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ان البحوث المنشورة في المجلة الطبية البيطرية العراقية متاحة الكترونيا ضمن موقع كلية الطب البيطري

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تعليمات النشر للباحثين

أولاً: ان المجلة الطبية البيطرية العراقية، مجلة علمية تصدر عن كلية الطب البيطري - جامعة بغداد - العراق. تعنى المجلة بنشر البحوث الاصيلة والقيمة ذات العلاقة بمختلف ميادين الطب البيطري ، الانتاج الحيواني ، علوم الحياة ، تقرير الحالات المرضية ، الملاحظات البحثية والمقالات الصغيرة باللغتين العربية والانكليزية.

ثانياً: يشترط في البحث المقدم للنشر ان لايكون قد نشر أو قبل للنشر في اي مجلة أخرى سابقاً و يتم تقييم البحث من ذوي الاختصاص قبل قبولها للنشر ويصبح ملكاً لها، ولا يحق للباحث سحب الاوليات في حالة رفض نشر البحث.

ثالثا: يقدم البحث من قبل الباحث الى سكرتارية هيئة تحرير المجلة الطبية البيطرية العراقية- كلية الطب البيطري- جامعة بغداد طلبا لتقييم ونشر بحثه في المجلة مصحوباً بثلاثة نسخ من البحث (ملونة في حال وجود اشكال او صور ملونة في متن البحث) مع مبلغ خمسة و عشرون الف دينار كدفعة من تكاليف تقييم ونشر البحث غير قابلة للرد ، او تقديم البحث من خلال البريد الالكتروني (iraqijvm@yahoo.com) ، وبعد تقييمه بالايجاب يقدم الباحث نسختين من البحث المعدل بعد التقييم ومحفوظة على قرص مدمج (CD)مصحوباً بباقي رسوم النشر المذكورة في الفقرة سادساً.

رابعا:- يطبع البحث وفق نظام ((*Microsoft Word 2007*)) وبمسافات مفردة بما في ذلك الجداول والاشكال والمصادر. يستعمل ورق بحجم A4 و يطبع بخط نوع Times New Roman لكل فقرات البحث وتكون أبعاد مسافة الطبع 15سم (العرض) ×24سم (الطول) وترقيم الصفحات وسط أسفل الصفحة.

يكتب عنوان البحث باللغتين العربية و الانكليزية واسم الباحث/الباحثين و مكان العمل مشفوعة بوسيلة الاتصال (رقم موبايل او بريد الكتروني) في ورقة منفصلة عند تقديم البحث لاول مرة مع مراعاة ادراج عنوان البحث ايضا باللغتين قبل الخلاصة العربية و الانكليزية. بعد تقييم البحث من قبل المقومين العلميين يرسل البحث المصحح من قبل الباحث و يطبع حسب التعليمات التالية: خط العنوان العربي والانكليزي غامق (16) تحته اسم الباحث/الباحثين بحجم (14) غامق تحته عنوان عمل كل باحث بحجم (12) عادي. خط العناوين الثانوية غامق (14) وسط الصفحة وكذلك خط متن البحث عادي (12) وترسل مع البحث في حالة وجود صور ملونة – الصور الاصلية.

خامسا: تنظيم فقرات البحث بفاصل مسافة سطر واحد بين العناوين الفرعية كما يلي:

العنوان: تحتوي الصفحة الأولى على عنوان البحث في الوسط تتبعها بفاصل سطر واحد اسم الباحث او الباحثين و عناوينهم وعنوان البريد الإلكتروني للباحث ألاول ورقم الهاتف المحمول.

الخلاصة: لاتزيد على 200 كلمة وتبين فكرة واضحة ومختصرة عن طريقة العمل والنتائج والاستنتاجات ويكتفي بذكر الارتفاع او الانخفاض معنوياً **بدون ذكر القيم**، تأتي الخلاصة الإنكليزية بعد الخلاصة العربية للبحوث المكتوبة باللغة العربية وبالعكس بالنسبة للبحوث المكتوبة باللغة الإنكليزية وتختم الخلاصة الانكليزية بالكلمات المفتاحية (Keywords).

المقدمة: تشمل مختصر الخلفية العلمية لموضوع البحث وتدرج فيه المعلومات الحديثة والهدف الذي من أجله أجري البحث.

المواد وطرائق العمل: تذكر معلومات وافية وتدعم بمصادر كافية حديثة وكذلك تدرج تعليمات التعامل مع الحيوانات والعناية بها، وتستعمل وحدات القياس المتري والعالمي في البحث. ويذكر البرنامج الاحصائي والطريقة الاحصائية المستعملة في تحليل البيانات وتعرف الرموز والمختصرات والعلامات الأحصائية المعتمدة للمقارنة.

النتائج و المناقشة: تعرض بدقة ويجب أن تقدم الاشكال والجداول والرسومات البيانية معلومات وافية مع عدم إعادة المعلومات في متن البحث، وترقم حسب ورودها في متن البحث و يشار الى الأهمية العلمية للنتائج ومناقشتها مع دعمها بمصادر حديثة. وتذكر الاستنتاجات بشكل مختصر في نهاية المناقشة.

الجداول و الاشكال: يشار الى الجداول و الاشكال بالارقام حسب تسلسل ورودها في متن البحث وتكون بارقام و حروف واضحة و لايزيد حجم الجدول او الشكل قياسات الورقة وتوضع الجداول كافة ، الاشكال كافة ، المخططات البيانية كافة ....الخ بعد نهاية فقرة النتائج و المناقشة وحسب تسلسلها.

الشكر و التقدير: تذكر الجهات المساندة والتي قدمت المساعدات المالية والعلمية والأشخاص الذين ساهموا في البحث ولم يتم إدراجهم كباحثين.

المصادر:

أ. يتم الاشارة الى المصادر في متن البحث بواسطة الأرقام وتسلسل حسب ذكر المصدر وعند وجود أكثر من مصدر واحد ترتب الأرقام وفق التسلسل الزمني للمصادر وتوضع بشكل أس مرفوع (Superscript). وتذكر ملخصات عناوين المجلات العلمية كما وردت تعليمات النشر الحالية.

ب. ترتب المصادر في نهاية البحث بأرقام وحسب تسلسل ظهورها في متن البحث بغض النظر عن الحروف الهجائية للباحثين وتعتمد الاسس التالية عند الكتابة.

اذا كان المصدر مجلة : يكتب الاسم الاخير للباحث (اللقب) ثم الحروف الاولى من أسمه ألاول والثاني ، السنة بين قوسين ،عنوان البحث ،أسم المجلة بالأختصار الشائع والمتبع علمياً ،المجلد ،العدد و رقم الصفحة مثال:

Williams, JC. (1997). Antiparasitic treament, current status and future. Vet. Parasitol., 127:61-77.

اذا كان المصدر كتاباً : ألاسم الأخير للمؤلف (اللقب) ثم الحروف الاولى من الأسم الاول والثاني ، السنة بين قوسين ، عنوان الكتاب ، الطبعة ، مكان النشر ، دار النشر و رقم الصفحات مثال:

Ingrkam, JL. and Ingrahan, CA. (2000). Introduction. In: Text of Microbiology. 2nd ed. Anstratia, Brooks Co. Thompson Learning, Pp:55.

أما اذا كان المصدر فصلاً من كتاب يذكر أسم المؤلف أو أسماء المؤلفين والسنة بين قوسين ، عنوان الفصل ، عنوان الكتاب كل كلمة تبدا بحرف كبير (Capital) ، أسم او أسماء المحررين ، مكان او جهة النشر و رقم الصفحة مثال:

Andersen, RM. (1998). Epidemiology of parasitic infections. In: Topley and Wilsons Infections. Collier, L. ; Balows, A. and Jassman, M. (Eds.), Vol. 5, 9th ed. Arnold a Member of the Hodder Group, London, Pp: 39 – 55.

أذا كان المصدر رسالة ماجستير أو اطروحة دكتوراه تكتب كما يلي . مثال :-

الاسم الاخير لصاحب الرسالة او الاطروحة ثم الاسم الاول و الثاني ،(السنة بين قوسين).، عنوان الرسالة أو الاطروحة ، الكلية، الجامعة، رقم الصفحة.

Kashifalkitaa, HF. (2008). Effect of bromocriptine and dexamethasone administration on semen characteristics and certain hormones in local male goats. PhD Thesis, College of Veterinary Medicine, University of Baghdad, Pp:87 – 105.

ملاحظة: يجب ان تكون نصف المصادر على الأقل منشورة بعد سنة 2002 ولاتتجاوز نسبة الكتب عن 10% من المصادر.

سادساً: تقبل البحوث بعد تقيمها وقبولها من قبل السادة المقيمين و بعد دفع أجور النشر البالغة 100000 دينار اذا كان الباحث بدرجة استاذ او 75000 دينار اذا كان الباحث بدرجة استاذ مساعد او 50000 دينار اذا كان الباحث بدرجة مدرس او مدرس مساعد من قبل الباحثين الذين وردت أسمائهم في البحث. وعشرة ألاف دينار ان زاد البحث على ستة أوراق (12 صفحة) واضافة 15000 دينار عن كل صفحة تحوي شكل او اكثر ملون.

سكرتارية تحرير المجلة الطبية البيطرية العراقية

العراق – بغداد – العامرية – كلية الطب البيطري – مكتب بريد الداودي / الكرخ ص . ب 28061

او جامعة بغداد – كلية الطب البيطري http://covm.uobaghdad.edu.iq

او البريد الالكتروني الخاص بالمجلة iraqijvm@yahoo.com

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المجلة الطبية البيطرية العراقية 36 (1): 1 –7 ،2012.

تأثير الاصابة بمرض الحمى القلاعية الـ F.M.D في الاداء التناسلي لثيران الهولشتاين لمركز التلقيح الاصطناعي في العراق

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الخلاصة

اجريت الدراسة في مركز التلقيح الاصطناعي قي العراق ، تم دراسة تاثير الاصابة بمرض الحمى القلاعية في بعض صفات السائل المنوي لـ 14 ثور هولشتاين مستورد متوزعين على ثلاثة مجاميع مختلفة الكفاءة التناسلية (عالية الكفاءة ، متوسطة الكفاءة وضعيفة الكفاءة) . اظهرت النتائج بان الاصابة بالمرض كان له تاثير معنوي (P < 0.05) على اغلب صفات السائل المنوي ،الحركة الجماعية ، الفردية وتركيز النطف / مل ، حيث حصل تدهوروبشكل معنوي (P < 0.05) في تلك الصفات خلال الشهرين بعد الاصابة بالنسبة للثيران العالية الكفاءة وخلال اربعة اشهر بالنسبة للثيران المتوسطة الكفاءة و جميع الاشهر للثيران الضعيفة ، كما ان المجموعتين الاولى والثانية عادت الى كفائتها التناسلية التي كانت عليها قبل الاصابة . لكن لم يلاحظ اي تاثير معنوي لتلك الاصابة على حجم السائل المنوي ولجميع المجاميع . تستنتج الدراسة بعدم جمع السائل المنوي من الثيران التي تتعرض للاصابة بهذا المرض الا بعد شفائها تماما ، اي بعد شهرين بالنسبة للثيران عالية الكفاءة وبعد اربعة اشهر للثيران متوسطة الكفاءة وتنبذ الثيران ضعيفة الكفاءة ولحظة اصابتها بالمرض لتجنب الفشل في القدرة الاخصابية للنطف عند استخدامها لاغراض التلقيح الاصطناعي فضلا عن عدم وجود جدوى اقتصادية من الاستمرار بتربيتها.

كلمات مفتاحية: الحمى القلاعية, الاداء التناسلي, الثيران, التلقيح الاصطناعي.

The effect of Foot and Mouth disease on reproductive performance of Holstein bulls in Artificial Insemination Center of Iraq

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Summary

This study was carried out in Artificial Insemination Center of Iraq to revealed FMD disease effect on some seminal attributer parameters of 14 imported Holstein bulls divided to three groups according to different reproductive efficiency (four High, five medium and five weak). Results showed that FMD disease had significant ($P < 0.05$) adverse effect on most seminal attributer parameters, mass, individual motility and sperm concentration / ml during post disease in first of two, four, all months of high, medium and weak semen quality bulls respectively .but semen volume didn't influenced significantly with this disease. So semen collection should be suspended until resume normal fertility of sperm, after two, four month of high and medium bulls respectively, and must be revealed weak bulls when disease happen to avoid the failure of conception from artificial insemination and there is no economic benefit to use or keep weak bulls.

Key words: FMD, reproductive performance, Artificial Insemination, Bull.

المقدمة

ان التلقيح الاصطناعي من الطرق المفضلة والسريعة في تحسين التراكيب الوراثية للحيوانات وتنفيذ التقنات الحديثة في تطوير وتحسين الثروة الحيوانية بشكل عام والأبقار بشكل خاص (1) . كما له دور في السيطرة على الامراض (2) . ولغرض نجاح التلقيح الاصطناعي فلابد من ان يكون هناك ثيران لها القابلية على انتاج نطف

قادرة على الاخصاب وتحتفظ هذه النطف بتلك القابلية بعد التخفيف والحفظ (4,3) النطف تتأثر بعوامل عدة منها العوامل الوراثية للحيوان (5) والعوامل لادارية واهمها الحالة الصحية حيث الاصابة بالامراض تؤثر سلبا على الحيوان وبالتالي قابلية الذكر الانتاجية للنطف (6 و7). واشار Yates (8) ان السبب الرئيسي لتلك الاصابات اما تكون اسبابها بكتريا او فايروسات او فطريات والتي تصيب بصورة مباشرة او غير مباشرة الجهاز البولي والتناسلي ، وبالتالي يكون السائل المنوي وسيلة لنقل الامراض (9و10) فان الرعاية الصحية والادارية لتلك الثيران واجراء التحصينات اللازمة ضد جميع الامراض من الاولويات المهمة التي يقوم بها مركز التلقيح الاصطناعي في العراق ،لان اصابة الذكر باي مرض حتى بعد علاجه ، يؤثر سلبا على عمره التناسلي (11) . ويعتبر مرض الحمى القلاعية F.M.D من أهم الأمراض التي تؤثر على اقتصاديات الإنتاج الحيواني (12) ، لذلك فقد اهتمت الدول بهذا المرض فعند الاصابة به لسبب او لاخر سوف يكون له تاثيرات سلبية على الحالة الصحية بشكل عام والحالة التناسلية بشكل خاص للحيوان (1). وان ثيران مركز التلقيح الاصطناعي في العراق وبالرغم من وجود برنامج وقائي ضد جميع الامراض ومنها مرض الحمى القلاعية لكن تعرضت للاصابة بهذا المرض وعليه وضع لها برنامج متكامل لتقليل شدة تاثير هذا المرض ، وتم ايقاف عملية جمع السائل المنوي ولمدة شهر، لكن بعد شفائها ولغرض التعرف على حدوث اي تاثير سلبي بعد الاصابة على كفائتها التناسلية وهل هذا التاثير وقتي ام دائمي واذا كان وقتي لاي مدى بعد الاصابة ،كما هدفت الدراسة الى التعرف الى العلاقة مابين الكفاءة التناسلية للثيران قبل الاصابة ودرجة تاثير تلك الاصابة عليها، ومن جدوى استخدام تلك الثيران او البعض منها لانتاج السائل المنوي لاغراض التلقيح الاصطناعي.

المواد وطرائق العمل

اجريت هذه الدراسة في مركز التلقيح الاصطناعي / ابي غريب حيث خضعت للدراسة 14 ثور هولشتاين فريزيان جميعها مستوردة من استراليا . وضعت تحت ظروف رعاية وادارة موحدتين ،.جميع الثيران مستخدمة بانتظام لجمع السائل المنوي بواسطة المهبل الاصطناعي مرة بالاسبوع من كل ثور، لغرض انتاج السائل المنوي المجمد حيث تم جمع (648) قذفة ، علما بان بعد كل عملية جمع يتم اجراء الفحوصات التالية : حجم السائل المنوي ، الحركة الجماعية للنطف (13) ، الحركة الفردية (14) تركيز النطف / مل (15) ، علما بان تلك الثيران تم تلقيحها ضد جميع الامراض ، لكن تعرضت للاصابة بمرض الـ F.M.D وتم ايقاف عملية جمع السائل المنوي لجميع الثيران وبعد شفائها تماما استأنفت عملية جمع وتقيم السائل المنوي شهريا ولمدة سنة كاملة. قسمت الثيران الى ثلاثة مجاميع ، حيث ان ثيران مركز التلقيح الاصطناعي عبارة عن ثلاثة مجاميع : المجموعة الاولى (عالية الكفاءة 4 ثيران) ، المجموعة الثانية (متوسطة الكفاءة 5 ثيران) و المجموعة الثالثة (ضعيفة الكفاءة 5 ثيران) . كما تم تحليل البيانات احصائيا حسب (16) .

النتائج والمناقشة

يتضح من الجدول (1) الذي يبين تاثير الاصابة بمرض الحمى القلاعية على صفات السائل المنوي للثيران عالية الكفاءة ، لوحظ بان الاصابة بهذا المرض ادى الى تاثيرسلبي في السائل المنوي لتلك الثيران حيث حصل انخفاض وبشكل معنوي ($P < 0.05$) في الحركة الجماعية ، الحركة الفردية وتركيز النطف (10^6نطفة/مل) خلال الشهر الاول والثاني بعد الاصابة حيث كانت قبل الاصابة (71.66 ،70.22 ،1250.44) ، واصبحت بعد الاصابة في الشهر الاول (50.33 ،43.03 ،833.45) والشهر الثاني (56.66 ،61.67 ،916.67) على التوالي. لم يتاثر حجم السائل المنوي بشكل معنوي بتلك الاصابة ، كما لوحظ من الجدول (1) ان هذه المجموعة عادت الى كفاءتها التناسلية بعد الشهر الثاني حيث سجلت كل من الحركة الجماعية ، الفردية وتركيز النطف (10^6نطفة/مل) زيادة معنوية ($P < 0.05$) في الشهر الثالث ، الرابع . الخامس ، السادس والسابع لغاية نهاية فترة البحث في الشهر الثاني عشر وعلى التوالي ، لكن يتضح من الجدول (1) بان حجم السائل المنوي لتلك المجموعة لم يتاثر معنويا بالاصابة . أما بالنسبة لمجموعة الثيران متوسطة الكفاءة الجدول (2) ان تلك الثيران تاثرت بالاصابة واستمر هذا التاثير حتى الشهر الرابع من الاصابة أذ كانت الحركة الجماعية ، الفردية والتركيز (106 نطفة/مل) قبل الاصابة (63.77 ، 1083.33،66.81) وانخفضت معنويا ($P < 0.05$) بعد الاصابة في الشهر الاول ، الشهر الثاني ، الشهر الثالث والشهر الرابع وعلى التوالي ، لكن حصل التحسن في تلك الصفات بعد الشهر الرابع وكما موضح في الجدول (2) أذ حصلت زيادة معنوية ($P < 0.05$) في الحركة الجماعية ، الحركة الفردية وتركيز النطف (106 نطفة/مل)، خلال الشهر الخامس . السادس والسابع لغاية نهاية فترة الدراسة وعلى التوالي .اما بالنسبة لحجم السائل المنوي جدول (2) لتلك الثيران كان متذبذب وغير واضح التاثر .

جدول (1) تاثير الاصابة عالية مرض الحمى القلاعية في بعض صفات السائل المنوي لثيران الهولشتاين الكفاءة

الاصابة بمرض الحمى القلاعية		حجم السائل المنوي (مل)	الحركة الجماعية (%)	الحركة الفردية (%)	تركيز النطف /مل ($10^6 \times$)
قبل الاصابة *		2.66 ± 10.66 a	5.76 ± 71.66 a	5.00 ± 70.22 ab	80.22 ± 1250,44 a
بعد الاصابة (شهر)	1	2.08 ± 8.88 a	5.33 ± 50.33 b	4.33 ± 43.03 c	77.13 ± 833.45 c
	2	1.55 ± 8.16 a	8.46 ± 56.66 b	4.06 ± 61.67 c	76.67 ± 916.67bc
	3	2.66 ± 9.66a	7.00 ± 70.02 a	6.09 ± 66.89 b	86.07 ± 1016.88 ab
	4	2.10 ± 10.01a	5.77 ± 70.77 a	4.55 ± 65.77 b	92.00 ± 1083.33 a
	5	1.50 ± 9.50 a	6.00 ± 68.77 a	7.55 ± 71.55a	70.04 ± 1110.89 a
	6	2.50 ± 8.50 a	7.21 ± 70.33 a	6.33 ± 66.07 b	104.33 ± 1077.33 a
	12 -7	1.66 ± 9.40 a	4.42 ± 69.78a	3.00 ± 72.55 a	96.33 ±1198.4a

الارقام تعني المتوسط ± الخطأ القياسي ، الاحرف المختلفة تدل على وجود فرق معنوي ($P < 0.05$) ضمن العمود الواحد . * اخذت القيم من السجل الخاص بالمركز.

جدول (2) تاثير الاصابة بمرض الحمى القلاعية في بعض صفات السائل المنوي لثيران الهولشتاين متوسطة الكفاءة

الاصابة بمرض الحمى القلاعية		حجم السائل المنوي (مل)	الحركة الجماعية (%)	الحركة الفردية (%)	تركيز النطف / مل ($10^6 \times$)
قبل الاصابة *		1.30 ± 9.22 a	4.00 ± 63.77 a	5.40 ± 66.81 a	70.20 ± 1083.33 a
بعد الاصابة (شهر)	1	2.00 ± 4.22 e	8.00 ± 30.88 d	4.80 ± 48.43 d	90.33 ± 533.88 d
	2	1.30 ± 7.94 abc	6.04 ± 40.66 c	5.00 ± 53.55 d	66.30 ± 766.72 bc
	3	2.10 ± 5.44 de	4.09 ± 44.03 c	8.22 ± 48.22 d	83.90 ± 853.34 b
	4	2.00± 7.29abcd	5.66 ± 45.66 c	5.09 ± 56.99 cd	90.00 ± 730.99 c
	5	1.90 ± 9.11 a	6.22 ± 60.01 ab	4.90 ± 61.02 abc	77.30 ±1000.76 a
	6	1.60 ± 6.44 cd	5.03 ± 55.77 b	6.10 ± 59.82 abc	67.65 ± 1087.65 a
	-7 12	2.80 ± 8.11ab	6.44 ± 60.22 ab	4.08 ± 64.11 ab	82.00 ± 1055 .44 a

الارقام تعني المتوسط ± الخطأ القياسي ، الاحرف المختلفة تدل على وجود فرق معنوي ($P < 0.05$) ضمن العمود الواحد * اخذت القيم من السجل الخاص بالمركز.

مجموعة الثيران ضعيفة الكفاءة وكما واضح من النتائج في الجدول (3) بان هذه المجموعة اثرت فيها الاصابة وبدرجة كبيرة اذ حصل انخفاض معنوي ($P < 0.05$) في الصفات : الحركة الجماعية ، الفردية وتركيز النطف

(10^6نطفة/مل) في جميع الاشهر بعد الاصابة ماعدا الشهر الخامس وفقط في الحركة الجماعية والفردية لم تتأثرتلك الثيران بالاصابة ، حيث يظهرمن الجدول نفسه بان الحركة الجماعية ، الفردية وتركيز النطف (10^6نطفة/مل) قبل الاصابة (58.24 ،60.34 ،753.88) وانخفضت معنويا ($P < 0.05$) بعد الاصابة في الشهر الاول ،الثاني ، الثالث ، الرابع ، الخامس (تركيز النطف فقط 365.23) ،السادس والشهر السابع ولغاية الثاني عشر على التوالي .

جدول (3) تاثير الاصابة بمرض الحمى القلاعية على بعض صفات السائل المنوي لثيران الهولشتاين ضعيفة االكفاءة .

الاصابة بمرض الحمى القلاعية		حجم السائل المنوي (مل)	الحركة الجماعية (%)	الحركة الفردية (%)	تركيز النطف / مل ($10^6 \times$)
قبل الاصابة *		1.33± 6.44 a	6.00± 58.23 a	3.66 ± 60.34 a	60.30 ± 753.88 a
بعد الاصـــــابة (شهر)	1	1.11 ± 5.33b	4.40 ±35.45 c	4.32 ± 40.55 b	68.40 ± 533.56 bc
	2	2.55 ± 5.49 b	3.12 ±38.12 bc	5.08 ± 35.66 c	70.00 ± 634.22 b
	3	1.80 ± 6.78 ab	4.05± 40.23 b	5.10 ± 43. 02 b	60.01 ± 577.78 bc
	4	1.22 ± 7.00 a	3.88 ± 39.83 bc	6.22. ±41.22 b	92.33 ± 498.33 c
	5	1.88 ± 7.02 a	4.32 ± 55.33 a	5.44 ± 60.01a	60.55 ± 365.23 d
	6	2.01 ± 6.32 ab	6.04 ± 30.22 d	6.50 ± 30.06 d	86.77 ± 300.55 d
	7-12	0.50 ± 7.00 a	3.03 ± 35.55 c	4.06± 40.00 b	52.99 ± 342.11 d

الارقام تعني المتوسط ± الخطأ القياسي ، الاحرف المختلفة تدل على وجود فرق معنوي ($P < 0.05$) ضمن العمود الواحد * اخذت القيم من السجل الخاص بالمركز.

اما بالنسبة الى حجم السائل المنوي لهذه المجموعة وكما واضح في الجدول (3) لم يتأثر معنويا بالاصابة . عند الرجوع الى الجداول (1،2،3) نلاحظ بان هناك تاثير سلبي للاصابة بمرض الـFMD في الكفاءة التناسلية لجميع الثيران ، واشار(11) بان الذكر عند اصابته باي مرض سيكون هذا المرض له تاثير سلبي في الاداء التناسلي ، واكد ذلك كل من (17 ،18 ،19 و20) , كما وجد (12) بان التطعيم ضد بعض الامراض ومنها FMD يحصل ارتفاع لدرجة حرارة الجسم وبالتالي تتاثر صفات السائل المنوي وتمتد لاشهر عدة . وبالتالي فان الاجهاد الحراري الناتج من الاصابة بالامراض او البيئة كلاهما يؤثران على عملية تكوين النطف وبالتالي يؤدي ذلك الى قلة حركة النطف وتركيز النطف (23,22,21) وارتفاع معنوي في تشوهات النطف (26,25,24) ،كما اشار كل من (27 و28) بان عملية تكوين النطف (Spermatogenesis) عندما تتاثر لابسط الظرورف تكون حيوية النطف خارج الجسم ضعيفة . ان عملية تكوين النطف وانتاج الهرمون الذكري كلاهما ذو حساسية عالية لاي زيادة بسيطة في درجة حرارة الخصية وكيس الصفن (29 ،30 و31) ، وبطبيعة الحال ان اي مسبب كان مرضي او بيئي يودي الى ارتفاع درجة حرارة جسم الحيوان فان هذا الارتفاع يؤثر سلبا بشكل مباشر او غير مباشر على كفائة السائل المنوي وقابليته على الاخصاب (23، 30 ،31 ،32 ،33) ، ومن المعلوم بان مرض الـ FMD الذي يعد من الامراض الاكثر تاثيرا في كفاءة الجسم الوظيفية ومنها التناسلية (34 ،35 و36) . يظهرمن الجداول (3,2,1) بان هناك اختلاف في درجة تاثير الاصابة في المجاميع الثلاثة من الثيران وان تاثيره كان الاكثر على الثيران ضعيفة الكفاءة والاقل على عالية الكفاءة ، وذكر كل من (37 و38) بان العوامل التي تؤثر سلبا في صفات السائل المنوي يكون اكثر تاثيرا في الثيران ضعيفة الاداء ، وهذا قد يرجع الى ان الحيوانات عالية الكفاءة تمتلك عدد اكبر من الخلايا النشطة و الفعالة المسؤولة عن انتاج النطف وعند حصول تلف في البعض منها نتيجة لاي مؤثر سلبي

،يكون هناك خلايا لها القابلية على تعويض هذا التاثير، او الخلايا التي تاثرت سلبا لها القابلية على تجديد قابليتها ذاتيا على الانتاج عكس خلايا الثيران التي هي بالاساس ضعيفة الاداء . اما بالنسبة لحجم القذفة وعدم تاثرها بالاصابة للمجاميع الثلاثة من الثيران هذا يعني بان الغدد التناسلية لم تتأثر بالاصابة ، لان 90% من البلازما المنوية تنتج من الغدد التناسلية (1) ، واشار كل من (27 و28) بان عملية تكوين النطف تحصل في درجة حرارة اقل من الجسم (2-3مº) وبالتالي فان عملية تكوين النطف تتاثر بدرجة عالية عند حدوث اي تغير في درجة حرارة الجسم مقارنة بعملية انتاج البلازما المنوية التي تنتج من قبل الغدد التناسلية الموجودة داخل جسم الحيوان ، كما اكد (39 ،40 و41) بان العوامل التي تؤثر سلبا في صفات السائل المنوي ليس بالضرورة ان تؤثر سلبا في حجم السائل المنوي ،تستنتج الدراسة بان التطعيم ضد مرض الـ FMD والذي سبب ارتفاع بدرجة حرارة جسم الثيران وبالتالي سبب تاثيرا سلبيا في جميع صفات السائل المنوي للثيران لكن حجم السائل المنوي لم يتأثربذلك.

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تأكيد التشخيص السريري لبعض امراض النقص الغذائي في المعز المحلي والشامي في مدينة بغداد

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الخلاصة

درست العلاقة بين العلامات السريرية وبعض المعايير الدمية والكيموحيوية في 126 حالة من المعز المحلي والشامي ومن كلا الجنسين شخصت تعاني من امراض النقص الغذائي من بين 230 فحصت ،وشمل الفحص السريري بالاضافة الى الفحص العياني للحيوان قياس درجة الحرارة ، تردد النفس والنبض وفحص الاغشية المخاطية والجلد والكسوة .في حين شمل فحص عينات الدم قياس عدد كريات الدم الحمراء ، كمية خضاب الدم ونسبة حجم خلايا الدم المضغوطة وكذلك تم قياس كمية النحاس ، المغنيسوم ، الفسفور والبوتاسيوم في مصل المعز .كان معدل درجات الحرارة في هذه المعز اقل منه في الحيوانات السليمة ، بينما كانت معدلات ترداد التنفس والنبض اعلى .واظهرت نتائج فحص الدم انخفاض في معدلات كريات الدم الحمراء وخضاب الدم وحجم خلايا الدم المضغوطة في المعز الذي يعاني من النقص الغذائي وكان الانخفاض في معدل كريات الدم الحمراء اشد في المعز المحلي . كذلك اظهرت النتائج وجود انخفاض في معايير كل من النحاس والمغنيسيوم والفسفور والبوتاسيوم عن معدلاتها الطبيعية في المعز المصاب .وكانت نسبة نقص النحاس هي الاعلى بين العناصرالمستهدفه في الدراسة تلاه عنصر الفوسفور ثم المغنيسيوم ثم البوتاسيوم. وكانت نسبة حالات النقص الغذائي عالية في ماعز محافظة بغداد , وفي العرق الشامي اعلى منه في المعز المحلي.

مفاتيح الكلمات:النقص الغذائي,المعاير الدميه و الكيموحيويه المعز,المعز النحاس الفسفور.

Conformation of the clinical diagnoses of some malnutrition diseases in local and Shammi goats in Baghdad province

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Summary

The relationship between the clinical singes and some of the hematological and biochemical values in 126 cases of local breed and Shammi breed goats (from both sexes) diagnosed as cases of malnutrition from 230 goats examined .The clinical examination include (beside the general inspection and case history) body temperature, respiratory and pulse rates, mucus membranes skin and coat. Examination of the blood samples included RBCs count, Hb concentration, PCV % and values of cupper, magnesium, phosphorus, and potassium. The results showed that the mean body temperature of these goats was lower than in normal goats, while the respiratory and pulse rates were higher .Examination of blood revealed decrease in the means of RBCs counts ,Hb concentration and PCV % in the clinically diagnosed cases , and the decrease was more sever in local goats . However the results indicated lower values of cupper, magnesium, phosphorus, and potassium in comparison with normal values in goats. The ratio of cupper deficiency was the highest followed by phosphorus, magnesium and potassium and the ratio of malnutrition was high in Baghdad province. It was in Shammi breed higher than in local breed.

Key words: goat malnutrition, goat hematological and biochemical, RBCs,Hb.

المقدمة

بينت (1) ان مسببات النقص الغذائي لدى الكائن الحي متعددة ، أهمها قلة العناصر المعدنية الموجودة في التربة والماء بالإضافة الى الاستهلاك البدني للجسم لهذه العناصر في بناء الخلايا. وذكر(2 و3)ان عنصر النحاس له دور في بناء الأنسجة المتقرنة للجسم وحالة الكسوة والمناعة وكعامل مساعد لعمل العديد من الانزيمات والنقص فيه يؤدي الى حالة الترنح المستوطن ، ان البوتاسيوم يحتاجه الجسم للتوازن الحامضي – القاعدي ،ويعد جزءاً مهماً في العديد من انزيمات الجسم ،وعنصر المغنيسيوم مهم للجهاز العصبي للمعز. واشار(4) الى اعتماد هذا العنصر كاحد العناصر الرئيسة للفعاليات الحيوية بالجسم ،حيث يؤدي نقصه الى حالة تصلب الحشائش (Grass Tetany) . فيما أشار(5) الى دور عنصر الفسفور في وظائف الجسم ،حيث يعد الفسفور ضروري لإنتاج الحليب ولنمو الجسم الهيكلي للمعز ويؤدي نقصه الى قلة الشهية (Anorexia) وقلة النمو. وأضاف (6) بان هنالك عوامل معينة تؤدي الى النقص الغذائي للعنصر في الجسم بالرغم من توفره بكميات كافية في علف الحيوان ،ومن هذه العوامل وجود عناصر اخرى تمنع او تقلل من امتصاص العنصر بالجسم.

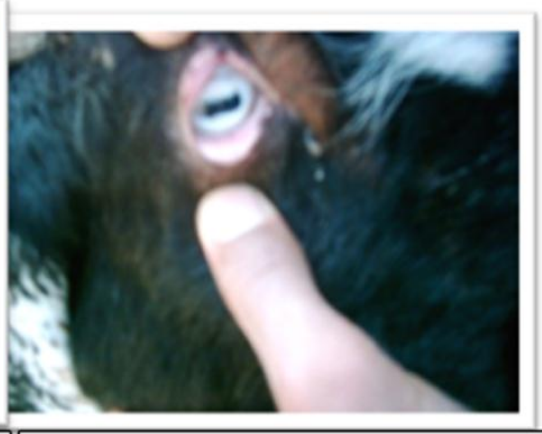
لقد أعتمد التشخيص السريري غالبا في تشخيص امراض النقص الغذائي دون تاكيد ذلك بقياس معايير العناصر المسببة لتلك الاعراض السريريه ومن هنا جاءت فكرة اجراء هذا البحث للربط بين العلامات والاعراض السريريه ومستوى العناصر الغذائية النادره كيموحيويا والمرتبطه بتلك العناصر في المعز المحلي والشامي بعد ان قام (7) بتثبيت معايير صورة الدم وبعض العناصرفي المعز السليم .

المواد وطرائق العمل

تم فحص 230 من المعز المحلي والشامي في منطقة بغداد اظهرت 126 منها علامات واعراض سريريه لامراض النقص الغذائي على ضوء الفحص العام وقياس درجات الحراره وترداد التنفس والنبض وفحص الاغشيه المخاطيه وفحص الجلد والكسوة باعتماد طريقة (8). ومن اجل ان يتم تأكيد انها تعاني من امراض النقص الغذائي تم الحصول على عينات من الدم لقياس عدد كريات الدم الحمراء وخضاب الدم وحجم خلايا الدم المضغوطة كما في تقنية (9) . وتم عزل مصل الدم طبقا (6) وتم قياس كمية النحاس وذلك بوحدة قياس µ/ lmol باستخدام جهاز قياس شدة الضوء بالامتصاص الذري (Atomic Absorption) باعتماد طريقة (10) وقدرت كمية المغنيسيوم بالمصل وبوحدة قياس mmol/l حسب طريقة (Calmagite) وذلك باستعمال جهاز الانكسار الضوئي الكهربائي (11).و قدرت كمية الفسفور بالمصل وبوحدة قياس mmol/l حسب طريقة (Colorimetric) (12),وقدرت كمية البوتاسيوم بالمصل وبوحدة قياس mmol/l حسب طريقة (Photometric Turbidimetric) والمستخدمة من قبل (13).

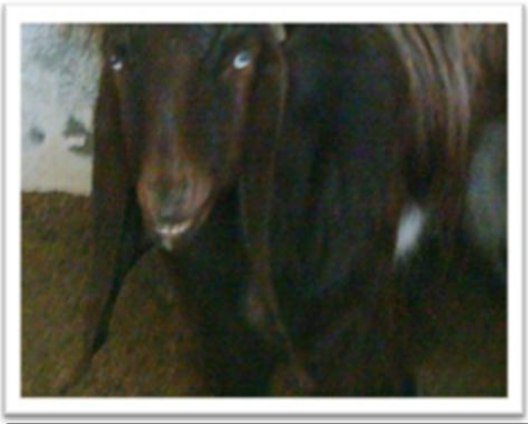
النتائج و المناقشة

أظهر الفحص السريري ان عدد حيوانات الذكور المصابة بالنقص الغذائي من السلالة المحلية هو 22 معز, و في الاناث 44 ، فيما كانت اعداد الذكور السوية ظاهرياً هي 19 والاناث 39 ، وكان عدد الذكور المصابة بالنقص العام من السلالة الشامية 14 , وفي الاناث كانت 46 ،فيما كان عدد الذكور السوية ظاهرياً هو 16 , والاناث 30 . وقد وجد ان عدد المعز الشامي المصاب بالنقص 60 حيواناً ومن السلالة المحلية 66 حيواناً بنسبة 6 . 56 % و 2 . 53% على التوالي . كانت الاغشية المخاطية في المعز الذي يشكو من النقص الغذائي شاحبة (صورة رقم 1)



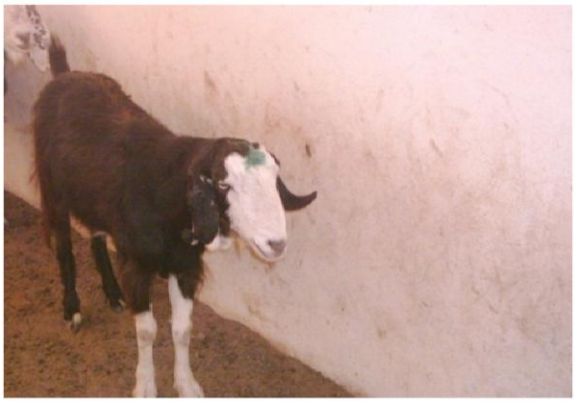
صورة رقم (1) توضح حالة شحوب الاغشية المخاطية في العين لماعز مصاب بالنقص الغذائي

صورة رقم (2) لحيوان ماعز توضح حالة زوال الخضاب من الشعر نتيجة نقص عنصر النحاس



صورة رقم (3) حالة نقص المغنيسيوم ويبدو الحيوان متهيج والعينان محدقتان مع التوسع المستمر.

صورة رقم (4) توضح حالة نقص الفسفور وتبدو حالة الكساح والتقوس بادي على أرجل الحيوان.



صورة رقم (5) توضح حالة نقص البوتاسيوم حيث يبدو الضعف العضلي والخمول بالاضافة للهزال على المعز.

. وكسوة المعز المصاب بنقص النحاس فقدت لونها من خلال زوال الخضاب (صورة رقم 2) . كما ظهرت المعز المصابة بنقص المغنيسيوم متهيجة ومحدقة العينين مع توسعهما (صورة رقم 3) . وكانت المعز المصابة بنقص الفوسفور تشكوا من الكساح وتقوس الارجل (صورة رقم 4) . في حين ظهر الهزال والضعف العضلي على المعز المصاب بنقص البوتاسيوم (صورة رقم 5) .

الجدول رقم (1) يبين معدلات درجات الحرارة في المعز المصاب حيث كانت في ذكوروانات المعز الشامي , وفي ذكوروانات المعز المحلي 38.3 و2 .38 و 38.19 و38.1 درجة مئوية على التوالي. وبلغت معدلات ترداد النبض في ذكور وأناث المعز الشامي ,وفي ذكور وأناث المعز المحلي 74.4 و75.8 و73.3 و 75 نبضة في الدقيقة على التوالي . وبلغ معدل ترداد التنفس في ذكور وأناث المعز الشامي ,وذكور وأناث المعز المحلي 33.5 و32.7و32.6 و32 في الدقيقة وعلى التوالي. وأظهرت النتائج الاحصائية وجود فرق معنوي بمستوى ($P < 0.05$) بين المعز السليم والمصاب ولكلا الجنسين في معدلات درجة الحرارة وترداد التنفس والنبض .

كان معدل كريات الدم الحمراء في ذكور المعز المحلي المصاب بالنقص هو 6.93 x مليون/ مل و معدله في الإناث المحلية المصابة بالنقص هو 7.15 x مليون/مل وكان معدلها في ذكور المعز الشامي المصاب بالنقص هو 7.23 x مليون/مل ومعدله في الاناث الشامية المصابة 7.13 x مليون/مل.وكان معدل خضاب الدم في ذكور المعز المحلي المصاب بالنقص الغذائي (6.42) غم/دسي لتر ،ومعدله لدى الإناث المحلية المصابة بالنقص (6.21) غم/دسي لتر ،وكان معدله في ذكور المعز الشامي (7.18) غم/دسي لتر ،ومعدله في الاناث الشامية (6.73) غم/ دسي لتر. وكان معدل حجم خلايا الدم المضغوطة في ذكور المعز المحلي المصابة (18.86) % ،ومعدله لدى الإناث المحلية المصابة (19.27) % وكان المعدل في ذكور المعز الشامي المصابة (19.35)% ،ومعدله في الاناث الشامية المصابة (18) % ، وأظهرت النتائج الإحصائية وجود فرق معنوي بمستوى ($P < 0.05$) بين معدلات اعداد كريات الدم الحمراء وكمية خضاب الدم ونسبة خلايا الدم المضغوطة في المعز السوية ظاهرياً والمصابة وفي كلا الجنسين (جدول رقم 2) .

جدول رقم(1) معدلات درجات الحرارة وترداد النبض والتنفس في المعز المصاب بامراض النقص الغذائي.

السلالة والجنس / المعـدل	شامي ذكر مصاب	شامي ذكر سوية ظاهريا	شامي إناث مصابة	شامي إناث سوية ظاهريا	محلي ذكر مصاب	محلي ذكر سوية ظاهريا	محلي إناث مصابة	محلي إناث سوية ظاهريا
درجة الحرارة Temp/c	38.3 b± 0.04	38.5a± 0.01	38.2 b± 0.04	38.5 a± 0.01	38.19 b± 0.07	38.55 a± 0.01	38.1 b± 0.03	38.5 a± 0.007
النبض pulse/min	74.4a± 0.13	72.3b± 0.12	75.8a± 0.17	73.7b± 0.21	73.3 a± 0.13	71.2 b± 0.10	75.06a± 0.18	71.7b± 0.16
التنفس R.R /min	33.5a± 0.22	25.3b± 0.17	32.7a± 0.16	25.5b± 0.14	32.6a± 0.29	20.8b 0.11	32 a± 0.23	23b± 0.17

(الحروف المختلفة تعني وجود فرق معنوي على مستوى احتمال ($P < 0.05$)

جدول رقم (2) معدلات كريات الدم الحمراء × مليون/مل, و خضاب الدم غم/دسي لتر,وحجم خلايا الدم المضغوطة % في المعز المصاب بأمراض النقص الغذائي.

السلالـة والجنس / المعــدل	شامي ذكر مصاب	شامي ذكر سوية ظاهرياً	شامي إناث مصابة	شامي إناث سوية ظاهرياً	محلي ذكر مصاب	محلي ذكر سوية ظاهرياً	محلي إناث مصابة	محلي إناث سوية ظاهرياً
كريات الدم الحمر	7.23b± 0.14	9.15a± 0.01	7.13b± 0.05	9.16a± 0.02	6.93a± 0.14	8.15b± 0.01	7.15b± 0.08	8.21a± 0.01
خضاب الدم	7.18b± 0.17	9.35a± 0.01	6.73b± 0.07	9.34a± 0.01	6.42b± 0.08	8.3a± 0.02	6.21b± 0.06	8.28a± 0.01
حجم خلايا الدم المضغوطة	19.35b± 0.48	31.5a± 0.12	18 b± 0.21	31.36a± 0.16	18.86b± 0.2	23.52a± 0.11	19.27b± 0.30	23.46a± 0.10

(الحروف المختلفة تعني وجود فرق معنوي على مستوى احتمال ($P < 0.05$)

بلغت معدلات قيم عنصر النحاس في ذكوروأناث المعز المحلي المصاب بالنقص 0.05 مايكرو مول/ لتر، و 0.07مايكرو مول/ لترعلى التوالي، وفي ذكوروأناث المعز الشامي المصاب بالنقص هو 0.07 و 0.06 مايكرو مول /

لترعلى التوالي (جدول رقم 3) .كما بلغت معدلات قيم عنصر المغنيسيوم في ذكوروأناث المعز المحلي المصاب بالنقص 0.94 و 0.67 مل مول /لتر على التوالي ،وكان معدل العنصر في ذكوروأناث المعز الشامي المصاب بالنقص 0.93 و 0.77 مل مول /لتر على التوالي. و بلغت معدلات قيم عنصر الفسفور في ذكوروأناث المعز المحلي المصاب بالنقص 1.94 و 1.18 مل مول /لتر على التوالي ، وبلغت معدلات قيم العنصر في ذكوروأناث المعز الشامي المصاب بالنقص 1.45 و 1.37 مل مول /لتر على التوالي(جدول رقم 3) .وبلغت معدلات قيم عنصر البوتاسيوم في ذكوروأناث المعز المحلي المصاب بالنقص4.21 و 4.20 مل مول /لترعلى التوالي ،وكانت معدلاته في ذكوروأناث المعز الشامي المصاب 3.65 و 5.37 مل مول /لتر على التوالي ، وأظهرت النتائج الإحصائية وجود فرق معنوي بمستوى ($P < 0.05$) بين معدلات هذه العناصر في ذكورواناث المعز السوية ظاهريا والمصابة ومن السلالتين (جدول رقم 3) . كانت معدلات درجات الحرارة في المعز المصابة بالنقص الغذائي اقل منه في السليمة في الذكور والإناث وفي السلالتين , وهذا يطابق ما ذكره (8) بانخفاض درجة الحرارة في الحيوانات الضعيفة التغذية بسبب انخفاض الفعالية الايضية بالجسم الذي يعود لقلة الغذاء المتناول أو المهضوم وفي معدلات النبض والتنفس تبين أن هذه المعدلات كانت في المعز المصاب أعلى في محاولة من جسم الحيوان لتعويض قلة الأوكسجين وفقر الدم الناتج عن النقص الغذائي(9).وهذا يرتبط مباشرة مع نتائج صورة الدم حيث كانت معدلات كريات الدم الحمراء و خضاب الدم وحجم خلايا الدم المضغوطة في المعز المصاب بالنقص الغذائي لكلا السلالتين اقل من معدلاتها الطبيعية التي ذكرها الباحثون(10,8 13,12, 11 , و14). وهو يؤكد ماذكره (6) بحصول انخفاض المعاييرالدمية عند فقر الدم بسبب سوء التغذية .لقد وجد ان درجة الانخفاض في عدد كريات الدم الحمراء لذكور المعز المحلي كان اعلى من ذكور المعز الشامي ،وهذا يعود الى ما اشار اليه (15) ان لسلالة المعز تأثير معنوي على عدد كريات الدم الحمراء بسبب الصفات الجينية التي تتمتع بها السلالات المختلفة.

ان شحوب الاغشية المخاطية وظهور الاعراض المرضية التي تجسد بما لا يقبل الشك وجود امراض النقص الغذائي تدعمها مؤشرات وجود فقر الدم في المعز المصاب تؤكده نتائج قيم معدلات عنصر النحاس في المصل حيث كانت أقل من معدلاتها الطبيعية بسبب انخفاض كمية النحاس المجهز للماعز او بسبب فقر الماء والتربة التي يرعى عليها الحيوان, او عندما تكون كمية النحاس المتوفرة للحيوان كافية الا ان وجود عوامل اخرى تحول دون افادة الجسم منه مثل نسبة عالية من عناصر المولبيدينيوم , الزنك , الكادميوم ،الحديد اوالسلينيوم (16, 17 و18) .وقد كانت معدلات النحاس متساوية في الذكور والاناث ولكلا السلالتين وهو يخالف ماذهب اليه (19) بتمتع سلالة المعز الشامي بصفات جينية تؤهلها للتفوق على سلالات المعز المحلي في المستويات الكيموحيوية . كذلك الحال في معدلات عنصر المغنيسيوم التي كانت أقل من معدلاتها العامة الطبيعية في مصل المعز المصاب بالنقص الغذائي وظهر على المعز المصاب علامات التهيج وتوسع حدقة العين وهذا يطابق ماذكره (20) .وكانت قيم المغنيسيوم في ذكور المعز الشامي والمحلي اعلى منها في الاناث الشامية والمحلية المصابة بالنقص الغذائي .ومن حالات الكساح وتقوس ارجل المعز المصاب بنقص الفوسفور تبين ان معدلات عنصر الفسفور في أمصالها كان أقل من معدلاتها الطبيعية (2), وكانت قيم الفوسفور في ذكور المعز الشامي والمحلي المصاب بالنقص الغذائي اعلى منها في الاناث وبفارق معنوي .وكانت معدلاته في ذكور المعز الشامي أقل منها في ذكور المعز المحلي ،فيما كانت هذه المعدلات في اناث المعز الشامي اعلى منها في اناث المعز المحلي . لقد تميزت حالات نقص البوتاسيم بالهزال والضعف والخمول وجائت نتائج مستوى هذا العنصر في مصولها واطئة وبفارق معنوي عن المعز السليم ومن كلا الجنسين والسلالتين (21) .وقد كانت قيم البوتاسيوم في ذكور المعز الشامي اقل منها لدى الاناث الشامية و اقل منها في ذكور المعز المحلي المصاب بالنقص الغذائي ،فيما كانت قيمه في الاناث الشامية اعلى من قيمه في الاناث المحلية المصابة بالنقص الغذائي (22). وتبين الدراسه بان محافظة بغداد تشكو من قلة في مستوى النحاس في التربة والمياه وهذا تأكيد لما اوردته(18) . ان معدلات نقص العناصر كانت في عنصري النحاس والفسفور اعلى منها من المغنيسيوم والبوتاسيوم ، وقد يعود السبب الى ماذكره (16) بوجود اكثر من عامل (العامل الاولي والثانوي) يؤدي لحدوث النقص بعنصر النحاس ، وكذلك بالنسبة لعنصر الفسفور فقد أورد (23) نفس السبب. وبذلك تصبح هذه الدراسة الاولى في تثبيت اهمية فحص قيم عناصر النحاس والفوسفور والمغنيسيوم والبوتاسيوم للحيوانات المشكوك باصابتها بامراض النقص الغذائي وتعطي دليل على مستويات هذه العناصر في امصالها ولاول مرة في منطقة بغداد.

جدول رقم (3) معدلات النحاس µmol/l والمغنيسيوم mmol/l والفوسفورmmol/l والبوتاسيومmmol/l في مصل المعزالمصاب بأمراض النقص الغذائي والسليم سريريا.

السلالـة والجنس / المعـــدل	شامي ذكر مصاب	شامي ذكر سوية ظاهرياً	شامي إناث مصابة	شامي إناث سوية ظاهرياً	محلي ذكر مصاب	محلي ذكر سوية ظاهرياً	محلي إناث مصابة	محلي إناث سوية ظاهريا
عنصر النحاس	0.07b ±0.04	0.27 a ±0.006	0.06 b ± 0.01	0.24a ±0.01	0.05b ±0.03	0.2a ±0.009	0.07b ±0.02	0.16a ±0.01
عنصر المغنيسيوم	0.93b ±0.07	1.24 a ± 0.006	0.77 b ±0.05	1.26a ±0.01	0.94 b ±0.04	1.16 a ±0.01	0.67 b ±0.01	0.99a ±0.02
عنصر الفسفور	1.45b ±0.32	3.54 a ± 0.01	1.37 b ± 0.03	3.07a ±0.09	1.94 b ±0.13	2.85 a ±0.009	1.18b ±0.06	2.30 a ± 0.05
عنصرالبوتاسيوم	3.65b ±0.32	5.66 a ± 0.01	5.37 b ±0.33	5.32a ±0.05	4.21 b ±0.33	4.36 a ± 0.08	4.20 b ±0.33	4.68a ± 0.08

الحروف المختلفة تعني وجود فرق معنوي على مستوى احتمال (P < 0.05)

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الفاعلية الحيوية لمادة عسل النحل المحلي في نمو عدد من الجراثيم الموجبة والسالبة لصبغة كرام

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الخلاصة

استهدفت الدراسة الحالية التحري عن النشاط الحيوي المضاد للجراثيم لتراكيز متدرجة من مادة العسل المحلي هي (125 ، 250 ، 375 ، 500 ملغم / مل) وقد اختبرت خمسة أنواع من الجراثيم اثنتان منها موجبة لصبغة كرام وهي العنقوديات الذهبية والمسبحيات وثلاثة جراثيم سالبة لصبغة كرام وهي الاشريشيا القولونية و السالمونيلا والزائفة الزنجارية باستخدام طريقة الانتشار عبر الاكار وطريقة التخفيف بالأنابيب . بينت نتائج الانتشار عبر الاكار بأن جراثيم العنقوديات الذهبية والمسبحيات هي الأكثر تحسساً لتخافيف العسل في حين أظهرت كل من جرثومتي الاشريشيا القولونية والسالمونيلا حساسية معتدلة فيما لم تظهر الزائفة الزنجارية أي تأثير يذكر كما وكان لدرجات حفظ الأوساط الزرعية (25˚ م ، 37˚ م) تأثيراً واضحاً على النتائج إذ تفوقت أقطار تثبيط النمو في الأطباق التي حضنت بدرجة (25˚ م) في اغلب النتائج المسجلة عن مثيلتها التي حضنت بدرجة حرارة (37˚ م) كما سجلت نتائج التخفيف بالأنابيب إن التركيز المثبط الادنىMIC كان (8، 8، 125، 64 ملغم / مل) لنمو العنقوديات الذهبية ، المسبحيات، الاشريشيا القولونية و السالمونيلا على التوالي في حين كان التركيز القاتل الادنى MBC للجراثيم المذكورة (32، 64 ، 250 ، 250 ملغم / مل) على التوالي للأنابيب التي حفظت بدرجة حرارة (25˚ م) أما الأنابيب التي حفظت بدرجة حرارة (37˚ م) فسجلت MIC (8 ، 16 ، 125 ، 250 ملغم / مل) على التوالي و MBC (32 ، 64 ، 125 و 250 ملغم / مل) على التوالي .

مفاتيح الكلمات: عسل محلي, الجراثيم الموجبة, الجراثيم السالبة, الفعالية الحيوية.

Biological activity of local honey bees in growth of some gram positive and negative bacteria

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Summary

The local study was targeting to investigate the biological antibacterial activity for gradiate concentrations of a local honey bees (125,250, 375 and 500 mg/ml) were tested for five type of bacteria, two of which are gram positive (staphylococcus*aureus* , *streptococcus spp.*) and three of them were negative bacteria (*Esherichia coli* , *salmonella spp.* , *pseudomonas aeruginosa*) by using agar well diffusion method and tube dilution method.

The results of agar well diffusion method showed that the bacteria of *Staphylococcus aureus* and *Streptococcus Spp*. were the most sensitive to honey dilutions, while *E.coli* ,*Salmonella Spp*. showed moderate sensitivity . *Pseudomonas aeruginosa*did not show any sensitivity. The result also showed that the temperature of incubation temperature of culture media (25and 37 C) had a marked effect on the result of local study, outperformed of diameters of growth inhibition in the cultures that were incubated at 25 C in most of the result recorded.The result of the tube dilution method, Minimum Inhibition Concentration (MIC) was (8 , 8 , 125 and 64) mg/ml for the growth of *S.aureus* , Streptococcus , *E.coli* and salmonella respectively , while the Minimum Bactericidal Concentration (MBC) for bacteria mentioned were (32 , 64 , 250 and 250) mg/ml respectively for tubes that incubated at 25 C while tubes which incubated at 37 C MIC recorded (8 , 8 , 125 and 250) mg/ml while MBC were (32 , 64 , 125 and 250) mg/ml respectively.

Keywords: honey bees, biological activity, gram positive, negative bacteria
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المقدمة

احتلت نواتج النحل في الآونة الأخيرة حيزاً مهماً من اهتمام الأطباء و الباحثين في العالم بصورة عامة والعراق بصورة خاصة لما تتمتع به هذه المنتجات من قيمة غذائية عالية من جهة وفعاليات حيوية وعلاجية منوعة من جهة أخرى وتشمل هذه النواتج الغذاء الملكي (Royal Jelly) وسم النحل (Bee venom) وحبوب الطلع(Pollen) وصمغ النحل (Bee glue) ومادة العسل (Honey bee) ويعد الأخير واحد من النواتج المهمة من الناحية الغذائية والطبية والعلاجية لما يحتويه من مجاميع كيمائية فاعلة .

العسل مادة طبيعية تنتج من قبل عاملات سلالات نحل العسل في معظم دول العالم والعسل الخام عادة ينتج في مزارع صغيرة ويترك على حالته الطبيعية بدون معاملة في الغالب (1) وأدرك المصريون والصينيون واليونانيون والإغريق وأهل اليمن وسكان وادي الرافدين المنافع العظيمة لعسل النحل وجعلوه في مقدمة أدويتهم لعلاج مرضاهم ووضعوه على قمة الأغذية القيمة التي كانوا يتغذون بها ورفعوه إلى مستوى الجواهر النفيسة التي كانوا يحتفظون بها ، إذ تم العثور على جرة من العسل في هرم توت عنخ آمون يقدر عمرها بأربعة الآلف سنة مازالت طيبة وزكية كما حفظت لكون العسل المادة الغذائية التي لا تتعرض للفساد بمرور السنيين (2) ومع شيوع استخدامه في الطب التقليدي عبر العصور وفي كل حضارات العالم القديم إلا إن استخدامه في الطب الحديث ما زال محدوداً لقلة المصادر والبحوث العلمية التي تدعم فوائده العلاجية (3) يستعمل العسل في مجال الطب التقليدي في علاج حالات الضعف العام (General weakness) واضطرابات القناة الهضمية وكمعزز غذائي للأمهات المرضعات وكمصدر للكالسيوم وفي معالجة فقر الدم ، كذلك يستخدم موضعياً في معالجة الماء الأبيض (ساد Cataract) والتهاب ملتحمة العين ، كما ويستخدم أيضاً في معالجة مختلف أمراض القرنية (4) . استهدفت الدراسة الحالية التحري عن الفاعلية المضادة للجراثيم لتخافيف متدرجة من مادة العسل المحلي وذلك من خلال دراسة تأثيرها على عدد من الجراثيم الممرضة الموجبة والسالبة لصبغة كرام والمعزولة من حالات مرضية مختلفة بالأطباق الزرعية وأنابيب الاختبار .

المواد طرائق العمل

تم جمع كمية من مادة العسل المحلي من عدة مناحل في مدينة الديوانية خلال شهر أيلول وتشرين الأول وتم تنظيفه من بقايا الشمع العالق به وذلك باستخدام شاش طبي معقم وبعدها وضع العسل المصفى في حاويات زجاجية نظيفة ومعقمة لحين استخدامه . حضرت أربع تراكيز متدرجة من مادة العسل المحلي وهي 125 ، 250 ، 375 ، 500 ملغم / مل باستخدام الماء المقطر إذ تم إذابة 10غم من مادة العسل في 20مل من الماء المقطر ليتم الحصول على التركيز 500 ملغم / مل ومنه حضرت بقية التراكيز المذكورة في أعلاه.تم استخدام خمسة أنواع من الجراثيم الممرضة لغرض دراسة فاعلية مادة العسل المحلي المنقى في المختبر وتم الحصول على العزلات الجرثومية من عدة مصادر وهي (المستشفى البيطري التعليمي في الديوانية ، مختبر الأحياء المجهرية/ كلية الطب البيطري – جامعة القادسية ، مختبرات وحدة بحوث الأمراض المشتركة / كلية الطب البيطري – جامعة القادسية) والمعزولة من حالات مرضية مختلفة وشخصت العزلات الجرثومية المذكورة أدناه في مختبرات وحدة بحوث الأمراض المشتركة / كلية الطب البيطري -جامعة القادسية (5 , 6) ثم حفظت العزلات المشخصة على أكار مائل من وسط نقيع الدماغ والقلب وهذه الجراثيم هي :

1- جرثومة المكورات العنقودية الذهبية *Staphylococcus aureus*
2- جرثومة المسيحيات القيحية *Streptococcus pyogens*
3- جرثومة الاشريشيا القولونية *E. Coli*
4- جرثومة السالمونيلا *Salmonella spp.*
5- جرثومة الزائفة الزنجارية.*Pseudomonas aeruginosa*

حضر العالق الجرثومي بتركيز (1×10^6) وحدة مكونة للمستعمرات / مل باستخدام طريقة أنابيب ماكفرلاند وحسب ما مذكور في (6) إذ تم تحضير أنبوبه ماكفرلاند وذلك بإضافة (0.05) مل من مادة (BaCl2) بتركيز 1% الى 95.5 مل من مادة حامض الكبريتيك (H2SO4) ثم قورنت كثافة العالق الجرثومي المحضر مع أنبوبة ماكفرلاند المذكورة أعلاه .

اجري هذا الاختبار باستخدام أكار فحص الحساسية Muller hinton agar الذي حضر بإذابة 38 غم في 1000 مل من الماء المقطر في وعاء زجاجي سعة 1000 مل بمساعدة اللهب ثم بعد تعقيمه بالمؤصدة Autoclave عند درجة حرارة 121˚ م وتحت ضغط 15 باوند / انج2 مدة 15 دقيقة ، صب الوسط الزرعي في أطباق بتري المعقمة وبواقع 20 مل من المستنبت لكل طبق ثم وضعت في الثلاجة لعدة دقائق للمساعدة على تصلب الوسط الزرعي ، تم نقل 0.1 مل من العالق الجرثومي المحضر في الفقرة السابقة إلى وسط فحص الحساسية ثم نشرت على سطح الطبق باستخدام الناشر الزجاجي (Spreader) وتركت الأطباق الزرعية بوضع مستوي لمدة 30 دقيقة لضمان التشرب الكامل للعالق الجرثومي في الوسط ، ثم عملت 5 حفر في كل طبق أربع منها محيطة أضيف إليها تراكيز مادة العسل المستخدمة قيد الدراسة وبواقع 0.1 مل لكل حفرة وواحدة مركزية وضع فيها المذيب المستخدم في تحضير التراكيز وهو الماء المقطر باستعمال ماصة دقيقة بعدها حضنت جميع الأطباق المحضرة بدرجة حرارة 37˚ م ودرجة حرارة 25˚ م مدة 24 ساعة وقرأت النتائج بعد ذلك بقياس أقطار تثبيط النمو حول الحفر باستخدام المسطرة .

تم تحضير سلسلة من التخافيف المتدرجة من مادة العسل المحلي في أنابيب الاختبار باستخدام المرق المغذي تراوحت قيمتها (2 ، 4 ، 8 ، 16 ، 32 ، 64 ، 125 و 250) ملغم / مل وبعدها لقحت جميع الأنابيب بما مقداره 0.1 مل من العالق الجرثومي الحاوي على (1×10^6) وحدة مكونة للمستعمرات / مل مع تحضير أنبوبه حاوية على المرق المغذي الملقح بالجرثومة قيد التجربة ممثلة سيطرة (1) وأنبوبة اختبار ثانية حاوية على المرق المغذي فقط ممثلاً سيطرة (2) وأجريت عملية إعادة التجربة في حالة عدم ظهور عكورة (Turbidity) في أنبوبة السيطرة (1) أو في حالة ظهورها في انبوبة السيطرة (2) ، بعدها حضنت جميع الأنابيب المذكورة في أعلاه عند درجة حرارة (37˚ م) لمدة 24 ساعة و حددت قيمة التركيز المثبط الأدنى (MIC) بأنها اقل تركيز من مادة العسل التي منعت ظهور عكورة واضحة للعيان في المستنبت الزرعي (المرق المغذي) فيما حددت قيمة التركيز القاتل الأدنى (MBC) بأخذ 0.1 مل من جميع الأنابيب التي لم تظهر عكورة وزراعتها على أطباق بتري الحاوية على الاكار المغذي وتم حضنها عند درجة حرارة 37˚ م مدة 24 ساعة وتم تحديد قيمة (MBC) بأنه اقل تركيز من مادة العسل الذي يقلل عدد المستعمرات الجرثومية بمقدار 99% من المزروع الأصلي .

النتائج و المناقشة

بينت نتائج اختبار حساسية الجراثيم المستخدمة قيد الدراسة وهي مجموعة الجراثيم الموجبة لصبغة كرام (العنقوديات الذهبية ، المسبحيات) ومجموعة الجراثيم السالبة لصبغة كرام وهي (الاشريشيا القولونية ، السالمونيلا ، الزائفة الزنجارية) لتراكيز متدرجة من مادة العسل المحلي في الإطباق الزرعية وهي (125 ، 250 ، 375 ، 500) ملغم / مل بأستخدام طريقة الانتشار عبر الاكار والتي تم بالإضافة الى ذلك اختبار تأثير درجة حرارة حضن الإطباق الزرعية عند درجات حرارة حفظ مختلفة باستخدام الحاضنة وهي (25˚م ،37˚ م) إن للتخافيف المختلفة تأثيرا واضحاً في تثبيط النمو الجرثومي لجميع الجراثيم المذكورة أعلاه عدا بعض الاستثناءات مع تباين هذا التأثير اعتمادا على ثلاث عوامل أساسية وهي عامل تركيز مادة العسل المستخدم ، درجة الحرارة التي استخدمت في حفظ الإطباق الزرعية في الحاضنة وعامل نوع الجرثومة المختبرة .

فقد اتضح من نتائج الدراسة والمدونة في الجدول (1) و (2) إن لمادة العسل تأثيرا مرتفعا على مجموعة الجراثيم الموجبة لصبغة كرام إذ ابتدء التأثير فيها من ادنى تركيز وهو 125 ملغم / مل وأعطت نتائج الإطباق الزرعية التي حفظت بدرجة حرارة 25˚ م أقطار من تثبيط النمو مقدارها (0 ± 13 ، 0 ± 8) ملغم / مل ضد نمو العنقوديات الذهبية و المسبحيات على التوالي و (0.33 ± 9.33 ، 0.33 ± 7.33) ملم على التوالي للإطباق الزرعية التي حفظت بدرجة حرارة 37˚ م وازدادت أقطار تثبيط النمو عند التركيز 500 ملغم / مل الى (0.66 ± 30.66 ، 0.33 ± 19.33) ملم ضد نمو العنقوديات الذهبية والمسبحيات على التوالي للإطباق الزرعية التي حفظت بدرجة حرارة 25˚ م و (0.66 ± 26.66 ، 0 ± 19) ملم على التوالي للإطباق الزرعية التي حفظت بدرجة حرارة37˚ م .

إما الجراثيم السالبة لصبغة كرام فقد كانت اقل تأثيرا بصورة عامة اذ لم يسجل التركيز 125 ملغم / مل أي قطر من تثبيط النمو ضد جميع الجراثيم السالبة لصبغة كرام في كل من الإطباق الزرعية التي تم حفظها في درجة حرارة 25˚ م و 37˚م أما التركيز الأعلى (500 ملغم / مل) فقد أعطت أقطارا من تثبيط النمو للجراثيم (الاشريشيا القولونية ، السالمونيلا) مقدارها (0.33 ± 17.33 ، 0.33 ± 14.33) ملم على التوالي للإطباق الزرعية التي حفظت بدرجة حرارة 37˚ م ، في حين لم تظهر جرثومة الزائفة الزنجارية أي حساسية تذكر اتجاهجميع تراكيز مادة العسل ولجميع الإطباق الزرعية والتي تم حفظها بدرجات حرارة (25˚ م ، 37˚ م) ومما تجدر الإشارة إليه الى ان الماء المقطر الذي استخدم كسيطرة سالبة لم يظهر هو الأخر أي تأثير يذكر في نمو جميع الجراثيم المختبرة قيد الدراسة . كما تبين إن هناك علاقة طردية بين تراكيز مادة العسل المستخدمة وقطر تثبيط النمو ضد الجراثيم الممرضة المذكورة (شكل 1, 2 ، 3 ، 4 و 5)

بين التحليل الإحصائي بأستخدام اختبار التباين مع اقل فرق معنوي بان كل من التركيز 375 ملغم / مل والتركيز 500 ملغم / مل قد اظهر تفوقاً معنوياً على جميع المضادات الحيوية المستخدمة كسيطرة موجبة وهي (الامبسلين ، السبروفلوكساسين و الجنتامايسين) في تثبيط نمو جرثومة العنقوديات الذهبية في حين اظهر التركيز 500 ملغم / مل تفوقاً إحصائيا على المضادان الحيويان الامبسلين و السبروفلوكساسين وتقارباً إحصائيا مع المضاد الحيوي الجنتامايسين (جدول 3 و 4) .

أظهرت نتائج حساب التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى (MBC) باستخدام أنابيب الاختبار عندما حفظت الأنابيب في درجة حرارة25˚ م إن التركيز 8 ملغم / مل سجل كأقل تركيز مثبط لمادة العسل اتجاه جرثومتي العنقوديات الذهبية و المسبحيات والتركيز 64 ملغم / مل للسالمونيلا و 125 ملغم /مل للاشريشيا القولونية فيما سجل التركيز (32 ، 64 ملغم /مل) كأقل تركيز قاتل لجرثومتي العنقودية الذهبية والمسبحيات على التوالي والتركيز 250 ملغم/مل لكل من جرثومتي الاشريشيا القولونية والسالمونيلا .

اما عند حفظ الأنابيب في درجة حرارة 37˚ م فقد سجل التركيز 8 ملغم / مل كأدنى تركيز مثبط اتجاه العنقوديات الذهبية والتركيز 16 ملغم / مل للمسبحيات ، في حين كان التركيز 32 ملغم / مل كأقل تركيز قاتل اتجاه نمو العنقوديات الذهبية والتركيز 64 ملغم / مل اتجاه نمو المسبحيات في حين كان التركيز 125 ملغم / مل

يمثل التركيز المثبط الأدنى والقاتل الأدنى لنمو السالمونيلا والتركيز 250ملغم / مل للاشريشيا القولونية . (جدول 5، 6) .

جدول (1) تأثير تراكيز مادة العسل المحلي على نمو كل من الجراثيم الموجبة والسالبة لصبغة كرام عند درجة حرارة حضن 25˚ م.

التركيز (ملغم/مل)	نوع الجرثومة ومعدل أقطار تثبيط النمو مقاسا بالملم ±الخطأ القياسي				
	العنقودية الذهبية	المسبحيات	الاشريشيا القولونية	السالمونيلا	الزائفة الزنجارية
125	13±0 Aa	13±0 Ab	0±0 Ac	0±0 Ac	0±0 Ac
250	0.33 19.66±Ba	11 ±0 Bb	10.33±0.33 Bb	10.33±0.33 Bb	0±0 Ac
375	24 ±0.57 Ca	15.33±0.33 Cb	13±0 Cc	13±0 Cc	0±0 Ad
500	30.66±0.66 Da	19.33±0.33 Db	17.33±0.22 Dc	14.33±0.33 Dd	0±0 Ae

- تشير الحروف الصغيرة المختلفة إلى وجود فروق معنوية بين القيم أفقياً وتحت مستوى احتمالية ($p<0.05$)
- تشير الحروف الكبيرة المختلفة إلى وجود فروق معنوية بين القيم عمودياً وتحت مستوى احتمالية($p<0.05$).

جدول (2) تأثير تراكيز مادة العسل المحلي على نمو كل من الجراثيم الموجبة والسالبة لصبغة كرام عند درجة حرارة حضن 37˚ م.

