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خصائص بعض الآفاق تحت السطحية لأراضي محافظة الأحساء بالمملكة العربية السعودية

سعد بن عبد الله البراك

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الأحساء، المملكة العربية السعودية

الملخص :

أجريت هذه الدراسة بغرض التعرف على الخصائص المورفولوجية، الفيزيائية والكيميائية للآفاق تحت السطحية في قطاعات بعض أراضي محافظة الأحساء، مع الرغبة في مقارنة هذه الخصائص بخصائص بعض الطبقات الموجودة في أحد التكتشفات الصخرية التابعة لتكوين الهفوف.

أظهرت النتائج أن خصائص بعض الآفاق تحت السطحية متأثرة بخصائص بعض الطبقات المتكشفة التي قد تمثل مادتها الأصلية، حيث تمثل هذا التأثير في خصائص التدرج اللوني، درجة الملوحة ونسبة الطين والرمل، كما بينت النتائج أيضاً أن نسبة الطين في الآفاق ذات التدرج اللوني (7.5YR) كانت هي الأعلى حيث كان متوسطها (٢٦٪) بينما كانت الأقل في الآفاق ذات التدرج اللوني (2.5Y) حيث وصل متوسطها إلى (١٥,٤٪) كما كانت الآفاق ذات التدرج اللوني (5Y) أكثر ملوحة من الآفاق ذات التدرج اللوني (7.5YR).

يؤكد البحث على أهمية التوسع في دراسة الآفاق تحت السطحية والطبقات التابعة لتكوين الهفوف، بحثاً عن مواد طينية منخفضة الملوحة، للاستفادة منها في تحسين قوام الأراضي الرملية المنتشرة كثيراً في أرجاء المحافظة.

مقدمة :

تعتبر محافظة الأحساء وعاصمتها الهفوف إحدى المحافظات الرئيسة الهامة المكونة للمنطقة الشرقية من المملكة العربية السعودية وتبعد الهفوف نحو ٧٠ كيلومتراً عن الخليج العربي كما وتبعد عن مدينة الظهران نحو ١٢٠ كم. هذا ويحد محافظة الأحساء شرقاً الخليج العربي ودولة قطر وغرباً صحراء الدهناء وشمالاً مدينة بقيق وجنوباً صحراء

[illegible]

المصدر : وزارة الزراعة والمياه ، ١٩٨٠م

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غالبية أنواع الأراضي والوحدات المورفولوجية المنتشرة بمحافظة الأحساء. ورغبة في توسيع قاعدة المعلومات المتعلقة بالآفاق تحت السطحية الموجودة بقطاعات أراضي الأحساء لتشمل كافة أرجاء الواحة وما حولها، فقد تم أخذ ما تم الحصول عليه من نتائج لتلك الآفاق وذلك من الدراسات السابقة التي قام بمعظمها الباحث وغيره من الباحثين عن واحة الأحساء وما حولها بهدف الوصول إلى بعض الاستنتاجات حول خواصها المختلفة بعد إخضاعها للمقارنة والتحليلات الإحصائية المعتادة. لذا فإن هذا البحث سيتناول دراسة الخواص المورفولوجية، الفيزيائية والكيميائية للآفاق تحت السطحية لبعض القطاعات الممثلة لأراضي الأحساء، بهدف التعرف على علاقة هذه الآفاق ببعض الطبقات الموجودة في التكتشفات الصخرية التابعة لتكوين الهوف العلوي.

الدراسات السابقة :

(١) جيو مورفولوجية محافظة الأحساء :

من الملاحظ أن الدراسات الجيومورفولوجية المتعلقة بشبه الجزيرة العربية تعتبر قليلة جداً مقارنة بالدراسات الجيولوجية ويمكن القول أن عدداً من الوحدات الجيومورفولوجية العامة بالمملكة موجودة بصورة جزئية ضمن محافظة الأحساء والتي تشمل منطقة الأحواض الرملية وهضبة الصمان ومنطقة السهل الساحلي للخليج العربي (Chapman, 1978) إضافة إلى الوحدات المحلية الأخرى كالواحات والسبخات.

تشكل الواحات الجزء الهام من منخفض الأحساء الذي يتألف بصورة عامة من سهول متموجة لا يتجاوز ارتفاعها في معظم الجهات 150 م فوق مستوى سطح البحر، والتي تتحدر بالتدرج نحو الشرق والشمال حيث تكثر به التلال الصخرية المنعزلة والأودية لاسيما في الغرب والمنخفضات وأحزمة من الكثبان الرملية.

هذا وقد أضاف (Hotzl et al, 1978) أن الأحساء تقع بين صحراء هضبة الصمان الصخرية في الغرب والكثبان الرملية التي تغطي السهل المحاذي لها في الشرق. وتتألف هذه الهضبة والتي تمتد من بقيق (شمال شرق) حتى حرض (جنوب غرب) من معقد رسوبي من

الطبقات الصخرية العائدة لعصري الميوسين الأعلى والبلايوسين الأدنى حيث تتكشف الطبقات العليا بدرجة واضحة في الجروف باتجاه الشرق بالقرب من الأحساء، تم تحديد الوحدات الجيومورفولوجية الموجودة بمحافظة الأحساء على النحو التالي:

- جروف صخرية وجبال.
- أراضي غير مستوية صخرية مارلية.
- سهول رملية غير مستوية.
- كثبان رملية هـ منحدرات رملية.
- سبخات وحوافها.
- سهول بيدمنت من الحجر الرملي والمارل.
- الواحة (Ministry of Agriculture and Water, 1980).

علماً أن الطبقات الصخرية المختلفة التي تظهر في الجروف الصخرية والجبال وسهول البيدمنت يمكن أن تمثل مواد الأصل لكثير من أراضي الأحساء إلا أنها لم تحظ بدراسة تبين خصائصها المورفولوجية أو محتواها من الطين أو من الأملاح الذائبة، وإن كان هناك من دراسات فقد تناولت فقط وصف للطبقات الموجودة في بعض الجروف والجبال الموجودة بالأحساء كجبل القارة ومن ذلك ما ذكره شابمان (Chapman, 1978) من أن جبل القارة يقع على بعد ١٠ كم شرق الهفوف ويغطي مساحة ١,٧ كم^٢ ويمثل هذا الجبل أحد البقايا المواجهة لهضبة الصمان ويعلو ٧٠ م عما يحيط به ويبلغ ارتفاع قمته عن سطح البحر ٢٠٥ م ويتألف من مارل وحجر رملي مارلي تابع لتكوين الهفوف والذي يعود عهده إلى العصر الميوسيني الأعلى والبليوسيني وهناك طبقات من السلت والطين تتداخل ضمن الحجر الرملي تتميز بألوانها الصفراء والحمراء والتي يعتقد أنها تمثل حفريات دقيقة من أصل نباتي هي جذور نبات الشورى mangrove (Hussain, et al, 2001).

