dm(gm/cm^3)
0.0	4.010619	4.45369	18.00619	4.4896	321.6275	2.29436	43.34	9.47	34.57	12.60
0.1	4.05369	4.05467	21.60619	5.33	355.1274	2.06392	49.06	3.39	35.99	11.54
0.3	4.25461	4.09540	23. 619	5.5513	411.5452	1.756822	54.74	9.48	27.99	7.77

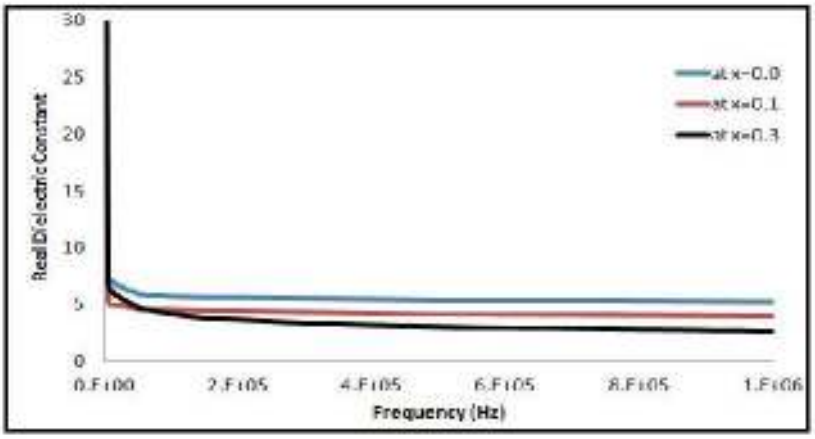

Figure (4): Dielectric constant ($\acute{\varepsilon}_r$) with frequency for $Ba_{2-x}Sr_x Ca_2Cu_3O_{10+\delta}$.

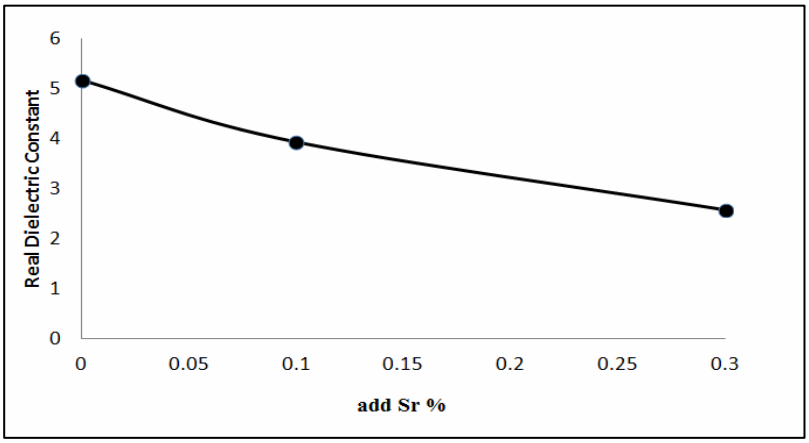

Figure (5): Dielectric constant ($\acute{\varepsilon}_r$) with Sr doping for $Ba_{2-x}Sr_x Ca_2Cu_3O_{10+\delta}$ at 1MHz

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Sr Substitution influence on the superconductivity characteristics of Ba2- xSrx Ca2Cu3O2n+4+δ System

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ABSTRACT

In this study, the specimens of $Ba_{2-x}Sr_x Ca_2Cu_3O_{2n+4+\delta}$ for x=0-0.3 were prepared by a SSR methods. Electrical resistivity was measured by linear –four point probe technique. Substitution of Sr to 0.0, 0.1 and 0.3will increase the transition temperature to T_c=88K, 99K, 115K, respectively with increasing oxygen content. While for the other specimens (x=0.1,x=0.3), the resistivity decreases slowly with decreasing temperature. The best nominal composition was found to be $Ba_{0.7} Sr_{0.3} Ca_2Cu_3O_{10+\delta}$ because of the highest value of T_c.

Keywords: Sr doping influence, superconductivity, characteristics of $Ba_{2-x}Sr_x Ca_2Cu_3O_{2n+4+\delta}$ system

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الملخص باللغة العربية

في هذه الدراسة، تم تصنيع عينات فائقة التوصيل $Ba_{2-x}Sr_x Ca_2Cu_3O_{2n+4+\delta}$ حيث x=0-0.3 بطريقة تفاعل الحالة الصلبة . تم قياس المقاومة الكهربائية بوساطة تقنية linear –four point probe . وقد أظهرت النتائج زيادة في درجة الانتقال عند تعويض السترنتيوم بدل الباريوم ، حيث T_c=88K, 99K, 115K عندما x=0.0, 0.1 and 0.3 على التوالي ، وكانت افضل نسبة يحدث عندها الانتقال تساوي T_c= 115K للمركب فائق التوصيل $Ba_{0.7} Sr_{0.3} Ca_2Cu_3O_{10+\delta}$.

INTRODUCTION

Materials, which have high-Tc superconductor (still below room temperature) were found to have superconducting activities above liquid nitrogen temperature (77 k) and exhibit very interesting and simplex relationships between their chemistry crystal structure and physical properties (1). The behavior of these materials is the presence of the planes containing copper and oxygen atoms chemically border to each other, and permit materials to conduct electricity very well. These classes of compounds called as cuprates and know as perovskite are a mixture of material oxide , and display physical properties of ceramics (2). Most of cuprite superconductors including Ba-based system, and have perovskite like structure, consist of insulating blocks and superconducting layers, which alternate each other (3). Ba-Sr-Ca-Cu-O has been found usually, have more oxygen atoms (as the structure in figure 1) (4).

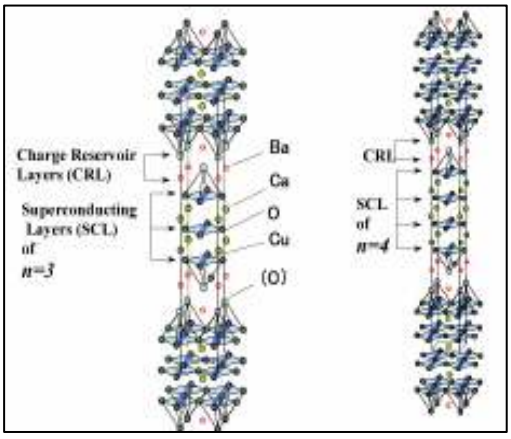

Figure (1): Crystal structures of (a) *n*=3, $Ba_2Ca_2Cu_3O_6$ (Ba-0223), (b) *n*=4, $Ba_2Ca_3Cu_4O_8$ (Ba-0234) (4)

The structural stability of the superconducting phases can be enhances by doping, and also by inserting extra oxygen (p > 0), which will create more holes in the perovskites. Layers [(CuO2) sheets] and will shorten the Cu-O bond length (5). Remarkable change associated with the superconductors was observed by partial compensation for Ba by Sr elements (6). Doping rates by Sr in this system will changes affects on the proportion of the high Tc super conduction phase, also affects on the physical properties of prepared sample and changes the resistivity and the offset and onset transition temperate (7).

EXPERIMENTAL WORK

The specimens of $Ba_{2-x}Sr_x\ Ca_2Cu_3O_{2n+4+\delta}$ for x=0-0.3were prepared by a SSR methods .Each sample is formed by mixing their materials in vortex mixer for 24 hr, then drying using electrical oven at 100 ˚C. Mixtures were placed in a steel die (of 1.5 cm in diameter and about 0.2 cm in thickness) and compacted at 5 MPa for one minute, using hydraulic press. Sintering process was carried out by using electric Furnace, samples were fired at heating rate of 5 ºC/min up to the firing temperature of (850) ºC and held at that temperature for 60 hours, when time is completed, the furnace was turned off and the samples were left in the furnace to cool down to room temperature. Electrical resistivity was measured by linear –four point probe technique which could be found from the relation (8-10):

$$\rho = 0.4532\ \frac{\nu}{I}\ \Omega.\ \text{cm}.$$

Where, v: is the voltage drop across the electrodes.
I: is the current passing through the sample.
As well as the energy gap superconducting compounds $Ba_{2-x}Sr_x\ Ca_2Cu_3O_{2n+4+\delta}$ for x= 0-0.3 accounted from $Eg=3.53K_BT_C$.
The oxygen containing (**δ**) was calculated from the following relation (11):

$$\delta = \frac{\left[\frac{M_A}{M_B}\right] - \left[\frac{3m_A}{CV}\right]}{\left[\frac{2m_A}{CV}\right] - \left[\frac{M_o}{M_B}\right]}$$

Where M_A: is the molar mass of the $Ba_{2-x}\ Sr_x\ Ca_2Cu_3O_{10+\delta}$. specimens
M_B: is the molar mass of $Na_2S_2O_3.(5H_2O)$ = 248.18
m_A: is the weight of the sample ≈ (40-45) mg.
M_o: is the atomic weight of oxygen = 15.999.
C: is the concentration of $Na_2S_2O_3.(5H_2O)$ = 0.015 gm /ml.
V: is the volume of $Na_2S_2O_3$ solution used in titration.

RESULTS

The result for the effects of Sr substitution on electrical resistivity measurements was investigated. The resistivity versus temperature for specimens with nominal composition $Ba_{2-x}\ Sr_x\ Ca_2Cu_3O_{10+\delta}$ for ($0 \le x \le 0.3$) sintered at 850°C for 60 hrs are shown in figure (2). It is found from (figure 1) for the compound at x=0.0 that $T_{c\ (onset)}$=101K and $T_{c(offset)}$=88K. For (x=0.1 and 0.3) the superconducting transition were sharp and they had $T_{c(offset)}$= 99K and $T_{c(offset)}$=115K, $T_{c(onset)}$=103K, and $T_{c(onset)}$=141K respectively. The sharp drop at the transition temperature is due to transition within the grains (12). The Sr addition effect on the (δ) was studied, to decide a good number magnitude of addition rate necessary to get the elevated ($T_{c)}$ in Ba- Ca-Cu-O system. Table (2) shows the variation

of both of δ and Tc for different Sr dopant.

$$\upsilon = 2\frac{n+\delta}{n} \quad (13).$$

Where υ *average Cu valence,* n is the number of CuO2 layer (n=3) and δ is the excess of the oxygen content.

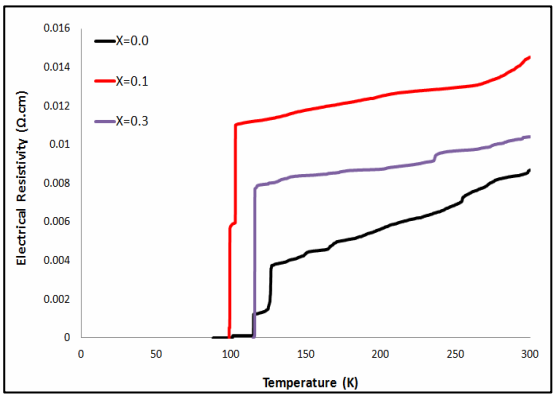

Figure (2): Temperature dependence of resistivity for $Ba_{2-x}Sr_x Ca_2Cu_3O_{10+\delta}$ sintered at Ts=850°C for 60 hrs.

Table (1): $Tc_{(Offset)}$, $Tc_{(onset)}$,transition width ΔT(K),Tc^{mid}(K),and E_g(ev) for $Ba_{2-x}Sr_x Ca_2Cu_3O_{10+\delta}$

Specimens	$T_{c(Offset)}$(K)	$T_{c(onset)}$(K)	ΔT(K)	$T_{c\ mid}$ (K)	E_g(ev)
$Ba_2 Ca_2Cu_3O_{10+\delta}$	88	101	13	94.5	0.026793
$Ba_{0.9} Sr_{0.1} Ca_2Cu_3O_{10+\delta}$	99	103	4	101	0.030142
$Ba_{0.7} Sr_{0.3} Ca_2Cu_3O_{10+\delta}$	115	141	26	128	0.035013

Table (2): oxygen content(δ) and the average Cu valence for different composition of $Ba_{2-x}Sr_x Ca_2Cu_3O_{10+\delta}$

X	δ	*v*	T_c (K)
0.0	0.012262	2.00817	88
0.1	0.048241	2.03216	99

CONCLUSION

Substitution of Sr to 0.0, 0.1 and 0.3 will increase the transition temperature to T_c=88K, 99K, 115K, respectively with increasing oxygen content. While for the other specimens (x=0.1,x=0.3), the resistivity decreases slowly with decreasing temperature. The best nominal composition was found to be $Ba_{0.7} Sr_{0.3} Ca_2Cu_3O_{10+\delta}$ because of the highest value of T_c.

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Study the effect of (Y2O3,SbO2) additives on the dielectrically properties of the [Hg-1223] compound

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ABSTRACT

The nominal composition of $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Y_xCa_2Cu_3O_{8+\delta}$, compounds with x=0 to 0.3 were prepared by solid- state reaction method and studied the dielectrically properties as a function of frequencies at room temperature (R.T). it was found that the dielectric constant for most samples systematically decrease from 65 to 35 by increase the frequency from 50 Hz to 1MHz. The value dielectric constant was increased with increasing the addition of SbO_2, it was showed that the $HgBa_{1.9}Sb_{0.1}Ca_2Cu_3O_{8+\delta}$sample has a high values of dielectric constant while the increasing the Y_2O_3addition in $HgBa_{2-x}Y_xCa_2Cu_3O_{8+\delta}$, compound produce decreasing in dielectric constant. A.C. conductivity ($\sigma_{a.c}$) of all specimens are changing from -14 (x=0) to -17, -20 and -46 relative to the doping of Y_2O_3, (x=0, 0.3, 0.3 and 0.2 respectively) while when doping by SbO_2 are changing from -14 (x=0.0) to -15 (x=0.3).

Keywords: Y_2O_3,SbO_2, [Hg-1223] compound

الملخص باللغة العربية

تم تصنيع المركب الأساسي $HgBa_2Ca_2Cu_3O_{8+\delta}$ والمركبات $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$, $HgBa_{2-x}Y_xCa_2Cu_3O_{8+\delta}$ بطريقة تفاعل الحالة الصلبة. وتم دراسة الخصائص العزلية للمركبات مع تغير التردد عند درجة حرارة الغرفة . وقد أظهرت النتائج أن ثابت العزل الكهربائي يتناقص من قيمة 65 إلى 35 بزيادة التردد من 50 Hz إلى 1MHz . كذلك لوحظ تزايد قيم ثابت العزل الكهربائي بزيادة إضافة SbO_2 عند تردد ثابت 50 Hz . وكان للمركب $HgBa_{1.9}Sb_{0.1}Ca_2Cu_3O_{8+\delta}$ أعلى قيم لثابت العزل، بينما تتناقص قيمة هذا الثابت بزيادة إضافة Y_2O_3 . وقد تغيرت التوصيلية الكهربائية المتناوبة لكل العينات من 14- إلى 17- عند نسبة إضافة تساوي 0.0 و 20-, 46- إلى إضافات أوكسيد اليتريوم Y_2O_3 ، بقيم (x=0, 0.3, 0.3, 0.2) ، بينما عند تغير نسبة التشويب من (x=0.0) إلى (x=0.3)، فإن قيمة التوصيلية الكهربائية المتناوبة تتغير من 14- إلى 15-.

INTRODUCTION

The discovery of Hg-based cuprates superconductors, with general formula $HgBa_2Ca_{n-1}Cu_nO_{2n+2+\delta}$ (n=1,2,3----8) (n is Cu-Olayers) (1) represents the most interesting homologues series out of all known high temperature cuprate superconductors. Undoubtedly the primary reason for this is the high critical transition temperature exhibited by this series (2). (Hg-1201), n=1,has (T_c) of 94 K , (Hg-1212) has T_c=127 K and n=3,(Hg-1223),has T_c = 135 K ,the T_c has been further increased up to 150-160 under high pressure (3). All the super phases of the $HgBa_2Ca_{n-1}Cu_nO_{2n+2+\delta}$ (n=1,2,3,…8) have system crystallizes with a tetragonal structure cell and perovskite layers (4). That is why these compounds are promising candidates for a number of possible applications. However, the scope of Hg-based superconductors' application is not all wide, because of difficulties in reproducible synthesis of samples containing only one superconducting phase; the toxicity of several substances that may be formed during the synthesis, chemical instability of the cuprates obtained (5). Unfortunately ,there are still problems concerning the phase stability, especially the presence of CO_2 and humidity. several reports show that the phase formation and superconducting properties of Hg-1223 are enhanced by means of cation substitutions.
The critical current density and phase formation of Hg-1223 can be improved at doping by high-valence type Re, Pb or other elements (6,7). Electrical insulators have very few free electrons to take part in normal electrical conductivity. Such a material has interesting electrical properties because of the ability of an electric field to polarize the material to create electrical dipole, thus insulating material moleculars are called (Non polar molecules) (8). As well as appearing dipole in a material in the presence of a field, dipoles may be present as a permanent feature of the molecular structure (9).
The dielectric constant is an essential property of dielectric materials. Hence, its determination is very important. There are many techniques have been developed to this end. The most used technique depends on the measurement of either reflection coefficients or resonant frequencies. The aim of the present study was to determine the effect of (Y_2O_3,SbO_2) additives on the dielectrically properties of the Hg-1223 compounds at room temperature at room temperature.

EXPERIMENTAL WORK

By using a sensitive balance to appropriate weights of pure powders of HgO, CaO, CuO, BaO, Y_2O_3 and SbO_2,synthesis the samples with chemical formula $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$, with x = 0 to 0.3 by solid state reaction method, as starting materials, according to the general chemical formulas:

$$HgO + (2\text{-}x)\ BaO + x\ (Y_2O_3 \text{ or } SbO_2) + 2CaO + 3\ CuO \rightarrow HgBa_{2-x}Sb_x(\text{or } Y_x)Ca_2Cu_3O_{8+\delta}$$

After the weight of each reactant, the powders were mixed together by using agate mortar to homogenization the mixture and to form slurry during the process of grinding for about 30-50 minute. The powder was pressed into disc-shaped pellets 15 mm in diameter and 3 mm in thickness, using hydraulic press under a pressure of 5 MPa for 1 min. The pellets were presented at (850) $^{\circ}$C for 24 hrs. by using electric furnace (Carbolite) at heating rate 5 ºC/min and cooling to the room temperature at the same rate of heating. The tests include the dielectric constants (ε'andε''), (tan δ) and (σ_{ac}) as a function of frequency applied field in the ranging from (50-1M) Hz ,at R.T.

RESULTS AND DISCUSSION

Dielectric constant for of the nominal composition of $HgBa_2Ca_2Cu_3O_{8+\delta}$,$HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$, x=0 to 0.3 as a function of frequencies at (R.T) are shown in figures (1-3). It is noticed from these figures that the values of dielectric constant are sharp fall and high at low frequencies and then gradually descend into the lower levels when the frequency at F $\geq 10^4$ Hz for all specimens. This difference in dielectric constant values due to the polarization, polarization categorized into several different types in terms of the displaced units: electronic polarization, ionic polarization, orientational polarization Space charge or interfacial polarization (10, 11). The only effect of polarization that keep pace with the high frequency domain is the electronic, so the dielectric constant data decrease with the increase of frequency. As it can be seen through these forms that additions of Y_2O_3 and SbO_2 drastically lowers the dielectric constant at low frequencies of $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ but at high frequencies (especially at 1 MHz) the lowering of ε is marginal. It may be noted that in the present case, at low frequencies, the addition of Y_2O_3 and SbO_2 claim to a decrease in the values of dielectric constant of $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$, and $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$,with a notes small different in the dielectric constant ratios between x=0.2 and x=0.3. The decrease in dielectric constant values when replacing Ba by Y is due to the difference in atomic size between the two elements.

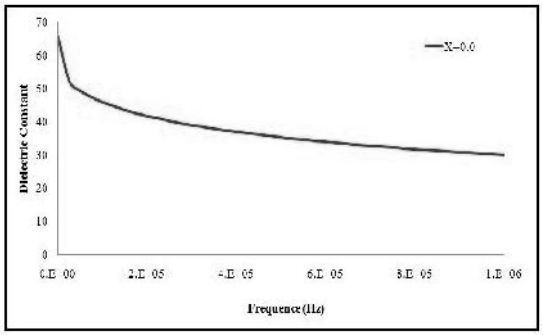

Figure (1): Dielectric constant (ε_r') with frequency for $HgBa_2\ Ca_2Cu_3O_{8+\delta}$ specimen

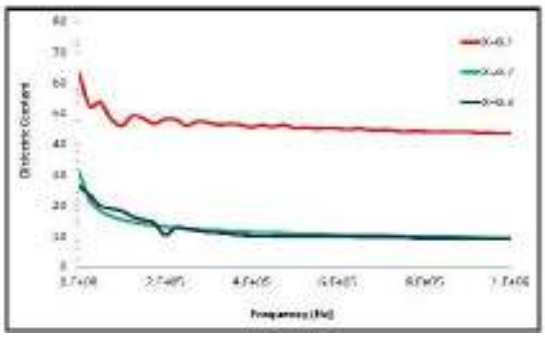

Figure (2): Dielectric constant (ε_r') with frequency for $HgBa_{2-x}Y_x\ Ca_2Cu_3O_{8+\delta}$ specimens

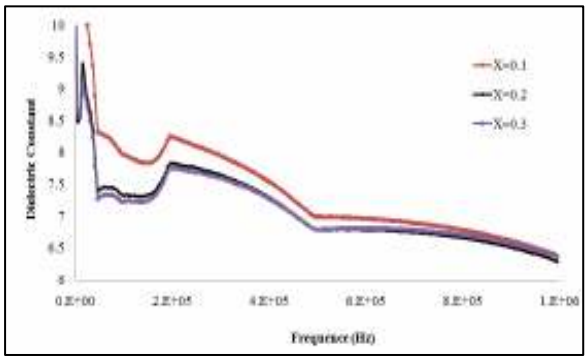

Figure (3): Dielectric constant (ε_r') with frequency for $HgBa_{2-x}Sb_x\ Ca_2Cu_3O_{8+\delta}$ specimens

Figure (4) shows the dielectric constant values as a function of Y_2O_3 and SbO_2 additions at 50MHz for $HgBa_{2-x}Y_x\ Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_x\ Ca_2Cu_3O_{8+\delta}$ specimens. It is obvious that the replacement of BaO by Y_2O_3 gave values of dielectric constant higher than the replacement BaO by SbO_2, this is due to the difference in volumes of the ionic and atomic size between the substituent. It was seen that decrease from567.13 and 65.5376 for $HgBa_{2-x}Y_x\ Ca_2Cu_3O_{8+\delta}$ (x=0.1 and x=0.3) specimens to 54.379 and 26.378 for $HgBa_{2-x}Sb_x\ Ca_2Cu_3O_{8+\delta}$ (x=0.1 and x=0.3) specimens respectively.

Figure (5) shows the specimens at 1MHz it was found thatdielectric constant values for $HgBa_{2-x}Y_x\ Ca_2Cu_3O_{8+\delta}$decreasing from 43.633 (x=0.1) to 9.031 (x=0.3) relative to the doping ofY_2O_3, while when doping by SbO_2increasing from 6.38055 (x=0.1) to 6.35988 (x=0.3).

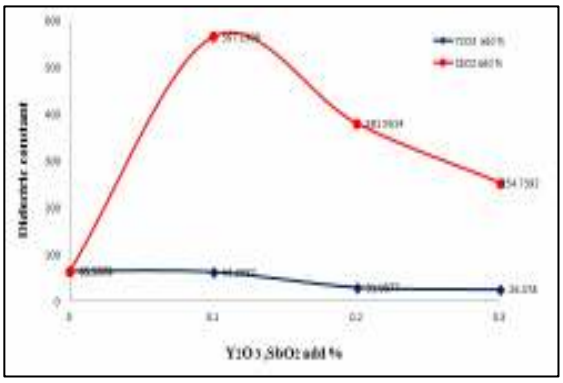

Figure (4): change Dielectric constant (ε_r') at 50Hz with doping for $HgBa_{2-x}Y_x\ Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_x\ Ca_2Cu_3O_{8+\delta}$ specimens

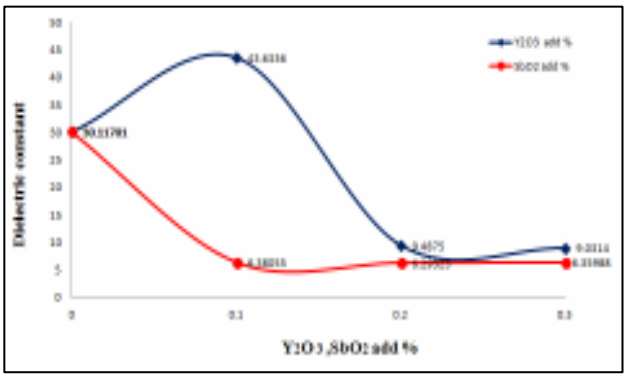

Figure (5): change Dielectric constant at 1MHz with doping for $HgBa_{2-x}Y_x\ Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_x\ Ca_2Cu_3O_{8+\delta}$ specimens

The increase (ε_r') value in $HgBa_{1.9}Sb_{0.1}\ Ca_2Cu_3O_{8+\delta}$ specimen to 567.1309 at 50 Hz, also raise of the dielectric constant value to 43.6336at 1 MHz ,which are illustrated in figures (4,5) due to the increase of the pores. while decreases (ε_r') values with increases of the doping from 0.0 to 0.3 for all specimens at 50 Hz and 1 MHz a leads to decrease the total pores. Ceramics are generally non-metallic inorganic compounds, e.g. oxides. These have excellent dielectric properties. The dielectric constant of most commonly used ceramics varies between 4 and 10. These are used in switches in plug holders, thermocouples, cathode heaters, vacuum type ceramic metal seals etc. (12).