التركيز (ملغم/مل)	نوع الجرثومة ومعدل أقطار تثبيط النمو مقاسا بالملم ±الخطأ القياسي				
	العنقودية الذهبية	المسبحيات	الاشريشيا القولونية	السالمونيلا	الزائفة الزنجارية
125	9.33±0.33 Aa	7.33±0.33 Ab	0±0 Ac	0±0 Ac	0±0 Ac
250	17.33±0.66 Ba	9.66±0.33 Bb	0±0 Ac	11.33±0.33 Bd	0±0 Ac
375	19.33±0.33 Ca	13.33±0.66 Cb	11.33±0.66 Bc	12.33±0.33 Bc	0±0 Ad
500	26.66±0.66 Da	19±0 Db	13.66±0.33 Cc	13±0 Bc	0±0 Ad

- تشير الحروف الصغيرة المختلفة إلى وجود فروق معنوية بين القيم أفقياً وتحت مستوى احتمالية ($p<0.05$)
- تشير الحروف الكبيرة المختلفة إلى وجود فروق معنوية بين القيم عمودياً وتحت مستوى احتمالية($p<0.05$)

جدول (3) تأثير عدد من المضادات الحيوية على نمو كل من الجراثيم الموجبة والسالبة لصبغة كرام عند درجة حرارة 25˚ م

نوع المضاد الحيوي وتركيزه	نوع الجرثومة معدل أقطار تثبيط النمو مقاسا بالملم ±الخطأ القياسي				
	العنقودية الذهبية	المسبحيات	الاشريشيا القولونية	السالمونيلا	الزائفة الزنجارية
الامبسلين (10 مايكروغرام/مل)	مقاومة	مقاومة	15±0	مقاومة	مقاومة
السبروفلوكساسين (5 مايكروغرام/مل)	18.33±0.33	16±0	19±0	مقاومة	19.66±0
الجنتامايسين (10 مايكروغرام/مل)	15±1	مقاومة	18.33±0.33	14.66±1	مقاومة

جدول (4) تاثير عدد من المضادات الحيوية على نمو كل من الجراثيم الموجبة والسالبة لصبغة كرام عند درجة حرارة 37ْم

نوع المضاد الحيوي وتركيزه	نوع الجرثومة معدل أقطار تثبيط النمو مقاسا بالملم ±الخطأ القياسي				
	العنقودية الذهبية	المسبحيات	الاشريشيا القولونية	السالمونيلا	الزائفة الزنجارية
الامبسلين (10 مايكروغرام/مل)	مقاومة	مقاومة	15.33±0.33	مقاومة	مقاومة
السبروفلوكساسين (5 مايكروغرام/مل)	18±1	14.66±0.33	20.33±0.33	مقاومة	19.66±0.33
الجنتامايسين (10 مايكروغرام/مل)	15±1	مقاومة	17.66±0.33	14.66±1	مقاومة

جدول (5) تأثير تراكيز مادة العسل المحلي على نمو كل من الجراثيم الموجبة والسالبة لصبغة كرام في الأنابيب عند درجة حرارة 25ْ م

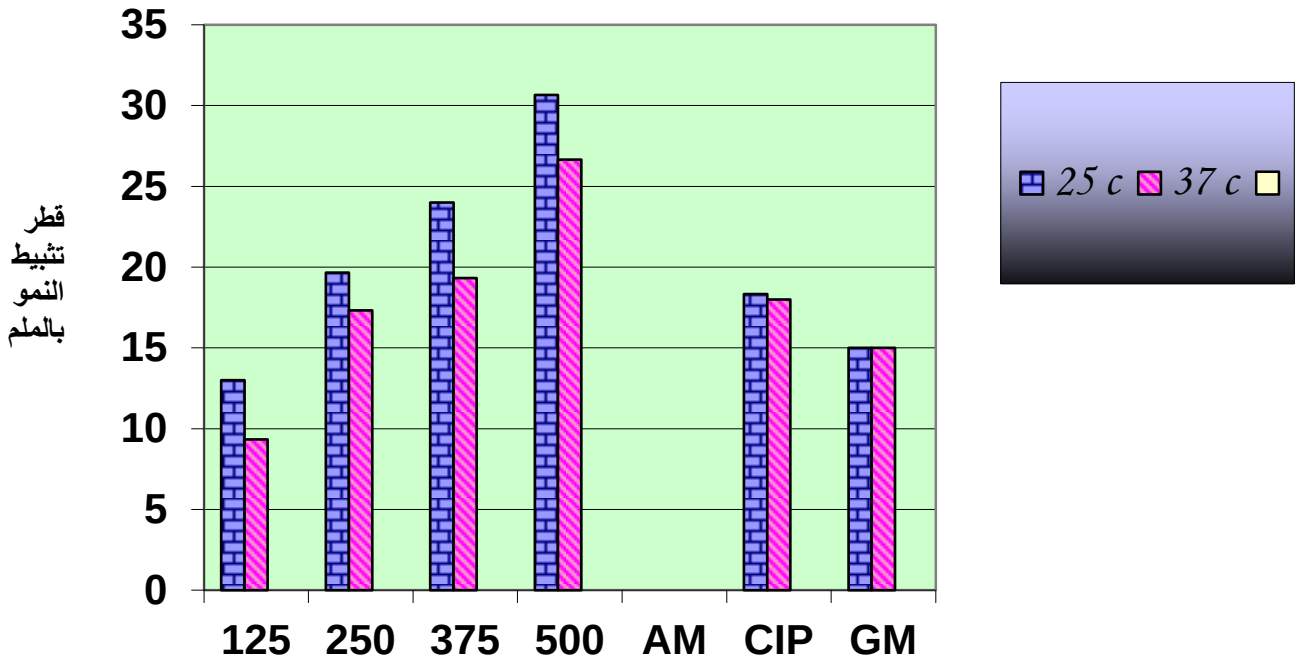
التركيز (ملغم/مل)	نوع الجرثومة ومعدل أقطار تثبيط النمو مقاسا بالملم ±الخطأ القياسي			
	العنقودية الذهبية	المسبحيات	الاشريشيا القولونية	السالمونيلا
2	-	-	-	-
4	-	-	-	-
8	+	+	-	-
16	+	+	-	-
32	++	+	-	-
64	++	++	-	+
125	++	++	+	+
250	++	++	++	++

(-) فعل غير مؤثر (+) فعل مثبط للجرثومة (++) فعل قاتل للجرثومة

جدول (6) تأثير تراكيز مادة العسل المحلي على نمو كل من الجراثيم الموجبة والسالبة لصبغة كرام في الأنابيب عند درجة حرارة 37ْ م

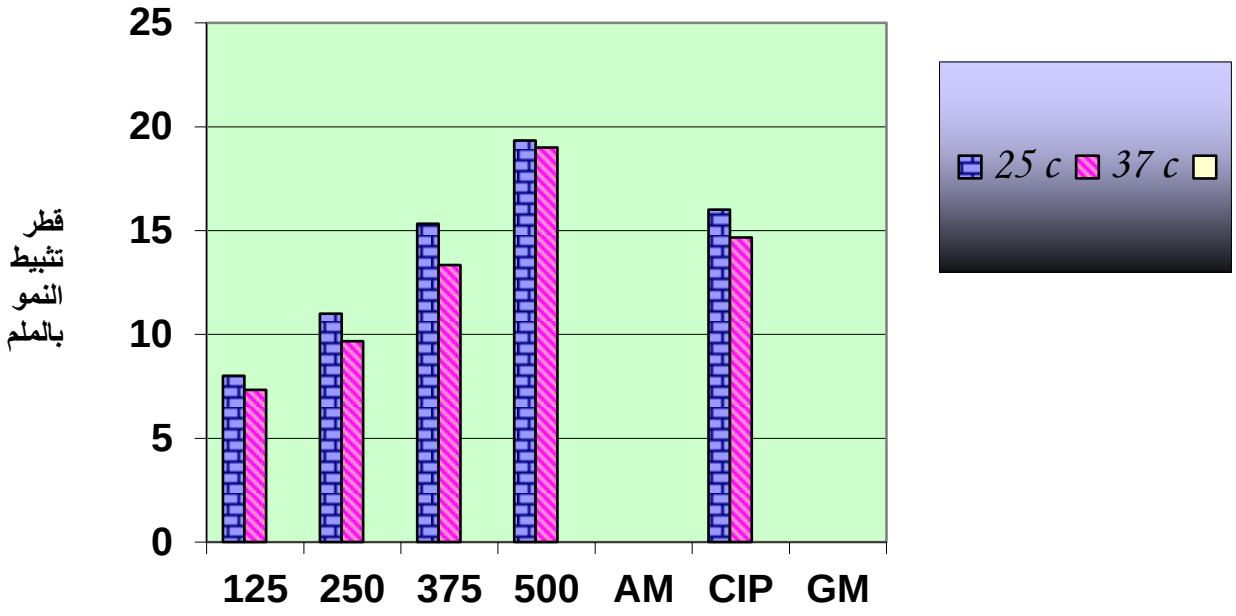
التركيز (ملغم/مل)	نوع الجرثومة ومعدل أقطار تثبيط النمو مقاسا بالملم ±الخطأ القياسي			
	العنقودية الذهبية	المسبحيات	الاشريشيا القولونية	السالمونيلا
2	-	-	-	-
4	-	-	-	-
8	+	-	-	-
16	+	+	-	-
32	++	+	-	-
64	++	++	-	-
125	++	++	-	+(++)
250	++	++	+(++)	+(++)

(-) تعني فعل غير مؤثر (+) تعني فعل مثبط (++) تعني فعل قاتل + (++) تعني إن التركيز مثبط وقاتل في الوقت نفسه



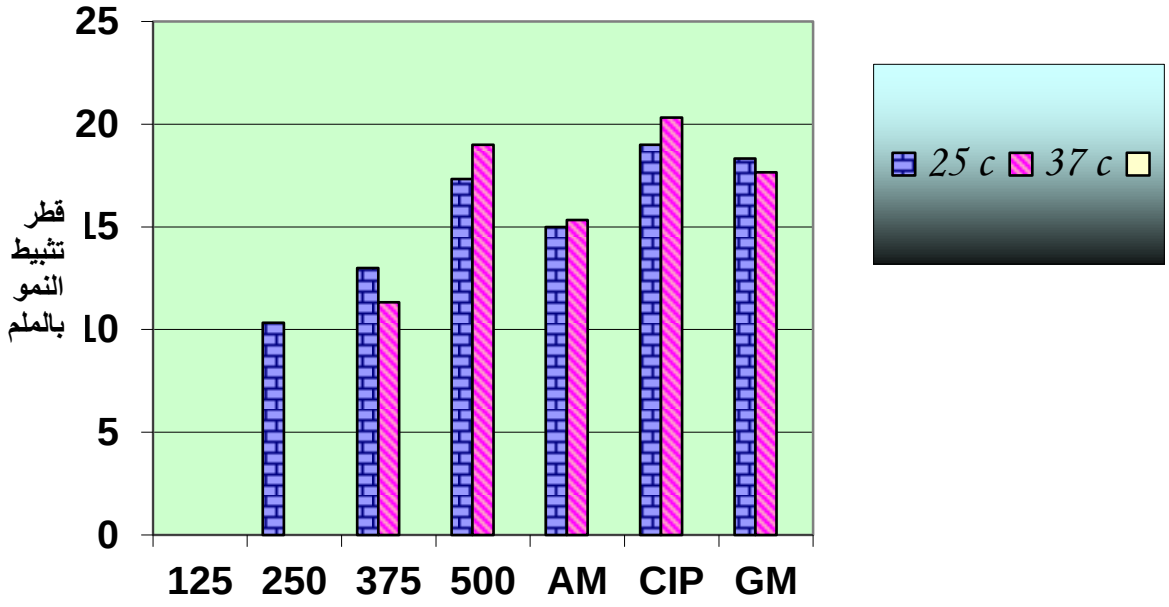
تركيز مادة العسل والمضادات الحيوية

شكل (1) العلاقة بين التراكيز المختلفة لمادة العسل المحلي والمضادات الحيوية ومعدل أقطار تثبيط النمو لجرثومة العنقوديات الذهبية .



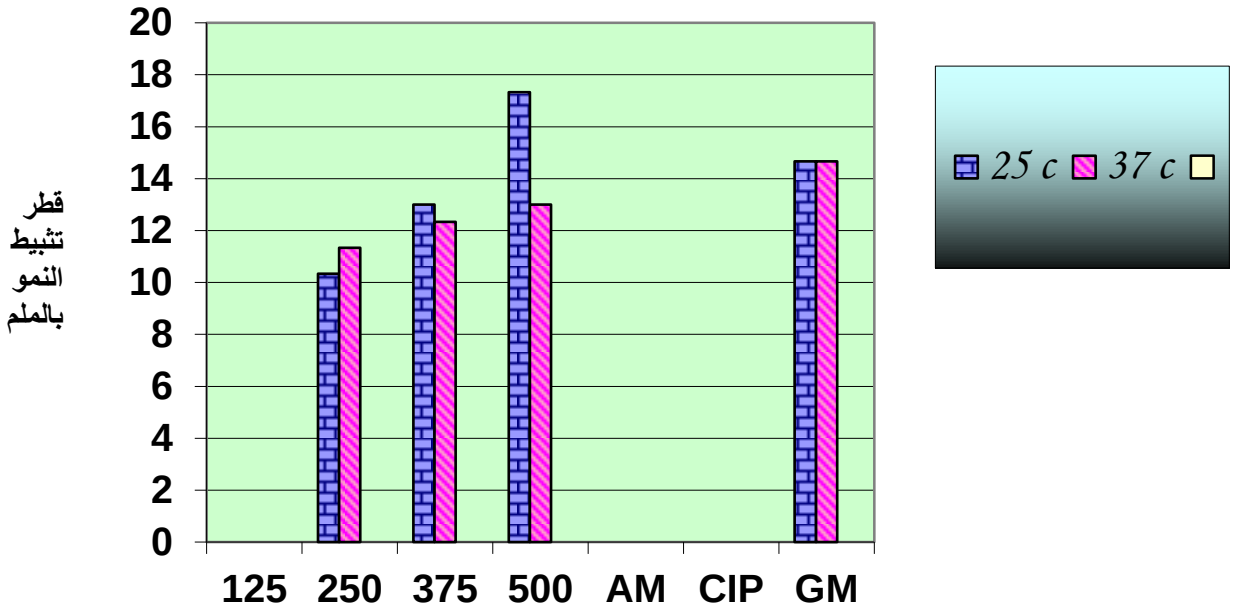
تركيز مادة العسل والمضادات الحيوية

شكل (2) العلاقة بين التراكيز المختلفة لمادة العسل المحلي والمضادات الحيوية ومعدل أقطار تثبيط النمو لجرثومة المسبحيات .



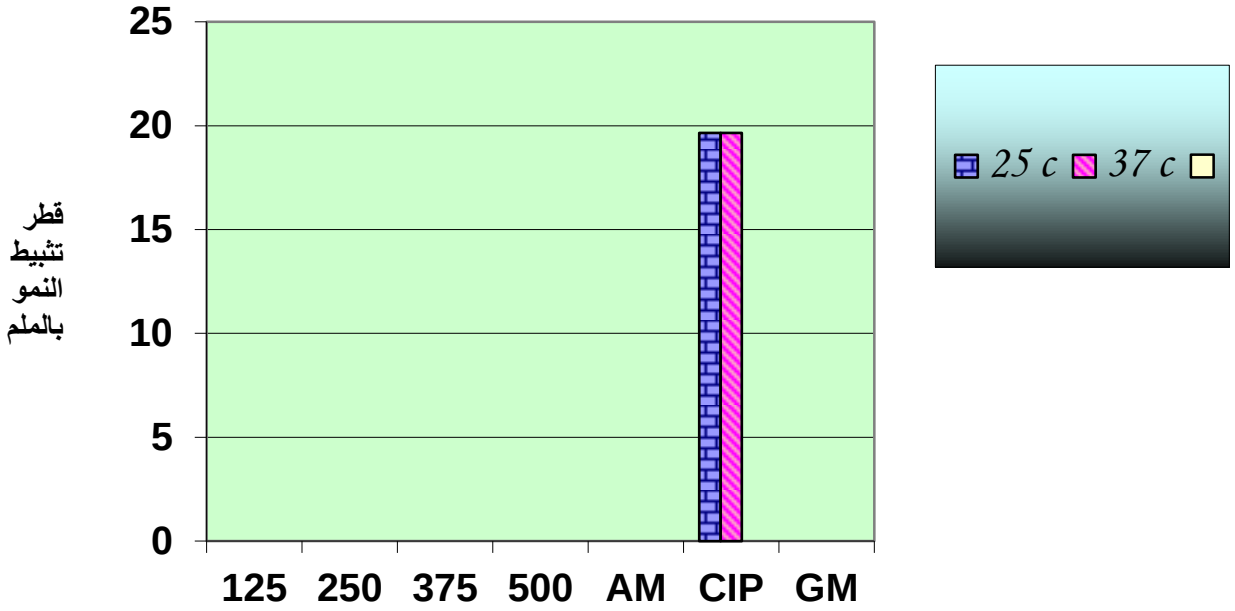
تركيز مادة العسل والمضادات الحيوية

شكل (3) العلاقة بين التراكيز المختلفة لمادة العسل المحلي والمضادات الحيوية ومعدل أقطار تثبيط النمو لجرثومة الاشريشيا القولونية .



تركيز مادة العسل والمضادات الحيوية

شكل (4) العلاقة بين التراكيز المختلفة لمادة العسل المحلي والمضادات الحيوية ومعدل اقطار تثبيط النمو لجرثومة السالمونيلا .



شكل (5) العلاقة بين التراكيز المختلفة لمادة العسل المحلي والمضادات الحيوية ومعدل اقطار تثبيط النمو لجرثومة الزائفة الزنجارية .

في هذه الدراسة اختبرت الفعالية الحيوية المضادة للجراثيم لمادة العسل المحلي الذي تم تحضيره بعدة تراكيز متدرجة وهي 125 ، 250 ، 375 ، 500 ملغم / مل ضد نمو وتكاثر خمسة أنواع من الجراثيم الممرضة والمعزولة من حالات مرضية مختلفة اذ كان نوعين منها موجبة لصبغة كرام وهي العنقوديات الذهبية *Staphylococcusaureus* والمسبحيات *Streptococcus spp.* وثلاثة أنواع سالبة لصبغة كرام وهي الاشريشيا القولونية *E . coli* السالمونيلا *Salmonella spp.* والزائفة الزنجارية *Pseudomonas aeruginosa*

استخدم لهذا الغرض طريقة الانتشار عبر الاكار (Agar well diffusion method) والذي تم من خلاله التعرف على أقطار تثبيط النمو الذي تحدثها تراكيز العسل المضاف إلى الحفر المعمولة في الوسط الزراعي وطريقة التخفيف بالأنابيب Tube dilution method والذي تم من خلالها حساب وتحديد قيمة التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى (MBC) لمادة العسل اتجاه الجراثيم التي تبدي فعالية واضحة في الإطباق الزراعية ، كما تم بالإضافة الى ذلك معرفة تأثير درجة الحرارة حفظ الإطباق الزراعية على النتائج من خلال حفظ الإطباق الزراعية بدرجتين حراريتين مختلفتين وهي (25مْ و37مْ) .

أوضحت نتائج اختبار الانتشار عبر الاكار أن التراكيز المختلفة لمادة العسل كان لها تأثيراً واضحاً وملموساً اتجاه مجموعة الجراثيم الموجبة لصبغة كرام وتأثيراً محدوداً اتجاه مجموعة الجراثيم السالبة لصبغة كرام (جدول 1 و 2) وهذا متفقمع ما توصلت اليه تقارير ودراسات أخرى أجريت في هذا الاتجاه على أنواع مختلفة من مادة العسل والذي تم جمعه من مناطق مختلفة من العالم اذ وجد الباحث Tajik (7) من خلال أختبار الفاعلية المضادة للجراثيم لمادة العسل الذي تم جمعه من مناحل مدينة Unmia في إيران على عدد من الجراثيم الممرضة الموجبة والسالبة لصبغة كرام ، بأن جرثومة العنقوديات الذهبية الموجبة لصبغة كرام كانت الأكثر تحسساً في حين كانت الاشريشيا القولونية السالبة لصبغة كرام الأقل تحسساً وهذه النتيجة أتفقت أيضا معتقارير ودراسات أخرى في هذا المجال والتي أختبرت فيها حساسية جرثومة العنقوديات الذهبية من قبل دراسات بكتريولوجية على مادة العسل ويرجع سبب ذلك إلى حساسية هذه الجرثومة المرتفعة اتجاه العسل (8 و 9) وعلى الرغم من إن سبب هذا التحسس المرتفع لهذه الجرثومة غير معروف على وجه الدقه ولكن يعتقد إن له علاقة مع البيئة الحامضية التي توفرها مادة العسل الطبيعي (9). كما درس الباحثانYagoubو El-Toum (10)فعالية ثلاثة أنواع من العسل وهي (Sidir , Sunflower , Sunut) ضد نمو عدد من الجراثيم وهي (*P. aeruginosa* , *S.aureus* , *E.coli* , *Klebsiellaaerugenes*)إضافة إلى خميرة المبيضة البيضاء ، كانت جرثومة العنقوديات الذهبية *S.aureus* الأكثر تحسساً اتجاه الأنواع الثلاثة في حين كانت الجراثيم *P.aeruginosa* , *K.aerugenes* , *E.coli* قليلة التأثر .العسل الأردني كان له تأثير تثبيطي واضح على نمو جراثيم *Shigelladysentery* , *Salmonella dublin* , *Bacillus subtlus*,*S.aureus* وقد بينت الدراسة إن الجراثيم موجبة كرام كانت الأكثر حساسية للتأثير التثبيطي لمادة العسل بالمقارنة مع الجراثيم سلبية كرام وقد يكون

لطبيعة التركيب الكيميائي للجدار الخلوي دورا في حساسية الجرثومة لتأثير العسل التثبيطي (2) . في نيجيريا أجريت دراسة من قبل الباحث Mogessie(11) اذ سجلت فيها فعالية العسل النيجري ضد نمو عدد من الممرضات المعزولة من الغذاء مثل*E.coli* , *S.enteritidis* , *S.typhimurium*وقد تم تثبيط نموها عند التركيز 15-20% في حين كان التركيز 10 % كافيا لتثبيط نمو جرثومة *S.aureus* ومما تجدر الإشارة اليه إلى إن جرثومة الزائفة الزنجارية قد أظهرت مقاومة مرتفعة اتجاه تراكيز العسل المحلي كافة وهذه النتيجة عززت النتائج التي تم التوصل إليها من قبل Efem (12) والذي سجل مقاومة جرثومة الزائفة الزنجارية للعسل كما وجد الباحثانYagoubو-El Toum(10) عدم تحسس جرثومة الزائفة الزنجارية اتجاه نوعين من العسل هي Sun honey و Sunflower honey بينما اظهر حساسية معتدلة اتجاه النوع Sidir honey وأعزا الباحث ذلك إلى إن الخضاب الخارجي exopigmentation المنتج من قبل هذه الجرثومة كان له دوراً معنوياً في بقاء ومقاومة جرثومة الزائفة إلى أجواء بيئية معينة . كما وتعزز نتائج دراستنا الحالية وبصورة عامة ما توصلت الية الدراسات العلمية العالمية التي أجريت على أنواع مختلفة من العسل والذي تم جمعه من دول مختلفة (13 , 14 و 15) أظهرت نتائج الدراسة تباين واضح لعامل التركيز المستخدم لمادة العسل المحلي في التأثير على جميع جراثيم الاختبار إذ أظهرت النتائج تبايناً واضحاً وملموساً بين التراكيز المستعمله في تأثيرها في نمو الجراثيم إذ في الوقت الذي لم تعطي معاملة السيطرة (الماء المقطر) أي تأثير يذكر في تثبيط النمو نجد أن زيادة تراكيز مادة العسل رافقه زيادة معنوية إحصائية وعند مستوى احتمالية ($P < 0.05$) في أقطار تثبيط النمو الجرثومي حتى بلغ أقصى تأثير له عند التركيز 500 ملغم / مل وقد يعود السبب في ذلك إلى إن زيادة التركيز المستخدم قد رافقه زيادة في المادة او المواد الفعالة ضد الجراثيم الموجودة في مادة العسل والذي ينعكس ايجابياً على النتائج في الأطباق الزراعية .

كما كان لعامل حفظ الأطباق الزراعية عند درجتين حراريتين هما 25مْ و37مْ تأثير واضحا على النتائج اذ أظهرت القراءة الإحصائية الى ان أقطار تثبيط النمو في الإطباق الزراعية التي حفظت بدرجة حرارة 25مْ مدة 24 ساعة قد أظهرت تفوقاً معنوياً احصائي على نتائج الإطباق الزراعية التي حفظت بدرجة حرارة 37مْ وللمدة الزمنية نفسها لمعظم التراكيز وعند معظم الجراثيم المختبرة عدا بعض النتائج التي لم تتأثر بدرجة حرارة الحضن ، ان تأثير هذا العامل قد يعود إلى ان حفظ الإطباق في درجة حرارة 25مْ قد يسهل من عملية انتشار تراكيز العسل في الطبق الزراعي مما أعطى فرصه اكبر للمادة الفعالة في الوصول إلى نقطة ابعد وبالنتيجة أعطت نتيجة ايجابية في هذا الاتجاه .

تعزى قدرة مادة العسل على تثبيط او قتل الجراثيم الى عوامل فيزيائية وكيميائية متنوعة فقد ذكر الباحث Molan(16) الى ان محتوى العسل من الكاربوهيدرات يعد احد العوامل التي تكسب مادة العسل فعالية مضادة للجراثيم والميكروبات بصورة عامة ، كما يحتوي العسل على lysozyme المعروف كعامل مضاد لنمو وتكاثر الجراثيم (17) والفلافونويدات الموجودة في العسل هي الأخرى تساهم وبالتآزر مع المركبات الأخرى في الفعل المضاد للجراثيم ولكن دورها في هذا الاتجاه محدودا نسبياً كما أشار الى ذلك الباحث (18) . كما عزلت عدة احماض اروماتية من أعسال نيوزلندية ووجد ان لها فعل مؤثر على نمو وتكاثر الجراثيم (16 و 19) .ذكرتتحقيقات أخرى في هذا الاتجاه الى ان الحامضية المنخفضة التي تتراوح بين 3.2-4.5 بجانب التناضحية العالية التي يتمتع بها العسل نتيجة محتواه السكري المرتفع له مسؤولية وبشكل معنوي على التأثيرات المضادة للجراثيم (20) كما عزلت عدد من المركبات الطيارة من نماذج عسل مختلفة اثبت فعاليتها ضد الجراثيم (21 و 22) ولكن مساهمتها الكمية في الفعل المضاد للجراثيم لمادة العسل لم يتم فحصها .

كما اثبتت دراسات أخرى الفعالية غير البيروكسيدية للعسل والمستخلصة بالمذيبات العضوية الا انها فشلت في معرفة طبيعتها الكيميائية (23 و 24). ذكرت مصادر عدة الى ان الفعل المضاد للجراثيم لمادة العسل يتمثل بالفعل التازري لعوامل عدة أهمها حامضية العسل ،الازموزية ، ووجود بيروكسيد الهايدروجين H2O2 والمواد الطيارة إضافة الى متبقيات شمع النحل ومادة البروبولس والرحيق وحبوب الطلع التي من الممكن ان تتواجد في العسل على هيئة متبقيات(25 ، 26 و 27) ومن العوامل الكيميائية المهمة في هذا الاتجاه والتي ثبت تواجدها في العسل هي مركبات Hydrogen peroxide(19) ومركب Cecropin-A و مركب Mellitin و مركب Methyl-3-4-5-Trimethoxybenzoate ،Methyl 3-5-Dimethoxy-4-hydroxybenzoate ومركب

3-5-Dimethoxy-4-hydroxybenzoic acid ، 3-4-5-methoxybenzoic acid ، nector (27 ، 28) .كما تحتوي بعض أنواع العسل على مشتقات التتراسايكلين derivativesTetracycline ، بيرو كسيدات Peroxides ، انزيم الاميليز Amylase ، الستربتومايسين Streptomycin ، السلفاثيازول Sulfathiazole ، تربينات Trepens ،alcohol Benzyl ، Benzoic acid(29 ، 30).

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تقييم الفعالية السمية والتثبيطية للمستخلصات المائية و الكحولية لبعض النباتات على نمو الجراثيم المرضية المعزولة من حالات الاسهال.

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الخلاصة

تضمنت هذه الدراسة عزل وتشخيص الانواع الجرثومية المتمثلة , *Escherichia coli*, *Salmonellatyphimurium* , *Shigelladysenteriae* من الاطفال المصابين بالاسهال من كلا الجنسين , وقد اعتمدت الاختبارات المجهرية والكيموحيوية ونظام API لتشخيص هذه الجراثيم ، والتي اظهرت مقاومتها للمضادات الحيوية التالية:- Ceftazidine, Cephalothin, Rifampicin, ,Tetracyclin , Ampicillin Vancomycimو درس تأثيرالمستخلصات الخام للنباتات (النومي البصره , الزنجبيل , عرق السوس , الينسون , الشاي , القهوة , الهيل , الكمون , الزعتر وبذور الحلبة) على نمو الجراثيم المعزولة من حالات الاسهال , وتم الكشف عن مكوناتها الكيميائيه وذات باها حامضي واعطى المستخلص الكحولي لنومي البصرة افضل فعاليه تثبيطية لنمو جرثومة *E.coli* بقطر تثبيطي 47 ملم.

كلمات مفتاحية :الزعتر،العرقسوس،الشاي الاسود، الينسون، النومي بصره، القهوة، الهيل، الكمون، الزنجبيل بذورالحلبة.

Evaluation of Toxicity and Antimicrobial Activity of Watery and Alcoholic extracts of some plant on growth of pathogenicBacteria isolatedfrom diarrhea.

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Summary

In This study, the bacteria Escherichia *Coli*, *Salmonella typhimurium* and *Shigella dysentarea* were isolated from diarrhea in infants. Microbiological and biochemical tests were conducted to identify these bacteria. In the Identification of bacterial Species the API-System was used. Sensitivity test of bacterial isolates revealed high resistance to many Antibiolics like Ampicillin, Tetracycline, Ceftazidine, Cephalothin ,Vancomycin and Rifamipicin.

The effects of extracts of (*Citrus aurantifolia ,Zingiber offienals, Glycyrrhiza glabra, Pimpinellaanisum, Camellia ,sinessis Coffeiaarabica, Elattariacar domomum, Cumminum cumminum, Thymus vulgaris and Trigonella foenum geoecum* in inhibiting bacteria isolated from diarrhea were studied , also indication of presence of chemical active components in extracts was observed . The Preliminary chemical tests revealed acidic PH of all extracts, and the best antibacterial was effect that of Citrus *aurantifolie* an lcoholic extract on growth of *E.coli* with inhibition zone diameter 47mm.

Key words:Glycyrrhiza glabra, Pimpinella anisum, Camellia Sinesis offiecinales Zingiber, Elettaria Cardomomum, Coffeiaarabica, Thymusvulgaris

المقدمة

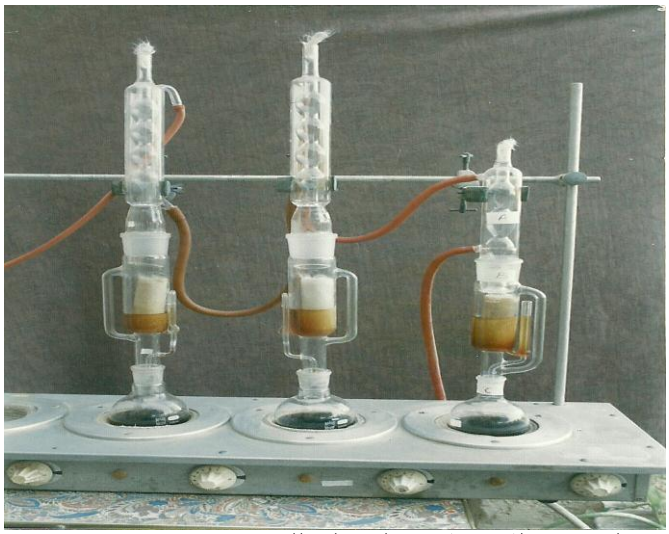
يشكل الاسهال مرضا رئيسيا لاعتلال الصحة وخاصة عند الاطفال والرضع اذ يسهم بحدوث سوء التغذيه ويبقى الاسهال من اكثر الصعوبات التي يواجهها الاطباء في العالم فضلا عن انه المسبب الرئيس للوفاة في سن الطفوله لاسيما في البلدان النامية .اشارت تقارير منظمة الصحة العالمية على مستوى العالم الى حدوث بليون حالة اسهال سنويا ينتج عنها 3,3 مليون من الوفيات (2و1) وتعد الجراثيم والطفيليات والفيروسات من المسببات الرئيسيه لحدوث الاسهال(3) . وقد اشارتتقارير البيئه الحديثة الى ارتفاع عدد الممرضات المعزوله من مزارع الاسهال من 29.1 % الى 52.8 %(4). وعدد من الانواع الجرثومية التقليديه التي اعتقد سابقا انها من المتعايشات في القناة

الهضمية (4) . يعرف الاسهال على انه الزيادة في فقدان السوائل والايونات مع البراز (5) . وقد يكون الاسهال حادا Acute او مزمنا chronic فالاسهال الحاد هو خروج السوائل بالبراز اكثر من الطبيعي عادة و اكثر من ثلاث مرات في اليوم ويستمر (5-6) ايام , اما الاسهال المزمن فهو مازادت مدته عن ثلاث اسابيع (3). ان الاسهال الحاد يسبب استنزاف الصوديوم والماء وكذلك فقدان البوتاسيوم مما يسبب اختلافا في توازن الحوامض والقواعد في الجسم اذ ينتج زيادة في حموضة الدم acidosis بسبب زيادة فقدان البيكاربونات مع البراز .اما في حالة الاسهال المزمن فيجدث حالة تدني تركيز البوتاسيوم في الدم Hypokalemic alkalosis .فالجفاف ليس حالة عدم توازن سوائل الجسم فحسب بل هو عدم انتظام في الايض و الذي يؤثر في الجسم (6) . تكون ميكانيكية تاثير الجراثيم المعوية عن طريق غزو الطبقة المخاطية للامعاء او افراز السموم المعوية , مما ينتج عنة تأثيرات حادة قد تؤدي الى حدوث اسهال دموي Bloody diarrhea وارتفاع درجة الحرارة مع حدوث التقيؤ وتستخدم المضادات الحيويه للمعالجة الا ان الاستخدام المتزايد و العشوائي ادى الى ظهور سلالات مقاومة للمضادات المستخدمة في الوقت الحاضر وهذه المقاومة ناتجة عن المقاومة الطبيعية او المقاومة المكتسبة اذ ان الجراثيم الممرضة تحتوي على بلازميدات تتاقلم مع مقاومة الادوية المتعددة و التي اصبحت مشكلة اساسية في معالجة امراض معديه خاصة بالانسان (7).لذا بدا التفكير بأستخدام بدائل اخرى والمتمثلة بالاعشاب الطبيعية فقد درست انواع من الاعشاب وتأثيرها على الكائنات المجهرية المختلفة .تهدف الدراسه الى ايجاد افضل انواع الاعشاب الطبيعية مثل (بذرة الحلبة , الكمون , الهيل , الزعتر , القهوة , الشاي , النومي بصره , الزنجبيل , العرقسوس والينسون) لمعرفة مدى تاثيرها التثبيطي على الجراثيم المسببة للاسهال.

المواد وطرائق العمل

اخضعت 37 عينة بمراكز الاطفال المصابين بالاسهال للفحوصات المختبرية المتعلقة بعزل وتشخيص الجراثيم المسببة اعتماداً على الصفات المزرعية والفحص المجهري والاختبارات الكيميوحيوية وفحوصات العدة التشخيصية API -20E كما استخدم التشخيص المصلي لتحديد الانماط المصلية الجرثومية وقد تمت مقارنة النتائج مع (9و8) كما تم فحص حساسيتها للمضادات الحيوية المتوفرة في السوق المحلي والمستخدمة للعلاج وفقاً لطريقة Kirby – baur disk method القياسية (10).تم الحصول على كميات من النبات من المعاشب الموجودة في الاسواق المحلية وشخصت اجناس النباتات في المعشب الوطني التابع لمديرية النبات في وزارة الزراعة في ابي غريب .طحنت النباتات كل على انفراد بواسطة مطحنة كهربائية (blender) وحفظت النماذج في اكياس بلاستيكية نظيفة بدرجة حرارة الغرفة لحين الاستعمال .

حضر المستخلص المائي للنباتات حسب طريقة (11) بوزن 100 غم من النباتات ووضعت في دورق سعته 1000 مللتر واضيف اليه 500 مللتر من الماء المقطر ثم وضع الدورق على سطح ساخن مع محرك مغناطيسي لكي يغلي 10 – 15 دقيقة ثم ترك المسحوق للنقع لمدة ثلاث ساعات ثم رشح المحلول اولاً بأستخدام الشاش ثم رشح مرة ثانية بأستخدام ورق الترشيح (Edero NO.2) , جمع المحلول الرائق في دورق خاص وتم تركيزه بأستخدام المبخر الدوار تحت درجة حرارة لاتتعدى 55 مئوي وضغط مخلخل لحين التخلص من الماء والحصول على المستخلص الخام. وحفظت المستخلصات في -20 مئوي الى حين الاستعمال واذيب المستخلص الخام بالماء المقطر لحساب التراكيز المطلوبة .حضر المستخلص الكحولي بأستخدام كحول الايثانول بتركيز 70 % للنباتات وحسب طريقة (12) بأستخدام جهاز السوكسليت لاستخلاص النبات والذي يتكون من مسخن كهربائي و دورق زجاجي و وحدة الاستخلاص الحاوية على كاس سيليلوزي (Thimble) ووحدة التقطير , اجريت عملية الاستخلاص بتسخين المذيب(50 مئوي) فيتصاعد البخار من الدورق الى وحدة التقطير بواسطة انبوب يربط بينهما , يتكثف بخار الماء في وحدة التقطير وينزل على شكل قطرات على الكأس السليلوزي الحاوي على مسحوق النباتات الجاف والموجود في وحدة الاستخلاص . تستمر هذه العملية حتى يمتلئ تجويف وحدة الاستخلاص وينسحب المذيب مع المواد النباتية الذائبة فيه بواسطة عملية السيفون الى الدورق وتتكرر العملية مرات عديدة وهكذا حتى يصبح المذيب المتجمع في وحدة الاستخلاص رائقاً كما في الشكل (1) ، ركزت المستخلصات بجهاز المبخر الدوار وحسبت نسب المستخلصات من وزن المادة الجافة .



شكل (1) جهاز السوكسليت المستخدم للاستخلاص الكحولي للنباتات.

تم الكشف عن بعض المكونات الاساسية للنباتات (13).حضرت التراكيز النهائية للمستخلصات المائية والكحولية للنباتات وكانت بتركيز(20% , 40 %,60%, 80%) اذ استخدم الماء المقطر المعقم لتحضير تراكيز المستخلصات .

استخدمت طريقة الانتشار بالحفر لملاحظة تأثير المستخلصات المائية والكحولية للنباتات على نمو الجراثيم المعزولة من حالات الاسهال .اذ لقح الوسط الزرعي الصلب بواسطة قطنة معقمة محملة بالعالق الجرثومي الحاوي على (1.5×10^8 خلية/ مللتر) عملت حفر على سطح الوسط الزرعي المزروع بواسطة ثاقب الفلين . ووضعت التراكيزالمحضرة لكل مستخلص بمقدار (0.1 مللتر) لكل حفرة واستخدم الماء المقطر للسيطرة وتركت الاطباق بدرجة حرارة الغرفة لمدة 20 دقيقة , ثم حضنت بدرجة حرارة 37 مئوية وبمعدل ثلاث مكررات لكل عزلة . حددت فعالية المستخلص بقياس قطر منطقة التثبيط حول كل حفرة بالمليمتر , وتم حساب المعدل للمكررات الثلاثة (14 , 15) .

استخدم في هذه الدراسة 16 فارا تراوحت اوزانها (30.8 – 35.2) غرام وبمعدل 33 غرام لدراسة الجرعة المميتة الوسيطة للمستخلص المائي والكحولي , قسمت هذه الفئران عشوائيا الى 4 مجاميع وبواقع 4 فئران لكل مجموعة واعطيت جرع متدرجة 5و10 و15 غرام / كيلو غرام من وزن الجسم . وذوبت بكمية من الماء المقطر بمقدار 0.1 مللتر 10 غرام من وزن الجسم ، وجرعت مجموعة بالماء المقطر لوحدة وعدت بوصفها مجموعة سيطرة (16) . كررت التجربة على نفس العدد من الحيوانات وبنفس الطريقة بأستخدام المستخلصات المائية والكحوليه وتمت مراقبة الحيوانات خلال 24 ساعة واجريت الصفة التشريحيه للحيوانات من كل مجموعة للتاكد من عدم وجود علامات نسجية ونزفية تدل على السمية .

النتائج والمناقشة

جمعت عينات البراز من الاطفال المصابين بحالات الاسهال من كلا الجنسين وتم اجراء الفحوصاتالتشخيصية الزرعية وكانت عينات البراز سائلة او نصف سائلة او مائية او مخاطية اودموية وبعد الحصول على المستعمرات المنفردة من حالات الزرع شخصت تبعا لشكل المستعمرة ولونها اذ شخصت المستعمرات اوليا على وسط الماكوني الصلب اعتمادا على شكل المستعمرة ولونها اذ كانت المستعرات العائدة لجنسي *Shigella*و*Salmonella* شاحبة اللون ودائرية الشكل ملساء بقطر 2-4 ملم في حين كانت المستعمرات العائدة لجنس *E.coli*ورديه اللون لكونها مخمرة لسكر اللاكتوز .كما استخدم وسط (اكار*Salmonella* – *Shigella*) للتفريق بين جنسي*Shigella* و *Salmonella* اذ تظهر مستعمرات جنس *Shigella* شاحبة اللون ودائريه الشكل وملساء في حين تظهر مستعمرات *Salmonella* بيضاء اللون ذات مركز اسود بسبب انتاج غاز كبريتيد الهيدروجين. وكذلك شخصت وفقا الى شكل الخلايا المجهرية ظهرت الخلايا في الفحص المجهري بعد معاملتها بملون غرام عصوية قصيرة ذات لون احمر وظهرت الحركة واضحة مع *Salmonella*و *E.coli*ومعدومة في *Shigella* وهذا يتفق مع نتائج (17).

تمت الاختبارات الكيموحياتية (18) , وكذلك كانت نتائج فحوصات العدة التشخيصية Apl-20Eمؤكدة للتشخيص السابق, كما استخدم التشخيص المصلي باستخدام المصول الاحادية والمتعددة التكافؤ لتؤكد تشخيص جنس*Salmonella*.

ابدت الجراثيم المعزولة تباينا في حساسيتها ومقاومتها للمضادات الحيوية المستخدمة اذ اكدت النتائج الحالية وبعد مقارنتها مع نتائج الباحثين الاخرين مقاومة معظم انواع البكتريا للكثير من المضادات الحيوية وهذا مطابق لما وجده (20,19) وهذا يعود الى الاستخدام العشوائي للمضادات الحيويةمما ادى الى ظهور احياء مجهرية مقاومة لهذه المضادات وتنتشر هذه المقاومة بين السلالات الجرثوميه بأليات مختلفة مما قد يسبب مشاكل من الناحية الصحية والاقتصادية في العديد من دول العالم كما في الجدول (1) .

جدول (1) قابلية انواع البكتريا العاندة لجنس *E.coli* , *S.typhi*, *sh.dysentarie* , على حساسية ومقاومة مضادات الحياة المختلفة :-

ت	نوع البكتريا	penicillin	Ampicillin	Rifampicin	Tetracyclin	vancomycin	gentamycin	Amikacin	ceftazidine	ciprofloxacin	Cephalothin
1	*E. coli*	R	R	I	R	R	S	S	R	S	R
2	*S.typhi*	R	R	R	R	R	I	S	R	S	R
3	*Sh.dysentarie*	R	R	I	R	R	R	S	R	S	R

R- Resistant ، I- Intermediate ،S-Sensitive

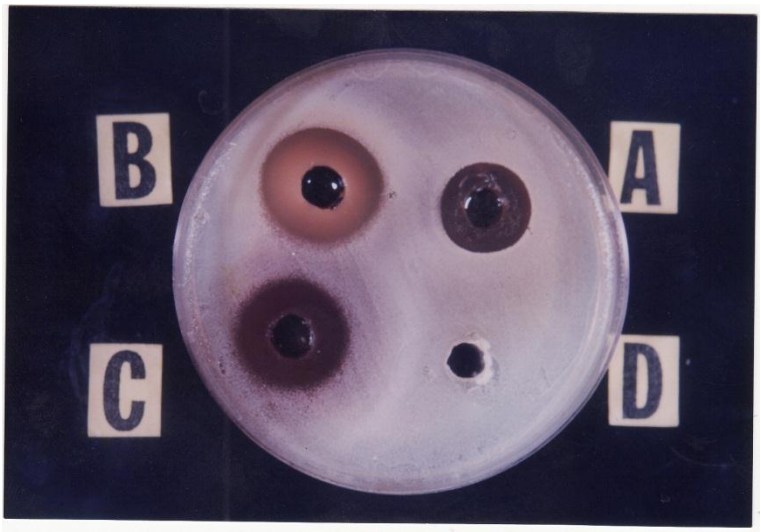
بلغت نسبة وزن المستخلصات المائية للقهوة , بذور الحلبة ,الهيل , النومي بصره , الزنجبيل , الكمون . عرق السوس , الينسون , الشاي والزعتر كالاتي
%8,6 , %6,2 , %3.1, %6,3, % 8,2, % 9%, %7,6, %10,%8,9, %8,5,%. بينما كانت نسبة وزن المستخلصات الكحولية 10%, 9% ,%5,4 , %6,5 , 9% , 11% , 8,2% 11,5% 10% , 10,4% على التواليولوحظ ان المستخلص الكحولي الخام للنباتات بعد تركيزه وتجفيفة اكثر من وزنة الناتج من المستخلص المائي وهذا يدل على ان المواد الاساسية المتواجدة في النباتات قابلية ذوبانيه في الكحول اعلى مما في الماء وكان لكل مستخلص من المستخلصات النباتية مواصفات خاصة تتميز عن مواصفات المستخلص الاخر من حيث اللون والطعم اذ تلون مستخلص نبات بذور الحلبة و الهيل و الزنجبيل و الكمون وعرق السوس و الينسون بلون بني فاتح وبطعم مائل للحلاوة بينما تميز مستخلص الشاي و النومي بصره و القهوة بلون بني غامق مائل للاحمرار و بطعم مائل للمرارة.اثبت التحليل الكيميائي العقاقيري من قبل الباحثين الذين درسوا الصفات الدوائية لبعض النباتات احتواء جذور وسيقان واوراق وازهار وبذور هذه النباتات على العديد من المواد الفعالة التي ادخلت بعد استخلاصها وتنقيتها في تركيب معظم الادوية والوصفات الطبية الحديثة مثل القلويدات و الكلايكوسيدات و الزيوت الطيارة والثابتة والراتنجات و الصمغيات و الصابونينات , الكومارينيات و التانينات (21) كما في الجدول (2) .

جدول (2) المكونات الاساسية والباها للمستخلصات النباتية

اسم النبات	الباها	الكلايكوسيدات	التانينات	الفينولات	الصابونينات	الراتنجات	القلويدات	الكومارينات	الفلافونات
نومي البصرة	6,5	+	+	+	+	+	+	+	+
الزنجبيل	5,6	-	+	-	+	-	+	-	-+
عرق السوس	6,4	-+	+	+	+	+	+	-	-
الينسون	5,8	-	+	+	+	+	+	-	+
الشاي	6,9	+	+	+	-	-	+	+	+
القهوة	5,4	-	+	+	+	-	+	+	+
الهيل	5,7	-	+	-	+	-	-	-	-+
الكمون	6,0	-	+	+	-+	+	+	+	+
الزعتر	5,0	-+	+	+	-	-	-	-	-
بذور الحلبة	6,6	-+	+	+	+	+	+	+	+

+ وجود المادة المراد الكشف عنها ، - عدم وجود المادة المراد الكشف عنها ،+- كمية قليلة جدا.

اعطت المستخلصات النباتية نتائج ايجابيه في تثبيط نمو البكتريا المسببه للاسهال كما في الجداول (5,4,3) والشكل رقم (2).



شكل رقم (2) الاقطار التثبيطيةللمستخلص المائي للشايعلى نمو جرثومة*E.coli - AB*- جرثومةC*Shigelladysentarie*- القطرالتثبيطي للمستخلص الشاي على نمو بكتريا *Salmonella Typhi*بتركيز 20%D-ماء المقطركسيطرة.

جدول (3) الاقطارالتثبيطة على نمو البكتريا العائدة لجنس *E.coli* بتاثير المستخلصات المائيه والكحولية لبعض النباتات(ملم).

ت	نوع المستخلص	20% مائي	40% مائي	60% مائي	80% مائي	control	20% كحولي	40% كحولي	60% كحولي	80% كحولي	control
-1	القهوة	28	32	34,3	38	-	30	33	35	40	-
-2	بذور الحلبة	-	-	-	-	-	-	-	-	-	-
-3	الهيل	-	-	-	-	-	-	-	-	-	-
-4	النومي بصرة	32	40	42	45	-	37	42	45	47	-
-5	الزنجبيل	14	15	20,3	22	-	17	20,6	25	30	-
-6	الكمون	-	-	-	-	-	-	-	-	-	-
-7	العرقالسوس	20	21,3	23	25	-	22	23	27	30	-
-8	الينسون	-	-	-	-	-	-	-	-	-	-
-9	الشاي	20	22	25	29	-	23	25,3	27	30	-
-10	الزعتر	-	-	-	-	-	-	-	-	-	-

جدول رقم (4) الاقطار التثبيطية على نمو البكتريا العائدة لجنس *Salmonella typhimurium* بتاثير المستخلصات المائية والكحولية لبعض النباتات (ملم).

نوع المستخلص	20% المائي	40% المائي	60% المائي	80% المائي	control	20% الكحولي	40% الكحولي	60% الكحولي	80% الكحولي	control
القهوة	28	30	35	40	-	31	35	37	40	-
بذور الحلبة	20	23	25	27	-	21,3	24	25	27	-
الهيل	-	-	-	-	-	-	-	-	-	-
النومي بصرة	30	38	40	42	-	35	40	43	45	-
الزنجبيل	20,3	25	27	30	-	23,3	27	29,6	32	-
الكمون	-	-	-	-	-	-	-	-	-	-
العرق سوس	-	-	12,6	16	-	13	15	17	20	-
الينسون	12	17	20	22	-	15	17	20,6	25	-
الشاي	25,3	28	32	40	-	27	29	33,6	40	-
الزعتر	-	-	-	-	-	-	-	-	-	-

جدول (5) الاقطارالتثبيطية على نمو البكتريا العائدة لجنس *dysentarieShigella* بتأثير المستخلصات المائية والكحولية لبعض النباتات (ملم).

نوع المستخلص	%20	%40	%60	%80	control	%20	%40	%60	%80	control
	المائي	المائي	المائي	المائي		كحولي	كحولي	كحولي	كحولي	
القهوة	28	30	33,3	34	-	32	34	36	39	-
بذور الحلبة	20,3	21	22	25,6	-	22	25	27	28	-
الهيل	-	-	15	20	-	-	-	15,3	21	-
النومي بصرة	30	33	35	38	-	33	35	38,3	40	-
الزنجبيل	-	-	-	-	-	-	-	-	-	-
الكمون	-	-	-	-	-	-	-	-	-	-
العرق سوس	-	-	-	-	-	-	-	-	-	-
الينسون	-	15,3	20	22	-	12,3	15,6	22	25	-
الشاي	27	29	30,3	32	-	28	29	30	33	-
الزعتر	-	-	-	-	-	-	-	-	-	-

و يعود التفاوت في درجة تاثير المستخلص على نمو الجراثيم اعتمادا على نوع الجرثومة و على نوع المواد الفعالة المتواجدة في النباتات فقد وجد ان المستخلصات النباتية اكثر تأثيرا على نمو الجراثيم المرضية من المضادات الحيوية وان التباين الحاصل في تركيز المستخلصات النباتية وبين درجة تاثيرها على نمو الجراثيم مرتبط مع زيادة التركيز اذ انه كلما زاد تركيز المستخلص زادت تراكيز المواد الفعالة فيه وبالتالي زيادة تثبيط نمو الجراثيم (22).ولقد اوضحت نتائج مستخلص الحلبة ان لها تأثير فعال على جرثومة *S.typhimurium* و *dysentarieShigella* وهذه الفعالية قد تعود الى وجود قلويدtrigonellin والكومارنيات و nicotenicacid اذ تمتلك هذه المواد فعالية تثبطية للجراثيم المرضية (23 و 24) ولم يكن له تأثير على جرثومة *E. coli* وجاءت هذه النتيجة مطابقة مع (19) . وسجل مستخلص النومي بصرة فعالية تثبيطية عالية على نمو الجراثيم وقد يكون ذلك لاحتواءه على مادة Morin, Naringenin , hesperatin , Sesellin وكلايكوسيدات التي تستعمل لمعالجة التهابات المعدة والامعاء وتزيد عصارات المعدة الهاضمة (23)

اما الزعتر والكمون والهيل فلا توجد له فعالية تثبيطيه على الانواع الجرثومية المرضية وانما هو طارد للرياح ويقوي جهاز الهضم والمبيض ومدر للحليب , بينما مستخلص العرق سوس كانت له فعالية تثبيطية لاحتواءه على الصابونينات , الكلايكوسيدات والكلكوز و السكروز والراتنجات والاسبارجين (23 و 25)

وكانت لفعالية الشاي والقهوة والينسون والزنجبيل تاثير تثبطي عالي على نمو الجراثيم المرضية المعزولة من حالات الاسهال لاحتواءها على التانينات التي لها فعالية تثبيطية لقدرتها على تحفيز الخلايا البلعمية وله فعالية لتحطيم البروتينات والتراكيب الاخرى المتواجدة على جدار الخلية الجرثومية التي تستخدمها الجرثومة للالتصاق (26) والقلويدات التي لها فعالية قاتلة للاحياء المجهرية لقدرتها على التاثير على الحامض النووي للخلية و انحشارها في اشرطته (27) وكذلك كان لها القدرة على تحطيم الغشاء البلازمي للخلية البكتيرية وتحطيم ماتحويه من بروتينات ودهون او انها قد تتداخل مع سلسلة التفاعلات الايضية اللازمة لنمو الكائن المجهري (28) . ولم نلاحظ اي علامات سمية ونزفية او نسجيه على الفئران المختبرية .

ذكر ان لهذه المستخلصات القدرة على تثبيط او قتل طيف واسع من الجراثيم وعدم الاقتصار على انواع محددة كما هو الحال في المضادات الحيوية لامتلاك هذه النباتات مركبات فعالة لها القابلية على الانتشار في الانسجة والنفاذ بكفاءة عالية والقدرة على اختراق الجدار الخلوي للبكتريا وتثبيط نموها من جهة اخرى ولا تتولد مقاومة تجاهها كما في المضادات الحيوية فضلا عن سهولة الحصول عليها ورخص ثمنها (29و30) .

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دراسة مرضية نسجية في بعض اسماك نهر دجلة المصابة بالطفيليات

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الخلاصة

جمع وفحص 69 نموذجا من اسماك نهر دجلة عند مدينة بغداد ومن ثلاث محطات هي (التاجي والشواكة والزعفرانية) للمدة من كانون الثاني إلى كانون الاول 2010 وفحصت اثناء مدة الدراسة الاعضاء الداخلية لخمسة أنواع من ألاسماك هي (الحمري *Barbus luteus*، الكرسين *Carassius carassius*، البلعوط الملوكي *Chondrostoma regium*، الخشني *Liza abu* والجري الاسيوي *Silurus triostegus*). بينت نتائج الدراسة إصابة إلاسماك المفحوصة بـ 39 نوعا من الطفيليات الداخلية والخارجية منها 21 نوعا من الأبتدائيات(ألاوالي) (خمسة من الهدبيات (*Eimeria cyprini*، *E. dogieli*، *E. spharica*، *Trichodina domerguei* ، *T. nigra*) وستة عشر نوعا من البوغيات (*Chloromyxium bychowiski*، *Myxidium monstrasum*، *M. pfeifferi*، *M. rhodei*، *Myxobolus bramae*، *M. cyprini*، *M. cyprinicola*، *M. drgajini*، *M. koi*، *M. macrocapsulari*، *M. mulleri*، *M. oviformes*، *M. parvus*، *M. poljanski* ، *M. pfeifferi* و *M. spharica*)) واثنا عشر نوعا من المخرمات (تسعة منها احادية المنشأ (*Ancylodiscodes silure*، *Dactylogyrus achmerovi*، *D. anchoratus* ، *D. dulkiti.* ، *D. formosus* ، *D. skarjabini*، *D. varicohrini*، *D. vastator* و *Diplozoon pavloviski*) وثلاثة انواع من الديدان المخرمة ثنائية المنشأ (*Ascocotyle coleostoma*، *Diplostomum commutatum* و *D. spathacum*)) ونوع واحد من الديدان الخيطية *Rabdicona* sp. ونوعان من الديدان شوكية الرأس (*Neochinorhynchus cristatus*، *N. iraqensis*) ونوعان من القشريات (*Dermoergaslus varicoleus*، *Ergasilus sieboldi*) ونوع واحد من الفطريات *Icthyophonus hoferi*. تضمنت الدراسة الحالية التغيرات المرضية النسجية للطفيليات (البوغيات الحيوانية والديدان شوكية الرأس) في أنسجة العضلات، الكلية، الغلاصم والأمعاء للأسماك المصابة، وقد تميزت آفات عضلات ألاسماك المصابة بالبوغيات (ألاوالي) بعدم إنتظام الحزم العضلية وتجزء الحزم المتنخرة وإستبدالها بنسيج ليفي، وتنخرها ونزف وإحتقان مع إرتشاح للخلايا الإلتهابية وحيدة النواة. فضلا عن وجود تغيرات تنكسية نخرية شديدة في بطانة النبيبات الكلوية مصحوبة بارتشاح خلايا وحيدة النواة مع وجود نخر في الخلايا الظهارية للصفائح الغلصمية الثانوية مصحوبة بفرط تنسج للخيوط الغلصمية مع توسع الشعيرات الغلصمية Telengiectasis . أما التغيرات المرضية النسجية للأمعاء المصابة بالديدان شوكية الرأس فتميزت بإنسداد تجويف ألامعاء بمقاطع الطفيلي و وجود حطام نخري وإختزال شديد للزغابات المعوية مع إرتشاح للخلايا الالتهابية وحيدة النواة.

كلمات مفتاحية:- شوكيات الرأس , الهدبيات , مرضي نسجي, اسماك دجلة.

Histopathological Study of Some Tigris River Fish Which Infected by Parasites

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Summary

A total of 69 fish samples were collected from three stations Tigris River namely (Al-Zaafaraniya, Al-Tagei and Al-Shawaka) at Baghdad city, during the period from January to December 2010). These fishes were belonging to five species which were *Barbus luteus, Carassius carassius, Chondrostoma regium, Liza abu* and *Silurus triostegus.* The microscopial examination revealed infection with 39 species of ectoparasites and endoparasites including twenty one from protozoans (five ciliate (*E.cyprini, E.dogieli, E.spherica, T.domerguei, T.nigra*) and sixteen sporozoa (*C.bychowski, Myxidium monstrasum, M.pfeifferi, M.rhodei, Myxobolus bramae, M.cyprini, M.cyprinicola, M.drgajini, M.koi, M.macrocapsulari, M.mulleri, M.oviformis, M.paljanski, M.parvus, M.pfeifferi* and *M.spherica*)),twelve trematodes (nine of them from Monogenea (*A.siluri , D.achmerovi , D.anchoratus, D.dulkiti, D.formosus, D.skarjabini, D.varicohrini,D.vasator* and *Diplozoon pavloviski*) three

digenea(*A.coleostoma,D.commutatum, D.spathacum*)), one nematode *Rabdicona* sp., two acanthocephala (*N.cristatus, N.iraqensis*), two crustaceans (*D.varicoleus, E.sieboldi*) and one from fungus *I.hoferi*. The present study included the histopathological changes which caused by *Myxobolus* on the site of infection (muscles, kidneys and gills) included muscular disorganization, necrosis, bleeding, hemorrhage and mononuclear cells infiltration, hyperplasia and telengiectasis on the gills secondary lamella. Also, the present study included the histopathological changes on the intestine which infected with *Neochinorhynchus iraqinesis* included closed of intestinal lumen with parasites section, debris necrosis, severe reduce of intestinal filament and mononuclear cells infiltration.

Key words:- Neochinorhynchus, myxobolus, histopathology, Tiger fish.

المقدمة

تعتمد صحة الكائنات الحية باختلاف انواعها على السيطرة على الامراض فضلا عن وجود علاقة متوازنة مابين تلك الكائنات الحية والظروف البيئية المحيطة بها لذلك اصبح من الضروري دراسة المسببات المرضية ومعرفة دورة حياتها ومن ثم تشخيصها وايجاد الطرق الكفيلة للسيطرة عليها (1). تعد الطفيليات من المسببات المرضية المهمة التي تواجه الأسماك ويكاد لايخلو مكان تعيش فيه الأسماك من وجود الطفيليات الخارجية والداخلية محدثه فيها خسائر جسيمه عن طريق الهلاكات أو التأثير في معدلات النمو والخصوبه (2) ، كما إن قسما غير قليل منها ينتقل الى الانسان عن طريق أكل ألأسماك غير المطبوخه جيدا والمخمجه بالطور اليرقي لتلك الطفيليات مثل الدودة الشريطيه *Diphylobothrium latum* (3). ان دراسة التأثيرات المرضية النسجية التي تحدثها طفيليات الأسماك على مضائفها وما يتبع هذه التأثيرات من ردود فعل من قبل المضيف تجاه الطفيلي كانت ومازالت من الدراسات التي جذبت انتباه العديد من الباحثين الى هذا الجانب، اد إن معظم الطفيليات تبدي تأثيرات مرضية مختلفة على مضائفها وإن هذه التاثيرات التي تسببها الطفيليات للاسماك تكون متنوعة فقد تلحق اضرارا ميكانيكية أو كيميائية أو تعمل على سلب جزء من غذاء الاسماك المضيفة ، إضافة الى إنها تقوم بدور حامل أو ناقل لمسببات مرضية اخرى ، وتكون إستجابة المضائف تجاه الطفيليات اما إلتهابية أو إضطرابات بالنمو (نمو غير طبيعي) أو إستجابة مناعية (4,3و5) . تناولت العديد من الدراسات إصابة ألاسماك بالبوغيات الحيوانية .لاسيما جنس *Myxobolus* لما تسببه من تغيرات مرضية نسجية وعيانية في أنسجة العضلات والكلى والغلاصم فضلا عن الهلاكات لاسيما في ألاعمار الصغيرة (6). إن الدراسة النسجية الدقيقة للمسبب المرضي يساعد في تشخيص المرض ومن ثم السيطرة عليه. ونظرا لقلة الدراسات في القطر التي تناولت التغيرات النسجية الناجمة عن الاصابات الطفيلية والفطرية في أسماك نهر دجلة فقد هدفت الدراسة الحالية الى: عزل المسببات الطفيلية ودراسة التغيرات المرضية النسجية لانسجة العضلات والكلى والغلاصم والامعاء المصابة بالطفيليات.

المواد وطرائق العمل

إستمرت مدة الدراسة سنة كاملة من كانون الثاني 2010 إلى كانون ألاول 2010 جمعت 69 سمكة من ثلاث مناطق (التاجي، الشواكة والزعفرانية) ، أخذت القياسات الحياتيه في المختبر وشملت الطول الكلي والقياسي والوزن والنوع. تراوحت أطوالها الكلية بين11-39 سم وبمعدل17.8سم وتراوحت أوزانها بين 21- 562غم وبمعدل 62.1 غم.

فحصت الأسماك من الخارج للكشف عن الإصابات الطفيلية الخارجية الكبيرة ثم حضرت شرائح زجاجية من الغلاصم والجلد والزعانف وفحصت مجهريا للتعرف على الطفيليات الخارجية الصغيرة ، ثم شرحت الأسماك بفتح الجهة البطنية وفحص جوفها الجسمي والأعضاء الداخلية عيانيا لمعرفة الطفيليات الخارجية والداخلية بعد ذلك تم عمل مسحات من الأعضاء الداخلية (الكبد، الكلية، الطحال، كيس الصفراء، القلب، المناسل والأمعاء) وفحصت مجهريا لمعرفة الطفيليات الداخلية الصغيرة. ولغرض دراسة التغيرات المرضية في الانسجة، تم اخذ 12 عينة من الاسماك المصابة وحضر منها 20 شريحة زجاجية من اعضائها المصابة(العضلات، الكلية، الغلاصم والامعاء)، وضعت النماذج في محلول الفورمالين المتعادل 10% لغرض تثبيت نماذج الانسجة وتهيئتها للتقطيع النسجي وتحضير الشرائح النسجية وذلك بتمريرها بجهاز التمرير Histokinate ثم طمرها بالبرافين Embedding of paraffin وتقطيعها بسمك 5-6 مايكروميتر بجهاز المشراح Microtome ثم صبغت المقاطع بصبغة الهيماتوكسلين والايوسين (7و8).

النتائجُ

اظهرت النتائج اصابة 25 نموذجا في محطة التاجي و29 نموذجا في محطة الشواكه و 11 نموذجا في محطة الزعفرانية ، كانت هذه الأسماك مصابه بـ 39 نوعا من الطفيليات الخارجية والداخلية وتبين إن سمكة الحمري *Barbus luteus* مصابه ب23 نوعا من الطفيليات الداخلية والخارجية وسمكة الخشني *Liza abu* والكرسين *Carassius carassius* والبلعوط الملوكي *Chondrostoma regium* كانت مصابة بـ13 نوعا لكل منهم

وسمكة الجري الآسيوي *Silurus triostegus* مصابة بـ 5 أنواع من الطفيليات.

جدول 1 انواع واعداد الاسماك المصادة وحسب مواقع الدراسة:

الاسم العلمي	محطة التاجي		محطة الشواكة		محطة الزعفرانية		المجموع
	مصابة	غير مصابة	مصابة	غير مصابة	مصابة	غير مصابة	
الحمري *B.luteus*	4	_	17	_	4	_	25
الكرسين *C.carassus*	7	3	_	1	1	_	12
بلعوط ملوكي *C. regium*	7	_	8	_	_	_	15
الخشني *L.abu*	7	_	_	_	6	_	13
الجري الاسيوي *S.triostegus*	_	_	4	_	_	_	4
المجموع	25	3	29	1	11	_	69

بينت الدراسة عن اصابة خمسة انواع من الاسماك بـ 39 نوعا من الطفيليات الداخلية والخارجية، وتم اختيار نوعين من الطفيليات لدراسة التأثيرات النسجية المرضية التي تسببها وهي كالاتي:

1-البوغيات الحيوانية Sporozoa:

اوضحت التغيرات المرضية النسجية لعضلات سمكة الكرسين *C.carassius* المصابة بطفيلي *M.cyprinicola* وجود تغيرات تنكسية شديدة في الحزم العضلية تميزت بعدم انتظام الحزم المتنكسة Muscular disorganization، مع استبدال النسيج العضلي المتنكس بنسيج دهني مصحوب بارتشاح شديد للخلايا الالتهابية ولاسيما خلايا وحيدة النواة mononuclear cells ، نزف و إحتقان الاوعية الدموية شكل (1).

كانت التغيرات المرضية النسجية لعضلات سمكة الكرسين *C.carassius* المصابة بطفيلي *M.oviformis* مشابهة لما ذكر سابقا باستثناء إنفصال وتجزء الحزم العضلية المتنخرة الفاقدة للتخطيط العضلي والتي اخذت صبغة حمضية غامقة Deep eosinophilis stain مع وجود الخزب وإستبدال النسيج العضلي المتنخر بنسيج ليفي (شكل 2).

اوضحت نتائج فحص المقاطع النسجية لكلية سمكة البلعوط الملوكي *C.regium* المصابة بطفيلي *M.cyprinicola* وجود نخر مصحوب بإنتعاف slonghing في الظهارة المبطنة للنبيبات الكلوية مع تراكم حطام خلوي في التجويف النبيبي الكلوي (شكل 3) ، مع وجود تغيرات وعائية في الكلى تميزت بنزف مع احتقان الاوعية الدموية وتثخن جدرانها لحصول فرط تنسج وسطي medial hyperplasia فضلا عن التنكس الشديد في النسيج الخلالي المجاور severe degeneration (شكل 4).

بين الفحص النسجي لكلية سمكة البلعوط الملوكي *C. regium.* المصابة بطفيلي *M.cyprinicola* وجود تغيرات نخرية وتنكسية شديدة لظهارة النبيبات الكلوية مصحوبةبإرتشاح شديد للخلايا وحيدة النواة ولاسيما اللمفية والبلازما والبلعمية مع تثخن المحفظة (شكل 5).

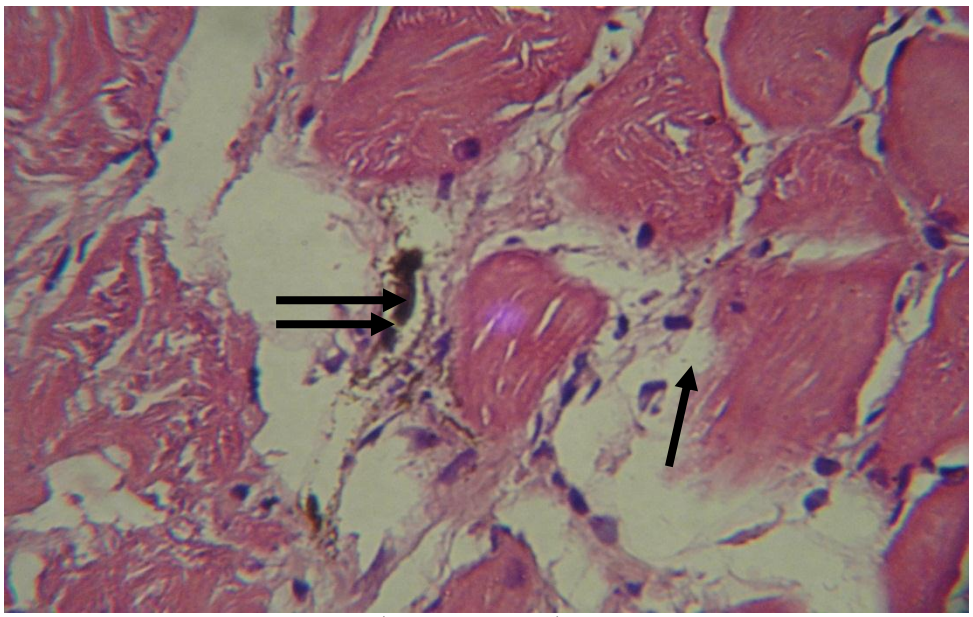
بينت نتائج الفحص النسجي لغلاصم سمكة البلعوط الملوكي *C.regium* المصابة بطفيلي *M.oviformis* وجود نخر في الخلايا الظهارية للصفائح الغلصمية الثانوية وإنفصال الظهارة عن الغشاء البلازمي نتيجة النخر الشديد مع احتقان ونزف شديد الصفائح الغلصمية gills lamella وحصول فرط تنسج Hyperplasia في بعض الخلايا الظهارية لهذه الخيوط الغلصمية مصحوبا بارتشاح شديد للخلايا الالتهابية لاسيما خلايا وحيدة النواة Mononuclear cells شكل(6).

تبين عند فحص المقاطع النسجية لغلاصم سمكة الكرسين *C. carasisus* المصابة بطفيلي *M. oviformis* وجود توسع الشعيرات الغلصمية الدموية telengiectasis في الصفائح الغلصمية الثانوية شكل (7).

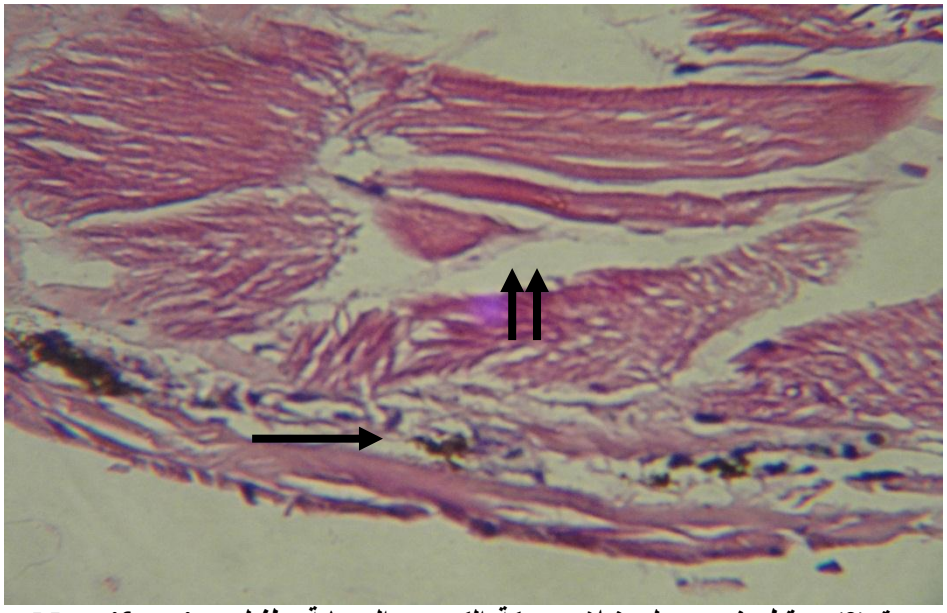
2 - الديدان شوكية الراس Acanthocephala:

تميزت التغيرات النسجية في امعاء سمكة الخشني *L.abu* المصابة بطفيلي *N.iraqinesis* وجود مقاطع تطور مختلفة للطفيلي في تجويف الامعاء مسببا انسدادها ومصحوبا بحطام نخري لظهارة الامعاء مع إستجابة إلتهابية (حمضات وعدلات) (شكل 8) فضلا عن وجود إنسلاخ وإختزال شديدين لظهارة الزغابات المعوية وإختزال شديد للزغابات المعوية (ضمور إنضغاطي)، وكذلك لوحظ ارتشاح خلوي كثيف مع وجود حطام نخري شديد في تجويف الامعاء المصابة بطفيلي *N. iraqinesis* وإنسلاخ وتوسف كامل للظهارة المعوية مصحوبا بخزب معوي(شكل

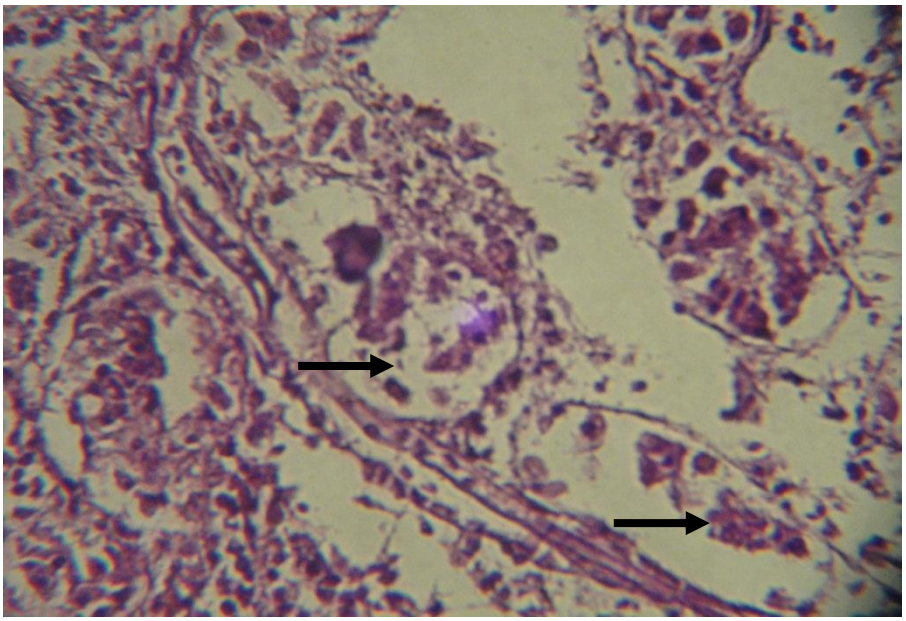
.(9



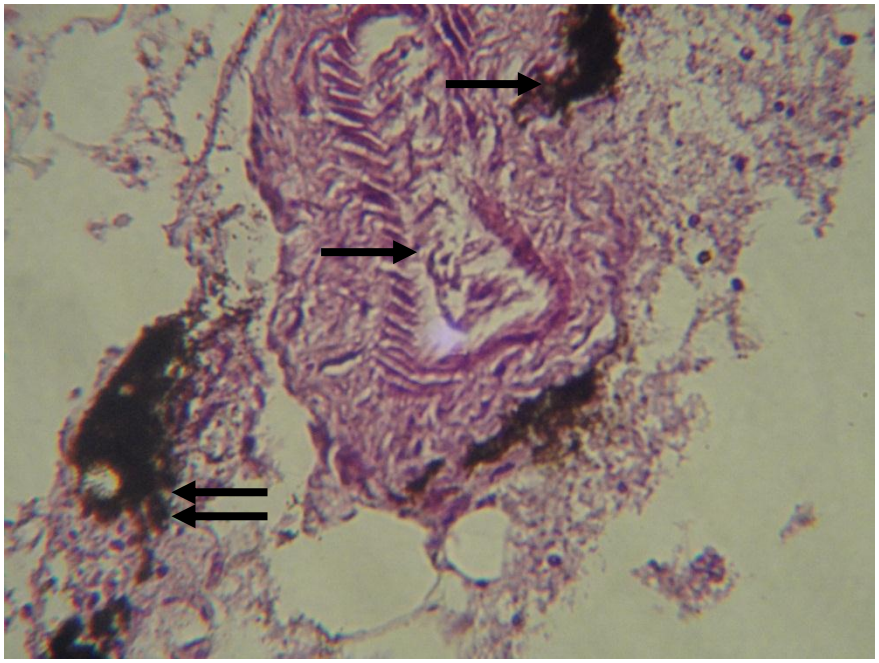
صورة (1): مقطع نسيجي لعضلات سمكة الكرسين المصابة بطفيلي *M. cyprinicola* يبين تجزء الحزم العضلية المتنخرة (⟶)مع وجود إرتشاح للخلايا وحيدة النواة في النسيج مابين العضلات ونزف واحتقان الاوعية الدموية الشعرية(⟹) (H & E stain x40).