لقد تم وصف تكوين الهفوف من قبل (Steineke and Koch, 1935) (powers et al, 1966) على النحو التالي :

تكوين الهفوف : عدسي الشكل وغير متماثل أو متجانس، إذ تتغير ليثولوجيته وسماكته

وتبلغ سماكته في المقطع النموذجي ٩٥م يتألف من الأسفل حتى الأعلى من أربع وحدات ليثولوجية هي:

١. كونغلواميرا متفاوتة اللون تتألف من جلاميد وحصى من الحجر الجيري أو السيليس مجموعة في أرضية سيليسية رملية الطبيعة بيضاء اللون سمكها ١٩م.
٢. حجر رملي جيري أبيض اللون يصبح حجر جيري كالكارنيتين رمادي اللون يبلغ سمكه ١٨م.
٣. تناوب من حجر رملي طيني رمادي فاتح اللون تتخلله سويات رملية غضارية حمراء اللون ويبلغ سمكه ٤٩م.
٤. كونغلواميرا رملية إلى مارلية رملية رمادية اللون حاوية على جلاميد من الحجر الجيري بيضاء سمكها ٩ أمتار

٢ (مواد الأصل والآفاق تحت السطحية :

نشأت أراضي الأحساء من رواسب متعددة ترسبت خلال فترات جيولوجية متعددة بواسطة بعض العمليات الجيولوجية المختلفة. فعلى سبيل المثال نجد أن الأراضي الرملية تسود في مناطق الكثبان الرملية، بينما تسود الأراضي الطينية في مساحات الحجر الطيني، ونظراً لكون منخفض الأحساء، يتكون من سهول متموجة ووديان، تتركز فيه واحات الأحساء، عليه يمكن القول أن مادة الأصل لأراضي الواحات المزروعة بالنخيل هي بصفة عامة رواسب وديان إذا ما صح الافتراض أن جزءاً كبيراً من الواحة كان وادياً في الزمن القديم امتلاً برواسب الوديان (Ministry of Agriculture and Water , 1980).

إلا أنه يصعب التعرف على نوع مادة الأصل الخاصة بأراضي الواحة المزروعة نظراً لمرور فترة زمنية طويلة على استغلال الإنسان لهذه المنطقة، ومما يزيد الأمر صعوبة، إقدام المزارعين بصورة مستمرة على إضافة الأسمدة العضوية المحتوية على نسبة عالية من الرمل إلى مزارعهم، وعليه فإن مادة الأصل للأراضي المزروعة بالواحة لا تعدو أن تكون محلية متبقية أو رملية أو رواسب وديان أو خليط من هذا وذاك، مع الأخذ في الاعتبار أن بعض

أجزاء الواحة المزروعة حالياً بالنخيل كانت في الماضي مدناً والعكس صحيح (البراك، ١٤١٤هـ).

يلاحظ أن الدراسات البيدولوجية السابقة المتعلقة بمورفولوجية ونشأة وتصنيف أراضي الأحساء لم تركز على خصائص الآفاق تحت السطحية لقطاعات تلك الأراضي بشيء من التفصيل ، بل تناولتها بصورة عرضية ، فمعظم هذه الآفاق والتي هي أشبه بالطبقات ، تتميز بأنها ملتحمة وتنتشر تحت ظروف متفاوتة من الطبوغرافيا ومادة الأصل والغطاء النباتي والظروف المناخية. ولهذه الطبقات عدة صور وتواجهها يسبب مشاكل زراعية متعددة أهمها المشاكل المرتبطة بعملية الصرف تحت السطحي وصعوبة اختراق الجذور النباتية وسوء التهوية ومن ثم التأثير السيئ على إنتاجية المحاصيل.

هذا ويطلق اصطلاح Pans على آفاق وطبقات التربة المندمجة بقوة أو الملتحمة أو المحتوية على نسبة عالية من الطين (Winter and Simonson, 1955) وهذه الطبقات إما أن تكون طبيعة تكوينها جيولوجية أو بيدولوجية طبقاً لعمليات التكوين المؤثرة. وهناك ثلاثة أنواع من هذه الطبقات (Soil Suvey Staff, 1993) وهي الطبقات الملتحمة بالسليكا Duripan والطبقات الطينية Argipan والطبقات الهشة Fragipan. الأولى تعبر عن طبقات أو آفاق ملتحمة كلية بالحديد أو بالحديد والمادة العضوية أو بالسليكا أو السليكا وكربونات الكالسيوم أو بيكربونات الكالسيوم فقط بينما تشير الطبقات الطينية إلى طبقات أو آفاق منضغطة تكونت نتيجة لعمليات تكوين التربة أو نتجت جيولوجياً من مادة أصل طبقية. والطبقات الهشة تعبر عن أفق منضغط غنى بحبيبات السلت أو الرمل أو كلاهما فقير في نسبة الطين.

توصل (العقل، ١٤٢٢هـ) في دراسته للخواص الكيميائية والبيدولوجية لبعض الظواهر المورفولوجية في ترب الأحساء إلى أن ترب الأراضي غير المستوية الصخرية تمتاز بوجود طبقات تحت سطحية شديدة الصلابة تختلف في سمكها ومكوناتها مع انتشار العقد أو التجمعات الداكنة اللون حيث وجد أن محتواها من الأملاح منخفض بينما محتواها من

الجبس مرتفع مع كميات من أكاسيد الحديد المتبلورة والمواد غير المتبلورة للسليكا والألومينا والتي تساعد في زيادة تصلب الطبقة وظهورها باللون الداكن.

وأضاف بأن الطبقات المتصلبة تحت السطحية يغلب على تكوينها وجود الجبس والجير مع وجود المواد غير المتبلورة للسليكا وأكاسيد الحديد الحرة بكميات أقل، هذا ويرتبط تكوينها بأصل القطاع الجيولوجي أكثر من التكوين البيدولوجي.

أجريت دراسة على أعماق كبيرة نسبياً لبعض الطبقات المتصلبة الموجودة في أراضي محطة الأبحاث والتجارب الزراعية بجامعة الملك فيصل على اعتبار أن وجودها يعطي تقريباً عمق التربة المتوفر لزراعة المحاصيل المختلفة حيث وجد أن عمق تلك الطبقات يتراوح بين أقل من متر إلى ما فوق ٣ أمتار في أراضي تلك المحطة كما تم تقدير معدل التسرب Infiltration rate في تلك الأراضي والذي تراوح بين ١٠،١ سم/ساعة في الأراضي الرملية والطينية على التوالي (Hussain,1984).

المواد وطرائق الدراسة :

تضم هذه الدراسة جزأين أحدهما نظري والآخر عملي حيث تمثل الجزء النظري في الأخذ بعين الاعتبار بعض نتائج الدراسات السابقة الآتية :

١. نتائج الدراسة التي قام بها الباحث حول خصائص بعض الأراضي في واحة الأحساء الشرقية (AL-Barrak, 1990) حيث تم عمل مقطع عرضي طوله ١٤ كم وذلك من الجزء الشمالي الشرقي لمدينة الهفوف وحتى جنوب قرية الجشة ولقد أخذت عينات من (٢٤) موقعاً على امتداد هذا المقطع العرضي على مسافات بينية قدرها ٥٠٠ م تقريباً بين كل قطاع وآخر حيث تم الاستفادة من جميع هذه القطاعات باستثناء القطاعات (١٠،١٤،٢٠) (شكل ١).