The results of Imaginary dielectric constant (ε_r'') are plotted in figures (6-8) with frequencies for of the nominal composition of $HgBa_2Ca_2Cu_3O_{8+\delta}$, $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_x$ $Ca_2Cu_3O_{8+\delta}$ specimens. In general, a dipole tends to align itself along the direction of applied electric field and for ac fields tends to follow the field and be in a phase with it. However, the interaction of this dipole with other dipoles in the medium prevents this and this leads to dielectric loss. This loss appears as heat. This dielectric loss is connected with (ε_r'') (12).

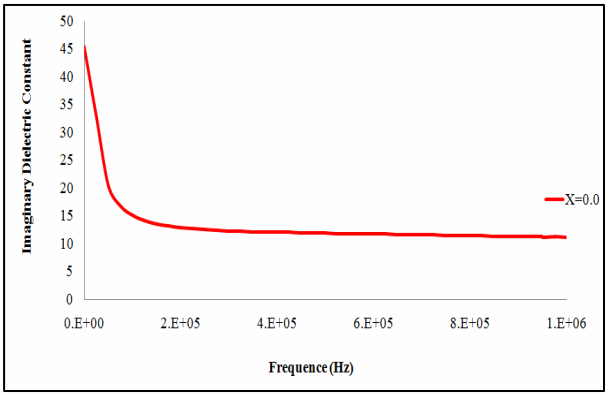

Figure (6):Imaginary dielectric constant (ε_r'') with frequency for $HgBa_2$ $Ca_2Cu_3O_{8+\delta}$ specimen

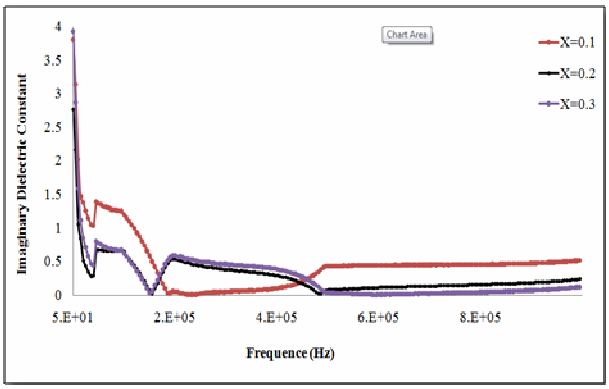

Figure (7):Imaginary dielectric constant (ε_r'') with frequency for $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ specimens

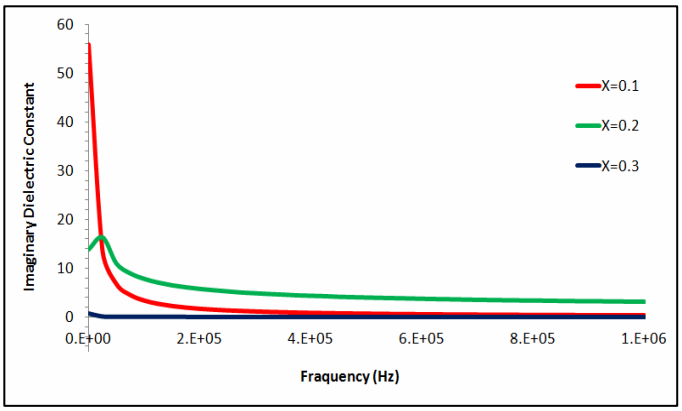

Figure (8):Imaginary dielectric constant (ε_r'') with frequency for $HgBa_{2-x}Sb_x$ $Ca_2Cu_3O_{8+\delta}$ specimens

The loss tangent (tanδ) with of applied frequency for of the nominal composition of $HgBa_2Ca_2Cu_3O_{8+\delta}$, $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_x$ $Ca_2Cu_3O_{8+\delta}$ specimens as shown in figures (9-11). It was noticed from the figures that," Ionization losses occurs in gases and solids having pores with entrapped gases. With the rise in field strength applied to a gas, a stage is reached when the gas molecules gets ionized due collisions. This leads to enhanced conduction leading to dielectric losses" (12).

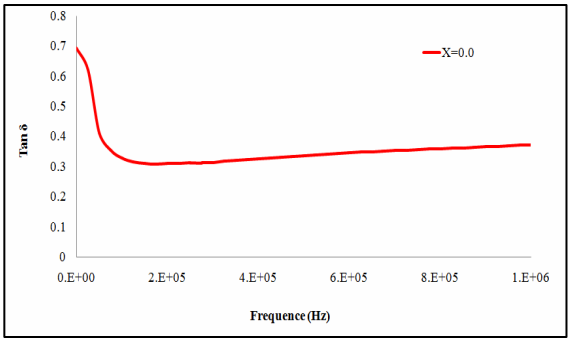

Figure (9): (tanδ) with frequency for $HgBa_2$ $Ca_2Cu_3O_{8+\delta}$ specimen

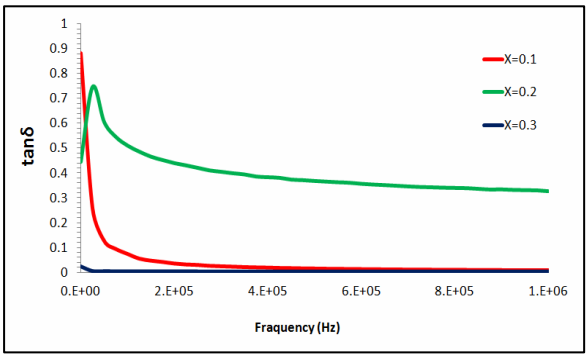

Figure (10): (tanδ) with frequency for $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ specimens

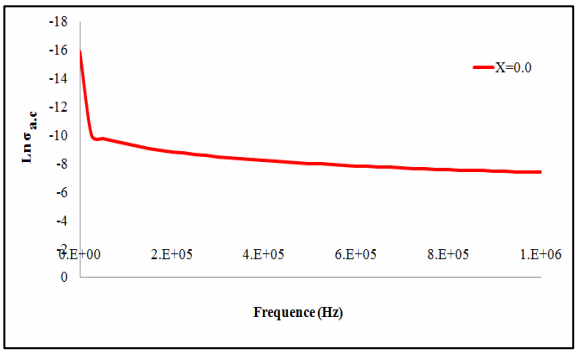

Figure (12): σ_{ac} with frequency for $HgBa_2$ $Ca_2Cu_3O_{8+\delta}$ specimen

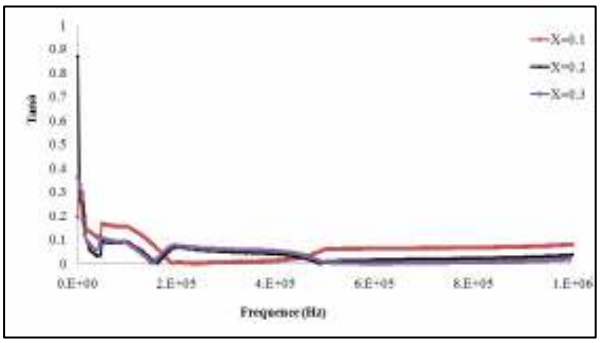

Figure (11): (tanδ) with frequency for $HgBa_{2-x}Sb_x$ $Ca_2Cu_3O_{8+\delta}$ specimens

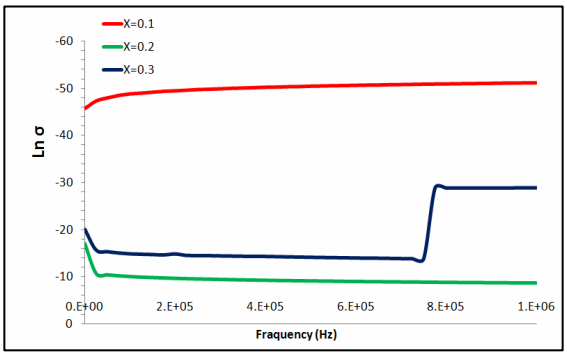

Figure (13):σ_{ac} with frequency for $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ specimens

The results of A.C. conductivity ($\sigma_{a.c}$) of the nominal composition of $HgBa_2Ca_2Cu_3O_{8+\delta}$, $HgBa_{2-x}Y_x$ $Ca_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Sb_x$ $Ca_2Cu_3O_{8+\delta}$ specimens are plotted as a function of the frequency , and defined by the figures (12-14). Figures (12 and 13) showed that A.C. conductivity ($\sigma_{a.c}$) of all specimens are changing from -14 (x=0) to -17, -20 and -46 relative to the doping of Y_2O_3, (x=0, 0.3, 0.3 and 0.2 respectively) while when doping by $SbO_2$are changing from -14 (x=0.0) to -15 (x=0.3) as shown in figure (14). For dc conditions, the current that passes through the capacitor will be determined by its dc conductivity. As the frequency increases, more and more of the bound charges will start to oscillate out of phase with the applied voltage and will contribute to σ_{ac} (13).

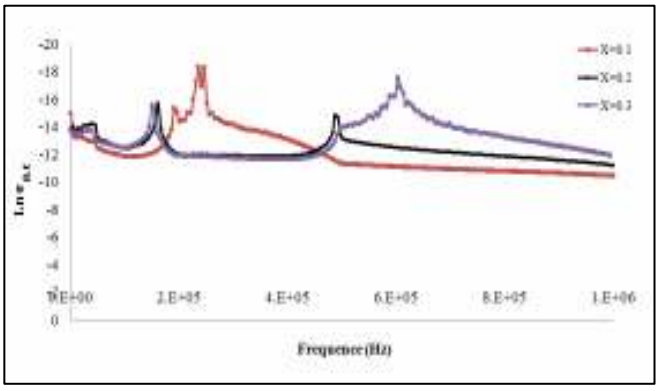

Figure (14): σ_{ac} with frequency for $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$ specimens

CONCLUSION

In the present paper, the authors have successfully synthesized he nominal composition of $HgBa_{2-x}$(Y and Sb)$_xCa_2Cu_3O_{8+\delta}$ compounds with x=0 to 0.3 have been prepared by solid state reaction process .We have investigated the effect of simultaneous doping of Y and Sb at Ba site of Bar-O layer in $HgBa_{2-x}Sb_xCa_2Cu_3O_{8+\delta}$ and $HgBa_{2-x}Y_xCa_2Cu_3O_{8+\delta}$ with special emphasis on correlation between dielectric constant properties and the observed A.C. conductivity. It was found that the dielectric constant for most samples systematically decrease from 65 to 35 by increase the frequency from 50 Hz to 1MHz. The value dielectric constant was increased with increasing the addition of SbO_2, it was showed that the $HgBa_{1.9}Sb_{0.1}Ca_2Cu_3O_{8+\delta}$ sample has a high values of dielectric constant while the increasing the Y_2O_3 addition in $HgBa_{2-x}Y_xCa_2Cu_3O_{8+\delta}$, compound produce decreasing in dielectric constant. A.C. conductivity ($\sigma_{a.c}$) of all specimens are changing from -14 (x=0) to -17, -20 and -46 relative to the doping of Y_2O_3, (x=0, 0.3, 0.3 and 0.2 respectively) while when doping by SbO_2 are changing from -14 (x=0.0) to -15 (x=0.3).

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قسم الدراسات والبحوث العربية

ARABIC STUDIES AND RESEARCHES SECTION

دراسة مرضية نسيجية للزوائد الأنفية للمرضى المصابين بالتهاب الجيوب الأنفية المزمن في مدينة الموصل / العراق

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الملخص باللغة العربية

هدفت الدراسة الحالية إلى التحري عن وجود الغشاء الحيوي biofilm داخل نسيج الزوائد الأنفية، والتعرف على الجراثيم الممرضة المكونة للغشاء الحيوي، بالإضافة إلى دراسة التغيرات النسيجية للزوائد الأنفية المصاحبة لالتهاب الجيوب الأنفية المزمن ، حيث تم جمع (11) عينة (4) منها للذكور و(7) منها للإناث المصابين بالتهاب الجيوب الأنفية المزمن وجمعت العينات من مستشفيات الجمهوري ، الربيع الأهلي والزهراوي الأهلي للفترة من شهر آذار/ مارس عام 2013 ولغاية شهر آذار/ مارس عام 2014 . جرى حفظ العينات في محلول الفورمالين الدارئ المتعادل (10%) ، وتم تحضير مقاطع نسيجية منها، ثم صبغت بصبغتي الهيماتوكسلين والأيودين وصبغة كرام النسيج.
أظهرت نتائج الفحص النسيجي وجود الغشاء الحيوي في بعض العينات، ووجود تغيرات نسيجية مرضية متمثلة بارتشاح الخلايا الالتهابية، وبخاصة الخلايا الحمضية واللمفية والبلعمية، بالإضافة إلى النخر والوذمة والنزف في الغشاء المبطن للتجويف للجيوب الأنفية، فضلا عن وضوح الإصابة الجرثومية، حيث تم عزل الجراثيم السالبة والموجبة لصبغة كرام من الزوائد الأنفية والتي شكلت نسبتها (11.43%).

الكلمات المفتاحية: غشاء حيوي، تغيرات نسيجية ، التهاب الجيوب الأنفية المزمن

Histological study of nasal polyps of affected patients with Chronic rhinosinsitis in Mosul/Iraq

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ABSTRACT

The aim of the present study was to find the presence of biofilm within the tissue of nasal polyps ,as well as to investigate the microorganisms that forming the biofilm .Also to study the histopathological changes of the nasal polyps that accompanying the chronic rhinosinusitis. A sample of (11) specimens were collected, (4) males and (7) females who suffered from chronic rhinosinusitis. Samples were collected from Al-Jamhory, Al-Rabea and Al-Zahrawy hospitals in Mosul City, for a period from March 2013 to March 2014. Specimens were preserved in (%10) neutral buffered formalin and histological sections were prepared and stained with H and E stain and Gram tissue stain. The results revealed the presence of histopathological changes represented by infiltration of inflammatory cells especially eosinophils, lymphocytes and macrophages in addition to necrosis , oedema, hemorrhage in mucous membrane of nasal sinus as well as presence of bacterial infection , in which G^+ and G^- bacteria has been isolated and it ratio is (%11.43).

المقدمـــــــة

تعرف الزوائد الأنفية على أنها كتل لحميـة تنـشأ مـن الغـشاء المخاطي المبطن للأنف والجيوب الأنفية، وعادة تكون مـصاحبة لالتهاب الأنف التحسسي، وكذلك تعد مـن مـضاعفات التهـاب الجيوب الأنفية المزمن (1). تتعرض الجيوب الأنفية للالتهـاب المزمن الذي يستمر لفترة أكثر من (12) أسبوعا، وهو أقل شيوعا من التهاب الجيوب الأنفية الحاد، ويحدث التهاب الجيوب الأنفيـة المزمن كتطور لحالة من حالات التهاب الجيوب الأنفيـة الحـاد، ويعتمد تشخيص الالتهاب المزمن علـى الأعـراض الـسريرية المتمثلة بانسداد الأنف وتحسسه، إفرازات الأنف الأمامي والخلفي، وقد يكون لون الإفرازات أصفرا مائلا إلى الاخضرار، مـع ألـم وضغط في الجيب المصاب ، وانخفاض حاسة الشم ويـتم تأكيـد التشخيص بالنـاظور الأنفـي Nasal endoscopy أو بالأشـعة المقطعية CT-Scan (2).

المواد وطـــرق العمـل

جمع العينات

تم الحصول على عينات الدراسة من (11) مريـضا، (4) ذكـور و(7) إناث، مصابين بالتهاب الجيوب الأنفية المزمن والخاضـعين إلى عملية الناظور الأنفي الـوظيفي Functional Endoscopy Sinus Surgery (FESS) من مستشفيات الجمهـوري والربيـع الأهلي والزهراوي في مدينة الموصل للفترة من شهر آذار/ مارس 2013 ولغاية شهر آذار/ مارس 2014 ، حيث تم جمع الخـزع النسيجية biopsy من المرضى الذين لديهم زوائد أنفية. وضعت الخزع في الملح الفسيولوجي phosphate buffer saline ونقلت العينات إلى المختبر، وغسلت عدة مرات بـنفس المحلـول ثـم حفظت في محلول الفورمالين الدارئ المتعادل (10%) Neutral buffer formalin.

تحضير المقاطع النسيجية من الزوائد الأنفية

حفظت عينات الدراسة بعد جمعها في محلول الفورمالين الـدارئ المتعادل (10%) لغرض تثبيتها لمدة (48-72) ساعة، بعد ذلك تم تقطيعها إلى قطع صغيرة بحجم ($1cm^3$) ، ثـم أجريـت عليهـا عمليات التمرير بالكحول الأثيلي بتراكيز تصاعدية -90-100%) (70-80، ثم بالزايلول، وصبت بالشمع على شكل قوالب وقطعت بجهاز المشراح microtome بسمك (4-5) مايكرون، ثم صبغت بالصبغة الروتينية الهيماتوكسلين والأيوسين، وفحـصت الـشرائح النسيجية بالمجهر الضوئي (3-5).

تحضير المقاطع النسيجية وصبغها بصبغة كرام النسيج

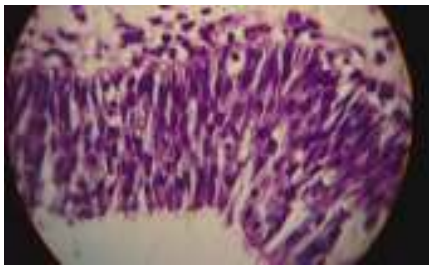
بعد جمع عينات الدراسة، حفظت في محلول الفورمالين الـدارئ المتعادل (10%) لغرض تثبيتها لمدة (48-72) ساعة بعد ذلك تم تقطيعا إلى قطع صغيرة بحجم ($1cm^3$) أجريت عليها التمريرات التصاعدية بالكحول الاثيلي (70-80-90-100%) ثم الزايلـول وبعد تقطيعها بالمشراح بسمك (4-5) مـايكرون صـبغت هـذه العينات بصبغة كرام النسيج حسب طريقة الباحث (5) ثم فحصت بالمجهر الضوئي.

النتـــــائج والمناقشـــــة

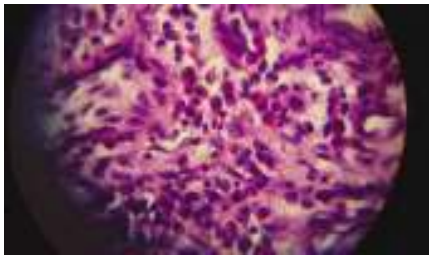
أظهرت نتائج الفحص النسيجي المجهري لعينات الدراسة والتي لم يتم عزل الجراثيم منها ولم يلاحظ وجود الغشاء الحيـوي فيهـا، وجود تغيرات نسيجية تمثلت بفرط التنسج hyperplasia، واختفاء الأهداب والارتشاح الكثيف للخلايا الالتهابيـة المتمثلـة بالخلايـا الحمضة والبلعمية واللمفية والبلازمية في طبقة السدى stroma وحول الأوعية الدموية (الأشكال 1-3). ويرجـع ظهـور هـذه التغيرات النسيجية إلى وجود عوامل عديدة أسهمت فـي تكـوين الزوائد منها التهاب الجيوب الانفية الزمن، الربو، التهاب الأنـف التحسسي (6) فـضلا عـن تحريـر الـسايتوكينات cytokines والكيموكينات chemokines الذي يؤدي إلـى جـذب الخلايـا الحمضية والقاعدية كما تحفز على تحرير الخلايا البدينـة mast cell للهستامين، الذي بدوره يؤدي إلى زيادة في سـرعة جريـان الدم، بالإضافة إلى زيادة في نفاذية الأوعيـة الدمويـة، وبالتـالي وصول الخلايا الالتهابية إلى منطقة الأذى (7).

اتفقت هذه النتائج مع ما وجده الباحث (1) ،فيما أظهرت عينـات أخرى تغيرات مرضية نسيجية تمثلت بالاحتقـان والتوسـع فـي تجويف الأوعية الدموية، فضلا عن ارتـشاح الخلايـا الالتهابيـة حولها. جميع هذه التغيرات ظهرت بسبب الحساسية، إذ لم يلاحظ ولم يتم عزل أي جراثيم أو غشاء حيوي من هذه العينات. وهذا ما أكده الزرع على الأوساط الزراعية الجرثومية ، وقد اتفقت هـذه النتائج مع نتائج دراسة كل من (1، 8).

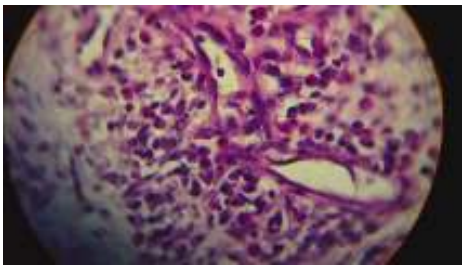
أظهرت العينات التي كانت موجبة للزرع الجرثومي على الأوساط الزرعية، والتي تم عزل الجراثيم منها وجـود تغيـرات نـسيجية تمثلت بالنزف الشديد ونخر الخلايا الظهارية والغـشاء المخـاطي للجيوب الأنفية، مع وجود الوذمة والخزب oedema، فضلا عـن ارتشاح البؤري للخلايا الالتهابية المتمثلة بالخلايا البلعمية واللمفية والبلازمية (الشكل 4). وفي عينـات أخـرى تمثلـت التغيـرات النسيجية بتضيق Stenosis في تجاويف الغدد الشمية مع وضوح الارتشاح الكثيف للخلايا الالتهابية البلعمية واللمفية والبلازمية بين الغدد الشمية (الشكل 5).



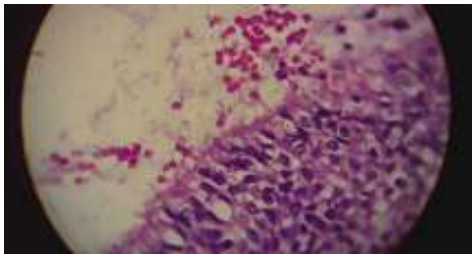
شكل (1): مقطع نسيجي في الغشاء المخاطي للزوائد الأنفية يوضح فرط التنسج للخلايا الظهارية المبطنة للغشاء المخاطي ،اختفاء الأهداب وارتشاح الخلايا الالتهابية (الحمضة،اللمفية،البلعمية) في طبقة السدى مصبوغ بصبغة H &E. قوة تكبير (100X)



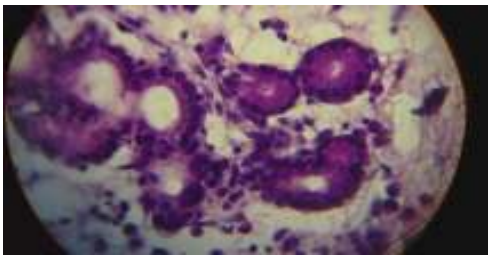
شكل (2): مقطع نسيجي يوضح الارتشاح الكثيف للخلايا الالتهابية الحمضة واللمفية والبلعمية البلازمية مصبوغ بصبغة H &E قوة تكبير (100X)



شكل (3): مقطع نسيجي في النسيج المخاطي للزوائد الأنفية يوضح توسع في تجويف الوعاء الدموي مع ارتشاح الخلايا الالتهابية حوله (الحمضة واللمفية والبلعمية) مصبوغ بصبغة H &E قوة تكبير (100X)

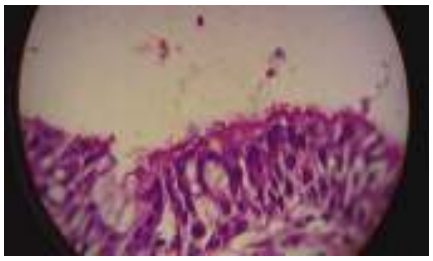


شكل (4): مقطع نسيجي يوضح النزف ونخر الخلايا الظهارية المبطنة للتجويف الأنفي بالإضافة إلى ارتشاح الخلايا الالتهابية (البلعمية واللمفية) مصبوغ بصبغة H &E قوة تكبير (100X)



شكل (5): مقطع نسيجي في الغشاء المخاطي للزوائد الأنفية يوضح تضيق في تجويف الغدد الشمية فضلا عن وضوح ارتشاح للخلايا الالتهابية (البلعمية واللمفية) بين الغدد الشمية مصبوغ بصبغة H &E قوة تكبير (100X).

إن سبب ظهور هذه التغيرات النسيجية نتيجة للإصابة الجرثومية والتي تقوم هذه الجراثيم بتحرير العديد من عوامل الفوعه أو الضراوة virulence factor، التي ترتبط بتكاثر الجراثيم في الجيوب الأنفية، والتي من خلالها تقوم الجراثيم بمقاومة الآلية الدفاعية للجسم فتحدث التغيرات النسيجية من خلال إنتاج الذيفانات والسموم toxins أو مواد الالتصاق adhesive factors بالسطح، فضلا عن إنتاجها لعوامل الإجهاد التأكسدي، وخاصة السوبر أوكسيد superoxide ، الذي يؤدي إلى حدوث التغيرات النسيجية المرضية وغيرها من عوامل الضراوة (9). وعند التحري عن وجود الغشاء الحيوي أظهرت النتائج وجود الغشاء الحيوي الذي كونته الجراثيم بنسبة (11.43%) من العينات كما في الشكل (6). اتفقت هذه النتائج مع ما وجده (3)، حيث وجد الغشاء الحيوي في المرضى الذين يعانون من التهاب الجيوب الأنفية المزمن، وباستخدامه نفس الصبغة، أي صبغة كرام المستخدمة في الدراسة الحالية، إذ لاحظ وجود الغشاء الحيوي بنسبة (62 %) من المرضى.