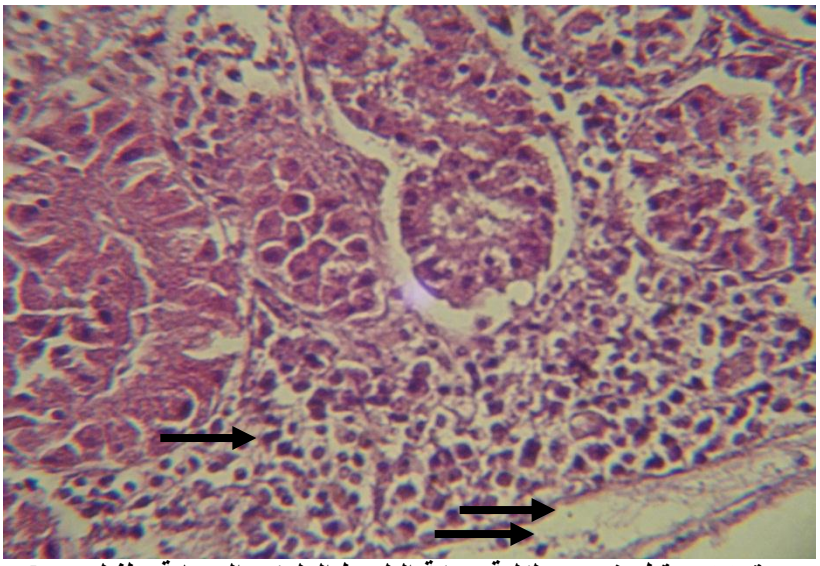
صورة (2): مقطع نسيجي لعضلات سمكة الكرسين المصابة بطفيلي *M. oviformis*يبين عدم إنتظام الحزم العضلية المتنكسة مع تجزئتها لوجود الخزب (⟹) وإستبدال النسيج العضلي المتنخر بنسيج ليفي (⟶) ((H&E stain x40.



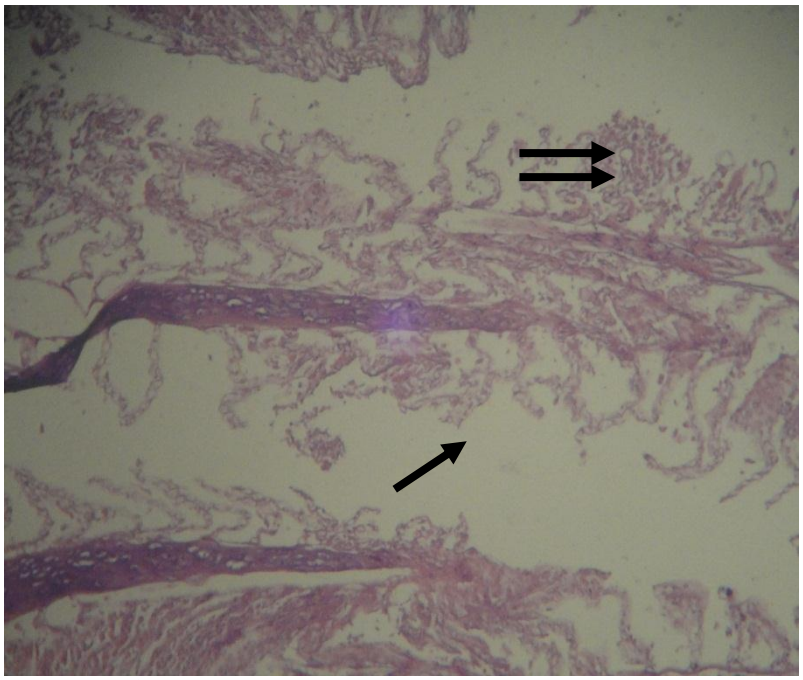
صورة (3): مقطع نسيجي لكلية سمكة البلعوط الملوكي المصابة بطفيلي *M. oviformis* يبين وجود نخر في الظهارة المبطنة للنبيبات النبيبي مع تراكم حطام خلوي في التجويف الكلوي (⟶) (H&E stain x40).



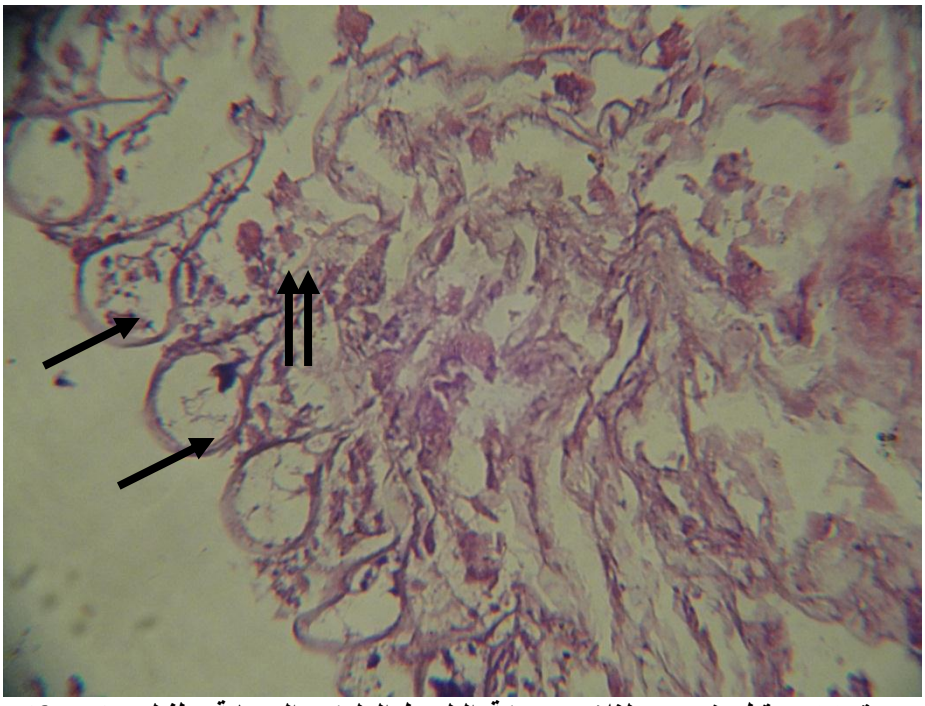
صورة (4): مقطع نسيجي لكلية سمكة البلعوط الملوكي المصابة بطفيلي *M. cyprinicola* يبين وجود تغيرات وعائية تميزت بإحتقان ونزف في الاوعية الدموية(⟶) مع وجود التكلس الشديد (⇉) (H&E stain x40) mineralization



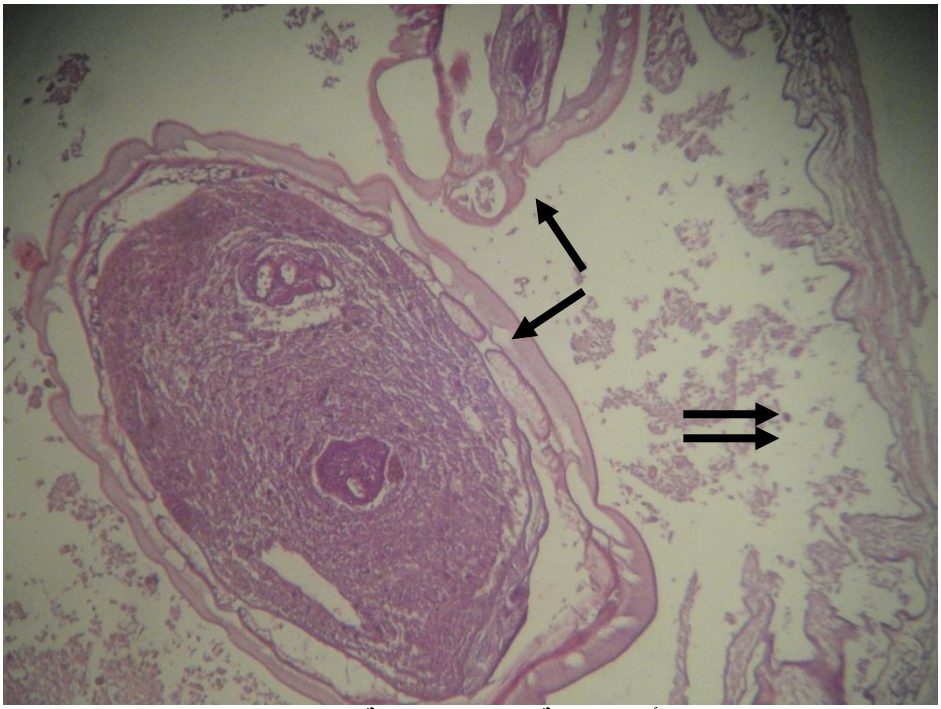
صورة (5): مقطع نسيجي لكلية سمكة البلعوط الملوكي المصابة بطفيلي *M. cyprinicola* يبين إرتشاح شديد للخلايا وحيدة النواة ولاسيما اللمفية والبلازما والبلعمية (⟶) مع تثخن المحفظة (⟹) (H&E stain x40).



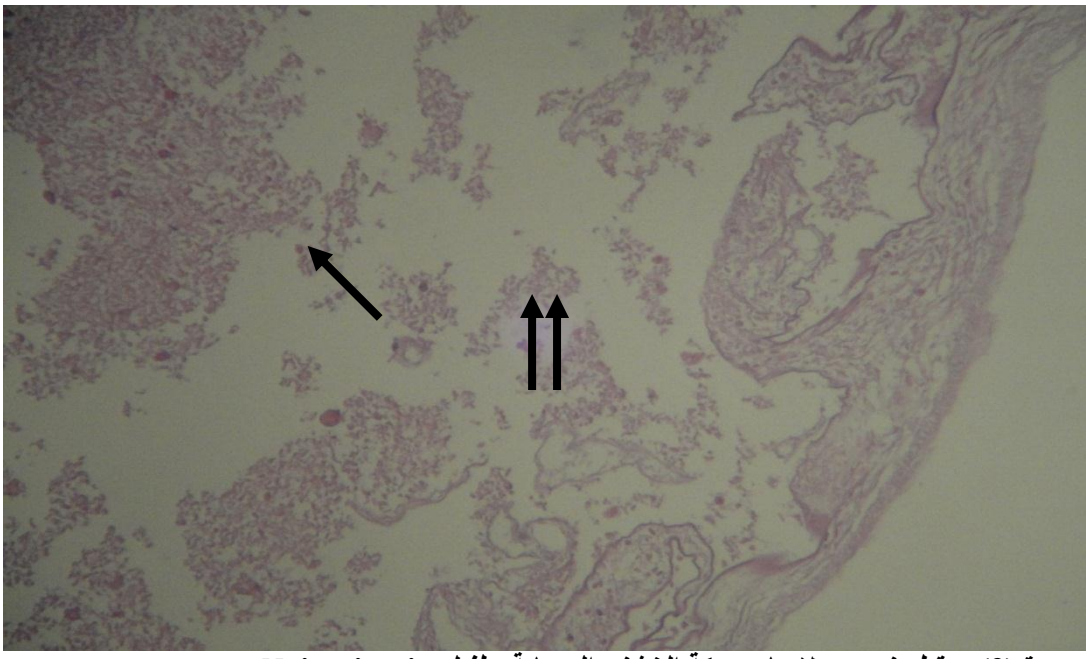
صورة (6): مقطع نسيجي لغلاصم سمكة البلعوط الملوكي المصابة بطفيلي *M. oviformis* يبين وجود نخر الخلايا الظهارية للصفائح الغلصمية الثانوية مع إنفصال الظهارة عن الغشاء البلازمي(⟶)، فرط تنسج hyperplasia للخلايا الظهارية في الخيوط الغلصمية ونزف وإحتقان الصفائح الغلصمية ، وجود رتشاح خلوي للخلايا التهابية (⟹) (H&E stain x20).



صورة (7): مقطع نسيجي لغلاصم سمكة البلعوط الملوكي المصابة بطفيلي *M. oviformis* يبين وجود توسع الشعيرات الغلصمية في الصفائح الغلصمية الثانوية (⟶) مع إحتقان ونزف في الصفائح الغلصمية الاولية (⟹).(H&E stain x40)



صورة (8): مقطع نسيجي لأمعاء سمكة الخشني المصابة بطفيلي *N. iraqinesis* يبين وجود مقاطع للطفيلي في تجويف الامعاء مسببا إنسداد التجويف المعوي(⟶)، إنسلاخ شديد لظهارة الامعاء إختزال شديد للزغابات المعوية (⟹) (H&E stain x40).



صورة (9): مقطع نسيجي لامعاء سمكة الخشني المصابة بطفيلي *N. iraqinesis* يبين وجود إرتشاح للخلايا الالتهابية (⟵)، حطام نخري في تجويف الامعاء إنسلاخ وتوسف كامل للظهارة المعوية خزب معوي (⟸) (H&E stain x40).

المناقشة

بين العزل الطفيلي ان نسبة الاصابة Prevalence of infection كانت 94,02% . تناولت الدراسة الحالية تسجيل لبعض الاصابات بالاوالي Protozoa ولاسيما الاصابة بالبوغيات المخاطية وفي الاعضاءالمختلفة للاسماك المصابة (العضلات، الكلية، والغلاصم). أشارت نتائج الدراسة إلى إن الإصابة بالبوغيات المخاطية تسبب تشوهات وعدم إنتظام الحزم العضلية skeletal deformation ناجمة عن نمو وتكاثر ألاكياس البوغية وتحطمها في النسيج العضلي لاسيما في النسيج الليفي مابين العضلات حيث سجلت الدراسة وجود نخر وتنكس الحزم العضلية وتبعثرها وفقدانها للتخطيط العضلي مع إرتشاح للخلايا الألتهابية وحيدة النواة mononuclear cells نتيجة للاستجابة الالتهابية الثانوية وقد جاء ذلك متفقا مع (9). كما بينت النتائج وجود آفات مرضية كلوية تميزت بانتعاف الظهارة المبطنة للنبيبات الكلوية مع إرتشاح شديد للخلايا البلعمية الكبيرة واللمفية وذلك نتيجة للاستجابة الالتهابية الناجمة عن وجود الكيس الحاوي على الأوالي، فضلا عن إن ألالية الدفاعية أدت الى تحطم وإنتشار البوغيات في النسيج مسببة إستجابة إلتهابية موضعية localized inflammatory reaction مع تجمعات للحطام الخلوي في التجويف كذلك حدوث تغيرات وعائية تميزت بإحتقان ونزف في الاوعية الدموية وقد جاءت هذه النتائج متفقة مع دراسات عديدة (6 و10). فضلا عن إن هذه الدراسات اشارت إلى إن الإصابة بالبوغيات تسبب أورام حبيبية في الكلى renal granuloma فضلا عن إلتهاب ألاوعية الدموية vasculitis وضمور النبيبات الكلوية. أما ألدراسة الحالية فقد بينت وجود نخر وتحطم في ظهارة الخيوط الغلصمية الثانوية secondary lamella فضلا عن إنفصالها مصحوبا بنزف وإحتقان شديدين مع حدوث توسع للشعيرات الوعائية الغلصمية telengiectasis وفرط تنسج في الخلايا الظهارية لهذه الخيوط إضافة إلى إرتشاح الخلايا الإلتهابية وحيدة النواة في قاعدة الخيوط الغلصمية mononuclear cells لعل ذلك يعزى الى ان الغلاصم تعد من ألاعضاء الحيوية في ألاسماك والغزيرة بالاوعية الدموية كما إنها وسط جيد لنمو وتكاثر وحدوث العوامل المرضية ولاسيما البكتيرية والفطرية secondary infectionعن طريق حدوث التبادل الغازي oxygen exchange وإنتقال المسببات المرضية عن طريق الدورة الدموية blood circulation وهذه النتائج متفقة مع (11, 12,13, 14و15).

وبينت التغيرات المرضية النسجية للأمعاء المصابة بطفيلي *N. iraqensis* لاسيما أمعاء سمكة الخشني *Liza abu*وجود إنسداد في تجويف الامعاء بمقاطع الطفيلي التي سببت تحطيم أو تدمير خلوي شديد للظهارة المعوية مع إختزال الزغابات مصحوبا بانسلاخ وتوسف desquementشديد للطبقة الظهارية وذلك نتيجة إلتصاق جزء الطفيلي الامامي (المخطم probosis) بظهارة الامعاء وإحداثه التخريش irritation الشديد لمكان وجود الطفيلي مكونا آفة تشغل حيزا space occupying lesion فضلا عن حدوث ألاستجابة الالتهابية موضعية شديدة ناجمة عن وجود الطفيلي وهذه النتائج جاءت متفقة مع (17,18و16). إن التاثيرات المرضية pathogenic effects لطفيلي *Neochinorynchus* ناتجة عن إلتصاق الطفيلي البالغ في القناة الهضمية وكذلك إلتصاق طور اليرقة المحاطة بمحفظة Acanthocephalon في ألانسجة، عموما إن التاثيرات المرضية تتموضع حول منطقة

إلتصاق الطفيلي البالغ وقد يصل التخريب إلى مناطق عميقة في ألانسجة أعمق من مناطق إلتصاق المخطم،عادة يميل الطفيلي الى الالتصاق بالظهارة المخاطية للامعاء وقد يمتد ألالتصاق إلى الطبقة العضلية محدثا ورم حبيبي granulomaوتليف شديد وهذا يحدث في حالة إختراق مخطم الطفيلي للطبقة العضلية للامعاء (16و19). وبينت الدراسات إن إختراق الطفيلي يختلف باختلاف المضائف(الاسماك) (19). في الحالات الشديدة سجلت حالات إلتهاب البريتون نتيجة ثقب القناة الهضمية للمضيف. وقد يصل الطور اليرقي لهذا الطفيلي (Cystacanths) الى ألاعضاء الحشوية (الكبد والطحال), وفي حالة الاصابة الشديدة لاصبعيات الاسماك يحدث الورم الحبيبي والتليف الشديد Fibrosis والضمور للاعضاء الداخلية Atrophy (16, 17و20). تستنتج الدراسه.تسجيل لبعض الاصابات بالاوالي ولاسيما الاصابه بالبوغيات المخاطيه في الاعضاء المختلفه للاسماك المصابه والتي تسسبب تشوهات وعدم الانتظام الحزم العضليه وان نسبة الاصابه بالطفيليات في اسماك دجله تصل الى 94%.

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دراسة بعض العوامل المؤثرةعلى كفاءة الكلورالتطهيرية لماء الشرب فى محطات التصفية لمدينة بغداد \ الكرخ

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الخلاصة

صممت هذه التجربة لدراسة العوامل التي يمكن ان تؤثر على كفاءة الكلور التطهيرية منفردة او مجتمعة مع بعضها وتأثير ذلك على المستوى القياسي والصحي لمياه الشرب لمدينة بغداد/ الكرخ، حيث جمعت 137 عينة ماء خلال الدراسة (تموز- تشرين الثاني 2007) من مساكن المواطنين في مناطق مختلفة لمدينة بغداد/ الكرخ، ولغرض توحيد وقت جمع نماذج الماء من حنفيات بيوت المستهلكين (بعد فتح الحنفية)، تم اختيار ثلاث اوقات (0، 5، 10 دقائق) من جريان الماء من الحنفية ومن خلال التحليل الاحصائي للنتائج استنتج بعدم وجود فروقات معنوية مهمة من الناحية الاحصائية ($P > 0.05$) بين قيم تركيز الكلور والعد الجرثومي الاكثر احتمالاً المسجلة في عينات ماء الشرب للاوقات 0، 5، 10 دقائق، واختير الوقت 5 دقائق كحل وسط لاكمال البحث على جميع العينات .

ومن خلال تحليل نتائج الدراسة الخاصة بالمسح الميداني لبيوت المستهلكين اتضح بان تركيز الكلور الحر في مياه الشرب عند المستهلك كان دون المستوى الصحي الموصى به من قبل منظمة الصحة العالمية خلال الشهرين الاوليين من الدراسة (تموز وآب) مما اثر سلباً على المستوى الصحي للماء، حيث سجلت اعلى قيم للعد الجرثومي الاكثر احتمالاً خلال تلك المدة، في حين انخفضت هذه القيم في الاشهر الثلاثة الاخيرة من الدراسة (ايلول، تشرين الاول وتشرين الثاني) بسبب زيادة تركيز الكلور الكلي في محطات تصفية الماء مما انعكس ايجاباً على تركيز الكلور الحر لدى المستهلك وتحسن المستوى الصحي القياسي للمستويات المقبولة عالمياً. كذلك درست بعض العوامل المؤثرة على كفاءة الكلور التطهيرية والتي اشتملت على كل من تركيز الكلور الحر في ماء الشرب ودرجة حرارة الماء والاس الهيدروجيني للماء وجهد الاكسدة والاختزال وعلاقة تلك العوامل مع بعضها ومن خلال التحليل الاحصائي للنتائج وجدت علاقة عكسية ($r= -0.72; P<0.01$) بين كفاءة الكلور التطهيرية وكل من تركيز الكلور الحر ووقت تماس الاحياء المجهرية مع الكلور، اما العوامل الاخرى فكان تأثيرها محدود او معدوم لانها تقع ضمن القياسات الموصى بها. ولغرض التعرف على واقع عمل محطات تصفية المياه لمدينة بغداد/ الكرخ والقياسات المعتمدة في تلك المحطات ومدى مطابقة ذلك للمواصفات القياسية، اعتمدت السجلات الرسمية لتلك المحطات لاسيما المدة الواقعة بين شهر تموز الى تشرين الثاني 2007، حيث سجلت قيم كل من تركيز الكلور الكلي والاس الهيدروجيني ودرجة حرارة الماء، حيث جمعت 16 عينة ماء خلال شهر تشرين الثاني وسجلت قيم نفس القياسات المشار اليها ولكن باستخدام الاجهزة الرقمية الحديثة التي استخدمت في المسح الميداني الاولي، وبعد تحليل نتائج قيم القياسات المعتمدة من سجلات المحطات اتضح بان معدل قيم تركيز الكلور الكلي في شهر تشرين الثاني كان 3.6 جزء بالمليون، في حين كان معدل قيم الاس الهيدروجيني للمدة نفسها 7.5، اما معدل قيم درجة الحرارة للمدة نفسها بلغ 21.7 مo وبالمقارنة مع معدلات قيم تلك القياسات والمأخوذة بالاجهزة الحديثة حيث كان معدل تركيز الكلور الحر في شهر تشرين الثاني 5.05 جزء بالمليون ومعدل قيم الاس الهيدروجيني 6.94 ومعدل درجة حرارة الماء 17.6 مْ , حيث اتضح وجود فروقات في قراءات تلك القيم. والسبب في ذلك يعود الى استخدام الباحث اجهزة رقمية حديثة وحساسة بالمقارنة مع الطريقة المستخدمة في تلك المحطات والتي تعتمد على العامل البشري في قراءة النتائج.

كلمات مفتاحية : الكلور, ماء الشرب , محطات التصفية

Evaluation of some Limiting Factors affecting Water chlorination at Baghdad / Al-Kurch District

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Summary

This study was designed to high light about the effect of some factors individually or in combination that shared in the reduction of the chlorine activity and efficiency for meeting the bacterial standards as a disinfection agent for drinking water at Baghdad city/ Al- kurch. To achieve the objectives (137) drinking water samples were collected from July up to the end of November 2007 from the houses of Baghdad's citizens/ Al-

kurch. Also studying the scientific nature of some municipal water supply at Baghdad/ Al- kurch, drinking water samples were collected from faucets after allowing the water to run for 0, 5 and 10 minutes. Statistical data showed that there was non significant difference in both the chlorine concentration and coliform counts in all samples that were taken after allowing the water to run for 0, 5 and 10 minutes and for that reason drinking water samples after allowing the water to run for 5 minutes were chosen as the best time for sampling in this research.

Data revealed that the free chlorine in drinking water was below the standards set by the World Health Organization (WHO) in the period from July up to the end of August 2007, whereas the highest Coliform counts in drinking water were established during the above mentioned months, while the coliform counts decreased in the period from September up to the end of November 2007 due to the utilization of higher concentrations of total chlorine in drinking water in municipal water supply, in addition to that, the effect of some variables such as quantity of free chlorine, temperature, pH and oxidation- reduction potential of water on the sanitizing efficiency of the chlorine were studied.

The statistical data revealed that there was a significant negative correlation ($P < 0.01$, $r = -0.072$) between the chlorine sanitizing efficiency with both the concentration of the free chlorine and its contact time with microorganisms while the effect of temperature, pH and oxidation- reduction potential of water showed non significant effect on the chlorine sanitizing efficiency.

In order to evaluate the sanitation program of the municipal water supply at Baghdad/ Al- kurch to ensure that the water treatment was being done properly by the employees and meeting the bacterial standards set by (WHO). All the official documents about the chlorine concentration, pH, temperature of water that were reported by the employees in the period from July up to the end of November 2007 were studied and compared to this results, for that reason 16 drinking water samples from the municipal water supply were collected and tested for the above mentioned parameters during November using the most sensitive advanced digital instrument (Chlorimeter). Data reported by the municipal water supply at Baghdad/ Al- kurch revealed that chlorine, pH and temperature of water were 3.6 PPM, 7.50 and 21.7C^{o}, respectively in November only. While the measurements were reported here 5.05 PPM, 6.94 and 17.6^{o}C respectively, during the same month by using the Chlorimeter and the only reason for such differences with our results was due to the use of a highly sensitive digital instrument by our research in comparison to the old methods and instruments that were used in the municipal water supply.

Key words: water chlorination, world health organization, coliform counts, sanitizing efficiency.

المقدمة

استخدم عنصر الكلور لغرض السيطرة والحد من انتشار الامراض المنتقلة عن طريق مياة الشرب، حيث يضاف الكلور الى الماء في محطات تصفية المياة لغرض التطهير باشكال وتراكيز مختلفة وهي: الشكل الغازي (Chlorine Gas) او السائل (Sodium hypochlorite) والشكل الصلب (Calicum hychloriite)، حيث ان اضافة أي شيء من اشكال الكلور يؤدي الى تحرر الكلور الحر (Free Chlorine) الذي يقوم بقتل او تقليل الاحياء المجهرية الموجودة في المياه وتحسين الصفات النوعية للماء(1). تعد معالجة وتوزيع المياه الصالحة للشرب والاستعمال اليومي واحدة من اعظم منجزات القرن العشرين، حيث اصبح بالامكان عن طريقها الحصول على مياه صحية وسليمة، ولغرض الوصول الى هذا الهدف، يتطلب ذلك تجاوز عدة معوقات تشتمل على حماية مصادر المياه من التلوث، والمعالجة المناسبة والملائمة للمياه الخام مع ضمان التوزيع السليم للمياه المعالجة وايصالها الى المستهلك عبر شبكات توزيع المياه(2) . لقد اوصت منظمة حماية البيئة الامريكية (EPA)، ولغرض الحد من تكاثر الاحياء المجهرية او منع تلوث مياه الشرب مرة ثانية اثناء عملية التوزيع بأن يكون

مستوى الكلور المتبقي في المياه المعالجة بحدود 4 جزء بالمليون كحد اعلى و الكلورامينات 4 جزء بالمليون و 0.8 جزء بالمليون ثنائي اوكسيد الكلور(3).

تعتمد فعالية عنصر الكلور في عملية التطهير (D_h) على تركيز الكلور (Cl_2) المضاف والوقت اللازم (t) للتطهير، هذه الفعالية تتأثر ايضاً بعدة عوامل مثل درجة حرارة الماء والاس الهيدروجيني و كمية المواد العضوية وسرعة ضخ الماء في شبكة توزيع المياه(4). تحتاج عملية تطهير المياه في محطات تصفية مياه الشرب من المسببات المرضية التي تنتقل عن طريق الماء، من 20 الى 30 دقيقة على الاقل، على ان تكون قيمة الاس الهيدروجيني (pH) بين 6-8، وتركيز الكلور الحر او المتاح بين 3-5 جزء بالمليون(5).

ومن اجل تحديد تركيز الكلور في عملية تطهير ماء الشرب المخصص لمدينة بغداد استناداً لتلك العوامل، ولعدم توفر الدراسات الخاصة بهذا الجانب تم اجراء هذه الدراسة والتي تشتمل على جانب الكرخ من مدينة بغداد بهدف دراسة تأثير بعض العوامل المؤثرة على كفاءة الكلور التطهيرية المستخدم في تطهير مياه الشرب في محطات تصفية مياه الشرب لمدينة بغداد/ الكرخ.

المواد طرائق العمل

حدد أفضل وقت لجمع عينات ماء الشرب لغرض تحديد تركيز الكلور الحر والعد الجرثومي الأكثر احتمالاً لبكتريا القولون. اشتملت هذه الدراسة على فحص 68 عينة ماء أخذت من حنفيات مساكن المواطنين في ثلاث اوقات (8 صباحاً و2 بعد الظهر و8 مساءاً بواقع 3 عينات في كل وقت) حيث اخذت 26 عينة في الوقت صفر دقيقة (بعد فتح حنفية الماء مباشرة) و21 عينة بعد كل من 5و10 دقائق من جريان الماء من الحنفية لتحديد تركيز الكلور الحر والعد الجرثومي الأكثر احتمالاً. وتم جمع عينات الماء من حنفيات مساكن المواطنين والاختبارات التي أجريت عليها اشتملت الدراسة على اجراء مسح ميداني لتحديد تركيز الكلور الحر والعد الجرثومي الاكثر احتمالاً لبكتريا القولون حيث جمعت 137 عينة خلال مدة الدراسة (تموز- تشرين الثاني 2007) من مناطق مختلفة من مدينة بغداد/ الكرخ. وقيس تركيز الكلور الحر في الماء بواسطة جهاز رقمي حديث (Pocket Colorimeter). وكذلك قيست قيم كل من درجة الحرارة الماء والأس الهيدروجيني ودرجة جهد الأكسدة والاختزال باستخدام جهاز واحد رقمي حديث.وبعدها الفحص الجرثومي لعينات الماء:

اعتمدت طريقة العد الجرثومي الأكثر احتمالاً لبكتريا القولون (Most Probable Number) (MPN) والمعتمدة عالمياً لكونها أكثر الطرائق شيوعاً في فحوصات المياه(6، 7، 8و 9).

حيث حضر وسط مرق الماكونكي بتركيزيين الاول تركيز عادي (Single strength) والثاني تركيز مضاعف (Double strength) وزع في قناني وانابيب للاختبار مع انبوبة درهم وعقمت بالموصدة ثم اضيف لكل 100 مليلتر من عينة الماء المراد فحصها 0.1 مليلتر محلول ثايوسلفات الصوديوم (3%) لمعادلة متبقي الكلور في الماء وابطال مفعول الكلور ضد الجراثيم في حالة وجوده، ورجت العينة لمزج محتوياتها، ومن ثم زرعت في وسط مرق الماكونكي، حيث زرع 50 مليلتر من الماء في 50 مليلتر من وسط الماكونكي في التركيز المضاعف وبواقع اسطوانة قياسية واحدة، وتم زرع 10 مليلتر من عينة الماء في كل من الانابيب الخمسة الحاوية على 10 مليلتر من مرق الماكونكي ذا التركيز المضاعف، و 1 مليلتر من عينة الماء في الانابيب الخمسة الحاوية في كل منها على 5 مليلتر من مرق الماكونكي ذا التركيز العادي، وحضنت لمدة 24-48 ساعة على درجة 37 مْ واعتمدت تحرر الغاز ونمو الجراثيم في الانابيب من خلال ملاحظة العكورة الحاصلة في الوسط والتي تدل على نمو الجراثيم وتحول لون الوسط من الاحمر الى الاصفر الباهت وعدّ النتيجة موجبة، رقمت الانابيب التي اعطت نتيجة موجبة للفحص واحصيت اعداد الجراثيم بالاعتماد على جدول خاص ورد في المصدر(8) حيث قدر العد الجرثومي الاكثر احتمالاً لبكتريا القولون (MPN) لكل 100 مليلتر.

استخدم اختبار الاندول للتأكد على وجود جراثيم القولون البرازية المقاومة للحرارة مثل E. coli واستخدم وسط EMB للتفريق بين E. coli و Enterobacter aerogens واجريت جميع الطرائق الاحصائية باستخدام (10) وجمع عينات الماء من محطات تصفية مياه الشرب لمدينة بغداد/ الكرخ والاختبارات التي أجريت عليها:

جمعت 16 عينة ماء من محطتين لتصفية مياه الشرب (Aو B) خلال شهر تشرين الثاني 2007، في الاوقات 9 صباحاً والواحدة بعد الظهر وأجريت الاختبارات الآتية عليها: قيس تركيز الكلور الكلي بجهاز قياس الكلور المستخدم في الفقرة اولاً (2-A). و قيست كل من قيم الأس الهيدروجيني ودرجة حرارة الماء بالجهاز المستخدم في الفقرة اولاً (2-B). ثم دراست العلاقة بين العوامل المؤثرة على الفعالية التطهيرية للكلور خلال مدة الدراسة.

شملت هذه الدراسة، دراسة العوامل التي تؤثر على الكفاءة التطهيرية للكلور بشكل انفرادي أو تآزري والتي شملت العوامل التالية:

1. العد الجرثومي الأكثر احتمالاً لبكتريا القولون.
2. درجة حرارة الماء.
3. الأس الهيدروجيني للماء.
4. جهد الأكسدة والاختزال للماء.

النتائج والمناقشة

تأثير وقت جمع عينات الماء (0 ,5 ,10 دقائق) على كل من تركيز الكلور الحر والعد الجرثومي الأكثر احتمالاً لبكتريا القولون خلال شهر أيلول2007. جمعت (68) عينة ماء بثلاثة أوقات زمنية مختلفة في اليوم الواحد وبواقع ثلاثة عينات في كل وقت أي بعد10,5,0 دقائق من جريان الماء من الحنفية وسجلت قيم كل من تركيز الكلور الحر والعد الجرثومي الاكثر أحتمالا كما هو موضح في الجدول رقم(1) .أشارت نتائج التحليل الاحصائي بأنه لايوجد فرق معنوي ($P< 0.05$) بين قيم تركيز الكلور الحر في عينات الماء بعد 10,5,0 دقائق من جريان الماء .

كذلك أشارت النتائج الى ان العد الجرثومي الاكثر احتمالا في عينات الماء للاوقات الثلاثة كانت متقاربة ولايوجد فرق معنوي بينهم ($P<0.05$) وأستنتج بأن وقت جمع العينات لايؤثر على كل من تركيز الكلور الحر والعد الجرثومي الاكثر أحتمالا لبكتريا القولون لذلك تم أختيار الوقت (5) دقائق لانجاز هذه الدراسة.

جدول رقم (1) يوضح تأثير وقت جمع عينات الماء 0 ,5 ,10 دقيقة على كل من تركيز الكلور الحر والعد الجرثومي الاكثر أحتمالا لبكتريا القولون خلال شهر ايلول 2007.

وقت جمع العينات/ دقيقة	تركيز الكلور الحر/ جزء بالمليون			العد الجرثومي/ جرثومة/ 100 ملل		
	المدى ادنى اعلى	المعدل	الانحراف القياسي	المدى ادنى اعلى	المعدل	الانحراف القياسي
0	1.39- 0.17	0.74	±0.43	20 - 0	8.9	±6.09
5	1.50- 0.16	0.82	±0.45	20 -0	7.4	±6.57
10	1.45- 0.12	0.79	±0.43	17 -0	7.8	±6.19

ان تحديد تركيز الكلور الحر على العد الجرثومي الاكثر احتمالاً لبكتريا القولون لعينات ماء الشرب المأخوذة خلال مدة الدراسة جمعت (137) عينة ماء من حنفيات مساكن المواطنين في الكرخ خلال مدة الدراسة والتي أمتدت من تموز الى نهاية تشرين الثاني 2007. تشير النتائج المدونة في الجدول رقم (2) بأن تركيز الكلور الحر في عينات ماء الشرب كان بأرتفاع مستمر أبتداءا من شهر اب ولغاية نهاية شهر تشرين الثاني حيث سجل ادنى مستوى في شهر تموز حيث بلغ المدى (0.16-0.02) جزء بالمليون في حين سجل أعلى مستوى في شهر تشرين الثاني وبلغ المدى بين (1.95-0.7) جزء بالمليون وأدى هذا الارتفاع في تركيزالكلور الحر الى الانخفاض الكبير في معدلات العد الجرثومي الاكثر احتمالا لبكتريا القولون حيث بلغ المدى في شهر تموز بين 11-180 جرثومة/100مليلتر ثم أنخفض ليبلغ المدى بين 0-5جرثومة/10مليلتر في شهر تشرين الثاني وأثبتت النتائج بأن تركيزالكلور الحر كان دون المستوى الذي أوصت به منظمة الصحة العالمية (0.5-1) جزء بالمليون خلال شهر تموز واب مما أثر سلبا على المستوى الصحي للماء وذلك بأرتفاع العد الجرثومي الاكثر احتمالا ووصل تركيز الكلور الحر الى التركيز الذي أوصت به المنظمة خلال الاشهر الثلاثة الاخرى من الدراسة مما أنعكس أيجابا على المستوى الصحي لماء الشرب وذلك بأنخفاض العد الجرثومي الاكثر أحتمالا لبكتريا القولون . وهذا ماأشارت اليه العديد من الدراسات التي أوصت بزيادة تركيز الكلور لضمان تركيز الكلور الحر بحد أدنى (2) جزء بالمليون في حالة تفشي الامراض التي تنتقل بواسطة مياه الشرب (9و11).

جدول رقم (2) يبين تأثير زيادة تركيز الكور الحر على العد الجرثومي المحتمل لبكتريا القولون في عينات ماء الشرب المأخوذة من مساكن المواطنين في مدينة بغداد/ الكرخ.

الاشهر	عدد العينات الكلي	عدد العينات المفحوصة	مدى تركيز الكلور الحر/جزء بالمليون		مدى العد الجرثومي المحتمل /100 مليلتر		النسبة المئوية للعينات المفحوصة/العدد الكلي
			أدنى	أعلى	أدنى	أعلى	
تموز	22	9 7	0.02 0.10	0.09 0.16	11 14	180< 35	%72.7
أب	13	3 9	0.08 0.12	0.09 0.28	20 10	30 30	%92.3
أيلول	68	25 10	0.12 1.12	0.98 1.50	0 0	20 14	%51.4
تشرين الاول	17	4 9	0.78 1.02	0.95 1.42	0 0	5 3	%76.4
تشرين الثاني	17	2 10	0.71 1.15	0.96 1.95	1 0	5 3	%70.5

تشير النتائج المدونة في الجدول رقم (3) الى ان معدل تركيز الكلور سجل أدنى مستوى له في شهر تموز حيث بلغ (0.09) جزء بالمليون ومن ثم أزداد ليصل الى أعلى مستوى في شهر تشرين الثاني حيث بلغ المعدل (1.30) جزء بالمليون وقد يعزى سبب هذا الارتفاع الى زيادة عنصر الكلور المضاف للماء من قبل الجهات الصحية لغرض السيطرة والحد من أنتشار مرض الكوليرا وقد يكون السبب نتيجة لانخفاض درجة حرارة الماء من (31) مْ في شهر تموز الى (25) مْ في شهر تشرين الثاني حيث يؤدي هذا الانخفاض الى تقليل تبخر عنصر الكلور من ماء الشرب , ويمكن ملاحظة قيم جهد الاكسدة والاختزال بأنها لم تتغير وكانت متقاربة خلال الاشهر الثلاثة الاولى من الدراسة (تموز واب وأيلول) ثم أنخفضت في شهري تشرين الاول والثاني حيث بلغت المعدلات (19.1mv) و(22.7mv)على التوالي ، في حين لم تتغير قيم الاس الهيدروجيني للماء تغيرا كبيرا خلال الدراسة حيث كانت المعدلات بين (7.5) في شهر تموز و(7.08) في شهر تشرين الثاني وهذه المعدلات ضمن المقاييس والمعايير الدولية والتي لاتؤثر على فعالية الكلور . ويمكن من خلال نفس الجدول ملاحظة أعلى قيمة للعد الجرثومي الاكثر أحتمالا وبلغ المعدل في شهر تموز حيث بلغ المعدل21 جرثومة/100 مليلتر وأنخفضت المعدلات لتسجل أدنى مستوى في شهري تشرين الاول والثاني حيث بلغت (1.46) و (1.75) جرثومة/100مل على التوالي وهذا الانخفاض أرتبط أرتباطا وثيقا مع زيادة تركيزالكلور الحر في الماء ونستنتج من هذا بوجود علاقة عكسية قوية ($r= -0.72, p<0.01$) بين تركيز الكلور الحر واعداد جراثيم القولون.
ومن خلال التحليل الاحصائي بأستخدام معامل الارتباط والانحدار الخطي وجد علاقة عكسية متوسطة ($r=-0.49, p<0.01$) بين تركيز الكلورالحر و درجةحرارة الماء ووجودعلاقة عكسية متوسطة ($r=-0.46, p<0.01$) بين تركيز الكلور الحر وجهد الاكسدة والاختزال، ووجود علاقة عكسية قوية ($r=-0.5, p<0.01$) بين تركيز الكلور والاس الهيدروجين ونستنتج بأن كل من درجة الحرارة والاس الهيدروجيني وجهد الاكسدة والاختزال لم يكن لها دور سلبي على كفاءة الكلور التطهيرية خلال فترة الدراسة حيث كانت المعدلات ضمن المعدلات المتعامل بها دولي(4و 9و 12).

جدول رقم (3) يبين معدلات العوامل المؤثرة على فعالية تركيز الكلور وثابت الانحراف (SD) خلال فترة الدراسة (تموز – تشرين الثاني)2007.

الاشهر	عدد العينات الكلي	عدد العينات المفحوصة	تركيز الكلور الحر /جزء بالمليون		العد الجرثومي الاكثر احتمالا لبكتريا القولون /100مل		درجة الحرارة		جهد الاكسدة والاختزال /mv		الاس الهيدروجيني	
			المعدل	ثابت الانحراف	المعدل	الانحراف القياسي	المعدل	الانحراف القياسي	المعدل	الانحراف القياسي	المعدل	الانحراف القياسي
تموز	22	16	0.09	0.03 ±	21.0	8.03 ±	31	1.00 ±	37.6	±2.04	7.50	0.07 ±
اب	13	12	0.16	0.06 ±	20.0	8.25 ±	30.1	1.09 ±	37.1	±1.64	7.48	0.10 ±
أيلول	68	35	0.82	0.04 ±3	8.23	6.12 ±	30.7	1.33 ±	35	±4.23	7.38	0.11 ±
تشرين الاول	17	13	1.09	0.02 ±4	1.46	1.16 ±	26.2	1.23 ±	19.1	±3.09	6.99	0.14 ±
تشرين الثاني	17	12	1.30	±0.035	1.75	1.60 ±	25	1.31 ±	22.7	±2.68	7.08	0.11 ±

تحديد تركيز الكلور الكلي والعوامل المؤثرة عل كفاءة الكلور التطهيرية (الأس الهيدروجيني ودرجة الحرارة) في بعض محطات تصفية مياه الشرب لمدينة بغداد/ الكرخ وأشتمل هذا الجانب من الدراسة على محطتين لتصفية مياه الشرب (AوB) والواقعة في جانب الكرخ لمدينة بغداد حيث أعتمدت القيم المسجلة يوميا في سجلات تلك المحطات خلال المدة الممتدة من تموزالى تشرين الثاني 2007. أظهرت النتائج المدونة في الجدول رقم (4) بأن معدلات كل من الاس الهيدروجيني ودرجة حرارة الماء التي تم تسجيلها خلال شهر تشرين الثاني بالاجهزة الرقمية الحديثة كما هي موضحة في الجدول رقم(4-2) كانت أقل بكثير من القيم التي سجلت من قبل كادر المحطات والتي وردت في الجدول رقم (4-1) بينماكان معدل تركيز الكلور الكلي خلال شهر تشرين الثاني بالاجهزة الرقمية الحديثة كما هو مدون في الجدول رقم (4-2) أعلى بكثير من المعدل الذي ورد في الجدول رقم (4-1) والذي تم تسجيله في محطات تصفية المياه بالاجهزة المتوفرة لديهم .وقد أعزيت الفروقات التي ظهرت بين تلك القيم خلال شهر تشرين الثاني الى دقة وحساسية الاجهزة الرقمية الحديثة التي أستخدمت من قبلنا بالمقارنة مع الطريقة المستخدمة في تلك المحطات والتي كانت تعتمد على كفاءة العامل البشري في قراءة النتائج.

جدول رقم (4) تركيز الكلور والاس الهيدروجيني ودرجة الحرارة لماء الشرب لمحطتي تصفية ماء الشرب لمدينة بغداد /الكرخ.(الجدول مقسم الى 4-1 و 4-2)

جدول رقم (4-1) قراءات القيم خلال المدة الواقعة بين شهر تموز وتشرين الثاني حسب سجلات المحطات.

الاشهر	تركيز الكلور الكلي/جزء بالمليون			الاس الهيدروجيني			درجةالحرارة		
	المعدل	المدى		المعدل	المدى		المعدل	المدى	
		أدنى	أعلى		أدنى	أعلى		أدنى	أعلى
تموز	1.81	0.6	2.8	7.6	7.5	7.9	31.1	28	34
اب	2.14	1.4	3.7	7.5	7.5	7.7	32.3	31	34
ايلول	3.5	0.3	5.0	7.6	7.5	7.9	30.5	27	32
تشرين الأول	4.2	4.0	5.1	7.5	7.2	7.9	26.5	23	31
تشرين الثاني	3.6	2.6	4.8	7.5	7.3	7.9	21.7	18	24

جدول رقم (4-2) قراءات قيم المحطات نفسها خلال شهر تشرين الثاني باستخدام الأجهزة الحديثة.

الأشهر	تركيز الكلور الكلي/جزء بالمليون			الاس الهيدروجيني			درجة حرارة الماء(مْ)		
	المعدل	المدى		المعدل	المدى		المعدل	المدى	
		أدنى	أعلى		أدنى	أعلى		أدنى	أعلى
تشرين الثاني	5.05	3.7	7.6	6.94	6.84	7.12	17.6	15.8	19

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- ✓ Ingrkam, JL. and lngrahan, CA . (2000). Introduction. In: Text of Microbiology. 2nd ed. Anstratia, Brooks, Co. Thompson Learning, P: 55.

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- ✓ Andersen, RM. (1998). Epidemiology of parasitic infections. In: Topley and Wilsons Lnfections. Collire, L. ; Balows, A. and Jassman, M. (Eds.), Vol, 5,9th ed. Arnold a Member of the Hodder Group, London, Pp: 39-55.

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Immunological Response of Bovine Mammary Cell Lines in Mastitis and Milk Hygiene

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Summary

Mammary adherent cells (MAC-T) were infected with six isolates of *Streptococcus uberis* (*S. uberis*). Three isolates were cases of mastitis in dairy cows and belonged to clonal complex 5, 143**,** which is associated with virulence and three were from cows with no clinical or laboratory evidence of mastitis. All these isolates belonged to clonal complex 86 which contains strains of low virulence. After incubation at 37^{o}C for 24 h, there were no significant differences in the number of adherent or internalized *S. uberis* between mastitis (M) and non- mastitis (NM) isolates ($p > 0.05$).

The levels of tumour necrosis factor (TNF-α), measured in treated MAC-T cells supernatant with *S. uberis* by ELISA, were significantly elevated in cultures infected with NM isolates compared with M isolates, after 10h ($p>0.05$) and 24h ($p>0.001$) respectively. Expression of TNF-α, TLR2, TLR4 and NFkB genes were examined by Real-Time PCR. There are highly significant differences in the timing of expression. The levels of TNF-α mRNA increased 36 fold after 6 hour of infecting cells with M strains, but not in NM strains of *S. uberis*. These results suggest a vital role for TNF-α, in the defence against *S. uberis* in the bovine mammary glands.

Key words: Mastitis, *S.uberis*, Cytokines, TNF, TLR, NFkB, PCR, Real Time PCR.
This study was performed within Department of Biotechnology, School of Applied Science at RMIT University, Melbourne, Victoria, Australia

الاستجابة المناعية لخلايا الضرع المختبرية في التهاب الضرع بالابقار وصحة الحليب

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الخلاصة

اصيبت خلايا الضرع بستة عزلات من المكورات السبحية المسببة لالتهاب الضرع. ثلاثة عزلات من حالات التهاب الضرع وتعود الى الاستنساخ المعقدة 5 و 143 التي لها علاقة بالضراوة في التهاب الضرع. ثلاثة عزلات من حالات التهاب الضرع وتعود الى الاستنساخ المعقدة 86 التي لها علاقة بأنخفاض الضراوة او التي لاتظهر علامات سريرية لالتهاب الضرع. لاتوجد اختلافات معنوية في اعداد البكتريا اللاصقة او الداخلة الى خلايا الضرع سواء كانت ضارية او غير ضارية. كان قياس معدل عامل نخر الورم بوساطة ELISA في مصل خلايا الضرع المختبرية المعرضة للمكورت السبحية مرتفع بصورة معنوية للبكتريا منخفضة الضراوة بعد 10 و 24 ساعة من التعرض الى الاصابة. التعابير الوراثية عامل نخر الورم والمستقبلات الشبيهة بعدد القتلى و العامل النووي للخلايا كابا ب فحصت بوساطة Real-Time PCR. ان معدل عامل نخر الورم مرتفع معنويا في البكتريا ذات الضراوة ليزيد على 36 مرة على البقية بعد 6 ساعات من التعرض للجرثومة ذات الضراوة المعنوية. اشارت النتائج الى وجود دور مهم يلعبه عامل نخر الورم في مناعة الجسم ضد البكتريا في الغدد اللبنية للابقار.

كلمات مفتاحية:ألتهاب الضرع، المكورات السبحية، عامل نخر الورم، المستقبلات الشبيهة، العامل النووي، البلمرة المتسلسل،الوقت الحقيقي للبلمرة المتسلسل.

اجريت هذه الدراسة في قسم تقنيات الحياة ، كلية العلوم التطبيقية ، جامعة ارمأيتي، ملبورن، فكتوريا ، استراليا

Introduction

Bovine mastitis is an inflammation of one or more quarters of the udder. This disease is still the most costly infection of dairy cattle for the milk industry (1). *S. uberis* is one of 'environmental pathogen' which is responsible for a significant proportion of clinical mastitis (2). It is considered as an 'effective pathogen' because cows are likely to develop intramammary infections if their udders are exposed to contaminated material, especially if they have damaged teat skin or open teat ends. *S. uberis* is passed in the faeces of cattle (and other ruminants) and can survive for up to 2 weeks in fresh dung or faecal-contaminated mud or straw (3). Infections due to *S. uberis* are predominantly in subclinical mastitis (95%) and up to 33% of clinical cases per year in the United States and 30% of clinical cases in UK (4 and 5). The clinical and subclinical mastitis caused by *S. uberis* ranged from 75% in Australia and New Zealand (6 and 7).

Analysis of *S. uberis* by pulsed-field gel electrophoresis (PFGE) from the same and different farms has shown that the species is highly diverse (8). Evidence is emerging from multi-locus sequence typing, however, that some clonal complexes (CCs) are highly associated with clinical and subclinical mastitis, while others are found in the environment or are isolated mainly from cows with low somatic cell counts (9). Strains belonging to global clonal complex (GCC) sequence type (ST) 5 and GCC ST143 are associated with clinical and subclinical mastitis, whereas GCC ST 83 are considered to have less capacity to cause mastitis (10, 11, 12). This laboratory has previously speculated that strains belonging to GCC ST5 and GCC ST143 possess factors promoting survival in the environment, invasion of host tissue, internalization of mammary epithelial cells or evasion of host immune responses (12).

MAC-T cells, an immortalized epithelial cell line isolated from bovine mammary tissue, were routinely cultured according to the recommended conditions (13). MAC-T cells were used widely in the experimental design instead of live animals. Bovine mammary epithelial cells (bMEC or MAC-T cells) are capable to produce neutrophil-mobilizing chemokines and pro-inflammatory cytokines such as Interleukin (IL)-6 and TNF-α upon bacterial stimulation (14, 15 and 16). MAC-T cells are crucial to delay the attacking bacteria while sending chemoattractant signals to circulate neutrophils and lymphocytes therapy facilitating generation of rapid stronger local innate immune defences mediated by infiltrating immune cells and ultimately antigen–specific protective immune responses (17, 18).

The innate immune system is the major contributor to acute inflammation induced by microbial infection or tissue damage. Innate immune cells including macrophages and dendritic cells (DCs) play important roles, nonprofessional cells such as epithelial cells, endothelial cells, and fibroblasts also contribute to innate immunity (19). The endothelial tissue and their pro-inflammatory cytokines play an important role during inflammation and caused reduction in the intracellular of *S. epidermidis* with bovine endothelial cells (20).

Toll-like receptors (TLRs) function is to distinguish antigens and to initiate an appropriate immune response (21). Toll-like receptors are key sensors of pathogen-associated molecular patterns (PAMPs) (22). Bovine mammary epithelial cells contribute to the innate immune response to intramammary infections by recognizing pathogens through specialized pattern recognition receptors. Toll-like receptor 4 (TLR4) is activated by lipopolysaccharide (LPS), a component of the outer envelope of Gram-negative bacteria (23).

The level of both tumour necrosis factor (TNF)-α and IL-12 were increased and played a role in regulation of the immune responses of bovine mammary gland in *S. aureus* infection (24). Real-time reverse transcriptase-polymerase chain reaction (RT-

PCR) was used to quantify interleukin-1 (IL-1), IL-1 receptor antagonist (IL-1Ra), tumour necrosis factor (TNF)-a, toll-like receptor 2 (TLR2), and toll-like receptor 4 (TLR4) (25). These various cytokines (TNF-α, IL-1ß, IL-6, IL-8, IL-4, IL-10 and IL-13) have synergistic and antagonistic effects in the immuno-inflammatory response of infections (26). TNF-α has ability to trigger directly or indirectly by inducing the production of secondary mediators. Recent results strongly suggest that the classical and alternative pathways to NF-kB activation have distinct regulatory functions, one that is mostly involved in innate immunity and the other in adaptive immunity. Recently, only a single NF-kB signaling pathway was known, whereby NF-kB activity is stimulated by pro-inflammatory cytokines, such as tumor necrosis factor-a (TNF-α) and interleukin-1 (IL-1), as well as by pathogen associated molecular patterns (PAMPs)(27).

The goal of this study was to compare strains of GCC ST3/ 143 with GCC ST 83 and investigate the mRNA expression of TNF-a, TLR2, TLR4 and NFkB immune components during the acute phase of mammary inflammation caused by *S. uberis*, and establish a new model design of vaccination against bovine mastitis.

Materials and Methods

Bacterial strains**:** Six *S. uberis* isolates from the milk of cows were used in this study. Three isolates 5851, 2520.1 and 2893.1 were from cows with clinical mastitis had previously been placed in GCC ST5 and 143 respectively. The other three isolates from cows without mastitis (milk somatic cell counts <250,000 /ml belonged to GCC St83 (12). One to two separate colonies were streaked on Columbia agar plates (CAB) incubated at 37°C for 18 h and then separate colonies were inoculated into a media contains beads. The cultures were stored at –80°C as stock cultures. One bead from the stock culture was streaked onto CAB and incubated overnight at 37 °C. Bacteria was harvested in sterile 20 ml Todd–Hewitt broth (THB), mixed in 150 rpm orbital shaker for 2 h at 37 °C. Bacterial suspension was washed three times with PBS, then estimated the number of bacterial counts by OD_{600} spectrophotometer (Invitrogen). Bacterial suspension was diluted with DMEM to a concentration of ~$1.3X10^7$ colony-forming units (CFU).

Invasion of MAC-T cells with *S. uberis***:** Dulbecco Modified Edward medium (DMEM) with 10% foetal bovine serum (FBS) plus antibiotics was removed from 24-well plates containing monolayer MAC-T cell. 1 ml of fresh DMEM medium only contains *S. uberis* ($1.3X10^7$ CFU/ml) per well in triplicate for each strain. Co-culture MAC-T medium were incubated for 60 minutes at 37°C and 5% CO_2. Mammary epithelial cell monolayers were washed three times with PBS, then added 1 ml/well fresh DMEM medium with 10% foetal bovine serum (FBS), 50 µg/mL of streptomycin, and 50 IU/mL (Invitrogen) 24-well plate was incubated for 24 h at 37°C, 5% CO_2. The MAC-T cells were detached after 24 h of incubation with 0.25% trypsine, checked the viability with counter (Invitrogen^{TM} countess, Automated Cell Counter) of cells and lyses with 0.25% Triton X-100 at a final concentration of 0.025% (v/v) in sterile distilled water. Serial dilutions of Lyses cells were streaked into CAB then incubated for 18 h at 37°C. The colonies were counted and multiplied by dilution factor. These experiments were repeated three times.

TNF-α test by ELISA**:** TNF-α tested by an ELISA procedure was followed (R&D research manufacture). MAC-T cells were infected with *S. uberis* for one hour. TNF-α protein level were measured in infected MAC-T supernatants harvested after incubation at 37°C and 5% CO_2 for 0 h, 3 h, 10 h and 24 h and stored at -20^{o}C until use. TNF-α was measured with ELISA followed (R&D research manufacture). Briefly, coated a 96-well

microplate with 100 µL per well of the diluted Capture Antibody. The plate was sealed and incubated overnight in refrigeration. Aspirated each well and washed with Wash Buffer for three times. The plate was inverted and blotted against clean paper towels. The plate was blocked Block Buffer. Then the plate was incubated at room temperature for 1 hour. The plate was washed again. The sample or standard was added to the well. An adhesive strip covered the plate and incubated 2 hours at room temperature. The plate was washed again. The Detecting Antibody was added to each well. The plate was covered with a new adhesive strip and incubated 2 hours at room temperature. Repeating the aspiration/wash was applied. Streptavidin-HRP was added to each well. The plate was covered and incubated for 20 minutes at room temperature in dark place. The plate was washed again. Substrate Solution was added to each well. The plate was incubated for 20 minutes at room temperature in a dark place. Stop Solution was added to each well, and thorough mixing. The plate was determined by optical density of each well immediately, using a microplate reader set to 450 nm.

RNA Isolation and Quantitative**:** *S. uberis* strains cultures of (5851 and 3327.3) were prepared as above-mentioned method in invasion of MAC-T cells with *S. uberis*. Stimulate MAC-T with DMEM only containing *S. uberis* ~1.3 X 107 for 1 h at 37oC 5% CO2, wash MAC-T with PBS three times. Fresh DMEM only was added and incubate for 0, 6, 12 and 24 h, MAC-T after which total RNA was determined and Gel electrophoresis.

RNA isolation from MAC-T cells**:** The cell culture medium was removed completely by aspiration. The cells were washed once with PBS pH 7.2. The RNA extraction was followed (Bioline instructions). Briefly, Added 1 ml of TRIsure (BIOLINE) to T75 ml tissue culture flask, scraped quickly by scrapper. The cells were collected in eppendorf tube by aspiration, and then added 0.2 ml chloroform, secure cap tube and shake it vigoursly by hand for 15 seconds. The tube was incubated at room temperature for 3-5 min, then centrifuge the sample at 12000 xg for 15 min. at 2-8^{o}C. The upper layer was collected into a new collection tube, and then added 0.5 ml Isopropyl alcohol to the collection tube incubated at room temperature for 15 minutes. Centrifuge the mixture 12000 x g for 15 minutes at 2-8^{o}C. The supernatant was removed and the pellet was washed once with 1 ml 70% ethanol. The sample was vortex and centrifuge at 7500 xg for 5 min. 2-8^{o}C. RNA was stored at -80^{o}C until used it further.

The complementary DNA: The complementary DNA (cDNA) was prepared by the following on ice: Mixing the reagents in PCR tube 1µg RNA (3 µl), Oligo $(dt)_{18}$ (1µl) 10mM dNTP (1µl) and diethyl pyrocarbonate-treated water (DEPC-H_2O) up to (10µl). The tubes were put in PCR machine, and then the mixture was incubated at 65^{o}C for 10 min. All tubes were placed in ice for 2 minutes. In the meantime these reagents 5x RT Buffer 4µl, RNase inhibitor 1µl, Reverse transcriptase (200u/ µl) 0.25 µl and DEPC-H_2O up to 10 µl. The last 10 µl reagents were added to 10 µl of the above reaction mix to a tube containing the primed RNA. Samples were incubated at 45^{o}C for 60 min and the reaction was terminated by incubating at 70^{o}C for 15 min. The samples were stored at -20^{o}C until next step real time PCR was be ready.

Real-Time PCR: 2 X SensiMix 25 µl final dilutions was 1X was added to 5 µl Template, then added 2 µl of each 10µM forward TLR2, TLR4, NFkB and TNF-α Primers final concentration 200nM. 2 µl of 10µM reverse Primers final concentration 200nM was added. RNase free water was added up to 50 µl. The mixture was placed in Real-Time PCR special tubes or 48 wells and sealed. The tubes or plate were put in Real-Time PCR machine which was programmed as follows. Cycle 1 was programmed at 95^{o}C for10 min. The 40th cycle was planned at temperature 95^{o}C for 10 seconds and 60^{o}C for 60 seconds acquire at end of step.

Statistical Analysis: Statistic for data was performed by student *t-test*, ANOVAs two way statistics.

Results and Discussion

Adherence, Invasion and Viability of MAC-T cells: There were no significant differences between M and NM isolates in their ability to adhere to (Figure 1) invade (Figure2), or kill (Figure 3), MAC-T cells after a 24 h incubation period at 37^{o}C.

MAC-T was infected with six M and NM strains of *S. uberis*. The viability of MAC-T cells in both M and NM of *S. uberis* strains were compared. Neither the adherent of M strain *S. uberis* counts to MAC-T cells or NM *S. uberis* counts were different in numbers after MAC-T cells infected with both strains of *S. uberis.* These results agreed with the results accomplished by (28, 29 and 30), these authors summarised that M and NM of *S. uberis* strains were adherent to MAC-T cells, but equivalent in the adherence counts between M and NM of *S. uberis* strains. The surviving of both M and NM of *S. uberis* strains were estimated by SPC after 24 h incubation in MAC-T cells, no significant differences between two strains. These results are supported by (1) who also mentioned that *S. uberis* survived intracellular for 120 h without loss of viability in MAC-T cells.

TNF- α Measurement by ELISA: In supernatant of MAC-T cells incubated with *S. uberis*, there was a steady increase in levels of TNF-α over the incubation period of 24 h. The levels TNF-α were elevated significantly ($P \leq 0.05$) in MAC-T cell's supernatant which was stimulated by NM strains of *S. uberis* more than M strains of *S. uberis* in 10 h incubation ($P \leq 0.05$). The difference was that TNF-α levels (yellow columns) were highly significant after 24 h incubation. The Level of TNF-α in stimulated MAC-T cells with M strains were increased after 10 h and 24 h incubation but less extent than NM strain. The negative control (blue columns) and positive control (red columns) were conducted in each experiment (**Figure 4**).The up-regulation of TNF-α in M *S. uberis* was converted from nanograms to picograms. TNF-α was 213.8 pg after 24 h incubation time of supernatant of MAC-T cells, in 10 h incubation time, the measurement of TNF-α was 15.1 pg, while in 3 h time was nearly close to 0 time measurement.

Activated macrophages release mediators, such as interleukin-1 (IL-1) and tumour necrosis factor (TNF) families appear to be uniquely important in initiation the next series of reaction. These cytokines have pleiotropic activity and act both locally and distally (31). In this study we found that TNF-α elevated after 10 h and 24 h of infected MAC-T with M and NM *S. uberis*. TNF-α was increased significantly in NM strains over M strains.

These results indicated that one or two of these 3 strains possessed influential antigenic activity to stimulate TNF-α more than the M strain. These results disagreed with the findings by (32) who designed their experiment in the macrophages of milk. The level of TNF-α in NM *S. uberis* strains up-regulated 10 folds in time 10 h and 24 h higher than in NM strains of *S. uberis*. The mRNAs tend to have abundant AU-rich elements in their 3'UTRs compared with mRNAs expressed at later time points (33). Therefore, control of mRNA decay may be as important as control of transcription in terms of the regulation of innate immune responses.

Real-Time PCR: Extraction RNA was measured by gel electrophoresis as shown in (Figure 5). The primer genes have been analysed by gel electrophoresis as revealed in (Figure 6). Normalise data from real time PCR were showed that TNF-α measurement has been much higher than TLR2, TLR4, and NFkB genes. Folds of TLR2, TLR4, and TNF-α treated with M of *S. uberis* strains was high. TNF-α level was 3.59 in 6 h and 0.209 in 12 h of incubation, while TNF-α Treated with NM 0.02 in 6 h and 0.002 in 12 h of incubation. TLR2 level treated with M of *S. uberis* was 0.15 in 6 h and 0.02 in 12 h of

incubation while TLR2 level treated with NM of *S. uberis* was 0.003 in 6 h and 0.02 in 12 h of incubation. TLR4 level treated with M of *S. uberis* was 0.06 in 6 h and 0.08 in 12 h of incubation while TLR4 level treated with NM of *S. uberis* was 0.02 in 6 h and 0.02 in 12 h of incubation. NFkB results in different time of incubation were below the TNF-α level so, in (**Figure 7)** was used sigmaplot graph to display lower columns in the graph for matching with TNF-α.

The inflammatory response is orchestrated by pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6. These cytokines are pleiotropic proteins that regulate the cell death of inflammatory tissues, modify vascular endothelial permeability, recruit blood cells to inflamed tissues, and induce the production of acute-phase proteins. Although TNF and IL-6 are mainly regulated at the transcriptional and translational levels, the production of IL-1b is regulated by a two-step mechanism. The first step is the expression of an IL-1β zymogen, pro-IL-1β, which is regulated by the synthesis of its mRNA in a TLR signal-dependent manner. However, IL-1b maturation requires cleavage of pro-IL-1b by a protease, caspase-1, which is activated independently of TLR signaling. The complex that activates caspase-1, called the inflammasome, is composed of Nod-like receptor (NLRs), Apoptosis associated speck-like protein containing a caspase recruitment (ASC), and caspase-1 (34).

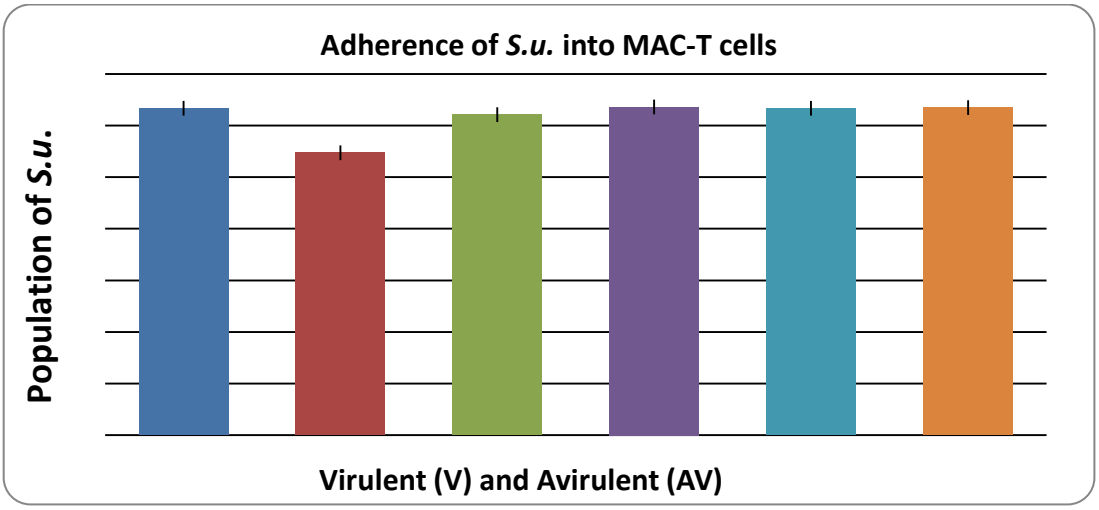

Figure1. Mean with SE number of Virulence and Non-Virulence of *S. uberis* adherent to MAC-T cells. Each point was the result of adhesion assays performed on these isolates of *S. uberis* either Virulence or Non-Virulence in triplicate.

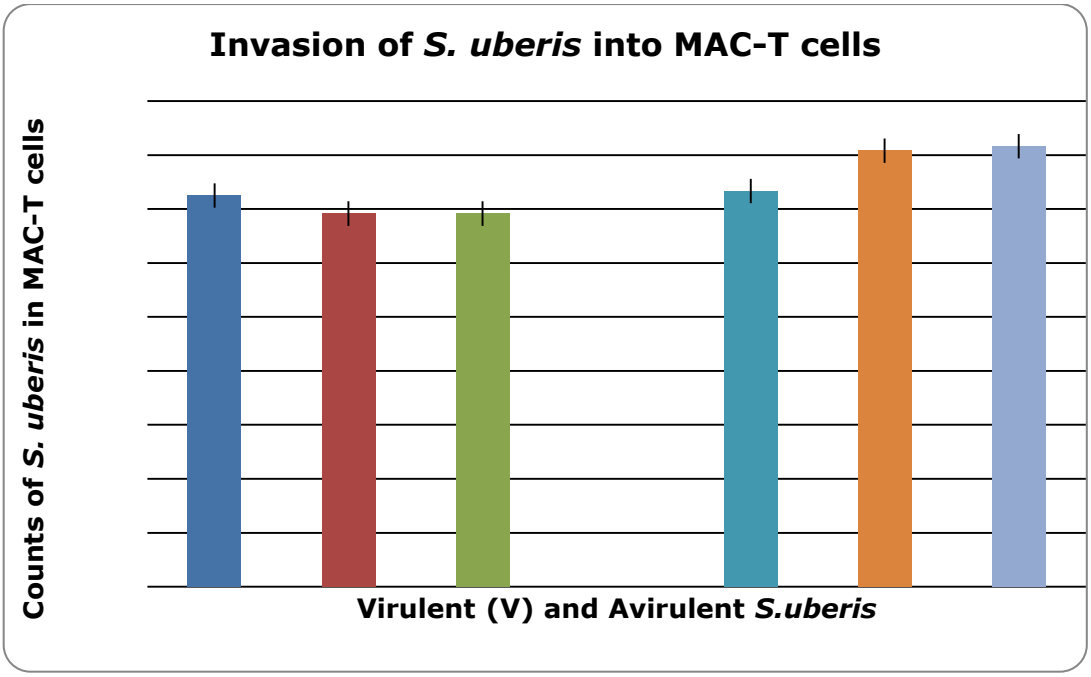

Figure 2. Mean with SE number of Virulence and Non-Virulence *S. uberis* invasion to MAC-T cells. Each point was the result of invasion assays performed on these isolates of *S. uberis* (either mastitis or non-mastitis associated) in triplicate.