٢. الدراسة التي قام بها الباحث أيضاً حول خصائص بعض الأراضي المتأثرة بالأملاح في الأحساء حيث درست تلك الأراضي في خمس مواقع مختلفة، أخذت قطاعاتها الخمسة بعين الاعتبار (AL-Barrak and Badawi,1988) (شكل ١).

٣. نتائج الدراسة التي قام بها (Abdelhai and Asif, 1981) على أراضي محطة الأبحاث والتجارب الزراعية والبيطرية التابعة لجامعة الملك فيصل والواقعة على طريق الهفوف - قطر. ولقد تمت الاستفادة من القطاعات ذات الأرقام (٨,٧,٦,٥,٤,٣) (شكل ١).
٤. نتائج الدراسة التي قامت بها الشركة الإستشارية الدولية المحدودة لتنمية المياه الجوفية (GDCIL) لصالح وزارة الزراعة والمياه (Ministry of Agriculture and Water, 1980) حول تصنيف أراضي الأحساء حيث تمت الاستفادة من القطاعات ذات الأرقام (٢,١٠,١٦,١٨,١٩,٢٧) (شكل ١).
٥. نتائج الدراسة التي قام بها العقل حول الخصائص الكيميائية والبيدولوجية لبعض الظواهر المورفولوجية في ترب الأحساء (العقل ، ١٤٢٢هـ) حيث تمت الاستفادة فقط من القطاعين الثالث والرابع (شكل ١).

تم تحديد واختبار تلك الآفاق على ضوء المعايير التالية :

١. اللون على اعتبار أن لون الأفق له ارتباط بلون الطبقات المتكشفة الموجودة في التلال المحيطة والتي لم تتعرض في الأغلب إلى تجوية كيميائية بدرجة كبيرة. فالآفاق تحت السطحية التي أخذت بعين الاعتبار يعتقد أنها كانت أقل عرضة للتجوية مقارنة بالآفاق السطحية التي من المعتقد أنها كانت قد تعرضت لدرجة أكبر من التأثيرات الناتجة عن فعل الإنسان مثل الحراثة أو الري أو التسميد والعمليات البيدوجينية الأخرى.
٢. نسبة الطين.
٣. درجة المقاومة.

وعلى ضوء ما ذكر أعلاه أمكن اختبار أو تحديد العينات الممثلة لتلك الآفاق تحت السطحية من بين مجمل العينات التي أخذت بعين الاعتبار في الدراسات المشار إليها. وتم وضعها في أربع مجموعات حسب درجة التدرج اللوني Hue الخاصة بكل أفق كما هو موضح في الجدول (١).

أما الجزء العملي من هذه الدراسة فقد تمثل في دراسة بعض الطبقات الموجودة في بعض التكتشفات الصخرية التابعة لتكوين الهفوف والمتمثلة في التتابع الطبقي أو الطبقات المشكلة للوجه الجنوبي من جبل القارة حيث تم الاستعانة بخريطة جيولوجية للوجه الغربي بالملكة العربية السعودية (Steineke et al, 1958) كما تم تحديد موقع الدراسة باستخدام جهاز تحديد المواقع GPS على النحو التالي (خط عرض $25^{\circ}23'25''$ شمالاً وخط طول $49^{\circ}41'40''$ شرقاً).

وصفت الطبقات مورفولوجياً طبقاً للإصطلاحات القياسية المذكورة في (Soil Suvey Staff, 1993) كما تمت الاستعانة بالوصف الخاص بالتتابع الطبقي لتكوين الهفوف من أحد البحوث المقبولة للنشر في جامعة الملك فيصل بالإحساء (Safarjalani, 2005) كما هو مدون في شكل (٢).

رغبة في إجراء مضاهاة مبدئية بين الطبقات المتكشفة من جبل القارة والآفاق تحت السطحية للقطاعات المدروسة تم اختيار عدداً من القطاعات القريبة من جبل القارة والتي تمت دراستها ضمن الدراسة الأولى المتمثلة في القطاعات أرقام ١٩ ، ١٦ ، ١٣ ، ١١ ذات التدرج اللوني 2.5Y بالإضافة إلى القطاعات أرقام ١٨ ، ١٧ ، ١٥ ، ١٢ ذات التدرج اللوني 5Y (جدول ٤).

تم اختيار جبل القارة كأحد التكتشفات الصخرية لتكوين الهفوف علماً أن هذا المتكشف الصخري يبرز على السطح في واحة الأحساء بالعديد من المواقع. وعلى اعتبار أن جبل القارة قد حظي بالكثير من الدراسات إضافة إلى كون خط الدراسة الأولى يمر بالقرب منه (٢,٢ كم جنوباً).

تم جمع عينات مناسبة من الطبقات الموصوفة من أجل التحليل المعملية وتم تجفيف العينات هوائياً وطحنها ونخلها لازالة الجسيمات الأكبر من ٢ ملم. تم إجراء التحليل الميكانيكي باستخدام الهيدروميتر (Day, 1965) قدر رقم الحموضة pH في مستخلص العجينة المشبعة باستخدام جهاز pH meter كما قدر التوصيل الكهربائي ECe في

مستخلص التربة المشبعة (Rhoades, 1982) تم تقدير كربونات الكالسيوم الكلية بالطريقة الوزنية (Allison and Moodie, 1965) كما تم تقدير الجبس بطريقة التجفيف بواسطة الأشعة تحت الحمراء (Elprince and Turjoman, 1983).

رقم العينة	لون التبقعات	لون النسيج	التتابع الليثولوجي	التشكيلة الصخرية
			الوحدة الصخرية الرابعة ٩, ١ م توضع كونغلومنتية رملية إلى مارلية رملية	
٦		5 Y 8/2	الوحدة الصخرية الثالثة (٤٨, ٦ م) صخر رملي غصاري أبيض اللون تتخلله رسوبيات رملية غصارية حديدية حمراء اللون	تشكيلة الهفوف (٩٥ م)
٥	5 Y 7/2	5 YR 5/6		
٤	5 YR 6/4	5 Y 8/2		
٣	2.5 YR 8/2	7.5 YR 6/4		
٢		5 Y 8/2	الوحدة الصخرية الثانية ١٨, ٢ م صخر رملي كلسي أبيض اللون الي كلسي كلكارينيني رمادي اللون	
١	5 YR 6/4	2.5 Y 8/2		
			الوحدة الصخرية الأولى ٩, ١ م توضع صخرية كونغلومنتية حصوية رملية	
			صخر كلسي غصاري مارلي	الدام

شكل (٢) : المستويات التي اخذت منها عينات الدراسة من الوجه الجنوبي لجبل القارة
المصدر : (Safarjalani, 2005)