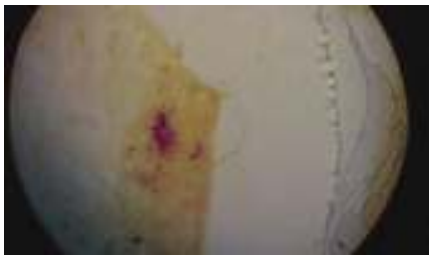


شكل (6): مقطع نسيجي في الغشاء المخاطي للزوائد الانفية يوضح وجود الغشاء الحيوي مصبوغ بصبغة H &E قوة تكبير (100X)

التحري عن وجود الجراثيم في نسيج الزوائد الأنفية باستخدام صبغة كرام النسيج:

أظهرت الدراسة الحالية إصابة الزوائد الأنفية بالجراثيم الموجبة لصبغة كرام G+ve ، حيث ظهرت بلون أزرق في النسيج كما موضح بالشكل (7) ، فضلا عن ظهور الجراثيم السالبة لصبغة كرام G-ve في عينات أخرى كما موضح في الشكل (8) .اتفقت هذه النتائج مع ما وجده (10)، حيث أثبت وجود جراثيم في الزوائد الأنفية.

شكل (7): الجراثيم الموجبة لصبغة كرام الموجودة في النسيج المصبوغ بصبغة كرام النسيج. قوة التكبير(100X)



شكل (8): الجراثيم السالبة لصبغة كرام باللون الأحمر الموجودة في النسيج مصبوغ بصبغة كرام النسيج قوة تكبير (100X) .

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التأثير الأليلوباثي لأوراق نبات الكونوكاربس *Concarpus lancifolius* الجافة ومستخلصها المائي في مؤشرات النمو الخضري والزهري لنبات الأقحوان *Calendula officinalis*

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الملخص باللغة العربية

أجريت تجربتين لدراسة تأثير أوراق نبات الكونوكاربس الجافة ومستخلصها في مؤشرات النمو الخضري والزهري لنبات الأقحوان. كانت الأولى تجربة حقلية في مشتل كلية الزراعة /جامعة الكوفة خلال الموسم الزراعي 2013-2014 ، حيث زرعت شتلات نبات الأقحوان *Calendula officinalis* في سنادين، وعُوملت بمسحوق أوراق نبات الكونوكاربس *Conocarpus Iancifolius* الجافة بالنسب التالية (0، 5، 10، 20 غم/ كغم تربة). ونفذت التجربة باستعمال تصميم القطاعات العشوائية الكاملة Randomized Complete Block Design وبثلاث مكررات، وبواقع خمس نباتات للوحدة التجريبية. وتمت المقارنة بين المتوسطات حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 5 %.

أما التجربة الثانية، فكانت التجربة المختبرية في مختبرات كلية الزراعة / جامعة الكوفة خلال الموسم الزراعي (2014)، لغرض دراسة التأثير الأليلوباثي للمستخلص المائي لأوراق نبات الكونوكاربس *Conocarpus Iancifolius* الجافة في إنبات ونمو بذور نبات الاقحوان *Calendula officinalis* ،وتضمنت التجربة زراعة بذور نبات الأقحوان *Calendula officinalis* في أطباق بيري ومعاملتها بالمستخلص المائي البارد والمغلي لأوراق نبات الكونوكاربس *Conocarpus Iancifolius* بتراكيز (0 ، 25 ، 50 ، 75 ،100 %)، ونفذت التجربة باستعمال تصميم تام التعشية(C.R.D) بثلاث مكررات لكل معاملة وبواقع (10) بذور للوحدة التجريبية.

وقد جاءت نتائج التجربتين كما يلي:

أولا: التجربة الحقلية: كان هناك تأثير تثبيطي في صفات النمو الخضري في تراكيز10، 20 غم/كغم تربة والذي سبب انخفاضًا معنويا في ارتفاع النبات (سم) ، وعدد الأوراق ،والمساحة الورقية (سم^2) ، والوزن الطري والجاف للمجموع الخضري (غم) ، مقارنة بنباتات المقارنة (المرشوشة بالماء المقطر فقط) والتي أعطت أعلى معدل للنمو الخضري. أما بالنسبة للنمو الجذري فقد أظهرت النتائج أن هناك تباينا معنويا في التراكيز المستخدمة في مؤشرات النمو الزهري مقارنة مع معاملة السيطرة.

ثانيا: التجربة المخبرية: أظهرت النتائج أن المعاملة بالمستخلصات المائية وبتراكيز مختلفة أدت إلى حصول اختزال معنوي في النسبة المئوية للإنبات(%)، وفي طول الرويشة وطول الجذير (سم). وأن مستخلص الأوراق المائي البارد كان له تأثير تثبيطي أكثر من تأثير المستخلص المائي المغلي. ولوحظ زيادة في تثبيط معايير النمو المدروسة مع زيادة التراكيز ولكل من مستخلص الماء البارد والمغلي، مقارنة مع معاملة السيطرة، وكان تركيز 25% مستخلص الماء البارد محفزا لكل من طول الرويشة والجذير.

الكلمات المفتاحية: نبات الكونوكاربس *Conocarpus Iancifolius*، نبات الأقحوان *Calendula officinalis*

The allelo pathic effect of *Conocarpus Iancifoliu* plant dry leaves and aqueous extract on some vegetative and flowering characteristics of *Calendula officinalis*

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ABSTRACT

Tw**o** experiments were conducted to study the effects of the dry leaves of *Conocarpus Iancifolius* plant and its aquatic extract on vegetative and flowering growth indicators for *Calendula officinalis plant.* The first one was a field experiment at faculty of Agriculture /Kufa University during the spring growing season of 2013 -2014 , where *Calendula officinalis plant* were cultivated in plastic pots and treated by dry leaves powder in ratio (0,5,10,20 gm/kgm soil). Randomized complete Block Design was used with three replications in five plants for experimental unit, using Duncan's multiples range test to compare means with probability of 0.05 level. Laboratory experiment was conducted at the laboratory of the faculty of Agriculture /Kufa University during the spring growing season of 2014 , to study the direct allelopathic effects of the water extracts for leaves of *Conocarpus* in germination of *Calendula officinalis* seeds in petri –dishes and treated by cold water (at room temperature) and boiling water extracts and prepared concentrations of 25% , 50%, 75% and 100% as well as the control treatment (water). Randomized complete Block Design was used with three replications.

The results of the two experiments were as follows:

1. for the field experiment: there was inhibitor effect in vegetative growth character in 10, 20 gm/kgm soil which ,causes inhibition in plant height ,number of leaves, leaves area, shoot fresh and dry weight(gm) compared with control treatment (distill water) which give higher value. Results showed the difference in flowering growth parameter campered with control group.
2. the lab experiments: the results revealed that the extract treatment caused significant reduction in germination percentage for seeds , plumule, radical growth and plumule . Inhibition effects were highest for the cold water leaf extract than boiled water leaf extracts. All concentrations of leaf extracts caused reduction in growth parameter but 25% concentration of extract by cold water caused an increase in Plumula length.

المقدمـــــــة

ينتمي نبات الأقحوان *Calendula officinalis* L. إلى العائلة المركبة Asteraceae التي تشمل العديد من نباتات الزينة المهمة اقتصاديا كالداليا ،الداوودي والجيربرا وغيرها، ويزرع الكثير منها لاغراض الزينة كالداليا والقديفة والأقحوان والأستر (1) ، إذ يعد من النباتات المألوفة والمرغوبة في حدائق الزهور، فهو يزرع إما في ألواح الأزهار أو في أحواض الأزهار، وقد يستفاد منه كأزهار قطف (2). موطنه الأصلي حوض البحر الأبيض المتوسط وجنوب أوروبا ، وهو من الأزهار الحولية الشتوية، فجذره وتدي أبيض مصفر مائل إلى بني فاتح، يبلغ طوله نحو 20 سم وقطره 7 ملم ، ويحمل العديد من التفرعات الجذرية (3). وتكمن أهمية نبات الأقحوان كونه نباتاً للزينة سريع النمو، وتعد أزهاره صالحة للقطف ومهمة في عمل الباقات الزهرية المتنوعة للمكاتب الرسمية والبيوت (4)، وقد أثبتت هذه النباتات نجاح زراعتها في الأصص، خاصة الأصناف المتقزمة منها، ويمكن زراعتها في الحدائق، كما يتميز النبات بتحمله الصقيع، ويحب النبات المواقع المشمسة، ويمكن التبكير بإنتاج أزهاره من خلال زراعته في المراقد الداخلية (5).

كما يعود نبات الكونوكاربس *Conocarpus Iancifolius* إلى العائلة Combretaceae ، ويضم نوعين معزولين *C. lancifolius* و *C. erectus* (6، 7). والنوع *C. lancifolius* هو موضوع البحث. موطنه الأصلي الصومال وجيبوتي واليمن وشرق أفريقيا وأرتيريا (8، 9)، كما يوجد في جنوب الجزيرة العربية والسودان والهند وباكستان وأستراليا (10، 11). وهو من النباتات ذوات الفلقتين، دائم الخضرة، كثير التفرعات. يصل ارتفاعه إلى أكثر من20 مترا، في حين يتراوح قطره ما بين 60–250 سم (11، 12). تكون الأوراق بسيطة رمحية متبادلة معنقة طولها 6.8–14.7 سم وعرضها 2–3.7 سم (13)، ومعدل مساحتها 22.4 سم وسماكتها 150ميكرون (14)، وتحتوي على العديد من الغدد الشعرية Hairy glands، بالإضافة إلى تراكيب إفرازية لاشعرية Non hair secretory structures ، ويكون تعريق الأوراق ريشياً وعرقها الوسطي بارزاً وسطحها مغطى بشعيرات مع وجود غدد رحيقية على جانبي السويق وحواف الأوراق (13). يعتبر من نباتات الزينة المهمة الأساسية في الحدائق ومشروعات التشجير ومواجهة ظاهرة التصحر، نظرا لسرعة نموه في المناطق الحارة (15)، بالإضافة إلى قدرته على مقاومة الجفاف وملوحة المياه والتربة. ينمو النبات بشكل جيد في الأراضي الطينية المزيجية والصحراوية الرملية، وقد انتشرت زراعته انتشارا مذهلا في دول الخليج والعراق، نظرا لنموه السريع وتقليمه وتشكيله وزراعته كسياج أخضر (16).

تعتبر ظاهرة الأليلوباثي ظاهرة بيئية تحدث بوجود مواد كيميائية سامة تسمى بالأليلوكيميائية Allelochemicals أو تسمى Phytotoxine ، تنتج من قبل النبات وتطرح إلى البيئة، فتؤثر في نمو وتكشف النباتات المجاورة له (17). وقد ذكرت الجحيشي (18) أن أهم الركبات الأليلوباثية التي شخصت هي الحوامض الفينولية القابلة للذوبان في الماء، القلويدات، الكلايكوسيدات، الفلافونات، الأحماض الأمينية غير البروتينية، الكومارينات، التربينات، التانينات، الستيرويدات، الزيوت والراتنجات. كما تتباين مركبات الأيض الثانوي اعتمادا على طبيعتها الكيميائية، إلا أن أكثرها أهمية هي المركبات الفينولية والمركبات القلوانية، والمركبات التربينية (19– 21). و أكدت نتائج دراسة الزبيدي (22) وجود المركبات الأليلوباثية في مختلف أجزاء النبات من الأوراق بالدرجة الأولى، ثم الجذور، السيقان، الأزهار، البذور، الثمار، الرايزومات، وحتى حبوب اللقاح. وقد اختبرت دراسة (23) المستخلصات المائية لأوراق نبات اليوكالبتوس والياس والدفلة المضافة لأطباق بتري وللتربة، والتي استنتجت أن تأثير مستخلص الأوراق المائي المغلي كان أكثر تثبيطا في طول الرويشة، في حين كان المستخلص البارد هو الأكثر تثبيطا في طول الجذير باستعمال أطباق بتري وفي السنادين. وكان تأثير المستخلصات أكثر تثبيطا في طول الرويشة والجذير في بادرات الرويطة منه في بادرات الحنطة، كما أظهرت مستخلصات أوراق الدفلة والتراكيز العالية من المستخلصات تثبيطا أكبر لطول الرويشة والجذير في كل من أطباق بتري وفي السنادين البلاستيكية. في حين وجد في دراسة (23) أن نبتة الكونوكاربس تحتوي على مادة التانين و تبلغ نسبة التانين في لحاء أشجار الكونوكاربس بحوالي (16– 18 %). وأكدت دراسة (24) على أن مادة التانين تستخلص من الخشب والأوراق وقلف الأشجار.

وبناء على ما تقدم، تم تنفيذ هذه الدراسة بهدف معرفة التأثير الأليلوباثي للأوراق الجافة ومستخلصها المائي لنبات الكونو كاربس *Conocarpus Iancifolius* في إنبات و نمو نبات الأقحوان *Calendula officinalis* .

المواد وطرق العمـــــل

أولا: التجربة المختبرية:

أجريت التجربة في مختبرات كلية الزراعة / جامعة الكوفة خلال الموسم الزراعي (2014)، لغرض دراسة التأثير الأليلوباثي للمستخلص المائي لأوراق نبات الكونوكاربس الجافة في إنبات بذور نبات الأقحوان، وذلك وفق الخطوات التالية:

1– جمع وتشخيص العينات: جمعت أوراق نبات الكونوكاربس من حدائق في كلية الزراعة / جامعة الكوفة في شهر تشرين الأول لسنة2013، بعدها تم تنظيفها ووضعها على ورق مقوى في غرفة ذات تهوية جيدة لتجف بشكل أولي، أما التجفيف النهائي فتم باستعمال الفرن الكهربائي بدرجة حرارة 45 مْ لمدة 48 ساعة، بعد ذلك طحنت الأوراق الجافة على هيئة مسحوق بواسطة المطحنة الكهربائية نوع(Moulinex)، وبعدها نخل المسحوق بمنخل قطر فتحاته 0.2 ملم، وحفظ المسحوق لحين الاستعمال.

2– طريقة تحضير المستخلصات المائية:

2–1 المستخلص المائي البارد: حضر المستخلص المائي البارد لأوراق نبات الكونوكاربس حسب طريقة كل من (21، 25). بعد ذلك، تم تحضير التراكيز (100,75,50,25,0%) ، ثم حفظت المستخلصات في دوارق زجاجية محكمة الغلق في الثلاجة.

2–2 المستخلص المائي المغلي: تم تحضير مستخلص الماء المغلي لأوراق نبات الكونو كاربس باستعمال خطوات الطريقة السابقة بحسب كل من (21، 25)، مع استبدال الماء البارد بماء مغلي بدرجة 100مْ إلى مسحوق المادة النباتية الجافة.

3– مصادر البذور وتهيئتها: تم الحصول على بذور نبات الأقحوان من حدائق كلية الزراعة في جامعة الكوفة، وتم إحضارها إلى المختبر لغرض عزلها وتنظيفها من الشوائب، ثم عقمت جميع البذور المستعملة بمادة كلوريد الزئبق بتركيز 0.1% لمدة عشر دقائق، ومن ثم تم غسلها بالماء المقطر (26).

4– الزراعة في أطباق بتري: زرعت (10) بذور من نبات الأقحوان على أوراق ترشيح رقم (Wathmann- No.1) موضوعة في أطباق بتري معقمة بقطر (10) سم، وأضيف لكل طبق (10) مل من مستخلص أوراق النبات وبالتراكيز (100,75,50,25,0)% ، وبمعدل ثلاثة مكررات لكل تركيز. ثم وضعت الأطباق في المختبر وبدرجة حرارة (25) مْ وذلك تبعا لطريقة (27). استمرت التجربة لمدة (15) يوما، إذ تم خلالها تسجيل المعايير الآتية:

– حساب النسبة المئوية للإنبات: تم تسجيل عدد البذور النابتة في اليوم العاشر من تاريخ الزراعة، وجرى تحويل القيم إلى النسبة المئوية حسب المعادلة التالية:

$$\text{النسبة المئوية للإنبات} = \frac{\text{عدد البذور النابتة}}{\text{العدد الكلي للبذور}} \times 100$$

– قياس معدل أطوال الرويشة والجذير بعد مرور15 يومـــــــاً من الزراعة: تم قياس معدل طول الرويشة من نقطة اتصالها مع الجذير وحتى النهاية لكل نبتة، ثم أخذ المعدل لجميع البادرات ضمن المعاملة .أما طول الجذير فقد تم قياس أطوال الجذور لكل نبتة، ثم أخذ المعدل لجميع البادرات ضمن المعاملة (28).

ثانيا: التجربة الحقلية:

أجريت التجربة الحقلية في مشتل قسم البستنة وهندسة الحدائق، كلية الزراعة في جامعة الكوفة خلال الموسم الزراعي -2014 2013 ، لدراسة التأثير الأليلوبائي لنبات الكونوكاربس في بعض مؤشرات النمو الخضري والزهري لنبات الأقحوان، بعد تهيئة الأرض. جمعت أوراق نبات الكونوكاربس من حدائق كلية الزراعة في جامعة الكوفة في شهر تشرين الأول لسنة 2013، ثم تم تنظيفها ، ووضعت على ورق مقوى في غرفة مهواة لتجف بشكل أولي، أما التجفيف النهائي فتم باستعمال الفرن الكهربائي بدرجة حرارة 65 مْ لمدة 48 ساعة، بعد ذلك طحنت الأوراق الجافة على هيئة مسحوق بواسطة المطحنة الكهربائية نوع (Moulinex)، وبعدها نخل المسحوق بمنخل قطر فتحاته 2ملم. ثم حضر خليط الزراعة المكون من الرمل النهري والطين بنسبة 1:1، وأضيف مسحوق أوراق نبات الكونوكاربس الجافة إلى السنادين بمقدار (0، 5، 10، 20 غم/كغم) ثم مزجت جيدا داخل السنادين وسقيت بالماء. زرعت شتلات نبات الأقحوان المنتجة بذوره من شركة Euro garden الأسبانية بتاريخ 2013/12/15 في سنادين قطرها 20 سم، وبواقع خمس نباتات للوحدة التجريبية. نُفذت الدراسة باستعمال تصميم القطاعات العشوائية الكاملة Randomized complete Block Design ، وبثلاثة مكررات، وتمت المقارنة بين المتوسطات حسب اختبار دنكن متعدد الحدود Duncans Mutiple test عند مستوى احتمال 5% (29).

القياسات والصفات المدروسة للتجربة الحقلية:

1– مؤشرات النمو الخضري : أخذت جميع القياســـــــــــات للصفات الخضرية في موسم النمو ولغاية وصول النبات إلى مرحلة التفتح الكامل للإزهار بشكل عشوائي من كل وحدة تجريبية بتاريخ 2014/4/15 وشملت:

– ارتفاع النبات (سم): تم قياس ارتفاع النبات ابتداء من مستوى سطح التربة إلى قمة النبات باستخدام المسطرة المترية لكل نبات وتم تسجيل معدلها.

– عدد الأوراق (ورقة.نبات$^{-1}$): تم حساب عدد الأوراق الكلي لكل نبات من النباتات المنتخبة من كل وحدة تجريبية وتسجيل معدلها.

– المساحة الورقية (سم^2): تم قياس المساحة الورقية اعتمادا على مساحة الورقة وعدد الأوراق بضرب عدد الأوراق في النبات بمساحة الورقة الواحدة.

حسبت المساحة الورقية بأخذ 5 أوراق وبصورة عشوائية من كل نبات من الوحدات التجريبية ولثلاث مكررات، وتم وزنها بعد فصل الأوراق عن الأعناق، ثم أخذت أقراص دائرية بمساحة 1 سم من كل ورقة ووضعت الأوراق الكاملة والدوائر الورقية معلومة المساحة في فرن كهربائي وعلى درجة 45 ° م لحين ثبوت الوزن الجاف للأوراق ، ثم تم حساب معدل مساحة الورقة حسب المعادلة التالية:

$$\text{مساحة الورقة (سم}^2\text{)} = \frac{\text{مساحة 30 قرصاً} \times \text{الوزن الجاف الكلي لأوراق النبات (غم)}}{\text{الوزن الجاف ل 30 قرص (غم)}}$$

– الوزن الطري للمجموع الخضري(غم.نبات$^{-1}$): تم حساب الوزن الطري للمجموع الخضري للنبات وذلك في نهاية التجربة، حيث جرى انتخاب ثلاثة نباتات من كل وحدة تجريبية من كل معاملة، قلعت النباتات من التربة وبعد تنظيفها فصل المجموع الخضري عن المجموع الجذري، وأخذ الوزن الطري باستعمال ميزان حساس وتم تسجيل معدلها.

– الوزن الجاف للمجموع الخضري(غم .نبات$^{-1}$): تم حساب الوزن الجاف للمجموع الخضري للنبات وذلك في نهاية التجربة ثم قطع النبات بالكامل وتمت إزالة الجذور ووضع النبات في غرف ذات تهوية جيدة لمدة 7-14 يوما ولحين ثبوت الوزن (29).

2– صفات المجموع الجذري:

– الوزن الجاف للمجموع الجذري: بعد فصل المجموع الجذري وغسله بالماء لإزالة الأتربة العالقة، وضعت العينات داخل أكياس ورقية مثقبة، ثم جففت في فرن كهربائي بدرجة حرارة 45 مْ ولمدة 48 ساعة ولحين ثبوت الوزن ولكل وحدة تجريبية.

– عدد الجذور: تم غسل جذور النباتات بالماء الجاري بعد قلعها من التربة، ثم تم حساب عدد الجذور الرئيسية في النبات واستخراج معدلها ولكل وحدة تجريبية.

3– صفات النمو الزهري:

– قطر الزهرة (سم): قيس قطر الأزهار لكل نبات من النباتات المنتخبة في كل وحدة تجريبية بواسطة مسطرة مترية بين أبعد نقطتين متقابلتين من قطر الزهرة واستخرج معدلها.

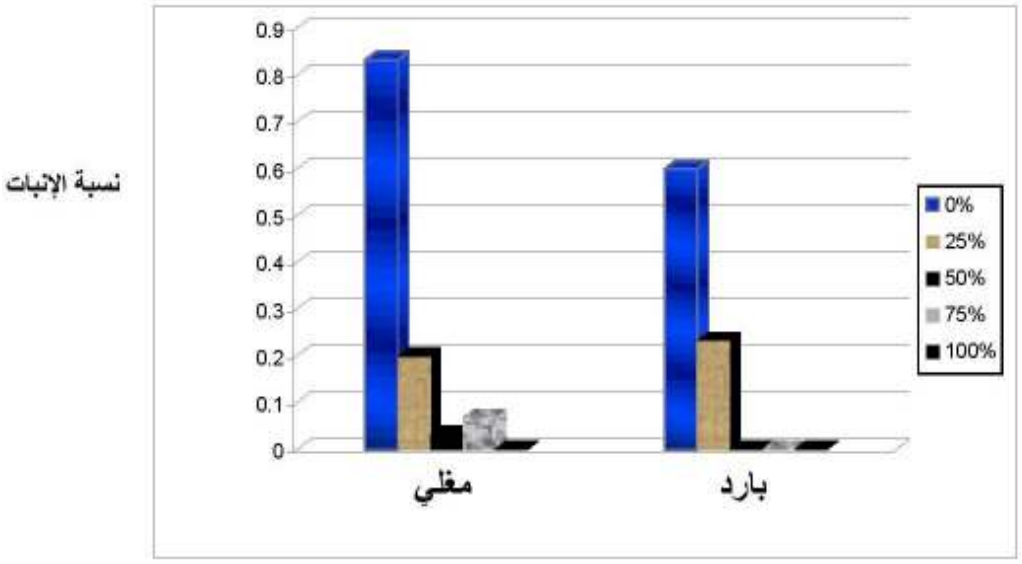
– عدد البتلات: تم حساب معدل عدد بتلات الأزهار في كل وحدة تجريبية.