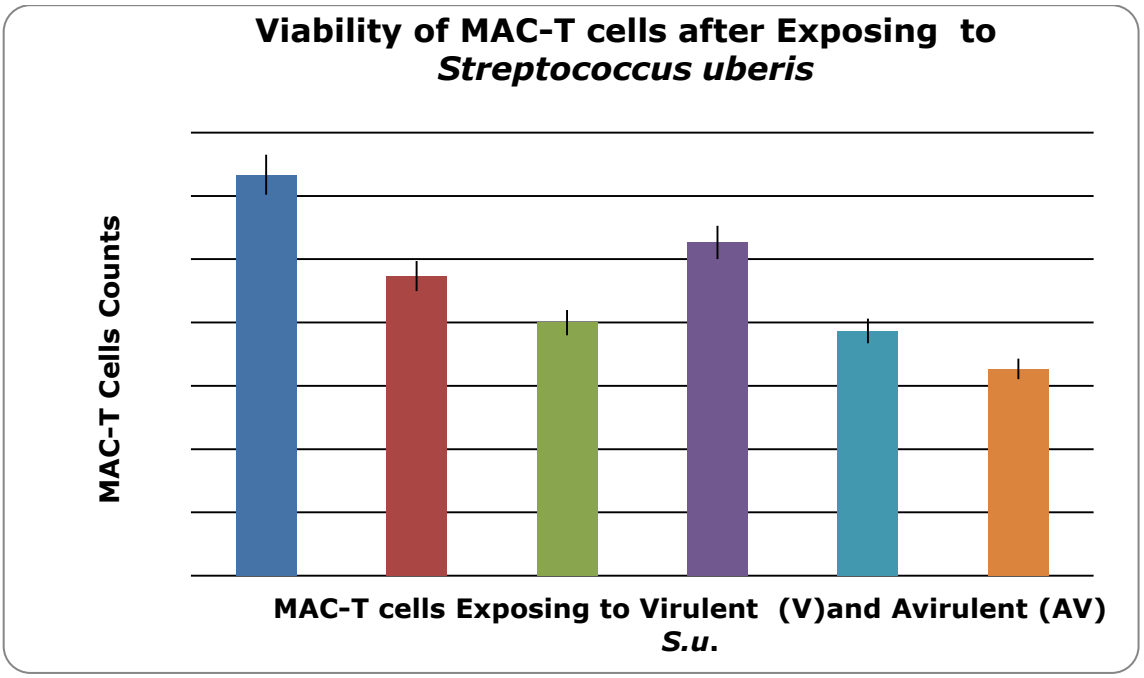

Figure 3. Mean with SE number of Virulence and Non-Virulence *S. uberis* to MAC-T cells viability. Each point was the result of killing performance of these isolates (either mastitis or non-mastitis associated) to MAC-T cells in triplicate.

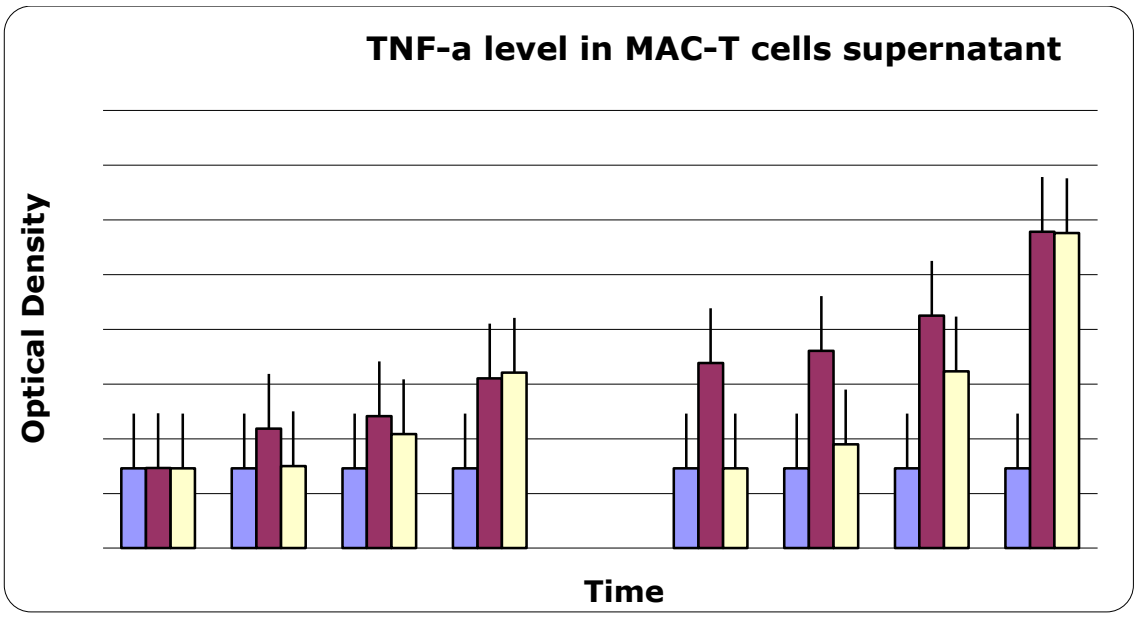

Figure 4. The level of TNF-α measurement level in Pictograms at time 0h, 3h, 10 h, and 24 h incubation period in both M and NM strains of *S. uberis*. Blue Columns are Negative control, red Columns are positive control, yellow columns are Mastitis left and non-mastitis right.

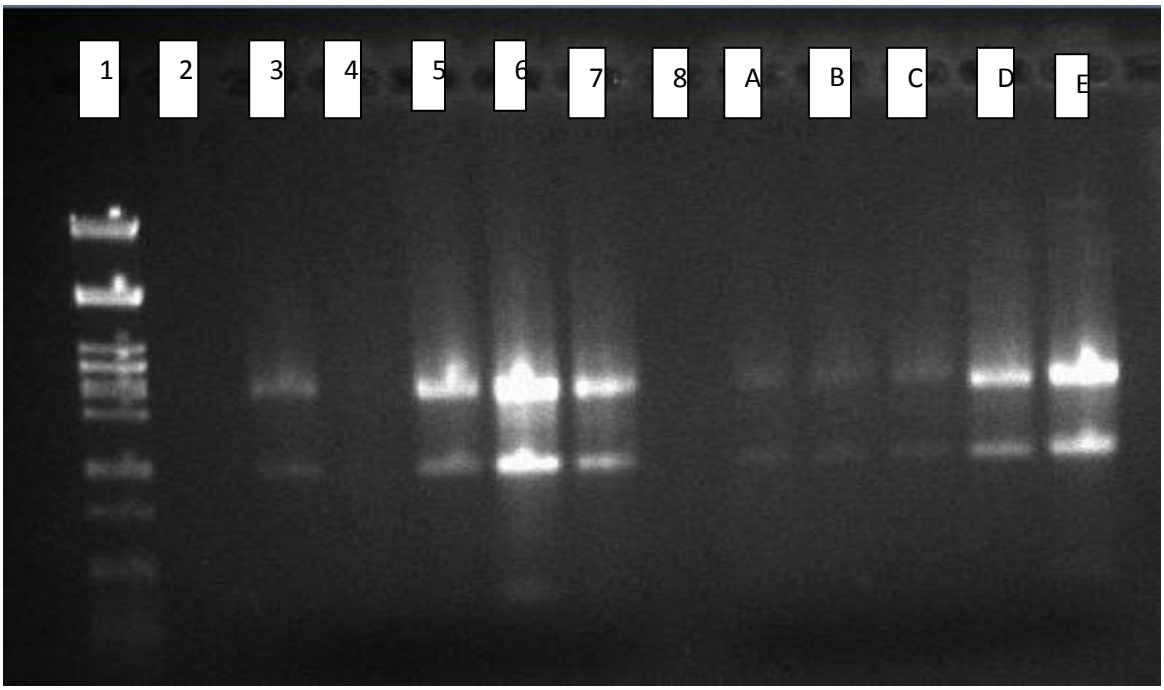

Figure 5. RNA extraction from MAC-T cells after stimulation with *S. uberis*. 1= Marker, 2= Empty, 3=M 0h, 4= M 3h, 5= M 6h, 6= M 12h, 7= M 24h, 8 = Empty, A= NM 0h, B= NM 3h, C= NM 6h, D= NM12h, E= NM24h.

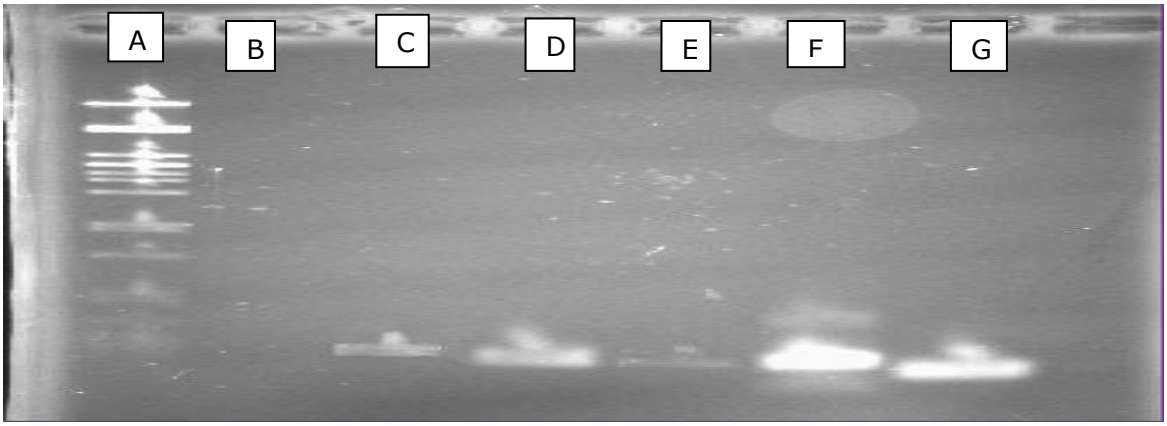

Figure 6. cDNA of RNA extraction from MAC-T cells after stimulation with *S. uberis*. A=Marker, B=NA, C=TLR2, D=TLR4, E=NFkB, F=GAPDH, F=TNF-α.

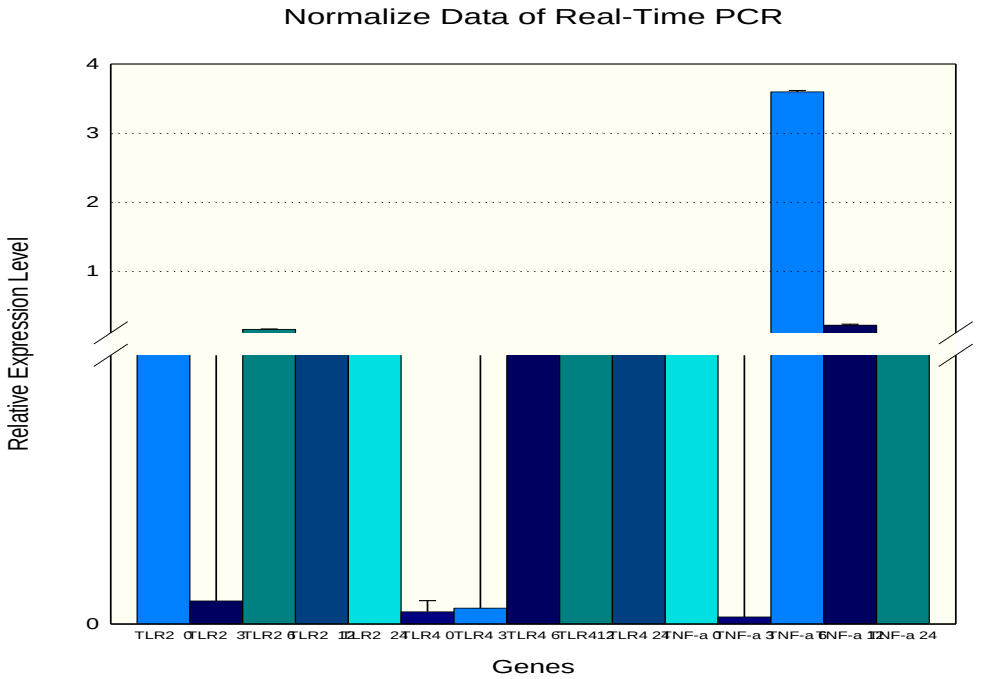

Figure7. Normalise data of mRNA, TLR2 0, 3,6, 12, 24 h - TLR4 0, 3,6, 12, 24 h, TNF-α 0, 3,6, 12, 24 h.

Table 1. Percentage of immune components after stimulation with Mastitis and Non-Mastitis of *S. uberis* strains.

Immune components	0h	3h	6h	12h	24h
TLR2 (M)	3%	3%	80%	10%	4%
TLR2 (NM)	2%	10%	6%	80%	2%
TLR4 (M)	1%	2%	40%	55%	2%
TLR4(NM)	2%	30%	33%	33%	2%
TNF-a (M)	1%	1%	90%	6%	2%
TNF-a(NM)	1%	1%	50%	45%	3%

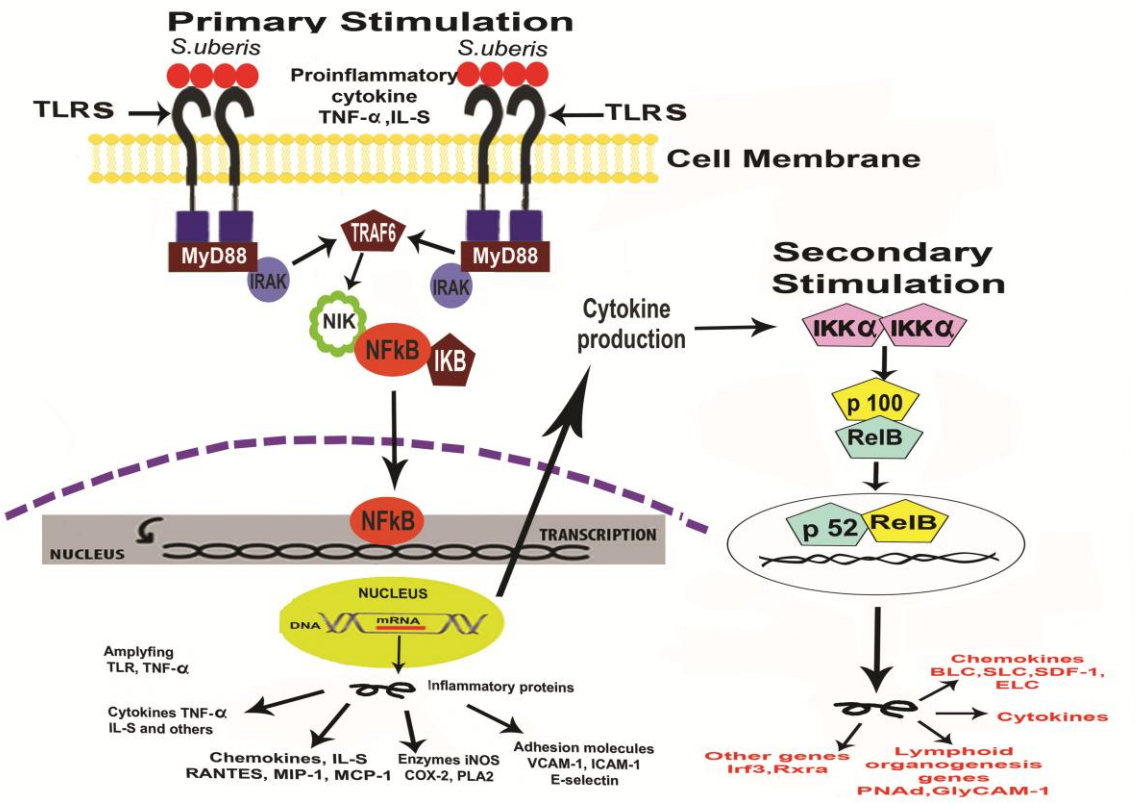

Diagram 1. Primary and secondary stimulation of innate immunity induced by mastitis and non-mastitis strains of *S. uberis.*

TNF-α, TLR2, TLR4, NFkB were determined and quantitative by Real-time PCR (Table 1) in this method we found that TNF-α was highly shifted in M strains of *S. uberis* than NM strains of *S. uberis.* TNF-α level was elevated 35.9 times, than others (Figure 7). Transcripts that increased in both cell lines by at least 20 fold included IL-8, CXCL6 and TNF-α, but in each case there was a much greater fold increase in the bMEC. IL-1b and b-defensin were markedly up-regulated by greater than 500 fold in bMEC but only 4.26 and 2.75 fold, respectively, in the MAC-T cells (17). Gram positive and gram negative bacteria fluctuate in their dose-dependent patterns of induction of TLR2 and TLR4 (35). Nearly all of the genes directly involved in the Toll-Like Receptor activation pathway (i.e. TLR2, TLR4, CD14, IRAK-1, IRAK-4, IRAK-M and TRAF-6) were expressed in both cell lines but in each case was largely unaffected by LPS treatment (17). In case of TLR2 and TLR4 were elevated in the M strains more than NM strains. The number of TLR2 copies correlated well with those of TLR4, indicating coordinated regulation of these two PRRs during infection of the udder (36). For that reason it thought that M strains were more reliable to induce TNF-α than NM strains of *S. uberis*. TLR4 is the major receptor for LPS and causes intracellular signal transduction (37). The initial stimulation of *S. uberis* induced innate immunity, while secondary stimulation induced cytokines which take part in pro-inflammatory responses or cure by production of anti-inflammatory immune components (Diagram 1). The function of TNF-α activates, as a synergistic in normal level and as an antagonistic or adverse reaction in high level.

In conclusion, the function of TNF-α activates as a synergistic in normal level and an antagonistic or adverse reaction in high level. TNF-α in the mRNA of MAC-T cells is induced by mastitis *S. uberis* strains after 6 h due to: Firstly, the cells being able to

produce TNF-α in the first 6 hours after infected with *S. uberis* due to stimulation of TNFR4 receptor which lead to inducing TNF-α. Secondly, mastitis strains of *S. uberis* have the ability to attack mammary tissue in the first 6 h and inhibit the secretion of cytokines to make the tissue medium safe for multiplication. Thirdly, MAC-T cells are defending themselves against bacteria by secreting cytokines to stop their action and sending a signal to other immunity cells and components to participate in the body defence. Fourthly**,** TLR2, TLR4 are involved indirectly in this process by activation of NFkB, for that reason low level of NFkB, TLR2 and TLR4 were detected in this process. Fifthly**,** NM strains of *S. uberis* have induced the TNF-α from MAC-T in supernatant, for that reason TNF-α in ELISA was detected in 12 and 24 hours. Sixthly**,** NFkB have appeared only during time 3 h after stimulation with M and NM. In M case NFkB was very low during this early stage while TNF-α level was the highest 36 folds and higher than TNF-α in NM stimulation. In NM case NFkB was cosiderably high while TNF-α was lower than M stimulation. Our results showed TNF-α had a negative correlation with NFkB. Seventhly**,** it recommond in future studies to design synthetic peptides of MAC-T cells TNF-α to use it as a vaccination against bovine mastitis as a trial proposal.

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Catalyzed Gas Phase Ammoxidation of 2, 3 and 4-picolines

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Summary

Catalyst gas phase ammoxidation of 2,3, and 4- picolines in a fixed bed reactor is described the catalyst applied composed of Vanadium (V) and tin (Sn) Oxides supported on Al_2O_3 as described in recent papers (1), (2). The effect of NH_3, O_2, contact time and temperature of the picoline mole ratios on the yield and conversion of the products was studied. The conversation and yield are in the rate of 2-picoline> 4-picoline > 3-picoline.

The applied catalyst was active even after 150 hours of reaction. The highest yield obtained of nicotinic acid was 90%, 88%, 87% 2-picoline, 4-picoline and 3-picoline respectively.

Key words: Catalyzed, Ammoxidation, 2,3,4 picolines ,NH3

امكسدة 3,2 و 4- بيكولين في الطور الغازي

راجحة اسماعيل خليل النعيمي
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الخلاصة

تمت دراسة تفاعلات الامكسدة 3,2 و 4- بيكولين في الطور الغازي في مفاعل ذو القاع الثابت. ان العامل المساعد المستخدم يتكون من اوكسيدي الفناديوم والقصدير ومحمول على اوكسيد الالمنيوم قد تم وصفه في بحوث سابقة (1),(2).
ان تاثير النسب المولية للامونيا والاوكسجين وزمن التماس ودرجة الحرارة الى البيكولين على ناتج الامكسدة ونواتج التفاعل الاخرى تمت دراسته وكانت نسبة التحويل 2- بيكولين >4-بيكولين> 3-بيكولين. واعلى نسبة للناتج من حامض النيكوتينك 90%, 88%, 87% ل 2- بيكولين, 4- بيكولين و 3- بيكولين على التوالي وكان العامل المساعد فعالا حتى بعد استخدامه 150 ساعة.

كلمات مفتاحية :- امكسدة , بيكولين, الامونيا.

Introduction

Ammoxidation is a valuable tool for one step synthesis of nitriles (3). The catalytic ammoxidation of hetroalkyl aromatics has been the subject of many patents (4, 5, and 6). Picolines consist of three structural isomers with methyl group at different positions from the nitrogen atom in their pyridine ring (7). The resulting nitriles are important intermediates to produce food industrial, pharmaceutical and petrochemical compounds such as nicotinamides, isonicotinamides and hydrazides (8). Both the conversion and the yield of the reaction depend on various factors such as the mole ratios of O_2, NH_3 and the pyridine derivatives in the reaction mixture, also the type of the catalyst, the reactor used and the temperature at which the reaction is curried out have influence to provide a catalyst with high yield and long life. Ammoxidation of 3- picoline over V2O5 / Ti O_2 showed a relationship between oxidation state of vanadium and ammoxidation activity (9- 12). A new structure of vanadium chromium composite oxide was reported (13). Incorporation of Sn to V_2O_5 / Al_2O_3 system make the catalyst more active and selective towards the formation of products in P-Xyline, O- Xyline and m- Xylines (1, 2).

Ammoxidation of isomeric picolines on Mo/PO catalyst with P/MO=1 was studied by (7, 14). Vanadium- modified zeolite, vanadium containing silico alumino phosphate were used as catalysts (15). The catalytic properties were influenced by chemisorptions of the reactants on the catalyst system (16). In this paper new results of using the catalyst described in earlier papers (1, 2), with development in the preparation technique for the ammoxidation of 2, 4 and 3- picolines to their corresponding nitriles with high yield, selectivity and long life for the catalyst, in an attempt to understand the influence of the methyl group position in the pyridine ring on the conversion of picoline isomers.

Materials and methods

The reactor used for the ammoxidation is the same as described in previous papers (1, 2). (Diag, 1). the same catalyst was applied to resulting reaction mixture was found to include picoline nitriles, amides, carboxylic acids and CO_2, CO, HCN. They were separated through fractional distillation under vacuum and estimated as raw products. Their identification was accomplished by IR spectroscopy, boiling point, melting point and refractive index.

The reactor consists of two Pyrex tubes with a side inlet for thermo couple to measure the temperature at different heights. The upper part is filled with glass severs used for preheating the reacting gasses. The lower tube consists of three different layers of glass, catalyst and glass respectively.

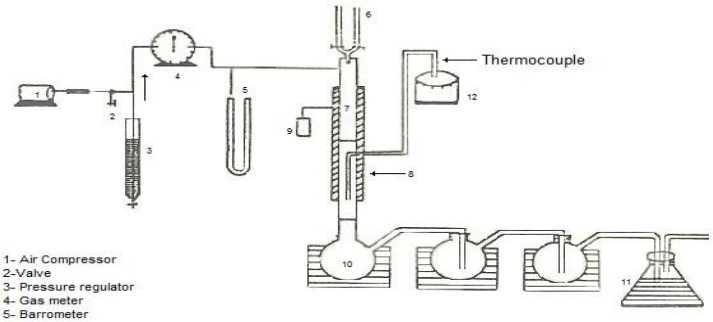


Diagram (1) Schematic Diagram of Ammoxidation Reactions devise used in the present work.

The catalyst is prepared from 40g NH_4VO_3 in 300ml water mixed with 120g of Sn $(NO_3)_2$ in 200 ml water. The PH was adjusted to be 10, the aqueous ammonia was added. The NH_4VO_3 suspension was stirred in a bath at 80-90° c for three hours. Water was removed by distillation using a rotary evaporator and the residue was placed in a drier at 120° c and dried. The product was calcinated at 900°c for 6 hours while passing air (17).

2, 3 and 4- picolines are supplied by Fluka Ac, Buchs, Switzerland. All the reactions are curried out using a self-build Pyrex reactor at the chemistry department, college of science, university of Baghdad.

Results and discussion

The ammoxidation of 2-, 3- and 4-picolines was carried out varying different conditions. These variations included the O_2/picoline, NH_3/picoline mole ratios, contact time and temperature. In each reaction run one parameter only was changed and the other kept constant

in order to determine its effect on the conversion and yield of the reaction. Since the reaction was highly exothermic, the system was cooled down to room temperature under a flow of pure nitrogen. A number of by products were formed such as CO, CO_2, and HCN in an addition to the main products. The obtained yield and conversion are listed in table (1) which shows the effect of reaction variables on the mole % conversion and yield of picolines studied to their sterile nitriles.

Effect of O_2 mole ratio on 2- picoline, 3-picoline and 4-picoline Ammoxidation. The dependence of the yield and conversion of the reaction on the mole ratio of oxygen is shown in (fig. 1) and table (1A). a- An increase in the oxygen to picoline mole ratio causes an increase in the yield of the nitrile formed and reached maximum at 6.0, 8.0, 8.0 mole ratios to 1 mole of picoline with a yield of 90%,88% and 87% for 2-,4- and 3-picolines respectively and a conversion of 70%, 69% and 68%. B- With further increase in O_2/picoline mole ratio, the conversion gradually decreases due to over oxidation of the picoline to oxides of carbon and water.

Effect of Ammonia on 2-, 3-, and 4-picoline Ammoxidation. The ammoxidation yield and conversion were found to increase on using aqueous ammonia solution (30%) instead of gaseous ammonia. The dependence of the reaction yield and conversion of 2-, 3- and 4-picolines on the ammonia quantity is shown in (fig. 2) Table (1B). They increase on increasing the ammonia mole ratio and reaches a maximum yield and conversion using 30, 35, 35 moles of ammonia for each mole of the picoline, and then decreased.

This result may be explained by the competition of the ammonia with the picoline on the same site of the catalyst surface. Another consequence is the decrease in the combustion of CO_2 gas.

The effect of contact time on yield of the main product and conversion is shown in (fig.3) and table (1C). The conversion reaches a maximum at about (1.5) sec. and then declines.

The yield increases gradually and reaches a maximum at (1.5) sec., then with further increase in contact time the yield decreases.

The effect of the reaction temperature on the mole conversion and yield of picoline isomers to their corresponding nitriles is shown in (fig.4) and table (1D).

With an increase in temperature the conversion and yield gradually increase and reach a maximum at 450 °C. Above this temperature such as at 500c° the conversion decreased due to complete oxidation of the picolines and the formation of CO_2 and the yield decreased also.

The effect of temperature on the activity of the catalyst is represented and showed in (fig.4) and table (1D). The catalyst prepared from ammonium vanadate and ammonium stannate showed the highest activity and selectivity with V_2O_5/SnO_2 mole ratio 2:1 on alumina heated to 900c° for six hours. Their mol ratio is 2: 1: 25 respectively.

The reaction mechanism of ammoxidation of picoline about the same as that of toluene. First picoline is oxidized on the surface of V_2O_5 to be stabilized on the surface as the reaction intermediate ion wich reacts with $NH_3$to form the nitrile. The reduced sites then reoxidized by oxygen to repeat the reaction.

The highest catalytic activity might be associated with the formation of $VSnO_4$ species. This is also supported by the influence of chemisorptions of the reactance on the catalytic properties of the catalyst system.

The reaction mechanism was investigated by kinetic and infrared studies. Alumina takes a role of converting the absorbed carboxilate ion into nitrile. The V_2O_5 supported on AL_2O_3 catalyst has a bifunctional activity. Increasing the V_2O_5 fraction causes an increase in the reaction yield. The selectivity of the catalyst depends on its chemical composition.

% conversion = (no. of reacted mole/ no. of starting material moles) x 100

% of yield = (no. of moles of produced nitriles / no. of moles of reacted material) x 100

Both % conversion and % yield for the studied picoline structural isomers 2-, 3-, and 4-picoline in the rate of 2-picoline > 4-picoline > 3-picoline. In other words the Ortho, Para isomers > the Meta isomer, which can be rationalized neither by steric effects nor by the thermo dynamic site of view. The highest yield obtained were 90%, 88% and 87% for 2-picoline, 4-picoline and 3-picoline respectively and so the highest conversion were 70%, 69%, 68%.

The catalyst was active even after 150 hours reaction. However the V_2O_5 and SnO_2 catalyst prepared here was found to be very effective to the ammoxidation reaction of picolines.

Table 1 effect of reaction variables on the conversion

Table 1A Effect of O_2/picoline mole ratio on the % conversion Of 2-,3- and 4- picolines to Nicotinic Acid.

	O2/picoline	Conversion%
2-picoline	1	30
	2.5	40
	4.0	50
	6.0	61
	8.0	70
3-picoline	10.0	45
	1	31
	2.5	44
	4.0	51
	6.0	57
	8.0	68
	10	49
4-picoline	1	29
	2.5	42
	4.0	53
	6.0	59
	8.0	69
	10.0	40

Table 1 B effect of NH_3 / picoline mole ratio on the % of 2-,3- and 4- picolines to Nicottinic Acid

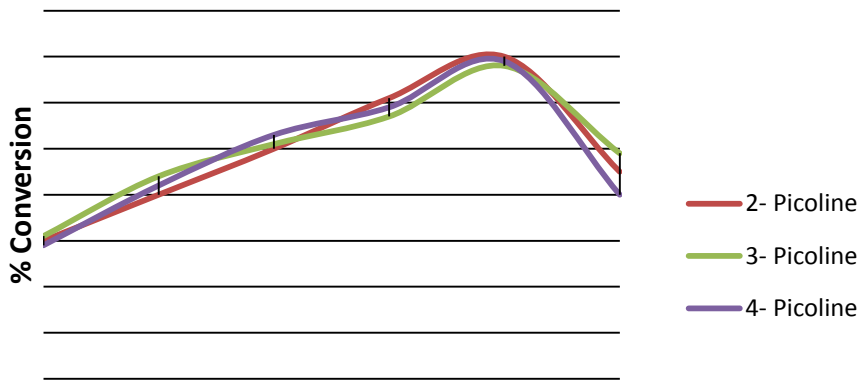
	NH_3/picoline	Conversion%
2-picoline	10	40
	15	50
	25	60
	30	70
	35	50
	40	44
3-picoline	10	30
	15	45
	25	55
	30	60
	35	68
	40	42
4-picoline	10	30
	15	40
	25	50
	30	60
	35	69
	40	40

Table1C Effect of the contact time (sec.) on on the %conversion and %yield of 2-,3- and 4-picolines to Nicotinic Acid

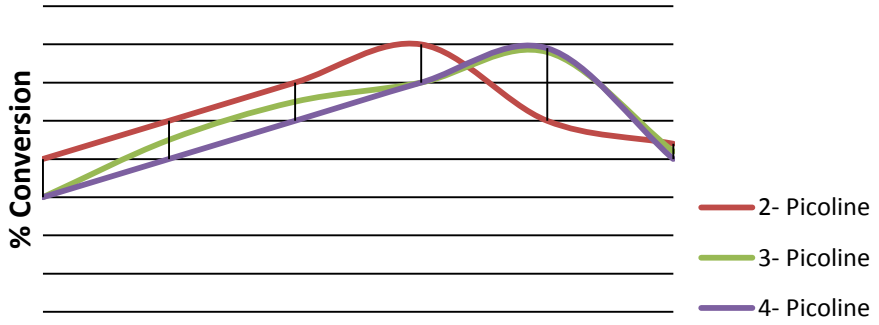
	Contact time / sec.	Conversion %	Yield %
2- picoline	0.4	30	40
	0.5	40	57
	0.6	50	62
	1.0	61	75
	1.5	70	90
	2.0	45	80
3- picoline	0.4	31	39
	0.5	44	50
	0.6	51	58
	1.0	57	70
	1.5	68	87
	2.0	49	74
4- picoline	0.4	29	38
	0.5	42	55
	0.6	53	62
	1.0	59	73
	1.5	69	88
	2.0	52	70

Table 1D effect of temperature °C the % conversion of 2-,3- and4-picolines to Nicotinic Acid

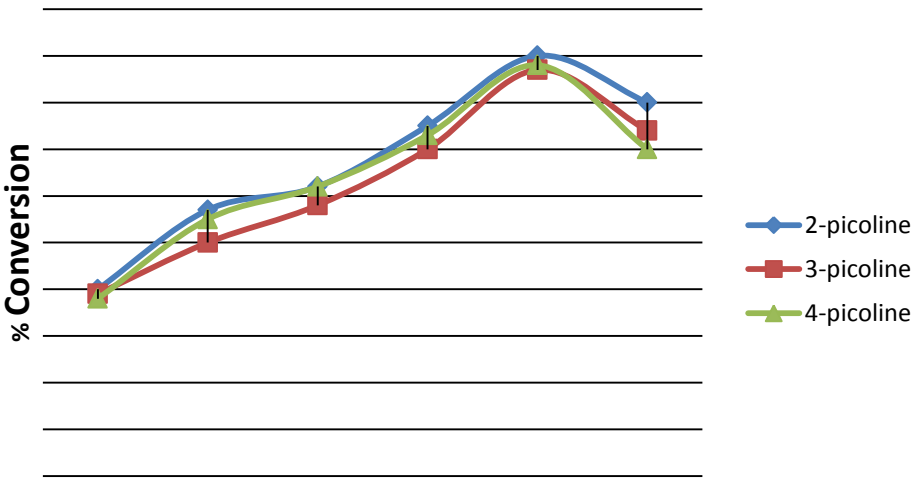
	Temperature	Conversion%
2-picoline	250	30
	300	40
	350	50
	400	61
	450	70
	500	45
3-picoline	250	31
	300	44
	350	51
	400	57
	450	68
	500	49
4-picoline	250	29
	300	42
	350	53
	400	59
	450	69
	500	52



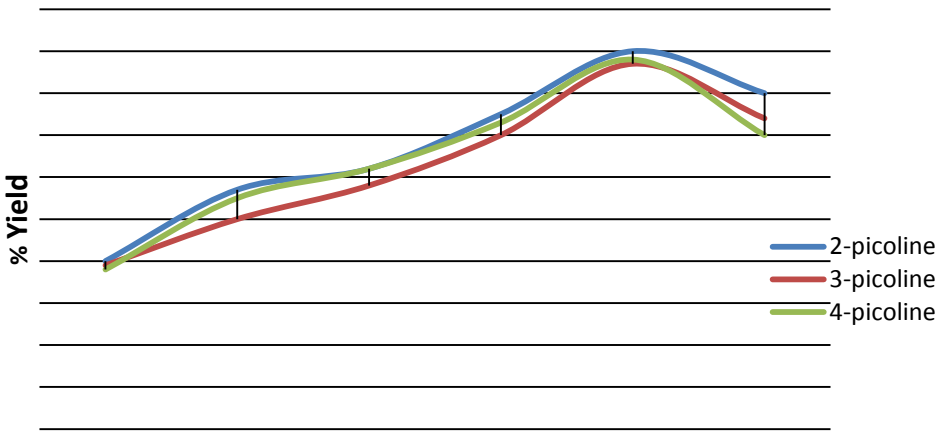
(Fig. 1) plot of %conversion of 2-,3- and 4-picolines vs. O2/picoline mole ratio



(Fig.2) plot of %conversion of 2-, 3- and 4- picolines vs. NH3/picoline mole ratio



(Fig. 3) plot of %conversion of 2-, 3- and 4-picolines vs. contact time (sec.)



(Fig. 4) plot of %yield of nicotinic acid from 2-, 3- and 4-picolines vs. contact time (sec.)

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The analgesic effects of L-arginine and its antagonist L-NAME in mice

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Summary

L-arginine-Nitric oxide pathway plays an important role in a series of neurobiological functions underlying behaviors including analgesic effect and has shown a role in pain feeling which is a mediator with modulation effect in dorsal root of ganglionic neurons of spinal cord. The goal of the present study is to clarify the influence of L-arginine-mediated nitric oxide (NO) on pain arbitration in both sexes of mice. The reactive time to thermal stimulus, latency period, tail withdrawal and the number of foot licking and flinching in hot plate test, tail flick and formalin tests were recorded. The results showed that L-NAME (nitric oxide synthase inhibitor) has had an antinociceptive activity demonstrated as prolonged reactive time to thermal stimulus, latency period for tail withdrawal and decreasing the number of foot licking and flinching in hot plate, tail flick and formalin tests. These findings might be attributed to that intensity of pain feeling is intercede due to interference of sex hormones in both sexes of mice. In addition, from the results of L-NAME on pain sensation, it may be concluded that L-arginine-nitric oxide pathway is extravital in male in comparison with female in pain sensation.

Key word: - L-arginine, analgesic, L- NAME, mice

التأثيرات التسكينية للأرجنين ومضاده L-NAME في الفئران

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الخلاصة

يلعب مسلك أل-ارجنين-أوكسيد النتريت دور مهم في الوظائف العصبية الحيوية ومنها السلوكيات التي تتضمن التأثيرات التسكينية فيلعب دوراً في الشعور بالألم حيث أظهر تأثيراً وسيطاً وتوليفياً في الجذر الظهري لأعصاب الحبل الشوكي العقيدية. تهدف الدراسة إلى توضيح تأثير أل-أرجنين وسيط أوكسيد النتريت في توليف الألم في كلا جنسي الفئران. سجل كل من الوقت الفعال للاستجابة الحرارية والفترة الفعالة لسحب الذيل وتقليل عدد مرات الإجفال ولعق القدم في اختبارات الصفيحة الساخنة كذلك تحريك الذيل والفورمالين لإدراك الألم.

لوحظ من نتائج التجربة: أن L-NAME يمتلك الفعالية المضادة للألم حيث لوحظت من خلال إطالة الوقت الفعال للاستجابة الحرارية والفترة الفعالة لسحب الذيل وتقليل عدد مرات الإجفال ولعق القدم في اختبارات الصفيحة الساخنة وتحريك الذيل والفورمالين. عزيت هذه النتائج إلى اختلاف درجة الشعور بالألم بين ذكور وإناث الفئران بسب تداخل الهرمونات الجنسية في هذا التأثير. إضافة إلى ذلك ونتيجة لتأثير L-NAME قد تختلف درجة الشعور بالألم نتيجة اختلاف فرط حيوية الإحساس بالألم في الذكور أكثر منه في الإناث.

كلمات مفتاحية:- التأثيرات التسكينية, أرجنين ,الفئران.

Introduction

Pain is a complex phenomenon that is difficult to describe and measure. It can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (1). Nitric oxide is produced within the central nervous system (CNS) from L-arginine by a constitutive neuronal form of NO synthase (nNOS), an enzyme which is localized in neurons of the central nervous system. A role of nitric oxide (NO) in nociceptive signaling was initially based on the localization of neuronal nitric oxide saynthase (nNOS) in the superficial dorsal horn and intermediolateral cell column (2and3), which led to the notion that nitric oxide

(NO) regulates both autonomic tone and sensory transduction at the spinal cord level. Some reports have shown that reduction of nitric oxide (NO) induces antinociception (4and5). Female mice could tolerate pain for a longer time than male. Inhibition of nitric oxide synthesis by administration of nitric oxide synthase (NOS) inhibitor (L-NAME), resulted in diminished perception of pain in male but not in female mice (6). This effect of L-NAME could be reversed by the administration of L-arginine (7). It has been shown that the inflammatory pain induced by epinephrine intradermaly injection in the hind paw of the rat is depended on sex hormones, and nitric oxide synthase inhibitors can antagonize pain only in male but not in female rats (8). It has been reported that sex is a factor that influences a variety of neurotransmitter systems and different mediators are important in the response to painful stimuli such as hot plate and tail flick in mice. The levels of stable metabolites of nitric oxide, nitrite and nitrate in the rat brain show sex differences i.e. female rats in comparison with males have lower levels in the cortex and hippocampus brain areas (9). In male mice, inhibition of nitric oxide synthase at the level of the brain but not at the spinal cord result in supraspinal analgesia. These results may suggest that sex steroid hormones such as estrogen have a role in the feeling of pain. The aim of this study is to clarify the influence of L-arginine-mediated nitric oxide (NO) on pain arbitration in both sexes of mice.

Materials and Methods:

Three hundred and twenty male and female mice (160 of each sex), weighing (25-30) gm. with an average of (27.5$\pm$0.02) gm. were used in the experiments of the present study. They were kept under suitable environmental conditions of (20-25) ^{o}C in an air-conditioned room, (12) hours light and nourished ad libitum. Pretreatment values were taken for all segments of the study for both sexes separately. Fifty male and fifty female mice were used to perform tail flick and hot plate tests. Five animals of each sex were given 160, 200 or/and 240 mg/kg, B.W. of L-arginine orally daily for 15 or 30 days. Other groups of five mice of both sexes were likewise treated with 100 mg/kg, B.W. of L-NAME intraperitoneally. Similar groups were given D.W. and served as control (figure 1). The tail flick test was described by (10). The noxious motivation was produced by immersing approximately (1) cm of the tip of the tail into a (56.0$\pm$0.5) C^{o} water bath and the latency period for tail withdrawal (second) was recorded, while the hot plate reactive time (second) to thermal stimulus was checked for certain different groups in hot plate apparatus which heated to (55$\pm$1) ^{o}C, as described by (11,12and13). Formalin test was applied by placing each mouse in a transport cage, (10) microliters of (2%) formalin were injected subcutaneously into the planter region of the right hind paw, which produced pain that can be measured in terms of nociceptive response namely flinching and licking of injected paw, the number of licking and flinching were recorded which measured immediately after formalin injection and continued for (60) min (14,15and16). Six groups of mice for each sex (5 mice) were treated in the same manner as in the tail flick and hot plate tests except one group was given normal saline intraperitoneally (figure 2).

Data were analyzed by using completely Randomized Design in factorial experimental (Two-way) ANOVA. For calculation the effect (SPSS package 2008) probability of ($P < 0.05$) were considered as significant differences.

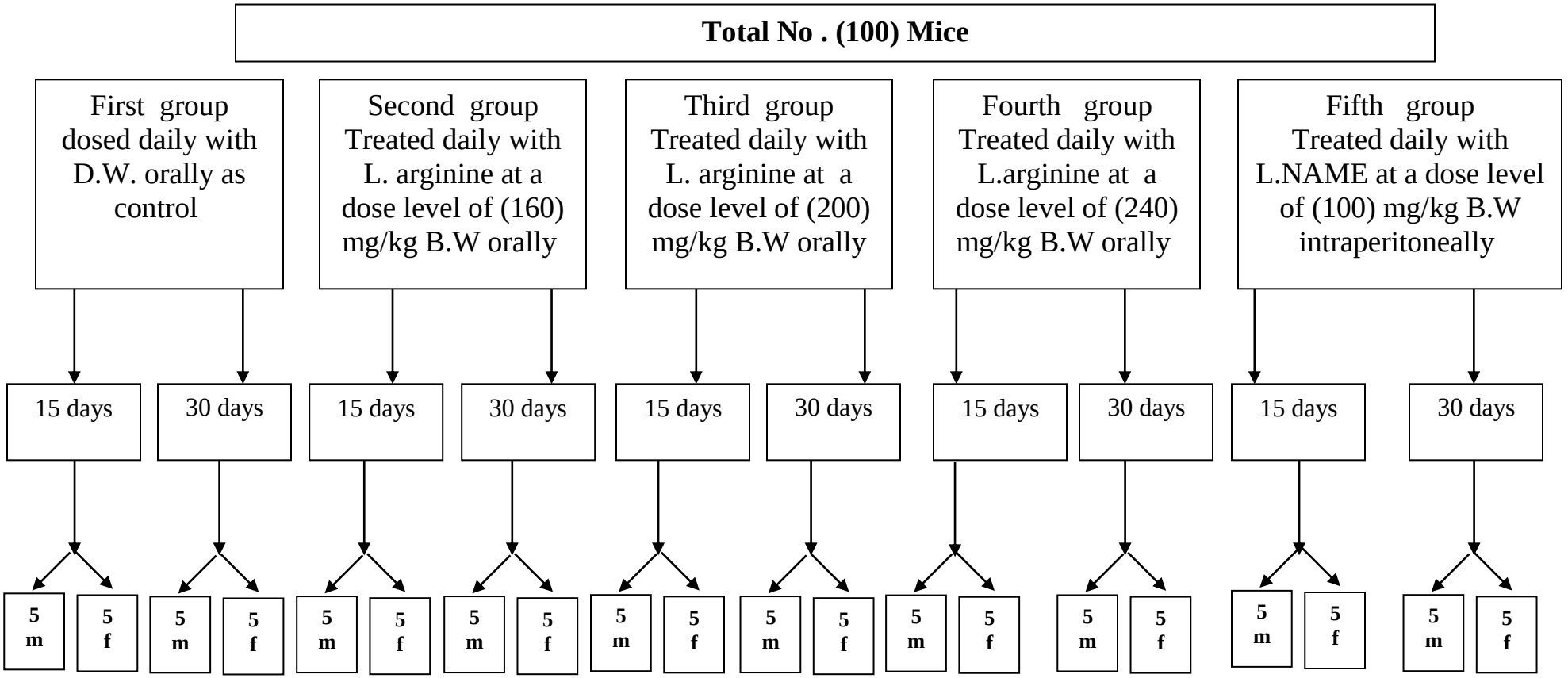
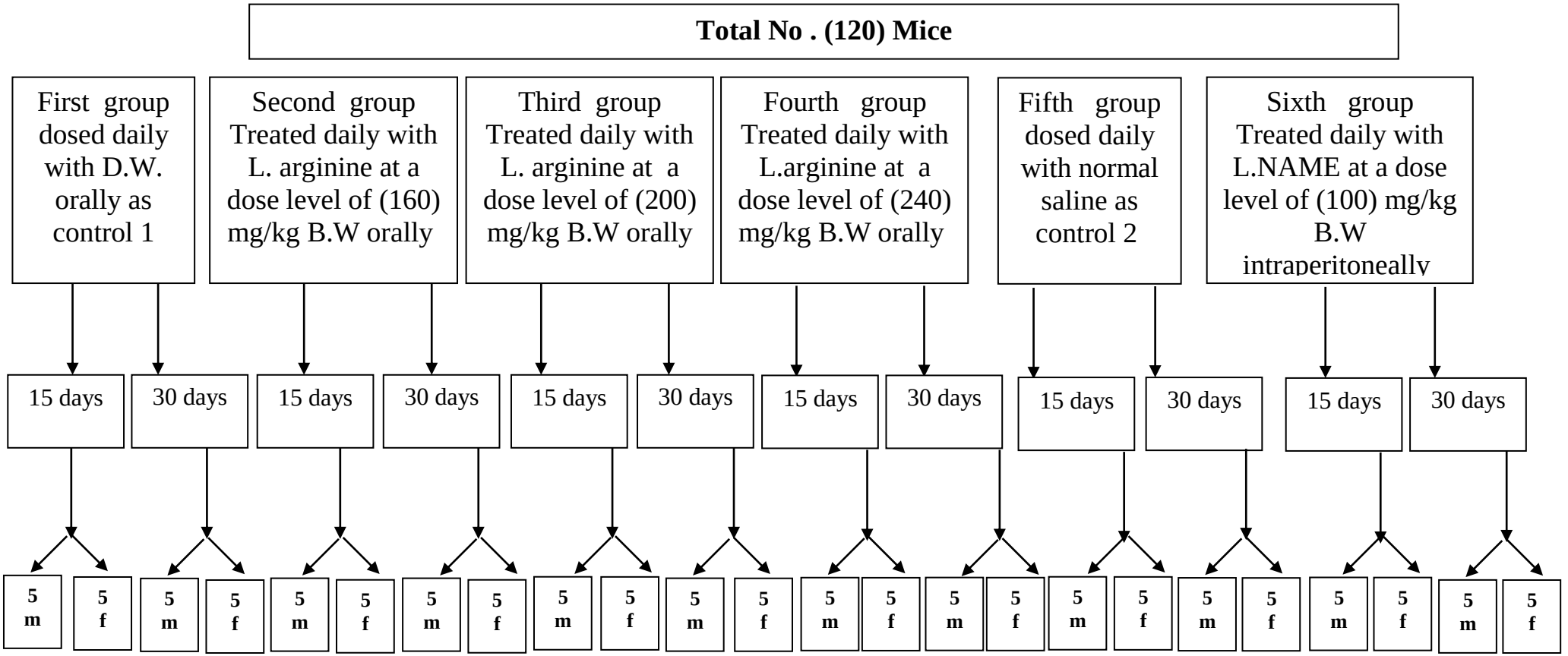


Figure (1): Experimental Design of Tail Flick and Hot Plate Test



m = male

f = female

Figure (2): Experimental Design of Formalin Test

Results and Discussion

The results of the effects of L-arginine and L-NAME on tail flick latency period table (1) and hot plate reactive time to thermal stimulus table (2) showed significant ($p<0.05$) differences between post-treatments (15 and 30) days as compared with control and pretreatment groups, which displayed decrease in L-arginine treated groups and increase in L-NAME treated groups. Results showed significant increase ($p<0.05$) in latency period displayed by females. Number of licking and flinching increase significantly ($p<0.05$) in mice treated with L-arginine, on the other hand L-NAME cause significant reduction ($p<0.05$) in number of licking and flinching in comparison with control groups table (3). There were significant ($p<0.05$) differences between early and late phase after formalin injection and significant gender differences in all treated groups, which was significantly lower in female than in male mice in the number of licking and flinching.

The results of this experiment point to the participation of L-arginine-nitric oxide (NO) pathway in mediation of acute pain in female mice is not significant as in male mice. It has been shown that the inflammatory pain induced by epinephrine intradermaly injection in the hind paw of the rat was dependent on sex hormones, because there were gender and sex hormone related differences in pain and nociception (17and18), which proposed that the modulatory effect of sex steroids on both nociceptive mechanisms of central and peripheral nervous system (CNS) that both oestrogen and androgen receptors are present on small-diameter dorsal root ganglion (DRG) neurons (19and20). Furthermore, sex hormones affect expression of protein kinase C (PKC), protein kinase A (PKA) and nitric oxide synthase (NOS) activity which were implicated in peripheral nociceptive mechanisms, and these messengers signaling pathways contribute to epinephrine induced hyperalgesia in males but not in females, due to suppression by oestrogen, which decreases epinephrine-induced hyperalgesia in females by suppressing contributions of protein kinase C (PKC) and protein kinase A (PKA) to pain signaling, (8). Furthermore, the gender differences in pain might be due to differences in nitrite and nitrate levels (nitric oxide metabolites) and activities founded in brain areas of cortex, hippocampus, corpusstriatum, midbrain and cerebellum which is higher in adult male than female rats (9). Several reports had suggested a role of L-arginine-NO-cGMP pathway in central and peripheral nociceptive processing (21,22and23), while nitric oxide (NO) mediated the N-methyl-D-aspartate (NMDA) produced facilitation of the nociceptive tail-flick reflexes which depends on the activity of spinal cord neurons (24and25). Nitric oxide (NO), which derived from L-arginine plays a role in nociceptive signaling due to localization of neuronal nitric oxide synthase (nNOS) in the superficial dorsal horn and intermediolateral cell column of spinal cord (2and3). Hot plate test was a marker test of supraspinal analgesia whereas, tail-flick was considered to be a measure of spinally mediated antinociception since the hot plate test was widely considered to be sensitive to drugs acting supraspinally (26and27). L-NAME, nitric oxide synthase inhibitor produce opioid-independent antinociceptive effects in the mouse, suggesting the role of NO-cGMP system in supraspinal transmission of nociceptive information and the antinociceptive in the mouse due to L-NAME is not antagonized by naloxone and is thus, independent of endogenous opioid release (21and22).In conclusion, the present data show that in male mice the inhibition of (NOS) in the brain but not in the spinal result in supraspinal analgesia. The response to the pain inhibition which was assessed by hot plate and tail-flick tests, may be sex steroid hormones related, like oestrogen interact with L-arginine-nitric oxide system and had involved a mechanism of pain feeling.

Table (1): The effect of L-arginine treated orally and L-NAME intraperitoneally daily on tail flick analgesic test latency period (second) in male and female mice.

Periods of treatment and sex / Groups	Pre-treatment		Post-treatment (15 days)		Pre-treatment		Post-treatment (30 days)	
	Male	Female	Male	Female	Male	Female	Male	Female
D.W. as control.	6.00 ± 0.30^{Bc}	9.00 ± 0.22^{Ac}	-	-	6.80 ± 0.33^{Bc}	9.60 ± 0.20^{Ac}	-	-
L-arginine (160) mg / kg B. W.	4.00 ± 0.01^{Ba}	6.06 ± 0.11^{Aa}	2.50 ± 0.20^{Cb}	4.66 ± 0.08^{Db}	4.00 ± 0.23^{Ba}	5.60 ± 0.11^{Aa}	2.60 ± 0.19^{Cb}	4.00 ± 0.09^{Db}
L-arginine (200) mg / kg B. W.	4.00 ± 0.01^{Ba}	6.20 ± 0.10^{Aa}	2.40 ± 0.03^{Cb}	4.70 ± 0.12^{Db}	4.00 ± 0.23^{Ba}	6.62 ± 0.10^{Aa}	2.30 ± 0.01^{Cb}	4.62 ± 0.04^{Db}
L-arginine (240) mg / kg B. W.	4.16 ± 0.08^{Ba}	6.00 ± 0.11^{Aa}	2.40 ± 0.04^{Cb}	4.74 ± 0.14^{Db}	4.20 ± 0.01^{Ba}	6.30 ± 0.10^{Aa}	2.34 ± 0.06^{Cb}	4.80 ± 0.08^{Db}
L-NAME (100) mg /kg B. W.	9.00 ± 0.11^{Bb}	7.72 ± 0.10^{Ab}	12.60 ± 0.24^{Ca}	10.40 ± 0.16^{Da}	10.16 ± 0.14^{Bb}	7.70 ± 0.70^{Ab}	13.00 ± 0.20^{Ca}	11.00 ± 0.11^{Da}

Values are presented as Mean ±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Capital letters denoted to (P<0.05) gender differences.
Number = 5mice/group.

Table (2): The effect of L-arginine treated orally and L-NAME intraperitoneally daily on hot plate analgesic test the reactive time to thermal stimuli (second) in male and female mice.

Periods of treatment and sex / Groups	Pre-treatment		Post-treatment (15 days)		Pre-treatment		Post-treatment (30 days)	
	Male	Female	Male	Female	Male	Female	Male	Female
D.W. as control	6.80±0.20^{Ac}	8.20±0.20^{Bc}	-	-	6.82±0.28^{Ac}	8.60±0.23^{Bc}	-	-
L-arginine (160) mg / kg B. W.	4.00 ±0.22^{Ba}	6.22 ±0.20^{Ab}	2.34 ±0.24^{Db}	4.80 ±0.09^{Cb}	4.80 ±0.28^{Ba}	6.00 ±0.23^{Ab}	2.30 ±0.30^{Db}	4.70 ±0.09^{Cb}
L-arginine (200) mg / kg B. W.	4.60 ±0.18^{Ba}	6.00 ±0.13^{Ab}	2.30 ±0.25^{Db}	4.76 ±0.10^{Cb}	4.40 ±0.28^{Ba}	7.00±0.44^{Ab}	2.20 ±0.28^{Db}	4.66 ±0.10^{Cb}
L-arginine (240) mg / kg B. W.	4.22 ±0.22^{Ba}	6.00 ±0.13^{Ab}	2.30 ±0.24^{Db}	4.72 ±0.09^{Cb}	4.16 ±0.27^{Ba}	6.20 ±0.40^{Ab}	2.24 ±0.25^{Db}	4.60 ±0.14^{Cb}
L-NAME (100) mg /kg B. W.	12.87 ±0.08^{Db}	10.30±0.34^{Ca}	19.80±0.73^{Aa}	12.00 ±0.20^{Ba}	12.60 ±0.25^{Db}	10.00 ±0.16^{Ca}	20.00 ±0.72^{Aa}	12.22 ±0.60^{Ba}

Values are presented as Mean±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Capital letters denoted to (P<0.05) gender differences.
Number = 5mice/group.

Table (3): The effect of L-arginine treated orally and L-NAME intraperitoneally daily on formaline analgesic test (the nociceptive response) in male and female mice.

Periods of treatment and sex / Groups	Nociceptive response (Number of licking and flinching)							
	Early phase (0-5) min. after injection				Late phase (15-45) min. after injection			
	Male		Female		Male		Female	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
D.W. as control 1 group before injection of formaline	34.20 ±2.20^{Aa}	35.00 ±1.60^{Aa}	29.20 ±2.30^{Ba}	30.00±1.62^{Ba}	21.00 ±1.74^{Ca}	21.00±1.40^{Ca}	12.00 ±0.94^{Da}	18.60±0.74^{Da}
L-arginine (160) mg / kg B. W.	40.00 ±0.71^{Ab}	39.60 ±0.71^{Ab}	33.20 ±1.04^{Bb}	34.20 ±1.30^{Bb}	23.40 ±3.04^{Cb}	23.60 ±0.63^{Cb}	21.20 ±1.81^{Db}	21.00 ±0.67^{Db}
L-arginine (200) mg / kg B. W.	40.00 ±0.81^{Ab}	41.00 ±0.93^{Ab}	33.00 ±1.25^{Bb}	34.00 ±1.14^{Bb}	24.20 ±3.06^{Cb}	24.60 ±3.01^{Cb}	21.00 ±3.05^{Db}	20.60 ±0.53^{Db}
L-arginine (240) mg / kg B. W.	40.20 ±0.72^{Ab}	41.00±0.85^{Ab}	34.30±0.54^{Bb}	35.00 ±1.12^{Bb}	23.60±2.70^{Cb}	24.40±2.29^{Cb}	21.20±2.60^{Db}	20.80±0.28^{Db}
normal saline as a control 2 group before injection of formalin.	34.00±2.10^{Aa}	34.00±2.60^{Aa}	28.80±2.30^{Ba}	28.60±1.60^{Ba}	22.00±3.90^{Ca}	21.40±1.70^{Ca}	18.80±2.26^{Da}	19.00±1.32^{Da}
L-NAME (100) mg /kg B.W.	19.20±2.23^{Bc}	18.40±2.30^{Bc}	24.40±1.60^{Ac}	24.00±1.6^{Ac}	10.20±2.23^{Cc}	10.00±2.10^{Cc}	16.00±1.60^{Dc}	16.80±0.24^{Dc}

Values are presented as Mean ±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Capital letters denoted to (P<0.05) gender differences in early and late phase and between early and late phase .
Number = 5mice/group.

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Prevalence of *Entamoeba histolytica* and *Giardia lamblia* in Children in Kadhmiyah Hospital

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Summary

In this study we collect 1520 stool samples during the period from September to December 2010 from children whom their ages between 1 month - 12 years. The results showed that the total infection of *Entamoeba histolytica* was 9.80% , and *Giardia lamblia* was 1.77%. And the male ratio that infected with *Entamoeba histolytica* was 9.83% , while the female ratio was 9.74%; and the male infected with *Giardia lamblia* was 1.51% , while the female ratio was 2.18%. The result showed that the high average of infection with *Entamoeba histolytica* and *Giardia lamblia* in age group from 1 month to 2 years. And there is no significance difference between gender and infectivity rate of *Entamoeba histolytica* and *Giardia lamblia* under $P \leq 0.05$. Also it showed that there were significant relation between Age group and infectivity rate of *Entamoeba histolytica* and *Giardia lamblia*.

Keywords: *Entamoeba histolytica, Giardia lamblia,* Prevalence, Age group, gender.

انتشار طفيلي *Entamoeba histolytica* و *Giardia lamblia* في الاطفال في مستشفى الكاظمية

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الخلاصة

جمعت 1520 عينة غائط من اطفال تراوحت اعمارهم ما بين شهر واحد – 12 سنة للفترة ما بين شهر ايلول – شهر كانون الاول لعام 2010. اظهرت النتائج ان نسبة انتشار طفيلي الزحار الاميبي *Entamoeba histolytica* كانت 9.80 % ، وانتشار طفيلي *Giardia lamblia* 1.77% من نسب العزلات الكلية.وكانت نسبة انتشار الاصابة بطفيلي *Entamoeba histolytica* في الذكور 9.83 % فيما كانت نسبة انتشار الاصابة بالطفيلي نفسه في الاناث 9.74 %. اما نسبة انتشار الاصابة بطفيلي *Giardia lamblia* في الذكور 1.51 % اما نسبة انتشار الاصابة بالطفيلي نفسه في الاناث 2.18 %. اظهرت النتائج كذلك ان اعلى نسبة للاصابة بطفيليات *Entamoeba histolytica* و *Giardia lamblia* كانت في الاطفال الذين تراوحت اعمارهم بين شهر واحد – 2 سنة. واظهرت نتائج التحليل الاحصائي عدم وجود فروق معنوية عند مستوى احتمالية ($P \leq 0.05$) عند دراسة العلاقة ما بين الجنس ومعدل الاصابة بطفيليات *Entamoeba histolytica* و *Giardia lamblia*. ، فيما كانت الفروق معنوية عند دراسة العلاقة ما بين الفئة العمرية ومعدل الاصابة بطفيليات *Entamoeba histolytica* و *Giardia lamblia*.

كلمات مفتاحية: طفيلي , الزحار الاميبي, الاطفال .

Introduction

The spread of Contagious and parasitic diseases of the digestive tract are facilitated by unsatisfactory sanitary conditions which result from the damage of plumbing and sewage systems (mostly as the effect of the warfare) (1). Every day 0.5 million tons of sewage is dumped into Iraqi rivers which contaminated the major source of drinking water in the country. This situation relatively leads to outbreak epidemics of various diseases (2) such as giardiasis and amoebiasis.

In 2001 it has been reported of 652314 cases of amoebiasis (2477 was infected out of 100 thousand people) and 563642 cases of giardiasis (2141 for every 100 thousand people) (3). The combined amoebiasis and giardiasis prevalence of infections in general population occur in all regions of the country, especially in the summer months.

Amoebiasis is still a big problem of human civilization at the beginning of 21st century, so every study in this field is valuable. Amoebiasis is caused by *Entamoeba histolytica*, a parasitic protozoan, which infects predominately human and other primates (4).

Entamoeba. histolytica is the most common form of enteric disease; it is the agent of amoebic dysentery. This parasite not only causes severe diarrhea but can cause abscesses in the intestine, liver, lung and other organs. Around 500 million people are infected worldwide while 75,000 die because of annually, and it ranks third on the list of parasitic causes of death worldwide behind malaria and schistosomiasis (5).

Giardiasis is an infectious disease that present all over the world but spread more in the third world countries like Iraq, where is bad sanitary and living conditions. It is a dangerous disease that affect children and adults and lead to malabsorbtion syndrome and weight loss in the infected persons. (6). *Giardia lamblia* is a waterborne protozoan parasite and a common cause of intestinal disease in all parts of the world. (7, 8 and 9). This primitive eukaryotic cell has two forms: the trophozoite and the cyst. The trophozoites spend their entire life within the intestinal lumen of their host, whereas cysts are released within fecal material, which constitutes the mode of spreading the infection from host to host. The infective dose in humans is between 10 and 100 cysts (10). *Giardia lamblia* can produce a wide spectrum of clinical manifestations, from asymptomatic to acute or chronic diarrhoea with malabsorption syndrome and weight loss (11). *Giardia lamblia* is considered to be an important cause of recurrent abdominal pain in children (12). So this study is aimed to investigate the prevalence of *Entamoeba histolytica* and *Giardia lamblia* in Kadhmiyah hospital for the period between September to December 2010.

Materials and methods

In this study 1520 stool sample collected from Kadhimiyah hospital for the period between September – December 2010 and the result concentrated on the prevalence of *Entamoeba histolytica* and *Giardia lamblia* and the relationship between these two parasites with gender and age. The stool samples were collected, examined macroscopically and microscopically to detect for the presence of *Giardia lamblia* and *Entamoeba histolytica* trophozoites and cysts stages. Data about age, sex and residence were recorded for each child on a special form (9). The results were analyzed statistically using the Chi-square test.

Results and Discussion

According to the gender the statistical analysis showed that there were no significant relation between gender and infectivity rate of *Entamoeba histolytica* and *Giardia lamblia* at ($P \leq 0.05$). The result showed that the number of patient infected with *Entamoeba histolytica* was 149 (9.80%) more than in *Giardia lamblia* 27 (1.77%). And the male is more infected with *Entamoeba histolytica* and *Giardia lamblia* than female. The percentage of infected male with *Entamoeba histolytica* was 91 (9.83%) and 58 (9.74 %) in female, while infected male with *Giardia lamblia* 14 (1.51%) and female infected 13 (2.18 %) (Table,1).

Table (1): Distribution of *Entamoeba histolytica* and *Giardia lamblia* according to the gender from the period between September – December 2010

	Male	Female	Total
Entamoeba	**91 (9.83%)**	**58 (9.74%)**	**149 (9.80%)**
Giardia	**14(1.51%)**	**13 (2.18%)**	**27 (1.77%)**
Non	**820 (88.64%)**	**524 (88.06%)**	**1344 (88.42%)**
Total	**925**	**595**	**1520**

The result showed that the high prevalence of *Giardia lamblia* recorded in September 14 patients (8 male, 6 female), while the lower prevalence was recorder in October and November in 4 patients. The high prevalence of *Entamoeba histolytica* recorder in September in 55 patients (32 male, 23 female) while the lower prevalence in December in 20 Patients (14 male, 6 female) [figure 1].

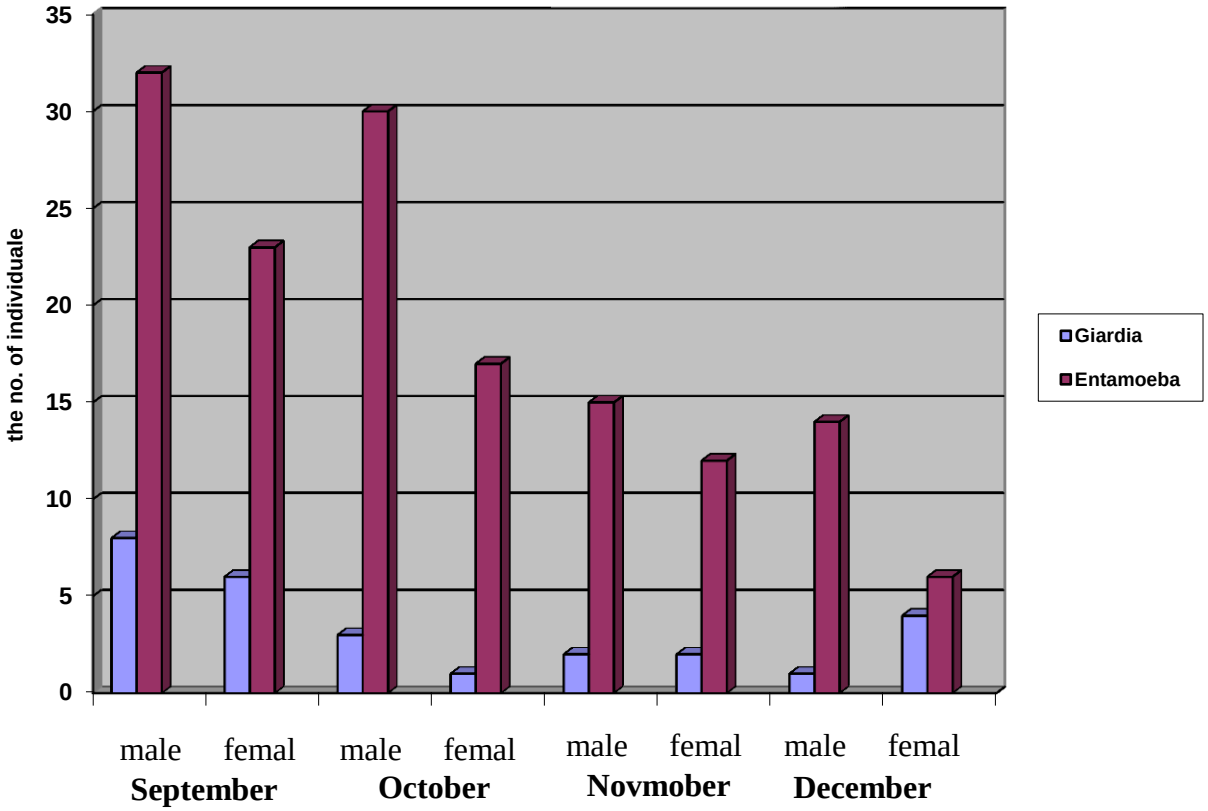

Figure (1): Distribution of *Giardia lamblia* and *Entamoeba histolytica* according to the gender from September – December 2010

According to the age the statistical analysis showed that there were significant relation between Age group and infectivity rate of *Entamoeba histolytica* and *Giardia lamblia* at ($P \leq 0.05$). It noticed the high prevalence of *Entamoeba histolytica* and *Giardia lamblia* in the age group 1 month -2 years (Table 2).

Table (2): Distribution of *Entamoeba histolytica* and *Giardia lamblia* according to the age group.

Age group (years)	*Entamoeba histolytica*	*Giardia lamblia*	Non infected	Total
1 month -2 year	99 (13.28%)	13 (1.74%)	633 (84.96%)	745
>2-4	28 (7.71%)	10 (2.75%)	325 (89.53%)	363
>4-6	12 (6.18%)	3 (1.5%)	179 (92.26%)	194
>6-8	5 (4.80%)	0 (0%)	99 (95.19%)	104
>8-10	2 (3.07%)	0 (0%)	63 (96.92%)	65
>10-12	3 (6.12%)	1 (2.04%)	45 (91.83%)	49

The results showed that there are differences to the prevalence between gender and two parasites. That the high prevalence of infection in male more than females. According *Giardia lamblia* this results agree with (9) who study the prevalence of *Giardia lamblia* in Duhok city and agreed with (13) whom they study frequency of *Giardia lamblia* infection in children with recurrent abdominal pain in Peshawar in India. According to *Entamoeba histolytica* this study agreed with (14) and disagrees with (15, 16 and 17).

The highest rate of the parasites was recorded at the age group 1 month – 2years then 2 – 4 years, and the reason of this high prevalence may be attributed to the low immunity against various pathogens as these age groups are comparatively less resistant to diseases as described in a previous study (18). And the other reason could be related to a number of factors such as poor health hygiene and toilet training, overcrowding, low socioeconomic status and climatic conditions (19). Additionally, the children feel free to play anywhere irrespective of the cleanliness or dustiness due to the absence of separate play grounds. The playing areas are main sources of diseases because waste materials of homes and industries are thrown there (17).

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Influence of crude extract of Hawthorn *crataegus oxyacantha* on some physiological aspects in mature male Rats exposed to hydrogen peroxide over load.

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Summary

This study was carried out to investigate the protective effects of 70% ethanolic alcohol extract of hawthorn (*crataegus oxycantha*) on some physiological functions of male rats exposed to 1% H_2O_2. Fifteen mature male Newzeland rats were randomly divided into three groups:- control group (C) ,two groups treated with 1% H_2O_2 alone (G1) or 1%H_2O_2 with crude extract of hawthorn(G2) orally daily for 30 days .Blood samples were taken at zero time and 30 days of the experiment .The present study declared an alteration in the lipid profile of the treated group (G2) at the end of treatment (30 days) manifested by asignificant reduction ($p<0.05$) in serumTC,TAG,LDL-C, VLDL-C concentrations. And elevation ($p<0.05$) in serum, HDL-C, as compared to the treated group (G1). Antioxidant status also exhibited significant ($p<0.05$) changes characterized by an elevation of serum GSH in group (G2). Histological study revealed that oral treatment with 1% H_2O_2 caused congestion of blood vessels of the heart with infiltration of inflammatory cells and odema between muscle fibers. It is concluded that treatment with hawthorn showed no clear pathological lesions.

Key words: Hawthorn , crataegus oxyacantha, hydrogen peroxide, Rat.

تأثيرالمستخلص الخام لثمار الزعرور على بعض الصفات الفسلجية الناتجة من فرط بيروكسيد الهيدروجين في ذكور الجرذان البالغة

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الخلاصة

أجريت هذه الدراسة لمقارنة الدور الوقائي للمستخلص الكحولي(70% من الكحول الأثيلي) لثمار الزعرور في بعض المؤشرات الفسلجية في ذكور الجرذان المعرضة للأجهاد التاكسدي بواسطة 1% من بيروكسيد الهيدروجين . تم أستخدام خمسة عشر جرذا نيوزيلانديا من الذكور البالغة قسمت عشوائيا الى ثلاثة مجاميع متساوية:- مجموعة سيطرة(C) أعطيت ماء الشرب الأعتيادي, ومجموعتين معالجة أعطيت ماء الشرب الأعتيادي مضافا اليه بيروكسيد الهيدروجين بتركيز 1% لوحده (G1) أو مضافا أليه المستخلص الكحولي لثمار الزعرور(G2) لمدة ثلاثين يوما . تم سحب عينات الدم للفترات 0 و 30 يوم من التجربة. أظهرت الدراسة الحالية تغيرا ملحوظا في دهون الدم للمجموعة المعاملة الثالثة بعد 30 يوما من المعالجة والتي تميزت بأنخفاض معنوي اكبر من 0.05 في الكوليسترول الكلي والدهون الثلاثية وكوليسترول الشحوم البروتينية واطئة الكثافة وكوليسترول الشحوم البروتينية ذات الكثافة الواطئة جدا , بالأضافة الى الأرتفاع المعنوي في تركيز الكلوتاثيون في مصل الدم . بينت نتائج الفحص النسيجي حدوث احتقان الأوعية الدموية في قلب الجرذان المعاملة ب 1% من بيروكسيد الهيدروجين مع وجود ارتشاحات التهابية خلوية ووذمة بين الألياف العضلية . لم تظهرالمعاملة بالمستخلص الكحولي للزعرور اي أفات مرضية .