النتائج والمناقشة:

تم تدوين نتائج التحاليل الفيزيائية والكيميائية للآفاق تحت السطحية لقطاعات أراضي واحة الأحساء وللتدرج الطبقي للوجه الجنوبي من جبل القارة في الجداول (١ ، ٢ ، ٣ ، ٤) يتبين من خلال المتوسطات الحسابية لأعماق تلك الآفاق أن الآفاق تحت السطحية ذات التدرج اللوني 2.5Y تتواجد على أعماق أكبر من الآفاق الأخرى تليها الآفاق ذات التدرج اللوني 5Y ثم الآفاق 7.5Y و 5Y (جدول ٢). فقد تراوحت تلك الأعماق بين ١١٠,٥ سم في الآفاق ذات التدرج اللوني 2.5Y إلى ٥٣ سم في الآفاق ذات التدرج 5Y وبمقارنة أعماق الآفاق تحت السطحية ذات التدرج اللوني 5Y و 2.5Y للقطاعات القريبة من جبل القارة (جدول ٤) بالترتيب الذي تتواجد عليه الطبقات ذات التدرج اللوني 2.5Y و 5Y في التتابع الطبقي للوجه الجنوبي من جبل القارة (شكل ٢ وجدول ٣) نجد أن المتوسط الحسابي لعمق الآفاق تحت السطحية ذات التدرج اللوني 2.5Y أكبر قليلاً من المتوسط الخاص بالآفاق ذات التدرج اللوني 5Y. حيث كان المتوسط في آفاق 2.5Y يساوي ١١٥ سم بينما كان المتوسط في آفاق 5Y يساوي ١١٢ سم (جدول ٤).

مما يعكس شيئاً من التقارب بين تلك الآفاق تحت السطحية وتلك الطبقات مما يعني الحاجة إلى إجراء دراسات مستقبلية تتناول مضاهاة تلك الآفاق المدروسة بنظيراتها المكونة للتتابع الطبقي الخاص بجبل القارة.

الملوحة : يتبين من الجدول رقم (١) والجدول رقم (٢) أن متوسط قيم التوصل الكهربائي ECe للآفاق تحت السطحية للأراضي المدروسة كانت أعلى من ٤ dSm^{-1} وبالتالي تعتبر متأثرة بالأملاح ، إلا أن الآفاق ذات التدرج اللوني 5Y كانت أكثر ملوحة من غيرها تليها الآفاق ذات التدرج اللوني 7.5Y فالآفاق 5YR ثم الآفاق 2.5Y ، حيث تراوحت قيم المتوسط الحسابي لها إلى ٢٦,٩ ، ٢٣,٨ ، ٨,٨ ، ١٤,٨ dSm^{-1} على التوالي (جدول ٢) ، وبما أن تركيز الأملاح في التربة يرتبط عادة بعدة عوامل منها على سبيل المثال محتوى مادة الأصل من الأملاح ودرجة القوام والبناء والتماسك وظروف الري والصرف

فيمكن تفسير النتائج أعلاه على ضوء تلك العوامل، فالآفاق ذات التدرج اللوني 5Y تتميز بقوامها الثقيل مقارنة بالأخرى ذات التدرج اللوني 2.5Y حيث نجد أن متوسط نسبة الطين في الأولى هو ٢٣٪ بينما متوسط نسبة الطين في الثانية هو ١٥,٤٪ (جدول ٢)، كما نجد أن متوسط نسبة الرمل في الأولى أقل من الثانية مما يعكس صعوبة غسل الأملاح في الأولى نتيجة لسوء الصرف المرتبط بضعف النفاذية. علماً بأن هذه الدراسة لم تتناول بقية الخواص المحددة لدرجة الصرف.

يتبين من النتائج الخاصة بطبقات جبل القارة الجدول (٣) أن قيم التوصيل الكهربائي كانت عالية جداً في أغلب الطبقات، كما كانت الطبقات ذات التدرج اللوني 5Y و 7.5Y أكثر ملوحة من الطبقات الصفراء (2.5Y, 5Y) حيث وصل التوصيل الكهربائي إلى ما لا يقل عن 500 dSm^{-1} في المجموعتين الأولى، بينما لم يتجاوز التوصيل الكهربائي في المجموعتين الثانيةين 193 dSm^{-1} وذلك نتيجة لما لوحظ في الحقل من احتواء نسيج أغلب الطبقات الموصوفة على تجمعات مختلفة من بلورات الملح.

وبمقارنة قيم التوصيل الكهربائي في الآفاق تحت السطحية للقطاعات القريبة من جبل القارة ذات التدرج اللوني 5Y و 2.5Y والتي وصل متوسطها إلى ١٦,٤ و 2.4 dSm^{-1} على التوالي (جدول ٤) بقيم التوصيل الكهربائي لطبقات جبل القارة ذات التدرج اللوني 5Y و 2.5Y والتي وصلت إلى ١٨٠ و 192.2 dSm^{-1} على التوالي. نجد أن الآفاق تحت السطحية ذات التدرج اللوني 2.5Y قد انخفضت فيها الملوحة بينما يحتمل أن الآفاق ذات التدرج اللوني 5Y قد ظلت متأثرة بمحتواها الأصلي من الأملاح كما هو الحال في طبقات جبل القارة المماثلة لها في التدرج اللوني، مما يعني أن عمليات تكوين التربة واستصلاحها قد ساهمت في خفض ملوحة الآفاق تحت السطحية لطبقات التدرج اللوني 2.5Y على الرغم من أن المحتوى الأصلي للطبقات المماثلة لها لونها من الأملاح كان عالياً (جدول ٣)، وذلك عند افتراض كون الآفاق تحت السطحية تنتمي إلى الطبقات المماثلة لها لونها.

رقم الحموضة : تراوحت قيم متوسطات رقم الحموضة (pH) في الآفاق تحت السطحية المدروسة بين ٧,٥ و ٧,٧ (جدول ٢) مما يشير إلى عدم وجود فروقات كبيرة بين تلك القيم وهذه القيم تقع بصورة عامة في مجال الترب المائلة نحو القلوية الخفيفة نتيجة لتأثير الأملاح المتعادلة وكربونات الكالسيوم في المحافظة على قيم رقم الحموضة ضمن الحد المتعادل تقريباً . أما بالنسبة لقيم الحموضة في طبقات جبل القارة فقد تراوحت بين ٦,٦ و ٧,٢ مع كون معظمها يقع ضمن المجال المتعادل باستثناء الطبقة ذات التدرج اللوني 5Y والتي وصل رقم الحموضة فيها إلى ٦,٦ أي المائل نحو الحموضة الخفيفة (جدول ٣).