النتـــــائج والمناقشـــــــة

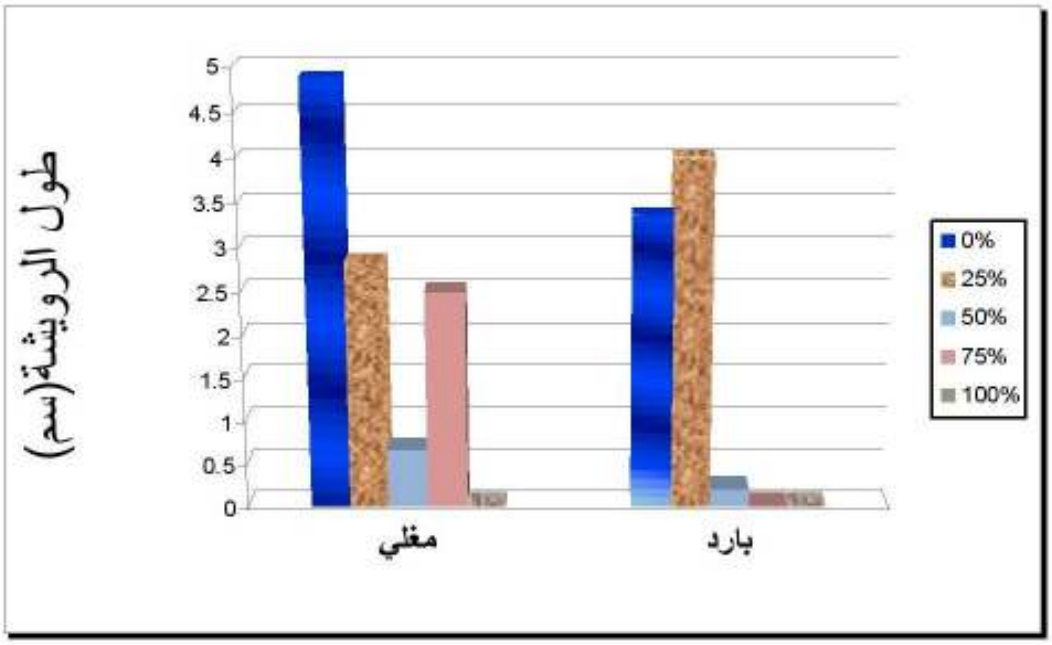
أولا: نتائج التجربة المختبرية:

يوضح الشكل (1) تأثير التداخل بين طرق الاستخلاص وتراكيزها لأوراق الكونوكاربس*Conocarpus Iancifolius* في النسبة المئوية لنبات الأقحوان *Calendula officinalis* ، حيث لوحظ انخفاض النسبة المئوية للإنبات بزيادة التراكيز، وبلغ أعلى معدل في النسبة المئوية (0.833%) في معاملة السيطرة، بينما انخفض معدل نسبة الإنبات في تركيز 50% للمستخلص المغلي، إذ بلغ (0.033%)، في حين لم يكن هناك أي نمو لتركيز 100%لمستخلص الأوراق الجافة للكونوكاربس المغلي والبارد على التوالي. يتضح من ذلك أن الانخفاض في نسبة الإنبات تتناسب تناسبا طرديا مع تراكيز المستخلصات النباتية، ويعود ذلك إلى التأثير التثبيطي للمستخلصات عند التراكيز العالية لما تحتويه من مواد مثبطة (كالفينولات والقلويدات) ويتفق هذا مع ما توصل إليه (30). كما أن نبات الكينوكاربس يحتوي الكثير من المركبات الأليلوبائية التي تعمل على تثبيط الإنبات (23، 31) .وكذلك لوجود بعض المركبات التي لها قابلية للذوبان في الماء الحار أكثر من قابلية ذوبانها في المستخلص البارد، مما يجعل هذا المستخلص يحتوي على بعض المركبات الكيميائية التي تقلل من سرعة الإنبات. وهذا يتفق مع دراسة كل من (32، 33) من أن المواد الأليلوبائية مثل الفينولات الذائبة في الماء تعمل كحاجز يعيق الإنبات ، وبالتالي يؤدي إلى خفض نسبة الإنبات في النباتات. في حين كان هناك تأثير معنوي في طول الرويشة للبادرات النامية بتأثير التداخل بين المستخلصات المائية البارد والمغلي وتراكيزها كما مبين في الشكل رقم (2)، حيث سببت تراكيز المـــستخلصات المائية اختزالا معنويا لطول الرويشة، وزيادة في درجة التثبيط بزيادة التراكيز، وبلغ أعلى معدل لطول الرويشة (4.12 سم) في معاملة السيطرة ، بينما انخفض معدل طول الرويشة في تركيز 50% للمستخلص المغلي، إذ بلغ (0.67 سم)، في حين لم يكن هناك أي نمو لتركيز 100% لمستخلص الكونوكاربس المغلي والبارد. ويعود السبب في ذلك إلى احتواء هذه المستخلصات على مركبات تعمل بتراكيزها العالية كمواد مضادة

لفعالية الجبرلين الذي يقوم بزيادة فعالية الإنزيمات المحللة للمواد الغذائية الموجودة في سويداء البذرة، وبذلك يقل وصولها إلى الأنسجة الفعالة في البذرة كالجذير والرويشة (34). ويعود السبب لإعطاء مستخلص الماء البارد أقل معدل لهذه الصفة إلى حدوث إذابة المواد التي تؤثر على فعالية الجبرلينات، وبزيادة تركيز مستخلص الماء البارد يزداد تركيز هذه المواد، بحيث يزداد تأثيرها الأليلوباثي على نمو النبات (34).



شكل (1): تأثيرالتداخل بين طرق الاستخلاص وتراكيزها لأوراق الكونوكاربس*Conocarpus Iancifolius* في النسبة المئوية لنبات نبات الأقحوان *Calendula officinalis*



شكل (2): تأثيرالتداخل بين طرق الاستخلاص وتراكيزها لأوراق نبات الكونوكاربس *Conocarpus Iancifolius* في طول الرويشة (سم) لنبات الأقحوان *Calendula officinalis*

يبين الشكل (3) تأثير تداخل تراكيز المستخلصات ونوع الاستخلاص في طول الجذير ، وأوضحت النتائج أن هناك اختلافات غير معنوية في طول الجذير باختلاف تراكيز المستخاصات، ويلاحظ زيادة درجة التثبيط بزيادة التراكيز، إذ كان أقل معدل لطول الجذير (0.33 سم) بتركيز 50% في مستخلص الماء المغلي مقارنة بـــكل من (3.9 و 3.81 سم) لمعاملة المقارنة، وقد كان هناك تثبيط في نمو الجذير في تركيز 100% للمستخلص المغلي والبارد على التوالي. ويعود سبب هذا التثبيط في كل من التراكيز 100,75,50 لكل من مستخلص الماء المغلي والبارد إلـــــى احتواء هذه المستخلصات على مركبات تصبح ذات سمية عالية عند استخدامها بتراكيز عالية، وإن مركبات التانين تأتي في مقدمة هذه السموم (35)، والتي تعمل على تثبيط طول المجموع الجذري، إذ تعمل على الارتباط مع الإنزيمات وتقلل فعاليتها، ولربما ارتبطت بإنزيمات خاصة بالتفاعلات الوسطية المؤدية لتكوين الأوكسين مما يؤدي إلى عرقلة تكوينه أو تكوينه بكميات قليله جدا لا تكفي لاستطالة الجذير (20، 33).

شكل (3): تأثيرالتداخل بين طرق الاستخلاص وتراكيزها لأوراق نبات الكونوكاربس*Conocarpus Iancifolius* في طول الجذير (سم) لنبات الأقحوان *Calendula officinalis*

ثانيا: نتائج التجربة الحقلية:

تبين النتائج الواردة في الجدول (1) أن الاختزال في الارتفاع يزداد بشكل ملحوظ مع زيادة تركيز مسحوق أوراق الكونو كاربس الجافة . وقد سجل أعلى اختزال في معدل الأرتفاع 7.00 سم عند تركيز 20 غم/كغم مقارنة مع 13.30 سم لمعاملة المقارنة في الفئة العمرية 50 يوم. في حين أن في الفئة العمرية 90 يوم كان هناك فروق معنوية في تركيز 20 غم/كغم عن معاملة السيطرة حيث بلغت 13.50 سم في تركيز 20 غم/كغم مقارنة مع معاملة السيطرة التي بلغ أرتفاع النبات عندها 26.30 سم والتي تفوقت معنوياًعن باقي التراكيز.

يتضح من النتائج أن ارتفاع النبات يقل بزيادة تركيز مسحوق أوراق نبات الكونوكاربس، مما يثبت التأثير التثبيطي للأوراق المختبرة بسبب احتوائها على التانينات والتي لها القابلية على التجزئة والتكسر بفعل الأحياء المجهرية المنتشرة في التربة، مكونة مركبات فينولية تعمل على تثبيط النمو الخضري لنبات الأقحوان.

جدول (1): تأثير مسحوق الأوراق الجافة لنبات الكونوكاربس في ارتفاع النبات (سم) خلال مراحل عمرية مختلفة

التركيز غم/كغم	عمر النبات (الايام)					معدل التركيز
	50	60	70	80	90	
0	13.30a	13.70a	15.70a	16.30a	26.30a	17.06a
5	11.33b	11.67b	12.00c	14.83b	16.67b	14.10b
10	11.67b	11.67b	13.67b	13.17c	14.33c	12.90c
20	7.00 c	8.67 c	11.33d	12.83d	13.50d	10.66d

**المعدلات التي تحمل الحروف الأبجدية نفسها ضمن كل عمود لا تختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05*

تشير النتائج الواردة في الجدول (2) إلى حدوث تباين في تأثير مسحوق أوراق الكونوكاربس في معدل عدد الأوراق، إذ قل معدل عدد الأوراق مع زيادة التراكيز المختلفة. وكان أقل عدد للأوراق في نبات الأقحوان عند التركيز 20 غم/كغم ، حيث بلغ 5.33 ورقة مقارنة مع 6.0 ورقة لمعاملة المقارنة عند الفئة العمرية 50 يوما، في حين استمر هذا الانخفاض في معدل عدد الأوراق لنبات الأقحوان لكافة التراكيز حتى بلغ 11.0 ورقة في الفئة العمرية 90 يوما، مقارنة مع معاملة السيطرة ، حيث بلغ معدل عدد الأوراق عندها 16.0 ورقة والتي تفوقت معنوياً عن باقي التراكيز.

جدول (2): تأثير مسحوق الأوراق الجافة لنبات الكونوكاربس في عدد الأوراق (ورقة/نبات) خلال مراحل عمرية مختلفة

التركيز غم/كغم	عمر النبات (يوم)					المعدل
	50	60	70	80	90	
0	6.0 b	6.67 bc	8.67a	9.0 a	16.0 a	9.26 a
5	6.67b	6.67 bc	6.67c	7.00 c	9.00 c	7.20b
10	7.00 a	7.67a	8.00a	8.67d	8.00b	7.86b
20	5.33 c	6.0 c	7.0 b	9.67 a	11.0b	7.80b

**المعدلات التي تحمل الحروف الأبجدية نفسها ضمن كل عمود لا تختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05*

توضح النتائج الواردة في الجدول رقم (3) أن المعاملة بمسحوق الأوراق الجافة لنبات الكونوكاربس أدى إلى حدوث تباين معنوي في معدل المساحة الورقية للنبات، إذ حقق كل من التركيزين 10، 20غم/كغم أقل معدل للمساحة الورقية بلغ 11.5، 14.9 سم^2 على التوالي مقارنة مع معاملة السيطرة حيث بلغ 21.7 سم^2.

جدول (3): تأثير مسحوق الأوراق الجافة لنبات الكونوكاربس في المساحةالورقية (سم^2)

التراكيز غم/كغم	المساحة الورقية(سم^2)
0	21.7 a
5	17.63 b
10	11.5 d
20	14.9 c

**المعدلات التي تحمل الحروف الأبجدية نفسها ضمن كل عمود لا تختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05*

بينت نتائج الجدول (4) وجود فروقات معنوية في معدل الوزن الطري للمجموع الخضري مع اختلاف التراكيزالمستخدمة . وقد أعطى التركيز 5 غم/كغم اختلافا معنوياً عن بقية التراكيز الأخرى، حيث بلغ 2.35 و0.78 غم للوزن الطري والجاف على التوالي، مقارنة بمعاملة السيطرة والتي بلغت 1.11 و0.42 غم للوزن الطري والجاف على التوالي. وقد تبع ذلك انخفاض معنوي في معدل الأوزان عند زيادة تركيز المسحوق إلـــى 10، 20غم/كغم ، حيث بلغت 1.41، 1.47 على التوالي للوزن الطري، في حين بلغت 0.47، 0.64 على التوالي للوزن الجاف.
أما عن تأثير مسحوق الأوراق الجافة لنبات الكونوكاربس في الوزن الجاف للمجموع الجذري، فقد أشارت نتائج جدول (4) إلى وجود تفوق معنوي بتركيز 5 غم/كغم في زيادة الوزن الجاف إلى 0.91 غم، فيما أعطت نباتات المقارنة انخفاضا في هذه الصفة، بلغ 0.71غم. وحيث لوحظ في الجدول نفسه أن أقل معدل في عدد الجذور كان عند التركيز10غم/كغم بلغ 78.0 جذرا والذي لم يختلف معنوياً عن التركيز 5غم/كغم ، الذي بلغ 78.67 جذر مقارنة مع 83.67 جذرا لمعاملة المقارنة.

جدول (4): تأثير مسحوق الأوراق الجافة لنبات الكونوكاربس في الوزن الطري والجاف للمجموع الخضري والجذري (غم.نبات−1) وعدد الجذور

التركيز غم/كغم	المجموع الخضري		المجموع الجذري	
	الوزن الطري	الوزن الجاف	الوزن الجاف	عدد الجذور
0	1.11 b	0.42 b	0.71 b	83.67 ab
5	2.35 a	0.78 a	0.91 b	78.67 b
10	1.41 b	0.47 b	1.22 a	78.0 b
20	1.47 b	0.64 ab	1.18 a	92.0 a

**المعدلات التي تحمل الحروف الأبجدية نفسها ضمن كل عمود لا تختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05*

تظهر النتائج الواردة في الجدول (5) تأثيرالتراكيز المختلفة من مسحوق الأوراق الجافة لأوراق الكونوكاربس في قطر الزهرة وعدد البتلات، حيث قل معدل قطر الزهرة مع زيادة التراكيزالمستعملة. وبلغ أقل معدل لقطر الزهرة 0.87 سم عند تركيز 20 غم/كغم مقارنة مع 1.03، 1.04 و 2.33 سم عند التراكيز(10، 5 أو0) غم/كغم من مسحوق الأوراق على التوالي.
وتظهر النتائج في الجدول نفسه أن إضافة مسحوق الاوراق الجافة لنبات الكونوكاربس أدى إلى وجود أختلافات معنوية في معدل عدد البتلات لنبات الأقحوان، إذ أعطت معاملة المقارنة أعلى معدل في عدد البتلات بلغ 9.0 مقارنة مع كل من التراكيز العالية التي أعطت معدل 8.0 و7.33 عند التركيز 10و 20 غم/كغم من مسحوق الأوراق على التوالي. في حين كان هناك انخفاض في معدل عدد البتلات للتركيز 5غم/كغم التي انخفضت معنوياً عن بقية التراكيز الأخرى.

جدول (5): تأثير مسحوق الأوراق الجافة لنبات الكونوكاربس في قطرالزهرة (سم) وعدد البتلات.

التراكيز غم/كغم	قطر الزهرة (سم)	عدد البتلات
0	2.33 a	9.0 a
5	1.04 b	6.67 d
10	1.03 b	8.0 b
20	0.87 c	7.33 c

**المعدلات التي تحمل الحروف الأبجدية نفسها ضمن كل عمود لا تختلف عن بعضها معنويا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05*

يتضح من نتائج مؤشرات النمو الخضرية والزهري و نسبة الإنبات أن مسحوق الأوراق الجافة ومستخلصاتها المائية لنبات الكونوكاربس قد أثرت سلبيا في نبات الأقحوان , ويعود السبب إلى أن نبات الكونوكاربس يحوي الكثير من المركبات الفينولات والتانينات التي تعمل على تثبيط الإنبات (24، 31)، حيث تم تشخيص العديد من هذه المركبات ذات الطابع الأليلوباثي والتي من أهمها الحوامض الفينولية مثل benzoic acid و cinnamic acid التي تتحرر من الأجزاء الخضرية والجذور (36). وذكر أن المواد الاليلوباثية مثل الفينولات الذائبة في الماء تعمل كحاجز يعيق الإنبات (33)، وبالتالي يؤدي إلى خفض نسبة الإنبات في النباتات. ويتضح من نتائج الدراسة الحالية أيضاً أن مسحوق الأوراق الجافة قد أثرت سلباً في الصفات الخضرية لنبات الأقحوان (الجداول 1− 5). وقد فُسر سبب تثبيط استطالة النباتات المعاملة بمستخلصات أوراق الكونوكاربس بأنه يعود إلى وجود مواد كيميائية ذات تراكيز عالية قد تعمل كمثبط لانقسام الخلايا وبالتالي اختزال في استطالة النبات أو من خلال التأثير على عمل الهرمونات المحفزة لانقسام الخلايا (37).
ربما يعود السبب الى تحرر المركبات الكيميائية عن طريق التطاير او الغسيل لكونها قابلة للذوبان في الماء او من التحلل الجزئي للاوراق المحضنة في التربة حيث يمكن لهذه المركبات ان تتحرر الى التربة للتراكم فيها او بفعل الكائنات الدقيقة (19).

وهذا التباين قد يعزى إلى حساسية الجزء النباتي، إذ إن بعض المركبات الأليلوباثية قد تؤثر على نمو الجزء الخضري دون التأثير على نمو المجموع الجذري، أو بالعكس، قد يتأثر نمو الجذر أكثر من نمو الجزء الخضري.

في حين أظهرت النتائج الواردة في التجربة الحقلية أن تأثير مستخلص الأوراق المائي البارد كان أكثر تثبيطاً من تأثيرالمستخلص المائي المغلي ويعود السبب إلى أن الاستخلاص بالماء البارد يؤدي إلى إذابة المواد التي تؤثر على فعالية الجبرلينات وبزيادة تركيز مستخلص الماء البارد يزداد تركيز هذه المواد بحيث يزداد تأثيرها الأليلوباثي على نمو النبات (38)، وأن تركيز 25% مستخلص الماء البارد قد حفز كلاً من طول الرويشة والجذير. وقد يعود السبب إلى امتلاك بعض مستخلصات النباتات بتراكيزها الواطئة طبيعة هرمونية مشابهة في تأثيرها للهرمونات المحفزة لنمو الأجزاء الخضرية مثل الجبريلين (39، 40).

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تأثير رش البنزل أدنين (BA) ومستخلص الكجرات على صفات النمو الخضري والزهـري لنبـات حلـق السبع *Antirrhimum majus*

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الملخص باللغة العربية

أجريت التجربة في كلية الزراعة– جامعة الكوفة في الموسم الزراعي 2014–2015 لدراسة تأثير رش البنزل أدنين ومستخلص الكجرات علـى صفات النمو الخضري والزهري لنبات حلق السبع *Antirrhimum majus* . نفذت تجربة عاملية بعاملين: الأول ثلاثة تراكيز من البنزل أدنين (0، 100، 200) ملغم.لتر$^{-1}$ والثاني ثلاث تراكيز من مستخلص الكجرات (0، 5، 10) غم.لتر$^{-1}$ وفق تصميم القطاعـات العـشوائية الكاملـة (R.C.B.D). تمت مقارنة المتوسطات حسب اختبار أقل فرق معنوي وعلى مستوى احتمال 0.05 .

أظهرت النتائج أن رش البنزل أدنين ومستخلص الكجرات والتداخل بينهما كان له أثر معنوي في صفات النمو الخضري والجذري والزهري، وظهر أن أعلى معدل كان عند التداخل بين 200 ملغم.لتر$^{-1}$ البنزل أدنين و10 غم.لتر$^{-1}$ مستخلص الكجرات الذي زاد من صفات النمو التالية (ارتفاع النبات، عدد الأوراق، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكربوهيدرات، محتوى الأوراق من الكلوروفيل الكلي، عدد الأزهارفي كل نورة، طول الشمراخ الزهري، قطر النورة الزهرية، قطر الزهرة، الوزن الرطب للجذور، الوزن الجاف للجذور) حيث أعطى (44.40 سم ، 59.67 ورقة.نبات–1 ، 5.49 غم، 11.83 ملغم.غم–1 وزن جاف، 38.09 ملغم.100 غم–1 وزن طري، 20.67 زهرة.نبات–1، 20.03 سم، 8.60 سم، 3.73 سم، 4.73 غم، 2.17 غم).

الكلمات المفتاحية: البنزل أدنين ، مستخلص الكجرات، حلق السبع

Effect of benzyladenine and roselle extract on some vegetative and flower growth characteristics of antirrhimum majus

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ABSTRACT

This study was conducted at the faculty of agriculture /University of Kufa on 2014-2015 season to evaluate the effect of Benzyladenine and Roselle Extract on some Vegetative and flower growth characteristics of Antirrhimum majus . A factorial experiment (3x3) was designed, first factor included three concentrations of Benzyladenine (0, 100 and 200) mg.L-1 and second factor included three concentrations of Roselle Extract (0,5,10) g.L-1 according to Randomized Completely Blok Design(R.C.B.D).

Results showed that, spraying with Benzyladenine, Roselle Extract and the interation between them illustrated significant effect on vegetative, root and flower growth characteristics, the interaction treatment200 mg.L-1 of Benzyladenine with 10 g.L-1 Roselle Extract in creased plant height, number of leaves, dry weight of shoots, leaf content of total chlorophyll, leaf content of the total soluble carbohydrates, number of flower, flower stalk, flower stalk dirmeter, dry weight of root. (44.40cm,59.67Leaf/plant,5.49g, 11.83mg-1dry weight, 38.09 mg.100gm-1 Fresh weight,20.67 flower.plant,20.03cm, 8.60cm, 3.73cm, 2.17gm).

المقدمـــــة

حلق السبع نبات حولي شتوي مـن عائلـة Scrophulariaceae يزرع في الحدائق بكثرة لجمال أزهاره الشبيهة بالفم المتجمعة في عناقيد وبألوان مختلفة جذابة، منها الأصفر والـوردي والأحمـر والأبيض والقرنفلي والقرمزي والمشمشي (1). النباتات منتـصبة ذات اوراق بسيطة متقابلة كاملة الحافـة (2). تعـد الهرمونـات النباتية من العوامل المهمة جدا في تنظيم الفعاليات الحيوية للنبات، ولها تأثيرات عديدة على تحسين وإنتـاج النباتـات (3)، ومنهـا السايتوكانينات من منظمات النمو التي تعمل على تنظيم التـوازن التنافسي بين الأوكسين والسايتوكاينين ، بالإضافة إلى تحفيز انقسام الخلايا، وتخصصها تتكون السايتوكانينات في الجذور ثـم تنتقـل بالسيقان والأوراق (4). وقد بينت دراسة (3) أن المستخلـصات النباتية تشابه الهرمونات النباتية في عملها، كونها تعمل بالاتجـاه ذاته وعلى مواقع الفعالية ذاتها في النسيج النبـاتي، منهـا نبـات الكجرات الذي ينتمي إلى نباتات مغطاة البـذور Angiosperms مـن ذوات الفلقتـين Dicotyledons مـن العائلـة الخبازيـة Malvaceae (5)، نباتات حولية عشبية قليلة التفرع (6)، تـزرع لغرض الحصول على الأوراق الكاسية الحمراء الداكنة والأخرى الحمراء الفاتحة. الأزهار في إبـط الأوراق، وأوراقهـا كاسـية متشحمة وسميكة (7). تحتوي الأوراق الكاسية علـى المركبـات الفينولية والكلايكوسيدات (8). وقد أشارت دراسـة (9) إلـى أن الأوراق الكاسية لنبات الكجرات غنية بفيتامين C ولهذا يعد مـن المصادر المهمة لهذا الفيتامين، كمـا يحتـوي علـى الكالـسيوم والفوسفور وكمياتها (2.30,2.78 ملغم. 100 غم) على التوالي. يحتوي النبات أيضا على كمية عالية من , Ascorbic acid Malic acid وهي المسؤولة عن الطعم الحامضي للمـستخلص المائي، كما تحتوي أوراقة علـى Tartaric acid،Citric acid بنسب تتراوح ما بين 3–4% (10)، كما تحتوي الأوراق الكاسية على المركب Proto Catehenic acid (PCA) (11). وقد بينت دراسة (12) عند تحليل الأوراق الكاسية بأنها تحتوي على نـسبة 25% من الكربوهيدرات وتحتوي على 6.2 من البروتين، وصبغة الانثوسيانين (13).

ونظرا لابتعاد الباحثين في العالم فترة غير يسيرة عن استخدام المستخلصات النباتية والاعتماد على الهرمونات المصنعة ، لذلك صار التوجه الحديث نحو استخدام المستخلصات النباتية في زيادة صفات النمو الخضري والزهري والجذري لاحتوائها على صفات مشابهة لمنظمات النمو النباتية . ولقلة الأبحاث في هذا المجال، فقد جاءت هذه الدراسة التي تهدف إلى استخدام ثلاث تراكيز من منظم النمو البنزل أدنين وثلاث تراكيز من مستخلص الكجرات لتحديد تأثيرها في تحسين صفات النمو الخضري والزهري والجذري لنبات حلق السبع.