مفاتيح الكلمات:- ثمار الزعرور, بيروكسيد الهايدروجين, الصفات الفسلجية, ذكور الجرذان

Introduction

Antioxidants can be defined as substances able to inhibit or delay the oxidative damage of protein, nucleic acid and lipid caused by dramatic increase of reactive oxygen species (ROS) (1) by inhibiting the initiation or propagation of oxidizing chain reactions (2). Antioxidants can be classified into endogenous antioxidants including Superoxide Dismutases (SOD)(3) Catalase(4), Peroxiredoxins (5), Thioredoxin and Glutathione system (6) which were involved in the neutralization of ROS species (7) and non-enzymaticExogenous antioxidants contained in a wide spectrum of herbs, fruits, and vegetables (8).

Plants have been used for several years as a source of traditional medicine to treat various diseases and conditions (9). A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can provide therapeutic effect (10). Phytochemical, especially Phenolics, in fruits and vegetables are suggested to be the major bioactive compounds for health benefits. Phenolics are one of the groups of nonessential dietary components that have been associated with the inhibition of atherosclerosis and cancer. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipooxygenase, and scavenge free radicals (11). The leaves, flowers, and berries of hawthorn contain a variety of bioflavonoid-like complexes, including oligomericprocyanidins (OPCc), vitexin, quercetin, and hyperoside (12, 13,14and15). The recommended daily dose of hawthorn is 160-900 mg of a native water-ethanol extract of the leaves or flowers (equivalent to 30-169 mg of epicatechin or 3.5-19.8 mg of flavonoids) (16 and 17). Its fruit has been used over the course of time as a diuretic, for dyspnea, and renal calculi. There are also studies that show its sedative and anxiolytic effects (18), and cardiotonic properties (19). So the antioxidant and the hypolipidemic activity of crataegus oxycantha was the aim of this study.

Materials and Methods

The fresh fruits of hawthorn were extracted with 70% ethanol according to (20). Fifteen mature (3-5 months) adult AlbinoWistar male Rats were randomly divided into three groups (each of 5) and treated as follows for 30 days :- Animals in group one had free access to food and water and served as control, group two(G_1) animals were subjected to ad libitum supply of drinking watercontaining 1% H_2O_2 (35% of hydrogen peroxide solution was diluted with water ,group three (G_2) animals were subjected to ad libitum supply of drinking water containing 1% H_2O_2 and received 300 mg / kg B.W. of crude ethanolic extract of Crataegus Oxyacantha dissolved in distilled water . Blood samples were collected by heart puncture technique at 0 time and 30 days of the experiment, serum collection by centrifugation (3000 rpm) for 15 minutes and frozen at -20C°until analysis. Serum samples were used for measuring the following parameters: - serum total cholesterol (TC) concentration was enzymaticlly measured using enzymatic assay kit (spain react) (21). Enzymatic estimation of serum triacylglycerol (TAG) concentration and serum high density lipoprotein cholesterol concentrations (HDL-C) were measured enzymatically using enzymatic kit) Linear chemicals) (22). Serum low density lipoprotein- cholesterol (LDL-C) concentration and serum very low density lipoprotein-cholesterol (VLDL-C)

concentration were calculated according to (23). Determination of Serum Reduced glutathione concentration (GSH) depended on the action of sulfhydryl groups (24). Stock standard solution of GSH (0.001M) was prepared by dissolving 0.0307 gm of GSH standard in a final volume of 100 ml of 0.4M Tris-EDTA-Na buffer (pH8.9). from stock solution 2, 5, 10, 40, 50, and 60 µM of standard GSH were prepared by following formula$N_1V_1=N_2V_2$ (Figure 1). The animals were then sacrificed for a histological examination, heart tissue sections were prepared according (25). Differences between experimental groups were statistically evaluated using two way analysis of variance (ANOVA) (26).

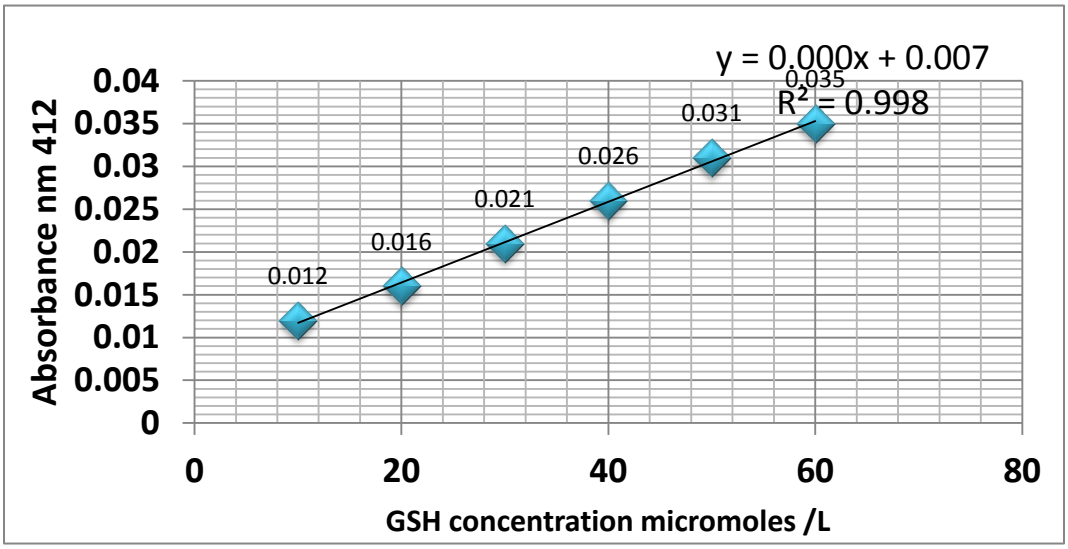

Figure (1) Standard curve of GSH concentration.

Results and Discussion

The effect of 70% alcoholic extract of *Crataegus oxyacantha* on lipid profile and antioxidant activity in mature male rats was shown in tables (1 and 2). Data pertaining to total cholesterol , TAG, HDL, LDL, VLDL concentration of rats in control group , group (G1) treated with 1% H_2O_2 and group (G2) treated with 1%H_2O_2plus 300 mg/ kg B.W. crud extract of *Crataegus oxyacantha* are depicted in table (1). The results showed after 30 days of treatment, significant (P< 0.05) decrease in serum TC concentration in the group (G2) as compared to the treated group (G1). There was significant (P<0.05) decrease in serum TAG in group (G2) as compared to the treated group (G1) and control group. There was significant (P< 0.05) increase in serum HDL concentration in group (G2) as compared to the treated group (G1), while there was significant (p<0.05) decrease in serum VLDL concentraton in group (G2) as compared to treated group (G1) and control. The results showed significant (P< 0.05) decrease in serum LDL concentration in treated group (G2) as compared to the treated group (G1). The mean values of serum reduced glutathione concentration in the control and treated groups along the experimental period are depicted in table (2). The statistical analysis indicated that the mean value of treated group (G2) significantly (P< 0.05) increased after 30 days of treatment as compared to control and treated group (G1). The result of the current study showed that oral administration of 1% H_2O_2 in drinking water for 30 days to adult male rats comparing to *Crataegus oxyacantha*, caused a case of dyslipidemia table (1) manifested by significant elevation in serum TC, TAG, LDL, VLDL concentration and significant depression in serum HDL concentration

which were similar to the findings on rat (27) and on japaness Quail (28). The potent oxidative effect of H_2O_2 which caused an oxidative damage manifested by free radicals and this lead to asubsequent complication and development of atherosclerosis (29 and 30). The present study demonstrated clearly that crude extract of hawthorn fruit possessed hypolipidemic activity, Hawthorn extract has been reported to lower plasma cholesterol concentration and other lipid profile in rats (31and 32) and rabbits (33). The mechanism for the hypocholesterolemic activity of hawthorn fruit may be either due to its inhibition of cholesterol and bile acid absorption or increased excretion of these neutral and acidic sterols (34). The beneficial effect of hawthorn was through reduction of intestinal cholesterol absorption via inhibition of the intestinal ACAT activity (35). These results, taken together, provide scientific evidence that hawthorn could be a useful natural ingredient for lowering plasma cholesterol concentrations in humans (36).

The elevation of serum TAG level in H_2O_2 treated group may contribute to the diffiency of lipoprotein lipase (the key enzyme determining the removal rate of TAG from plasma), associated with increased output of lipoprotein from the liver (37). Tincture of hawthorn inhibited oxidation of LDL and VLDL (38). Crataegus up- regulates hepatic LDL receptors, resulting in greater influx of plasma LDL – cholesterol into the liver by preventing the accumulation of cholesterol in the liver and cholesterol degradation to bile acids , promoting bile flow and suppressing cholesterol biosynthesis (32and39) .On the other hand, the investigation showed depression in the HDL concentration in group (G1) treated with 1% H_2O_2 while group (G2) treated with 1% H_2O_2 plus 300 mg / kg B.W. crude extract of hawthorn showed significant increase in this parameter. High density lipoprotein (HDL) plays an essential role in plasma lipid transport. It provides a reservoir of C, a lipoproteins, which are required for the metabolism of chylomicrons and very low density lipoproteins (VLDL), and acts as a scavenger of surplus unesterified cholesterol from these lipoproteins. HDL is also the major vehicle for the transport of cholesterol from peripheral cells to the liver for excretion and catabolism (40). Supplementation of hawthorn (4 weeks) with high cholesterol diet resulted in marked decrease in total cholesterol and LDL-lipoprotein, and more importantly with an increase in HDL-lipoprotein (41).

The current study showed decrease in the concentration of reduced glutathione (GSH) in group (G1) treated with 1% H_2O_2 and significant elevation in group (G2) administrated 1% H_2O_2 with hawthorn. Hyperlipidemia induced oxidative stress lead to decreased level of GSH in blood (42). The oxidative stress lead to increase oxidation of GSH into bisulphoric phase GSSG by inhibition pathway of pentose phosphate shunt which limits NADPH production that is necessary for activity of glutathione reductase enzyme which is important in GSH reproduction from oxidized phase (GSSG) (43). Hawthorn contains abundant amount of antioxidants such as chlorogenic acid, epicatechin, hyperoside and quercetin (54) which may be useful in alleviating the adverse effects associated with low-density lipoprotein (LDL)-cholesterol oxidation in atherosclerosis (45). Many studies show that hawthorn exhibited antioxidant activity associated with its flavonoids, polyphenol, procyanidin and this activity may be attributed to its effective inhibition of oxidative processes, efficient scavenging of O2- and possible increasing GSH biosynthesis (46 and 47). The histological structure of normal heart of control group is show in figure (2). After 30 days of oral gavage of 1% H_2O_2 the treated group (G1) showed pathological changes in the heart manifested as congested myocardial blood vessels with neutrophils in their lumen as well as odema between the myocardial muscle fibers , it also showed inflammatory cells between muscle fibers and lumen of blood vessels (figures 3and 4) .The histological

changes in the heart of animals of group (G2) treated with 1% H_2O_2 plus 300 mg/ kg B.W. of crude extract of *Crataegus oxyacantha* showed no clear pathological lesions (figure 5).

Table (1):-Effect of crude extract of *crataegus oxyacantha* on lipid profile in male rat (mg/dl)

	Group Treatment Orally for 30 days	Control Group(Distilled water)	G1 1% H_2O_2 with water	G2 1% H_2O_2+300mg/ kg B.W. of plant extract with water
Zero time	Total cholesterol conc.	67.61 ± 2.89 Aa	73.03 ±1.97 Aa	67.96 ± 3.11 Aa
	Triacylglycerol conc.	66.58 ± 2.23 Aa	65.26 ± 2.19 Ba	66.64 ± 2.44 Aa
	HDL conc.	51.43 ± 3.19 Aa	46.96 ± 1.31 Aa	47.71 ± 2.05 Aa
	LDL conc.	29.50 ± 5.06 Aa	39.12 ± 2.53 Aa	33.58 ± 2.34 Aa
	v-LDL conc.	13.32 ± 0.45 Aa	13.05 ± 0.44 Ba	13.33 ± 0.49 Aa
After 30 days treatment	Total cholesterol conc.	50. 85 ± 1.24 Bb	64.61 ± 3.37 Ba	49.86 ± 3.38 Bb
	Triacylglycerol conc.	65.29 ± 3.54 Ab	124.01± 6.91 Aa	49.84 ± 3.32 Bc
	HDL-c conc.	56.67 ± 1.12 Aa	42.63 ± 3.56 Ab	53.20 ± 5.52 Aa
	LDL- c conc.	7.24 ± 2.15 Bb	46.79 ± 5.36 Aa	14.38 ± 4.68 Bb
	v-LDL –c conc.	13.06 ± 0.71 Ab	24.80 ± 1.38 Aa	9.97 ± 0.66 Bc

Values expressed as means ± SE.n=5 / group
Small letters denote between groups differences, p< 0.05 vs cnotrol.
Capital letters denote within groups differences, p<0.05 vs control.

Table (2):- Effect of crude extract of *Crataegus oxyacantha* on glutathione concentration (µmol/l)

Group	Zero time	30 days treatment
Control	30.40 ± 1.60 Aa	27.20 ± 1.36 Ab
G1	30.00 ± 1.90 Aa	27.60 ± 0.75 Ab
G2	29.60 ± 1.60 Ba	41.20 ± 0.80 Aa

Values expressed as means ± SE.n=5 / group
Small letters denote between groups differences, p< 0.05 vs control.
Capital letters denote within groups differences, p< 0.05 vs control.

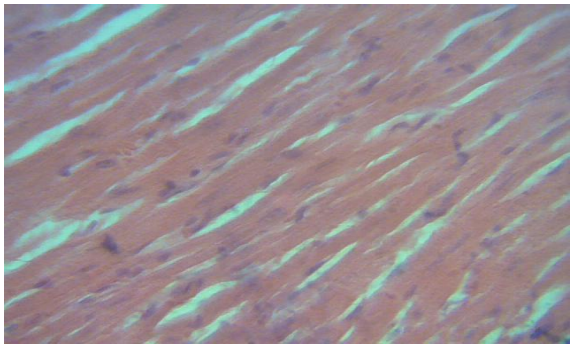

Figure 2:- Histological section of normal heart of rat (H and E, 40^X)

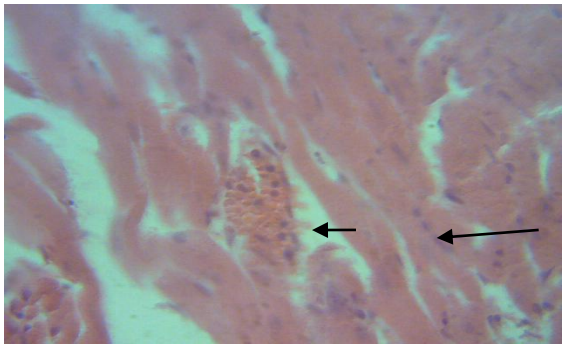

Figure 3:- Histological section of heart of rat shows congested blood vessels with neutrophils in their lumen (⟶) (H and E, 40^X)

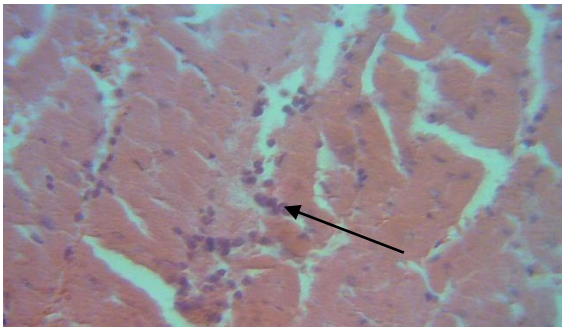

Figure 4:- Histological section of heart of rat shows inflammatory cells between the muscle fibers and lumen of blood vessels (⟵) (H and E, 40^X)

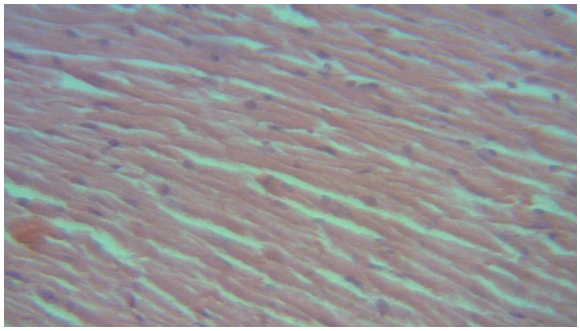

Figure 5:- Histological section of heart of rat treated with1% H_2O_2+300mg/kg B.W. of *crataegus oxyacantha* shows no clear pathological lesion (H and E, 40^X)

The histological changes in the heart showed congested myocardial blood vessels with neutrophils in their lumen as well as odema between the myocardial muscle fibers, but no lesion was observed in group treated with hawthorn. It could be concluded from this study that *Crataegus oxyacantha* has hypolipidemic and antioxidant activity.

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Detection of bovine viral diarrhea –mucosal disease (BVD-MD) in buffaloes and cows using ELISA

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Summary

Out of 210 tissue samples (lung and lymph nodes) collected from buffaloes examined by ELISA specific to(BVDV) Bovine Viral Diarrhea Virus antigen ten (10) samples were positive out of 160 lung tissue collected with (4.7%)and (3)positive sample were detected out of 50 lymph node tissue samples collected with (1.4%). Totally 13 positive samples with (6.1%) in buffaloes examined. The total positive tissue samples were divided to three age group. Group age one from (birth -6 month), group age two from (6-18 month) and group age three (18 month up). The positive samples were (1),(10) and (2) with (0.47%),(4.7%)and (0.95%)respectively that indicate the presence of BVD antigen in buffaloes (Bubals bubalis) .Out of (210) sera samples collected from buffaloes examined by ELISA specific to (BVDV) antibody (83) positive sera samples with (39.5%) in buffaloes. The positive serum samples were divided into three age groups, group age one from (birth -6 month), group age two from (6-18 month) and group age three (18 month up). The positive samples were (3), (60) and (20) with (1.4 %), (28.5%) and (9.5%) respectively in buffaloes. Out of (60) sera samples collected from cows examined by ELISA specific to (BVDV) antibody 21/60 positive samples with (35%) in cows.The positive serum samples divided to three age group ,group age one from (birth-6 month),group age two from(6-18 month) and group age three (18 month up). The positive samples (1), (14), and (6) with (1.6%), (23.3%) and (10%) respectively. The result above indicated the presence of the disease as persistent infection (PI) in group one, (MD) in group two and (BVD) in group age three.

Key words: (BVD) Bovine Viral Diarrhea, Buffaloes (Bubalus bubalis) , cow, ELISA, (MD) Mucosal Disease.

التحري عن وجود مرض الإسهال الفيروسي في الجاموس والأبقار باستخدام اختبار المقايسة المرتبطة بالإنزيم المناعي (الأليزا)

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الخلاصة

جمعت (210) عينة نسيج من الجاموس منها (160 عينة رئة و50 عينة عقدة لمفاوية) فحصت باختبار المقايسة المناعية المرتبطة بالإنزيم المناعي الخاصة بمستضد مرض الإسهال الفيروسي ألبقري ظهرت منها العينات في نسيج الرئة (10)عينات موجبة وبنسبة (4.7%) وفي نسيج العقد اللمفاوية 3 عينات موجبة وبنسبة(1.4%). بأجمالي (13) عينة موجبة وبنسبة (6.1%) في الجاموس (Bubalus bubalis) . قسمت العينات النسيجية الموجبة إلى ثلاث مجاميع اعتمادا على العمر المجموعة الأولى من (الولادة-6أشهر) والمجموعة الثانية من (6-18 شهر)والمجموعة الثالثة من (18 شهر فما فوق) ظهرت نتائج العينات الموجبة كالأتي {(1),(10),(2) وبنسب (0.47%) (4.7%) (0.95%)} على التوالي.
جمعت (210) عينه مصل الدم من الجاموس فحصت باختبار المقايسة المناعية المرتبطة بالإنزيم المناعي الخاصة بأضداد فيروس الإسهال الفيروسي ألبقري (BVDV) ظهرت (83) عينه موجبه بنسبة (39.5%) .قسمت عينات مصل الدم الموجبة إلى ثلاثة مجاميع اعتمادا على العمر المجموعة الأولى من(الولادة -6

اشهر) المجموعة الثانية من(6-18شهر) المجموعة الثالثة من (18 شهر فما فوق) فحصت باختبار المقايسة المناعية المرتبطة بالأنزيم المناعي الخاص بأضداد المرض .ظهرت العينات الموجبة في المجاميع الثلاث (3) , (60) ,(20) بنسب ((1.4%),(28.5%),(9.5%)على التوالي مما يشير إلى وجود الأضداد في دم الجاموس (Bubalus Bubalis). جمعت 60 عينه من مصل دم الأبقار فحصت باختبار المقايسة المناعية المرتبطة بالأنزيم المناعي الخاص بأضداد فيروس الإسهال الفيروسي ألبقري (BVDV) ظهرت (21) عينه موجبة بنسبة (35%) .قسمت عينات مصل الدم الموجبة اعتمادا على العمر المجموعة الأولى من (الولادة -6اشهر). المجموعة الثانية من (6-18 شهر)المجموعة الثالثة من (18 شهر فما فوق) فحصت باختبار المقايسة المناعية المرتبطة بالأنزيم المناعي ظهرت العينات الموجبة (1),(14),(6) وبنسب (1.66%)(23.33%),(10%) على التوالي ,تشير هذه النتائج لوجود أضداد فيروس الإسهال الفيروسي ألبقري . تشير هذه الدراسة وجود المرض بأشكاله الثلاث في المجموعات الثلاثة وكما يلي (PI) في المجموعة الأولى (MD) في المجموعة الثانية (BVD) في المجوعة الثالثة.

مفاتيح الكلمات: الإسهال الفيروسي , الجاموس ,الأبقار , الأليزا.

Introduction

Bovine viral diarrhea virus (BVDV) is an important economically disease causing severe economic looses . It is an RNA virus , member of the genus Pestivirus of the family Flaviviridae consist of two geno types, bovine viral diarrhea virus type 1 (BVDV1) and bovine viral type 2 (BVDV2).

BVDV infect mainly cattle and can infect sheep .goat and buffaloes (1 and 2). BVDV is a significant pathogen associated with gastrointestinal ,respiratory and reproductive disease (3) multiple clinical forms of the infection that vary from mild subclinical to fatal mucosal disease BVDV is a very common agent affecting livestock production throughout the world serological studies have shown that the presence of antibodies to BVDV in cattle is (60-70 %), (4), and (47%) in buffaloes(5). Fatal mucosal disease MD is caused by combination of cytopathic (cp) and non-cytopathic (ncp) biotypes of the virus (6). BVDV usually causes early embryonic death ,respiratory disease ,diarrhea ,congenital malformation , reproductive failures ,lameness , immunosuppression and (MD) mucosal disease (7) . Intrauterine infection with (ncp) biotype at early stage of gestation lead to birth of persistently infected (PI) calves , fatal infection with BVDV may result of calf immunotolerant to BVDV with an in apparent persistent infection which are serving as source of infection by shedding large quantities of virus life along with various body excretion .PI animals are difficult to identify , because of thrire normal appearance . ELISA test to detect viral RNA (Antigen) is becoming a popular screening method for detection of BVDV (3).

In IRAQ BVD virus in cow was proved by antibody detection by (8), later BVDV in cow was isolated by Al-Rodhan (9).

Materials and Methods

Samples collected from water buffalo (Bubalus bubalis) and cows from slaughter houses around Baghdad city (Abo-grab, Al-Shula, Al-Fudaiylia and khan-Tharey). Two hundred ten (210) tissue samples collected include 160 lung tissue samples and 50 lymph node tissue samples were taken to investigate the presence of the antigen in buffaloes (Bubalus bubalis) . Two hundred ten (210) blood samples were collected to detect the presence of antibody in buffaloes and 60 blood samples collected from cows. All samples were stored at (-20C)

ELISA Kits: Antigen capture ELISA (ACE) kits and Antibody ELISA kits . ELISA Kits purchased from belgium BIO-X diagnostics.

Method: ELISA procedure for Antigen and Antibody diagnosis of BVDV were performed according to instruction

Results and Discussion

In buffaloes ELISA antigen test was carried on (210) tissue samples collected (160- lung tissue and 50 lymph nodes tissue) positive samples were 13/210 with (6.1%).positive lung tissue samples were (10) with (4.7%) and positive lymph node tissue samples were (3) with (1.4%). the positive tissue samples were divided according to the age to three age groups. In group age one (birth-6 months) 1/210 one positive samples with (0.47%) in group are two (6-18 months)10/210 ten positive samples with(4.7%), in group age three 918 month up)2/210 two positive samples with (0.95%). The three age groups showed different clinical signs , group age one showed poor growth respiratory infection, diarrhea and death, group age two showed skin lesion blindness, depression, loss appetite and group age three showed mild or /no clinical finding or good health but shedding the virus (Table,1).

ELISA antibody test was carried on (210) sera samples in buffaloes (83) samples positive with (39.5%). The positive sera samples were divided to three age group according to the age ,group age one from (birth -6 months) 3/210 three positive samples with (1.4%), group age two from (6-18 months)60/210 sixty positive sera samples with (28.5%), group age three (18 months up)20/210 twenty samples with (9.5%). According to antibody validity these age groups classified as low or no antibody titer in group age one while high antibody titer in group age two and medium antibody titer in group age three tables 2. Out of the total serum samples collected from cows examined by ELISA 21/60 twenty one positive samples with (35%), these positive sera samples were divided into three groups ,group age one from (birth-6 months) 1/60 one positive samples with (1.6%), group age two (6-18 month) 14/60 fourteen positive samples with (23.3%), group age three (18 months up) 6/60 six positive samples with (10%) . According to antibody validity and age these three age groups classified as low antibody titer or non in group age one high antibody titer in group age two and medium antibody titer in group age three (Table,3). The proposed clinical diagnosis for the antibody result of buffaloes and cows was (PI) for age one. (MD) for age group two and (BVD) for age group three.

Table (1): Bovine viral diarrhea Antigen detection by ELISA according to age and type of tissue and the main clinical signs in buffaloes.

No. of tissue samples collected	Type of tissue samples	No. Of Collected samples	Pos.	%	Total positive samples	%	The age groups	Pos.	%	Main clinical signs
210	Lung	160	10	4.7	13	6.1	One day -6 months	1	0.47	Poor growth Respiratory signs Diarrhea death
	Lymph node	50	3	1.4			6-18 months	10	4.7	Skin lesion Blindness Depression Loss apatite Death
							18 months up	2	0.95	No symptom may be in good health Chronic case

Table 2: Bovine viral diarrhea antibody detection by ELISA according to age with validity and the proposed diagnosis in buffaloes.

No. of tissue samples collected	Positive samples	Per. %	Age	Pos.	Per %	Antibody titer and validity	Pr0p0sed diagnosis
210	83	39.5	One day-6 month	3	104	Low or/ no antibody titer	PI
			6-18 month	60	28.5	High antibody titer	MD
			18 month up	20	9.5	Medium antibody titer	BVD

Table 3: Bovine viral diarrhea antibody detection by ELISA according to age with validity and the proposed diagnosis in cows.

No. of tissue samples collected	Positive samples	Per.%	Age	Pos.	Per%	Antibody titer and validity	Proposed diagnosis
60	21	35	One day -6 months	1	1.6	Low or/no antibody titer	PI
			6-18 months	14	23.3	High antibody titer	MD
			18 months up	6	10	Medium antibody titer	BVD

Antigen and antibody detection of BVD-MD by ELISA in buffaloes and cows indicated the presence of the disease in IRAQ and in more than one form as can be seen from the tables of the results. This study detects the presence of BVD-MD virus antigen in lung and lymph node tissue with 6.1%. BVD-MD virus was present in lung tissue (4.7%) and lymph node tissue (1.4%) in this investigation, and was detected in similar tissue by (10 and11). Dividing the thirteen 13 positive cases of the antigen detection according to the age group revealed that the second age group (6-18 months) was the mostly affected such result agree with (12). The clinical finding of the three age group were not similar but different according to the form of the disease affecting the buffaloes, first and second age groups showed the most sever signs while many of the third age group were symptomless ,some in good health such finding also were mentioned and agree with (13).

In India (14) detected a total of 23.3%positive antibody cases by ELISA kits while it was 39.5 in this investigation and was (52.5%) in the investigating conducted by (15) the 6-18 months age group was the highest in the percentage, such deferens's in results could occur taking in to consideration the previous

disease situation and outbreaks of the disease if occurred, which could affect the total result of such investigations.

The validity were expected since the first age group were mostly infected during pregnancy and yielding low titer or no antibody, high antibody titer were found in the second age group, while the third age group were grown animals whom have experienced the disease and react to it in a mild form or chronic with titer in between the first and second age group such results agree with (16 and 17). Correlating between the clinical finding and antibody titer mentioned a proposed clinical diagnosis was made to identify the forms of the disease uncounted during this investigation. It was found that three forms were uncounted and could be suspected no similar researchers were found to be compared with using ELISA kits to detect antibodies in cows the second age group result (18) with23.3%was the highest percentage this result agree with (19).

The validity titer manifested in cows the second age group (6-18 months) being the highest no similar researchers were found to be compared with.

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Use of turmeric (*Curcuma longa*) on the performance and some physiological traits on the broiler diets

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Summary

This study was conducted at the poultry farm ,Veterinary Public Health .,College of Veterinary Medicine, to study the effect of Tumeric (Curcuma longa) on broiler performance and some physiological traits .Two hundred fifty day-old (Rose308) broiler chicks were all located randomly to five treatments from 1-42 days of age, with tow replicate pens (25 birds /pen) per treatment .

Chicks were fed the following :- Diet (1)Using basal diet free from herbal plants kept as control , Diet (2) Basal diet + 0.25% of Curcuma longa (250 gm/100kg of feed) Diet (3) Basal diet + 0.50% of Curcuma longa (500 gm/100kg of feed).Diet (4) Basal diet + 1% 0f Curcuma longa (1000 gm/100kg of feed).Diet (5) Basal diet plus 1.5 % of Curcuma longa (1500 gm/100kg of feed).Results revealed that the inclusion of turmeric at the levels of 0.50% in the diets improved body weight ,feed conversion ratio ,there were a significant difference in feed consumption . At the same time there was no significant difference for edible parts, were as found significant difference ($P< 0.05$) for dressing percent for all treatments treat with compare for control group . At the same time there was no significant difference in PCV, RBC, Hb, WBC while there was significant difference in H/L ratio, Albumin and globulin.

Key words: - turmeric, *Curcuma longa*, broiler, diets.

استعمال الكركم (Curcuma longa) في العليقة وتاثيره على الأداء الانتاجي وبعض الصفات الفسيولوجية لفروج اللحم

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الخلاصة

أجريت هذه الدراسة في حقل الدواجن التابع لفرع الصحة العامة البيطرية / كلية الطب البيطري /جامعة بغداد ، لدراسة تأثير الكركم (Curcuma longa) على الاداء الانتاجي وبعض الصفات الفسيولوجية لفروج اللحم . 250 فرخ لحم بعمر يوم واحد من نوع (Rose308) تم توزيعها بشكل عشوائيا إلى خمس معاملات من عمر 1-42 يوم مع مكررين لكل معاملة وبواقع (25 طير / مكرر)، غذيت الافراخ كالتالي: المعاملة الاولى (1) معاملة السيطرة وقدمت فيها للافراخ عليقة قياسية خالية من اي اضافة، المعاملة (2) عليقة قياسية +0.25% من مسحوق الكركم (250غم/كغم) ،المعاملة (3) عليقة قياسية +0.50% من مسحوق الكركم (500غم/كغم) ، المعاملة (4) عليقة قياسية + 1%من مسحوق الكركم (1000غم/كغم),المعاملة (4) عليقة قياسية + 1.5% من مسحوق الكركم (1500 غم / كغم علف). أظهرت النتائج أن أضافة الكركم على مستوى 0.50 ٪ ساهم في تحسين وزن الجسم ، كفاءة التحويل الغذائي ولايوجد فروقات معنوية في استهلاك العلف في الوقت نفسه فانه لا يوجد فروقات معنوية في الأجزاء الصالحة للأكل ، بينما يوجد فروقات معنوية على مستوى معنوية ($P<0.05$) لنسبة التصافي لكل المعاملات مقارنة مع مجموعة السيطرة .ولاتوجد فروقات معنوية لصفات الدم الخلوية لكن هنالك فرق فقط في نسبة H/L وفي الالبومين والكلوبيولين لمصل الدم.

الكلمات المفتاحية:- الكركم, عليقة, فروج لحم.

Introduction

A variety of feed additives are being included in poultry diet to derive maximum growth of broiler chickens. Use of in-feed-antibiotics not only increases the cost of production but

also leads to residues in meat and eggs (1) and develops antibiotic resistance in microbes (2). Turmeric powder (*Curcuma longa*) has long been used as antibacterial agent, antioxidant, growth stimulant etc for centuries in India.

Feed is responsible for about (65-70%) of overall poultry production costs, led to an increase number of studies on alternative dietary products that improves bird performance and lower production costs.

Since the 1950s, antimicrobial additives are the most frequently used as a performance enhancers in animal production and their positive results are observed even in high-challenge conditions. Since the 1990s, for a ban use of some antibiotics as growth promoters and the growing trend of the public to consume natural products, plant extracts have been selected as alternatives to antibiotic growth promoters.

First study that evaluated the antibacterial activities of plant extracts was carried out in 1881; however, they started to be used as flavors enhancers only during the next decades. With the emergence of antibiotics in the 1950s, the use of plant extracts as antimicrobial agents almost disappeared. Several studies in literature assessing the use of plant extracts, individually or in combination, as antimicrobials, antioxidants, or digestibility enhancers in animal feeds. Researches results on the factors affecting their action, such as plant variety, harvest time, processing, extraction, as well as the technology employed in synthesis commercial product and dietary inclusion levels show controversial results , warranted the need of further research and standardization for the effective use of plant extracts as performance enhancers, when added to animal feeds.

This article aims at presenting plant extracts as alternatives to antibiotics, Antioxidants, explaining their main modes of action as performance flavor, dig enhancer in broiler production.

Curcuma longa or turmeric is a medicinal plant widely used and cultivated in tropical regions. Plant extracts were found to have antifungal (3), immunomodulatory (4),antioxidative (5) and antimutagenic (6) activities.

Some of pharmacological activities of *Curcuma longa* as nematocidal (7), described by (8) and anti- inflammatory. Moreover, (6) proved the protective effect of *Curcuma longa* food additives on aflatoxin-induced mutagenicityand hepatocarcinogenicity.

Turmeric has been used as a coloring and flavouring agent and spice in many foods. Spices are the natural food additives contribute immensely to the taste and flavour of our foods. Smallpox, chickenpox, insect bites, as a food purifier and anthelmintic (9).

Turmeric has been subjected to chemical analysis which yielded essential oils (2.4-4%) and fatty oils (1.7-3.3%). Apart from curcumin, some other murcuminoids, fats, minerals,fibers, vitamins, proteins and carbohydrates, with total ash content of 4.7-8.2% (10 and 11). In spite of the fact that spices have been extensively consumed for centuries, occasional doubts have been expressed regarding the safety of some of them.

Fortunately the safety of turmeric and its yellow coloring agent, curcumin, are approved by many organizations and researcher (12); the joint FAO/WHO Expert Committee on Food Additives, JECFA,(13); Department of Biochemistry and Nutrition of Central Food Technological Research Institute of India, CFTRI (14 and 15).

Turmeric (*Curcuma longa*), a medicinal plant native to the Asian subcontinent, is known to possess antimicrobial and antioxidant properties. The curcuminoids, yellowish pigments present in turmeric powder, have shown protective effects against AFB1 (6). The most recent dietary approach to prevent mycotoxicoses in poultry is the combined use of antioxidants and adsorbents (16). The poultry industry has undergone remarkable change

and growth over the last 30 years, and it will continue to expand in the next coming years to meet a higher demand for low-cost, healthy and convenient products. In addition to the expanding market, commercial poultry is being genetically selected for ever increasing growth performance and efficiency. This selection for increased growth rate has resulted in changes in gastrointestinal development during growth of the animal (17). Apparently, young poultry are more susceptible to enteric pathogens today than they were 30 years ago. These pathogens have been of concern to the poultry industry because of lowered productivity, increased mortality, and contamination of poultry products for human consumption (18).The main purpose of this study was to evaluate the effect of turmeric longa L. on the performance and other physiological properties on broiler chicks.

Materials and Methods

This experiment was carried out in the poultry farm of veterinary collage ,Baghdad University .the experiment were lasted long continued for 42 days starting at 3-1-2010 to 14-2-2010 .(250) days old chicks (Rose 308) were divided randomly into 5 treated groups 50 bird .each ,chicks subdivided into 2 replicates of 25 birds each.

Chicks were fed the following :- Diet (1)Using basal diet free from herbal plants kept as control ,Diet (2) Basal diet + 0.25% of Curcuma longa (250 gm/100kg of feed) Diet (3) Basal diet + 0.50% of Curcuma longa (500 gm/100kg of feed).Diet (4) Basal diet + 1% 0f Curcuma longa (1000 gm/100kg of feed).Diet (5) Basal diet plus 1.5 % of Curcuma longa (1500 gm/100kg of feed). Chicks were reared in (1.5m×1.5m) floor pens of a thick litter system of wood shavings about 7 cm the feeding program including starter diet that have been used until 21 days of age and a finisher diet processed to the end of 42 days .All diets were formulated with the same batch of ingredients within a period had the same composition .Diets were designed to meet or exceed requirements by the National Research Council (19) for broilers at this age.Feed and water was provided at ad-libitum during the experiment, two phase feeding program involved in supplying starter to the first 21 day of age diet to the chicks.

Table (1) The composition of the experimental basal diets

Ingredient (%)	Starter 1-21 day	Finisher 22-42 day
Yellow Corn	51	53.3
Soybean meal (45% protein)	30	25
Wheat	13.8	15
Oil	1	2.5
Premix*	2.5	2.5
Salt	0.3	0.3
Methionine	0.1	0.1
Lysine	0.1	0.1
Di- Calcium phosphate	1.2	1.2
Calculated chemical analysis		
ME(Kcal/kg)	3000	3086
Crude protein %	21.3	19.5
Calcium %	0.69	0.52
Avialable phosphore	0.74	0.69
Methionine	0.33	0.31
Lysine	1.19	1.08

***Premix:- (2.5%) Provided the following (per Kg of complete diets)367500 IU, 133500IU Vit.D3,1920 mg Vit.E,83.42 Vit.K3,50mg Vit.B1,150 Vit B2, 500 mg Vit.B3,177,5 mg Vit.B6,0.8mg Vit B12,600mg Vit.PP,24.5 mg folic acid,27 mg Biotin,5767.5 mg choline,2667mg Fe,333.75 mg Cu,3334.06 mg Mn,203 mg Co 2334.38 mg Zn,100.75 mg Ca,10 mg Se,65446.46 mg Ph,36667.5 mg DL-Methionine ,200.02 mg Ethoxyquin,50mg Flavophospholipol, 30 g fish meal,1800 g wheat bran.**

Chicks were vaccinated against Newcastle disease (ND) and infectious bronchitis (IB). Body Wight was determined through the above diets periods feed intake was recorded for the corresponding periods. At the end of the experiment, three chicks of each replicate were randomly selected and weighted to obtain live body weight. were slaughtered using sharp knife for complete bleeding , feather birds were plucked .Head ,viscera and shanks were removed .Carcass was left for an hour to remove excess water and allowed for overnight cooling at 4 ± 2 C then weighed . Dressing percentage was calculated free from giblets (Heart ,gizzard ,liver) and the weight of each organ was calculated as percentage of the carcass weight .blood sample were taken from the brachial vein using a syringe . Samples were used for the determination of various hematological parameters including (PCV, WBC and RBC counts, hemoglobin (Hb) concentrations and hetrophile/lymphocytes ratio), glucose and cholesterol concentration. Data were analyzed using the General Linear Model Procedure of SAS,(20).Duncan multiple range test was used to detect the differences ($P<0.05$) among different group means.

Results and Discussion

Table (2) revealed the effect of adding Turmeric to the diet on the performance of broiler chickens (body weight gain, feed intake, feed conversion) for a six weeks age. It had been found that the higher body weight gain was observed in birds fed diet contained Turmeric at level (T3) followed by birds in (T5,T4) respectively as compared with treats (T1 and T2), feed intake showed a higher increase in (T3) as compared with other treats, on the other hands ,feed conversion (T4) birds was the best as compared to control group (T1) and other treats group .,the improvement in body weight gain ,feed intake ,feed conversion traits .during the (6) weeks of experiment were attributed to the presence of active compounds in turmeric, mainly antioxidant activity of it (5) that stimulates protein synthesis by bird enzymatic system.

In fact the above results are in agreement with (21) who observed a significant ($P<0.05$) increase in weight gain in broiler fed turmeric (1%) over those of control group .,on the contrary (22) stated that broiler fed with turmeric (0.25,0.50,0.75 and 1%) levels did not significantly affect body weight gain.

Table (3) showed the effect of turmeric on blood characteristics of broiler (PCV, RBC, Hb, WBC, H/L Ratio) and Albumin Globulin traits, it revealed that there are no significant differences among treats for (PCV, RBC, Hb, WBC) traits. H/L ratio, Level of albumin was significantly ($p<0.05$) lower in treats. group as compared with control group, it seems that various organs in treated group did not revealed any inflammatory reaction hence heterophillia in these groups may be as result of influx of granulocytes from marrow.

Thus heterophillia that appeared may be due to increase granulopiosis as result of Turmeric supplementation to the diet .,Further H/L constitute the first line of defense with efficient Chemotactic response, it suggested that birds of treated group were better equipped for the non –specific cellular response when invaded by foreign agents viable or innate .,on the other hands, lowered albumin level ,had no adverse effect on the colloidal state of blood as well as capillary permeability.

Significantly ($P<0.05$) higher level of globulin, suggested that birds of treated group had potential for better humeral immune status, as that in control group. Table (4) indicates the effect of turmeric in broiler diet on the qiblets triats and mortality (i.e Dressing ,Liver

,Gizzard, Heart%) at (6)weeks of age ,results showed a reduced mortality percent of treated group as compared with control group ,this may be due to the action of active compounds in turmeric such as antioxidant ,antimicrobial, antimutagenic…… ect) that keep birds in good health condition, moreover, results in table(4) also showed no significant ($P<0.05$) differences in giblets of treated group as compared with control group.

Mainly in Gizzard, but clear decrease in dressing percent , increase in liver percent in control group as compared with the treated group, these results concur with (23) who reported that feeding broilers of turmeric did not alter the percentage of (liver, gizzard, heart) traits. The above results raised form this study on turmeric supplementation in the broiler diet could be attributed to turmeric effect on birds giblets organs, turmeric included in diet a maximum of (1.5%) might be the reason for no change in structure of body giblets organs that may be used as (Viz turmeric) an alternative to feed antibiotics for improving giblets organs in broiler and improve their performance.

Table (2) Effect of Turmeric (Curcuma longa) on the performance of broilers ±SE.

Weeks / Treatments	3 weeks			6 weeks		
	Body weight gain (gm)	Feed intake (gm)	Feed conversion	Body weight gain (gm)	Feed intake (gm)	Feed conversion
Control T_1	874± 13.4 c	1555.7± 43.6 c	1.78± 2.7b	2552± 41.6 c	5359± 52.4 a	2.1± 1.2 a
0.25% T_2	1126± 16.8 b	1790.3± 52.7 b	1.59± 1.8 a	2743± 33.9 b	5376± 47.5 a	1.96± 1.9 a
0.50% T_3	1378± 18.7a	2135.9± 51.9 a	1.55± 2.3 a	3011± 36.4 a	5389± 49.7 a	1.79± 1.7 b
1% T_4	1198± 19.6ab	2012. 6 ± 46.7 ab	1.68± 1.8 a	2848± 37.7 b	5154± 51.8 a	1.81± 1.6 b
1.5% T_5	1278± 20.8a	2083.2± 42.8 a	1.63± 2.1 a	2885± 32.7 b	5019± 58.6 a	1.74± 1.7 b

abc,Means in the same colum with no common supercript differ significantly , $P<0.05$.

Table (3) Effect of turmeric (Curcuma longa) on mortality , Dressing %,liver %,gizzard% and heart% of broiler ± SE.

abc, Means in the same Colum with no common superscript differ significantly , P<0.05

Measures / Treatments	PCV %	RBC Cell/mm^3	HB g/100ml	WBC Cell/mm^3	H/L Ratio	Albumin g/100ml	Globulin g/100ml
Control T_1	31.4± 0.42 a	3.42± 0.20 a	8.94± 0.22 a	23.40± 0.28 a	0.35± 0.03 a	2.63± 0.03 a	2.71± 0.06 c
0.25% T_2	32.2± 0.31[a]	3.38± 0.17 a	9.05± 0.16 a	23.72± 0.31 a	0.31± 0.02 b	2.57± 0.04 b	2.68± 0.07 c
0.50% T_3	31.5± 0.29 a	3.41± 0.19 a	8.95± 0.20 a	23.31± 0.29 a	0.31± 0.02 b	2.54± 0.05 b	2.84± 0.06b
1% T_4	31.7± 0.35 a	3.42± 0.17 a	8.94± 0.24 a	23.45± 0.26 a	0.30± 0.03 b	2.54± 0.04 b	2.91± 0.07 a
1.5% T_5	32.1± 0.29 a	3.61± 0.22 a	8.93± 0.31 a	23.38± 0.23 a	0.29± 0.04b	2.51± 0.03 b	2.78± 0.08 b

Table (4) Effect of turmeric (Curcuma longa) on blood characteristics of broiler ±SE.

Measures / Treatment	Mortality %	Dressing %	Liver %	Gizzard %	Heart %
Control T_1	7.9±2.6 a	73.6±1.85 b	3.4±1.2 a	2.8±0.63 a	0.03±0.44a
0.25% T_2	5.3±3.1 b	74.6±1.92 a	2.9±1.4 b	2.7±0.04 a	0.04±0.48a
0.50% T_3	2.9±2.7 c	77.8±1.85 a	2.8±1.6 b	2.9±0.05 a	0.01±0.45a
1% T_4	4.3±3.5 b	75.7±2.1 a	2.9±1.5 b	2.8±0.04 a	0.9±0.42a
1.5% T_5	4.8±3.2 b	75.8±2.6 a	3.0±1.4 b	2.9±0.06 a	0.03±0.44a

A,b,c Means with different superscript in the same row differ significantly P<0.05.

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Hematological and Neurotoxic Effects of Endosulfan Pesticide on Common Carp *Cyprinus carpio*

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Summary

The present study including determined blood picture by measuring red blood cells count, hemoglobin concentration, packed cell volume and white blood cells in common carp *Cyprinus carpio*, as well as description behavior and growth of carp fish .In order to estimate LC50 used 240 fingerlings of common carp *Cyprinus carpio* were exposed to 0.0008µg/L, 0.0010µg/L, 0.0011µg/L, 0.0012µg/L, 0.0013µg/L, 0.0014µg/L and 0.0015µg/L. The LC_{50} of endosulfan was 0.0012µg/L for 48h of exposure Fish behavioral were recorded that showed abnormalities after exposure to the various endosulfan concentrations such as increase swimming activity, hypersensitivity, jerky movement, violent movements, loss of equilibrium, hyperactivity, increase operculum movement, frequent jumping, swimming at the water surface, erratic swimming, spiraling, convulsion, escape attempts from the aquarium with respiratory stress and decrease in respiratory rate as well as a significant decrease at ($P<0.05$) in body weight of all treated groups. The result of blood picture showed a significant reduction in red blood cells count, hemoglobin concentration, packed cell volume values while the number of white blood cells was showed a significant increase in its values.
Key word : Endosulfan , Hematology, Pesticide,*Cyprinus carpio*

التأثيرات الدمية والسلوكية العصبية لمبيد الاندوسلفان في اسماك الكارب العادي *Cyprinus carpio*

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الخلاصة

هدفت الدراسة الحالية معرفة تأثير مبيد الأندوسلفان في أسماك الكارب الأعتيادي عن طريق وصف سلوك ونمو الأسماك ودراسة الصورة الدمية المتضمنة حساب عدد كريات الدم الحمرو تركيز خضاب الدم و حجم الخلايا المرصوصة وعدد كريات الدم البيض فضلا عن وصف سلوك ونمو الاسماك. لغرض تحديد التركيز المميت الوسطي أستعمل 240 أصبعية من الأسماك للتراكيز0.0008 و 0.0010 و0.0011 و0.0012 و0.0013 و 0.0014 و 0.0015 µg/L ، وكان التركيز المميت الوسطي 0.0012µg/L أثناء 48 ساعة.أظهرت النتائج زيادة فعالية الأسماك وفرط التحسس وحركة متقطعة وحركة انفعالية وفقدان التوازن وزيادة حركة الغطاء الغلصمي والقفز المتكرر والسباحة على سطح الماء بصورة لولبية غير طبيعية والتشنج وصعوبة التنفس.أظهرت نتائج التحليل الأحصائي أنخفاضآ معنوياً بمستوى $P<0.05$ في معدل أوزان الأسماك.أظهرت نتائج حساب عدد الخلايا الحمر وتركيز خضاب الدم وحجم الخلايا المرصوصة أنخفاضآ معنوياً في معاييرها بينما سجلت عدد الخلايا البيض زيادة معنوية في قيمها.
مفاتيح الكلمات : الدمية , الاندوسلفان , اسماك الكارب.

Introduction

Pesticides are well recognized as an economic approach for controlling pests in agriculture and horticulture. Endosulfan ($C_9H_6Cl_6O_3S$), an OC compound belonging to cyclodiene group is extensively used as a broad- spectrum pesticide to treat a wide variety of invertebrate pests and bird repellent on fruit crops in more than 70 countries (1). Endosulfan is an organochlorine insecticide and acaricide, and acts as a contact poison in

a wide variety of insects and mites. Endosulfan is persistent, with a half-life in water of up to six months under anaerobic conditions (2), and a half-life in soil under aerobic conditions of up to six years (3). Fish can be exposed to the insecticide when dissolved in water by absorption through the gills, skin and by contaminated food. Fish gills are the first organ that adsorbs the toxicants and contaminants which are then distributed via blood system into other organs. Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure.

Intentional misuse of endosulfan for killing fish (4). Endosulfan is very toxic to fish, even compared to other organochlorine pesticides such as dichlorodiphenyl-trichloroethane (DDT) (5).The present work aims to study the acute toxicity of endosulfan through the following: Determining LC0 , LC50, LC100 and safety concentration (SC) values for 48h. Study the clinical signs (behavior of fish such as movement, food intake and survival rate and the growth, food ratio). Study the effect on hematological picture which including: RBCs, WBCs, Hb and PCV.

Materials and Methods

The study was conducted at the Fish diseases laboratory, Veterinary Medicine College, University of Baghdad. A total of 240 Fingerlings of common carp *Cyprinus carpio* ranging 12-14 cm in total length and 40-50 gm in weight, were obtained from Al-Talbe hatchery and acclimated to laboratory conditions for 15 days before the commencement of the experiment. The fish acclimatized in aquarium measuring 80x40x30 cm and were supplied with oxygen using tap water in the aquarium to reach 70 L. Physico-chemical characteristics were measured such as temperature ranges between 21-24C°, pH ranges between 7-7.8 , Fish were fed twice daily with commercial pellets with ratio of 4% of initial body weight per day, having 28% crude protein.

Determination of median lethal concentration of endosulfan seven different concentrations of treatment of endosulfan was used; each concentration was added 3 times to each group. The concentration at which 50% mortality of fishes occurred after 48h was selected as the medium lethal concentration (LC_{50}). The LC_{50} concentration for 48h was calculated by the probit analysis method. In this study, LC_0 and LC_{100} were determined also and the observation of toxic symptoms such as movement, respiration, swimming, food intake and response to the outer effects was recorded.

The concentrations that using in this experiment were 0.0008µg/L, 0.0010µg/L, 0.0011µg/L, 0.0012µg/L, 0.0013µg/L, 0.0014µg/L and 0.0015µg/L. During the experiment period the observation of toxic symptoms such as stress, movement, respiration, swimming, responses to the outer effects. Blood collection was done via cardiac puncture technique, blood was withdrawn directly from heart after the experiment end by a sterile disposable syringe (needle gauge 23), and blood was transferred into a tube containing EDTA solution for hematological tests, and counting (RBC, WBC and PCV).

Results are expressed as M± SE. Statistical analysis of data was performed on the basis of one- way analysis of variance (ANOVA I) for experiment Group differences were determined using least significant difference (LSD).

Results and Discussion

Median Lethal Concentration Measurement: The estimated LC_{50} by (Probit method) for endosulfan of *Cyprinus carpio* was shown in Table (1). In the acute toxicity test, approximately 1 h after exposure to the various lethal endosulfan concentrations, the fishes showed behavioral abnormalities such as: increase jerking, frequent jumping, erratic swimming, spiraling, and convulsion, escape attempts from the aquarium, loss of

equilibrium, molting, color changes and paralysis. Results showed that no mortality of fishes in the control treatment. The acute toxicity of endosulfan concentrations on *Cyprinus carpio* at different exposure period and the mortality percentages are shown in Figur (1).

The mortality of the fish indicated that the toxicity and mortality of endosulfan increased with endosulfan concentrations and exposure. At the low endosulfan concentration (0.0010 and 0.0011µg/L), mortality was found after 24 h of exposure, While at higher concentrations (0.0015 µg/L), mortality occurred after 1 h of exposure. However, the mortality of *Cyprinus carpio* exposed to either low or high concentrations of endosulfan remained constant after exposure for 48 h. The present study determined the LC_{50} of endosulfan (0.0012µg/L) and LC_0 (0.0008µg/L), LC_{100} (0.0015µg/L), SC (0.0006µg/L) during 48h.

Table 1: Show LC_{50} of endosulfan on *Cyprinus carpio* by Probit method

Conc.µg/L	Log Conc.	Fish No.	Survival Fish	Mortality	Mortality %	Probit No.
0.0008	0.90	10	10	0	0	0
0.0010	1	10	8	2	20	4.16
0.0011	1.04	10	6	4	40	4.75
0.0012	1.07	10	5	5	50	5.00
0.0013	1.11	10	3	7	70	5.52
0.0014	1.14	10	1	9	90	6.28
0.0015	1.17	10	0	10	100	7.50
Control	…….	10	10	0	0	…….

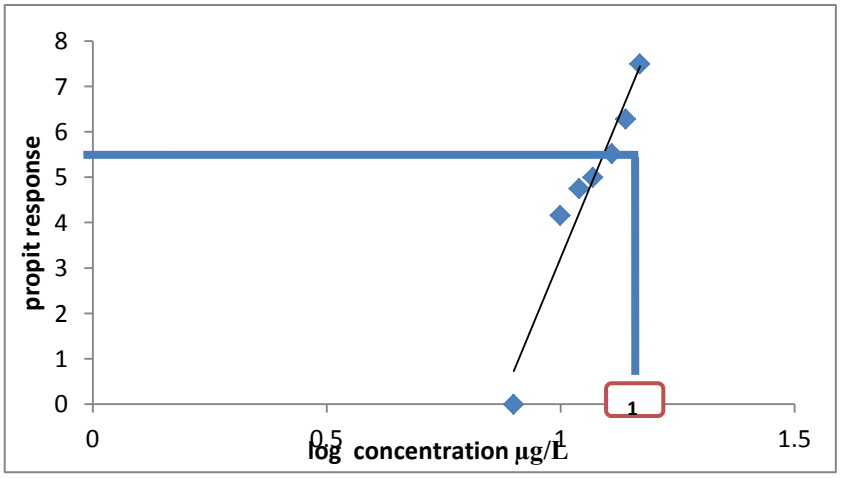

Fig. 1: Linear relationship between probit response and log concentration of median lethal concentration for endosulfan in 48h.

The present study determined the acute toxicity of endosulfan on the *Cyprinus carpio* during 48 h. The LC_{50} of endosulfan was 0.0012µg/L.In another study, LC_{50} has been established as 0.0016µg/L found in *Cyprinus carpio* (6), The endosulfan had 96h LC_{50} of 0.93 µg/l found in *Macrobrachium rosenbergii*, post larvae (7) and grass shrimp *Palaemonetes pugio* where 96-h LC_{50} was 0.62- 1.01 µg/L, with a range of 0.35-1.43µg/L (8).

Fishes behavioral showed abnormalities approximately 1 h. after exposure to the various endosulfan concentrations such as increase swimming activity, hypersensitivity, jerky movement, violent movements,loss of equilibrium, hyperactivity, hyper-excitability, increase operculum movement, frequent jumping, swimming at the water surface, erratic swimming, spiraling, convulsion, escape attempts from the aquarium, hitting to the walls of the aquarium before finally sinking to the bottom (Fig.2). The exposed fishes exhibit tremors and gradual weakening of reflexes leading to imbalance in posture and loss of equilibrium (Fig.3). In due course of time a few fishes start drowning but by sudden somersaulting, regain normal posture and balance temporarily.

Finally, however, they succumb to poison with mouth and operculum wide open, molting of body color changes from silvery white to pale white. At lower concentration, however, the changes in behavior are conspicuous. Results showed fish loss appetite of food. Abnormalities behavior observed in all treated groups but the severity of signs increased with a high concentration of endosulfan.

Fig. 2: Show fish sinking to the bottom of the aquarium

Fig.3: Show imbalance in posture and loss of equilibrium of fish

Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and neuromuscular junctions (9).

Surfacing phenomena as observed in the fish treated with lethal concentration of endosulfan indicates hypoxic condition. Such surfacing might be to procure definite proportion of its oxygen requirement from the atmosphere (10). Bradbury and Coast (11) reported signs of endosulfan poisoning in fish, which included loss of schooling behavior, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, increased gill mucus secretions, flaring of the gill arches, head shaking and restlessness before mortality.

Signs of acute Endosulfan poisoning include frequent jumping and erraticopercular movement followed by convulsions (12). Sunderam, (13) found the 96 hr LC50 values vary from 0.014 µg/L for the harlequin fish *Rasborahetero morpha* to 14 µg/L for catfish *Claria sbatrachus*. The 96 hr LC50 values for Australian fish are 2.2 µg/L for the native firetail gudgeon *Hypseleotris gallii*, 2.4 µg/L for the eastern rainbow fish *Melanotaenia duboulayi* and silver perch *Bidyanu sbidyanus*, 0.5 µg/L for golden perch *Macquaria ambigua* and 0.2 µg/L for bony bream *Nematolosaerebi* (13).

The LC50 values for exotic species that inhabit the Murray-Darling basin are 3.1 µg/L for mosquito fish *Gambusia affinis*, 0.1 µg/L for carp *Cyprinus carpio* and 1.6 µg/L for rainbow trout *Oncorhynchus mykiss* (14 and 13).

Endosulfan has long been known to be extremely toxic to many fish species, with the harlequin fish *Rasborahetero morpha* apparently most sensitive (24 hour LC50 = 0.02 µg/L). High excitability, loss of equilibrium and spasmodic movement has been reported as general symptoms of endosulfan poisoning in fish (13).

The decrease in the consumption of oxygen is probably the result of alterations of energy metabolism (15). Some studies of the pathological effects caused by chronic exposure to chemical substances evidenced the gradual destruction of gills filaments, killing the fish by asphyxia (16).

Body weight variations are the most sensitive indication of potential toxic effects studied. The physiological indicator may be affected if food is limited or if food consumption of the fish is impaired due to other stress factors.Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. This was more noticed in sub-lethal exposure periods. For these animals, it might be profitable to decrease their food uptake under toxic environmental conditions to lower the energetic costs of digestion. Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods, it can have a clear impact on growth and reproduction (17).

The effects on growth or survival rate were observed in fathead minnows *Rasborahetero morpha* continuously exposed for 60 days under flow-through conditions to endosulfan concentrations between 0.04 and 0.4 µg/L (13). Treatment at the high dose of endosulfan induced lower body weights and body weight gains and abdominal cramping in other fish. A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations. Dembele, (18) indicated that the abnormalities in fish behavior observed in exposure with OC insecticides could be related to failure of energy production or the release of stored metabolic energy, which may cause severe stress, leading to the mortality of the fish. The stress response in some situations may lose its adaptive value, which can result in the inhibition of growth, reproductive failure and immune suppression (19). Body weight may be reduction due to Liver and kidney damage in fish (20).

Results of RBCs count (Cells/µL x10^6) in blood of *Cyprinus carpio* which exposed to repeated different concentrations of endosulfan insecticide for three months are shown in Table(2).There was a significant reduction in RBCs count at ($p<0.05$) of all treated groups values when compared with the control group values. The highest reduction in RBCs count was recorded in T5 group value which treated with a concentration of 0.0005 µg/L while the lowest reduction in RBCs count was recorded in T1 group value which treated with a concentration of 0.0001 µg/L. Results of PCV (%) in blood of *Cyprinus carpio* which exposed to a repeated different concentration of endosulfan insecticide for three months are shown in Table (2). Results showed significant reductions in PCV (%) at ($p<0.05$) of all treatments groups values when compared with the control group value.

The highest reduction in PCV (%) was recorded in T5 group value which treated with a concentration of 0.0005µg/L while the lowest reduction in PCV% was recorded in T1 group value which treated with a concentration of 0.0001µg/L. The results of hemoglobin (Hb) (gm /dl) are shown in Table (2). There was a significant decrease ($p<0.05$) in values in all treated groups along the period of the experiment in comparison with the control group value in blood of *Cyprinus carpio* which exposed to a repeated different concentration of endosulfan insecticide. The highest reduction in Hb concentration was recorded in T5 group value which treated with a concentration of 0.0005µg/L while the lowest reduction in Hb concentration was recorded in T1 group value which treated with a concentration of 0.0001µg/L. Results of WBCs count (Cell/µLx10^3) in blood of *Cyprinus carpio* which exposed to repeated different concentration of endosulfan insecticide for three months are shown in Table (2). There were significant elevations at ($p<0.05$) in T5 group value as compared with the control group value. While There were no significant differences at ($p<0.05$) among T1, T2, T3 and T4 groups values also, when compared with the control group and T5 group values respectively.

Table 2: The results of hematological tests (WBCs count, RBCs count, Hb concentration and PCV) of *Cyprinus carpio* which exposed to a repeated different concentration of Endosulfan pesticide during experiment period.

Hematological tests				
Test Groups	**WBCs count Cells/µLx10^3**	**RBCs count Cells/µLx10^6**	**Hb concentration (gm/dl)**	**PCV (%)**
Control	**25.4±0.9 B**	**2.6±0.01 A**	**8.4±0.1 A**	**31.0±0.5 A**
T1 0.0001µg/ L	**25.9±1.2 AB**	**2.42±0.005 B**	**7.8±0.1 B**	**29±0.4 B**
T2 0.0002µg/ L	**26.7±1.2 AB**	**2.23±0.004 C**	**7.1±0.1 C**	**26±0.4 C**
T3 0.0003µg/ L	**27.3±1.1 AB**	**2.04±0.004 D**	**6.4±0.1 D**	**24±0.3 D**
T4 0.0004µg/ L	**28.2±1.1 AB**	**1.89±0.007 E**	**5.9±0.1 E**	**22±0.3 E**
T5 0.0005µg/ L	**28.9±1.1 A**	**1.72± 0.006 F**	**5.4±0.1 F**	**20±0.3 F**

- **L.S.D. value: RBCs=0.014, PCV=0.9,Hbc. =0.3, WBCs=3.1**
- **Figures represent mean ± standard error.**
- **Different capital letters represent significant difference between groups vertically at $p<0.05$.**
- **n =10**

The alterations of the haematological parameters could be used as an important tool for the assessment of pathological conditions of animals. The changes in the haematological parameters of fish are a helpful biomarker for evaluating their health status (21). The endosulfan induced reduction in the blood parameters recorded in the present study. This may be due to haemolysis and/or haemorrhage caused actions of pollutants to the fish (22). In the present study, the haematological parameters were negatively correlated with the endosulfan concentration and the experiment length.

This results agreement with Jenkins,(23) stated that the reduction in the haematological parameters after sub-lethal endosulfan exposure is the result of the inhibition of erythropoiesis, haemosynthesis, osmoregulatory dysfunction or an increase in the rate of erythrolysis. Although the level of hemoglobin reduced in *Cyprinus carpio* exposed to endosulfan, the erythropenia could be associated with a possible hemolysis (24). Banik, (25) reported that *Anabas testudineus* exposed to 0.00125% of endosulfan are unable to absorb iron efficiently from the intestine leading to a decrease in the hemoglobin formation and then anemia. On the other hand, the reduction in erythrocyte numbers could be related to possible oxidative damage to hemoglobin caused by the lipid peroxidation observed in fish exposed to the low concentration of endosulfan (23)

A reduction in haematological values, indicated anemia in the pesticide exposed fish may be due to erythropoiesis, haemosynthesis and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs (26). In the present study, the decrease in RBCs count during the chronic treatment might be resulted from severe anemic state or haemolysing power of toxicant (endosulfan) particularly on the red cell membrane. Pesticides were found mainly in the erythrocytes (particularly in the erythrocyte content) and plasma and not in the leucocytes, platelets or stroma indicating that they mainly bind with hemoglobin. The reduction in erythrocyte count, PCV and haemoglobin of *Cyprinus carpio* in the present study can be attributed to the following factors, haemodilution of blood due to the damage of fish organs (27) and the haematological parameters PCV, RBCs and Hb, whose changes can be interpreted as a compensatory response that improves the O_2 carrying capacity to maintain the gas transfer, also indicates a change in the water blood barrier for gas exchange in gill lamellae (28).

Erythrocyte level was found to be depressed in fishes subjected to stressful conditions. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and the nature of the changes shows a release of erythrocytes from the blood depots (24).

Inhibition of erythropoiesis and an increase in the rate of erythrocyte destruction in hematopoietic organs are the cause of decrease in RBCs count. Joshi, (29) found a significant decrease of RBCs, Hb and packed cell volume (PCV) in endosulfan exposed fish species and indicated the toxic effect of endosulfan on spleen, liver and anterior kidney.

The decrease in the haemoglobin content in the present study resulted from rapid oxidation of hemoglobin to methaemoglobin or release of O_2 radical brought about by the toxic stress of endosulfan. It is increasingly recognized that xenobiotics which is capable of undergoing redox cycling can exert toxic effects *via* the generation of oxygen free radicals. Matkovics, (30) observed *Cyprinus carpio* a quick decrease in haemoglobin content in response to parquet toxicity .

The PCV values decrease when a fish loses its appetite, or it is diseased or poisoned by pesticides (31). The reduction in the PCV values indicates that the fish suffers from anemia or hemodilution. In addition, an alteration in the fish metabolism would also lead to decrease values of hemotocrite in *Cyprinus carpio* (32).

Similar to the present study, a significant decrease in the red cell count was reported in fingerlings *Cyprinus carpio* exposed to concentrations from 0.26 to 0.78 µg.L of endosulfan (33).

In the present study, although significant variations were observed in terms of leucocyte concentration, the progressive trend of leucocytosis observed in *Cyprinus carpio* exposed to both concentrations of endosulfan could be considered as a cellular response of tissue alteration generated by the insecticide (34) or a high sensitivity of neutrophils to environmental changes (35). The increase in WBCs count in the present study indicates the stress condition of the fish caused by endosulfan which might produce gill damage and other organs.

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Effect of L-carnitine administration to pregnant mice on some reproductive hormones and organs

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Summary

Carnitine is quaternary ammonium compound and required for the transport of fatty acids from the cytosol into the mitochondria for the generation of metabolic energy. The aims of the present study were to assess the effects of L-carnitine administration to pregnant mice on some parameters of reproductive performance and pregnancy outcome.

One hundred and five pregnant female mice Swiss albino strain mice age: 12-14 weeks were used in this study. Pregnant mice were divided randomly into three equal groups including control group (administered distilled water; DW), low dose group (T1) administered 0.5 mg/Kg L-carnitine and high dose group (T2) administered 1 mg/Kg L-carnitine. Daily administration of D.W. or L-carnitine was continued from day 1 (day post-sexual mating) until parturition. Hormone assay involving follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E_2), litter size, percentage of female sex, weight of the reproductive system and endometrial thickness were assessed.

Assessment of levels of serum reproductive hormones appeared that the FSH and LH and E_2 for both treated groups were increased significantly ($P<0.05$) as compared to the control group. Moreover, significant increment ($P<0.05$) in the weight of reproductive system, litter sizes and a significant increment ($P<0.05$) in the thickness of endometrium for both treated groups was observed as compared to the control group.

Conclusion: administration of 0.5 mg/Kg L-carnitine to pregnant mice had beneficial effects on pregnancy and offspring outcomes.

Key words: L-carnitine, mice, reproduction, pregnancy, FSH, LH, Estrogen.

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تاثير اعطاء مادة ال-كارنيتين الى حوامل الفئران على بعض صفات الكفاءة التناسلية

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الخلاصة

يعرف ال-كارنيتين على انه مركب رباعي المونيوم وهو ضروري لانتاج الطاقة الايضية من خلال نقل الاحماض الدهنيه من سيتوبلازم الخلية الى بيوت الطاقة. لذلك تهدف الدراسة الحالة الى معرفة تأثيراعطاء ال-كارنيتين للأناث الفئران الحوامل على بعض الصفات التكاثرية وناتج الحمل.استخدمت الدراسة مئة وخمسة أنثى فأر نوع الابرص السويسري عمر:12-14 اسبوع، والتي قسمت عشوائيا الى ثلاث مجاميع متساوية و تتضمن مجموعة السيطرة (عوملت بالماء المقطر فقط)، مجموعة الجرعة المنخفضة (T1):عوملت بـ 0,5 ملغم/كلغم ال- كارنيتين ومجموعة الجرعة العالية (T2):عوملت بـ 1 ملغم/كلغم الل -كارنيتين خلال فترة الحمل. وبعد الولادة تمت دراسة مستوى الهرمونات لكل من FSH و LH وE_2 وحجم الولادة ووزن الاعضاء التكاثرية بالاضافة الى قياس سمك طبقة بطانة الرحم للامهات ونسبة الاناث في المواليد.

اظهرت الدراسة الحالية زيادة معنوية ($P<0.05$) في كل من مستوى الهرمونات التناسلية في مصل الدم المقاسة (FSH و LH و E_2) ووزن اعضاء التناسلية وحجم المواليد وكذلك سمك بطانة الرحم لكلا مجموعتي

المعاملة بالمقارنه مع مجموعة السيطرة. نستنتج من نتائج الدراسة الحالية بأن التجريع بالجرعة المنخفضة لمادة أل-كارنيتين للامهات الحوامل له تاثير مفيد للحمل ولناتج المواليد.

كلمات مفتاحية:- كارنيتين , حوامل, فئران, التناسلية, استروجين.

Introduction

Carnitine, or 3-hydroxy-4-N-trimethylaminobutyrate, is a ubiquitous molecule within mammalian tissues, which was first discovered in the skeletal muscle extracts in the early twentieth century (1). The crucial role of L-carnitine in metabolism was not elucidated until 1955, and its deficiency was not described until 1972 (2). Carnitine was certified as an essential nutrient of multifunction for the body (3). A trimethylated amino acid, roughly similar in structure to choline, L-carnitine is a cofactor required for transformation of free long-chain fatty acids into acylcarnitines, and for their subsequent transport into the mitochondrial matrix, where they undergo beta-oxidation for cellular energy production (4).

In normal animals, the excretion of unchanged carnitine in urine seems to be the main pathway of loss. This excretion is increased in thyrotoxic and decreased in hypothyroid patients (5). Also, in normal animals carnitine is lost mainly by excretion in the urine (6). The absorption and deposition of dietary carnitine in human found that carnitine absorption is dependent on the intake amount. Approximately 54-87% of dietary carnitine is absorbed in the intestine and enters the bloodstream of rats and human being (7 and 8). Carnitine uptake from blood into tissues takes place via an active transport process against concentration gradient. Furthermore, tissue carnitine concentration is 20-50 folds higher than in plasma (9 and 10). Carnitine biosynthesis accounts for one third to one half of the total carnitine sources when omnivorous diet is consumed (11).

After oral administration of radioactive-labeled carnitine in rats, labeled trimethylamine N-oxide and butyrobetaine were found in urine and feces, respectively (12). Carnitine degradation in mammals was restricted to the non-absorbed carnitine in the intestinal tract, whereas absorbed or intravenously administered carnitine and endogenous carnitine were mostly eliminated in urine (13), and also excreted in milk (14). The European Food Safety Authority has made an extensive safety evaluation and concluded that up to 2 g L-carnitine or the equivalent 3 g L-carnitine tartrate are regarded safe for daily consumption (15).

Animal studies had revealed no harm to the fetus but that no adequate studies in pregnant women had been conducted. L-carnitine had been given to pregnant women late in pregnancy with resulting positive outcomes (16). Therefore, the aims of the present study were to assess the effects of L-carnitine administration to pregnant mice throughout all gestation days on some reproductive hormones and pregnancy outcome.

Materials and Methods

One hundred and five mature Swiss albino strain female mice age: 12-14 weeks; weight 25-28 g were used which obtained from animal house at Institute of Embryo Research and Infertility Treatment/Al-Nahrain University. Each female in the metestrus phase was caged with mature healthy male mouse, and the occurrence of vaginal plug was considered as the first day of pregnancy. The pregnant females were isolated in the cages alone. Pregnant mice were divided randomly into three equal groups (each group contains 35 pregnant mice) including control group (administered distilled water), low dose group (T1) administered 0.5 mg/Kg L-carnitine and high dose group (T2) administered 1 mg/Kg L-carnitine. Daily administration of D.W. or L-carnitine was continued from day 1 (day post-sexual mating) until parturition.