التحليل الميكانيكي للحبيبات : أوضحت النتائج أن نسبة الطين في الآفاق ذات التدرج اللوني 7.5YR كانت هي الأعلى حيث وصل متوسطها إلى ٢٦٪ بينما كانت الأخفض في الآفاق ذات التدرج اللوني 2.5Y حيث وصل متوسطها إلى ١٥,٤٪ وبعبارة أخرى نجد أنه ضمن الآفاق الصفراء ، فإن الآفاق ذات التدرج اللوني 5Y أكثر طيناً من الآفاق ذات التدرج اللوني 2.5Y بينما نجد أنه ضمن الآفاق الحمراء المصفرة فإن الآفاق ذات التدرج اللوني 7.5YR أكثر طيناً من الأخرى ذات التدرج اللوني 5YR (جدول ٢).

وبالنظر إلى نتائج التحليل الميكانيكي للحبيبات الخاص بطبقات جبل القارة نجد أن نسبة الطين في الطبقة ذات التدرج اللوني 7.5Y هي الأعلى حيث بلغت ٤٧٪ بينما كانت النسبة في الطبقة ذات التدرج اللوني 5Y هي الأخفض والتي بلغت ١٣٪ ، في حين كانت نسبة الطين في الطبقة ذات التدرج اللوني 2.5Y متقاربة مع طبقات 5Y (جدول ٣).

يتبين من النتائج الخاصة بالآفاق تحت السطحية للقطاعات القريبة من جبل القارة (جدول ٤) ، أن متوسط نسبة الطين في الآفاق ذات التدرج اللوني 2.5Y متقاربة مع متوسط نسبة الطين في الآفاق ذات التدرج اللوني 5Y والتي بلغت ١٣٪ و ١٥٪ مما يدل بصورة عامة على أن نسبة الطين في الآفاق تحت السطحية في لأراضي واحة الأحساء ترجع إلى طبيعة وتركيب مادتها الأمية أكثر من عمليات التكوين المحلية (جدول ٣).

تابع جدول (١) • نتائج بعض التحاليل الفيزيائية والكيميائية للأفلاق تحت السطحية حسب درجات ألوانها

رقم القطع	رقم الدراسة	العمق (سم)	مستخلص العينة المشبعة			التحليل الميكانيكي			كربونات الكالسيوم	الجبس
			ECdSm -1	pH	% للرمل	% للصلت	% للطين			
5Y										
3A	1	AA	0.1	AA	13	6.0	AA	AA.1	1.0	
AA	1	AB	AA	BA	17	8	LA	AA.2	1.0	
AA	3	0.7	0.5A	1.7	1A	0.6	31	1.7	3	
VA	3	0A	LA.3	0.8	60	0.1	1A	AA.7	AA.7	
0.1	3	VA	70.8	3.8	1A	63	0.8	70A	A	
A	3	0.6	3AA	7.8	AA	AA	11	36.7	AA.1	
AA	1	LA	1.31	AA	LA	61	0	3.11	0.0	
VA	1	03A	AA.1	LA	7A	70	3	AA.1A	0.1	
AA	1	1A	AA	7.8	0A	AA	AA	70A	0.0	
0.1	1	A3	AA	AA	70	AA	0.8	703	A.0	
AA	1	0.8	03	0.7	1A	AA	AA	AA.A	3.A	
b	1	0A	1.6	1.8	60	LA	71	30.3	70	
7	1	0.31	AA.8	1.8	7A	0.3	AA	AA.10	AA.8	
A	1	0.8	3.6	1.8	0.8	AA	7A	1.1A	0.0	
L	1	0.61	1.8	0.7	1A	60	AA	AA.03	AA.01	
0	1	0A	0.8	7.8	7A	6A	AA	LA.63	1.0	
3	1	LA1	0.8	7.8	01	3A	10	AA.00	1.0	
A	1	0.8	AA0	1.7	LA	LA	7A	LA.10	0.0	
0	A	AA	0.01	AA	13	03	31	LA.61	A.0	
3	A	6A	03	AA	3A	AA	60	AA.1	3.0	
A	A	0.11	AA	AA	LA	LA	71	LA.71	0.0	
1	A	AA1	AA	1.8	LA	61	AA	AA.11	6.0	
1	A	LA	66	1.8	61	7A	AA	AA.A0	7.1	
7	A	0A	AA.8	LA	60	0.1	1A	AA.7	LA.8	
A	A	1A	1.2A	LA	AA	71	2.0	703	L	

جدول (٢) مدى ومتوسط بعض خواص التربة في الآفاق تحت السطحية لأراضي الأحساء

الخاصة	5YR			7.5YR			2.5Y			5Y		
	القيمة الصغرى	القيمة العظمى	المتوسط الحسابي	القيمة الصغرى	القيمة العظمى	المتوسط الحسابي	القيمة الصغرى	القيمة العظمى	المتوسط الحسابي	القيمة الصغرى	القيمة العظمى	المتوسط الحسابي
المق/سم	٢٠	٧٨	٥٣	٥	١٠٠	٦٣	٥٠	٢٠٥	١١٠,٥	٢١	٢٤٥	٨٦
التوصيل الكهربائي EC	٤,٣	٢٥,١	١٤,٨	٣,٢	٦٢	٣٣,٨	١,٥	٣٩,٦	٨,٧	١,٣	١٠٥	٣٦,٩
رقم الحموضة PH	٧,٢	٨,٠	٧,٦	٦,٩	٨,٦	٧,٥	٧,٢	٨,٣	٧,٧	٧,١	٨,٥	٧,٥
رمل %	١١	٩١	٦١	٣	٨٥	٥٤	٣٥	٨٧	٦٢	١٥	٧٦	٤٣
سلت %	١	٨٣	١٧,٣	٥	٥٤	١٧,١	١	٦٠	٢١	١٠	٦٨	٣٣,٥
طين %	٦	٥١	٢١	١٠	٥٩	٣٦	٥	٣٠	١٥,٤	٤	٥٥	٣٣
كربونات الكالسيوم %	١١,٥	٣٧,٤	٢٣,٥	٢,٨	٦٢,٨	٢١	٤,٧	٦٩	٣١,٦	٨,١	٨٩,٤	٣٨,٧
الجبس %	٣,٣	٢٤	٩,٤	٠,٥	٤٠,٣	٨,٨	٠,١	٢٥	٣,٩	٠,٤	١٠,٣	٢,٣

جدول (٣) بعض الخصائص الفيزيائية والكيميائية لعينات من الطبقات المتكشفة من الوجه الجنوبي لجبل القارة

رقم العينة	اللون	مستخلص العينة المشبعة			التحليل الميكانيكي			كربونات الكالسيوم %	الجبس %
		pH	ECdSm ⁻¹		% للرمل	% للصلت	% للطين		
٦	5Y 8/2	٦,٦	٥٨,٥		١٥	٧٢	١٣	٤٢,٣	١٨,٤
٥	5YR 5/6	٧,١	٥٠٠,٠		٣٧	٥٠	٣٣	٢٥,٨	٤,٣
٤	5Y 8/2	٦,٦	١٨٠,٤		٤٥	٤٢	١٣	٣٢,٨	٠
٣	7.5YR 6/4	٦,٧	٦٨٧,٥		١١	٤٢	٤٧	٢٨,٧	١٠
٢	5Y 8/2	٧,٠	١٣٤,٣		٥٧	٣٠	١٢	٣٢,٥	٣,١
١	2.5Y 8/2	٧,٢	١٩٢,٢		٦١	٢٤	١٥	٣٦,٨	٠