المواد وطرق العمـــــل

نفذت تجربة عامليـة بتـصميم القطاعـات العـشوائية الكاملـة R.C.B.D بثلاث مكررات، احتوى كل مكرر على تسع معاملات وثلاث سنادين للوحدة التجريبية في كلية الزراعة – جامعة الكوفة خلال الموسم الزراعي 2014–2015 على نبات حلـق الـسبع. زرعت البذور من إنتاج Euro-graden الإسبانية صنف الطويل من 40– 60 سم على شكل دايات في الظلة الخشبية، حيث كانت الظروف الجوية فيها غير مسيطر عليهـا بتـاريخ 2014/10/1. نقلت الشتلات الجاهزة للشتل بتاريخ 2014/11/21 إلى أصـص بلاستيكية قطرها (15 سم) بعد ظهور أربع أوراق حقيقية بواقـع شتلة واحدة لكل إصيص مع إجراء كافة عمليات الخدمـة للنبـات كلما دعت الحاجة. تم الرش البنزل أدنين من إنتاج شركة Green River الهندية بثلاث تراكيز (0، 100، 200) ملغم.لتر$^{-1}$ بواقـع رشتين، بينهما أربعة أسابيع: الأولى كانت بتاريخ 2014/12/21 مع رش معاملة المقارنة بالماء المقطر لمعاملة المقارنة، فـصلت المعاملات بقطع كرتونية لتجنب الرذاذ المتطاير. أجريت عمليـة الرش بمستخلص الكجرات بـثلاث تراكيـز هـي (0، 5، 10) غم.لتر$^{-1}$. حضر المستخلص المائي لـلأوراق الكاسـية لنبـات الكجرات، إذ تم أخذ (10، 5 غرام) كل على حدة مـن مـسحوق الأوراق الكاسية، ووضعت في دورق زجاجي سـعة 2000 مـل وأضيف إليه 1000 مل ماء مقطر ومن ثم تم نقع الخلـيط لمـدة (24) ساعة، ثم وضع بعد هذه الفترة في خلاط كهربائي لمدة (5) دقائق , ثم نقل الخليط إلى اسطوانة زجاجية مدرجة (Cylinder) وترك لمدة ساعتين، ثم فصل الرائق من المستخلص عـن المـواد الراسبة التي تمثل بقايا الأوراق الكاسية، بعدها تم تنقية المستخلص الرائق عن طريق تمريره عبر ورق الترشيح، وعد المـستخلص الذي تم الحصول عليه كامل القوة (Stock 100%)، وتـم حفـظ المستخلص في دوارق زجاجية محكمة الغلق في الثلاجة لحـين الاستعمال، ومنه تم تحضير التركيز (10) غـم مـن مـستخلص الكجرات وإكمال الحجم إلى لتر من الماء المقطر. وهكذا لبقيـة التراكيز وتم الرش حتى البلل الكامل بواقع رشتين الرشة الأولـى بعد 30 يوما من زراعة البذور، والثانية بعد 10 أيام من الرشـة الأولى ورشت معاملة المقارنة بالماء المقطر. والجدول (1) يوضح مخطط التجربة.

جرى تحليل النتائج باستخدام تحليل التباين (ANOVA)، وقورنت المتوسطات حسب اختبار أقل فرق معنوي L.S.D وعلى مستوى احتمال 0.05 (14).

جدول (1): مخطط التجربة

ت	المعاملة	المستوى
1	T1	0 ملغم.لتر$^{-1}$ البنزل ادنين x 0 غم.لتر$^{-1}$مستخلص الكجرات
2	T2	0ملغم.لتر$^{-1}$ البنزل ادنين x 5 غم.لتر$^{-1}$مستخلص الكجرات
3	T3	0ملغم.لتر$^{-1}$ البنزل ادنين x 10 غم.لتر$^{-1}$مستخلص الكجرات
4	T4	100ملغم.لتر$^{-1}$البنزل ادنين x 0غم.لتر$^{-1}$مستخلص الكجرات
5	T5	100ملغم.لتر$^{-1}$البنزل ادنين x 5غم.لتر$^{-1}$مستخلص الكجرات
6	T6	100ملغم.لتر$^{-1}$البنزل ادنين x 10 غم.لتر$^{-1}$مستخلص الكجرات
7	T7	200ملغم.لتر$^{-1}$البنزل ادنين x 0 غم.لتر$^{-1}$مستخلص الكجرات
8	T8	200ملغم.لتر$^{-1}$البنزل ادنين x 5 غم.لتر$^{-1}$مستخلص الكجرات
9	T9	200ملغم.لتر$^{-1}$البنزل ادنين x 10غم.لتر$^{-1}$مستخلص الكجرات

في نهاية التجربة وبتاريخ 2015/5/10 تم حساب مؤشرات النمو التالية التي أخذت من ثلاث نباتات لكل وحدة تجريبية وهي:

أولا: صفات النمو الخضري وتضمنت :

1– ارتفاع النبات(سم): تم قياس ارتفاع النبات من محل اتـصال الساق الرئيس بالتربة وحتى أعلى قمة للنبـات بواسـطة شـريط القياس.

2– عدد الاوراق (ورقة.نبات$^{-1}$): تم حساب عدد الأوراق الكليـة لكل نبات واستخرج المعدل.

3– الوزن الجاف للمجموع الخضري(غم.نبات$^{-1}$): تـم تجفيـف النباتات طبيعيا في غرفة ذات تهوية مع التقليب المستمر من (7–14) يوما لحين ثبوت الوزن ثم أخذت الأوزان لكل معاملة.

4– محتوى الأوراق من الكربوهيدرات الكلية الذائبة (ملغـم.غـم وزن جاف): تم تقدير الكربوهيدرات في الأوراق لكل نبات وذلك بسحق (1غم) من المادة مع 10 مل من الماء المقطـر، وفـصل الراشح عن الراسب بجهاز الطـرد المركـزي بـسرعة 1500 دورة/دقيقة. بعدها أخذ 1 مل من الراشح وأضيف له 1مـل مـن كاشف الفينول 5% و5 مل من حامض الكبرتيك المركز، ثم ترك ليبرد عند 25 م. بعدها تمت قراءة الامتصاص الضوئي بواسـطة

جهاز Spectrophotometer بطول موجي 488 نانوميتر حسب طريقة (15).
5-محتوى الأوراق من الكلوروفيل الكلي (ملغم.100غم$^{-1}$وزن طري): تم تقدير الكلوروفيل الكلي حسب طريقة (16).

ثانيا: صفات النمو الجذري:
1- الوزن الرطب للجذور (غم): تم فصل المجموع الجذري عن الساق وأخذ الأوزان الرطبة بواسطة الميزان الحساس، واستخرج المعدل.
2- الوزن الجاف للجذور (غم): تم تجفيف الجزء الجذري طبيعيا مع التقليب المستمر، ثم أخذت القياسات واستخرج المعدل.

ثالثا: صفات النمو الزهري:
1-عدد الأزهار(نورة.نبات$^{-1}$): تم حساب عدد الأزهار في كل نورة زهرية واستخرج المعدل.
2- طول الشمراخ الزهري (سم): قيست أطوال الشماريخ الزهرية للنباتات من نقطة اتصال الشمراخ الزهري بالساق إلى قاعدة الزهرة بواسطة المسطرة، ثم استخرج المعدل لكل معاملة.
3- قطر نورة الشمراخ الزهري(سم): تم قياس قطر النورة الزهرية بين أوسع منطقتين بواسطة القدمة Vernier.
4- قطر الزهرة (سم): تم قياس قطر الزهرة لجميع الأزهار المتكونة على النبات بواسطة القدمة بين أبعد نقطتين واستخرج المعدل لكل معاملة.

النتـــــائج والمناقشـــــة

أولا: تأثير الرش بالبنزل أدنين BA في صفات النمو الخضري لنبات حلق السبع:

يتضح من جدول (2) أن الرش بالتركيز 200 ملغم.لتر$^{-1}$ BA أدى إلى زيادة معنوية في صفات النمو الخضري والصفات الكيميائية، منها ارتفاع النبات ، عدد الأوراق، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكربوهيدرات ، محتوى الأوراق من الكلوروفيل الكلي، إذ بلغ وعلى التوالي 40.43 سم، 53.78 ورقة.نبات$^{-1}$، 4.51 غم.نبات$^{-1}$، 10.01ملغم.100غم وزن طري، 36.43 ملغم.غم وزن جاف ، مقارنة بمعاملة المقارنة والتي أعطت أقل نتائج (34.00سم، 40.22 ورقة.نبات$^{-1}$، 3.43غم. نبات$^{-1}$ ، 7.76 ملغم.100 غم وزن طري، 28.35 ملغم.غم وزن جاف). ويرجع سبب هذه النتائج إلى دور البنزل أدنين BA الذي تحتاجه جميع النباتات بكميات ضئيلة جدا، ويكون في صورة نشطة حيويا خلال مرحلة النمو الخضري. ويعتبر البنزل أدنين ذا نشاط حيوي يختلف عن الفعالية البيولوجية لكل من الجبرلينات والأوكسينات لأنها تعمل على زيادة حجم الخلايا باستطالة عرضها، فضلا عن أنه يشجع خلايا الكمبيوم الوعائي على الانقسام والنمو، و ينشط البنزل أدنين بناء كل من RNA والبروتين في الخلايا ، ويشجع نشاط إنزيمات معينة ، وتكوين نواتج تفاعلاتها (17، 18)، ولذلك يحافظ على ثبات تركيب tRNA ويزيد من قوة ربط الحامض الأميني أثناء عملية الانتقال، مما يشجع النمو الخضري (19). وقد لوحظ من خلال نتائج دراسة (20) عند رش نبات الورد الشجيري Rosa hybrida بالبنزل أدنين وبالتراكيز(0، 50، 100) ملغم.لتر-1 حصول زيادة معنوية في طول النبات، والوزن الجاف والطري للمجموع الخضري. ووجدت دراسة (21) أنه عند رش الكاينتين على أوراق نبات الدارسينا Dracaena hookeriana بتراكيز (0، 50، 75) ملغم.لتر-1 أن النباتات المعاملة بالتراكيز 75 ملغم.لتر-1 أعطت أفضل النتائج من حيث زيادة ارتفاع النبات ومحتوى الأوراق من الكلوروفيل الكلي. كما وجدت دراسة (22) أن رش نبات الورد الشجيري بالبنزل أدنين أدى إلى زيادة الوزن الجاف للنمو الخضري.
ومن نتائج دراسة (23) على نبات الفوجير عند رش النبات بتراكيز مختلفة من البنزل أدنين (0، 100، 200، 400) ملغم.لتر-1 ، تبين أن الرش بتركيز 200 ملغم.لتر-1 أدى إلى زيادة معنوية في عدد الأوراق ، والتركيز 100 ملغم.لتر-1 زاد من الوزن الجاف للمجموع الخضري والجذري للنبات. لوحظ من دراسة (24) على نبات البزاليا العطرية عند رشه بالبنزل أدنين بالتراكيز (0، 10، 20) ملغم.لتر-1 ،حيث تفوقت النباتات التي رشت بالتراكيز 10 ملغم. لتر-1 في محتوى الأوراق من الكربوهيدرات الذائبة الكلية . ويتبين من تأثير الرش بالبنزل أدنين BA في صفات النمو الجذري لنبات حلق السبع من جدول (3) أن الرش بالتركيز 200 ملغم.لتر-1 BA أدى إلى زيادة معنوية في صفات النمو الجذري، منها الوزن الرطب للجذور والوزن الجاف للجذور وعلى التوالي، إذ أعطى 4.13 غم, 1.40 غم مقارنة بمعاملة المقارنة والتي أعطت أقل القيم ، حيث بلغت 3.32 غم, 0.66 غم . ويرجع السبب إلى دور البنزل أدنين في زيادة حجم الخلايا باستطالة عرضها وليس طولها سواء كانت الأعضاء الخضرية أو الجذور، فضلا عن أنه يشجع خلايا الكمبيوم الوعائي على الانقسام والنمو (2)، إذ تنتج السايتوكانينات في الأنسجة المرستيمية أو الأنسجة التي لها القدرة على استعادة النشاط في النمو. وقد أمكن التأكد من أن السايتوكانينات يتم إنتاجها جزئيا (أي بعض السايتوكانينات وليس كلها) في قمم الجذور، وتنتقل إلى أعلى النبات عن طريق أوعية الخشب (25).
إن الموقع الرئيسي لإنتاج السايتوكانينات هي قمم الجذور(26). ومن نتائج دراسة (23) على نبات الفوجير عند رش النبات بتراكيز مختلفة من البنزل أدنين (0، 100، 200، 400) ملغم.لتر-1، تبين أن التركيز 100 ملغم.لتر-1 زاد من الوزن الجاف للمجموع الخضري والجذري للنبات. وقد أوضحت نتائج دراسة (27) عند معاملة نبات الكروتون بالبنزل أدنين بالتراكيز (0، 20، 40) ملغم.لتر-1 تفوق التركيز 20 ملغم . لتر-1 في زيادة الوزن الجاف للمجموع الجذري وطول الجذر. وقد ذكرت دراسة (28) في تجربة لبيان تأثير رش تراكيز من البنزل أدنين هي (0، 50، 100، 150) ملغم.لتر-1 على نبات الكروتون ، حيث أدى رش النبات بالتركيز 150 ملغم.لتر-1 إلى زيادة الوزن الجاف للمجموع الجذري وطول الجذر. وفيما يتعلق بتأثير الرش بالبنزل أدنين BA في صفات النمو الزهري لنبات حلق السبع، يتضح من جدول (4) أن الرش بالتركيز 200 ملغم.لتر-1 BA زاد معنويا من صفات النمو الزهري عدد الأزهار و طول الشمراخ الزهري و قطر نورة الشمراخ و قطر الزهرة، إذ بلغ 17.78نورة.نبات-1 ، 18.30 سم، 7.91 سم، 3.17 سم مقارنة بمعاملة المقارنة، والتي أعطت أقل القيم بلغت 10.11 نورة.نبات-1 ، 13.77 سم ، 6.89 سم، 2.47 سم . ويعود السبب إلى دور البنزل أدنين من العوامل الداخلية المهمة في النباتات المزهرة لدفع مرحلة نموها الخضري إلى مرحلة النمو الزهري، مع المحافظة على عدم سقوط الأعضاء الزهرية خلال عمليتي التلقيح والإخصاب (2). وفي دراسة أجريت على نبات الجبسوفيليا Gypsophlla paniculata من قبل (29)، وجد أن رش النباتات بالتركيز 300 ملغم.لتر-1 من البنزل أدنين أدى إلى زيادة قطر الزهرة. ووجدت نتائج دراسة (30) أن رش البنزل أدنين على نبات القرنفل بتركيز 70 ملغم.لتر-1 أدى إلى حدوث زيادة معنوية في قطر الأزهار. ولاحظت دراسة (31) لنبات القرنفل أن الرش بالتركيز (0، 50، 100) ملغم.لتر-1 قد أحدث زيادة معنوية في صفات النمو الزهري تفوقت معنويا عند التركيز 100 ملغم.لتر-1 في زيادة قطر الزهرة وطول الساق الزهري.

جدول (2): تأثير الرش بالبنزل أدنين BA ومستخلص الكجرات والتداخل فيما بينها في صفات النمو الخضري لنبات حلق السبع

الكلوروفيل	الكربوهيدرات	الوزن الجاف للمجموع الخضري	عدد الاوراق	ارتفاع النبات		
28.35	7.76	3.43	40.22	34.00	0	بنزل ادنين (BA)
32.50	8.30	3.60	43.44	35.71	100	
36.43	10.01	4.51	53.78	40.43	200	
3.201	1.028	0.321	2.101	2.014	L.S.D. 0.05	
32.32	7.42	3.08	42.11	34.24	0	مستخلص الكجرات
28.85	8.29	3.62	42.11	34.76	5	
36.10	10.36	4.84	53.22	41.14	10	
3.201	1.028	0.321	2.101	2.014	L.S.D. 0.05	
28.87	6.37	2.45	33.67	30.60	0	0	بنزل ادنين(BA) × مستخلص الكجرات
30.06	6.44	2.67	35.67	31.53	5	
38.04	9.45	4.11	57.00	40.60	10	
21.74	7.74	3.38	39.67	33.43	0	100	
31.65	8.38	3.54	42.00	34.53	5	
33.17	8.76	3.94	44.67	36.30	10	
34.43	9.17	4.46	47.33	37.97	0	200	
35.77	10.09	4.58	52.67	41.07	5	
38.09	11.83	5.49	59.67	44.40	10	
5.217	2.347	0.607	4.401	4.233	L.S.D. 0.05	

جدول (3): تأثير الرش بالبنزل أدنين BA ومستخلص الكجرات والتداخل فيما بينها في صفات النمو الجذري لنبات حلق السبع

الوزن الجاف للجذور	الوزن الرطب للجذور			
0.66	3.32	0		بنزل ادنين(BA)
0.96	3.81	100		
1.40	4.13	200		
0.352	0.751	L.S.D. 0.05		
0.54	3.16	0		مستخلص الكجرات
1.00	3.78	5		
1.47	4.32	10		
0.352	0.751	L.S.D. 0.05		
0.46	2.97	0	0	بنزل ادنين(BA) × مستخلص الكجرات
0.54	3.22	5		
0.62	3.29	10		
0.70	3.33	0	100	
0.90	3.66	5		
1.42	4.36	10		
0.82	3.65	0	200	
1.42	4.56	5		
2.17	4.73	10		
0.842	1.201	L.S.D. 0.05		

جدول (4): تأثير الرش بالبنزل ادنين BA ومستخلص الكجرات والتداخل فيما بينها في صفات النمو الزهري لنبات حلق السبع

			عدد الازهار	طول الشمراخ الزهري	قطر نورة الشمراخ الزهري	قطر الزهرة
بنزل ادنين(BA)	0		10.11	13.77	6.89	2.47
	100		12.33	14.80	7.04	2.93
	200		17.78	18.30	7.91	3.17
L.S.D. 0.05			1.002	1.487	0.154	0.274
مستخلص الكجرات	0		11.78	14.08	6.31	2.27
	5		12.11	14.60	7.56	3.02
	10		16.33	18.19	7.98	3.28
L.S.D. 0.05			1.002	1.487	0.154	0.274
بنزل ادنين(BA) × مستخلص الكجرات	0	0	7.67	10.93	5.60	1.90
		5	8.00	11.90	5.50	2.37
		10	19.67	19.40	7.83	2.53
	100	0	11.33	13.73	7.43	2.67
		5	12.00	14.60	7.93	3.17
		10	13.00	15.47	7.30	3.23
	200	0	11.33	16.63	7.63	2.83
		5	17.00	17.90	7.70	3.27
		10	20.67	20.03	8.60	3.73
L.S.D. 0.05			1.475	1.877	0.357	0.409

ثانيا: تأثير الرش بمستخلص الكجرات في صفات النمو الخضري لنبات حلق السبع:

يتضح من جدول (2) أن الرش بالتركيز 10غم.لتر−1 من مستخلص الكجرات أدى إلى زيادة معنوية في صفات النمو الخضري، منها ارتفاع النبات وعدد الأوراق والوزن الجاف للمجموع الخضري و محتوى الأوراق من الكربوهيدرات ومحتوى الأوراق من الكلوروفيل الكلي، إذ بلغ 41.14 سم و 53.22 ورقة.نبات−1 و 4.84 غم.نبات−1 و10.36 ملغم.100 غم وزن طري و 36.10 ملغم.غم وزن جاف مقارنة بمعاملة عدم التسميد والتي أعطت 34.24 سم و 42.11 ورقة.نبات−1 و 3.08 غم.نبات−1 و 7.42 ملغم.100 غم وزن طري و 32.32 ملغم.غم وزن جاف وعلى التوالي. ويرجع السبب في دور مستخلص الكجرات إلى احتواء أوراقة الكاسية على أحماض عضوية وبروتينات وكربوهيدرات وعناصر غذائية (32). كذلك تحتوي الأوراق الكاسية على الفسفور الذي يلعب دورا في الكثير من التفاعلات الإنزيمية، فهو يدخل في تركيب الأحماض النووية مثل DNA و RNA و tRNA و rRNA ، بالإضافة إلى دخوله في تركيب الإنزيمات اللازمة لتفاعلات الطاقة المختلفة في عمليات التنفس والتمثيل الضوئي، كذلك يدخل في تركيب المركبات الفسفورية الغنية بالطاقة ATP و ADP وفي تركيب بعض الدهون التي تدخل في تركيب الأغشية الخلوية ، حيث يوجد بتراكيز عالية في المناطق المرستيمية، ويشترك في تمثيل البروتينات النووية، ويعمل على التبكير في النضج . كذلك يلعب الكالسيوم دورا في تكوين الجدران الخلوية وخاصه الصفيحة الوسطى بهيئة بكتات الكالسيوم التي تعمل مع بكتات المغنسيوم على لصق سلاسل السيللوز بعضها البعض أثناء تكوين الجدران الخلوية، كذلك في تركيب الأغشية الخلوية، وله دور في الانقسام الخلوي، ويدخل في تركيب وثبات الكروموسومات، حيث يتراكم معظم الكالسيوم في الأوراق (2). وقد وجدت دراسة (33) أن مستخلص الكجرات عمل على تأخير الشيخوخة للمجموع الخضري للنباتات التي رشت به، وإطالة عمر النبات وزيادة كمية الكلوروفيل. وأشارت دراسة (34) إلى أن مستخلص الكجرات يحافظ على مستوى البرولين وأن رش النبات يؤدي إلى خفض ارتفاع النبات، وبذلك يعمل عمل معوقات النمو، ولمستخلص الكجرات تأثير على الحاصل ومكوناته لتحسين كفاءة استهلاك الماء (35)، كذلك احتواء الأوراق الكاسية على فيتامين C الذي يؤثر في نمو النبات، ويوجد أولا في الأوراق ثم ينتقل إلى الجذور، مما يؤدي إلى زيادة كبر في حجم الأوراق، حيث تشترك الفيتامينات في التفاعلات الكيمياوية الحيوية التي تقوم بتحويل الغذاء إلى طاقة، وتعتبر أساس استمرار الوظائف المختلفة للجسم وبناء أنسجة جديدة (2). وقد بينت نتائج تأثير الرش بمستخلص الكجرات في صفات النمو الجذري لنبات حلق السبع من خلال جدول (3) أن الرش بمستخلص الكجرات اثر معنويا على صفات النمو الجذري: الوزن الرطب للجذور و الوزن الجاف للجذور، إذ بلغ 4.32 غم و 1.47 غم مقارنة بمعاملة عدم التسميد والتي أعطت 3.16 غم و 0.54 غم. ويعود السبب في دور مستخلص الكجرات احتواء أوراقه الكاسية على أحماض عضوية وبروتينات وكربوهيدرات وعناصر غذائية، بالإضافة إلى زيادة كمية الكلوروفيل، وهذا يؤدي إلى تكوين مجموع جذري أفضل (33). كذلك يشجع الفسفور على نمو الجذور وخاصة الجذور العرضية (2).

فيما يتعلق بتأثير الرش بمستخلص الكجرات في صفات النمو الزهري لنبات حلق السبع، يتضح من جدول (4) أن الرش بمستخلص الكجرات أثر معنويا في صفات النمو الزهري، ومنها عدد الأزهار وطول الشمراخ الزهري وقطر نورة الشمراخ الزهري و قطر الزهرة، إذ بلغ 16.33 نورة.نبات و 18.19 سم و 7.98 سم و 3.28 سم مقارنة بمعاملة المقارنة والتي أعطت أقل القيم، حيث بلغت 11.78 نورة.نبات و 14.08 سم و 6.31 سم و 2.27 سم وعلى التوالي. ويرجع السبب في دور مستخلص الكجرات إلى احتوائه الفسفور الذي يتركز في الأزهار بنسبة 100.1 % (2).

ثالثا: تأثير الرش بالبنزل أدنين ومستخلص الكجرات والتداخل بينهما:

يبين جدول (2) أن المستوى 200 ملغم.لتر−1 بنزل أدنين و10 غم.لتر−1 مستخلص الكجرات أثر معنويا في صفات النمو الخضري، ومنها ارتفاع النبات وعدد الأوراق والوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكربوهيدرات ومحتوى الأوراق من الكلوروفيل الكلي، إذ أعطى 44.40 سم و59.67 ورقة.نبات−1 و5.49 غم.نبات−1 و11.83 ملغم.100 غم وزن طري و38.09 ملغم.غم وزن جاف مقارنة بمعاملة المقارنة والتي أعطت أقل القيم، حيث بلغت 30.60 سم و33.67 ورقة.نبات−1 و2.45 غم.نبات−1 و6.37 ملغم.100 غم وزن طري و28.87 ملغم.غم وزن جاف على التوالي. ومن خلال جدول (3) ، يتضح وجود زيادة معنوية في صفات النمو الجذري للتداخل بين البنزل

أدنين عند المستوى 200 ملغم.لتر−1 ومستخلص الكجرات 10 غم.لتر−1 ، إذ أعطى 4.73 غم و2.17 غم مقارنة بمعاملة المقارنة (عدم التسميد) ، والتي أعطت أقل القيم 2.97 غم و0.46 غم وعلى التوالي. ويبين جدول (4) أن التداخل بين البنزل أدنين بالمستوى 200 ملغم.لتر−1 ومستخلص الكجرات بالمستوى 10 غم.لتر−1 أثر معنويا في صفات النمو الزهري، ومنها عدد الأزهار وطول الشمراخ الزهري وقطر نورة الشمراخ وقطر الزهرة، حيث بلغت 20.67نورة.نبات−1 و20.03 سم و8.60 سم و3.73 سم مقارنة بمعاملة عدم التسميد، والتي أعطت أقل القيم التي بلغت 7.67 نورة.نبات−1 و10.93 سم و5.60 سم و1.90 سم.