Low and high doses of L-carnitine were prepared by dissolving one crushed tablet (1000 mg tablet; Harbin Yeekong Herb Inc.; Australia) in 100 mL and 50 mL of distilled water; respectively. Each pregnant mouse was orally administered 0.05 mL from one of previous two solutions throughout pregnancy period.

At end of gestation period, 105 pregnant mice were delivered. Litter size and percentages of the female to male new born pups were determined. From 30 delivered mice, blood samples were taken under light anesthesia using diethyl ether (Fluka; Germany) by heart puncture using 2 mL syringe attached to 21-gauge needle and put in 1.5 mL tube and left for 10 minutes. serum were separated from blood using centrifugation for 2500 RPM for 8 minutes and preserved in refrigerator freezer at -20 ^{o}C untill the time of the hormone analyses (FSH, LH and E_2) using radioimmunoassay (RIA) technique at Biochemical tests laboratory, Institute of Embryo Research and Infertility Treatment.

Reproductive organs consisting ovaries, uterine horns and vagina were taken and cleared from attached adipose tissues. Weight of whole reproductive system was assessed using sensitive balance (BL-2105; Germany). Then, tissue of uterine horn was fixed and processed for histological sectioning to measure thickness of the endometrium according to procedure was mentioned by (17).

4. Statistics:

Data analyses were conducted using Statistical Analysis Package for Social Sciences (SPSS, version 14). All values were presented as mean and standard error of mean (Mean ± S.E.M). To compare among means of three groups, multiple analysis of variance (MANOVA) analysis and student t-test were used. Significance was set at $\boldsymbol{P} \leq 0.05$ (18).

Results and Discussion

Significant increment ($P<0.05$) in the weight of reproductive organs for both treated groups was assessed as compared to the control group. For the same parameter, non significant differences ($P>0.05$) were noticed between both treated groups. Litter sizes for both treated groups were increased significantly ($P<0.05$) as compared to the control group. However, non significant ($P>0.05$) differences were assessed for the litter size between both treated groups. Also, non significant ($P>0.05$) differences were observed in the female sex ratio among the control and both treated groups (Table 1).

Figure (1) shows the changes in the endometrial thickness for the control and both treated groups. Significant increment ($P<0.05$) in the thickness of endometrium was observed for both treated groups as compared to the control group. However, non significant ($P>0.05$) differences were appeared between both treated groups.

The results showed that the gonadotropins (FSH and LH) and estradiol (E_2) for both treated groups (T1 and T2) were elevated significantly ($P<0.05$) as compared to the control group. However, non significant differences ($P>0.05$) were reported for levels of all serum reproductive hormones between both treated groups (Table 2).

Table 1: Litter size, percentage of female sex and weight of reproductive organs for pregnant mice# administered two doses of L-carnitine throughout pregnancy (No.=35 pregnant mice/group; Mean± S.E.)

Groups	Parameters		
	Litter size	Percentage of female sex	Weight of reproductive organs (g)
Control group	6.72 ± 0.11 *	0.76 ± 0.09	4.23 ± 0.041 *
Low dose group (T1)	8.64 ± 0.12	0.73 ± 0.09	6.61 ± 0.024
High dose group (T2)	8.81 ± 0.08	0.70 ± 0.05	6.82 ± 0.031

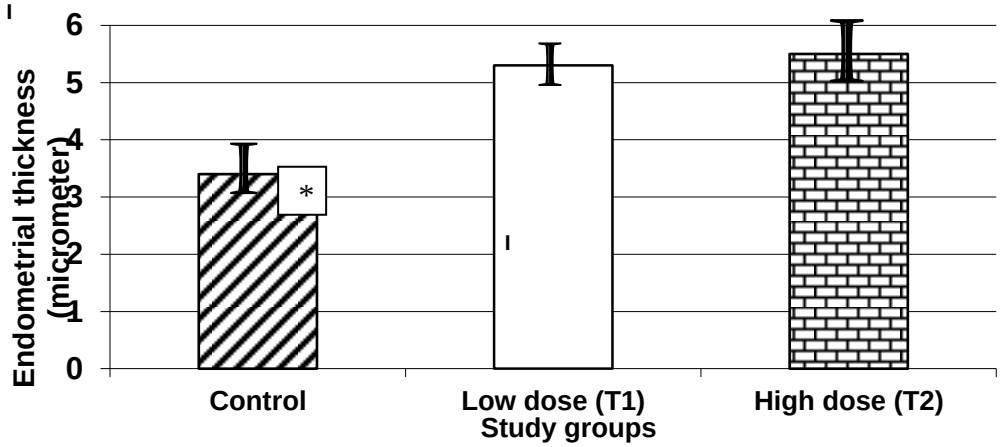

Figure 1: Endometrial thickness for pregnant mice# administered two doses of L-carnitine throughout pregnancy (No.=10 pregnant mice/group; Data are Mean± S.E.)

Table 2: Levels of serum FSH, LH and E_2 for pregnant mice# administered two doses of L-carnitine throughout pregnancy (No.=10 pregnant mice/group; Data are Mean± S.E.)

Groups	Reproductive hormones		
	FSH (mIU/mL)	LH (mIU/mL)	E_2 (Pg/mL)
Control group	3.41 ± 0.020 *	1.30 ± 0.031 *	6.81 ± 0.012 *
Low dose group (T1)	5.10 ± 0.027	2.32 ± 0.022	8.42 ± 0.018
High dose group (T2)	5.22 ± 0.020	2.41 ± 0.026	8.34 ± 0.020

In the present study, a significant increment ($P<0.05$) in the weight of reproductive system and endometrial thickness was assessed for both L-carnitine treated groups as compared to the control group. The recently study, the intrauterine milieu is a complex mixture of substances originating from serum and endometrium that support blastocyst growth and development (19). Therefore, use of LC in patients with anorexia nervosa has been shown to accelerate body weight gain, normalize gastrointestinal function, and improve physical performance. Although LC biosynthesis increases during embryonic development, its levels are still much lower than those measured in adults (12). Thus, if carnitine food intake is reduced, the biosynthesis of carnitine can account for more than 90% of the body requirements (20).

Table 1 and Figure 1, changes in the weight of reproductive organs and endometrium thickness may be as a result of changes in the number of implantation sites and metabolism in several body organs and systems. There is experimental evidence that

LC stimulates the activity of the pyruvate dehydrogenase (PDH) complex by decreasing the intramitochondrial acetyl-CoA/CoA ratio through the trapping of acetyl groups (21). The simultaneous reduction of acetyl-CoA levels in the cytosol further contributes to activate the glycolytic pathway (22). In general, L-carnitine transports long-chain fatty acids into the mitochondria where they are oxidized (metabolized). Once oxidized enhance the mitochondrial production of adenosine triphosphate (ATP). Enhancing ATP production, improves the metabolic efficiency in the tissues involved (23). In hearts containing raised concentrations of carnitine, there was a significant increase in glucose oxidation (24), subsequently, leads to increase ATP production and tissue formation.

Results show significant differences ($P<0.05$) were reported in the litter size between the control group and both treated groups. Previous researches had shown the addition of LC to maternal gestation diets increased body weight gain (25), plasma insulin like growth factor-II (26) of gestation mothers and increased total number of new born and born alive (27). Although LC is supplied exogenously as a component of the diet and can also be synthesized endogenously, evidence suggests both primary and secondary deficiencies do occur. On the other hand, carnitine deficiency can be acquired or a result of inborn errors of metabolism (16). Carnitine degradation in mammals is restricted to the non-absorbed carnitine in the intestinal tract, whereas absorbed or intravenously administered carnitine and endogenous carnitine are mostly eliminated in urine (13), and also excreted in milk (14).

Although much is known concerning the utilization and/or metabolism of specific nutrients, such as glucose and amino acids, by embryos before hatching from the zona pellucida (28 and 29). More recently, the impact of select nutrients on development of hatched blastocysts is limited, and this is especially true for species in which hatched blastocysts must undergo extensive elongation before implantation (29). Furthermore, a decrease in the production of free radicals, less tissue damage and reduced muscle soreness after exercise and a better utilization of fat as energy source during recovery (30). Carnitine had also an antioxidant capacity and decreases oxidative stress (31).

In conclution that administration of low concentration of L-carnitine to pregnant mothers had beneficial effects on pregnancy and offspring outcomes.

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Neurotoxic effect in lactating mice pups received oseltamivir phosphate (tamiflu) through milk from dosed nursing mothers during lactation period

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Summary

The present study was aimed to evaluate neurotoxic effects of oseltamivir phosphate in lactating pups of orally dosed mice mothers during lactation. Twelve recently parturited female albino mice were divided equally into three groups, one control and two treated groups, each group consists of 4 dosed dams and 8 chosen pups .The nursing dams of T1 and T2 dosed daily orally with 1mg/kg and 5mg/kg oseltamivir phosphate respectively representing the therapeutic dose and 5 fold dose of drug while control group dosed with distilled water. Lactating mice pups of all groups examined for the following parameters: First parameter was body weight changes and gain: In which T1group showed significant increase in mice pups body weight gain after 14 day of treatment in comparison with control group and T2. Second parameter was clinical symptoms observation /daily, all treatment groups that showed neurotoxic symptoms appeared from 1st dose and extended along the next few days of treatment to be gradually disappeared and completely lost within the last days of treatment in dose dependent manner.These neurotoxic symptoms were weakness, convulsions ,lay on back or side, extended body, incoordination ,extended limbs and limbs stiffness. Third parameter was gross and histopathological studies which demonstrate that the brain was the most affected organ beside extensive lesions in liver, kidney, stomach and small intestine of treated groups in dose dependent manner.

In conclusion of this study revealed that Oseltamivir phosphate produce neurotoxic effect in mice pups through indirect administration by nursing mothers dosing during lactation period and the level of toxicity was in dose dependent manner.

Key words: - oseltamivir phosphate, neurotoxic, lactation period, mice.

التأثيرات العصبية السمية للاوزيلتامفير فوسفيت (تاميفلو) في جراء الفئران المهقاءالراضعة لحليب امهات معالجه خلال فترة الرضاعة

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الخلاصة

اجريت الدراسه لتقييم التأثير العصبي السمي لعقار الأوزيلتامفير فوسفيت على جراء الفئران خلال فترة الرضاعه عن طريق المعامله الفمويه للأمهات المرضعه . تم تقسيم إثنا عشر من أمهات الفئران الوالده حديثآ إلى 3 مجاميع ,كل مجموعه مكونه من 8 جراء راضعه و4 أمهات معالجه مرضعه ,إشتملت مجاميع الدراسه على مجموعة سيطره واحده ومجموعتي علاج (T2,T1) جُرعت فموياً ويومياً 1ملغم/كلغم و5ملغم/كلغم من عقار الأوزيلتا مفيرفوسفيت لتمثل مجموعتي الجرعه العلاجيه و مجموعة خمسة أضعاف الجرعه العلاجيه على التوالي.

بينما جرعت مجموعة السيطره بالماء المقطر.خضعت جراء الفئران المقهاء لجميع المجاميع لفحوصات تسجيل المعاير التاليه :- ألمعيار الأول: وزن الجسم المتغير والمكتسب لجراء الفئران المهقاء:- في هذه الدراسه أظهرت مجموعة (T1) زياده في وزن الجسم المتغير والمكتسب بعد 14 يوم معالجه عند المقارنه مع مجموعة السيطره ومجموعة T2. ألمعيار الثاني :المراقبه اليوميه للعلامات السريريه لجراء الفئران المهقاء :- أظهرت جميع المجاميع العلاجيه بعض العلامات العصبيه منذ الجرعه الأولى تناسبت طرديا مع الجرع المعطاة وقد أمتدت العلامات العصبيه لتشمل الأيام اللاحقه من فترة التجريع حتىبدأت بالأختفاء و بشكل تدريجي حتى إختفت بشكل كامل خلال الأيام الأخيرة من فترة المعالجه.وقد أشتملت العلامات العصبيه السميه مايلي(وهن,تشنجات,,الإستلقاء الظهر او الجانب, إستطالة الجسم ,عدم التوازن,أطراف متصلبه وأطراف ممتده). المعيار الثالث: دراسة التغيرات العيانيه والمجهريه لمجاميع حيوانات التجربه :أظهرت الدراسه وجود آفات عيانيه ومجهريه كانت على أشدها في الدماغ حيث كان أشد الأعضاء تأثراً مع وجود آفات عيانيه ومجهريه في الكبد,الكلى,المعده والأمعاء الدقيقه.هذه الآفات كانت تتناسيب طرديآمع مقدار الجرعه المعطاة.

نستنتج من هذه الدراسة إن عقار الأوزيلتا مفيرفوسفيت يسبب تأثيراً سمياً عصبياً في جراء الفئران المهقاء المجرعه بشكل غير المباشر (عن طريق الأمهات المرضعة) بعقار الأوزيلتا مفيرفوسفيت خلال فترة الرضاعة وكانت هذه تأثيرات السميه العصبيه تتناسب طردياً مع مقدار الجرع المعطاة.

مفاتيح الكلمات:- للاوزيلتامفير فوسفيت , العصبية , الفئران.

Introduction

Oseltamivir phosphate (OP) is an orally administered anti-influenza agent of the neuraminidase inhibitor class. The ethyl ester prodrug oseltamivir is administrated orally as a phosphate salt and converted by hepatic esterases to the active metabolite oseltamivir carboxylate (OC) (1). OC specifically binds and inhibits the influenza virus neuraminidase enzyme that is essential for viral replication (2). In this way, oseltamivir limits the spread of influenza virus subtypes A and B within the infected host. When used as treatment, oseltamivir reduces the severity and duration of symptoms (3), while prophylactic administration prevents their onset (4). In recent years, abnormal or delirious behaviors have been reported with a low incidence in young individuals with influenza who were also receiving oseltamivir (5). Cases arose most commonly in Japan but were also observed in Taiwan, Hong Kong, North America, Europe, and Australia. No causative association could be demonstrated, and similar events were also reported in the absence of oseltamivir (6 and 7).

Nevertheless, health and regulatory authorities in Japan and elsewhere have amended the product label to include precautions on the use of oseltamivir in young persons. These actions, and the associated media coverage, have fostered renewed interest in the central nervous system (CNS) tolerability of oseltamivir(5). The aim of this study to evaluate neurotoxic effects of oseltamivir phosphate in lactating pups.

Materials and methods

Chemicals : Oseltamivir phosphate (flufly®)75 mg tablets provided by Julphar Ras Alkhima-UAE. The stock solution (1mg/ml) of oseltamivir phosphate were prepared by dissolving 1capsule (75)mg oseltamivir phosphate in 75 ml distilled water .(1)ml of stock was dilute with (9)ml of distilled water to prepare the concentration of 0.1mg/ml with dose volume of 0.1ml/10gm BW that used for dosing in therapeutic dose of 1mg/kg BW administrated toT1 .While 1ml of stock was diluted with 1ml of distilled water to prepare the concentration of 0.5mg/ml administrated with dose volume of 0.1ml/10 gm BW for 5 fold of therapeutic dose(5)mg/kg.BW administrated to T2. While control group administrated distilled water for the period of 14 day lactation. Total number of (12)pregnant female mice that kept after parturition with their of chosen (24) nursing pups ,weighed (3-4)gm that were divided equally into three groups, Two treatment group T1,T2 and one control group each consist of (4) nursing mother and(8) of their chosen lactating pups. The animals were raised and bred in the animal house of college of veterinary medicine/Baghdad University where the research was done. The animals were kept in cages of (20x15x15) cm^3 dimensions in average of one nursing mother with their

nursing pups in each cage, in optimum conditions of breeding at (22±3) ºC With (14/10) Hours (Light/Dark) cycle Standard pilliets and water Provided ad li-itum (8). When pups of treatment groups and control group reach 7 day of age, the mothers of control group was dosed orally, daily with distilled water for period of 14 day .While nursing mothers of treatment groups T1,T2 were dosed daily with therapeutic dose(1mg/kg).BW and fivefold the dose (5fd)5mg/kg.BW respectively for the rest period of lactation (14 day) to detect the neurotoxic effect of oseltamivir phosphate on mice pup during the lactation period and 7day after the termination of the nursing mothers treatment (at the end of lactation period). Pups of experimental groups (T1, T2and C) were tested for following parameters: Body weight gain/weekly1st and 14 day of treatment (at end of lactation). Clinical symptoms observation/daily. Gross and histopathological examination of organs and tissues /7 day after treatment termination. These organs involved brain, liver, kidney stomach and small intestine done according to (9).

Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group differences were determined using least significant differences (LSD) as described by (10).

Results and Discussion

The result of body weight gain showed that at 14 day of treatment T1 recorded significant increase in body weight gain ($P\leq0.01$) which was 7.4 gm in comparison with control group and T2 in which body weight gain 5.6gm and 4.4 gm respectively. Table (1)

Table :(1) Body weight changes/grams of nursing pups received OP indirectly from daily dosed nursing mothers with different doses during lactation period.

Period / Group n=8 pups	1 day of treatment M±SE	14 day of treatment M±SE	Body weight gain M±SE
C D.W	3.2±0.2 A b	8.8±0.37 AB a	5.6±0.24 B
T1(1mg/kg)	2.8±0.2 A b	10.4±0.4 A a	7.4±0.4 A
T2(5mg/kg)	3.2±0.2 A b	7.6±0.4 B a	4.4±0.24 B

T1= Pups of 4 nursing mothers, in which nursing mothers dosed with therapeutic dose (T.D) 1mg/kg.BW.
T2= Pups of 4 nursing mothers , in which nursing mothers dosed with 5 fold (5FD) therapeutic dose 5mg/kg.BW.
C=Pups in which nursing mothers dosed distilled water (D.W)
M±SE represent mean ± standard error
-Different small letters represent significant differences within groups ($P\leq0.01$)
-Different capital letters represent significant differences between groups ($P\leq0.01$).

This result may be due to effect of OP and/or OC on dopamine and/or other catecholamine. Its reported that dopamine-deficient mice grew normally up to around 10 day after birth, but at postnatal day (P10–14) they were distinguishable during subsequent development, they lost more body weight and gradually weakened, the phenotypic abnormalities of these mutant mice revealed that dopamine is essential for animal development and survival during the juvenile stage(11).Also this result may be

occur through effecting the appetite by alteration in dopamine levels .Its estimated that dopamine also play a major role in the regulation of appetite (12),also may be as a result of interference with growth hormone release .Other suggestion is due to the reported gross and histopathological effect of drug in our study on stomach and intestine of treated groups in dose dependent manner that may affect absorption of food or effect the appetite positively in low doses and negatively in high doses. Clinical observation of lactation study showed that T1 and T2 showed neurotoxic symptoms appeared 1.5-2 hours after lactation .These symptoms appeared from the first dose and extended along the next days of treatment in dose –depended manner .The intensity and number of affected animals reach to peck for T1 in the 3rd and 4th day of treatment and for T2 in the 3rd 4th ,5th and 6th day of treatment then gradually reduced to be completely disappeared in the last 4 days of treatment in T1 and in last 2 days of treatment in T2 .These clinical symptoms include weakness ,convulsions, Lay on back or side, irritability and itching ears by hind limbs ,extended body, incoordination, extended limbs and limbs stiffness. Table (2).

Table (2): clinical symptoms of mice pups dosed with two different doses by nursing mother of oseltamivir phosphate during lactation period.

GROUP n=8 pups	Day of treatment / Clinical symptoms	1st	3rd	5th	7th	9th	11th	14th
D.W Control	_	_	_	_	_	_	_	_
T1 Pups of 4 nursing mothers dosed with therapeutic dose (1mg/kg .BW) of OP	weakness	++3 +5	++5 +3	++6 +2	+8	+8	_8	_8
	Convulsion	_2 +6	+8	-6 +2	+8	_8	_8	_8
	Lay on back or side	4	4	-	-	-	-	-
	Itching ears by hind limbs	3	1	_	_	_	_	_
	Extended body	-8	3	-5	-5	-5	-5	-5
	Incoordination	+2 _6	_8	_8	_8	_8	_8	_8
	Extended limbs	+8	+3 _5	_8	_8	_8	_8	_8
	Limbs stiffness	+8	+3 _5	_8	_8	_8	_8	_8
T2 Pups of 4nursing mothers dosed with (5 fold) of therapeutic dose (5mg/kg.BW) of OP	Weakness	++3 +5	++6 +2	++6 +2	++3 +5	+3 _5	+2 _6	_8
	Convulsions	+8	+8	+2 _6	+2 _6	+8	_8	_8
	Lay on back or side	4	4	-	-	-	-	-
	Itching ears by hind limbs	_	4	3	1	_	_	_
	Extended body	2	5	_8	_8	_8	_8	_8
	Incoordination	+ 3	+2_6	+2 _6	+8	+8	8-	_8
	Extended limbs	+3 _5	+3 _5	+2 _6	_8	_8	_8	_8
	Limbs stiffnes	+3 _5	+2 _6	+2 _6	_8	-8	_8	-8

1-8=number of pups showing toxic symptoms.

- (NON), + (SLIGHT), ++ (OBVIOUS), +++ (SEVER)

This result may be due to effect of OP and/or its metabolite OC on dopamine and/ or another catecholamine or may be other neurotransmitters of central nervous system like serotonin, GABA, glutamine and Ach. Primarily we think that OP and/or OC has ability to penetrate blood brain barrier(BBB).This penetration occur in time when BBB is still immature during lactation period and may be interfere with dopamine and /or another catacholamins and even other CNS neurotransmitters. It's reported that in developing mice BBB maturation is complete around the third postnatal week (13). Developing of elimination system of OP and/or OC beside gradual maturation of BBB may be were the main causes that reduced the observed clinical symptoms within last days of treatment. Its estimated that brain level of OP in pups were 1500 times that of adult animals exposed to the same dose(14).It's important to know that up to one fourth of OP is distributed via circulation and enters tissue though the BBB(15).We agree with recent study done by Yoshino(16) who used wibester rats in his study in which these rats received OP at dose 25 and 100 mg/kg.BW I/P the result indicate that increase extracellular dopamine with 156% and 223% of pre-administration level in prefrontal cortex (PFC) also was significantly greater than vehicle administrated rats and ataxia was observed. Also the neurotoxic symptoms observed in our study may be due to the extensive damage in brain of treated groups that involved cerebellum and also cerebrum. Weakness may be due to cerebellum damage .Studies shows that cerebellum dysfunction leading to delay initiation of movement and do not prevent it (17).This delay in movement may be appeared as weakness. Incoordination may be due to cerebellum and/or vestibular dysfunction caused by OP and/or OC effect .The cerebellum modulation and coordination of muscular activity are important in skilled voluntary movement as well as in the movement and posture equilibrium (18)while its damage may lead to incoordination and ataxia. Extended limbs may be due to effect of OP and/or OC on CNS neurotransmission, this cause that muscles receives impaired signals that lead to excessive muscle relaxation. Limbs stiffness may be attributed to imbalance between the two opposing muscles of limbs may be one of them more active than another. Extended body and back head noticed in T1 and T2.This result may be due to toxic effect of OP and/or OC on cerebellum causing more cerebellum dysfunction and more increase in stretch reflex that control muscle tone(19) . In recent study(2010) reported that When oseltamivir is administered in extremely high doses (500–1000 mg/kg) to young juvenile rats, central nervous system toxicity and death occurred in some animals. Mortality was not observed in older juvenile rats, suggesting a possible relationship between neurotoxicity and an immature blood-brain barrier. To assess potential neurologic adverse effects of oseltamivir use in infants, a retrospective chart review was performed in infants less than 12 months of age who received oseltamivir, amantadine, or rimantadine. The result revealed the occurrence of adverse neurologic events during therapy among subjects treated with oseltamivir versus those treated with the adamantanes but without significant difference .This is the largest report to date of oseltamivir use in children less than 12 months of age. Neurologic events were not more common with use of oseltamivir compared with that of the adamantanes (20).

The pathological changes study showed the presence of gross and histopathological lesions in dose dependent manner in all the examined organs, mainly were in the brain and to less extent in liver, kidney, stomach and small intestine. Brain of T1 macroscopically showed edema and slight congestion Fig (1) .while T2 showed severe congestion of meninges and hemorrhage. Microscopically brain of T1 showed Shrinkage of neurons with focal areas of hemorrhage Fig (2) in addition to congestion of blood vessels of cerebrum and meninges with focal gliosis. The cerebellum showed edema with degenerative changes and complete dissolution of purkinji cells. While T2 showed sever congestion of blood vessels of cerebral and cerebral meninges. Liver of T1 macroscopically showed enlargement and friability. Fig (3), while liver of T2 showed Hepatomegaly with rounded

edges and pale color with multiple areas of necrosis (gray-white) in color. Microscopically liver of T1 showed dilatation and congestion of blood vessels .Massive necrosis of hepatic parenchyma and increase in apoptosis, severe hemorrhage with infiltration of large numbers of neutrophils, Focal aggregation of neutrophils and lymphocytes in hepatic parenchyma with infiltration of neutrophils in sinusoids were also seen .The portal blood vessels and sinusoids contain serum protein, while T2 showed extensive areas of necrosis and heamorrhage. Neutrophils aggregation in parenchyma ,dilatation and congestion of blood vessels and sinusoid containing neutrophils in their Lumina with infiltration of mononuclear cells in portal areas and formation of granulomas beside blood vessels Fig (4). Kidney of T1 macroscopically showed slight congestion and atrophy, while T2 showed Atrophied kidney. Fig(5).Microscopically T1 showed Perivascular cuffing, congestion of blood vessels ,shrinkage of glomerular tuft .multiple areas of severe cortical hemorrhages with mononuclear cells infiltration in the interstitial tissue and around glomeruli with slight periglomerular fibrosis and proliferation of parietal layer of capsule with vacuolation of glomerular tuft ,also there was necrosis and apoptosis Fig(6 and7),while T2 showed Degeneration and necrosis of epithelial cells forming epithelial casts, congestion of blood vessels .severe destruction of renal tissue with hemorrhage. Stomach of T1 and T2, macroscopically, .showed thickened, corrugated mucosa Fig (8). While Microscopically stomach of T1 and T2 showed that in the non glandular regions: There was papillary proliferation of epithelial lining with marked hyperkeratosis Fig (9).The glandular region showed severe congestion of blood vessels of mucosa. Macroscopically small intestine of T1 showed edema Fig(10),while small intestine of T2 showed congestion of serosa surface ,with areas of hemorrhage, microscopically small intestine of T1 showed marked hyperplasia of lymphoid tissue with increase in cellularity of mucosa while T2 showed increase in numbers of goblet cells with increase in mucin secretion ,also infiltration of inflammatory cells in the lamina propria of mucosa Fig(11). All pathological changes were in dose dependent manner.

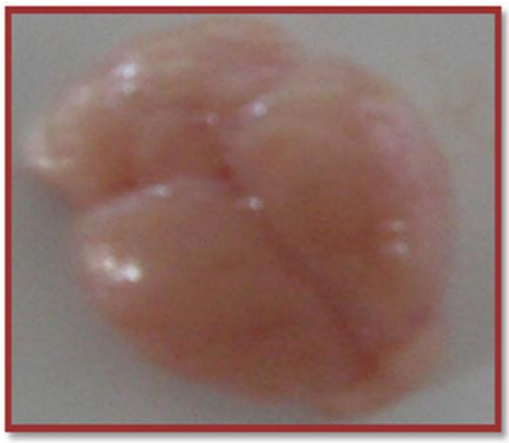
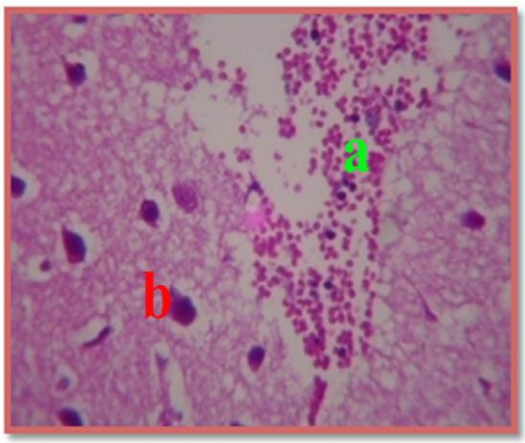
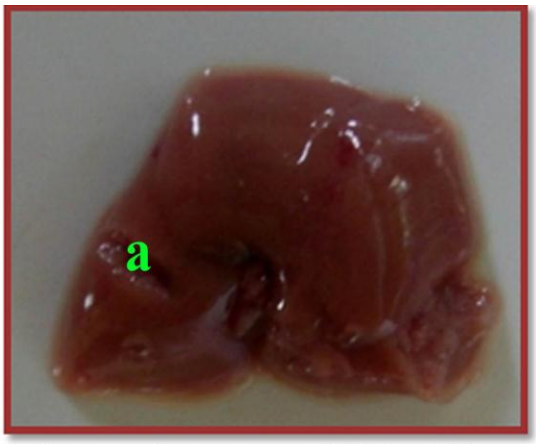


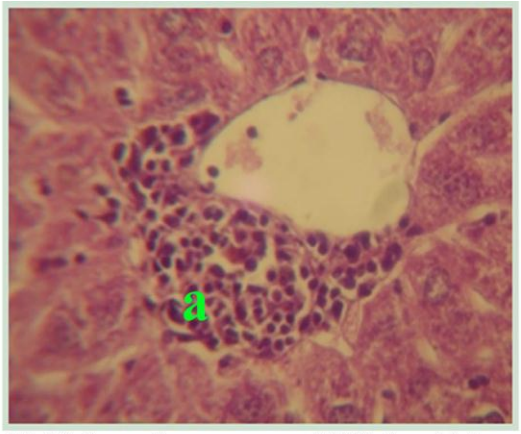
Fig (1) Macroscopical section of brain of mouse pup of T1 in which nursing mother treated with 1mg/kg.BW/dayof oseltamivir phosphate for 14 day of lactation period. Shows edema and slight congestion.



Fig(2) Histopathological section of brain cerebrum of mouse pup of (T1) in which nursing mother treated with 1mg/kg BW/day of oseltamivir phosphate for 14 day during lactation period shows shrinkage of neurons (a) with focal areas of severe hemorrhages (b) (H&Ex400).

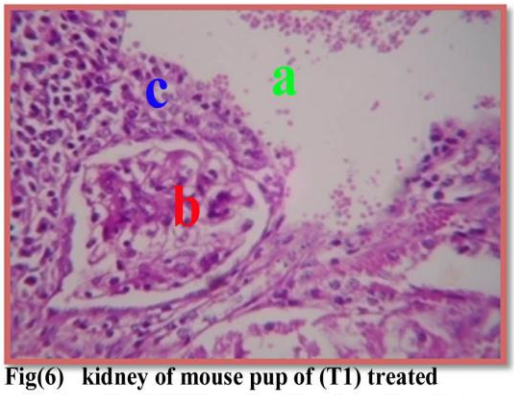


Fig(3) liver of mouse pup of T1 in which nursing mother treated with 1mg/kg.BW /day of oseltamivir phosphate for 14 day of lactation period shows enlargement and friablity.

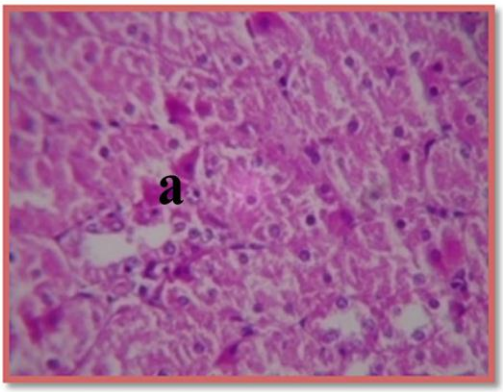


Fig(4) liver of mouse pup of (T2) in which nursing mother treated with 5mg/kg .BW/day of oseltamivirphosphate during lactation period for 14 day shows formation of granuloma beside the congested blood vessels (H&Ex400).

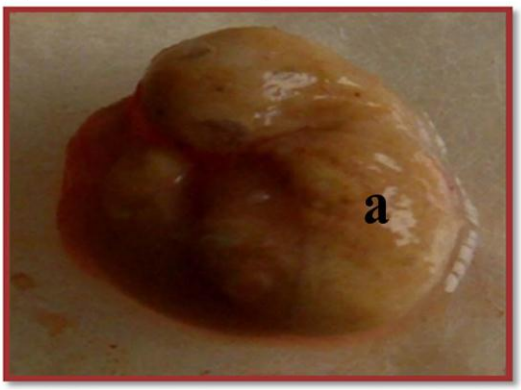
Fig (5) kidneys of mouse pup of T1 in which nursing mother treated with 1 mg/kg .BW /day of oseltamivir phosphate for 14 day Shows atrophy , with focal areas of necrosis.



Fig(6) kidney of mouse pup of (T1) treated with 1mg/kg .BW/day of oseltamivir phosphate for 14 day during lactation period shows severe cortical hemorrhage (a) with mononuclear cells infiltration in interstitial tissue, around glomeruli (b)and slight periglomerular fibrosis and poliferation of parietal layer of capsule (c)with vacuolation of glomerular tuft(H&E40X).



Fig(7) kidney of mouse pup of (T1) in which nursing mother treated with 1mg/kg.BW/day of oseltamivir phosphate for 14 day during lactation period shows severe necrosis and apoptosis(a) (H&Ex400).



Fig(8) Stomach of mouse pup of T1 in which nursing mothertreated with 1 mg/kg .BW /day of oseltamivir phosphate for 14 day shows thic kened ,corugated mucosa(a) .

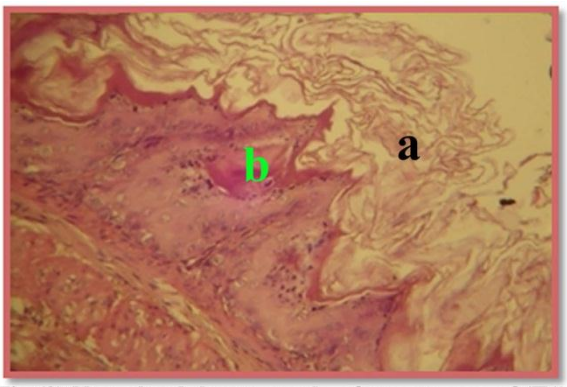
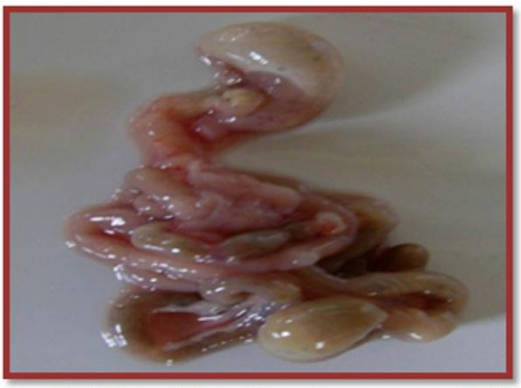


Fig (9) Non-glandular stomach of mouse pup of (T1) in which nursing mother treated with 1mg/kg .BW/day of oseltamivir phosphate for 14 day during lactation period shows papillary proliferation of epithelial lining (a) with marked hyperkeratosis(b) (H&E40X) .



Fig(10)Small Intestine of mouse pup of T1in which nursing mother treated with 1mg/kg. BW/day of oseltamivir phosphate for 14 day of lactation period shows edema .

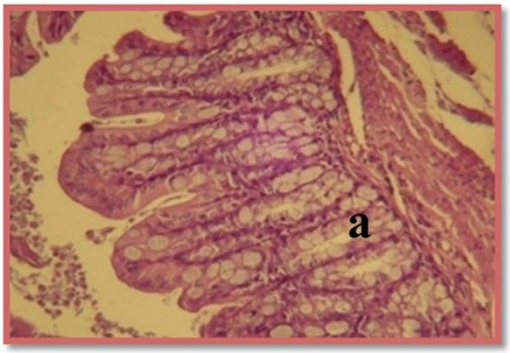


Fig (11) Intestine of mouse pup of (T2) in which nursing mother treated with 5mg/kg .BW/day of oseltamivir phosphate for 14 day during lactation period shows increase in numbers of goblet cells (a) with increase mucin secretion (H&E40X) .

In our study the gross and histopathological lesions of examined organs may be due to excessive exposure to OP and/or its metabolite OC. Gross and histopathological lesions of brain may due to penetration of OP and/or OC cross BBB and cause damage to multiple areas of brain ,we thought that OP may be more implicated in damage of brain structures and may be effected other organs. Its estimated that brain level of OP in pups were 1500 times that of adult animals exposed to the same dose (14). Because OP contain phosphate salt that may cause extensive damage and toxicity in the brain, when the BBB is still immature during the first days of study, as reported by James Farrelly from center of drug evaluation and research in (2000) who performed studies using rats and marmosets dosed orally for one month and he found tha t lesions were chronic progressive nephropathy,corticomedullary mineralization,tubular minerli-zation,tubular vacuolation, basophilic tubules and focal nephropathy,he suggest that the lesion of kidney were due to excessive exposure to more toxic prodrug OP where rats could not hydrolyze the OP sufficiently to its metabolite that will cause accumulate excessive amount of phosphate this would negatively affect the dietary calcium/phosphate ratio in species known to be sensitive to this type of change ,this consequencely would lead to mineralization of kidney, he showed no histopathological changes in liver of treated animals with OP .When he used marmosets as a labratory animals in his studies he showed that no histopathological changes seen in liver,kidney and bones except in GI in which OP was extremely irritant in primate more than of rodents ,in marmosets the lesions exist only in GI represented by severe gastric mucosal

inflammation ,atrophy ,hemorrhage ,erosion and ulceration are associated with 1000mg/kg. the ratio of OP:OC was 1:3 while in marmosets was 1:15 in urine analysis showed may be rodent could not hydrolyze OP efficiently like primates, OP is pro-ester drug we expect that its easily penetrate BBB and cause damage in brain in dose dependent manner. This not exclude the effect of OC .We thought that nursing mothers may be play an important role in transporting excessive amounts of OC to lactating pups within lactation period ,when metabolism system of adult mice more developed than of neonates.

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Evaluate the effect of different doses for grape seed extract in mice

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Summary

The aim of this study was to evaluate the effect of different doses for grape seeds extract in internal organs of mice. Histopathological, cytogentical and haematological studies were done to evaluate this effect. The study was done on (24) mice divided into four groups (6/group) for one month. Group one treated with PBS considered as control Negative, Group two treated with (100mg/kg B.W.) of grape seed extract, Group three treated with (200mg/kg B.W.) of grape seed extract, Group four treated with (300mg/kg B.W.) of grape seed extract. The results showed that (200, 300mg/kg B.W.) cause significant decrease in hematological, cytogentical parameters and severe histopathological changes while at dose (100mg/kg B.W.) induce immune pathological response organ with amelioration of haematological and cytogentical parameters. In conclusion the effect of grape seed extract was dose dependent that more severe toxicopathological changes appeared in haemtological, cytogentical and histopathological results at doses (200 and 300mg/kg B.W.) while at dose (100mg/kg B.W.) induce immunopathological response internal organs with amelioration of haematological and cytogentical parameters.

Key words: Grape, toxicity, grape seed, Mice.

تقييم التاثير العلاجي بجرعات مختلفة لخلاصة بذور العنب في الفئران

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الخلاصة

هدفت الدراسة الى تقييم التاثير العلاجي بجرعات مختلفة لخلاصة بذور العنب على التغيرات المرضية النسجية والوراثة الخلوية والدموية . استخدمت في الدراسة (24) فأرقسمت الى(4) مجاميع احتوت على اعداد متساوية من الحيوانات وعولجت لمدة شهر وكما يلي: مجموعة (1) اعطيت PBS واعتبرت مجموعة سيطرة, مجموعة (2) اعطيت (100mg/kg B.W.) من خلاصة بذورالعنب, مجموعة (3) اعطيت (200mg/kg B.W.) من خلاصة بذورالعنب , مجموعة (4) اعطيت (300mg/kg B.W.) من خلاصة بذورالعنب. اظهرت النتائج ان الجرع (200,300) قد سببت نقصان معنوي في المقاييس الدموية والوراثة الخلوية مع حدوث تغيرات مرضية نسجية شديدة في الانسجة اما عند اعطاء جرعة .(100) فقد اظهرت الحيوانات تحسنا في نفس المعايير المذكورة اعلاه واستجابة مناعية لانسجة الاعضاء الداخلية . ننستنتج مما سبق ان تأثير مستخلص بذور العنب يعتمد على الجرعة المعطاة من التغيرات الدموية والوراثة الخلوية والمرضية النسجية.

الكلمات المفتاحية : سمية بذور العنب ,المركبات السامة لبذورالعنب , تأثير بذورالعنب.

Introduction

Grape is one of the most commonly consumed fruits in the world. It has various biological functions due to it rich polyphenol ingredients, (1). Grape seed extract is the primary commercial source of group of powerful antioxidants known as oligomeric

proanthocyanidins (OPCs). Also generically called pycnogenol, a class of flavonoids (2). Proanthocyanidin, a type of polyphenol is found a bundantly in grape seed extract and has anti-inflammatory, anti-arthritic and anti-allergic properties. Grape seed extract had antioxidant and free radical scavenging activity. Flavonoids found in red wine have been reported to protect the heart by inhibiting the oxidation of LDL "bad"cholesterol (LDL oxidation which can lead to hardening of the arteries or atherosclerosis) (3). According to our knowledge there is few researches about the effect of different doses for grape seed on internal organs (lung, liver, kidney, spleen, heart and brain) so the present work accomplished this task.

Materials and Methods

Local grapes cultivated in Iraq were collected from the local market and classified as *Vitis vinifera* by the herbarium of the Biology Department, College of Science, Baghdad University. Shed and dried at room temperature. 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 number (199) in (19/01/2011). plant was (*Vitis vinifera*), a member of the family (Vitaceae *)* genus (Vitis) species (Vinifera), according to certificate. According to (4) the ethanolic extract of plant was prepared as follows:-1-aliquots of 50 gm of the powdered seed of plant were suspended in 250 ml of 70% ethanol alcohol in Erlyn Myer flask and stirred on cold magnetic stirrer overnight.2-After72hr., the sediments were filtered by gauze and then by filter paper.3-Steps (1) and (2) were repeated 4-5 times.4-The pooled extract was dryness y oven 40 °C.5-The weight of yield resulted from that amount of powdered plant was measured.6-The yield was kept at -20°C until the time of use. For following experiments, 3.5 gm of powdered plant extract was dissolved into 100 ml PBS (as solvent),the suspension then filtered and sterilized by using 0.4*m*m sterile Millipore filter and kept in deep freeze (-20 °C) until use.

Heamatology was determined as following according to the (5). Test was performed by using of hemoglobin kit (Drapkin´s reagent) then uses 5 ml of Drapkin´s with 20 µl of blood, left for 5 min then read on 540 nm. Packed cell volume was measured by using micro-hemocrit capillary tubes. Haymes solution was used to dilute the blood for counting RBCs by hemocytometer method. Thoma's solution was used to dilute blood to count WBCs by hemocytometer method as mentioned before in RBCs counting, but here the only difference was the type of pipette.

One drop of blood was dripped on a slide to differential Counting of WBCs (%) then this drop was spread with one stroke and after getting dried, the slide was dyed with Wright giemsa stain and left for10 minutes then washed with tap water. The slide was examined under a microscope.

Ammonium oxalate solution was used to dilute blood to count platelet (x 10^6 cell/ L) by hemocytometer method. Cytogenetical was determined as following according to the (6). After the bone was washed, both ends of bones were cutting and the bone holding vertically above test tube, 5 ml at 37C˚ sterile PBS injected in bone and collected in test tube. Cell suspension mixed with 1ml of colchicin for about 10 minutes and then the tube was put in incubator at 37°C. The tubes centrifuged which contain bone marrow at 2000 rpm for 10 minute. The suspension was with draws and the sediments mixed very well and then 10 ml of 37°C KCl was added gently at the beginning and incubated for about 40 minutes. After that tubes centrifuge at 2000 rpm for 10 minute and withdraw the suspension, the sediments mixed well and fixed by fresh glacial acetic acid: methanol (1:3 v/v) (5ml). Then centrifuge at 2000 rpm for 10 minutes, the washing process return for 6 hours and then the

sediment suspend in 1-2 ml. The suspension mixed by pipette and then dropped the suspension on oil free slide at distance (30-50cm) and the slides were left to dry. The slide stained by giemsa stain for about (15) minute and then after dry, the slides were examined under light microscope. LD50 of grape seed extract was determined as following according to the Dixon (7).

The study involves 24 mice which were divided in to 4 groups each group contains 6 animals treated as following: 1- Group one: gavage orally by stomach tube with PBS 4 time/ week this group considered as control negative. 2- Group two: gavage orally by stomach tube 4 time/week 100 mg /kg .B.W. grape seed extract for 4 weeks .3- Group three: gavage orally by stomach tube 4 time/week 200 mg /kg .B.W. grape seed extract for 4 weeks. 4- Group four: gavage orally by stomach tube 4 time/week 300 mg /kg .B.W. grape seed extract for 4 weeks.

Histopathology According to the (8), the animals were sacrificed and postmortem examination done. Specimens were taken from all internal organs; the tissues have been kept in 10% formalin immediately after removal. After 48 hours then tissue sections were embedded in paraffin blocks, and sectioned by microtome at 5µm. All tissues were stained with hematoxylin and eosin stain and the histopathological changes were examined under light microscope.

Statistical analysis of data was performed by using Statistical Package for Social Science, (9) Version 16, and for determination of significant differences using ANOVA one way and two way. Group differences were determined using least significant difference (LSD) test at $P<0.05$ (9).

Results and Discussion

The calculated LD50 for grape seed extract was 2000mg /kg. B.W.

Haematological effect of grape seed extracts: The result of Hb (mg/dl) is shown in (Table 1). The result of group treated with grape seed at dose (100mg/kg B.W. orally) Hb was (14.10±0.27) showed increase in Hb values when compared with control group which Hb was (13.50±0.10). In addition the result showed significant decrease ($p<0.05$) in Hb values in groups treated with ethanolic extract at dose (200, 300mg/kg B.W. orally) Hb was (11.30±0.19, 11.10±0.15) when compared with control group which Hb was (13.50±0.10).

The result of P.C.V (%) are listed in (Table 1).There were increase of P.C.V (%) in group treated with ethanolic extract of grape seed at dose (100mg/kg B.W. orally) P.C.V was (45.00±0.51) when compared with group control which P.C.V was (43.67±0.33). In addition the result in groups treated with grape seed at dose (200 and 300mg/kg B.W. orally) P.C.V was (36.67±0.88, 36.00±0.32) showed significant decrease ($p<0.05$) in P.C.V (%) when compared with control group which P.C.V was (43.67±0.33).

The data of RBCs count are shown in (Table 1). The group treated with grape seed at dose (100mg/kg B.W. orally) RBCs was ($6.3.x10^6$±0.08165) showed increase in RBCs count but no significant difference when compared with control group which RBCs was ($5.6\ x10^6$±0.04082). In another way the result of this study showed significant decrease ($p<0.05$) of RBCs count in group treated with grape seed at dose (200 and 300mg/kg B.W. orally) RBCs was ($4.4\ x10^6$±0.09082, $3.6\ x10^6$±0.0505) when compared with control group which RBCs was ($5.6\ x10^6$±0.04082)

The data of WBCs count are shown in (Table1). The results showed increase in WBCs count but no significant difference of group treated with grape seed at dose (100mg/kg B.W. orally) was ($7.1x10^3$±0.07743) when compared with control group which

WBCs was (6.6±0.1 $x10^3$1547). In another way the result of group treated with grape seed at dose (200 and 300mg/kg B.W. orally reached (5.3 $x10^3$±0.05132, 5.00$x10^3$±0.3333) showed decrease in WBCs count but no significant difference when compared with control group which WBCs was (6.6 $x10^3$±0.11547).

The data of platelet count are explained in table (1). Group treated with grape seed at dose (100mg⁄kg B.W. orally) was (9.8 $x10^4$±0.77451) showed increase in Platelet count but no significant difference when compared with control group which platelet was (9.5 $x10^4$±0.11536). In another way platelet count showed significant decrease ($p<0.05$) in groups treated with grape seed at dose (200 and 300mg⁄kg B.W. orally) was (5.4 $x10^4$±0.05513 and 5.1 $x10^4$±0.11000) when compared with control group which platelet was (9.5 $x10^4$±0.11536).

The result of differential count are listed in (Table 1). The study showed increase in numbers of lymphocyte (%) in group treated with ethanolic extract of grape seed at dose (100mg⁄kg B.W. orally) was (62.00±1.50) when compared with control group which lymphocytes was (61.67±1.40). In addition the result of groups treated with grape seed at dose (200,300mg/kg B.W. orally) was (38.00±0.58, 30.45±0.29) showed significant decrease ($p<0.05$) when compared with control group which lymphocytes was (61.67±1.40).

The result of group treated with grape seed at dose (100mg⁄kg B.W. orally) was (33.00±1.53) showed increase in numbers of neutrophils (%) compared with control group which neutrophils was (32.00±0.36). In another way the result showed significant increase ($p‹0.05$) in numbers of neutrophils (%) in groups treated with grape seed at dose (200 and 300mg⁄kg B.W. orally) was (58.00±0.58 and 66.55±0.27) when compared with control group which neutrophils was (32.00±0.36).

The study showed no significant difference of group treated with grape seed at dose (100mg⁄kg B.W. orally) eosinophils count was (2.00±0.30) when compared with control group which was (2.33±0.33). In addition the result of groups treated with grape seed at dose (200 and 300mg/kg B.W. orally) showed increase was (1.67±0.19 and 1.55±0.27) when compared with control group which eosinophils was (2.33±0.33).

The result showed decrease in numbers of monocytes of group treated with grape seed at dose (100mg⁄kg B.W. orally) was (3.00±0.40) when compared with control group which monocytes was (4.00±0.47). In addition the result of group treated with grape seed at dose (200 and 300mg/kg B.W. orally) showed significant decrease ($p<0.05$) was (2.33±0.21 and 2.00±0.50) when compared with control group which monocytes was (4.00±0.47).

Table (1) The result (mean ± SE) of some Haematological parameter (HB (g/dl), pcv%, WBC (dl), RBC (dl), Platelet (dl) and differential count (%).

Groups / Parameter	Control (-)	Treated with plant at dose (100mg/kg B.W. orally)	Treated with plant at dose (200mg/kg B.W. orally)	Treated with plant at dose (300mg/kg B.W. orally)
HB	13.50±0.10 A	14.10±0.27 A	11.30±0.19 B	11.10±0.15 B
P.C.V%	43.67±0.33 A	45.00±0.51 A	36.67±0.88 C	36.00±0.32 C
RBCs	5.6±0.04082 A	6.3±0.08165 A	4.4±0.09082 B	3.6±0.32 B
WBCs	6.6±0.11547 A	7.1±0.07743 A	5.3±0.05132AB	5.00±0.33333AB
Platelet	9.5±0.11536 A	9.8±0.77451 A	5.4±0.05513 B	5.1±0.11000 B
Differential count Lymphocyte	61.67± 1.40 A	62.00± 1.50 A	38.00± 0.58 B	30.45 ± 0.29 B
Neutrophil	32.00 ± 0.36 D	33.00 ± 1.53 D	58.00 ± 0.58 B	66.00 ± 0.27 B
Eosinophil	2.33 ±0.33 A	2.00 ±0.30 A	2.67± 0.19 A	2.55 ± 0.27 A
Basophile	-	-	-	-
Monocyte	4.00±0.47 A	3.00±0.40 A	1.33±0.21 B	1.00±0.50 B

Different letters means significant (p<0.05) between groups

The present study found that treatment of grape seed extract at dose (100mg/kg B.W. orally) significantly improved the hematological parameters compared with animals treated at dose (200,300mg/kg B,W orally) these beneficial effect of grape seed extract on these parameters probably add to the long list of known pharmacological actions of grape seed extract that have been recorded by author (10) due to chemical structure(s) are mainly responsible for the antioxidant activities of grape extracts.

In another way the results demonstrated grape seed extract at dose (200 and 300mg/kg B.W. orally) induced a significant decrease in erythrocyte and leukocytes count, hematocrit, platelet count and lymphocyte percentage may be due to inhibition or defective hematopoiesis, these findings are agreed with those obtained from other studies (11and12), a prolonged prothrombin time is indicative of abnormalities of factors V,VII or X, prothrombin, fibrinogen or due to the presence of an inhibitor (13).

Cytogenetics effect of grape seed extracts on bone marrow: Mitotic and Blast Index

The data of table (2) explain the effect of ethanolic extract of grape seed on MI in group treated with grape seed at dose (100mg/kg B.W. orally) was (16.1±0.4) showed significant increase ($p<0.05$) in MI when compared with control group which MI was (14.0±0.1). While groups treated at dose (200 and 300mg/kg B.W. orally) was (13.2±0.1and 12.1±0.4) showed significant decrease ($p<0.05$) in MI when compared with control group which MI was (14.0±0.1). The result showed significant increase ($p<0.05$) in BI value in all groups treated with ethanolic extract of grape seed at dose (100, 200 and 300mg/kg B.W.

orally) was (38.33±0.8, 32.67±0.3 and 29.33±0.2) when compared with control group which BI was (27.33±0.1).

Table (2) showing (mean ± Se) the effect of grape seed extract on mitotic and blast index.

groups Test	MI*	BI**
Control (-)	0. 14.0±0.1**C**	**27.33±0.1 D**
Treatmen with plant at dose (100mg/kg B.W. orally)	**16.1±0.4 A**	**38.33±0.8 A**
Treatment with plant at dose (200mg/kg B.W. orally)	**13.2±0.1 AB**	**32.67±0.3 B**
Treatment with plant at dose (300mg/kg B.W. orally)	**12.1±0.4 B**	**29 29.33±0.2 C**

***MI=Mitotic index ** BI =Blast index**

Different capital letters means significant (p<0.05) results between weeks, L.S.D=1.03

The results with treated with grape seed extract at dose (100mg/kg B.W. orally) showed significant increase in mitotic index and blastic index and this agreement with (14).The grape seed contain flavonoids, catechin was one of active constituents of grape seed, stimulated the proliferation of mouse bone marrow cells (15). While result treated with grape seed extract at dose (200 and 300mg/kg B.W. orally) showed decrease gradually in mitotic index and blastic index and this agreement with (16) who showed that the higher doses affected the mitotic process in the normal lymphocyte but the chromosomal changes might be intacted. When mice were given grape seed extract in their diet at different doses, they had better contact hypersensitivity response and less immunosuppression (16).

Pathological Study:

Control group: There are no clear macroscopic findings.

Pathology of organs in healthy female mice treated with ethanolic extract of grape seed at dose (100mg/kg B.W. orally).

Brain: Histopathological lesion of brain showed focal aggregation of microglial cells (gliosis) forming nodular like structure (fig1). Lung: Histopathological changes showing perivascular and peribronchiolar lymphocytic cuffing (fig2). Heart: Histopathological changes characterized by marked hyperplasia of lymphoid tissue of pericardial adipose tissue. Liver: Histopathological lesion showing proliferation of kupffer cells with infiltration of lymphocytes within the dilated sinusoids. Other sections showed formation of early granuloma within hepatic parenchyma consisting of mononuclear cells aggregation (3). Kidneys: Histopathological changes showing perivascular lymphocytic cuffing in the interstitial blood vessels (fig4). Spleen: Histopathological changes charactersized by marked hyperplasia of lymphoid tissue in white pulp (fig5). Pancreas: Histopathological lesion for pancreas was marked by hyperplasia of islets of langerhans (fig6). In addition to hyperplasia of lymphoid tissue of the organ. Stomach: Histopathological changes showing in non-glandular region showed no pathological changes. While the glandular region was undergoing focal infiltration of mononuclear cells in the mucosa (fig7). Intestine: Histopathological lesion showing there is extensive hyperplasia of lymphoid tissue (fig8). Bone marrow: Histopathological charactersize moderate hyperplasia of hemopoietic tissue with increase in numbers of megakaryocytes(fig9).

Pathology of organs in healthy female mice treated with ethanolic extract of grape seed at dose (200mg/kg B.W. orally).

Brain: Histopathological lesion for brain showing severe congestion of blood vessels of cerebral and cerebellar meninges (fig 10). In addition perineuronal and perivascular edema of cerebrum. Lung: Histopathological section showed hemorrhage, congestion of alveolar blood capillaries and pulmonary blood vessels with pneumonic area. Heart: Histopathological changes showing marked fibrosis of myocardium with infiltration of mononuclear cells (fig 11). Liver: Histopathological characterize there is extensive areas of necrosis and apoptosis (fig22). Infiltration of mononuclear cells in the portal areas. Kidney: Histopathological lesions showing severe necrosis of epithelial lining of proximal and distal convoluted tubules (fig 12). Spleen: Histopathological changes showing deposition of small amount of amyloid like substance with deposition of hemosiderin pigment. Stomach: Histopathological lesion showing non-glandular region showed marked hyperplasia and hyperkeratosis of mucosa (fig213). Intestine: Histopathological lesion showing infiltration of mononuclear cells in mucosa with slight fibrosis. Pancreas: Histopathological characterize tissue section showed extensive necrosis of pancreatic acini and islet of Langerhans (fig 14). Bone marrow: Histopathological changes showing depletion of hemopoietic tissue with increase in numbers of blood sinuses.

Pathology of organs in healthy female mice treated with ethanolic extract of grape seed at dose (300mg/k B.W. orally).

In addition to previous histopathological changes seen in treated group with grape seed extract. There are the following changes: Brain: Histopathological lesion showing shrinkage of neurons (dark blue), edema of the cerebrum. The cerebellum showed edema between the molecular and granular layer with degeneration of numerous purkinji cells and complete dissolution of the others (fig 16). Lung: Histopathological sections showed sever hemorrhage, congestion of alveolar blood capillaries and pulmonary blood vessels with large pneumonic area. Heart: Histopathological changes showing sever lesion that there is wide areas of fibrosis of myocardium with highly infiltration of mononuclear cells. Liver: Histopathological characterized hyperplasia intrahepatic bile ducts forming papillary projection (fig 17). Kidney: Histopathological lesion showing severe periglomeruler infiltration of inflammatory cells mainly mononuclear cells (18A) with cystic dilation of renal tubules containing hyaline cast fig (18B). Spleen: Histopathological changes showing increase in amount of amyloid like substance leading to pressure atrophy of lymphoid tissue with deposition of hemosiderin pigment (fig 19). Pancreas: Histopathological characterized the main microscopic changes were the infiltration of mononuclear cells around pancreatic duct with slight fibrosis (fig20). Stomach: Histopathological lesion for stomach showing extensive fibrosis of mucosa leaving only few mucous glands. Intestine: Histopathological changes showing similar pathological changes as seen in the previous period of treatment. Bone marrow: Histopathological characterized showed depletion of hemopoietic tissue with increase in numbers of blood sinuses.

The pathological changes due to (100 mg/kg B.W.) Showed brain gliosis and that due to aggregation of microglial cell (17) explained that the flavonoids has a direct effect on glial cells by inducing activation of astrocytes and microglia and release of tumor necrosis factor alpha (TNF-a). Flavonoids are generally thought to be having antioxidant and free radical scavenging effects .Flavonoids are also known to had neuroprotective actions (18).

Active compound like antioxidant, flavonoid, catechine and alkaloids which may be act as immune stimulant and increased splenocyte proliferation (19). The perivascular and peribronchiolar lymphoctic cuffing in the lung, kidney and hyperplasia of lymphoid tissue

of white pulp of spleen were due to the immune stimulant and increased splenocytes proliferation caused by active compound of the plant (19). Pancreas showed regeneration of islet of langerhans and this agreement with (20) Referred that patients with end-stage diabetic nephropathy showed corrected thiol deficiency, increased T cell activation, and reduced tumor necrosis factor–α, thus normalizing immunoregulatory defect (20). Also agreement with (21) in the pancreas grape seed acts mainly as chemoprotectant and can stimulate recovery after intoxication.

Bone marrow undergo proliferation of stem cell with presence of large numbers of megakaryocytes .The hyperplasia of hemopoietic tissue may be due to hemolytic anemia and it is a usual physiological response in order to increase the production of blood cells by the bone marrow with immature forms which appear in the circulation suggesting that compensatory erythropoiesis had been suppressed. In other words the bone marrow of toxicated group far from being depressed is in fact producing red cells at a considerable faster rate than normal and this is of a great value in helping the animal maintaining its hematological indices to meet the continuous blood loss. Stimulation and toxic effect resulting in exhaustion and suppression of hemopoietic tissue, and that agreed with (21).

Infiltration of mononuclear cells especially in liver, stomach and formation of early granuloma in liver, this may be attributed to the active compound like antioxidant flavonoid and alkaloids which may act as immune stimulant. The patient was given a better chance at survival if the cancer tissue showed infiltration of inflammatory cells, in particular lymphocytic reactions. The results suggested some extent of anti-tumor immunity is present in colorectal cancers in humans, in (22) published apaper findind tumour infiltrating lymphocytes to be quite significant in human colorectal cancer (22).

While at dose (200mg/kg B.W.) the predominant feature of organs was the necrosis and may be due to increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow. Apoptosis also was due to connected to slight alterations within the plasma membrane causing the dying cells to be attractive to phygocytic cells (23).

Cerebellum there is severe congestion of blood vessels of cerebellar meninges that agreed with (24), that may be due to penerat of the extract the blood brain barrier. In intestine was infiltration of mononuclear cells in mucosa with slight fibrosis may be due the effect of extract on immune system (23). Histopathological examination of kidney sections showed necrosis of the epithelial lining of proximal and distal convoluted tubules may be to direct toxic effect of extract this result was agreed with (25). The hyperplasia and hyperkeratosis of the mucosa of stomach with marked changes that was attributed to the irritant and toxic effects of grape seed on the mucosal and submucosal layers of the organ. The available human and animal data suggest that gastro-intestinal tract is a sensitive target of toxicity (26).

Dose (300mg/kg B.W.) showed in cerebellum edema between molecular and granlur layer with degeneration of many purkinji may be attributed to the increase in the permeability of the blood brain barrier leading to disturbances in the blood dynamics and escape of fluids to the nervous tissues and that agreed with (27). Other pathological changes seen in liver was hyperplasia of intrahepatic bile ducts which may be due to cholestasis that agreed with (28). In the kidney was severe periglomerular infiltration of inflammatory cells and cytic of renal tubules with formation of hyaline cast that can be attributed to the damage which affect the renal parenchyma these results was in agreement with (29). Deposition of amyloid fibril protein (Amyloid light chain) type is associated with some form of monoclonal B-cell proliferation (30) Other pathological changes seen in pancreas was fibrosis that agreed with studies now suggest the pathogenesis of fibrosis is tightly

regulated by distinct macrophage population that exert unique functional activities throughout the initiation, maintenance and resolution phases of fibrosis (31).

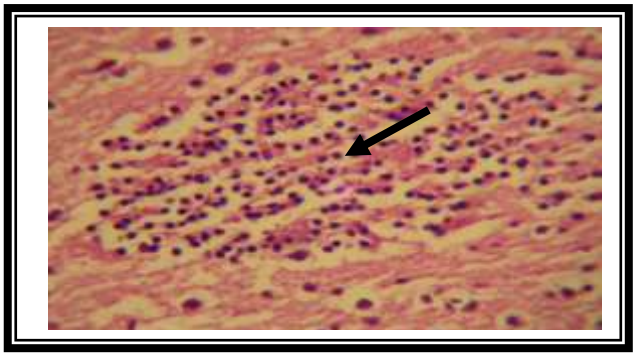
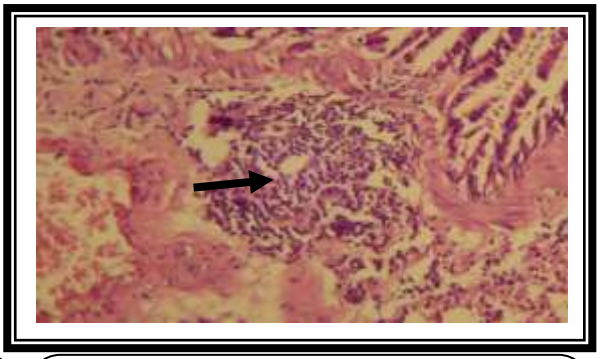
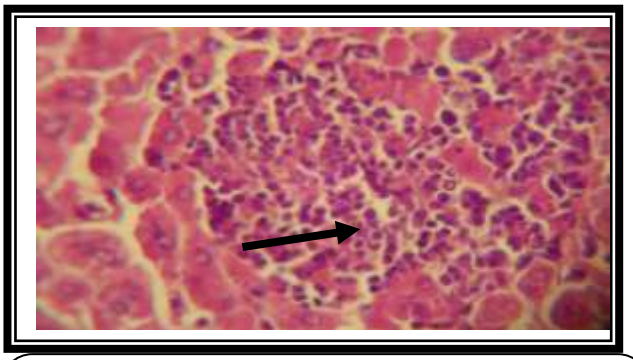


Fig (1): Histopathological section of Brain treated with grape seed at dose (100mg/kg b.w orally) showing focal gliosis (H&E X400).



Fig(2): Histopathological section of Lung treated with ethanolic extract of grape seed at dose (100mg/kg b.w orally) showing perivascular and peribronchiolar lymphocytic cuffing (H&E x400).



Fig(3) :Histopathological section of Liver treated with ethanolic extract of grape seed at dose (100mg/kg b.w orally) showing formation of early granuloma within hepatic parenchyma consisting of mononuclear cells aggregation (H & E x400).

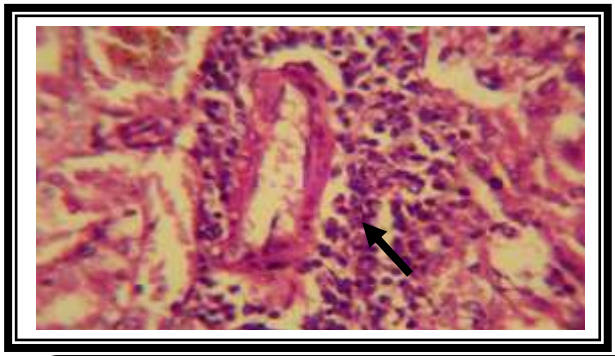


Fig (4): Histopathological section of Kidney treated with ethanolic extract of grape seed at dose (100mg/kg b.w orally) showing perivascular lymphocytic cuffing (H&E x400).

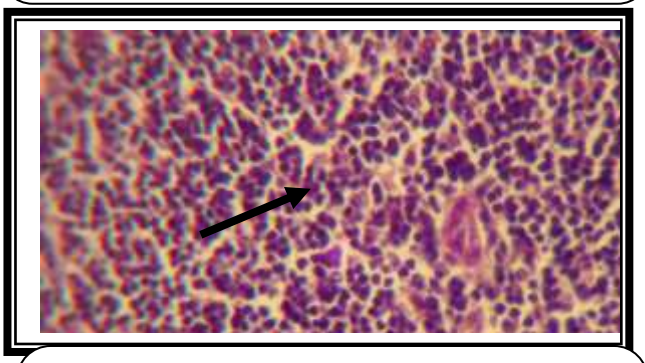


Fig (5): Histopathological section of Spleen treated with ethanolic extract of grape seed at dose (100mg/kg b.w orally) showing hyperplasia of lymphoid tissue of white pulp with deposition of hemosiderin pigment (H&E x400).

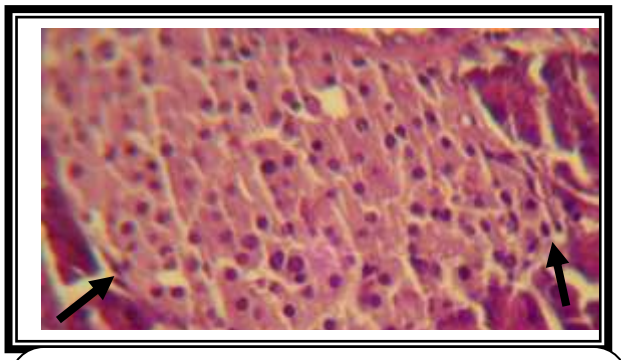


Fig (6): Histopathological section of Pancreas treated with ethanolic extract of rape seed at dose (100mg/kg b.w orally) showing regeneration of islets of Langerhan's (H&E x400).

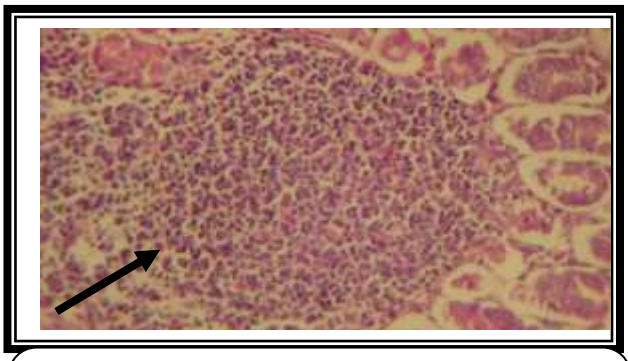
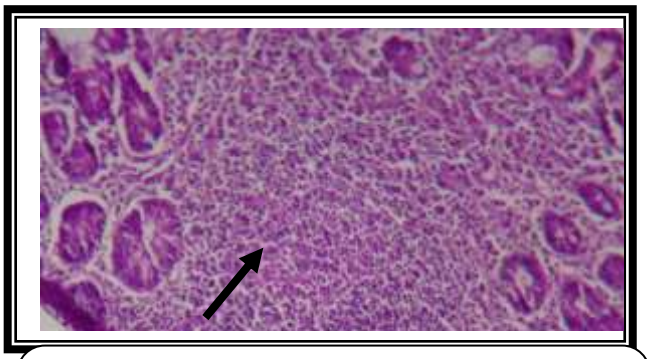
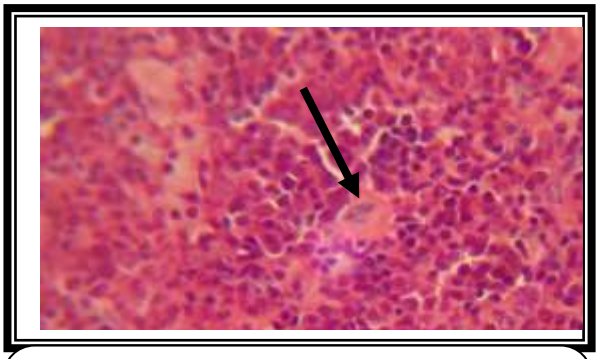


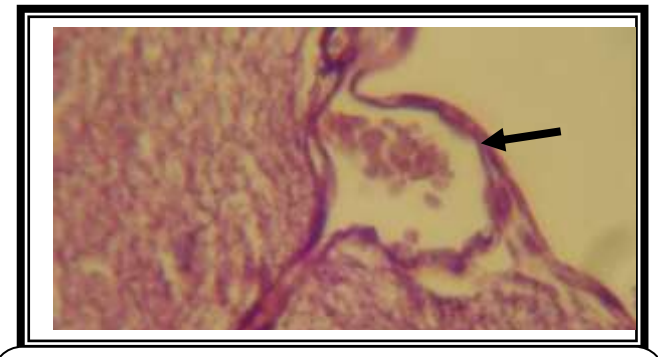
Fig (7): Histopathological section of Stomach (glandular region) treated with grape seed at dose (100mg/kg B.W orally) showing focal aggregation of mononuclear cells in mucosa (H&E x400).



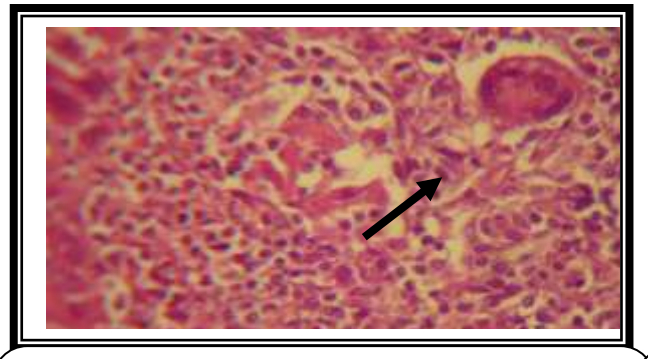
Fig(8) :Histopathological section of Intestine treated with ethanolic extract of grape seed at dose (100mg/kg b.w orally) showing hyperplasia of lymphoid tissue (H & E x400).



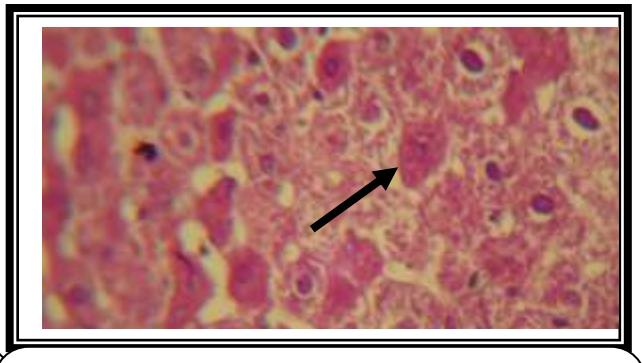
Fig(9) :Histopathological section of bone marrow of mouse treated with ethanolic extract of grape seed at dose (100mg/kg b.w orally) showing hemopoietic tissue with increase in numbers of megakaryocytes (H & E x400).



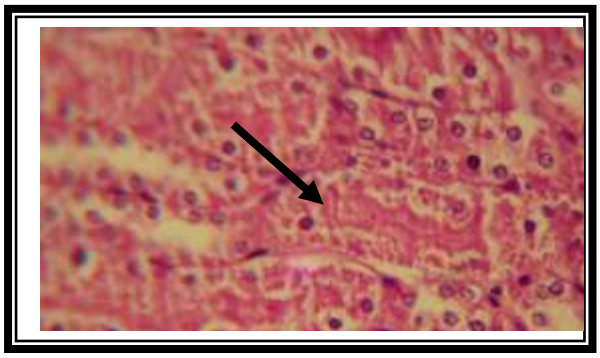
Fig(10) :Histopathological section of Brian treated with ethanolic extract of grape seed at dose (200g/kg b.w orally) showing severe congestion of blood vesscls of cerebellar meninges (H & E x400).