جدول (٤) بعض الخصائص الفيزيائية والكيميائية للآفاق تحت السطحية لبعض القطاعات القريبة من جبل القارة

رقم القطعة	المق/سم	مستخلص المعينة المشبعة				التحليل الميكانيكي				كربونات الكالس % وم	الجبس %
		ECdSm ⁻¹	pH	للمل %	للطين %						
					للسلت %	للطين %					
2.5Y											
الدراسة الأولى											
١٩	٥٠	١,٥	٣٥	٦٠	٥	٥	٥٠	٢٥			
١٦	٢٠٥	٢,٢	٤٥	٣٥	٢٠		٦٩				
١٣	٦٠	١,٧	٧٠	١٤	٦١		٤٠				
١١	١٤٥	٤,١	٧٠	٢٠	١٠		٢٤,٩				
المتوسط الحسابي	١١٥	٢,٤	٥٥	٣٢	١٣		٣٨,٥				
5Y											
الدراسة الثانية											
١٨	٢٤٥	١٦,٧	٣٨	٥٨	٤		٦٦,٢				
١٧	٦١	١,٣	٧٥	١٣	١٢		٢٥,٨				
١٥	٧٤	٢,٧	٥٨	١٢	٣٠		٤٥,٨				
١٢	٧٠	٤٥	٦١	٣٧	١٢		٧٧,٢				
المتوسط الحسابي	١١٢	١٦,٤	٥٨	٣٧	١٥		٥٣,٧				

أما بالنسبة لنسبة الرمل فكانت منخفضة في الآفاق تحت السطحية ذات التدرج اللوني 5Y والتي بلغ متوسطها ٤٣٪ بينما كانت مرتفعة في الآفاق ذات التدرج اللوني 2.5Y والتي بلغ متوسطها ٦٢٪ (جدول ٢) وهذه العلاقة مماثلة لتلك التي ظهرت بالنسبة للطين في الآفاق الصفراء ولكن بصورة عكسية. احتوت الطبقات التابعة لجبل القارة ذات التدرج اللوني 2.5Y على أعلى نسبة من الرمل (٦١٪) بينما تراوحت نسبة الرمل في الطبقات ذات التدرج اللوني 5Y بين ١٥ - ٥٧٪ (جدول ٣). كانت نسبة الرمل في الآفاق تحت السطحية للقطاعات القريبة من جبل القارة ذات التدرج اللوني 2.5Y والتي بلغ متوسطها ٥٥٪ متقاربة مع نسبة الرمل في الآفاق ذات التدرج اللوني 5Y والتي بلغ متوسطها ٥٨٪ (جدول ٤).

لقد أظهرت النتائج تقارباً في محتوى الآفاق تحت السطحية ذات التدرج اللوني الأحمر المصفر من السلت حيث كان متوسط نسبته في الآفاق ذات التدرج اللوني 5YR هو ١٧,٣٪ بينما كان متوسط النسبة في آفاق 7.5YR ١٧,١٪ أما بالنسبة للآفاق الصفراء فقد وصل متوسط نسبته ٣٣,٥٪ في آفاق 5Y بينما كانت النسبة ٢١٪ في آفاق 2.5Y. (جدول ٢) وبصورة عامة أظهرت نتائج السلت في طبقات جبل القارة نفس الاتجاه كما كان متوسط نسبة السلت في آفاق القطاعات القريبة من جبل القارة ذات التدرج اللوني 5Y أعلى قليلاً منها في الآفاق ذات التدرج اللوني 2.5Y حيث بلغ ١٥٪ و ١٣٪ على التوالي (جدول ٤).

كربونات الكالسيوم : بصورة عامة كان متوسط نسب كربونات الكالسيوم في الآفاق الصفراء أعلى منها في الآفاق الحمراء المصفرة كما كانت النسب متقاربة بين الآفاق الحمراء المصفرة حيث بلغ المتوسط ٢٣,٥٪ في آفاق 5YR و ٢١٪ في آفاق 7.5YR ، بينما بلغ متوسط النسب ٣٨,٧٪ و ٣١,٦٪ في الآفاق ذات التدرج اللوني 5Y و 2.5Y على التوالي (جدول ٢).

وعند مقارنة نسبة كربونات الكالسيوم في الآفاق تحت السطحية للقطاعات القريبة من جبل القارة بمحتوى طبقات جبل القارة من كربونات الكالسيوم نجد أنها كانت أخفض بصورة عامة في الطبقات ذات التدرج اللوني 5Y و 2.5Y من متوسط قيمها في

الآفاق تحت السطحية والتي بلغ متوسطها في الآفاق 5Y و 2.5Y ٥٣,٧٪ و ٣٨,٥٪ على التوالي (جدول ٤ و٣). تراوحت نسب كربونات الكالسيوم في الطبقات ذات التدرج اللوني 5Y بين ٤٢,٣٪ و ٣٢,٥٪ ، كما بلغت نسبة كربونات الكالسيوم ١٦,٨٪ في الطبقة ذات التدرج اللوني 2.5Y مما يعكس تفاوتاً في محتوى الطبقات الصفراء من كربونات الكالسيوم (جدول ٣). وعلى الرغم من أن متوسطات نسب كربونات الكالسيوم في الآفاق تحت السطحية لأراضي الأحساء كانت أعلى نسبياً من نسبة كربونات الكالسيوم في طبقات جبل القارة إلا أن نسبتها في تلك الطبقات كانت أعلى من القيم الصغرى للكربونات في الآفاق تحت السطحية (الجدول ٢ و٣). وقد يعزى ارتفاع قيم كربونات الكالسيوم في الآفاق تحت السطحية عن مثيلاتها لونها في طبقات جبل القارة إلى كونها موزعة بصورة أكثر انتشاراً في الآفاق تحت السطحية وذلك نتيجة لتأثير عوامل وعمليات تكوين التربة المختلفة.

الجبس : كانت متوسطات نسبة الجبس في الآفاق تحت السطحية ذات التدرج اللوني الأحمر المصفر أعلى منها في الآفاق ذات التدرج اللوني الأصفر حيث وصل متوسط نسبة الجبس في آفاق 5YR إلى ٩,٤٪ بينما وصل متوسط النسبة في آفاق 5Y إلى ٢,٣٪ (جدول ٢) أما بالنسبة للآفاق تحت السطحية للقطاعات القريبة من جبل القارة فقد كان متوسط نسبة الجبس في الآفاق ذات التدرج اللوني 2.5Y متقارباً مع المتوسط الخاص بالآفاق ذات التدرج اللوني 5Y حيث بلغا ٦,٨٪ و ٥,١٪ على التوالي (جدول ٤).

أما طبقات جبل القارة ذات التدرج اللوني 2.5Y و 5Y فقد أظهرت تذبذباً في محتواها من الجبس (جدول ٣).