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دراسة تأثير اللبن اللاكتيكي المحضر بواسطة بكتيريا العصيات اللبنية على الإصابة بطفيلي الزحار الأميبـــي في الفئران البيضاء

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الملخص باللغة العربية

في هذه الدراسة، أمكن الحصول على (13) عزلة من بكتيريا *Lactobacillus casei* من منتجات اللبن والجبن المحليين ومن معي الفئران، وقد أثبت من خلال الفحوصات المناسبة ان هناك عزلتين من بينها منتجة للبكتريوسين والذي كان مقاوما لدرجات منخفضة من الأس الهايدروجيني pH، كما أمكن تشخيص طفيلي*Entamoeba histolytica* من غائط أشخاص مصابين به ، ومن المراجعين لمستشفى سامراء العام. وقد أثبت من خلال دراسة تأثير اللبن اللاكتيكي المحضر بواسطة بكتيريا *L. casei* والمنتجة للبكتريوسين على الإصابة بطفيلي الأميبا المحلة للنسيج في الفئران البيضاء. وقد أظهرت النتائج أن لهذا اللبن تأثيرا إيجابيا في جعل البيئة المعوية قادرة على مقاومة الإصابة بهذا الطفيلي، وهذا أمكن الاستدلال عليه أيضا من خلال المقاطع النسيجية التي تم عملها لكل من الأمعاء والكبد والطحال.

الكلمات المفتاحية: بكتيريا العصيات اللبنية، اميبا الزحار، البكتريوسين، اللبن اللاكتيكي

Study the effect of lactic acid producing bacteriaon the infecting *Entamoeba histolytica* in mice

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ABSTRACT

Throughout the present study, strains of. *Lactobacillus casei* were isolated from; Yoghourt, cheese and gut of mice, two of them are bacteriocin producer. The two strains which are resistant to low pH were employed to produce lactic milk. The effects of lactic milk on mice experimentally infected with *Entamoeba histolytica* were studied. The mice in comparison to control showed resistant to infection to this parasite, This was confirmed through histological examination of the intestines, liver and spleen.

المقدمـــــــة

يعد داء الأميبا (amoebiasis) واحدا من أكثر الأمراض الطفيلية شيوعاً في العالم (1)، والذي ينتج من الإصـابة بطفيلـي الأميبـا *Entamoeba histolytica* وهو أحد الأوالي المعوية التي تصيب الإنسان (2). وهنالك حوالي 500 مليون إصابة في العـالم تنـتج عنها الوفاة بمعدل 100 ألف حالة سنويا (3، 4).

وقد أشار Gilliland (5) إلى أهميـة إضـافة بعـض الأحيـاء المجهرية للأغذية لجعلها صحية، وافترض أن استهلاك منتجـات لبنية متخمرة تحتوي على بكتيريا حامض اللاكتيك قـادرة علـى الاستقرار في القناة الهضمية وباستطاعتها أن تحـل بـديلا عـن البكتيريا المعوية غير المرغوب فيها في الأمعاء. إلا أنه ليس هناك أي تأثيرات ضارة للـمعززات الحيويـة Probiotic bacteria على الفئران خصوصا جنس *Lactobacillus* حتى إذا جرعـت بشكل مباشر.

وقد استخدمت بكتيريا حامض اللاكتيـك Lactic acid (LAB) bacteria في الأغذية بوصفها بادئات تخمير (7)، وتوجـد فـي العديد من الأغذية، ويظهر الحامض فيها بـشكل طبيعـي، مثـل الألبان ومنتجات اللحوم والخضروات (8). وفي بداية العقد الأخير من القرن العشرين، بدأت دراسة تأثير الحفظ بهذه البكتيريا تحظى باهتمام الباحثين، حيث وجد أن هذه البكتيريا تنتج موادا يمكـن أن تستعمل لحفظ الأغذية، وذلك من خلال خفـض الحموضـة عـن طريق إنتاج هذه المركبات، فضلا عن دور البكتريا نفـسها، إذ إن مختلف هذه المركبات تعتبر مـضادة لنمـو الأحيـاء المجهريـة وخصوصا البكتريوسينات (9). ومن أهم الأنـواع العائـدة لهـذا الجـــنس *Lactobacillus casei* و *Lactobacillus acidophilu* (10)، حيث تمتلكان خصائص علاجية لما تبديانه من فعل تضادي للعديد من الأحياء المجهرية. وقد جاء في دراسة (11) أن لبكتريا *Lactobacillus casei* إمكانية تثبـيط طيـف واسع مـن البكتريـا المرضـية منهـا *Shigella dysenteria* و *Shigella flexneri* و *E.coli* و *Salmonella typhi* و *Pseudomonas aeruginosa*, مما شجع على استخدامها فـي الحفاظ على التوازن الطبيعي للنبيت المعوي وفي علاج حـالات الإسهال المختلفة (12).

هدفت هذه الدراسة إلى تحديد إمكانية استخدام اللـبن اللاكتيكـي المحضر بواسطة بكتريا *Lactobacillus casei* في منع الإصابة بطفيلي *Entamoeba histolytica* في الفئران البيضاء.

المواد وطرق العمـــــل

جمع عينات الطفيلي:

تم جمع عينات البراز للحـصول علـى طفيلـي *Entamoeba histolytica* من مراجعي مستشفى سامراء العام والذين يعـانون من إسهال شديد إلى متوسط الشدة، وفي معظـم الحـالات كـان المرضى يعانون من الإسهال الدموي. وقد تم جمع العينـات فـي عبوات معقمة ذات فتحة واسعة مزودة بسداد محكم للحفاظ علـى رطوبة العينة ومنع جفافها. وفحصت العينات خلال نصف سـاعة من وصولها المختبر، مع الحرص على تجنب الأجـزاء الحاويـة على على الدم والمخاط كونها تحجب الناشطات عند الفحص، إذ غالبا ما يشير وجودها إلى حدوث إصابة بطفيلي الأميبا للنـسيج، كما تشير إلى وجود أعداد كبيرة من الناشطات الأميبية الملتهمـة لكريّات الدم (13).

التشخيص المختبري للطفيلي:

– **الفحص المجهري المباشر:** فحصت عينات البراز مجهريا تحت قوة تكبير 40X باستعمال طريقة الشرائح الرطبة (wet mounts) المصبوغة وغير المصبوغة وكذلك بطريقة التركيز حسب ما ذكر في (14).

– **حساب عدد الأطوار المتكيسة للطفيلي:** تـم احتـساب أعـداد الأطوار المتكيسة التي استعملت في تجريع الحيوانات المختبريـة بواسطة عداد الكريات وبالاعتماد على طريقة Hemocytometer الدم (15).

جمع عينات بكتريا *Lactobacillus casei*:

جمعت نماذج من الألبان والأجبان المحلية ومعي الفئران البيضاء، ووضعت في الثلاجة لحـين اسـتخدامها فـي عـزل بكتريـا *Lactobacillus casei*، ثم وضعت هذه النمـاذج فـي أنابيـب اختبار حاوية على الوسط الغـذائي MRS الـسائل (De Man Rogosa Sharp broth) والمعقم، ثم وضـعت الأنابيـب فـي الثلاجة لحين نقلها إلى المختبر (16)، كما تم الحصول على نماذج بكتيرية مرضية والتي استخدمت بعد ذلك لاختبار فحـص إنتـاج البكتريوسين وهي: *Escherichia coli* و *Staphylococcus aureus*.

الأوساط الغذائية المستخدمة:

حضرت الأوساط الزرعية حسب تعليمات الشركة المنتجة وهي : وسط MRS السائل والصلب ووسط MRS الصلب الحاوي على 0.02% أزيد الصوديوم (17)، كما استخدمت أوساط أخرى مثل وسـط آكـار المـاكونكي MacConkey Agar (Oxoid) Medium ووسط المرق المغـذي Nutrient Broth (Oxoid) Medium ووسط الآكار المغـذي Nutrient Agar (Oxoid) Medium ووسط ماء الببتون. وقد حضرت حـسب التعليمـات الواردة من الشركة المصنعة.

التشخيص المختبري:

– **الصفات الزرعية والشكلية:** زرعت النماذج المأخوذة على وسط MRS الصلب وحضنت الأطباق عند درجة 37مْ لمـدة (18 – 24) ساعة. فحصت الأطباق لمـشاهدة المـستعمرات البكتيريـة وأخذت مسحة من المستعمرات المنفردة على شـريحة زجاجيـة وصبغت بصبغة كرام لمعرفة شكل البكتريا.

– **الفحوصات البيوكيميائية:** درست الفحوصـات البايوكيمياويـة التالية لتوصيف العزلات وتشخيصها: فحص الكتـاليز ، فحـص تكون الامونيا من الارجنين، وفحص تخمر الكربوهيـدرات (18، 19).

– **اختبار قابلية العزلات لإنتاج البكتريوسين:** تم تنمية العـزلات العصوية المنتخبة في وسط MRS السائل في أنابيب اختبار بنسبة 1% من المزروع المنشط لكل عزلة، إذ حـضنت فـي درجـة حرارة 37مْ ولمدة 48 ساعة وفي ظروف لا هوائية بعـد ضـبط الأس الهيدروجيني للوسـط عنـد 5.8 (باسـتخدام هيدروكـسيد الصوديوم 0.5 عياري وحامض الهيدروكلوريك 0.5 عياري). كما تم تنمية بكتريا الاختبار في أوساط سائلة بنسبة 1% من المزروع المنشط لكل بكتريا، وحضنت في الدرجة الحرارية المناسبة لهـا ولمدة كافية. وقد استخدمت طريقة الانتشار في الحفـر لاختبـار قابلية العزلات لإنتاج البكتريوسين (20)، والتي يمكـن شـرحها بالشكل التالي:

تم تقدير الفعالية التثبيطية للبكتريوسين المنتج من بكتريا حـامض اللاكتيك المعزولة بطريقة الانتشار في الحفر (Well diffusion method)حسب طريقة (21)، وذلـك للكـشف عـن الفعاليـة التثبيطية للبكتريوسين في رواشح مزارع العزلات المختلفة . تـم تسخين الراشح في حمام مائي بدرجة حرارة 60م^5 و لمدة نـصف ساعة، ثم اختبرت فعاليته التثبيطية ضد بكتريا *Staphylococcus aureus* للتأكد من أن الراشح البكتيري هو بكتريوسين وليس مادة تثبيطية أخرى، وبعدها رسبت البكتريا باسـتخدام جهـاز الطـرد المركزي على 6000 دورة/ دقيقة ولمدة 15 دقيقة، تم تعديل الأس الهيدروجيني للراشح على 1– 10 باستخدام 10 N HCl و 10 N NaOH وتم قياس فعالية البكتريوسينات بطريقة الحفر باستخدام البكتريا *Escherichia coli*.

تم أخذ لتر (1000 مل) من حليب الأبقار الطازج وتم تنقيته وترشيحه. أضيف سكر الكلوكوز أو العسل 1٪، ثم لقح ببكتريا *Lactobacillus casei* 1×10^5 وحضن بدرجة 37م لمدة 12 ساعة. كررت العملية ثلاث مرات لحين ظهور التخثر، ثم تم تعبئته في أوان وخزن بدرجة 5 ُم لحين الاستعمال (22، 23). وقد أعطي اللبن اللاكتيكي (باستخدام بكتريا *Lactobacillus casei* المنتجة للبكتريوسين والمعزولة من معي الفئران) عن طريق زرقه في الفم مباشرة بواسطة محقنة خاصة أعدت لهذا الغرض بواقع مللتر منه مرتين يوميا.

الحيوانات المختبرية:

استخدمت الفئران البيضاء نوع *Mus musculus* من سلالة BALB/C ومن كلا الجنسين، والتي تراوحت أوزانها من 23 – 25 غم ، وتم الحصول عليها من الشركة العامة لصناعة الأدوية في سامراء.

الإصابة المختبرية للفئران بالطفيلي:

جرعت الفئران المختبرية بـ 1 مليلتر من المعلق الحاوي على 10^3 كيس / مل و باستخدام محقنة تجريع فموي stomach syringe معقمة سعة 1 مليلتر. وتم فحص غائط الفئران المجرعة بعد 10 أيام للتأكد من مدى حصول الإصابة.

تصميم التجربة:

استعمل 24 فأرا من الذكور وقسمت إلى أربع مجموعات أساسية بصورة عشوائية :
المجموعة الأولى: تضمنت 6 فئران جرعت باللبن المحضر ببكتريا *Lactobacillus casei* لمدة أسبوع.
المجموعة الثانية : تضمنت 6 فئران والتي جرعت بأكياس الأميبا فقط.
المجموعة الثالثة: تضمنت 6 فئران جرعت باللبن المحضر ببكتريا *Lactobacillus casei*, وبعد مضي أسبوع من التجريع المستمر باللبن، تم تجريعها بأكياس طفيلي *Entamoeba histolytica*.
المجموعة الرابعة: تضمنت 6 فئران تمثلت بمجموعة السيطرة، حيث لم يتم إصابتها بالطفيلي، ولم تجرع باللبن المحضر.
تم فحص البراز لجميع الفئران بالمجموعات الأربعة بعد عشرة أيام من الإصابة للتأكد من جدوث الإصابة.

حساب عدد البكتريا في براز الفئران:

جمع براز الفئران لحساب عدد بكتريا *Lactobacillus* وبكتريا العائلة المعوية Enterobacteriaceae حيث نقل البراز الى انابيب معقمة وحفظ بدرجة 4م . اخذ 1 غم براز من كل مجموعة وعملت تخافيف لغاية 10^6 في 0.15% من ماء الببتون. تم حساب عدد الخلايا البكتيرية باستخدام طريقة الصب. تم احتساب بكتريا *Lactobacillus*على وسط MRS الصلب المحضن بظروف لاهوائية ووسط الماكونكي بظروف هوائية لاحتساب بكتريا العائلة المعوية (24).

الاختبارات النسيجية:

عملت هذه الطريقة حسب طريقة (25)، وذلك بأخذ (الأمعاء – الكبد – الطحال) من الفئران وحفظها بالفورمالين، ومن ثم عملت مقاطع نسيجية وصبغة بصبغة الهيماتوكسلين والأيوسين، وشوهدت تحت المجهر الضوئي تحت تكبير X10 X40.

التحليل الاحصائي:

استخدم اختبار F وحسب المعدل الحسابي لمقارنة النتائج بين مجاميع الفئران المعاملة ومجموعة السيطرة (26).

النتـــائج والمناقشـــة

العزل والتشخيص لبكتريا *Lactobacillus casei*:

تم عزل (13) عزلة عائدة لبكتريا *Lactobacillus casei*, توزعت إلى (4، 4، 5) عزلات من منتوج اللبن المحلي و منتوج الجبن المحلي ومن معي الفئران على التوالي, وهذا يتفق مع ما توصلت إليه دراسة كل من (27، 28)، حيث تم عزل هذه البكتريا من منتجات الألبان والأجبان. كما أشار كل من (29، 30) إلى إمكانية عزلها من البراز والأنسجة المعوية للحيوانات وخصوصا الفئران. وتم عزل بكتريا *L.casie* من مصادر مختلفة منها النبيت الطبيعي للإنسان وخاصة الأطفال (31)، وتميزت العزلات بشكلها العصوي المستقيم تحت المجهر وكانت أيضا موجبة لصبغة كرام وغير متحركة وغير مكونة للأبواغ ، وسالبة لفحص الكاتاليز وفحص تكوين الأمونيا من الحامض الأميني الآرجينين، كما كان شكل المستعمرة البكتيرية محدبا وكريمي اللون ولماعا، ومخمرة لكل من سكر الكلوكوز والفركتوز و المانوز والمانيتول، وغير مخمرة للرافينوز والارابينوز، وهذا ما يثبت عائديتها إلى الجنس البكتيري *Lactobacillus* كما جاء في (32، 33).

التحري عن إنتاج البكتيريوسين:

أوضحت الدراسة أن هناك عزلتين منتجة للبكتروسينات، واحدة كانت عزلة من معي الفئران وأخرى من منتجات اللبن المحلي وهذا يتفق مع دراسة (34)، التي اشارت الذي أشار الى إنتاجية هذه البكتريا للبكتريوسين كما أظهرت العزلات قدرتهما على إنتاج الحامض و خفض الأس الهيدروجيني pH للحليب حيث كانت بحدود (5) ،وهنا يجب الإشارة إلى إن انخفاض الأس الهيدروجيني pH بمستويات معينة يؤدي إلى زيادة نشاط هذه البكتريا وقدرتها على إنتاج البكتريوسين. فقد كان النمو البكتيري وإنتاج البكتريوسين عند الأس الهيدروجيني (5 و6) في أفضل مستوياته، كما كانت فعالية البكتريوسين التثبيطية ثابتة عند هاتين الدرجتين، ولكنها قلت في مستويات الأس الهيدروجيني المنخفضة والعالية جدا (2،8) وهذا يتفق مع ما ذكره (35)، كما أشارت دراسة (36) إلى أن البكتريوسين المنتجة من بكتريا حامض اللاكتيك وخصوصا من قبل *Lactobacillus acidophilus* ذات فعالية تثبيطية عالية في الأس الهيدروجيني (5) وتقل في الأس الهيدروجيني (7).

تشخيص طفيلي *Entamoeba histolytica*:

تم تشخيص طفيلي *Entamoeba histolytica* في غائط الأشخاص المصابين من خلال الفحص المجهري المباشر، إذ شخصت أكياس طفيلي الأميبا الحالة للنسيج من خلال شكلها الكروي والحاوي على أربع أنوية (37).

تأثير اللبن اللاكتيكي على الإصابة بطفيلي *Entamoeba histolytica*:

لقد تم حساب معدلات اعداد بكتريا *Lactobacillus casei* والأنواع العائدة للعائلة المعوية Enterobacteriaceae في النبيت الطبيعي للفئران في مجموعات الفئران الأربعة، وقد تبين أن هناك زيادة معنوية في أعداد بكتريا *Lactobacillus casei* في معي فئران المجموعة الأولى المجرعة باللبن اللاكتيكي، حيث بلغ 6×10^3 CFU/ gm مقارنة بالسيطرة الموجبة (المجموعة الرابعة)، حيث كانت$5،4\times10^3$ CFU/ gm، في حين حصل نقصان في العائلة المعوية ولكن بشكل غير معنوي، حيث بلغ $4،5\times10^3$ CFU/ gm مقارنة بمجموعة السيطرة $5،6\times10^3$ CFU/ gm(جدول رقم 1). وهذا ينطبق على ما توصلت إليه دراسة (38)، كما توافقت النتائج مع نتائج (39) حيث أمكن من عزل كل من بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* من حليب البقر الطازج، وتم استخدام هذه العزلات

في تجريع الفئران لملاحظة تأثيرها على النبيت الطبيعي فيها، حيث لوحظ انخفاض معدلات البكتريا العائدة للعائلة المعوية بالمقارنة مع السيطرة (7,5.5) ، في حين زادت معدلات بكتريا Lactobacilli مقارنة مع السيطرة (5.3,8.2). وقد تعود هذه القدرة على التثبيط لإنتاجها لمواد أيضية مختلفة، فضلا عن البكتريوسينات ذات التأثير القاتل للأنواع الغير منتجة لها، بوصفها أحد أنواع المركبات المضادة للأحياء المجهرية (40)، كما أشارت دراسات سابقة إلى قابلية الأنواع العائدة لجنس Lactobacillus على الالتصاق بالخلايا الطلائية المعوية، حتى وإن زرعت في المزارع النسيجية، وبالتالي قدرتها على المنافسة على المكان من خلال الالتصاق بالأغشية المخاطية المعوية Intestinal mucosa (41).

أما المجموعة الثانية والتي تضمنت تجريع مجموعة الفئران بأكياس الطفيلي، فقد بينت النتائج ازديادا في عدد الأكياس في غائط الفئران خلال فترة الإصابة ولمدة 10 أيام، مما يعني حدوث الإصابة وتحققها، كما تم حساب معدل أعداد بكتريا حامض اللاكتيك والعائلة المعوية والتي كانت cfu (3.5,3.8) على التوالي، والتي يلاحظ من خلالها انخفاض معدلات النوعين من البكتريا عن مستوياتها مقارنة بمجموعة السيطرة، أو حتى عند مقارنتها بالمجموعة الأولى، والتي افترضت ارتفاع معدلات بكتريا حامض اللاكتيك وانخفاض معدلات بكتريا العائلة المعوية، ولكن ليس بهذا المستوى. وما يفسر هذا الانخفاض هو أن هذا الطفيلي يعيش على سطح الطبقة المخاطية وفي خبايا القولون، ويبدأ في التعايش مع الفلورا الطبيعية المتواجدة في الأمعاء، ومن ثم يعاني من تغيرات نتيجة التداخل مع هذه البكتريا، كما تزداد ضراوتها مع وجود أنواع بكتيرية مثل *Esherichia coli* (42) و *Salmonella paratyphi*. كما لوحظ خلال تجربة دراسة (43) بعد نصف ساعة من معاملة طفيلي *Entamoeba histolytica* مع كل من *E.coli* و Lactobacilli عند إجراء التجربة في الطبق خارج الجسم الحي بأن الطفيلي قد التهم حوالي 90% من بكتريا *E. coli* ، في حين التهم حوالي 60% من الأنواع العائدة لجنس Lactobacilli. ومن هنا يتبين تأثير الطفيلي على العائلة المعوية اكثر من بكتريا حامض اللاكتيك. وهذا ما أشار إليه (43)، حيث تبين تفاعل *E. Histolytica* مع *E. coli* أكثر من الأنواع العائدة لبكتريا Lactobacilli.

أما المجموعة الثالثة، وهي محور البحث والتي جرعت فيها الفئران باللبن المحضر بكتريا *Lactobacillus casei*، وبعد مضي أسبوع من التجريع المستمر باللبن، تم تجريعها بأكياس طفيلي *Entamoeba histolytica*. وقد لوحظ عدم حدوث الإصابة بالطفيلي بعد متابعة الفئران بعد فترة التجريع، وكذلك لوحظ حدوث تغيير بزيادة أعداد بكتريا *Lactobacillus casei* وانخاض معدلات بكتريا العائلة المعوية مقارنة بالمجموعة الأولى، حيث بلغت $(4,6.2)\times 10^3$ على التوالي كما مبين في الجدول (1). وهنا تجدر الإشارة إلى انه قد تم استخدام العلاج ببكتريا Lcatobacilli وخصوصا النوع *L.casie* في علاج العديد من الطفيليات، فقد استخدمت الأخيرة في علاج كل من طفيليات *Trichinella spiralis* و *Trypanosoma cruzi* و *Babesia microti* في الفئران (44)، مما يعني بوجود تأثير لها في منع الإصابة بالطفيليات، كما يجب الإشارة إلى ان الأكياس الرباعية النواة لطفيلي *E. histolytica* تتحفز بالبيئة القاعدية لتجويف الأمعاء الدقيقة، ليتحلل جدارها بفعل إنزيم التربسين Trypsin ، وتحدث عملية الإفلات من الكيس Excystation كأول خطوة لإمكانيتها إحداث الإصابة، والتي تنتهي بالتضاعف في خبايا الطبقة المخاطية للأمعاء الغليظة، وحدوث التفاعل الأيضي مع البكتريا المعوية (6)، وكما هو معلوم ، فإن بكتريا Lactobacilli تنتج أنواعا متعددة من الأحماض العضوية ، التي ترفع من مستوى حموضة الأمعاء (Intestinal pH) (45)، والذي يمكن أن يفسر عدم تحقق الإصابة بالطفيلي لعدم توفر البيئة الملائمة لإفلات الاكياس الرباعية، كما أن لبكتريا Lactobacilli والمنتجة للبكتريوسين خاصة دورا في تعزيز الجانب المناعي للمضيف، فقد اشارت نتائج دراسة (46) إلى قدرة بكتريا المعززات الحيوية Probiotic bacteria وخصوصا الجنس Lactobacilli على استحثاث إنتاج السايتوكينات Cytokines والكيموكينات Chemokines من الخلايا الطلائية للأمعاء في الفئران. وبما أن هذه السايتوكينات والكيموكينات مرتبطة بالاستجابة المناعية للمضيف، فبالتالي ستؤدي إلى تحسنها وزيادتها، مما يعني زيادة مقاومة الأنسجة المعوية لأغلب الممرضات التي تهاجمها. وبالإشارة إلى أن الطفيلي يعيش في القولون ويتعايش مع الفلورا الطبيعية المتواجدة في الأمعاء وخصوصا أفراد العائلة المعوية، وبزيادة ضراوته مع وجود أنواع بكتيرية مثل *Esherichia coli* و *Salmonella paratyphi*، وبما أن بكتريا *L. casie* وفق النتائج التي تم التوصل إليها فإنها تقوم بخفض أعداد أفراد العائلة المعوية، وبالتالي فإنها تزيد من مقاومة المضيف للإصابة بالطفيلي.