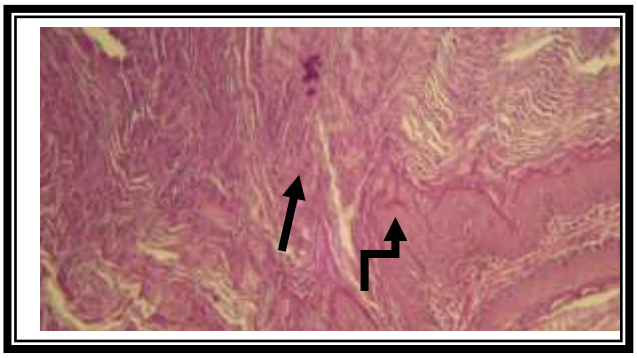
Fig(11) :Histopathological section of Intestine treated with ethanolic extract of grape seed at dose (200mg/kg b.w orally) showing infiltration of mononuclear cells in mucosa with slight fibrosis leading to atrophy of glands (H & E x400).



Fig(12) :Histopathological section of Liver treated with ethanolic extract of grape seed at dose (200g/kg b.w orally) showing extensive areas of necrosis and apoptosis (H & E x400).



Fig(13) :Histopathological section of Kidney of mouse treated with ethanolic extract of grape seed at dose (200g/kg b.w orally) showing severe necrosis of epithelial lining of proximal and distal convoluted tubules (H & E x400).



Fig(14) :Histopathological section of Stomach treated with ethanolic extract of grape seed at dose (200mg/kg b.w orally) showing hyperplasia( ) and marked hyperkeratosis of mucosa( ) (H & E x400).

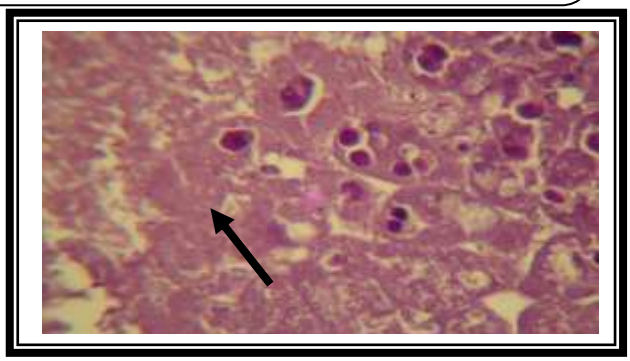


Fig (15): Histopathological section of Pancreas treated with ethanolic extract of grape seed at dose (200mg/kg b.w orally) showing extensive necrosis of pancreas (H&E x400).

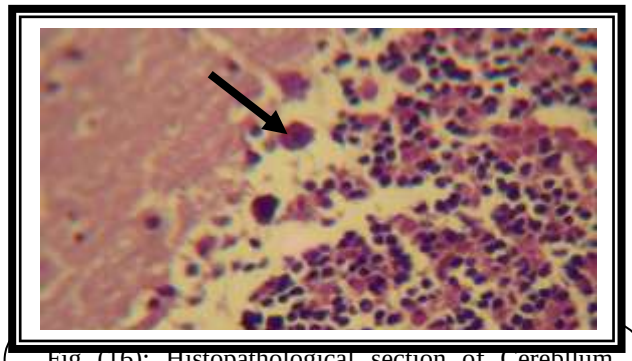
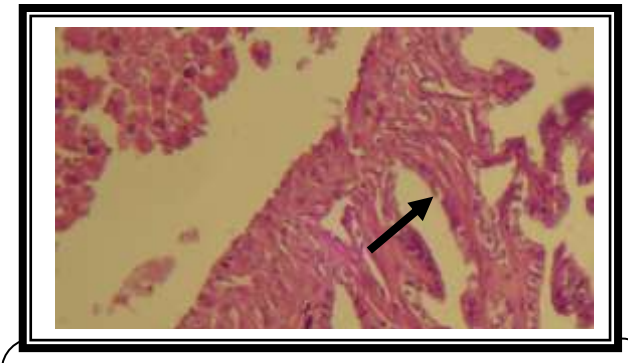
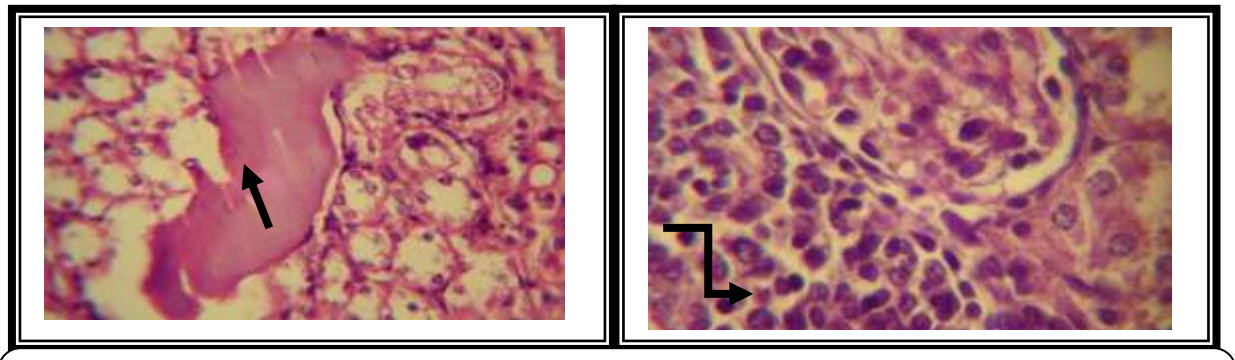


Fig (16): Histopathological section of Cerebllum treated with ethanolic extract of grape seed at dose (300mg/kg b.w orally) showing edema between molecular and granular layer with degeneration of many purkinji and complete dissolution of the others (H&E x400).



Fig(17) :Histopathological section of Liver treated with ethanolic extract of grape seed at dose (300mg/kg b.w orally) showing hyperplasia of intrahepatic bile ducts formting papillary projections (H & E x400).



Fig(18) :Histopathological section of Kidney of mouse treated with ethanolic extract of grape seed at dose (300mg/kg b.w I/P) showing severe periglomerular infiltration of inflammatory cells (↳)and cystic of renal tubules with formation of hyaline cast (→) (H & E x400).

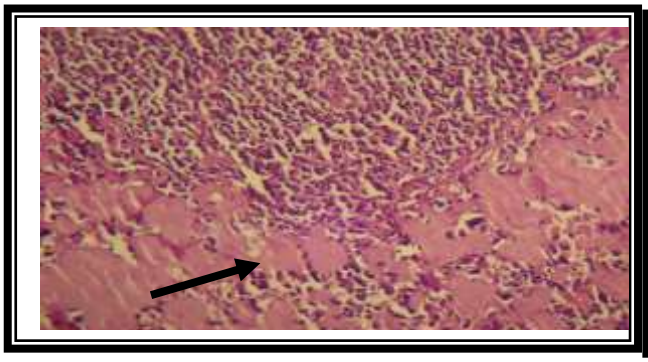


Fig (19): Histopathological section of Spleen treated with ethanolic extract of grape seed at dose (300mg/kg b.w orally) showing deposition of amyloid like substance causing pressure atrophy to lymphoid tissue (H&E x400).

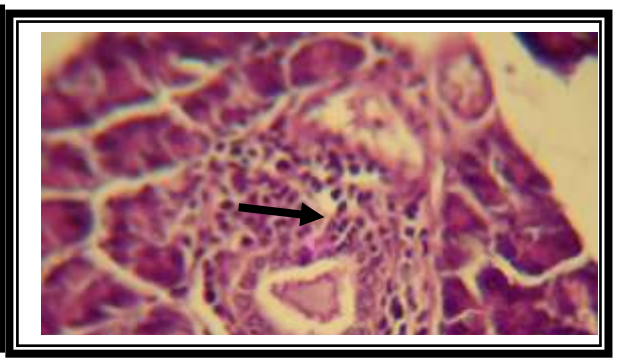


Fig (20): Histopathological section of Pancreas treated with ethanolic extract of grape seed at dose (300mg/kg b.w orally) showing infiltration of mononuclear cells around pancratic duct with slight fibrosis (H&E x400).

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The effect of L-arginine and its antagonist L-NAME and Methylene blue on sensory and cognitive function in mice

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Summary

The present study was done to focus light on possible enhancement of the functional performance of male mice and female in neuronal behaviors by using L-arginine as a precursor of nitric oxide (NO). The results showed increase of latency period to reach the novel object in L-arginine treated groups and decrease in both L-NAME and methylene blue treated groups in both periods of treatment (15 and 30) days and were more prominent in male than in female mice as compared with control groups. Similar results were observed in passive avoidance latency period to enter the dark compartment. There was a reduction in latency period to reach the alternative arm of T-maze test in L-arginine treated groups and increase in both L-NAME and methylene blue treated groups in both periods of treatment (15 and 30) days in both genders. It could be concluded that L-arginine-NO pathway plays an important role in improving memory in male more than female mice.

Key words:- L-NAME, Methylene blue, sensory, cognitive

تأثيرات ال-ارجنين ومضاداته L-NAME وMethylene blue على الوظائف التحسسية والإدراكية في الفئران

فريد جميل الطحان و مهند عبد الستار علي البياتي و سلمى جميل عسكر
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الخلاصة

أنجزت الدراسة الحالية لتسليط الضوء على إمكانية تسريع الإنجاز الوظيفي لذكور وإناث الفئران في السلوكيات العصبية باستخدام ال-أرجنين كواهب لأوكسيد النتريت (NO).أظهرت النتائج زيادة في الوقت الفعال للوصول إلى الهدف الغريب غير المألوف في المجاميع المعالجة بال-أرجنين ونقصان في كلا مجاميع المعالجة بالـL-NAME والـmethylene blue في كلا فترتي المعالجة (15 و30) يوم، وكانت أكثر جلياً في الذكور مقارنة في إناث الفئران مقارنة بمجاميع السيطرة. لوحظت نفس النتائج في الفترة الفعالة للتجنب السلبي passive avoidance للدخول للحجيرة المعتمة. هناك اختزال في الفترة الفعالة للوصول إلى الذراع البديل في اختبار T-maze في المجاميع المعالجة بال-أرجنين وزيادة في كلا المجاميع المعالجة بالـL-NAME و methylene blue في كلا فترتي المعالجة (15 و30) يوم وفي كلا الجنسين. من الممكن أن نستخلص أن مسلك ال-أرجنين-أوكسيد النتريت ذو فرط حيوية في تحسين الذاكرة في الذكور أكثر منه في إناث الفئران.

كلمات مفتاحية : ال-ارجنين, التحسسية, الإدراكية.

Introduction

Behavior is the adjustment of the animal to its environment under specified conditions. It simply means what animal does, how it does, and usually in response to stimuli from the environments (1 and 2). There were different pharmacological remedies used to treat the behavioral disorders such as cognitive impairment. L-arginine is one of modifiers of behaviors. It acts as neuromodulator in the central and peripheral nervous systems from which nitric oxide (NO) is derived that acts as a retrograde messenger in the central nervous system (3). Nitric oxide (NO) is diffusible molecules endowed with inter cellular messenger properties in several biological

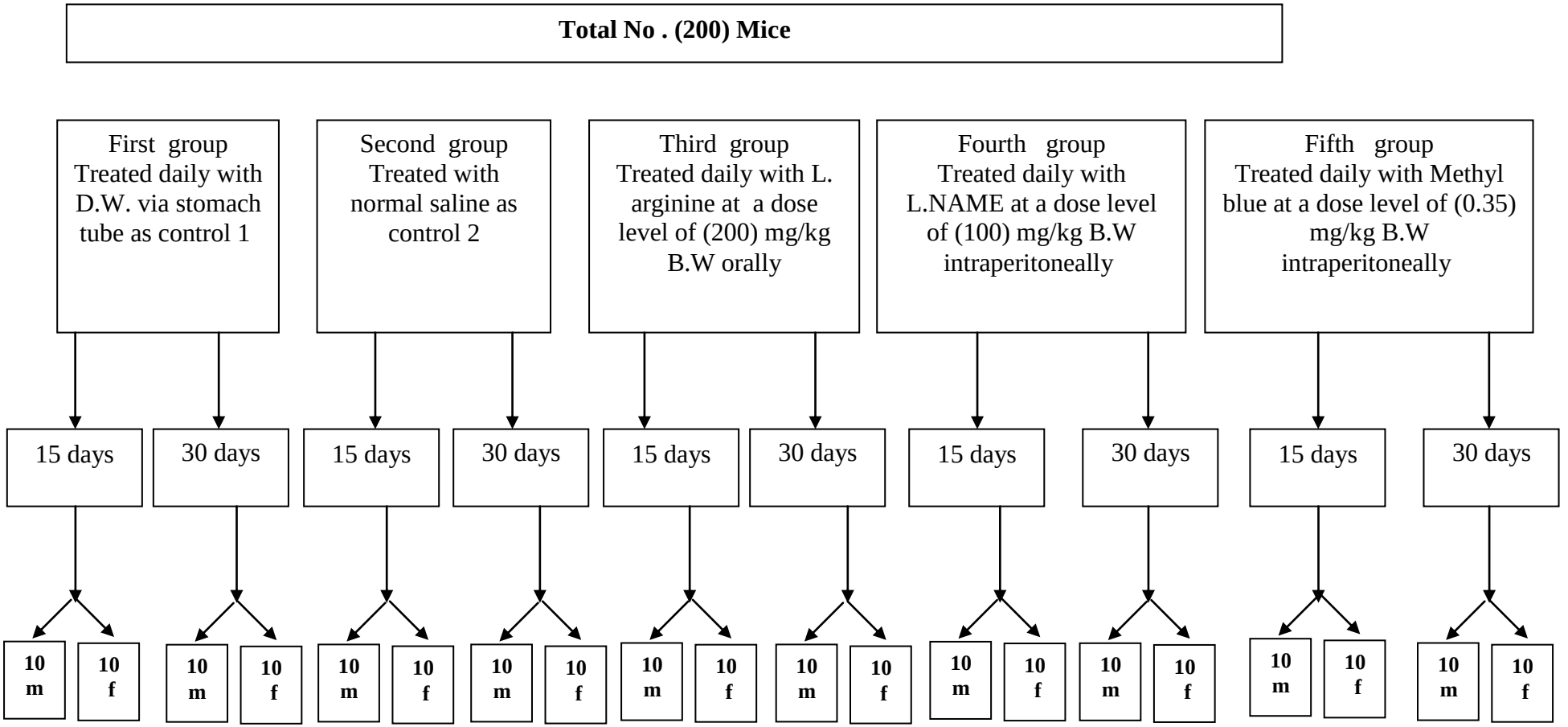
systems including the brain (4). This molecule plays an important role in learning and memory (5). Learning and memory field has focused on hippocampus, amygdale and cerebral cortex as critical sites in the brain where the plasticity underlying learning and memory occurs (6). Many reports suggested that L-arginine-NO system is part of the mechanisms underlying learning and memory. Long-term potentiation (LTP) in the hippocampus and long-term depression (LTD) in the cerebellum to be involved with learning and memory (7and 8). Because calcium-dependent activation of Ras-pathway of neural activity-dependent long-term changes in nervous system, (NO) may be a key mediator linking activity to gene expression and long-term plasticity (9). In mice, the formation of long-term memory (LTM) requires an increase in intracellular cyclic adenine monophosphate (cAMP) and requirement of the cAMP-dependent protein-kinase (PKA) that phosphorylates the transcription factor, cAMP - response element- binding protein (CREB) (10). The roles of (cAMP) pathway in the formation of long-term memory (LTM) are often supplemented by other signaling pathways, most notably by the (NO-cGMP) signaling pathway (11and12). The objective of this study was done to focus light on possible enhancement of the functional performance of male mice and female in neuronal behaviors by using L-arginine as a precursor of nitric oxide (NO).

Materials and Methods

One hundred male and 100 female white albino mice, weighing 25-30 gm. with an average of 27.5±0.02 gm. were used. They were kept under suitable environmental conditions of 20-25 ^{o}C. in an air conditioned room, (12) hours light and nourished *ad libitum*. Ten animals of each sex were given 200 mg/kg B.W. of L-arginine orally daily for 15 or 30 days. Other groups of ten mice of both sexes were likewise treated with 100 mg/kg B.W. of L-NAME and methylene blue 0.3 mg/kg B.W. intraperitoneally (13). Similar groups were given D.W. and normal saline, which served as control groups (Figure 1). The study consists of three experiments; the first one is object recognition test which is non spatial memory task based on spontaneously exploratory activity which used to test the novel object recognition capabilities of a mouse. In this task, mice are presented during a sample phase (learning) with either one or two identical objects and after a variable retention interval, animals are returned to the open-field and exposed to a novel object along side an identical copy of the object they had explored previously that is now familiar (14). The second experiment is passive avoidance task which described as a mouse has to choose between responding to obtain appositive reinforcement (entering to a dark compartment) and not responding to avoid an electric shock (not entering the dark compartment). The latency to refrain from entering into the dark compartment serves as an index of conditioned suppression and the ability to avoid, and allows memory to be assessed while the third experiment T-maze test is a spatial task in which animal learn to alternate between arms based on their memory of the previously visited arms. The latency to visit the correct choice (visited alternate arm) was the measure recorded (14).

Data were analyzed by using completely Randomized Design in factorial experimental (Two-way) ANOVA. In any experiment used two or three factors according to type of experiment. For calculation the effect of factors on dependent traits using (SPSS package 2008). To compare between treatments used Duncan, (1955) for multiple ranges. All the data were analyzed by using the procedures of (15). A probability of ($P< 0.05$) was considered as significant differences.

Figure (1): Experimental Design of Sensory and Cognitive Function



m = male

f = female

Results and Discussion

The effects of L-arginine and its antagonist L-NAME (NO synthase inhibitor) and methylene blue (soluble guanylyl cyclase inhibitor) on the object recognition time latency to reach the novel object table (1) and on the passive avoidance test latency period to enter the dark compartment table (2) for both genders, revealed significant differences at ($P<0.05$) between treated groups (15 and 30) days and control groups, which displayed increase in L-arginine and decrease in both L-NAME and methylene blue.

The results of T-maze test table (3) revealed significant decrease at ($P<0.05$) in the latency period to reach the alternative arm in L-arginine treated groups and increase in L-NAME and methylene blue treated groups as compared with control groups.

The results of novel object recognition which depended on the hippocampus and cortex brain areas (16) might be attributed to the role of L-arginine-NO pathway on cognition through enhancing and improving learning and memory due to, increase in synaptic transmission after stimulation of an excitatory pathway in the cortex and hippocampus called as long-term potentiation (LTP) by L-arginine-NO pathway and found to increase long-term potentiation by activating soluble guanylyl cyclase (sGC) and ultimately cyclic guanosine monophosphate (cGMP) which inturn increased nitric oxide (NO) formation in the cortex and hippocampus (7,17and18). Furthermore, L-arginine-NO-pathway might be involved increase release of excitatory neurotransmitters glutamate and acetylcholine through (cGMP)-dependent mechanism in the brain cortex and hippocampus which inturn supports synaptic plasticity through the critical role of glutamate, acetylcholine and nitric oxide in pathways associated with long-term potentiation, therefore this effect of L-arginine-NO pathway on these excitatory neurotransmitters might be involved in improving memory performance and enhanced cognitive function (19,12and 21). The passive avoidance test was used to evaluate emotional, learning and memory, L-arginine-NO pathway was modulated latency period time to enter the dark area. These results presumably due to the important role of L-arginine-NO pathway on cognitive through increasing nitric oxide (NO) concentrations, due to increase the activity of nitric oxide synthase (NOS) in the cortex, amygdala and hippocampus brain areas of adult mice. On other wise, L-arginine-NO-pathway might be stimulate release of argininevasopressin (AVP), neurohypophysial hormone, which facilitates memory, and nitric oxide (NO) synthesis evoked the vasodilatation caused by (AVP), and improvement of learning and memory due to at an angiotensin II (22), which increases regional cerebral blood flow by dilating cerebral arteries in rabbit and rodents (18 and 22). Although angiotensin II stimulate mostly the acquisition, while argininevasopressin stimulate consolidation of memory processes, both peptides have been found to facilitate recall of information in a passive avoidance test (24 and 25). In the T-maze test the simple capital (T) shape design incorporates a single choice point with only two alternatives, in which mice model was used to alternate between arms based on their memory of the previously visited arms. T-maze test and other behavioral tests of learning and memory are visuospatial tests of cognitive function and animals with impaired visual acuity may perform poorly on these tasks because of poor vision (26). The results in this test might be attributed to the effect of L-arginine-NO pathway on cognitive due to, provoked visual responses through L-arginine-NO pathway by modulation N-methyl-D-aspartate (NMDA) receptor-mediated excitation within the dorsal lateral genicular nucleus (dLGN), a major target of output from retina, which involved in visually specific pathways beside modulatory pathway which influence the activity of (dLGN) cells in arousal states (27,28 and 29). On other wise, the highly diffusible gas

nitric oxide (NO) may act in the proximity of the synaptic area of the parabrachial terminals of the brain stem, diffusing to act on retinogeniculate or other synapses utilizing (NMDA) receptors, thus release of nitric oxide may facilitate visual transmission in the thalamus affecting the functional activity of neuronal population in an extended volume of tissue, (29). Furthermore, the gender differences in learning and memory might be attributed to levels and activities of nitrate and nitrite (NO metabolites) which found higher in adult male brain areas of cortex, hippocampus, midbrain and cerebellum than female rodents (30).

In conclusion, the present data show that L-arginine-NO pathway is more prominent in enhancing and improving learning and memory in male than female mice.

Table (1): The Object Recognition time (minute) in L-arginine treated orally male and female mice, L-NAME intraperitoneally and with Methyl blue intraperitoneally daily (Object Recognition Test).

Periods of treatment and sex / Groups	15 days		30 days	
	Male	Female	Male	Female
D.W. as control 1.	3.52±0.20 Aa	1.25±0.25 Ba	3.01±0.19 Aa	1.35±0.20 Ba
Normal saline as control 2.	3.00±0.20 Aa	1.20±0.25 Ba	3.21±0.19 Aa	1.21±0.25 Ba
L-arginine (200)mg/ kg B.W.	6.31±0.84 Ab	4.00±0.47 Bb	6.00±0.62 Ab	4.33±0.29 Bb
L-NAME (100)mg/ kg B.W.	1.38±0.16 Ac	0.44±0.21 Bc	1.30±0.12 Ac	0.40±0.14 Bc
methyl blue (0.35)mg/ kg B.W.	1.33±0.18 Ac	0.35±0.23 Bc	1.24±0.10 Ac	0.43±0.20 Bc

Values are presented as Mean ±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Capital letters denoted to (P<0.05) gender differences.
Number=10mice/group

Table (2): The Passive Avoidance latency period (minute) in L-arginine treated orally male and female mice, L-NAME intraperitoneally and Methylblue intraperitoneally daily (Passive Avoidance Test).

Periods of treatment and sex / Groups	Male mice						Female mice					
	15 days			30 days			15 days			30 days		
	Habituation day	Training day	Testing day	Habituation day	Training day	Testing day	Habituation day	Training day	Testing day	Habituation day	Training day	Testing day
D.W as control 1	5.26 ± 0.60^{Ba}	3.16 ± 0.48^{Ca}	18.20 ± 3.20^{Aa}	5.00 ± 0.60^{Ba}	3.00 ± 0.46^{Ca}	18.61 ± 3.20^{Aa}	5.00 ± 0.25^{Ba}	3.20 ± 0.20^{Ca}	19.00 ± 1.83^{Aa}	4.62 ± 0.25^{Ba}	2.34 ± 0.20^{Ca}	18.81 ± 1.83^{Aa}
Normal saline as control 2.	5.20 ± 0.60^{Ba}	3.20 ± 0.48^{Ca}	18.80 ± 3.20^{Aa}	5.26 ± 0.60^{Ba}	3.00 ± 0.48^{Ca}	18.81 ± 3.20^{Aa}	4.62 ± 0.25^{Ba}	3.00 ± 0.20^{Ca}	19.21 ± 1.83^{Aa}	4.82 ± 0.25^{Ba}	3.16 ± 0.20^{Ca}	18.61 ± 1.83^{Aa}
L-arginine (200)mg/ kg B.W.	1.69 ± 0.20^{Bb}	1.04 ± 0.26^{Bb}	26.55 ± 3.80^{Ab}	1.00 ± 0.22^{Bb}	1.00 ± 0.21^{Bb}	26.14 ± 2.50^{Ab}	2.00 ± 0.13^{Bb}	1.21 ± 0.18^{Bb}	26.00 ± 1.80^{Ab}	1.66 ± 0.12^{Bb}	1.32 ± 0.20^{Bb}	25.52 ± 1.84^{Ab}
L-NAME (100)mg/ kg B.W.	7.60 ± 1.50^{Bc}	5.21 ± 1.40^{Cc}	3.23 ± 0.22^{Ac}	7.00 ± 1.05^{Bc}	5.61 ± 1.30^{Cc}	3.16 ± 0.27^{Ac}	7.02 ± 1.03^{Bc}	5.00 ± 1.18^{Cc}	3.00 ± 0.22^{Ac}	6.14 ± 0.92^{Bc}	6.05 ± 1.92^{Bc}	3.24 ± 0.92^{Ac}
Methyl blue(0.35)mg/ kg B.W.	7.00 ± 1.50^{Bc}	5.00 ± 1.40^{Cc}	3.00 ± 0.22^{Ac}	6.70 ± 1.05^{Bc}	5.00 ± 1.07^{Cc}	3.05 ± 0.27^{Ac}	7.39 ± 0.92^{Bc}	5.17 ± 0.91^{Cc}	3.20 ± 0.24^{Ac}	7.00 ± 1.08^{Bc}	6.17 ± 0.94^{Bc}	3.00 ± 1.06^{Ac}

Values are presented as Mean ± SE.
Small letters denoted to ($P < 0.05$) different between treated groups.
Capital letters denoted to ($P < 0.05$) different between experimental days of certain period of treatment.
Number = 10 mice / group.

Table (3): The T-maze latency period (second) in L-arginine treated orally male and female mice, intraperitoneally with L-NAME and intraperitoneally with Methyl blue daily (T-Maze Test)

Periods of treatment and sex / Groups	15 days		30 days	
	Male	Female	Male	Female
D.W. as control 1.	8.60 ±1.40[a]	8.48 ±1.10[a]	8.70 ±1.40[a]	8.46 ±1.10[a]
Normal saline as control 2	8.62 ±1.40[a]	8.44 ±1.12[a]	8.70 ±1.44[a]	8.62±1.20[a]
L-arginine (200)mg/ kg B.W.	2.66 ±0.40[b]	2.60 ±0.46[b]	2.70 ±0.33[b]	2.64 ±0.40[b]
L-NAME (100)mg/ kg B.W.	17.30 ±2.40[c]	17.27 ±2.18[c]	17.32 ±2.40[c]	16.93 ±2.60[c]
Methyl blue (0.35)mg/ kg B.W.	17.39 ±2.60[c]	16.97 ±0.80[c]	17.00 ±2.33[c]	17.20 ±2.76[c]

Values are presented as Mean ±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Number = 10 mice/group

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The effects of dry yeast levels on some water parameters

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Summary

This study was carried out at fish laboratory of Animal Production Department, college of Agriculture, university of Sulaimaniya using commercial dry yeast probiotics (Saccharomyces cerevisiae) in three concentration (3%, 5% and 7%) to study their effects on growth performance of common carp fingerlings(Cyprinus carpio) fed diets and some of water parameters.The experiment was included nine treatments each in three replicates (plastic aquaria) in which 10 fingerlings common carp of the same size and weight (3.5 gram) were stocked in each aquarium. The actual experimental feeding trials durated for three months. Results indicated that thethird treatment(7% concentration of the yeast) giving more weight gain, growth rate and best relative growth weight.Monthly samples of water were taken from each pond for measuring water quality control, temperature and dissolved oxygen, BOD_5, pH, EC (dc/m), TDS, No_2 (mgl^{-1} No_2-N), No_3 (mgl^{-1} No_3-N).

Key words: Yeast,water parameter, Saccharomyces cerevisiae , Cyprinuscarpio

تأثير مستويات مختلفة من الخميرة الجافة في بعض قياسات الماء

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الخلاصة

أجريت هذه الدراسة في مختبر الاسماك من قسم الإنتاج الحيواني، كلية الزراعة، جامعة السليمانية باستخدام الخميرة الجافة التجاري (المعزز الحيوي) (*Saccharomyces cerevisiae*) في ثلاث تراكيز (3 ٪ و 5 ٪ و 7 ٪) لدراسة تأثيرها على أداء نمو إصبعيات الكارب الشائع (*Cyprinus carpio*) وبعض القياسات البيئية لمياه التجربة. وقد شملت التجربة تسع معاملات بثلاث مكررات (أحواض بلاستيكية) واحتوت كل منها على 10 إصبعيات من أسماك الكارب العادي (3.5 غرام) في كل حوض. استمرت التجارب الفعلية التجريبية مدة ثلاثة أشهر. وأشارت النتائج الى ان المعاملة الثالثة (تركيز 7 ٪ من الخميرة اعطت اكثر زيادة وزنية ، وأفضل معدل نمو والوزن النسبي. تم اخذ العينات من المياه شهريا من كل حوض لقياس درجة الحرارة والأوكسجين المذاب وBOD_5 وpH و EC (dc/m) و TDS وNo_2 (mgl^{-1} No_2-N) وNo_3 (mgl^{-1} No_3-N).

كلمات مفاتحية:الخميره الجافه,قياسات الماء,اصبعيات الكارب,احواض بلاستيكيه.المعزز الحيوي.

Introduction

Freshwater fish habitat is constrained by water quality, food supply, human interference, and other parameters (1). Water quality generallymeans the component of water which must be present foroptimum growth of aquatic organisms. The determinantof good growth in water body includes dissolved oxygen,hardness, turbidity, alkalinity, nutrients, temperature, etc.Conversely, other parameters like biological oxygendemand, and chemical oxygen demand indicate pollutionlevel of a given water body. In most water bodies, variouschemical parameters occur in low concentrations (2). Fish are extremely sensitive toenvironmental factors such as oxygen levels, water salinity, temperature, etc. Given that fish are poikilotherms, water temperature is a major determining factor in fish biology. The most obvious effect of high temperatures is an increase of the embryonic development rate without affecting the relative timing of formation of the anatomical structures.The major water quality factors that are important in freshwater aquaculture

systems and methods to monitor them are described in this publication. Water quality determines not only how well fish will grow in an aquaculture operation, but whether or not they survive. Fish influence water quality through processes like nitrogen metabolism and respiration. Knowledge of testing procedures and interpretation of results are important to the fish farmer (3and4).Because the quality of water environment is considered the main factorscontrolling fish quality and subsequentially its growth and production.

The probiotics of live microbes have shown their effectiveness to mitigate the effects of stress , resulting in a greater production. (5) concluded that yeast have a positive effect on fish performance when cultured under stress condition of lowering dietary protein, leading to improving growth and feed efficiency. In contrast, (6) found that growth and feed conversion of juvenile dentex were not significantly influenced by probiotics which is in agreement with the findings, Shelby(7) found that the probiotic used with juvenile channel catfish diet had lack effect on specific growth promoting or immune stimulating aspects.

The main aim of the present study is to investigate the effect of using different dry yeast levels on some water parameters and fish quality in order to determine which one could be most suitable for use to yield the best quality of fish for human consumption. These are achieved by determination of some Physico-chemical properties of water during different seasons.

Materialsand Methods

The experiment was conducted for 12 weeks, using common carp (*Cyprinuscarpio*, 3.5 g average weight) fingerlings obtained from dukan hatchery. The experimental system was in 100 L plastic tanks. For water quality control,temperature and dissolved oxygen, BOD_5, pH, EC(dc/m), TDS, No_2 (mgl^{-1} No_2-N), No_3 (mgl^{-1} No_3-N) were measured monthly analyses were done of total nitrite, nitrate and pH levels, using standard methods (8).

The tested commercial dry yeast probiotics (*Saccharomyces cerevisiae*) were used to study their effects on growthperformance of common carp (*Cyprinuscarpio* fingerlings.The animals were allowed to adapt to the experimental system for a week and fed with a conventional diet after which time the different treatments(control treatment without any addition, first treatment addition of 3% of the dry yeast, second treatment the addition of 5% of the dry yeast and the third treatment the addition of 7% of the dry yeast) were randomly assigned to the tanks with three replicates per treatment.Feed was manually administered ad libitum twice / day for 12 weeks. A daily record was kept of feed offered. Bulk weight was measured weekly to follow growth in weight and calculate survival and feeding ration. Briefly, the fish were taken from each tank using a net previously disinfected. This was then passed over fabric towels to eliminate excess moisture and the fish weighed on an electronic balance.Feeding level of all experimental diets was 6% of the total biomass of thefish per day. The amount of feed was divided into two equal portions and distributed by hand in one side of theaquaria two times daily at 9 a.m, 2p.m. The performance parameters included total weight gain, total daily gain (TDG), total specific growth weight (SGW).

At the laboratory, hydrogen ion potential of each water sample from each site was measured again in the Lab. temperature using a portable (pH) meter (pH 330i/SET-WTW Company-Germany), as described by (9). Before each sampling process, the calibration of instrument was done by specific standard buffer solutions (4, 7 and 9), prepared by manufacture company.

The ability of water to conduct an electric current is known as specific conductance and depends on the concentration of ions in solution. At the laboratory electrical

conductivity of water sample from each site (station) was measured again using a portable (EC) meter (LF318/SET- WTW Company-Germany), as described by (10). Before each sampling, the calibration of instrument was done by specific standard solutions (0.1N KCl), given by manufacture company. Final result corrected at (25C°) and expressed in ($\mu S.cm^{-1}$).

Dissolved oxygen (DO) was measured directly at the lab using a special oxygen-sensitive membrance electrode (InoLab. OXi730WTW Company-Germany). Results were expressed in (mg $O_2.l^{-1}$).

The main principle underlying for determination (BOD_5), is the measurement of oxygen content before and after incubation for five days (20°C) as described in (APHA, 1998). According to the below equation:

$BOD_5 = DO_o - DO_5$ mg $O_2.l^{-1}$

The most highly oxidized form of nitrogen compounds, it is commonly present in surface and ground water, because it is the product of the aerobic decomposition of organic nitrogenous matter (11). In laboratory, the nitrate concentration of each water samples was estimated by using a special nitrate-sensitive membrane electrode (Ino lab.pH/Ion/Cond.750-Multiparameter laboratory, WTW company-Germany). The results were expressed in (mg l^{-1}).

Nitrite is an unstable, intermediate stage in the nitrogen cycle and is formed in water either by the oxidation of ammonia or by the reduction of nitrate. Thus biochemical processes can cause a rapid change in the nitrite concentration in water sample (11). In natural water, nitrite is normally present only in low concentrations. In laboratory, the concentration of nitrite in each water sample was determined according to method that given by (WTW company. Photospektral lab. Germany). 0.2mg of nitrite reagent (Sulfanilic acid) was added to (5ml) of water sample, shaking vigorously to dissolve the solid substance, then check (pH), specific range (2.0-2.5), for adjusting (pH) use dilute (sodium hydroxide or suluric acid), then leave the solution for (10 minutes) for reaction time. The extinction of the solution in (1cm) cuvette was measured at wavelength of (543nm). The results were expressed in (mg l^{-1}).

T.D.S is defined as one of the most important physical parameter of water. In the present study, the value of (T.D.S) in each water sample was measured depending on the method given by (12) using the following equation:

T.D.S= EC × F

Where:

EC= Electrical conductivity in ($\mu S.cm^{-1}$).

F= Factor equal to 0.64

Results and Discussion

The presented data in Table (1) showed that the weight gains at the start of the experiment for all groups were found to be 0.24, 0.32, 0.07 and 0.07 g, respectively.Where there were no significant (P> 0.05) differences among these fish groups. The obtained results showed that the third treatment had a significant (P<0.05) effect on body weight of common carp *Cyprinuscarpio* all over the experimental period. It could be recommended that this additive at above mentioned level are the most effective for improving the body weight of common carp *Cyprinuscarpio*. The achieved results of dietary yeast effect which illustrated in Table (1) on body weight of common carp *Cyprinuscarpio*were supported byAbdelhamid (13and 14). Kobeisy (15) reported that 10% dietary live yeast increased body weight of *Oreochromis niloticus* significantly. For these reasons, (15 and 16) found that the growth performance of tilapia and common carp was better when active yeast fed than inactive yeast. Rumsey (17) showed that brewer's dried yeast

(*Saccharomycescerevisiae*) diet supported the best growth of rainbow trout (*Oncorhynchusmykiss*).

However, the obtained results could be explained by some factors as dietary live yeast (LY) improved growth performance due to LY is a source of protein (18). Live yeast acts as a source of enzymes, i.e amylase, protease and lipase which may improve food digestion and consequently food utilization. Moreover, LY is a very good source of vitamin B6 (39.8 mg/kg dry matter) (19). Vitamin B6 may act as a stimulator of growth hormone.Tryfiates (20) showed that growth hormone (GH) concentrations in both pituitary gland and serum were low in vitamin B6-depleted rates. Therefore, vitamin B6 (pyridoxal 5-phosphate, PLP) acts as a coenzyme of dopa decarboxylases enzyme and dopamine stimulates GH secretion. The low PLP levels limit the availability of active decarboxylase thus causing low GH. In addition, yeast has high nitrogen content (21). Moreover, replacement of fish meal by dietary yeast did not affect the growth performance of trout and turbot (22), tilapia (*Oreochromismossambicus Peters*) fry (23). Nile tilapia (*Oreochromisniloticus*) (24),European sea bass (*Dicentrarchuslabrax*) (25) and sea bream (*Sparusaurata*) (26). On the contrary no positive role in growth was observed in fish fed dietary yeast. (27and 28).

Data presented in Tables (2and 3) showed that the specific growth rate of *C. carpio* had remarkable increase in fish groups fed the high levels of yeast (7%) at most intervals from the beginning of the experimental period until the eleven week. the present results reveal that the level of 7% dietary yeast had a pronounced effect on RGR (% /day) of *C. carpio* (Table 3). These results are supported by (29) who reported an improvement in specific growth rate of 13% in rainbow trout fed a diet containing nucleotides (source of yeast). Lara-Flores(24) mentioned that the addition of yeast to the diets produced the best specific growth rate of Nile tilapia (*O. niloticus*).

Generally, the data illustreated in Table (4) showed that 7% yeast were more effective in enhancement of body weight of common carp *Cyprinuscarpio*. These results are in agreement with the findings of (30) found that 10% brewer's yeast was optimal level, where more than this level (20 and 30%) performed worse body weight. At the same trend, similar positive effects of dietary Pronifer, algae and yeast on average body weight gain of tilapia fish were recorded by different workers (15, 31, 32 , 33and 34) .

Additionally dietary 5, 10 and 20% live yeast increased body weight gain by 10 %, 37% and 61 % in *Oreochromisniloticus* groups, respectively (15). Li(35) found that the hybrid striped bass fed the diets supplemented with 1% and 2% dried brewer's yeast (Brewtech) had up to 20% more weight gain compared to fish fed the basal diet. (28) showed that enhanced weight gain was generally observed in fish fed diets supplemented with 1% and 2% Grobiotick AE compared to those fed the basal diet ($P<0.05$). Li(36) fed 1% and 2% brewer's yeast and 2% GroBiotic A had significantly higher weight gain than fish fed the basal diet for hybrid striped bass. On the contrary, there was no significant difference in percent weight gain (WG), among the treatments in tilapia feeding on torula yeast (23).

The water quality criteria illustrated in Table (5) were suitable for rearing the common carp *Cyprinuscarpio*. The analysis was based on the samples taken from fish aquariums. The parameters are presented in (Table 5). pH range was 7.97- 8.06; TDS range was 236.80 to 256.00 mg/l. BOD (mg/l) ranged from 1.27- 1.87mg/l, DO was from 3.03- 5.31 mg/l, nitrate values ranged from 0.28 - 0.41 mg/l, nitrite values ranged from 0.90- 2.91 mg/l . Generally, the parameters analyzed fell within the desirable and acceptable limits. Although, there were values higher than the acceptable limit, the situation can be remedied by change of water in the ponds. However, significant pollution of the fish ponds was not indicated from the result of the parameters analyzed. These values are suitable for rearing the common carp *Cyprinuscarpio* ,(2 and 37).

Water quality study is essential for setting base line conditionsand standards.Against these standards results offurther studies can be evaluated. The results of this studyare presented in (Table 5). A total of sevendifferent physiochemical parameters were analyzed. Theanalysis was based on the samples taken from fish aquariums. The parameters arepresented in(Table 5). pH range was 7.97- 8.06; TDS range was 236.80 to 256.00 mg/l. BOD (mg/l) rangedfrom 1.27- 1.87mg/l, DO was from 3.03- 5.31 mg/l,nitrate values ranged from 0.28 - 0.41 mg/l, nitrite values ranged from 0.90- 2.91 mg/l . Generally, the parameters analysed fellwithin the desirable and acceptablelimits. Although, therewere values higher than the acceptable limit, the situationcan be remedied by change of water in the ponds.However, significant pollution of the fish ponds was notindicated from the result of the parameters analyzed.

The desirable range for pond pH is 7.97- 8.06 andacceptable range is 5.5 - 10.0 (37). The range of the pH obtained from this study was7.97- 8.06 (Table 5). This agrees with (37). Thus, good pond productivity and fishhealth can be maintained. Furthermore, a similar rangewas obtained by Kamed (38) who reported arange of 7.3 - 8.3.

Total dissolved solid varied from 236.80 to 256.00 mg/l. Thehighest value being 256 mg/l and the least value being 236.80 mg/l. Farmers use feeds to supplement pond nutrients.Among others the feeds have been reported to increasetotal dissolved solids (39). This may have been responsible forthe variation from pond to ponds in thisstudy. The result is supportive of the findings of thisstudy.

Biochemical oxygen demand varied significantly amongthe ponds. The highest value was 1.87 and least was1.27 mg/l. These are all below FEPA standard (40). TheFEPA limit is 30 mg/l. This is suggestive that the pondwater is not polluted and the fishes are not beingnegatively affected. However, permissible limit is 4 mg/l. Accumulation of low BOD results in organismsbeing stressed, suffocated and death (3). This was not observed in the ponds under study.This is a measure of amount of gaseous oxygen dissolved in an aqueous solution that plays a vital role in the biology of cultured organisms (41).The DO (mg/l) obtained from this study was in the rangeOf3.03- 5.31mg/l. These values agree with those of(41). In the present results, the higher value of DO in the third treatment water may be due to the abundance of yeast that increase photosynthetic activity leading to production of large amount of DO. The nitrate level in this study was in the range of 0.28 - 0.41 mg/l. The desirable limit is 0 - 2 mg/l and acceptablelimit less than 4 mg/l (22). These desirableand acceptable limits are lower than those from previousstudy and therefore not in consonance with the result ofthis study. The high values suggested that there is thepresence of pollutants like bacteria and pesticides. Nitrate concentration in agriculture drainage fish ponds water was significantly higher than that of irrigation ponds water. This may be due to that agriculture drainage water is rich in nitrate content. Moreover, the high level of ammonia in agriculture drainage ponds water may be nitrified to nitrate due the high concentration of the available Do (4).

In conclusion, the overall mean pH values were significantly higher at agriculture drainage ponds water compared to irrigation ponds water. This may be due to the higher concentration of yeast in the third treatment. This may be due to the increase in pH value in water with high photosynthetic rate and the depletion of carbon dioxide. Autotrophic activity increases pH through Co_2 absorption, while heterotrophic activity decreases pH through respiration, since the autotrophic and heterotrophic processes affect the measured variables in opposite ways.

Table (1): The effect of dry yeast levels on common carp fingerlings gain weight (gm)

Treatment	Weeks											General mean
	1	2	3	4	5	6	7	8	9	10	11	
Control	0.24abc ±0.01	0.14bc ±0.10	0.11bc ±.0.04	0.09bc ±0.01	2.03a ±0.00	1.58abc ±0.03	2.07a ±0.10	1.87abc ±0.02	1.95ab ±0.02	1.22abc ±0.02	1.02abc ±0.03	1.12a ±.0.01
First Treatment	0.32abc ±0.29	0.17abc ±0.09	0.31abc ±0.26	1.04abc ±0.77	0.75abc ±0.62	0.23abc ±0.05	1.28abc ±0.01	1.38abc ±0.54	1.62abc ±0.21	1.42abc ±0.28	0.87abc ±0.31	0.85a ±0.31
Second Treatment	0.07c ±0.01	0.14bc ±0.10	0.27abc ±0.09	0.15bc ±0.11	0.58abc ±0.51	0.83abc ±0.60	1.04abc ±0.61	0.83abc ±0.39	1.72abc ±0.31	0.89abc ±0.24	0.88abc ±0.17	0.67a ±0.29
Third Treatment	0.07c ±0.01	0.24abc ±0.04	0.28abc ±0.16	1.69abc ±0.82	1.54abc ±0.37	1.16abc ±0.10	1.80abc ±0.39	0.67abc ±0.03	1.96a ±0.48	0.51abc ±0.20	1.28abc ±0.75	1.02a ±0.30
General mean	0.17d ±0.09	0.15d ±0.09	0.22cd ±0.19	0.64bc ±0.73	1.01b ±0.69	0.74bc ±0.42	1.14ab ±0.76	0.95b ±0.49	1.30a ±0.89	0.76b ±0.48	0.76b ±0.75	

Control without any addition, First treatment(3% dry yeast),Second treatment(5% dry yeast),Third treatment(7)% dry yeast.

Table (2): The effect of dry yeast levels on common carp fingerlings daily growth rate (gm/day):

Treatment	Weeks											General mean
	1	2	3	4	5	6	7	8	9	10	11	
Control	0.03bcd±.	0.02cd±.	0.06abcd±.	0.01cd±.	0.29ab±.	0.23abcd±.	0.30a±.	0.27abcd±.	0.28abc±.	0.17abcd±.	0.15abcd±.	0.16a±.
First Treatment	0.05 abcd ±0.04	0.02bcd ±0.01	0.04 abcd ±0.04	0.15abcd ±0.11	0.10abcd ±0.09	0.03bcd ±0.01	0.18abcd ±0.00	0.20 abcd ±0.08	0.23abcd ±0.03	0.22 abcd ±0.02	0.13 abcd ±0.05	0.12a ±0.04
Second Treatment	0.01d ±0.00	0.02cd ±0.01	0.04bcd ±0.01	0.02cd ±0.02	0.08abcd ±0.07	0.12 abcd ±0.09	0.17abcd ±0.09	0.12 abcd ±0.05	0.25abcd ±0.04	0.13 abcd ±0.03	0.13 abcd ±0.02	0.10a ±0.04
Third Treatment	0.01d ±0.00	0.03bcd ±0.01	0.04 abcd ±0.02	0.24abcd ±0.12	0.22abcd ±0.05	0.17 abcd ±0.01	0.26abcd ±0.06	0.10 abcd ±0.00	0.28ab ±0.07	0.07 abcd ±0.03	0.18 abcd ±0.11	0.15a ±0.04
General mean	0.03d ±0.02	0.02d ±0.01	0.04cd ±0.02	0.11bd ±0.08	0.17b ±0.07	0.14bc ±0.04	0.23ab ±0.05	0.17b ±0.04	0.26a ±0.05	0.15bc ±0.03	0.15b ±0.06	

Control without any addition, First treatment (3% dry yeast), Second treatment (5% dry yeast), and Thirdtreatment (7) % dry

Table (3): The effect of dry yeast levels on common carp fingerlings relative growth rate(%):

Treatment	Weeks											General mean
	1	2	3	4	5	6	7	8	9	10	11	
Control	6.88bc ±.	3.75c ±.	2.84c ±.	2.26c ±.	49.88a ±.	25.9abc±.	26.95abc ±.	19.18 abc±.	16.78 bc ±.	8.99 bc ±.	6.90 bc ±.	15.48a ±.
First Treatment	13.60 bc ±12.92	4.31 bc ±1.56	9.90 bc ±8.84	22.75abc ±15.14	12.08 bc ±8.68	4.69 bc ±2.41	22.92abc ±7.10	17.88abc ±2.61	19.18abc ±3.06	13.84 bc ±0.87	7.14 bc ±0.84	13.48a ±5.82
Second Treatment	2.46c ±0.54	4.40 bc ±3.14	8.75 bc ±2.79	4.12c ±3.00	14.61 bc ±12.37	16.99 bc ±9.91	18.61abc ±8.10	13.30 bc ±5.91	27.85ab ±5.51	10.89 bc ±2.50	11.02 bc ±3.91	12.09a ±5.24
Third Treatment	1.91c ±0.31	6.69 bc ±1.21	7.87 bc ±5.02	41.91a ±21.03	26.49abc ±6.01	16.23 bc ±2.13	20.58abc ±2.65	6.86 bc ±1.37	17.29 bc ±2.73	4.18c ±1.74	11.39 bc ±7.95	14.67a ±4.74
General mean	6.21c ±4.59	4.79c ±1.97	7.34c ±5.55	17.76ab ±13.06	25.77a ±9.02	15.95abc ±4.81	22.27ab ±5.95	14.31abc ±3.30	20.28ab ±3.77	9.48bc ±1.70	9.11abc ±4.23	

Control without any addition, First treatment (3% dry yeast), Second treatment (5% dry yeast), and Thirdtreatment (7) % dry yeast

Table (4): The effect of dry yeast levels on common carp fingerlings total growth performance:

Treatment	Characteristics		
	Total weight gain(gm)	General mean of total daily growth weight(gm/day)	General mean of total relative growth weight(%)
Control	12.32 ± . a	0.15± . a	353.01 ± . a
First Treatment	9.37 ± 2.22 a	0.12 ± 0.03 a	286.73 ± 24.14 a
Second Treatment	7.39 ± 2.59 a	0.09 ± 0.03 a	249.17 ± 79.27 a
Third Treatment	11.20±1.06 a	0.13 ±0.01 a	318.94 ± 40.39 a

Control without any addition, First treatment(3% dry yeast), Second treatment(5% dry yeast), Third treatment(7)% dry yeast

Table(5): the effect of the yeast addition on some water parameters

Control	Do(mg/l)	BOD5	Ph	EC(dc/m)	No_2 (mgl^{-1} No_2^{-N}	No_3 (mgl^{-1} No_3^{-N}	TDS
Treatment	3.09±.524 a	1.31±. 7 a	7.97±.035 A	.40±.01 a	.41±.35 a	2.15±.42 a	256.00±6.4 a
First Treatment	3.18±.312 a	1.43±.48 a	8.06±.036 a	.37±.014 a	.38±.18 a	.90±.17 a	236.80±8.9 a
Second Treatment	3.03±.29 a	1.87±.53 a	7.85±.22 a	.40±.07 a	.35±.16 a	2.91±.81 a	254.93±3.85 a
Third Treatment	5.31±2.12 a	1.27±.37 a	7.94±.07 a	.40±.03 a	.28±.14 a	2.27±.46 a	253.84±8.26 a

Control without any addition, First treatment(3% dry yeast), Second treatment(5% dry yeast), Third treatment(7)% dry yeast

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The immunomodulatory effect of Neem (*Azadirachtaindica*) seed aqueous, ethanolic extracts and *Candida albicans* cell wall mannoproteins on immune response in mice vaccinated with Brucella Rev-1

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Summary

In the present study, the immunomodulatory effect of Neem (Azadirachtaindica) seed aqueous, ethanolic extracts and Candida albicans cell wall mannoproteins on the immune response of mice vaccinated with Brucella Rev-1 vaccine was investigated. The study was conducted on two main groups(160 mice for each group), Each group was divided into eight subgroups 20 mice for each (I: treated with distilled water, II: treated with the Brucella Rev-1 vaccine, III: treated with mannoproteins, IV: treated with neem aqueous extract, V: treated with neemethanolic extract, VI, VII and VIII: treated with mannoproteins, neem aqueous extract and neemethanolic extract, respectively, then they were vaccinated with Brucella Rev-1). All these treatments were carried out on day 1 and then vaccinated with brucella Rev-1 vaccine on day 4 .Then the mice were tested as follows , on day 8 after vaccination(serum IFN-γ level), day 21 for (anti-Brucella antibody titer). The doses of both plant extracts and mannoproteins represented 10% of the calculated LD50 (neem extracts: 3.8096 g/Kg mannoproteins: 5.7144 mg/Kg), which were given subcutaneously. Mice of the second main group were injected with the immune suppressive drug prednisolone (5mg/Kg) 5 days prior to the treatments, which carried out on mice of the first main group. The results demonstrated clear immunomodulatory effects (improvement of non-specific, humoral immunity) of the tested immunomodulators in mice vaccinated with Brucella Rev-1 as compared with mice that were not treated with Neem extracts or mannoproteins. In this regard, The interferon-γ showed a significant increase ($P \leq 0.01$) serum level in immunomodulator-treated and -vaccinated mice in comparison with negative and positive groups, and again group VII showed the highest increase. The anti-Brucella antibodies assessed by indirect Immunoflourescent test also showed a significant increase titer in immunomodulator-treated and -vaccinated mice in comparison with negative and positive groups .

Conclusion: The aqueous and ethanolicNeem seed extract reported the highest enhancement in all immunological parameters employed in comparison with mannoproteins of Candida albicans cell wall.

Key words: Neem,Candida albicans, Brucella Rev-1,immunomodulatory.

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تأثيربذور نبات النيم و مانوبروتينز الجدار الخلوي للمبيضات البيضاء Candida albicansكمحفز مناعي للفئران الملقحة بلقاح البروسيلا-Brucella Rev-1

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الخلاصة

درس تأثير بعض المحورات المناعية المتمثلة بالمستخلصين المائي والكحولي لبذور نبات النيم (Azadirachtaindica) والمانوبروتينز (Mannoproteins) المستخلصة من جدار خلايا المبيضات (Candida albicans) على الاستجابة المناعية للفئران الملقحة بلقاح البروسيلا العترة Brucella Rev-1.ضمت الدراسة مجموعتين رئيسيتين(160فأرفي كل مجموعة) وأحتوت كل منها على ثمان مجاميع فرعية(20فأر في كل مجموعة) (I: معاملة بالماء المقطر، II: معاملة باللقاح Brucella Rev-1، III: معاملة بالمانوبروتينز، IV: معاملة بالمستخلص المائي للنيم، V: معاملة بالمستخلص الكحولي للنيم، VIو VII و VIII: معاملة بالمانوبروتينز والمستخلصين الما ئي والكحولي لنبات النيم، على التوالي ومن ثم باللقاح). أجريت جميع هذه المعاملات في اليوم الأول وأعطيت لقاح البروسيلا في اليوم الرابع وضحي بالحيوانات في اليوم 8 (المستوى المصلي للأنترفيرون جاما)، في اليوم 21 (عيارية أضداد البروسيلا). أعطيت المحورات المناعية تحت الجلد بجرعة معادلة لـ 10% من نصف الجرعة المميتة (المستخلصين الما ئي والكحولي لبذور نبات النيم:3.9086 ملغم/كلغم؛ مانوبروتينز5.7144ميكروغرام). وقد حقنت فئران المجموعة الثانية بمادة البريسلون (Prednisolone)بجرعة (5ملغم/كلغم) كمثبط مناعي قبل أجراء المعاملات الأنفة الذكر والتي أجريت في حيوانات المجموعة الرئيسية الأولى. أظهرت النتائج تأثيرات واضحة للمحورات المناعية المستخدمة في الدراسة وفي المجاميع الممنعة بلقاح البروسيلا ومن خلال تحسن الاستجابة المناعية غير النوعية والمناعة الخلطية مقارنة مع المجاميع غير المعاملة بالمحورات المناعية، وأشارت النتائج إلى ارتفاع واضح في المستوى المصلي لأنترفيرون جاما في المجاميع الممنعة والمعاملة بالمحورات المناعية بالمقارنة بمجاميع السيطرة السالبة والموجبة وسجلت أيضا المجموعة VII أعلى مستوى. وعند قياس عيارية أضداد البروسيلا بواسطة الوميض الاشعاعي غير المباشر، أظهرت المجاميع الممنعة والمعاملة بالمحورات المناعية ارتفاع مستوى هذه الأضداد مقارنة بمجاميع السيطرة السالبة والموجبة وبفوارق إحصائية معنوية.

مفاتيح الكلمات: النيم ,للمبيضاتالبيضاء , بلقاحالبروسيلا ,كمحفزمناعي

Introduction

Understanding the immune system may help in improving immunization protocols inhuman, as well as, animals to develop vaccines, which play an important role in the preventive medicine and provide a rational basis for devising new therapeutic strategies for immune mediated diseases (1). In this regard, immunomodulators are plants and plant products, or biological materials that mediate the effectors mechanisms of the immune system through immune stimulation to a given antigen or potentiate the effectiveness of a vaccine (2). Scientists have began to adopt vaccine strategies that are based on the maximization of antigen presentation for major histocompatibility complex (MHC) class I or class II molecules due to the importance of these molecules in immune response, especially those materials that act as immunomodulators (1).Materials of fungal and/or plant origins have been the interest of different investigators around the globe with their aims to establish the immunomodulator potentials of these materials. Some risks associated with attenuated or killed whole-organism vaccines can be avoided with vaccines that consist of specific purified macromolecules derived from pathogens or in combination with plant materials (2,3and4).The plant extracts, derivatives or their products, have also been the interest of investigators as immunomodulators to overcome the disadvantage of biological and chemical immunomodulators. One of these plants is Azadirachtaindica, which is more popular with the name neem, and has the advantage to

be a medicinal plant with a wide range of applications in folkloric medicine (5). Furthermore, recent investigations demonstrated several biological and pharmaceutical potentials; for instance, anti-viral, anti-bacterial, anti-parasitic, anti-cancer and immune stimulant properties of the Neem (4, 5, 6, 7, 8, and 9). Brucellosis is one of the most wide spread infectious disease in the world that causes fetal death as a single agent in human and animals being. The disease is widely distributed in different countries of the world among humans and animals, and there is a positive correlation of infection between animal and human populations (3). Until now there is no effective available vaccine for protection against brucellosis, although there have been many trials to use combinations of immunomodulators and vaccines to immunpotentiate the immune mechanism in recipient animals (8, 10, 11, 12,13,14,15 and 16). In agreement with such scope, the present study came to add some understanding about the role of biological (Candida albicans cell wall mannoproteins) and a medicinal plant (neem seed aqueous and ethanolic extracts) materials in potentiating the immune response (immunomodulators) against brucellosis in mice vaccinated with Brucella Rev-1 vaccine .

Materials and Methods

All experiments was done on 320 male and female albino mice (Balb-c).Their age range at the start of experiments 6-8 weeks. They were housed in bio-clean hoods at 20-25ºC with light: dark periods of 14:10 hours. They had free access (*ad libitum*) to food (standard pellets) and water, and their average weight was 22 ± 3 grams at the start of experiments. Before carrying out the experiments, the mice were left in separate cages for one week to experience the acclimatization period. The study was conducted on two main groups(160 mice for each group), Each group was divided into eight subgroups 20 mice for each (I: treated with distilled water, II: treated with the *Brucella* Rev-1 vaccine, III: treated with mannoproteins, IV: treated with neem aqueous extract, V: treated with neemethanolic extract, VI, VII and VIII: treated with mannoproteins, neem aqueous extract and neemethanolic extract, respectively, then they were vaccinated with *Brucell a* Rev-1). All these treatments were carried out on day 1 and then vaccinated with brucella Rev-1 vaccine on day 4 .Then the mice were tested as follows , on day 8 after vaccination(serum IFN-γ level), day 21 for (anti-*Brucella* antibody titer). The doses of both plant extracts and mannoproteins represented 10% of the calculated LD_{50} (neem extracts: 3.8096 g/Kg mannoproteins: 5.7144 mg/Kg), which were given subcutaneously. Mice of the second main group were injected with the immune suppressive drug prednisolone (5mg/Kg) 5 days prior to the treatments, which carried out on mice of the first main group. The following kits were used in the experiments of the study: Mouse IFN-γ ELISA quantitative determination (Bender Med Systems, Austria) / Rat anti-mouse IgG conjugated with fluorescin (Bethyl, USA) Two extracts of Plant Neem Seeds (aqueous and ethanolic) were prepared(10). The doses of both extract were prepared after the determination of the LD_{50} (17) .The Mannoproteins were prepared from the cell wall of a *Candida albicans* isolate (18). The isolate, which was obtained from the vaginal swab of a healthy woman, was supplied by the Central Health Laboratory (Iraq/Baghdad). The *C. albicans* sample was maintained on yeast extract peptone glucose agar supplemented with amino acids (18) .The dried lyophilized seed of *Brucella*Melitensis Rev-1 strain was supplied by the Central Veterinarian Laboratory Iraq/Baghdad, and this laboratory received the strain from the Food and Agriculture Organization. Indirect Fluorescent Antibody Test (IFAT) .The IFAT was used to assess anti-*Brucella* antibody titer in the sera of mice that were immunized with *Brucella* Rev-1 vaccine in different treatment regimens (3). The procedure of WHO was adopted to determine such titer. Quantitative Determination of Interferon-γ Serum Level was carried out using a mouse IFN-γ ELISA kit (Bender Med Systems, Austria), which is an enzyme-

linked Immunosorbent assay for quantitative detection of murine interferon-γ (IFN-γ) in murine serum. The values of the investigated parameters were given in terms of means ± standard errors (S.E.), and differences between means were assessed by analysis of variance (ANOVA), least significant difference (LSD) and Duncan test, using the computer programmer SPSS (Statistical Package of Social Sciences) version 7.5. The difference was considered significant when the probability value was equal or less than 0.05. A further estimation was also given; it was treated efficiently (19), which were calculated according to the following equation:

Treatment efficiency (%) = $\left(\frac{A - B}{B}\right) \times 100$ A=Treated groups, B=Negative control group.

Results and Discussion

After the tabulated procedures and calculations presented in this study, it was found that the LD_{50} of *C. albicans* cell wall mannoproteins was (5714.4 µg /kg), while the corresponding LD_{50} for neem seed aqueous or ethanolic extract was (3809.6 mg/kg). Based on these findings, 10% of the $LD_{50}$of each material (mannoproteins: 5.7144mg/kg/bodyweight; aqueous or ethanolicneem extract: 3.8096mg/kg/body weight) was considered as the immunomodulator dose in the study (17). The sera of animals in groups I, III, IV and V of both treatments (with or without prednisolone treatment) showed no anti-*Brucella* antibodies at the start titer 1:16, while the other groups showed some variations. In mice of group II of both treatments, three mice out of four (75%) showed a positive Immunoflourescent reaction at the titer 1:32, while in mice without prednisolone treatment of groups VI, VII and VIII, a positive reaction was observed at the titer 1:64 (50, 75 and 100%, respectively).The latter groups of mice with prednisolone treatment showed 100% positive reaction at the titer 1:32 (Table-1).

Table -1: Anti-*Brucella* antibody titer by indirect Immunoflourescentin sera of treated mice.

Groups	Number of Mice with a Positive Anti-*Brucella* Antibody Titer / 4 Animals									
	Without Prednisolone Treatment					With Prednisolone Treatment				
	1:16	1:32	1:64	1:128	1:256	1:16	1:32	1:64	1:128	1:256
I	0	0	0	0	0	0	0	0	0	0
II	4	3	0	0	0	4	3	0	0	0
III	0	0	0	0	0	0	0	0	0	0
IV	0	0	0	0	0	0	0	0	0	0
V	0	0	0	0	0	0	0	0	0	0
VI	4	444 4	2	0	0	4	4	0	0	0
VII	4	4	3	0	0	4	4	0	0	0
VIII	4	4	4	0	0	4	4	0	0	0

Anti-brucella antibodies showed an increased titer in all immunized groups treated with the immunomodulators used in the study, especially group V111 (Brucella vaccine + ethanolicneem extract solution) as compared to the control group that received vaccine only. Such observation suggests that the immunomodulation involved the humoral immune response, although the pathway may be through the modulation of macrophages and T lymphocytes as both types of cells are required to enhance the B lymphocytes to produce immunoglobulin (1). Such findings came to confirm previous results reported by (8, 6, 16, 17 and 18). Also the results agreed indirectly with (6), who demonstrated that mice and rats immunized with breast tumor antigen (BTA) and a neem leaf preparation (NLP) have a higher antibody response. They also suggested that the use of NLP in vaccination was involved in the induction of a T_H1 response, as evidenced by the enhanced secretion of IFN-γ and decreased release of IL-10 from spleen cells. The results of IFN-γ Serum Level were given in (table-2), while the treatment efficiency for each group of treated mice was presented in (figure-1). A significant increased serum level of IFN-γ was observed in groups IV, VI, VII and VIII (114.00 ± 22.90 , 130.00 ± 37.00, 170.00 ± 10.00 and 126.70± 1.76pg/ml, respectively) of mice with prednisolone treatment as compared to group I (43.33± 23.40pg/ml), and such differences were associated with treatment efficiencies of 163, 200, 292 and 192%, respectively. In mice with prednisolone treatment, similar results were obtained, and group VIII recorded the highest significant increased level of IFN-γ, but their values were significantly showed mean value significantly lower (P≤0.01)as a result of breakage down of the immune response by immunosuppressed drug prednisolone.

Table-2: Interferon-γ serum level by ELIZA in treated mice.

Groups	Interferon-γ Serum Level (mean ± S.E.; pg/ml)*		Probability**≤
	Without Prednisolone Treatment	With Prednisolone Treatment	
I	43.33 ± 23.40 [c]	24.67 ± 4.40[c]	0.01
II	85.00 ± 14.00[bc]	74.67 ± 2.85[ab]	0.01
III	44.67 ± 14.70 [c]	29.00 ± 11.53	0.01
IV	114.00 ±	91.33 ± 14.7 [a]	0.01
V	44.67 ± 17.30 [c]	38.33 ± 15.3[bc]	0.01
VI	130.00 ±	88.00 ± 19 [a]	0.01
VI	170.00 ± 10.00	80.67 ± 4.9 [a]	0.01
VI	126.70 ± 1.76[ab]	73.67 ± 11.7[ab]	0.01

***Different letters: Significant difference (P≤0.05) between means of the same Colum **The comparison is between means of the two columns (horizontal comparison).**

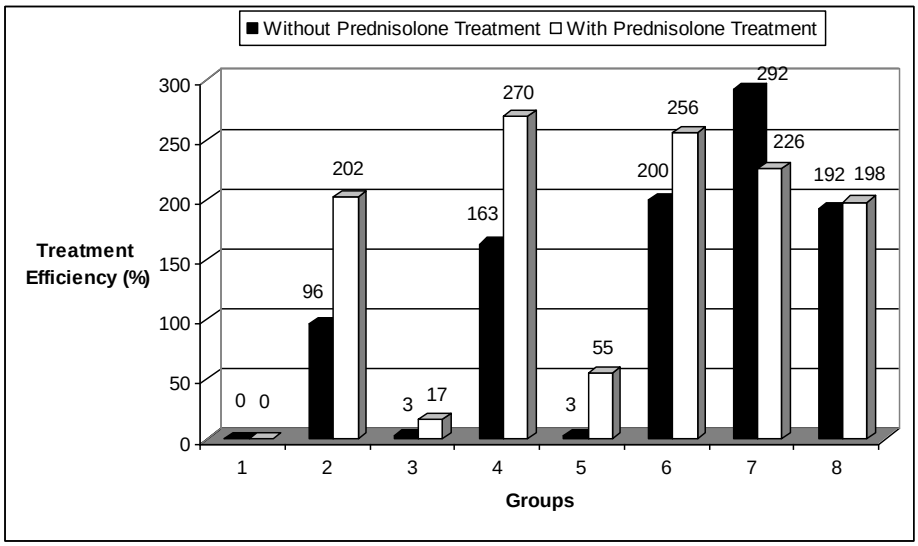

Figure 1: Treatment efficiency of IFN-γ serum level in treated mice

One of the cytokines is IFN-γ, which was evaluated in the present study through different experiments in regard to *Brucella* vaccination and immunomodulators (mannoproteins and neem extracts). The results demonstrated a significant increased serum level of IFN-γ in mice treated with the tested immunomodulators especially groups without prednisolone treatment because of the immunosuppressed effect of prednisolone. Such findings highlight the importance of ethanolic and aqueous neem seed extracts, as well as, mannoproteins of *C. albicans* cell wall as immunomodulators, especially when we consider the immunological importance of IFN-γ in enhancing the cellular immune response, which is important in controlling *Brucella* infection. However, the pathway by which these materials can act as immunomodulators, especially for the neem extracts, is not well understood, but investigators interested in the immunomodulation of neem extracts suggested that such extracts are rich in chemical constituents that may act positively on the immune system and enhance the immune response (5, 7, 8, and 16). Furthermore, these investigators agreed that the action of neem extracts is a cytokine-mediated activation, and the cytokine most often implicated is IFN-γ, which enhances both oxygen-dependent and oxygen-independent killing mechanisms in phagocytes. A further inspection of this implication has been recently addressed, in which neem leaf glycoprotein (NLGP)-mediated immune activation and associated immune polarization was investigated, and NLGP-induced activation was reflected in up regulation of early activation marker CD69 on lymphocytes, Monocytes, and dendritic cells. Activation is also denoted by CD45RO enhancement, with a decrease in CD45RA phenotype and CD62L (L-selectin). A also suggested that NLGP-activated T cells secrete a greater amount of the T_H1 cytokine IFN-γ and a lower amount of the T_H2 cytokine IL-4. The antigen-presenting monocytes and dendritic cells are also involved through the up regulation carried out by IL-12 and tumor necrosis factor-α (1). The aqueous and ethanolicneem seed extract reported the highest enhancement in all immunological parameters employed in comparison with mannoproteins of *C. albicans* cell wall, with the exception of DTH parameter, in which the latter immunomodulators showed the higher level of immunomodulation. In recent years the understanding and importance of antigen-specific immune responses after vaccination has completely changed. In the past the focus for monitoring a vaccine-specific immune reaction was principally based on the humoral branch of the immune system, and the efficacy of vaccines, as assessed by the induction of protective immunity was mainly correlated with antibodies and antibody titers. However, this correlation is often failed and other parts of

the immune system have also to be considered; namely the innate immune system and the cellular branch of the antigen-specific immune system. The innate immune system plays its main role in the effective activation of the antigen-specific immune response, in antigen-uptake and antigen-presentation. Furthermore, in order to achieve an effective vaccination, the activation of all T-cell subpopulations is of advantage, but more important is the generation of antigen-specific memory T and B lymphocytes(1and 2).

Conclusion, that the neem seed extracts (aqueous and ethanol) and the cell wall mannoproteins of *C. albicans* might be a potential immune adjuvant for inducing active immunity against *brucella*, and may act as Immunopotentiators through increasing microsomal proteins. These proteins have a binding activity to antigens, and such binding helps in extending the half-life of the antigen by a gradual release of it over a long period

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Study the Pathological Effects of the Combination of Estrogen and Progesterone Hormones on Some Organs Experimentally Induced in Mice

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Summary

The aim of this study was design to investigate the pathological changes for one month after therapeutic and toxic doses of subcutaneous injection of estrogen and progesterone combination hormones in mice, on the target organs testis and epidydimus in males and uterus and ovary in females. As well as the effects on non-target organ of Brain, liver spleen, intestine, stomach, kidney and lung in both sexes. The results showed sever pathological changes in male's testis and epidydimus and in females, uterus and the ovary. It is characterized by some pathological changes in toxic group less severity than in the therapeutic group.

Also, in non-target organs brain and spleen of toxic group of males and females showed some pathological changes while therapeutic group almost appear normal. The liver and kidney were affected in both groups (therapeutic and Toxic) in males and females. Other organs like intestine stomach, Lung doesn't showed any change in both groups.

Keyword:-estrogen, progesterone, toxic, therapeutic.

دراسة التاثيرات المرضيه المستحدثه تجريبياً لخليط هرموني الاستروجين والبروجسترون على بعض الاعضاء في الفئران

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الخلاصة

هدفت هذه الدراسة الى التحري عن التغيرات النسجيه المرضية الناجمة عن حقن هرموني الاستروجين والبروجسترون في الفئران لمدة شهرتحت الجلد، تم أخذ الخصي والبربخ في الذكور والارحام والمبايض في الاناث وكذالك تاثيرها على الدماغ والكبد والطحال والامعاء والكلى و المعدة والرئة في كلا الجنسين . نتائج التجربة تشيرالى وجود تغيرات شديدة في المجموعة السمية, اما في المجموعة العلاجية كانت اقل حدة. اما الاعضاء الاخرى الغير مستهدفة كالدماغ، والطحال، والكبد، والرئة، والمعدة ،والامعاء، والكلى، فقد وجدت التجربة وجود تغيرات مرضية في الدماغ، والطحال في المجموعة السمية في كلا الجنسين، الامر الذي لم يلاحظ في المجموعة العلاجية. اما الاعضاء الاخرى كالكبد، والكلية فقد وجدا متأثرين في كلا المجموعتين، وفي كلا الجنسين. بالنسبة للأعضاء الاخرى كالمعدة، والامعاء، والرئة فلم تظهر اية تغييرات نسجية.

مفاتيح البحث: استروجين, بروجسترون, الفئران.

Introduction

Steroid hormones are divided into five groups according to receptor which they bind, glurocorticoid, mineral corticoid, androgen, estrogen and progesterone. Steroid hormone help in control of the inflammation, immune function, salt, water and balance and development of sexual characteristic and ability to withstand illness and injury. These steroid hormones are generally synthesized from the cholesterol in the gonads and adrenal gland. These hormones are lipid soluble and they can pass through receptor (1).