الاستنتاجات والتوصيات :

يمكن من خلال هذه الدراسة البحثية المبدئية والأولية التوصل إلى بعض الاستنتاجات والتوصيات التالية :

١. إن خصائص بعض الآفاق تحت السطحية لقطاعات أراضي واحة الأحساء كانت متأثرة بخصائص بعض الطبقات التابعة لتكوين الهفوف والتي قد تمثل مادتها الأصلية، حيث تمثل هذا التأثير في خواص التدرج اللوني ودرجة الملوحة ونسبة الطين والرمل.
٢. فيما يتعلق بنسبة كربونات الكالسيوم فقد كانت منخفضة إلى حد ما في الطبقات المتكشفة مقارنة بالآفاق تحت السطحية أما بالنسبة لمحتوى الآفاق تحت السطحية من الجبس فقد كان متقارباً في الآفاق ذات التدرج اللوني الأصفر (2.5Y و 5Y) كما أظهرت الطبقات المتكشفة من جبل القارة تذبذباً في محتواها من الجبس.
٣. على ضوء تقسيم الآفاق تحت السطحية لقطاعات أراضي الأحساء إلى أربع مجموعات حسب التدرج اللوني، فإن الآفاق ذات التدرج اللوني الأحمر المصفر (7.5YR) كانت أكثر طيناً من المجموعات الأخرى بينما كانت الآفاق ذات التدرج اللوني الأصفر (5Y) أكثر ملوحة من آفاق المجموعات الأخرى أما بالنسبة للطبقات المتكشفة ذات التدرج اللوني.
٤. الأصفر (2.5Y , 5Y) فكانت درجة ملوحتها أعلى مما هي في الآفاق تحت السطحية المماثلة لها لوناً.
٥. يوصى الباحث بأهمية التوسع في هذا النوع من الدراسات لحصر المزيد من مساحات الأراضي في محافظة الأحساء للوقوف على الخصائص البيدولوجية لآفاقها تحت السطحية كي تصبح النتائج المتحصل عليها أكثر تمثيلاً للواقع مع مراعاة أن تشمل الدراسة مستقبلاً العديد من المواقع التي يبرز فيها المتكشف الصخري لتكوين الهفوف بواحة الأحساء حتى تتم عملية المضاهاة بصورة أفضل.
٦. الأخذ بعين الاعتبار أهمية البحث عن طبقات قريبة من السطح تحتوي على مواد طينية منخفضة الملوحة وبكميات اقتصادية كي تفي باحتياجات المزارعين المستقبلية من تلك المواد، بغرض استخدامها وإضافتها لتحسين قوام الأراضي الرملية المنتشرة في غالبية أرجاء المحافظة، أو لعمل ترب صناعية مناسبة لغرض التشثيل.

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Characteristics of Some Subsurface Horizons of Al-Ahsa Soils, Saudi Arabia

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Abstract :

This research was conducted to study the morphological, chemical and physical characteristics of the subsurface horizons of Al-Ahsa soils with an attempt to compare their characteristics with that of some miopliocene rock strata outcrop (Hofuf formation). Results indicated that the properties of some subsurface horizons were influenced by the properties of some strata beds which may represent their parent materials. Properties such as color hues, ECe and percentages of clay and sand were the most influenced.

In addition, the results showed that the subsurface horizons with 7.5 YR hue had the highest clay content with an average of 26%, whereas those with 2.5 hue with 5Y hue were more saline than those of 7.5YR hue.

Further research work should be carried out in more details to search for low saline clayey materials to be used for improvement of the texture of sandy soils spreading all over the district of Al-Ahsa

English Section

الاستنتاج :

أثبتت الدراسة أن اليموكينون التي تكون الشق النشط للحبة السوداء أو ما تسمى بحبة البركة (النجيلا ساتيفا) قد أوقفت نمو ميكروب الفيوسريم سولاني المعزول سريرياً مقارنة بالامفتيرسين بي على نفس الميكروب.

مقارنة النشاط المضاد للفطريات للثيموكينون كمادة فعالة في الحبة السوداء (النجيلا ساتيفا) الامفوتيرسين بي ضد ميكروب الفيوسريم سولاني

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الملخص :

الهدف من البحث:

أجريت البحث لمقارنة نشاط الثيموكينون التي تكون الشق النشط للحبة السوداء أو ما تسمى بحبه البركة (النجيلا ساتيفا) ضد ميكروب الفيوسريم سولاني المعزول سريريا مع نشاط الامفوتيرسين بي على نفس الميكروب

طريقة البحث :

تم فصل ميكروب الفيوسريم سولاني المعزول سريريا من إصابة بالظفر في ذكر يافع ولا يعاني من اعتلال بالمناعة خلال مزرعة فطرية روتينية على الدرماسيل الاجار. تم تحديد الميكروب عن طريق الفحص الظاهري للمستعمرات الميكروبية والفحص المجهرى. وللحساسية ضد المضادات التالية من الثيموكينون والامفوتيرسين بي : ١ و ٠.٥ و ٠.٢٥ و ٠.٠٦٢ و ٠.٠٣١ ملي جرام لكل ميلي لتر. كما تمت على مجموعة أخرى بدون مضادات كمجموعة ضابطة. تم قياس النمو في اليوم العاشر من التلقيح كنسبة مئوية لمنع النمو

النتائج :

أثبتت الدراسة ان الثيموكينون قد أوقف نمو الميكروب بنسب ٢.٠ و ٣ و ١٨.٣ و ٥٩.٣ و ١٠٠٪ تبعا للتركيزات التالية من الثيموكينون ٠.٠٣١ و ٠.٠٦٢ و ٠.١٢٥ و ٠.٢٥ و ٠.٥ و ١ ملي جرام لكل ملي لتر مقارنة بالامفوتيرسين بي الذي منع نمو الميكرو بنسب مئوية ٦.٢ و ٢٤.٥ و ٣١.٢ و ٣٩.٧ و ٥٤.٤ و ٧٢.٤ لنفس التركيزات من الامفوتيرسين. وقد لوحظ أيضا عدم منع كامل للنمو في أي تركيز من الامفوتيرسين بي .

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Fusarium is one of the most resistant fungi to the arsenal of modern antifungal agents. Current therapeutic approaches for fusariosis are suboptimal, resulting in exceedingly high mortality rates (Anaissie, 1989; Martino, 1994; Boutati & Anaissie, 1997; Girmenia *et al.*, 2000; Torres & Kontoyiannis 2003). The mainstay in the treatment of fusariosis has traditionally been amphotericin B. However, the in vitro susceptibility of *Fusarium* species to amphotericin B is, at best, mediocre (Anaissie, 1989; Boutati & Anaissie, 1997). The activity of amphotericin B in animal models of fusariosis is also limited (Anaissie *et al.*, 1992; Guarro *et al.*, 1999). In fact, only high doses of liposomal amphotericin B have been shown to be active against *Fusarium* species in animal models using immunocompetent mice (Ortoneda *et al.*, 2002).