جدول (1): معدلات أعداد البكتريا (CFU/ gm) لمجموعات الفئران المعاملة

البكتريا	المجموعة الأولى ×CFU 10^3	المجموعة الثانية ×CFU 10^3	المجموعة الثالثة ×CFU 10^3	المجموعة الرابعة (سيطرة) ×CFU 10^3
Lactobacillus casei	6	3.8	6.2*	4.5
Enterobacteriaceae	4.5	3.5	4	5.6

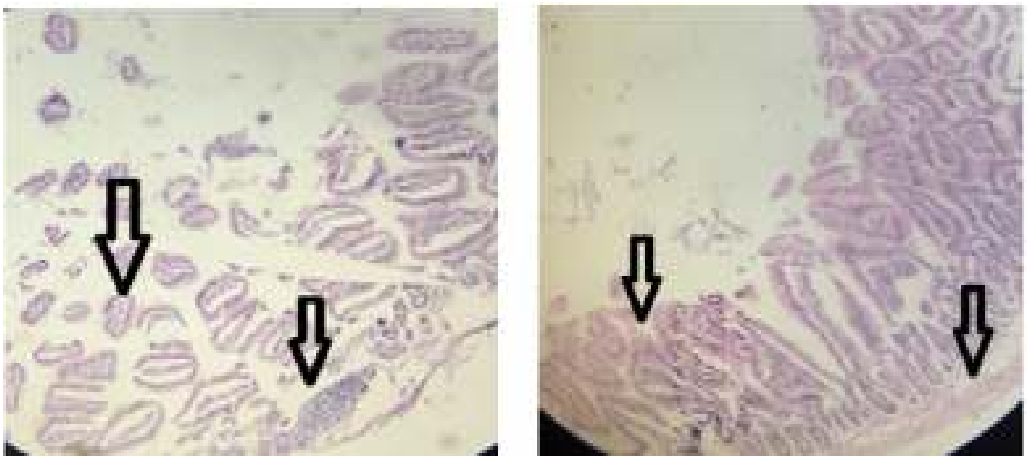
** : تأثير معنوي*

تأثير التجريع باللبن اللاكتيكي على الأنسجة المعوية:

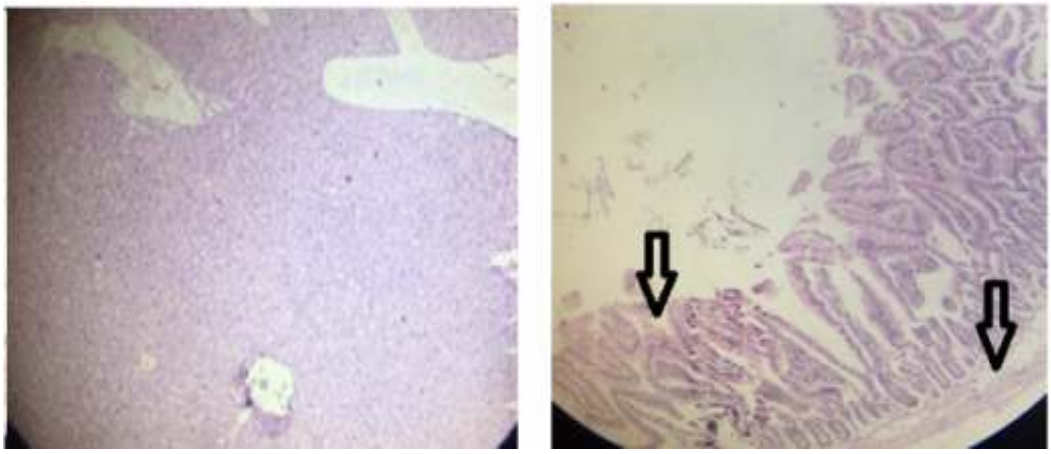
عملت مقاطع نسيجية للامعاء الفئران باعتبارها محل الإصابة الرئيسي, وكذلك بسبب بطء حركة الغذاء فيها وكثرة التعرجات (28). كما تم أخذ عينات من الكبد والطحال لعمل مقاطع نسيجية لها، نظرا لانتقال الإصابة أحيانا إلى هذه الأعضاء، مسببة خراج الكبد الأميبي وتضخم الطحال (4).

وتظهر الاشكال (1–3) الفرق بين تأثير المجموعة التي جرعت باللبن اللاكتيكي قبل التجريع بالطفيلي والمجموعة التي جرعت بالطفيلي فقط على كل من الأمعاء والكبد والطحال في الفئران المعاملة، والذي يتضح من خلالها التأثير الإيجابي للتجريع باللبن اللاكتيكي على الأنسجة المذكورة من تأثير الإصابة بالطفيلي، وهذا ما بينه (47)، في بيان تأثير التجريع ببكتريا *L. casie* على التغذية السيئة، وبالتالي على المجتمع البكتيري للأمعاء والأنسجة المبطنة له في الفئران، حيث لوحظ بعد يومين من التغذية انخفاض الأنواع العائدة للعائلة المعوية وفي نفس الوقت زيادة في بكتريا *L. casie* ، مع تحسن في الطبيعة النسيجية والزغابات المعوية، مقارنة بالسيطرة التي غذيت بالتغذية السيئة، والتي انخفضت فيها أعداد بكتريا *L.casie* ، وكذلك باقي الأنواع اللاهوائية. كما تلعب التغذية دور المفتاح في إعادة التوازن للنبيت الطبيعي للأمعاء، فسوء التغذية يؤدي إلى اضطراب الحواجز البيئية، وبالتالي يؤدي إلى تضرر الأنسجة المبطنة للأمعاء (47).

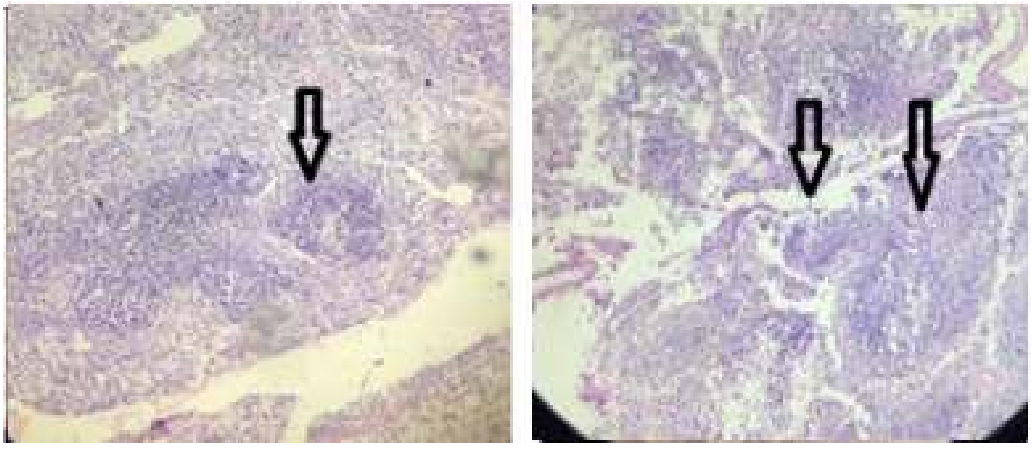
يساعد التجريع باللبن اللاكتيكي ببكتريا *L. casie* على تكوين جانب وقائي جزئي ضد الإصابة ببكتريا *E. coli*, حيث توصل (39) إلى أن تأثير النوع *L. casie* كان أفضل من النوع *L. acidophilis* لتكوين الجانب الوقائي أعلاه، كما تم عمل مقاطع نسيجية تبين من خلالها أن التجريع بهذه الأنواع من البكتريا لا يؤثر سلبا على الأنسجة المبطنة للأمعاء، بل يؤدي إلى تحسن أدائها وتنسجها.



شكل (1): الفرق بين تأثير المجموعة التي جرعت باللبن اللاكتيكي قبل التجريع بالطفيلي والمجموعة التي جرعت بالطفيلي فقط على أمعاء الفئران المعاملة



شكل (2): الفرق بين تأثير المجموعة التي جرعت باللبن اللاكتيكي قبل التجريع بالطفيلي والمجموعة التي جرعت بالطفيلي فقط على أنسجة كبد الفئران المعاملة



شكل (3): يوضح الفرق بين تأثير المجموعة التي جرعت باللبن اللاكتيكي قبل التجريع بالطفيلي والمجموعة التي جرعت بالطفيلي فقط على طحال الفئران المعاملة

الشـــكر والتقديـــر

تم تنفيذ هذه الدراسة بمنحة ودعم مقدمين من كلية العلوم التطبيقية في جامعة سامراء.

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الأحماض الأمينية والدهنية وبعض الفيتامينات في قشور الباذنجان

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الملخص باللغة العربية

هدفت هذه الدراسة إلى التحري عن الأحماض الأمينية والدهنية وبعض الفيتامينات في قشور الباذنجان باستخدام جهاز كروماتوكرافيا السائل ذي الأداء العالي HPLC لتقييم الفوائد فيها.
أظهرت التحليلات الكيميائية احتواء القشور على العديد من الأحماض الأمينية وكانت أعلى نسبة فيها لحامض الفالين ثم للبرولين، ثم يليها كل من: فنيل الأنين، ميثيونين، تايروسين ، الأنين، تربتوفان، هستدين ، كلوتامين ، ارجنين، لايسين. بينما الأقل منها كانت الأحماض: ليوسين، اسبارجين، سيستين، كلوتاميك ، سيرين، والأقل تواجدا من الأحماض في القشور كان حامض اسبارتيك. وأشارت النتائج إلى عدم وجود كل من :حامض كلايسين ، ثيريونين ، ايزوليوسين. أما الأحماض الدهني، فقد كانت أعلى نسبة لحامض أوليك ثم لينوليك. والأقل منها: إلفا–لينولينيك ، الاراكيديك ، مايرستيك ، ستيريك، في حين كان أقل الأحماض تواجدا فيها حامض البالمتيك. وأظهرت النتائج وجود عدد من الفيتامينات في قشور الباذنجان ،إذ كانت أعلى نسبة للثايمين ثم حامض نيكوتينيك ويليها كل من: فوليك، حامض الآسكوربيك ، بايريدوكسين، وكان الفيتامين الأقل تواجدا هو حامض البانتوثينيك ، كما افتقرت القشور إلى سيانوكوبالامين.

الكلمات المفتاحية: قشور الباذنجان، HPLC، فيتامينات، أحماض أمينية، أحماض دهنية

Amino acids and fatty acids with some vitamins in eggplant peels

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ABSTRACT

The present study was conducted to investigate and estimate the presence and values of amino acids, fatty acids and vitamins in the eggplant peel by using high performance liquid chromatographic (HPLC).The results showed that, the eggplant peel contains various amino acids at different concentration. Valine, gave the highest concentration, followed by phenylalanine and proline. Low concentrations were observed for: Methionine, Alanine , Tyrosine, Tryptophan, Histidine, Glutamine, Arginine and Iysine. Then the lowest concentration was obtained for; Leucine, Asparagine, Cystine, Glutamic acid, serine. Both Glycine and Threonine were not found. In regards to fatty acid, highest concentration was observed for; Oleic acid, Linoleic acid,α -Linolenic acid, Arachidic acid followed by lower concentration for Myristic acid, Stearic acid and Palmitic acid. Regarding to vitamins, the highest concentration was recorded for Thiamine, then Nicotinic acid followed by Folic acid, Pyridoxine, Pantothenic acid and Ascorbic acid. No Cyanocobalamin was found.

المقدمـــــة

يعد نبات الباذنجان (Eggplant) *Solanum melongena* من محاصيل الخضر الرئيسة في الكثير من بقاع العالم، إذ يعرف باسم Brinjal في الهند والصين، وباسم Aubergine في بعض الدول الأوربية. ويتبع العائلة الباذنجانية *Solanaceae* (1). تضم العائلة مجموعة كبيرة من الأجناس التي تنتشر في المناطق الاستوائية والمعتدلة من العالم، حيث تشتمل على 75جنساً و 2000 نوعاً منتشرة في أنحاء العالم(2). ويعد غذاء شعبيا في معظم المناطق الاستوائية ومنطقة الشرق الأوسط. وللباذنجان أكثر من 30 صنفاً بمختلف الأشكال والألوان، وتنتشر زراعته في مختلف بلدان العالم لكونهِ نباتا محايدا للضوء (3)، ويعد أحد محاصيل الخضر الصيفية في العراق، ومن المحاصيل في الزراعة المحمية، ويزرع من أجل ثماره التي تؤكل بعد طبخها أو تستعمل في عمل المخللات والمعلبات، كما تحفظ بالتجميد. ويزرع في الربيع ليعطي حاصلا في فصل الصيف، ويستهلك بكميات كبيرة منه نظراً لقيمته الغذائية، وقد سمي محصول الرجل الفقير في الهند (2)، ويعتقد أن موطنه الأصلي هو وسط الهند وجنوب شرقي الصين، ومن هناك انتشرت زراعته في أفريقيا وإسبانيا والمناطق الأخرى من العالم (4). وتظهر أهميته من خلال مساهمته في تزويد جسم الإنسان بمركبات الطاقة المهمة للبناء (الكربوهيدرات، البروتينات والدهون)، ويحتوي على كميات كبيرة من فيتامين A، كما يعد مصدرا جيدا لفيتامين B و C والعناصر المعدنية (5). و يحتوي كل (100) غم من ثمار الباذنجان على المكونات التالية: ماء 87.2% ، بروتين 1%، شحوم0.3%، كربوهيدرات 6.5%، ألياف 1.5%، رماد 0.5 ، فيتامين C 10 ملغم ، فيتامين A0.05ملغم ، فيتامين B2 0.05 ملغم، فيتامين B1 0.04 ملغم (6)، كالسيوم 18.0 ملغم، مغنيسيوم 16.0ملغم، فسفور 47.0 ملغم، حديد 0.9 ملغم، صوديوم 3.0 ملغم، نحاس0.17 ملغم، بوتاسيوم 2.0 ملغم (7). ومن فوائده الطبية العديدة يستعمل غذاء وعلاجا للشفاء من بعض الأمراض (8)، وعدد غير قليل منه يستعمل في الزينة (2). تستخدم ثمار الباذنجان ولا سيما الأبيض منها لتساعد في علاج مرض البول السكري و تخفيف آلام الأسنان (7) . كما تشير العديد من الدراسات إلى أن لثمار الباذنجان استخدامات طبية متعددة لمعالجة آلام الكبد كونه ينشط تحولات الكاسترين ويخفض فعاليته (2)، إذ يمكن استعماله في حالات الإسهال وفي خفض نسبة الكولسترول في دم مرضى السكري، والربو، والكوليرا، وعسر البول (9، 10).

هدفت هذه الدراسة إلى تحديد محتوى قشور الباذنجان من الأحماض الأمينية والدهنية وبعض الفيتامينات لمعرفة القيمة الغذائية لها.

المواد وطرق العمـــــل

مصدر وتهيئه النموذج:

بعد الحصول على الباذنجان من الأسواق المحلية في مدينه سامراء. ثم تم التحقق من تحديد الصنف في مختبرات كلية الزراعة /جامعة تكريت. وقد تم تقشير الباذنجان على عمق يتراوح ما بين (4.5-5) ملم، ثم تم حساب الاستخلاص لقشور الباذنجان والتي وجدت بحدود 18% من الوزن الكلي، تم تجفيفها في الظل لأكثر من ثلاثة أيام، وأجريت عملية الطحن باستعمال المطحنة الكهربائية للحصول على مسحوق ناعم (Powder) يمرر من خلال منخل بقطر ذو فتحات (1) ملم، ثم حفظت النماذج في أكياس بلاستيكية محكمة بعيدة عن الرطوبة.

فصل واستخلاص الأحماض الأمينية من القشور:

استخلصت الأحماض الأمينية بطريقة التجانس ((11) Homogenization)، وتم أخذ (50) غم من القشور وأذيبت في 100 مل من الميثانول (75%)، وأجريت للمزيج عملية تجانس. بعد ذلك تم نقل المزيج المتجانس إلى قنينة التكثيف الراجع (Reflux)، وأجريت عملية التكثيف الراجع عند درجة حرارة 80مه لمدة 15 دقيقة. بعد ذلك أجريت عملية تصفية للمزيج ونقل الراشح إلى قنينة (250) مل، ثم أهمل الراسب وأكمل الحجم في القنينة إلى العلامة بالماء المقطر. وتركت القنينة طوال الليل بدرجة حرارة 20 مه ليسمح للأحماض الأمينية بالترسيب. تم تجفيف الراسب بعملية الترشيح تحت ضغط مخلخل (Vacuum Filtration)، ثم نقل إلى قنينة وأكمل الحجم بواسطة المذيب المجهز للترحيل في جهاز HPLC . وتم تثبيت طريقة سريعة وحساسة بتحليل الأحماض الأمينية المستخلصة على عمود الطور المعاكس في الجهاز، و ظروف الفصل التالية:

- الشركة والموديل (6AVP) Koyoto Japan (Shimadzu).
- أبعاد العمود (50×4.6 mm l.d).
- الطور الناقل (Solvent(**A)** 5%methanol in 0.01 N sodium acetate buffer pH (7.0)
Solvent **(B)**methanol.
- الطور الصلب (C. 18 shimpack ODS).
- حجم الجزيئات (3um).
- سرعة الجريان (1 ml/min).
- نوع وعدد المضخات (Two Shimadzu model LC-6Apumps).
- أنبوب الحقن(20µl).
- الطول الموجي (280 nm).
- نوع الكاشف (SPd-6AV Uv-visible detector Equipped with flowCell8µ.

تحضير الكاشف Ortho phthalaldehyde:

أذيب (50) ملغم من مادة من (OPA) Ortho phthalaldehydeفي 50 مل من 2-Mercaptoethanol ثم أضيف إليه 1.5 مل من الكحول الإثيلي المطلق، وعدل الأس الهيدروجيني للمحلول إلى 9.5 باستخدام 0.4 مول منظم البورات Borate buffer. ثم حفظ المحلول في الظلام واستخدم خلال 24 ساعة (12).

تحضير مشتقة الأحماض الأمينية:

تم مزج 10 مايكروملليتر من مشتقة OPA مع (20) مايكروملليتر من محلول الأحماض الامينية القياسية أو النموذج لغرض تشخيص والتعيين الكمي للأحماض الأمينية. وبعد الخلط والرج لمدة دقيقة واحدة تم إضافة 50 مايكروملليتر من 0.1 M من خلات الصوديوم pH 7.0 لغرض تعديل الأس الهيدروجيني لإكمال تفاعل المشتقة، وتم الحقن بمقدار 20 مايكروملليتر من النموذج المحضر لغرض التحليل. تم التعين النوعي للأحماض الأمينية بمقارنة زمن احتجاز كل حامض أميني مع نظيره في المادة القياسية. وتم حساب تركيز كل حامض أميني في المستخلص مقارنة مع تركيز الحامض في المادة القياسية، والتي أجريت تحت نفس ظروف الفصل.

فصل الأحماض الدهنية في القشور:

تم استخلاص الأحماض الشحمية من القشور وذلك بإجراء عملية التقطير البخاري (Steam distillation) للنماذج. وتتلخص الطريقة بأخذ 1 غم من النماذج ووضعها في قنينة زجاجية وتعريضها لتيار بخاري حيث يعمل البخار على استخلاص المركبات الأروماتية (aromatic compounds) من النماذج ونقل المزيج المتصاعد إلى جزء التكثيف، حيث تم تكثيفه باستخدام تيار من الماء البارد، وتكون في النهاية مزيج من الأحماض الدهنية والماء. بعد ذلك تم فصل الأحماض الدهنية عن الماء بطريقة FAME والموصوفة من قبل Christie (13)، حيث تم

أسترة الأحماض الدهنية بمجوعة المثيل باستخدام ميثوكسيد الصوديوم (Sodium methoxide)، بعد ذلك تم أخذ 50 µl من عينة الزيت المستخلصة وأضيف إليها 950 µl من الهكسان، إذ يعمل الهكسان على إذابة الأحماض الدهنية المؤسترة، ومن ثم أخذ مزيج الهكسان والأحماض الدهنية للتحليل بجهاز HPLC ، وحسب الظروف المثبتة الآتية:

- الشركة والموديل (Koyoto Japan (6AVP) Shimadzu).
- أبعاد العمود (50×4.6 mm l.d).
- الطور الناقل (Acetonitrile: acetone (59:41 ,V/V.
- الطور الصلب (C. 18 shimpack ODS).
- حجم الجزيئات (3um).
- سرعة الجريان (1 ml/min).
- نوع وعدد المضخات (Two Shimadzu model LC-6Apumps.)
- أنبوب الحقن (20µl).
- الطول الموجي (280 nm).
- نوع الكاشف (Uv-visible detector (SPd-6AV Equipped with flowCell8µ.

فصل الفيتامينات من قشور الباذنجان:

تمت إذابة 10غم من قشور الباذنجان في مزيج الميثانول/ماء (60:40 حجم /حجم) وتم إذابته باستخدام الموجات فوق الصوتية Ultra Sonic ولمدة 30 دقيقة. بعد ذلك أجريت عملية ترشيح للمزيج بواسطة ورقة ترشيح (Whatman) وبسمك 0.5 um وذلك لإزالة الألياف والمكونات الأخرى .

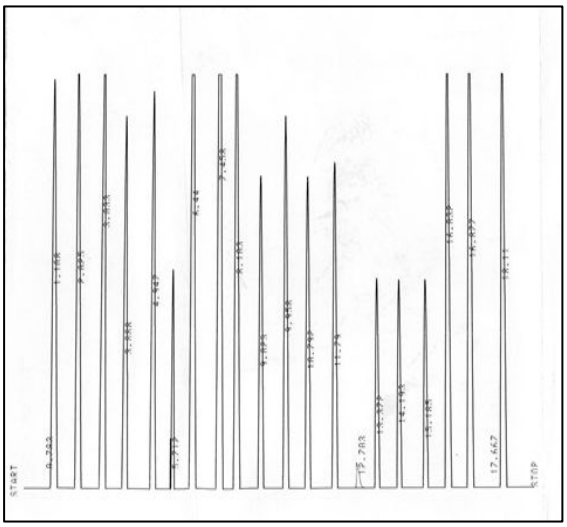
أجريت عملية تركيز للراشح وذلك بالتعريض للنيتروجين وتركيز الراشح بحدود 0.5 مل ومن ثم أكمل الحجم إلى 1 مل وذلك بإضافة محلول الطور الناقل. وفصلت الفيتامينات على عمود الطور المعكوس بعد اخذ 20µl من كل عينة لغرض التحليل. وتم كشف الفيتامينات المفصولة على كاشف الأشعة فوق البنفسجية عند طول موجي 220 نانوميتر، وحسب ظروف الفصل المبينة الآتية:

- الشركة والموديل (6AVP) Koyoto Japan (Shimadzu).
- أبعاد العمود (50×4.6 mm l.d).
- الطور الناقل 10 mm Octylsulfonate in phosphate buffer pH 2.5 acetonitrile 40:60 V/V.
- الطور الصلب (phenomenex C. 18 shimpackODS).
- حجم الجزيئات (3um).
- سرعة الجريان (1.2 m1/min).
- نوع وعدد المضخات (Two shimadzu model LC-10Apumps.)
- أنبوب الحقن (20µl).
- الطول الموجي (220 nm).
- نوع الكاشف (Uv-visible detector SPd-10AV) Equipped with flowCell 8μ.
- جهاز السيطرة (Automatic system controller (SIL-6A

النتائج والمناقشة

فصل الأحماض الأمينية:

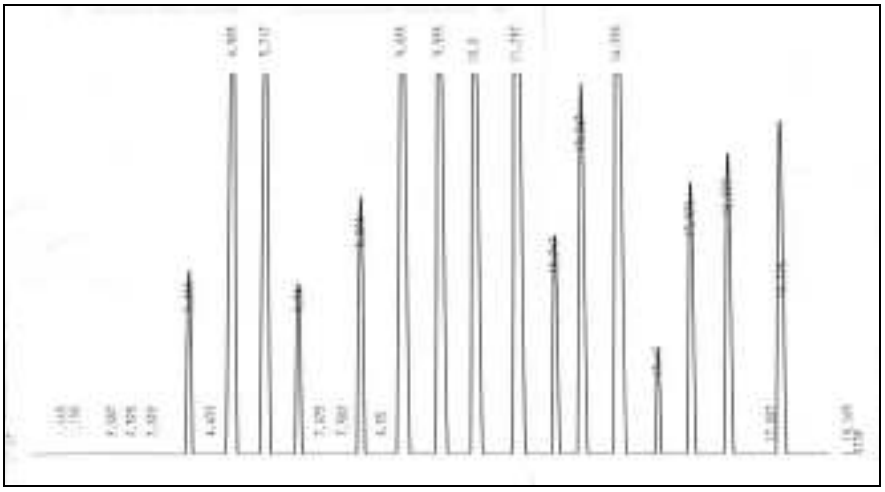
يبين الشكل (1) الأحماض الأمينية القياسية المفصولة بواسطة جهاز HPLC لغرض المقارنة وحساب تركيزها في القشور, وكان عدد هذه الأحماض 17 حامضاً أمينيا. كما يبين الجدول (1) احتواء قشور الباذنجان على العديد من الأحماض الأمينية، وكانت أعلى نسبة فيها لحامض الفالين 6.752µg/ml ثم µg/ml 3.553 للبرولين ثم يليها كل من : فنيل الأنين 3.286, ميثيونين 2.963 , تايروسين 2.540 , الانين 2.410 , تربتوفان 2.083 , هستدين 1.941 , كلوتامين 1.482 , ارجنين 1.220, لايسين 1.021µg/m. بينما الأقل منها لكل من ليوسين (0.992) , اسبارجين (0.970) , سيستين (0.813) , كلوتاميك (0.432) , سيرين (0.401) µg/ml والأقل تواجدا من الأحماض في قشور الباذنجان كان حامض اسبارتيك (0.355)µg/ml. وأشارت النتائج إلى افتقار القشور لكلٍ من حامض كلايسين, ثيريونين , ايزوليوسين, ويبين شكل (2) الأحماض الأمينية المفصولة من قشور الباذنجان.