Estrogen is steroid hormone produced in the ovary (2) and its known as hormone of female, its essential for development of accessory genital organ and

secondary sexual characteristic (3,4). The synthesis and secretion of estrogen stimulated by follicle stimulating hormone FSH which is in turn controlled by the hypothalamus gonad tropic releasing hormone (GnRH), high level of estrogen suppress the release of GnRH providing negative feedback control of hormone(2).

Progesterone is sex steroid hormone which is conjugated with estrogen to regulate the function of accessory sex organ during an ovarian cycle (5). It's secreted mainly from the corpus luteum in the ovary and placenta (3). Also was produced from the cortex of the adrenal gland in both male and female (6).

Smaller amount of progesterone are also produced in the testes and glial cells of the brain in both sexes (7). Progesterone receptors are located in the uterus and mammary gland in female (8). These receptors are found in the testis and prostate in male (9).

Progesterone prepare the uterus for the implantation of fertilized ovum and promote the secretary changes in the endmoetrium (3), and stimulate the endometrial gland to enlarge and increase secretion of water, salt and glycoprotein for the nutrition of embryo (10 and 11). On the other hand prevent the contraction of uterus during gestation by blocking the oxytocin receptor and still quiescent during the period of pregnancy (10) and suppress the immunity in order to prevent the rejection against embryo (12).

Materials and Methods

Thirty six mice from both sexes (18 males and 18 females) were used. The animals were diet locally and water was *adlibittum* a long the period of experiment 30 days. Estrogen hormone (Vetastrol by Abuaihan Pharmaceutical Co, Iran), 10 ml each ml contain 2 mg Oesteradiol benzoate, and progesterone (Vetagesterone, Abuaihan Pharmaceutical Co, Iran), 10 ml, each ml contain 25 mg progesterone were used in this experiment. The animals were divided into three equal groups as the following:-
Therapeutic group: - which contain 12 animals 6 males and 6 females which were received 16 mg estrogen and 20 mg progesterone (13). Toxic group: - which contain 12 animals 6 males and 6 females which were received 32mg estrogen and 40 mg of progesterone (13). Control group: which contain 12 animals 6 males and 6 females which are treated with sun flower oil only in the same manner.

Progesterone was prepared for injection after diluted with sun flower oil for subcutaneous injection (13) all animals were subcutaneous injection daily for one month. Animals were sacrificed by ether inhalation and post mortum examination was done for all the organs. The histopathological sample of 1-2 cm were taken from the lesion kept in formalin 10% for fixation processed routinely staining (Hematoxyllin and eosin stain) and all the lesion were be recorded.

Results and Discussion

The animals were sacrificed after one month of injection and histopathological examination of organs mention above recorded that: - The testis of male of toxic group showed loss of spermatogonia (Fig 1). While testis of therapeutic group were showed less severity represented by incomplete spermatogenesis with presence of multinucleated spermatid giant cell in the lumen of semineferrous tubules (Fig 2). The epidydimus of toxic group showed empty of sperms (Fig 3). The uterus of toxic group showed hyperplasia of epithelial lining cells of endimetrium with papillary projection extend to the lumen of the uterus (Fig 4).

Also other section showed eosinophilic homogenous substances appear in the lumen of endometrial gland (Fig5). While in the theruptuic group the lesion showed less severity characterized by moderat hyperplasia of epithelia and presence of protinicious exudates with poly morphonuclear cell and mononuclear cell in their lumen. (Fig 6).

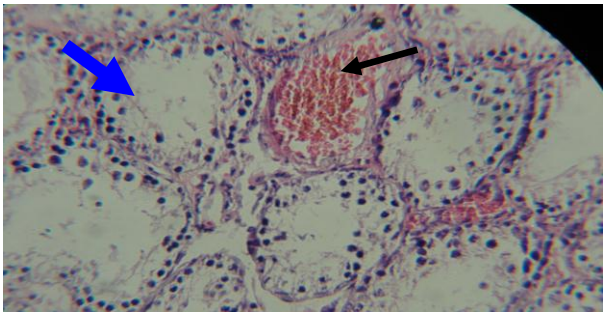
The ovary of toxic group showed increase number of primary and secondary follical with congestion of stromal blood vessels (Fig 7).

Other non target organ like brain, the toxic group showed focal glyosis in both male and female (Fig. 8) and prineuronal odema in other area (Fig 9). While the therapeutic group showed no clear pathological changes.

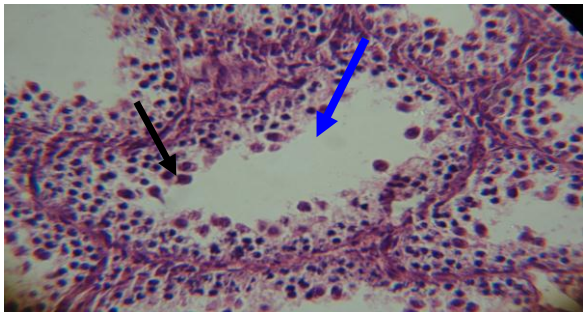
Spleen of toxic group of both male and female showed deplesion of white pulpe with increase number of megakaryocyte (Fig 10).While therapeutic group the spleen appear normal.

Liver of toxic group showed mononuclear cell aggregation in the portal area around bile duct and congestion of blood vesselse with necrotic area by pyknotic of nuclei or dispear (Fig 11). Therapeutic group: liver showed mononuclear cell aggregation in the portal area around bile duct with congestion of blood vessels (Fig 12).

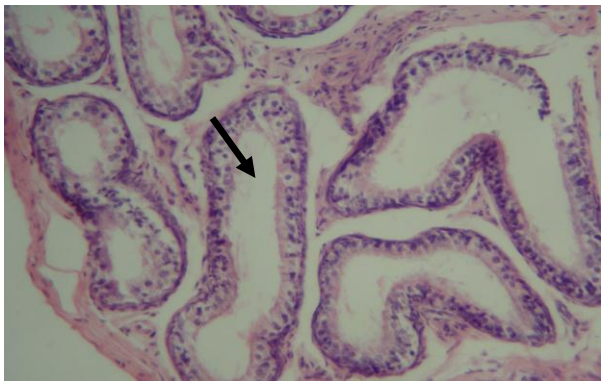
Kidney of toxic group showed hemorrhage of cortical renal tubules (Fig 13). Kidney of therapeutic group showed perivascular mononuclear cell aggregation (Fig 14).



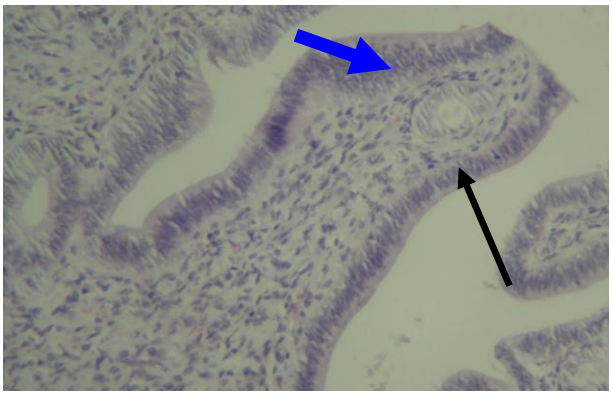
Figure(1): Histopathological section of testis of mice in toxic group treated with (estrogen and progesterone) showed testicular degeneration, and absence of spermatogonia (➡) and congestion of blood vessels(➡) after one month (H&E) 40x.



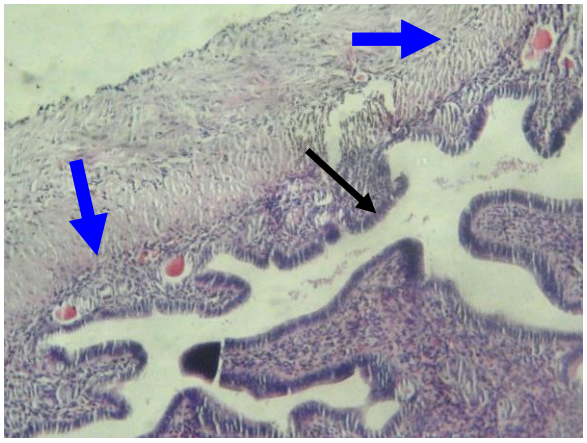
Figure(2): Histopathological section of testis of mice in therapeutic group treated with (estrogen and progesterone)showed incomplete spermatogenesis(➡) with presence of multinucleated giant cell in the seminiferous tubules(➡) after one month (H&E)40x.



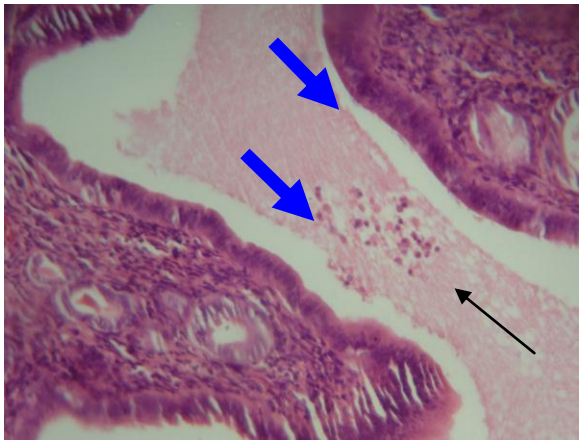
Figure(3): Histopathological section of epididymus of mice in toxic group treated with (estrogen and progesterone) appear testicular degeneration with empty and vaculation of epithelial lining cell(➡) after one month(H&E) 40x.



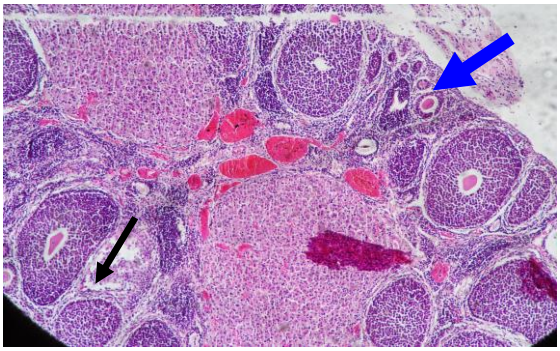
Figure(4): Histopathological section of uterus of mice in toxic group treated with (estrogen and progesterone) showed hyperplasia of epithelial lining cell of endometrium(➡) with papillary projection extend to the lumen(➡)(H&E) 40x



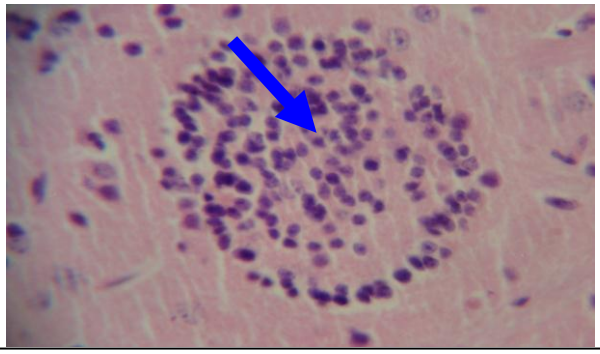
Figure(5): Histopathological section of uterus of mice in toxic group treated with (estrogen&progesterone)showed osinophilic haemogenous substances appear in the lumen of endometrial gland(➡) with papillary projection extend to the lumen (➡) (H&E) 20x.



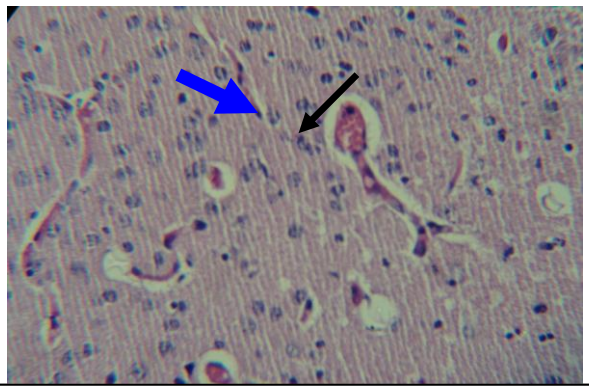
Figure(6): Histopathological section of uterus of mice in theraputic group treated with (estrogen and progesterone)showed protinicious exudate with polymorpho and mononuclear cell in their lumen and sub epithelial layer(➡) with moderate hyperplasia of epithelial lining of endometrium(➡) (H&E) 40x.



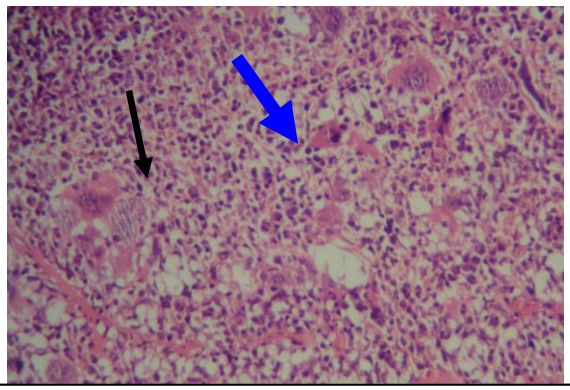
Figure(7):Histopathological section of ovary of mice in toxic group treated with (estrogen and progesterone) showed increase number of primary (➡) and secondary follical(➡) after one month .(H&E)40x.



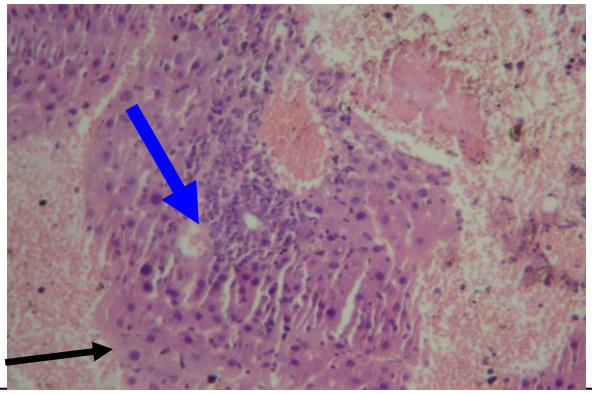
Figure(8):Histopathological section of brain of mice in toxic group treated with (estrogen and progesterone) showed focal glyosis (➡).(H&E)40x.



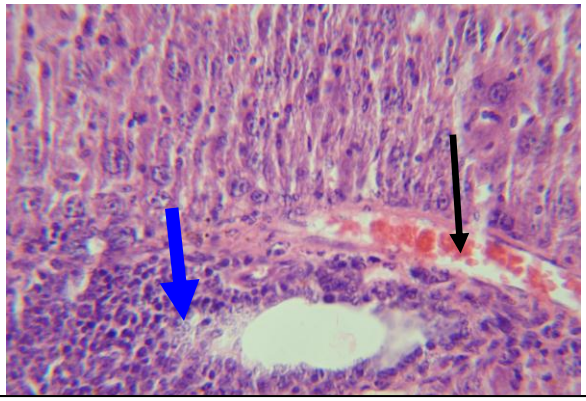
Figure(9): Histopathological section of brain of mice in toxic group treated with (estrogen and progesterone) showed privascular odema( ) with congestion ( ) after one month (H&E) 40x.



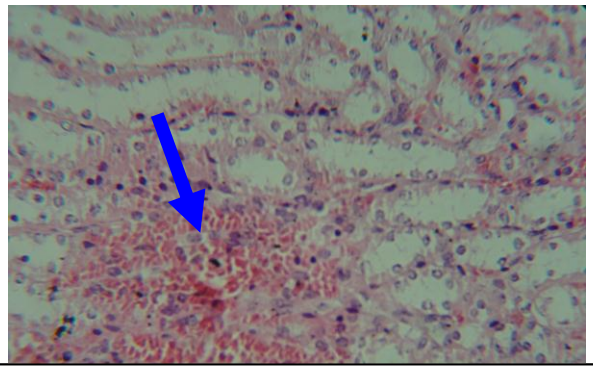
Figure(10): Histopathological section of spleen of mice in toxic group treated with (estrogen and progesterone) showed depletion of white pulpe ( )with increase number of megakaryocyte( ) after one month (H&E) 40x.



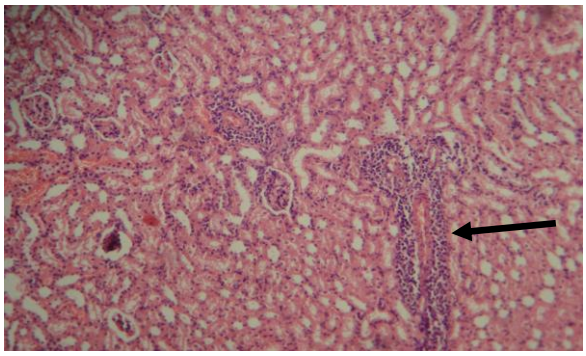
Figure(11): Histopathological section of liver of mice in toxic group treated with (estrogen and progesterone) after one month showed mononuclear cell aggregation in the portal area around bile duct( ) with necrotic area by pyknotic of nuclei or disappear( ) after one month (H&E) 40x.



Figure(12):Histopathological section of liver of mice in thereputic group treated with (estrogen and progesterone) showed mononuclear cell aggregation in the portal area around bile duct( ) with congestion of blood vessel( ) after one month (H&E) 40x.



Figure(13): Histopathological section of kidney of mice in toxic group treated with (estrogen and progesterone) showed haemorhagic of cortical renal tubule(➡) after one month (H&E) 40x.



Figure(14): Histopathological section of kidney of mice in theraputic group treated with (estrongen and progesterone) showed perivascular mononuclear cell aggregation after (➡)one month. (H&E) 40x.

The actions of steroid hormone are thought to be mediated through their binding to steroid receptor (14). Which is divided into two types α and β receptor (15). Present study are focus on the pathological effect of both hormones on the target and non – target organ on both male and female in mice.

The target organ of male represented by the testis in toxic group, the organ showed absence of spermatogenesis, this process result called azoospermia which mean complete absence of sperms. This result was in agreement with (16 and17) whom reach azoospermia after administration with estrogen also. This result was agreed with (18and 19). Which return this result to the effect of high dose of progesterone. They reported that the inhibition of the spermatogenesis may be result from the inhibition of LH and FSH hormones responsible for the activity of sertoli cell leads to suppression of spermatogenesis (azoospermia). Other group (therapeutic group) showed incomplet spermatogenesis with presence of spermatid multinucleated gaint cell, these cell indicating that there is testicular degeneration occurs in the semineferrous tubules of testis. These cells occur after spermatocyte degeneration which appears to be secondary change resulting from distrupt sertoli cell to germ cell association these result was in agreement with (20).

Epidydimus of the toxic group appear empty of sperms, this result occurred may be due to the high doses of both hormones.

Other target organ of the female of toxic group represented by the uterus, the lesion showed hyperplasia of epithelial lining cell of endometrium with papillary projection extend to the lumen of the uterus, this lesion consider preneoplastic change as many author think like (21), who consider the proliferative lesion of uterus epithelial like hyperplasia are preneoplastic (22). The effect estrogen may be occur as a result of progesterone due to high dose of this hormone and the papillary projection of endometrial epithelia lining cell with increase number of endometrial gland. These lesion said to be the classical response to progesterone (23) and progesterone cause increase dilatation of endometrial gland (16). These feature might be attributed to the high number of progesterone receptor present on the endometrial lining to which progesterone bind after diffusion through cell membrance and then bind to progesterone response element in the nucleus resulting in activation of mRNA transcription allowing increase division rate (24and25).

Proliferative phenomena have been assignees as uterine preneoplastic changes are lesion as some scientist (21and 22) mentioned.

In the therapeutic group which has less severity characterized by moderate hyperplasia of epithelid lining cell restricted to the superficial layer of the endometrium epithilia as (23) mention. Koss, (26) consider this lesion as a classical response of this organ to the stimulation caused by estrogen due to high number of estrogen receptor in it.

The ovary of toxic group showed increase number of primary and secondary follicles but never reach the maturation also no ovulation will occure. This occure due to elevated levels of estrogen also effect these growing follical inducing cystic dilatation of them. These result are strongly similar to the result of (16,26 and 27). In addition to that (28) which found that the follicular cyst in ovary of rats of their studies which were receiving environmental estrogen also they got complete absence of corpus luteum. While (29) thought that these follicals produces further estrogen and cause endometrial hyperplasia.

Other non- target organ like brain of toxic group of both male and female showed focal glyosis and perineuronal and perivascular odema. This is return to the presence of progesterone receptor in the glial cell (7). The high dose of progesterone in this group cause proliferation of glial cell (15). They consider progesterone receptor is gene transcription, also the presence of perineuronal odema may be a result from high dose of progesterone. Other author refers to the same thing they refer to the present of estrogen which induced in sizable quantities in the brain (30). While the brain of therapeutic group appear normal without any change.

The spleen of toxic group of male and female showed depletion of white pulpe with increase number of megakargocyte, this result agree with (31and 32). Who suggest that progesterone and estrogen cause inhibition cellular immunity by prolong exposure to both hormones (11). Revealed that progesterone cause depression of lymphoid tissue. Similarly to (33) which found that progesterone inhibit the activity of lymphocyte and monocyte.

The liver of toxic group of both sexes showed mononucleur of poly morphonculer cell aggregation in the portal area around bile duct with congestion of blood vessles with necrotic area characterized by pyknotic of nuclei or dispear while in therapeutic group the lesion showed mononuclear cell aggregation in portal area around bile duct with vacculation of hepatocyte, this occur duo to increase of fat level in the blood(lipidemia)duo to metabolism disturbance of fat and due to formation of lipoprotein in blood circulation (34). While the aggregation of inflammatory cell around the central vein return to the effect of progesterone which cause increase proliferation of leukocyte (35). While necrosis may occur duo to high dose (toxic effect) of both hormones.

The kidney of toxic group of male and female showed Hemorrhagic of cortical renal tubules, this result from high dose of both estrogen and progesterone. While in the therapeutic group the kidney showed preivascular mononuclear cell aggregation this associated with the stimulation of the steroid hormone (estrogen and progesterone).

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New Formulation of Trimethoprim Injectable Solution for Veterinary Use

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Accepted:15/11/2011.

Summary

The aim of this study was prepared new formulation of trimethoprim injectable solution in the Physiology and pharmacology department /College ofVeterinary Medicine, University of Baghdad.Trimethoprim injection formulation is antibacterial and used against a number of bacteria, protozoa and Rickettsia. Data was collected about the materials used in the preparation of the formula from the well-known pharmacopeia, including the specification, physical and chemical properties of active ingredient, the additive and preservative that must be used. Three pilot formulae were prepared from analar chemical ingredient from which one formula was chosen and tested to approve its quantitative and qualitative specification. Quantitative evaluation, stability of the formula was also tested under different storage environmental condition of low and high temperature at different periods through and one year and through the questionnaire field. Questionnaire proved the product, stability and therapeutic efficiency. The composition obtained a certificate of acceptance from the Veterinary State Company and Veterinary Drug Research and production Centre as new preparation Formula of Trimethoprim Injectable Solution for Veterinary use.

***Key words*: Trimethoprim, Veterinary, therapeutic, bacteria, protozoa, Rickettsia**

تركيبة جديدة للترايمثوبريم محلول معد للحقن البيطـــري

عروبة محمد سعيد إبراهيم دريد عبد الهادي عباس
فرع الفسلجة والأدوية / كلية الطب البيطري ـ جامعة بغداد – العراق

الخلاصة

الهدف من هذه الدراسة هو تحضير تركيبة جديدة من الترايمثوبريم كمحلول معد للحقن قد تم تحضيره من قبل الباحثين في فرع الفسلجة والأدوية / شعبة الأدوية / كلية الطب البيطري - جامعة بغداد . مستحضر ترايميثوبريم/ حقن من المضادات البكتيرية ويستخدم ضد عدد من البكتريا والبروتوزوا والركتسيا. تم جمع بيانات حول المواد المستخدمة في إعداد الصيغة الدوائية المعرفة في دساتير الأدوية العالمية، بما في ذلك المواصفات والخصائص الفيزيائية الكيميائية للمواد الفعالة، فضلا عن إضافة المواد الحافظة. وقد أعدت ثلاث تركيبات تجريبية من المكونات الكيميائية والغرض منها الوصول إلى التركيبة النهائية المعتمدة والتي تم اختيارها بعد اختبارها وموافقتها للمواصفات الكمية والنوعية. التقييم الكمي تم اختباره مع استقرار الصيغة التركيبية تحت ظروف التخزين البيئية المختلفة من درجات الحرارة المنخفضة والعالية وفي فترات مختلفة خلال عام واحد. ومن خلال الاستبيان الحقلي الذي أجري على المستحضربرهن ثباتيته وكفاءته العلاجية. وقد حصلت التركيبة على شهادة القبول من الشركة العامة للبيطرة ومركز بحوث إنتاج الأودية البيطرية كمستحضر تركيبي جديد للترايمثوبريم للحقن وللاستعمال البيطري.

كلمات مفتاحية : ترايمثبريم، بيطري، علاجي، بكتريا، بروتوزا، ريكتسيا.

Introduction

Trimethoprim is a synthetic antibacterial combination product.That considered as bacteriostatic antibiotic, it belongs to the class of chemotherapeutic agents known as dihydrofolatereductase inhibitor. Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis oftetrahydrofolic acid. Which is an essential precursor in the *de novo* synthesis of the intermediate Thymidine monophosphate (dTMP), a precursor of DNA metaboliteThymidine triphosphate(1)

Bacteria are unable to take up folic acid from the environment and are thus dependent on their own synthesis. Inhibition of the enzyme deprivesthe bacteria of nucleotides which are necessary for DNA replication causing, in certain circumstances, cell lethality.Trimethoprim combination with Sulphadimidine antibiotic at 1:5 ratioinhibits an earlier step in the folate synthesis pathway **(figure 1)**

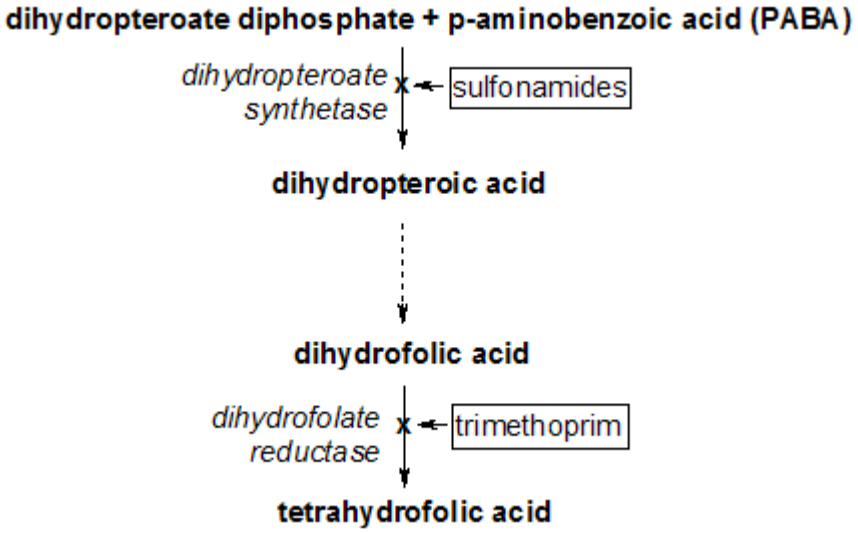

Figure1. Mechanism of action of trimethoprim

This combination, results in an in-vitrosynergistic antibacterial effect by inhibiting successive steps in folate synthesis. This claimed benefit was not seen in general clinical use (2). The combinations use has been declining due to reports of sulphadimidine or sulfamethoxazolebone marrow toxicity, resistance and lack of greater efficacy in treating common urine and chest infections, and side effects of antibacterial sulfonamides (3).

Sulphadimidine /trimethoprim injection is effective against susceptible strains of *Streptococcus pneumoniae, Escherichia coli, Haemophilusinfluenzae, Morganellamorganii, Proteus mirabilis,*indole-positive Proteus species (eg, *Proteus vulgaris*), Klebsiella species, Enterobacter species, *Shigellaflexnerii*, and*Shigellasonnei*. These agent is labeled for the treatment of *Pneumocystis jiroveci* (formerly *P. carinii*) pneumonia; enteritis caused by *S. flexneri* or *S. sonnei*; and severe or complicated urinary tract infections caused by *E. coli*, *M. morganii*, *Klebsiella* species, Enterobacter species, or Proteus species when oral therapy is infeasible and the organism is not susceptible to single antimicrobial agents (4).

The combination of Sulphadimidin and Trimethoprim acts synergistic and usually bactericidal against many Gram-positive and Gram-negative bacteria like *E. coli, Haemophilus, Pasteurella, Salmonella, Staphylococcus* and *Streptococcus spp.*

Gastrointestinal, respiratory and urinary tract infections caused by TMP and Sulphadimidin sensitive bacteria, *E. coli, Haemophilus, Pasteurella, Salmonella, Toxoplasmosis , Staphylococcus* and *Streptococcus* spp., in horse, foal, calves, cattle, pig (5).

Trimethoprim (Bp.): Trimethoprim, BW 56.72.2, 4-Diamino -5- (3,4.5-Trimethoxyben zyl) pyramidin. (14H18N4O3=290.3).A white odourless powder with a very bitter taste. M.P. about 200 soluble 1 in 2500 of water, 1 in 300 of alcohol, 1 in 55 of chloroform and 1 in 80 of methyl alcohol, practically in soluble in ether. A 1% suspension in water has a pH of 7.5 to 8.5.

Trimethoprim affects the nucleoprotein metabolism of cells similarly to pyrimethamine by interference in the folic – folinic acid systems. Its effects are

considerably greater on the cells of microorganism than on mammalian cells. Sulfonamides act on the same biological pathway at a different point and the effects of Trimethoprim and sulfonamides are synergistic. Trimethoprim has been used in the treatment of malaria and may be of value in infection caused by strains of malaria parasites resistant to pyrimethamine although. It is less effective than pyrimethamine against sensitive strain, for human it may be given alone usually in dose of up to 1.5gm. daily for 7 days. Or in conjunction with Sulfonamides, when a single 500mg dose. (6)

Sulphadimidine (BP.): Sulphadimidine (I.P); Sulphadimethyl pyrimidine, Sulphamethazine, Sulphamerthazine (U.S.P.) Sulphadimerazine, Sulphadimezium. (7)

Materials and Methods

-Materials and Chemical compounds:

Trimethoprim, Sulphadimidine, Sodium Hydroxide, Sodium Sulphite, Methylparaben, Propylparaben, Sodium Nitrite, Hydrochloric acid Chloroform, Anhydrous sodium sulphate, Prechloric acid, Crystal violet, and Glacial Acetic

- Apparatus :

Sensitive balance, Magnetic stirrer, Millipore filtration, pH meter, Different glassware (Separator funnel, Volumetric Beaker, Cylinder, volumetric flask, Conical flask, Stirrer, ptri-dish, brown color vial) Starch – Iodine paper Autoclave and Incubator

Procedure: Weighing the material to produce 100 ml. injectable solution formula. Trimethoprim 4.0gm., Sulphadimidin 20.0gm., Sod. hydroxide 4.5gm., Sod. sulphite 0.1 gm., Methylparaben 0.07 gm., Propylparaben 0.03gm., Ethylalcohol 99% 10 ml. ,and Distilled water add to 100 ml.

Adding distilled water in volume beaker up to 50 ml adding the above materials as following:

4.5 gm Sod. hydroxide with continuous mixing , 0.1gm Sod. sulphite with continuous mixing, 20gmSulphadimidinwith continuous mixing.

Mixing distilled water and ethanol up to 50 ml in volume beaker and then step wise add (4 gm) Trimethoprim with continuous mixing, mixing the step (b) with (c) until clear solution, Adjusting the pH by using the buffer, Filtration the final product by using paper (0.2)M., packing the final product in volume bottle of 100 ml., Send sample to the quality control for estimation.(8,9,10,11,12). Table (1,2)

N.B. all step done under complete sterilized technique and under U.V light cabinet.

Composition: Each l00 ml contain

-Trimethoprim 4 gm

- Sulphadimidine 20 gm

Table 1: Quality Standard of Sulphadimidin / Trimethoprim Injection 15 February 2009

Quality Standard according to BP2002	
Moisture	**≤2%**
PH	**10.0~11.0**
Color of Solution	**≤Y_4**
Related Matters	**≤0.5%**

Table 2: Specification of Sulphadimidine / Trimethoprim Injection

Description	**clear brown solution**
Acidity or alkalinity	**pH 10 – 11(Quality Standard BP2002)**
Package	**l00 ml**
Storage	**under 25C^{o}**
Content of trimethoprime	**(95 –110) %**
Content of Sulphadimidin	**(95 –110) %**

Dosage: For intramuscular administration:
Horse, cattle : 0.15 ml/ kg body weight,
Calves, goats, sheep, foal and swine: 0.2ml / kg body weight
Dog and cat : 0.3ml / kg body weight
Once or Twice daily for 2-3 days
Withdrawal times: For meat: 12 days, For milk: 4 days.
Packaging: 10 ml/vial, 50 ml/vial and 100 ml/vial.
Assay:
1. Sulphadimidine:- The following steps was done

keep the temperature below 15C^{o}during all the procedure. Using volume equivalent to 0.5g. of Sulphadimidin in a conical flask. Add 75 ml, of water, and 10 ml, Conc. HCl, then mix and titrate with 0.1 M. Sod. nitrite until dark blue colour appears immediately after the addition drop of solution on starch–iodine paper. Each ml of 0.1).Sod.nitrite is equivalent to (0.02783)gm. of $C_{12}H_{14}N_4O_2S$ (7)

2. Trimethoprim:- The following steps was done

Transfering 10 ml, injectable solution to separator funnel and adding 5 ml, of 20% NaOH, that extracted three time with 30 ml, chloroform. Filter each extract chloroform layer through anhydrous sod. sulphate and collecting in 250 , conical flask. That keep for evaporate to dryness. Then dissolve it in 40 ml, glacial acetic and titrate with 0.1 N prechloric acid using crystal violet as indicator to the green – blue end point. Each ml. Of 0.1 N prechloric acid is equivalent to (0.0293)g mtrimethoprime(8).

The Stability of trimethoprim injectable solution (formula was stable) after storage at room temperatures and temperatures 50C^{o} with different time periods. Quantitative analysis was studied using (HPLC) high-performance liquid chromatography**.** Samples were also examined visually for color change or precipitation. More than 98% of the initial concentration remained after storage (9) (table 3)

Table (3): Chemical analysis of Active ingredient *Trimethoprim injectable solution formula*

Type of analysis	Temperature	pH	Active ingredient %	Date
Fix methods Trimethoprim Sulphadimidine	RT	10.2	90.96% 99.03%	
Zero time Trimethoprime Sulphadimidin	RT	10.4	100.0 % 98.03 %	14.1.2007
After 1 month Trimethoprim Sulphadimidine	RT 50 c^o	10.0	100.1 % 98.05 %	14.2.2007
After 6 months Trimethoprim Sulphadimidine	RT 50 c^o	10.0	100.5% 98.08 %	14.8.2007
After over 1 year Trimethoprim Sulphadimidine	RT 50 c^o	10.0	101.0 % 99.0 %	14.1.2008

RT: Room Temperature

Antimicrobial activity of trimethoprim injectable solution (Formula)

Measurement of antimicrobial activity:

The antimicrobial activity of trimethoprim formula has been measured by the use of agar well diffusion techniques. This technique done by takes plates of agar mixed with cultures of particular bacteria, *Staphylococcus aureus* being the most commonly used, *Corynbacterium, Clostridia,.E.coli, Klebsella, Shigella, Salmonella, Proteus, Brucella* and *Pasteurella*. Wells are cut into the agar and solutions of the test material applied at varying concentrations of trimethoprim formula, the diameter of the zone of bacterial growth inhibition is measured after incubation of the plates at 37C^o for 24 hrs.(13, 14) (Table 4) .

Table (4): Diameter Zone inhibition (mm) of Trimethoprimat different concentration (ug/ml) against different microorganism

Microorganism	Concentration ug/ml				
	1.25	2.5	5	10	20
S. aureus	10 ± 0.2	12 ± 0.12	14 ± 0.3	18 ± 0.4	21 ± 0.12
Corynbacterium	9 ± 0.12	11 ± 0.02	13 ± 0.04	19 ± 0.4	23 ± 0.03
Clostridia	8 ± 0.2	10 ± 0.11	12 ± 0.11	18 ± 0.11	21 ± 0.04
E.coli	12 ± 0.3	13 ± 0.12	15 ± 0.3	19 ± 0.03	23.5± 0.20
Klebsella	10 ± 0.2	12 ± 0.02	14 ± 0.02	18 ± 0.04	20 ± 0.11
Shigella	11 ± 0.13	13 ± 0.10	15 ± 0.3	19.5 ± 0.4	23 ± 0.22
Salmonella	12 ± 0.4	14 ± 0.11	17 ± 0.01	20.5± 0.14	24 ± 0.02
Proteus	10 ± 0.2	12 ± 0.02	14 ± 0.3	18 ± 0.01	21 ± 0.01
Brucella	11 ± 0.11	13 ± 0.10	16 ± 0.02	21 ± 0.02	22 ± 0.11
Pasteurella	9 ± 0.10	10 ± 0.11	12 ± 0.3	16.5± 0.01	19 ± 0.02

While the Specification of final product of Trimethoprim, Sulphadimidin, Sod. Hydroxide and Sod. Sulphite showed in table (5)

Table (5): Specification of final product

Substance name	Specification	Description	Solubility
Trimethoprim	**BP 20l0**	**White, odourless powder with a very bitter taste.**	**Soluble 1 in 2500 of water 1 in 300 of Alcohol 1 in 55 of chloroform 1 in 80 of methyl alcohol Practically soluble in ether**
Sulphadimidin	**BP 20l0**	**White or creamy-white crystals or powder, odourless or almost odourless**	**Very slightly soluble in water Soluble in 120 part of water**
Sod. Hydroxide	**BP 20l0**	**White sticks, pellets fused masses, scales, dry hard brittle and showing a crystalline**	**Completely or almost completely soluble in 7 part of water**
Sod. Sulphite	**BP 20l0**	**White, crystalline powder, odourless or almost odourless**	**soluble in water Practically soluble in ether**

Results and Discussion

The results of evaluation (table 1) of the effectiveness and stability of the formula product at room temperature and at time zero, immediately after the preparation without storage, indicated that the effectiveness was very good for active ingredient and was stable during the last six months of follow-up (table 3). When the product stored at room temperature and 50C^{o}, observed an increasing in the pharmacological activity of the preparation, this was noted when an older drug, and this determines the old of product at a temperature not exceeding 25C^{o} and that old of the product up to (1) year (15) . The stability and quantitative analysis of trimethoprim in this solution after storage at room temperatures and 50^{o}C also studied, by using high-performance liquid chromatography (table 3). Samples were also examined visually for signs of changing in color or precipitation. More than 98% of the initial concentration remained after storage at 50^{o}C, for 160 days. Examination of the stability data suggested that trimethoprim degradation was a zero-order process, although a first-order process could not be excluded.

Extrapolation of data from a logarithmic plot yielded a zero-order trimethoprim degradation rate constant at 25C^{o} of 0.0113 day-1. (16)The time for 25% trimethoprim degradation at 25C^{o} would be 845 days. No precipitation was observed, clear brown solution during storage. The extent of color change was associated with the degree of trimethoprim degradation. Trimethoprim, when prepared in the non aqueous solution, is stable at 25 C^{o}. and antimicrobial activity of trimethoprim after storage at room temperatures and 50 C^{o} was nearly stable for different time period. The preparation of trimethoprim injectable solution as antibacterial formula can be used against a number of species of bacteria, protozoa and Rickettsia in particular and other cases in general(10, 12, 17).

Trimethoprim (3,4,5-trimethoxybenzylpyrimidine) inhibits dihydrofolic acid reductase 50,000 times more efficiently in bacteria than in mammalian cells. This enzyme reduces dihydrofolic to tetrahydrofolic acid, a stage in the sequence leading to the synthesis of purines and ultimately of DNA. Sulfonamides and trimethoprim each can be used alone to inhibit bacterial growth. If used together, they produce sequential blocking, resulting in a marked enhancement (synergism) of activity. Such mixtures of sulfonamide

(five parts) plus trimethoprim (one part) have been used in the treatment of pneumocystis pneumonia, malaria, *shigella enteritis*, systemic salmonella infections, urinary tract infections, and many others. *Streptococci, Staphylococci, Corynbacterium, Clostridia, E. coli, klebsella, Salmonella, Proteus, Brucella and Pasteurlla* (11, 13, 18 and19).

Pyrimethamine also inhibits dihydrofolatereductase, but it is more active against the enzyme in mammalian cells and therefore is more toxic than trimethoprim. Pyrimethamine plus sulfonamide or clindamycin is the current treatment of choice in toxoplasmosis and some other protozoal infections (15, 16 and20,).

Sod. Hydroxide act to maintain the pH of the product. And prevent the degradation that gets during testing and after packing. The materials are part of a portfolio of product and the other solvent (21).

Clinical evaluation of the formula therapeutic effect was done in Central Veterinary Hospital of Baghdad in treatment of diseased sheep. The formula approved its activity as listed in the attached evaluation certificate, also the formula stability and quantitative evaluation was done inthe Veterinary quality control and approved its specification. All these result was listed in the certificate of approval for the formula from Veterinary State Company and research center which accept the formula as new preparation for Veterinary therapeutic use.

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Evaluation of selected parameters of rat liver injury following repeated administration of oseltamivir for different periods

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Summary

The effects of oseltamivir administration, an anti influenza viruses A and B, on some functional parameters of rat liver were investigated, to evaluate the possible hepatotoxic effect. Eighteen (18) wister male albino rats with body weight ranged 150-190 gm were divided into three groups, the first group(T1) was treated orally with 1mg/kg.BW as therapeutic dose of Oseltamivir for 7consuctive days. The second group (T2) was treated with the same dose for six weeks, while the control group dosed distill water. The results revealed, there was a significant increase in the onset of barbiturate sleeping time and a significant $p \leq 0.05$ decrease of the duration of barbiturate sleeping time of the T2 rats . The liver enzymes activity revealed a significant decrease in ALT in T1 rats and significant increased $p<0.05$ in the T2 rats, while the AST activity showed only significant increased $p<0.05$ in the T2 treated rats. The activity of ALP was $p<0.05$ significantly increased in the rats of treated groups. The blood sugar was significantly decreased $p<0.05$ only in the T2rats. Cholesterol level was significantly $p<0.05$ increased in T2 treated rats, while the serum of both treated groups showed a significantly increase $p<0.05$ in the triacylglycerol concentration.

The HDL level was significantly decreased $p<0.05$ only in theT1 rats. The treated T2 rats showed a significant decrease $p<0.05$ in the LDL, while the VLDL level revealed a significant increase $p<0.05$.The total serum protein level was significantly increased $p<0.05$ in the rats of T2. Liver histopathological lesions of the T1rats revealed large amount of suppurative exudates, severe dilation and congestion of central veins and sinusoids with activation of kupffer cells. The liver of T2 rat showed multiple areas of focal necrosis, fibrous thickening of Glisson capsule with vacuolar degeneration of hepatic parenchyma. In:conclusion, Oseltamivir has hepatotoxic effect in rats treated with therapeutic dose 1mg/ kg.BW. orally in different periods.

Key words:- Oseltamivir, Liver injury ,Rat.
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تقييم بعض معايير إصابة الكبد في الجرذان المجرعة لفترات مختلفة بعقار الأوسلتيمافير

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الخلاصة

تم تقييم بعض معايير وظائف الكبد في الجرذان المعامله بعقار الأوسلتمافير المضاد للأنفلونزا A,B.أستعملت 18 من ذكورالجرذان المهقاء بأوزان تراوحت150-190 وقسمت إلى ثلاث مجاميع بصورة متساوية وجرعت جرذان المجموعة الاولى(T1) بالجرعة العلاجية لعقار الأوسلتيمافير 1ملغرام / كيلوغرام من وزن الجسم لمدة سبعة أيام, أما جرذان المجموعة الثانية(T2) كانت فترة التجريع لمدة ستة أسابيع وبنفس الجرعة, أما المجموعة الثالثة فعدت سيطرة وجرعت ماء مقطر. وقد أظهرت النتائج,أن فحص وقت النوم المستحث بالثايوبنتال الصوديوم أظهر زيادة معنوية في فترة الكمون مع نقصان معنوي في فترة النوم $p\leq 0.05$ لجرذان المجموعة الثانية.أما فحص نشاط إنزيمات أذى الكبد فقد أظهرت نقصانا معنويا$p<0.05$ في نشاط ALTلجرذان المجموعة الأولى . في حين أظهرت جرذان المجموعة الثانية زيادة معنوية قي نشاط نفس الأنزيم وعلى نفس المعنوية.بينما سجل نشاط انزيم

AST زيادة معنوية $p<0.05$ في جرذان المجموعة الثانية, في حين سجل نشاط إنزيم ALP زيادة معنوية في كلتا مجموعتي المعالجة.و أظهر سكر الدم نقصانا معنويا $p<0.05$ في جرذان المجموعة الثانية. في حين كانت هناك زيادة معنوية $p<0.05$ في مستوى الكولسترول في مصل دم جرذان المجموعة الثانية ,أما مستويات الدهون الثلاثية فقد سجلت زيادة معنوية $p<0.05$ قي مجموعتي المعالجة بالعقار.أما فحص مستويات نمط الدهون البروتينية في الدم فقد أظهرت نقصانا معنوياً$p<0.05$ في مستوى الدهون البروتينية عالية الكثافة(HDL) في جرذان المجموعة الأولى ,وسجلت نقصانا معنويا $p<0.05$ في مستوى الدهون البروتينية واطئة الكثافة(LDL) في جرذان المجموعة الثانية , أ ما مستوى الدهون البروتينبة الواطئة الكثافة جدا(VLDL) سجلت زيادة معنوية $p<0.05$ فقط في جرذان المجموعة الثانية أما فحص مستوى بروتين مصل الدم أظهر زيادة معنوية$p<0.05$ في جرذان المجموعة الثانية .

الفحص النسجي المرضي للكبد :- أظهر نضح تقيحي , توسع واحتقان الأوردة المركزية والجيبيات ونشاط خلايا كفر في جرذان المجموعة الأولى , أما أكباد جرذان المجموعة الثانية فقد أظهرت بقع تنخرية متعددة , تثخن ليفي لمحفظة كلايسون مع تغيرات فجوية في الخلايا البرنكيمية.تستنتج الدراسة ان لعقار الأوسيلتمافير تأثير سمي في أكباد الجرذان المعاملة بجرعة علاجية 1ملغم/كغم من وزن الجسم ولفترات مختلفة.

الكلمات المفتاحيه: الاوسلتيمافير ,الجرذ , الاصابات الكبد .

Introduction

Oseltamivir is a potent and selective inhibitor of the neuraminidase enzyme of the influenza viruses A and B(1),and it is used in the treatment and prophylaxis of both influenza viruses(avian and swineinfuenza) (2). Oseltamivir is rapidly hydrolyzed to its active form oseltamivircarboxylase (Ro 64-0802), by human carboxylesterase 1(hCES1) in the liver (3). After oral administration, oseltamivir is readily absorbed from the gastrointestinal tract. The active metabolite is detectable in plasma 30 minutes after dosing (4). Oral oseltamivir has generally been well tolerated in patientswith influenza given the standard oral dosage of 75mg bid. Nausea (9.9%) and vomiting (9.4%) were the mostfrequently reported adverse events. Similar results were seenin children. Other events such as diarrhea, bronchitis, abdominal pain, dizziness and headache were seen in lessthan 7% of recipients. Gastrointestinal events generally appear within the first one to two days and resolve thereafter (5). The oseltamivir ethyl ester is well absorbed and rapidly metabolized to active oseltamivir carboxylate. The bioavailability of the oseltamivir carboxylate is about80% after oral administration of the prodrug (6 and 7).The liver is prone to xenobiotic – induced injury because of its central role in xenobiotic metabolism., its portal location within the circulation, and its anatomic and physiologic structure (8).Drug –induced liver injury (DILI) is a major health problem that challenges not only health care professional, but also the pharmaceutical industry (9). Because of the significant patient morbidity and mortality associated with DILI, the USA Food and Drug Administration (FDA) have removed several drugs from the market (10).This work is set out to provide information on the effect, if any, of repeated administration of oseltamivir at the required therapeutic dose 1mg/kg.BW on selected functional indices of rat liver. This was evaluated by monitoring some functional tests for rat liver. The aim of the study is to evaluate the possible hepatotoxic effect of oseltamivir in male albino rats.

Materials and Methods

Preparation of Oseltamivir solution by dissolving one capsule-Oseltamivir (Flufly)® capsule 75 mg.(Julphar-RasALkhimah-UAE) in 75 milliliters of distilled water to achieve concentration of 1mg/ml. Each 100 gm of body weight of rat dosed 0.1 ml of drug solution which contained 0.1mg of Oseltamivir as therapeutic dose.

Wister male albino rats with body weight ranged 150-190 gm were procured from the animal house of the College of Veterinary Medicine .University of Baghdad,

maintained in an air conditioned room (25±1)°C with a 12 hours light : 12 hours dark cycle. Standard pellet diet and water were provided ad –labium (11).

Barbiturate sleeping time :- eighteen rats divided into three equal groups(T1, T2, control) subjected to barbiturate induced sleeping time test which performed according to the method of(12).The T1 group dosed 1mg/kg.BW orally(by orogastric tube) as therapeutic dose of Oseltamivir for 7consuctive days. The T2 group also dosed the same dose but for six weeks, while the control group dosed distilled water. All groups injected IP with 25 mg/kg. Thiopentalsodium (THIOPENTAL) ® 1gm obtained from Egyptian international pharmaceutical industries CO A.R.E an ultra-short barbiturate. The time elapsed between the loss and voluntary recovery of the righting reflex was recorded as sleeping time. Sleep latency was also recorded.

Clinical study:- eighteen rats divided equally into three groups (T1, T2 and control) subjected to blood biochemical analysis. The both two treatment groups were dosed in the same pattern mentioned above, while the control group dosed distilled water. Blood collected through heart puncher post treatment from anesthetized animals of all groups by ketamine HCL (Ketamin10%)® obtained from KEPRO- HOLAND and xylazine HCL(XYLAZIN 2%) obtained from CEVA-GERMANY . The serum obtained by centrifugation of all blood samples with 5000rpm for five minutes.

All the animals of groups T1 and T2 were sacrificed at the end of dosage period .Livers obtained and preserved in 10% formalin saline, then sent to laboratory of medicinal city hospital for histopathology processing according to (13).

Blood biochemical analysis:-The activities of aspartateaminotraseferase (AST), alanineaminotranseferase (ALT) and alkalinephosphatase(AP) assessed by using commercial available kit (RANDOX- UK). The serum cholesterol was assessed by using commercially kit (Biomaghreb), which is enzymatic colorimetric test depending on hydrolysis and oxidation (14, 15, and 16).Serum triacyglycerol were estimated by using diagnostic kit (BIOLAB SA – FRANCE), depending on the formation of colored complex (quinoneimine) (17). HDL, LDL and VLDL were assessed by using commercially kit (Biomagreb) depending on precipitation of LDL, VLDL and chylomicron fractions (18 and19). Total serum bilirubin, direct bilirubin and indirect serum bilirubin were assessed by using available commercial kit (BIOLAB SA – FRANCE), which is depending on formation of azobilirubin(17) .Blood glucose was assessed by Trinder reaction (14).Which is depending on the formation of red quinonimine by using available commercial kit (LINEAR chemicals – SPAIN).Total serum protein assessed by biuret reaction, chelae formed between the Cu^{++} ion and the peptide bonds of protein in alkaline solution to form violet complex. Photometrically measured at 540 nm wave length (20).

Statistical analysis was conducted according to SPSS version 13.00. The ANOVA one way used for significances assessment between groups. The P – value <0.05 considered as statistically significant. LSD multiple range tests was carried out for comparing between means (21).

Results and Discussion

Barbiturate sleeping time:-There was significant increase $p \leq 0.05$ in the onset of barbiturate sleeping time of the animals that received therapeutic dose of oseltamivir for six weeks(T2) in comparison with the pretreatment onset of the same group and with both T1 treated group which received therapeutic dose of oseltamivir for one weekT1 and the control one which was received distilled water(table1),while the duration of barbiturate sleeping time revealed significant decrease $p<0.05$ in the same pattern of the onset time ,(table1).

ALT AST and ALP serum activity:-The liver injury enzymes activity revealed significant decrease $p<0.05$ in ALT of the animals of T1 treated group(9.16±0.70)U/L and significant increase in theanimals of T2 treated group(50.00±1.89)U/L in comparison with the animals of control group(18.8±1.35) (table,2). The AST activity showed only significant increase $p<0.05$ in the animals of T2 group (113.33±3.80)U/L in comparison with both T1 treated group and the control one. The activity of ALP were significantly increased $p<0.05$ in both treated group animals T1 (120.50±32.18)U/L and T2 (78.16±12.18)U/L in comparison with the control one (30.40±2.73)U/L,(table,2).

Random blood sugar: - The random blood sugar is significantly decreased $p<0.05$ only in the animals of T2 treated group (98.50±18.06)mg/dL in comparison with the animals of T1 treated group and the control one, (table 3).

The cholesterol level is significantly increased $p<0.05$ in the animals of T2 treated group (125.50±10.42)mg/dL in comparison with both T1 treated group animals and the control one .while the animals of both T1 and T2 treated groups showed significantly increase $p<0.05$ in the triacylglycerol level between them and in comparison with the control one (Table 4).

The result of HDL level is significantly decreased $p<0.05$ only in the animals of T1 treated group(27.00±2.86)mg/dL in comparison HDL level in the animals of both T2 treated group(45.50±7.20)mg/dL and the control one(40.50±2.68) mg/dL (table,5).The animals of T2 treated group showed significant decrease $p<0.05$ in the LDL (30.66±13.04)mg/dL in comparison with the LDL level of both T1 treated group(64.90±6.97)mg/dL and the control one(73.64±4.93)mg/dL(table,5). While the results of VLDL level revealed only significant increase $p<0.05$ in the animals of T2 treated group(63.66±0.61)mg/ dL in comparison with VLDL level in the animals of both T1 treated group(5.43±0.55)mg/ dL and the control one(6.14±0.75)mg/dL,(table,5).

The total serum protein level is significantly increased $p<0.05$ only in the animals of T2 treated group(620.00±18.38)mg/dL in comparison with its level in the animals of both T1 treated group(278.66±9.10)mg/dL and the control one (327.40 ±40.29) mg/dL (Table,6).

The results of total serum bilirubin (T.S.B) level revealed significant decrease $p<0.05$ only in the animals of T2 treated group(1.15±0.17) mg/dL in comparison with its level in the animals of both T1 treated group(3.08±0.32)mg/dL and the control one(3.68±0.66)mg/dL(table,7),while the level of direct serum bilirubin revealed significant increase $p<0.05$ in the animals of T1 treated group(2.73±0.21)mg/dL in comparison with its level in the animals of both T2 treated group(0.66±0.17)mg/dL and the control one(0.74±0.15)mg/dL(table,7) , but there is a significant decrease $p<0.05$ in the level of indirect serum bilirubin of both T1 treated group animals(0.35±0.10)mg/dL and T2 treated group animals(0.33±0.14)mg/dl in comparison with control group(2.90±0.64)mg/dL(table,7).

The histopathological lesion in liver rats received therapeutic dose 1mg/kg.BW for one week revealed large amount of suppurative exudates contains large number of neutrophils in the pre-hepatic area (figure 1).There are severe dilation and congestion of central veins and sinusoids with activation of kupffer cells were also had seen (figure 2).Furthermore there is focal infiltration of mononuclear cells in the liver parenchyma with vacuolation of hepatocytes (figure 3). The liver of rats treated with one 1mg/kg.BW oseltamivir for six weeks revealed multiple areas of focal necrosis characterized by pyknosis or disappearance of nuclei of hepatic cells (figure 4).There is fibrous thickening of Glisson capsule with vacuolar degeneration of hepatic parenchyma(figure 5).In addition there is a slight fibrous thickening in the wall of central vein.

The barbiturate induce sleeping time is the one of the tests performed to evaluate liver toxicity. The sleep latency and the elapsing between the loss and voluntary recovery changes impose as measurement of liver toxicity. The increasing of the onset and decreasing of the duration of sleeping time in the animals ,that had dosed 1mg/kg.BW oseltamivir for six weeks may indicate liver injury ,which is confirmed by histopathological lesion of liver (necrosis, fibrous thickening of Glisson capsule, vacuolar degeneration of hepatic parenchyma .The liver is richest source of drug metabolism (22).Carboxylesterases are a class of enzyme that play important roles in the hydrolytic metabolism of drugs and other xenobiotics , patient with liver condition such as cirrhosis shows decreased capacity of hydrolytic biotransformation(23). It can say that the oseltamivir dosed for six weeks interfere with liver biotransformation of thiopental sodium and led to increase the onset and decrease the duration of sleeping time, and may through enhancing the hydrolysis pathway. The liver performs different kinds of biochemical, synthetic and excretion function, so the biochemical indices that had performed are useful parameters to indicate impairment of functional capability of the liver. There is no single test for measuring liver function, because all the functions of the liver are not equally or simultaneously affected in hepatobilliary disorder (24).

Aminotransferases (ALT, AST) and alkaline phosphatase tests are helpful screening tool to detect injury of hepatocyte. The significant increase serum activity of ALT, AST and ALP in the rats dosed 1mg/kg.BW oseltamivir for six weeks ,may be due to sever hepatic injury(necrosis , vacuolar degeneration).While the significant increase of ALT serum activity in the rats that dosed 1 mg/kg.BW for one week is may be due to mild liver injury(inflammation ,vacoulation).The elevation of AST and ALT serum activity is moderate and not severe ,because the elevation range (3-20) times considered as moderate in acute and chronic hepatitis ,autoimmune hepatitis and drug induced hepatitis(25).The decline in serum activity of ALT and AST of rats that dosed 1mg/kg.BW oseltamivir for one week, may indicate recovery of poor prognosis in fulminant hepatic failure(26and27).

Alkalinephosphatase is found histochemically in the microvlli of bile canaliculi and sinusoidal surface of hepatocytes (27). So the significant increase in ALP activity of rats dosed 1mg/kg. BW of oseltamivir for one and six week is due to the suppuration which is found in the livers. Rosalk and Mcintyre (27) found that elevation of ALP activity may be seen in infiltrative liver disease, abscesses, granulomatous liver disease and hepatitis .Oseltamivir has been associated with hepatitis and abnormal liver function testes(28). Wael and Mohamed (29) found that oseltamivir dosed with 2.2mg/kg.BW to rats for five days, caused acute liver toxicity, modest reduction in hepatic activity in both genders, but with elevation ALP activity.

The liver is consider the site of glycolysis and glucogenesis, so the estimation of blood sugar may be one of the helpful tool for detection liver injury .The significant decrease in random blood sugar in the rats dosed 1mg/kg.BW oseltamivir for six weeks maybe an indicator of the liver damage, which may resulted from the repeated exposure to oseltamivir, in comparison with the rats exposed to oseltamivir for one week and rats of control group, which were have no blood sugar changes. Recent studies have shown that hypoglycemia is associated with many acute illnesses (30). The liver illness induced by oseltamivir is clearly appear in histopathological finding of rats treated for six weeks which represented by necrosis and vacuolar degeneration. About 80% of body cholesterol is produced by the liver, while the rest comes from food , after meal dietary cholesterol absorbed from the intestine and stored in the liver. The liver is able to regulate cholesterol activity in blood stream and can secrete cholesterol if it is needed by the body(31).Cholesterol is excreted by the liver via bile into digestive tract (32) .So the significant increase in the cholesterol and VLDL of rats dosed 1mg/kg.BW oseltamivir

is due to hepatotoxic effect of oseltamivir and/or its metabolite ,which represented by necrosis ,fibrosis and vacuolation. The increases in LDL-cholesterol also found in male rats which were administered 2.2mg/kg.BW oseltamivir for five days (29). We are assume that the significant increase of triacyglycerol, VLDL and decrease of HDL level may be due to shortage in lipoprotein lipase (LPL) production (enzyme that hydrolyzes triglycerides and conversion of VLDL to IDL then to LDL) , While the site of the LPL synthesis in the parenchymal cell(hepatocyte) (33).The shortage of LPL production is due to pathological effect of repeated therapeutic dose of oseltamivir for six weeks on liver .The important signs of liver dysfunction are elevated LDL ,reduced HDL and elevated triacylglycerol**.**

The serum protein test is one of the tests of the liver biosynthesis capacity (25). Chemotherapy drugs can be damage the liver as side effect, and it is one of the liver dysfunction causes. The liver dysfunction is one of important causes of total serum protein increasing. This concept is confirmed by the significant increase in serum protein level of the rats that had dosed 1mg/kg.BW of oseltamivir for six weeks. Albumin is the major protein in serum, Albumin synthesis is affected not only in liver disease , but also by nutritional status , hormonal balance and osmotic pressure(27).We are suggest that oseltamivir dosed to the rats as phosphate salt for six weeks ,may led to change the osmotic pressure due to high phosphate supplement with the drug, and resulted increase serum protein level. Where rats could not hydrolyze the oseltamivir phosphate to its metabolite (oseltamivir carboxylase) that will lead to accumulate extensive amount of phosphate this would negatively affect the calcium /phosphate ratio (34).

The bilirubin in the body is a careful balance between production and removal of the pigment in body, serum bilirubin is a test of the liver capacity to transport organic anions and metabolize drugs (25). Serum bilirubin could be lowered by drugs like salicylates, sulphonamides and free fatty acids which displace bilirubin from its attachment to plasma albumin (35). Parenchymal liver diseases or incomplete extra hepatic obstruction due to biliary canaliculi give lower serum bilirubin value (36).The decrease in total serum bilirubin level of the rats treated orally for six weeks with 1mg/kg.BW oseltamivir is may be due to the necrosis of hepatocytes and vacuolar degeneration of hepatic parenchyma which had observed in their's livers.

Oseltamivir induced liver injury in the rat treated with therapeutic dose for seven consecutive days and six weeks through infiltration of mononuclear cells and neutrophils in parenchyma , congestion of central vein ,which are considered as indicators of drug induced liver injury by larrey(37) who found ,the drug induced liver injury can affect both parenchymal and nonparenchymal cells of liver , including acute and chronic hepatitis, fibrosis, cirrhosis, cholestasis, steatosis, as well as sinusoid and hepatic artery /vein damage.

Direct hepatotoxicity is often caused by the direct action of a drug or more often reactive metabolite of drug against hepatocytes, Acute hepatitis is defined as marked increase in aminotraserferases coinciding with hepatocellular necrosis (38), these findings is compatible with histopathological changes that found in the rats of both treated groups which represented necrosis, Pyknosis and disappearance of hepatocyte nuclei, and confirmed with increases of the aminotraseferases serum activity . Hepatocyte stress and or/damage could result in the release of signals that stimulate activation of other cells , particularly that of innate immune system including kupffer cells (KC),natural killer (NK)cells, These cells contribute to the progression of liver injury by producing proinflammatory cytokines , such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ and interleukin(IL) IB produced during DILI are involved in

promoting tissue damage (39,40,41). We are also found there was activation of kupffer cells especially in the liver of rats treated with 1mg/kg.BW oseltamivir for six weeks.

We suppose that the inflammatory reaction(neutrophils and mononuclear cells infiltration, suppurative exudates) in the liver caused by oseltamiver dosed to the rats has an important role of its hepatotoxic effect, science Michael (38) found the damaged hepatocytes triggers the activation of the other cells ,which can initiate an inflammatory reaction and /or adaptive immune response .These secondary events may overwhelm the capacity of the liver for adaptive repair and regeneration , there by contributing to the pathogenesis of liver injury. The toxicities of oseltamivir phosphate to the rat liver were mild and considered of slightly increase in liver weigh,and plasma activity of glucose .chlestrol,total blood protein(34).These findings is not in agreement with present results of significant increase in cholesterol, blood glucose and total serum protein .

Table (1):-Barbiturate (Thiopental sodium) sleeping time /minute in rats dosed therapeutic dose 1mg/kg.BW of Oseltamivir for different experimental periods.

Group	Onset		Duration	
	Pretreatment M±SE	Post-treatment With oseltamivir M±SE	Pretreatment M±SE	Post-treatment With oseltamivir M±SE
T1 N=6	5.50 ±0.92 A a	3.33±0.55 A a	47.50±3.81 A a	48.33±6.01 Aa
T2 N=6	4.83±0.98 Aa	33.33±11.15 B b	56.33±8.02 A a	23.66±5.42 B b
C N=6	6.60±2.33 Aa	6.66±2.15 Aa	55.83±8.50 A a	55.60±8.20 Aa

-T1=therapeutic dose 1mg/kg.BWof oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks.C= dosed distilled water.
N=number of rats.
-The different capital letters mean the difference is significant between groups at activity P≤ 0.05.
- The differentsmall letters mean the difference is significant within group groups at activity P≤ 0.05.

Table (2):- The serum activity of liver enzymes (ALT , AST ,ALP) U/L in rats dosed orally therapeutic dose 1mg/kg.BW of oseltamivir for different experimental periods.

Group	ALT U/L M ± SE	AST U/L M ± SE	ALP U/L M ± SE
T1 N=6	9.16±0.70A	7.83± 0.40A	120.50± 32.18 A
T2 N=6	50.00±1.89B	113.33±3.80B	78.16±12.18B
C N=6	18.8±1.35C	10.60±1.77A	2.73 ± 34.40C

- T1=therapeutic dose 1mg/kg.BWof oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks.
-C= control dosed distilled water.
N=number of rats
-The capital letters mean the difference is significant between groups at activity P≤ 0.05.

Table(3):- Random blood sugar mg/dL of rats dosed orally therapeutic dose 1mg/kg.BW of oseltamivir for different experimental periods .

Group	Blood glucose mg/dL M ± SE
T1 N=6	139.00 ± 18.06 A
T2 N=6	98.50 ± 6.94 B
C N=6	132.40 ± 6.82 A

- T1=therapeutic dose 1mg/kg.BW of oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks.
-C= control dosed distilled water.
N= number of rats
-The capital letters mean the difference is significant between groups at activity P≤ 0.05.

Table(4):-The serum cholesterol mg/dL and triacylglycerol mg/dL of rats dosed orally therapeutic dose 1mg/kg.BW of oseltamivir for different experimental periods.

Group	Cholestrol(mg/dL) M ± SE	Triacylglycerol mg/dL M ± SE
T1 N=6	93.16 ± 2.45 A	56.50 ± 10.23 A
T2 N=6	125.50 ± 10.42 B	320.66 ± 11.36 B
C N=6	91.18 ± 6.28 A	30.84 ± 3.75 C

- T1=therapeutic dose 1mg/kg.BW of oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks
. -C= control dosed distilled water.
N=number of rats.
-The capital letters mean the difference is significant between groups at activity P≤ 0.05.

Table(5):- The serum HDL (mg/dL) , LDL (mg/dL) and VLDL (mg/dL) of rats dosed orally therapeutic dose 1mg/kg.BW of oseltamivir for different experimental periods.

Group	HDL mg/dL M ± SE	LDL mg/dL M ± SE	VLDL mg/dL M ± SE
T1 N=6	27.00±2.86 A	64.90±6.97 A	5.43±0.55 A
T2 N=6	45.50± 7.20 B	30.66±13.04 B	63.66± 0.61 B
C N=6	40.50±2.68 B	73.64±4.93 A	6.14± 0.75 A

- T1=therapeutic dose 1mg/kg.BW of oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks. .
-C= control dosed distilled water.
N=number of rats.
-The capital letters mean the difference is significant between groups at activity P≤ 0.05.

Table (6):- The total serum protein (mg/dL) of rats dosed orally therapeutic dose 1mg/kg.BW of oseltamivir for different experimental periods.

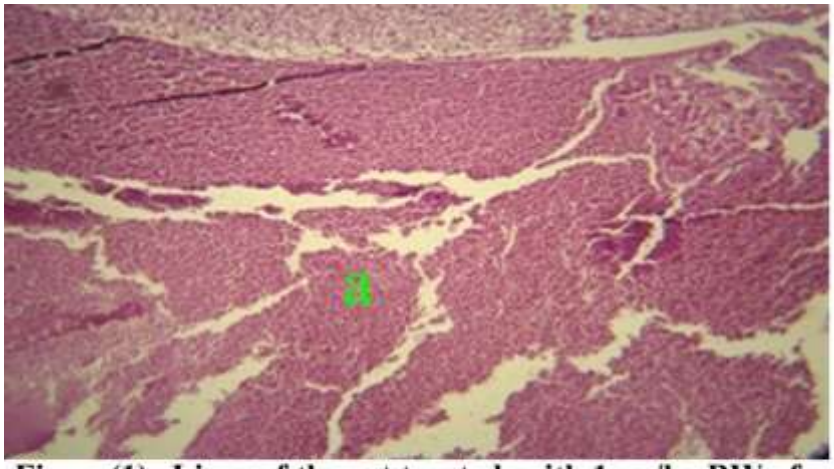
Group	Serum protein mg/dL M ± SE
T1 N=6	278.66 ± 9.10 A
T2 N=6	620.00 ± 18.38 B
C N=6	327.40 ± 40.29 A

- T1=therapeutic dose 1mg/kg.BW of oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks.
-C= control dosed distilled water.
N=number of rats.
-The capital letters mean the difference is significant between groups at activity P≤ 0.05.

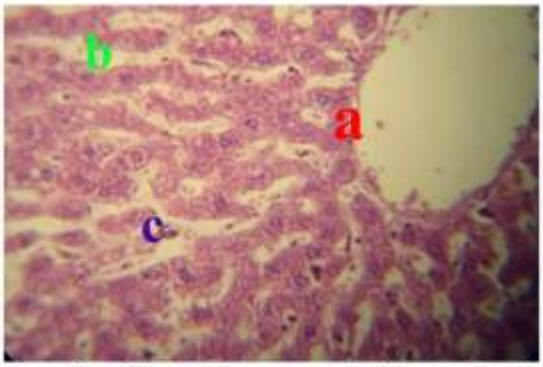
Table (7):- The total serum bilirubin (TSB mg/dL), direct serum bilirubin mg/dL and indirect serum bilirubin mg/dL of rats dosed orally therapeutic dose 1mg/kg.BW of oseltamivir for different experimental periods .

Group	T.S.B(mg/dL) M ± SE	Direct serum bilirubin(mg/dL) M ± SE	Indirect serum bilirubin(mg/dL) M ± SE
T1 N=6	3.08 ± 0.32 A	2.73 ± 0.21 A	0.35 ± 0.10 A
T2 N=6	1.15 ± 0.17 B	0.66 ± 0.17 B	0.33 ±0.14 A
C N=6	3.68 ± 0.66 A	0.74 ± 0.15 B	2.90 ± 0.64 B

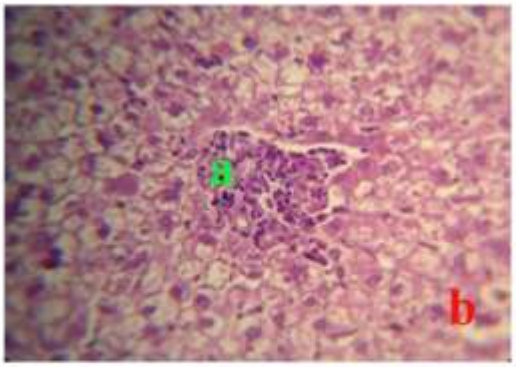
- T1=therapeutic dose 1mg/kg.BW of oseltamivir for one week.
-T2=therapeutic dose 1mg/kg.BW of oseltamivir for six weeks.
-C= control dosed distilled water.
N=number of rats.
-The capital letters mean the difference is significant between groups at activity P≤ 0.05.



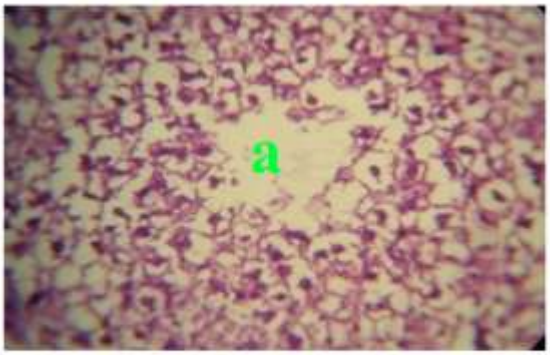
Figure(1):-Liver of the rat treated with 1mg/kg.BW of oseltamivir for one week shows deposition of suppurative exudates contain large numbers of neutrophils in the pre-hepatic area (a) (H&E 400X).



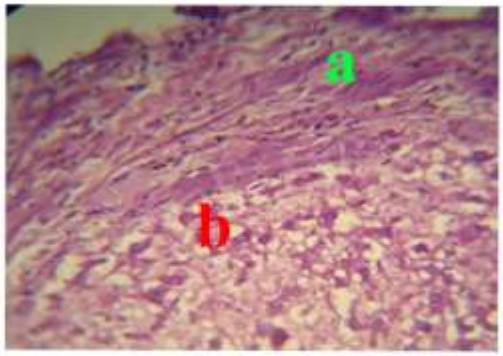
Figure(2) :- Liver of the rat treated with 1mg/kg.BW of oseltamivir for one week shows severe dilation and congestion of central vein (a), and sinusoid(b)with activation of kupffer cells (c) (H&E 400X).



Figure(3):-Liver of the rat treated with 1mg/kg.BW of oseltamivir for one week shows focal infiltration of mononuclear cells in the hepatic parenchyma (a) with vacuolation of hepatocytes (b) (H&E 400).



Figure(4):- Liver of rat treated with 1mg/kg.BW of oseltamivir for six weeks shows focal necrosis characterized by pyknosis or disappearance of nuclei of hepatic cells (a) (H&E 400X) 400X).



Figure(5):- Liver of rat treated with 1mg/kg. BW of oseltamivir for six weeks shows fibrous thickening of the Glisson capsule (a) with vacuolar degeneration of hepatic parenchyma (b) (H&E400X).

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