شكل(1): الأحماض الامينية القياسية المفصولة باستخدام دليل (OPA) Ortho phthalaldehyde في جهازHPLC

جدول (1): تركيزالأحماض الأمينية في قشور الباذنجان

ت	الأحماض الأمينية	تركيز(µg/ml)
1	كلوتاميك	0.432
2	اسبارتيك	0.355
3	سيرين	0.401
4	اسباراجين	0.970
5	كلوتامين	1.482
6	هستدين	1.941
7	سايستين	0.813
8	كلايسين	--
9	ثريونين	--
10	برولين	3.553
11	ألانين	2.410
12	تايروسين	2.540
13	ميثيونين	2.963
14	فالين	6.752
15	تربتوفان	2.083
16	فينيل الانين	3.286
17	ليوسين	0.99
18	ايزوليوسين	--
19	لايسين	1.021
20	أرجينين	1.220



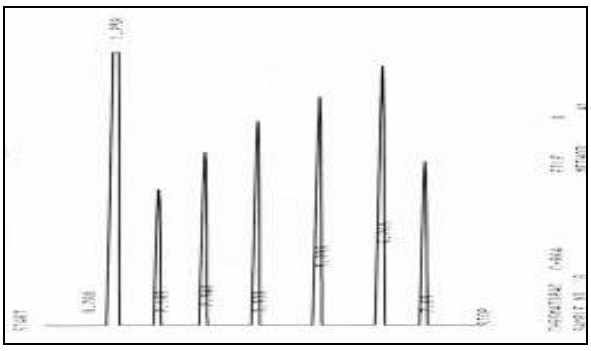
شكل (2): الأحماض الأمينية في قشور الباذنجان المفصولة باستخدام دليل Ortho phthalaldehyde (OPA) في جهازHPLC

فصل الأحماض الدهنية:

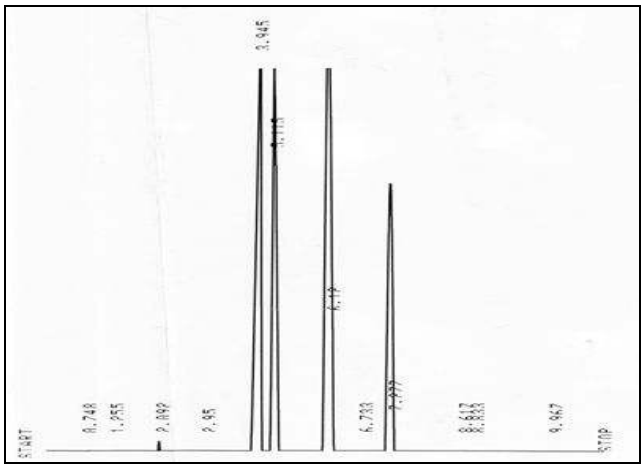
يبين الجدول (2) الأحماض الدهنية المفصولة بواسطة جهاز HPLC وحساب تركيزها في نماذج المعاملات، وكانت أعلى نسبة للأحماض الدهنية في قشور الباذنجان هي لحامض أوليك (µg/ml31.93) ثم تليها (µg/ml 29.011) لينوليك. والأقل منها الفا–لينولينيك (µg/ml 27.937), الاراكيديك (µg/ml26.358) , مايرستيك (µg/ml17.832), ستيريك (µg/ml9.554). وأقل الأحماض تواجدا في قشور الباذنجان كانت لحامض البالمتيك (µg/ml 8.917). حيث يلاحظ من شكل (3) عدم ظهور القمم الامتصاصية في كل من حامض ستيريك , مايرستيك , بالمتيك وهذا يفسر بأن تركيز هذه الأحماض كانت قليلة جدا في القشور مما أدت إلى عدم ظهور القمة. يبين شكل (4) الأحماض الدهنية المفصولة في القشور بواسطة جهازHPLC, حيث تم حساب تركيز جميع الأحماض الدهنية في النماذج من خلال مقارنه زمن الاحتجاز للأحماض الدهنية القياسية مع زمن الاحتجاز في النماذج وتطبيق المعادلة الخاصة بذلك.

جدول (2): تراكيز الأحماض الدهنية في قشور الباذنجان

ت	الأحماض الدهنية	تركيز(µg/ml)
1	حامض البالمتيك	8.917
2	حامض مايريستيك	17.832
3	حامض ستيريك	9.554
4	حامض الاوليك	31.93
5	حامض لينوليك	29.011
6	α–حامض لينولينك	27.937
7	حامض الاراجيديك	26.358



شكل (3): الأحماض الدهنية القياسية المفصولة في جهاز HPLC



شكل (4): الأحماض الدهنية في قشور الباذنجان المفصولة في جهاز HPLC

بعض الفيتامينات الذائبة في الماء:

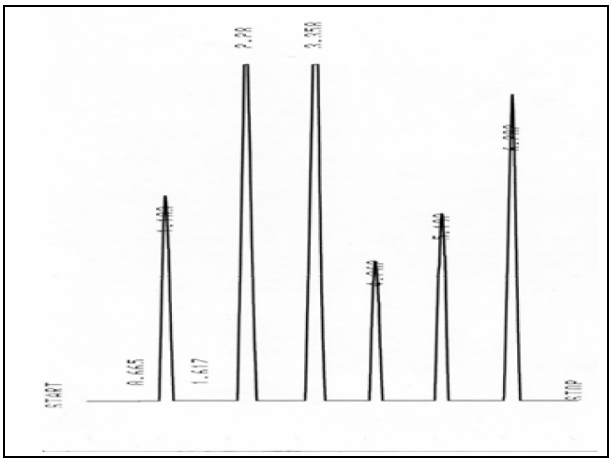
يبين جدول (3) الفيتامينات المفصولة بواسطة جهاز HPLC وحساب تركيزها في القشور. كما يبين شكل (5) الفيتامينات القياسية المفصولة بواسطة جهاز HPLC .

ان أعلى نسبة هو للثايمين (µg/ml 8.287) وثم µg/ml (7.406) حامض نيكوتينيك ويليها كل من فوليك (µg/ml 4.543) , حامض الآسكوربيك (µg/ml 3.757), بايريدوكسين (µg/ml 3.338) والأقل تواجدا هو حامض البانتوثينيك (µg/ml 3.014) و يفتقر إلى سيانوكوبالامين.

ومن الجدير بالذكر أن معظم البحوث المتوفرة لم تشر إلى نسبة الأحماض الأمينية والدهنية والفيتامينات المذكورة في قشور الباذنجان.

جدول (3): نسبة بعض الفيتامينات في قشور الباذنجان

ت	الفيتامينات	تركيز(µg/ml)
1	حامض الاسكوربيك	3.757
2	حامض نيكوتينيك	7.406
3	ثيامين	8.287
4	بايريدوكسين	**3.338**
5	حامض بانتوثينيك	**3.014**
6	حامض فوليتك	**4.543**
7	سيانوكوبالامين	--



شكل (5): الفيتامينات في قشور الباذنجان المفصولة في جهازHPLC

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تحضير كاسترد محلى بدبس التمر وتقييمه تغذويا وحسيا

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الملخص باللغة العربية

يعتبر العراق من البلدان المنتجة للتمور التي تستهلك بشكل مباشر او بعد تصنيعها. ونظرا لما لها من قيمة غذائية وعلاجية عالية فقد اجريت دراسات عديدة لإدخالها في تصنيع بعض المنتجات الغذائية. وتهدف هذه الدراسة الى استخدام دبس التمر كمصدر للسكر في تحضير كاسترد ذو قيمة صحية وغذائية عالية لتجنب الضرر الصحي لسكر المائدة. تم تقييم الخصائص الحسية والقيمة الغذائية للمنتج ومقارنته بكاسترد تجاري. أظهرت النتائج أن الكاسترد المحضر بإضافة الدبس قد أعطى درجات حسية مرتفعة وان قيمته الغذائية وطعمه أفضل مقارنة مع مثيله التجاري.

الكلمات المفتاحية: كاسترد، دبس، كركم، تقييم حسي، سكر

Making custard with dates syrup, and evaluating it nutritionally and organolepticall

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ABSTRACT

Iraq is one of the producing countries of dates, consumed directly or after processing. Because of high nutritional and therapeutic value of dates, many studies has been conducted for inclusion them in the manufacture of some food products. This study aimed to use dates syrup as a source of sugar in the preparation of custard with a healthy and high nutritional value to avoid health disadvantages of table sugar. The sensory properties and nutritional value of the product was evaluated and compared with commercial custard. The results showed that the addition of dates syrup has given a high degree of sensory and nutritional value and tasted better compared with that of the commercial.

المقدمـــــة

يعد النخيل (*Phoenix dactylifera* L.) من أشجار الفاكهة الرئيسية في العراق (1). يستهلك إنتاجه من التمور بشكل مباشر او بعد تصنيعه ويمتاز بخصائصه العلاجية بجانب قيمته الغذائية العالية (2), حيث تعتبر التمور مصدرا مهما لتزويد العناصر المعدنية والفيتامينات في النظام الغذائي المتوازن (3).
أثبتت الدراسات أن استهلاك التمور قد تفيد في السيطرة على نسبة السكر والدهون في الدم لدى مرضى السكري (4) و (5). وفي الآونة الأخيرة، تم اثبات العديد من الفوائد العلاجية للتمور ومشتقاتها. فقد ثبت ان لسكرياتها المتعددة فعالية مضادة للأورام (6)، ولها خواص مضادة للأكسدة وخصائص مضادة للطفرات الوراثية (7). وهذه الفاكهة معروفة في العلاج الشعبي لمختلف الأمراض المعدية وأمراض السرطان (8). ففي الطب التقليدي الهندي يعطى مستخلص التمور الجافة للمرأة بعد الولادة كمواد محفزة للمناعة immunostimulants (9). ووجد أيضا ان المستخلص المائي للتمور يمنع اكسدة الدهون والبروتين (10). وعلاوة على ذلك، خلص الباحثون إلى اعتبار التمور غذاء مثاليا تقريبا، لكونها توفر مجموعة واسعة من المواد الغذائية الأساسية والفوائد الصحية (11).

منتجات التمور

نظرا لفوائد التمور العديدة حرص الباحثون على استخدمها لانتاج العديد من المنتجات الغذائية مثل مربى التمر (12) والمشروبات الغازية (13)، والزبادي المطعم (14)، وشكولاتة التمر (15) ووفر التمر Date wefer (16)، والحليب المطعم (17).

الكاسترد

يعد الكاسترد احد تحضيرات الطبخ الذي يعتمد على طبخ خليط من الحليب او الكريم ومادة مثخنة. ويختلف قوام الكاسترد حسب كمية المادة المثخنة المضافة , ولذلك فقد يكون خفيفا أو سميكا. الأكثر شيوعا استخدامه كحلويات بعد الطعام, وعادة يحتوي على السكر والفانيلا. ويضاف له بعض الأحيان الطحين او نشا الذرة او الجيلاتين. يطبخ عادة على نار هادئة او يستخدم حمام مائي عند الشي في الفرن. والكاستر حساس للحرارة لان ازديادها بمقدار 3 الى 6 درجات مئوية يمكن ان يؤدي الى التخثر والطعم المطبوخ. وبصورة عامة يبدأ بالنضوج على 70م, ولا يجور ان تتجاوز درجة الحرارة 80 م (18), وان استعمال الحمام المائي يجعل انتقال الحرارة بطيئا ويسهل إزالة الكاسترد من الفرن قبل تخثره (19).
يشير الاتحاد الدولي لمرض السكري، أن المرض يصيب حاليا 415 مليون شخص في جميع أنحاء العالم (20). وترجع زيادة حدوث مرض السكري أساسا إلى تغير نمط الحياة الحديثة وتغير النظام الغذائي الذي يميل نحو الأطعمة المكررة خاصة السكر والدهون. كما وجد ان السعرات الحرارية المتأتية من استهلاك السكر تساهم في مشكلة السمنة، وهو عامل خطر لبعض الأمراض المزمنة مثل مرض السكري. يتجه بعض الناس إلى مواد التحلية الاصطناعية، وهي مواد كيميائية تسبب مشكلات صحية أكثر مما تعالج. هذه المواد الكيميائية تهاجم الأجهزة الحيوية في الجسم التي يمكن أن تؤدي إلى مضاعفات خطيرة بعد الاستعمال لفترة طويلة. لذلك اتجه الباحثون الى استعمال مواد تحلية طبيعية مثل نبات ستيفيا *Stevia rebaudiana* وهو عشبة معمرة لها فوائد صحية وتمتاز بحلاوة تفوق حلاوة السكروز 300 مرة وخالية من السعرات (21). وقد استعمل باحثون مسحوق ستيفيا لتصنيع الكاسترد ومنتجات لبنية أخرى وظهر أن المنتجات الجديدة قد نالت قبولا ممتازا من قبل المحكمين والمستهلكين واعتبروا المنتجات ملائمة لمرضى السكر ولطالبي تخفيف الوزن (22). مسحوق الكاسترد منتج غذائي يصنع من نشاء الذرة مع إضافة النكهة واللون، ومع أو بدون إضافة مواد البيض الصلبة ، والفيتامينات، والمعادن. يمكن أن يستعمل مسحوق الكاسترد بمثابة مكمل لتغذية الرضع، وكوجبة افطار للكثيرين ويمكن اعتباره غذاء مفضل للمرضى (23). ولأهمية الكاسترد عمل بعض الباحثين على إدخال مواد محلية في تصنيعه مثل نشأ cassava وهو نبات معروف في نايجيريا (24). كما قام باحثون آخرون بتدعيم الكاسترد بمنتجات فول الصويا ليكون المنتج ملائما لبعض المستهلكين الذين يعانون من عدم القدرة على تحمل اللاكتوز والمتحسسين من بروتينات الحليب (23) و(25).
تم دراسة مكونات التمور وقيمتها الغذائية من قبل كثير من الباحثين (26) و(27), كما درست مكونات الدبس وعصير التمر (28) وفوائد اضافتها للمنتجات الغذائية (29). فمن دراسة لـــ 16 نوع من التمور تبين انها تحتوي كمعدل من الوزن الرطب على 42.4 % رطوبة و 1.5% بروتين و0.14 % دهن و1.16 % معادن و 54.9% كاربوهيدرات. بالاضافة الى انها غنية بالبوتاسيوم (26). ويكون الكلوكوز والفركتوز حوالي 70% الى 80% من كاربوهيدرات التمور وتمتاز هذه السكريات بسهولة امتصاصها من قبل الجسم (30) و (31).
ولغرض إضافة قيمة نوعية إلى الكاسترد ودعمه بالعناصر الغذائية الموجودة في التمور وجعله أقل خطرا على مرضى السكري، تم إجراء هذه الدراسة لتصنيع الكاسترد وتحليته بالدبس وتقييمه حسيا وتغذويا مقارنة بالكاسترد التقليدي المحلى بسكر المائدة. رغم توفر الكاسترد على نطاق واسع في المحلات التجارية. الا أن هذه الدراسة أخذت بنظر الاعتبار تبسيط طريقة العمل بحيث يمكن تنفيذها منزليا مما يوفر من ميزانية العائلة، ويقدم منتجا بنوعية أفضل لكونه لا يحتوي على أي مواد حافظة، ولا نكهات صناعية ولا مواد تسبب الحساسية بالإضافة الى كونه أعلى في القيمة الغذائية، ومكوناته الأساسية متوفرة.

المواد وطرق العمل

تم شراء كافة المواد المستعملة في البحث من السوق المحلية. استعمل الحليب المعقم بطريقة UHT من انتاج شركة KDD / الكويت, والذي يحتوي 3.2% دهن و 8.5% مواد صلبة غير دهنية. ودبس تمر الزهدي (تركيز 68–70%) انتاج شركة تعليب كربلاء للاغذية المحدودة / العراق الذي يحتوي على معدل 69.33 % كاربوهيدرات كلية. وخلاصة الفانيلا انتاج شركة مازدا المحدودة / الهند. ونشاء الذرة إنتاج شركة الخليج للصناعات التحويلية / الإمارات العربية المتحدة, يحتوي 91.4% كاربوهيدرات ويعطي 381.25% سعرات. وصبغة الكركم E100 انتاج شركة Xi'an Nate Biological Technology المحدودة / الصين. ومسحوق الكاسترد التجاري انتاج شركة لافانت للمنتجات الغذائية / الاردن, مكون من نشأ وسكر وفانيلا ونكهة الموز وصبغة E102 ويحتوي 11% دهن و 73% كاربوهيدرات و5% بروتين و 0.02% ألياف ويعطي 411% سعرة حرارية.

طريقة تحضير الكاسترد بالدبس

تم إجراء تجارب أولية لتحديد أفضل نسبة خلط من الدبس والحليب والنشا وبقية المكونات من الكركم كمادة ملونة والفانيلا، وظهر ان استعمال 20غم دبس و125غم حليب و7غم نشا تكفي لإعطاء القوام المناسب والطعم الحلو المقبول، وعلى هذا الأساس تم حساب كمية المكونات اللازمة لعمل 2كغم من الكاسترد. استعمل قدر معدني لعمل الكاسترد حيث تم إضافة النشا الى الحليب المعقم على حرارة المحيط بالتدريج مع الخلط المستمر لتمام الإذابة ثم يضاف الدبس وبقية المكونات مع الخلط المستمر، ويوضع على نار هادئة مع استمرار الخلط حتى بداية ازدياد السمك. يفرغ في كؤوس زجاجية شفافة، بمقدار 20غم تقريبا لكل كأس, وتترك الكؤوس تبرد على حرارة المحيط قليلا ثم توضع في الثلاجة لمدة نصف ساعة لتقدم بعدها للتقييم الحسي في نفس اليوم.

تحضير الكاسترد التجاري (عينة السيطرة)

تم عمل الكاسترد التجاري حسب تعليمات الشركة المنتجة وذلك بإذابة محتويات علبتين (320غم) في لترين من الحليب المعقم على حرارة المحيط ثم تسخينه ببطء مع التحريك المستمر حتى التماسك, ثم يفرغ في كؤوس بمقدار 20غم لكل كأس وتترك تبرد على حرارة المحيط ثم تترك في الثلاجة لمدة نصف ساعة وتقدم للتقييم الحسي.
تم اجراء ثلاثة مكررات من الكاسترد بالدبس والكاسترد التجاري مع ترك فاصل زمني لمدة 24 ساعة بين مكرر وآخر.
استعمل 9-point hedonic scale لتقدير إحساس الفم -month feel والطعم واللون والنكهة والمظهر والقبول العام للكاسترد (32). تم اختيار ثمانية مقومين من أساتذة قسم علوم الأغذية و40 من طلبة المرحلة الرابعة من القسم بعد تدريبهم والتأكد من رغبتهم بإجراء التقييم وقابليتهم لتمييز خواص الغذاء الحسية كما وصف Iwe (33).

القيمة الغذائية

تم حساب القيمة الغذائية للمنتج الجديد بناء على تقدير مكونات الدبس حسب ما ذكرها العكيدي (34) وما مدون على علب الدبس من قبل الشركة المنتجة، بالاضافة الى تقدير المواد الصلبة الكلية الذائبة عن طريق قياس درجة بركس بواسطة جهاز رفركتومتر. تم اعتماد المعلومات المثبتة على علب الحليب المعقم من قبل الشركة المنتجة، وكذلك الحال لمكونات الكاسترد التجاري. ويبين الجدول (1) مكونات المواد المستعملة في الدراسة.

جدول (1) النسبة المئوية لمكونات المواد المستعملة بالبحث حسب ما مثبت على علاماتها التجارية

المواد	دهن	كربوهيدرات	بروتين
الحليب المعقم	3.2	5.5	3.6
دبس	0.1	69.33	0.25
نشا	0.0	91.4	0.0
كاسترد تجاري	11.0	73.0	5.0

التحليل الإحصائي

تم تحليل النتائج إحصائيا باستعمال t test calculator وهو برنامج على الشبكة العنكبوتية لشركة GraphPad (35).

حساب الطاقة

تم حساب الطاقة الحرارية (السعرات) التي يجهزها المنتج على اساس كل غرام من الكاربوهدرات او البروتينات يعطي 4 سعرات وكل غرام من الدهون يعطي 9 سعرات (36).

النتـــــائج والمناقشـــــة

التقييم الحسي:
يعرض الجدول (2) نتائج التقييم الحسي حسب الـ 9-point hedonic scale الذي يحدد أقل درجة وهي (1) لخيار (لم يعجبني نهائيا Dislike extremely) و 9 درجات لخيار (يعجبني جدا Like extremely). وهو مصمم لقياس رغبة المستهلكين للمادة الغذائية. ومن درجات التقييم الحسي يظهر أن خواص الكاسترد المصنع بالدبس كانت مقاربة جدا لخواص الكاسترد التجاري المحلى بسكر المائدة في كافة مواصفاته. أما بخصوص لون الكاسترد بالدبس فقد حصل على أدنى تقدير ويرجع ذلك الى عدم ظهور اللون الأصفر الواضح مقارنة بلون الكاسترد التجاري. ومن المعروف أن لون الدبس مع لون الكركم المضاف يعطي لون كريمي فاتح وليس أصفر مما جعل المحكمين يعطون درجة أدنى لصفة اللون, مع ذلك هي صفة مقبولة وليست مرفوضة. ومن الناحية الصحية يفضل استعمال الكركم لكونه ملون طبيعي وله فوائد طبية عديدة ويحتوي على Curcumin وهي المادة الفعالة الأساسية التي تمتاز بكونها مضاد أكسدة قوي جدا ومضاد للالتهابات (37). في حين أن الصبغة المستعملة في الكاسترد التجاري (E102) هي مركب صناعي (Tartrazine) وهو قد يسبب حساسية لبعض الأفراد (38).

جدول (2) : نتائج التقييم الحسي والتحليل الإحصائي لنوعي الكاسترد

الصفات	Group	Mean	SEM	SD	SED
اللون	كاسترد بالدبس	7.10	0.11	0.84	0.120
	كاسترد بالسكر	8.00	0.07	0.44	
المظهر	كاسترد بالدبس	7.91	0.25	0.85	0.079
	كاسترد بالسكر	8.00	0.19	0.64	
النكهة	كاسترد بالدبس	7.63	0.22	0.76	0.079
	كاسترد بالسكر	7.92	0.13	0.64	
القوام	كاسترد بالدبس	7.84	0.21	0.66	0.079
	كاسترد بالسكر	7.95	0.11	0.63	
الطعم	كاسترد بالدبس	7.90	0.15	0.72	0.071
	كاسترد بالسكر	7.82	0.29	0.61	
القبول العام	كاسترد بالدبس	7.60	0.24	0.70	0.073
	كاسترد بالسكر	7.81	0.17	0.62	

SED= standard error of difference,
SEM= Standard Error of the Mean

تبين نتائج التحليل الإحصائي عدم وجود فروق معنوية للخواص الحسية لنوعي الكاسترد.

القيمة الغذائية

تم حساب النسبة المئوية لمكونات الكاسترد المحلى بالدبس والكاسترد المحلى بالسكر من خلال مكونات المواد الداخلة في الخلطة وباستعمال البيانات المذكورة في الجدول رقم (1). يبين الجدول رقم (3) النسب المئوية لمكونات نوعي الكاسترد والسعرات الحرارية الكلية لهما.

جدول (3): النسبة المئوية لمكونات الكاسترد المحلى بالدبس والكاسترد التجاري المحلى بالسكر

المنتج	كربوهيدرات	بروتين	دهن	السعرات
كاسترد بالدبس	17.855	2.993	2.644	107
كاسترد بالسكر	14.553	3.787	4.288	91

يظهر من الجدول (3) تقارب تركيب المنتوجين في المكونات الغذائية الأساسية الى حد كبير. مع ذلك فان الكاسترد المحلى بالدبس يتفوق كثيرا على الكاسترد التجاري المحلى بالسكر. فوجود الدبس يعطي دعما تغذويا ممتازا بسبب محتوى الدبس من المعادن وخاصة الكالسيوم والحديد والزنك مما يضيف توازن صحي للمعادن في الجسم (39). ويعتبر الدبس خلاصة لفاكهة التمر يحسن وجوده طعم الحلويات. وينسجم هذا مع ما وجده باحثون في إمكانية تحسين طعم الحلويات التي تدخل الصويا في مكوناتها وذلك بإضافة خلاصة بعض الفواكه (40). وان استعمال الدبس يجنب المستهلك الأضرار الصحية الناتجة عن استهلاك السكروز. فقد أصدرت منظمة الصحة العالمية توجيها جديدا يوصي البالغين والأطفال بتخفيض استهلاكهم اليومي من السكريات الحرة إلى أقل من 10% من إجمالي استهلاكهم للطاقة، لأن ذلك يقلل من خطر زيادة الوزن والسمنة وتسوس الأسنان، ولا يشير التوجيه إلى السكريات الموجودة في الفواكه والخضروات الطازجة وتلك الموجودة طبيعيا في الحليب، لأنه لا يوجد ما يفيد بأن استهلاكها يترك آثارا ضارة (41).

الاستنتاجات والتوصيات

يمكن اعتماد هذه الدراسة بالإضافة الى الدراسات المماثلة كأساس للتوجه الى مزيد من البحوث في مجال إدخال الدبس في إنتاج أغذية أخرى. ونوصي بأن تتبنى مصانع وشركات تصنيع الأغذية تطوير تصنيع مسحوق الكاسترد الخالي من السكروز مع الإشارة في التعليمات الملصقة على علب المنتج الى استعمال الدبس في تحلية الكاسترد لغرض الحصول على فوائد صحية إضافية.

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