Further investigations of usefulness of *N. sativa* and its active principles in the treatment of opportunistic fungal infections like fusariosis should be considered.

Table (1)

Percentage inhibition of growth of *Fusarium solani* with different concentrations of thymoquinone and amphotericin B after 10 days of incubation.

Concentrations (mg/ml)	% inhibition of growth		*P-values from students "t" test
	Thymoquinone (Mean \pm se)	Amphotericin B (Mean \pm se)	
0.031	0 \pm 0	6.2 \pm 0.76	<0.01
0.062	2.0 \pm 1	24.5 \pm 0.92	<0.001
0.125	3.0 \pm 0.25	31.2 \pm 0.65	<0.02
0.25	18.3 \pm 1.45	39.7 \pm 1.22	<0.001
0.5	59.3 \pm 3.93	54.4 \pm 2.72	>0.1
1.0	100 \pm 0	72.4 \pm 2.12	<0.001

*P-values for the differences between the means \pm se of corresponding concentrations of thymoquinone and amphotericin B.

Discussion:

N. sativa has been used for many ailments in ancient Greek, Indian and Arabic medicines for treatment of warts, collar-studd abscesses, ringworm infections and gastrointestinal infections(Al-Jishi, 2000). In the present study we observed a dose related anti-fusarium activity of thymoquinone, an active principle of *N. sativa*. There were 0, 2.0, 3.0, 18.3, 59.3, and 100% inhibitions of growth of *Fusarium solani* with 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg thymoquinone/ml on 10th day of incubation. Previous reports also showed concentration-dependent inhibitions of growth of Gram-positive & Gram-negative bacteria, and *Candida albicans* by *N. sativa* seed and hexane-extracted *N. sativa* oil (Hanafi & Hatem, 1991; Al-Syed et al., 1994).

In the present study amphotericin B showed activity against *F. solani* but there was no complete inhibition at any concentration of the drug tested. Lewis et al. have also reported shallow concentration-effect curve and low efficacy of amphotericin B against *F. solani* (Lewis et al., 2005). The amphoteric properties and high protein binding of this drug may partially explain this effect (Bekersky et al., 2002). The relatively steep concentration-effect curve of thymoquinone against *F. solani* resembles to that of voriconazole reported in another study (Lewis et al., 2005). Perhaps, the kinetic properties of thymoquinone resemble to voriconazole but needs further investigations.

Preparation of Reagents & Media:

Thymoquinone (Sigma, USA) and amphotericin B (Sigma, USA) were separately dissolved in 4 ml of dimethyl sulphoxide (DMSO) (Sigma, USA) and then serially diluted in dermasel agar to give final concentrations of 1.0, 0.5, 0.25, 0.125, 0.06 & 0.031 mg/ml. Four plates of each concentration were prepared. Four plates of dermasel agar containing the same concentrations of DMSO as in the treated plates, alone were used as a control.

Susceptibility Testing:

Susceptibility testing was carried out as previously described (Ali-Shtayeh & Abu-Ghdeib, 1999). A mycelial disc of clinical isolate of *Fusarium solani*, 5 mm in diameter, cut from the periphery of 7 days old culture in dermasel agar was aseptically inoculated onto each set of above mentioned plates. The inoculated plates were incubated at 30°C for 10 days. The growth was examined on 4th and 7th days and finally reported on 10th day of inoculation and results interpreted by measurement of the mean diameter of the growth. The percentage inhibitions of *Fusarium solani* with different concentrations of thymoquinone and amphotericin B were then calculated by taking its growth on non-drug dermasel agar as 100%.

Statistical Analysis:

The results of thymoquinone and amphotericin B were compared statistically using students "t" test and P-values determined for the differences between the means \pm se of corresponding concentrations of thymoquinone and amphotericin B.

Results:

Percentage inhibitions of growth of *Fusarium solani* with 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg thymoquinone and amphotericin B /ml on 10th day of incubation are shown in table 1. Growth on the control plates on respective days was considered as 100%. There was complete inhibition of growth at 1.0 mg thymoquinone/ml and there was no complete inhibition of growth at any concentration of amphotericin B. At higher concentrations (0.5-1.0 mg/ml), thymoquinone, giving a steep dose-effect relationship, more effectively inhibited the growth of a clinical isolate of *Fusarium solani* as compared to amphotericin B, which gave a rather shallow dose-effect relationship. However, amphotericin B was better at lower concentrations (0.031-0.25mg/ml).

volatile oil, ether extract and its active principle thymohydroquinone has been reported in the literature against a number of bacteria (including *Staphylococcus aureus*, *Pseudomonas aeruginosa* & *Escherichia coli*) and fungi like *Candida albicans* & *Aspergillus niger* (Topozada et al. 1965; El-Fataty, 1975; Hanafi & Hatem, 1991; Morsi, 2000; Al-Jabre et al., 2003).

Fusarium solani is a filamentous mold. Even though filamentous molds are ubiquitous in the environment, only over the past two decades have such saprophytic fungi emerged as a major threat in patients with compromised host defenses, such as those with hematologic malignancies and bone marrow transplant recipients (Anaissie et al., 1989; Marr et al., 2002; Kontoyiannis & Bodey, 2002). *Aspergillus* is by far the most common mold causing severe infections. However, *Fusarium* spp., have been increasingly recognized as lethal pathogens in these patients after invasive aspergillosis (Anaissie et al., 1989; Marr et al., 2002; Nelson et al., 1994; Martino et al., 1994; Boutati & Anaissie, 1997; Girmenia et al., 2000).

The skin and respiratory tract are the primary portals of entry for *Fusarium* infection (Nelson et al., 1994; Guarro & Gene, 1995; Musa et al., 2000). Localized skin and nail infections have also been associated with subsequent dissemination of *Fusarium* species when the patient becomes neutropenic during the course of immunosuppressive treatment (Gupta et al., 2000). Hospital water distribution systems have recently been implicated as sources of nosocomial fusariosis (Anaissie et al., 2001).

Keeping in view the antibacterial and anti-aspergillus activity we thought that *N. sativa* or some of its active principles might have useful activity against *Fusarium solani*, a relatively resistant opportunistic fungus. In this study, the activity of thymoquinone against *Fusarium solani* was compared to that of amphotericin B *in vitro*.

Materials and Methods:

Fusarium solani was isolated from a nail clipping from an adult immunocompetent male with clinical diagnosis of onychomycosis. The specimen was cultured on dermasel agar (Oxoid) in the Department of Microbiology, College of Medicine, King Faisal University, Dammam, Saudi Arabia. The plates were incubated at 30°C for 10 days. The growth was identified as *Fusarium solani* by colonial morphology and by microscopy after staining with lactophenol cotton blue.

Comparison of Antifungal Activity of Thymoquinone and Amphotericin B Against *Fusarium solani* in-vitro.

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Abstract:

The activity of thymoquinone, an active principle of *Nigella sativa*, and amphotericin B was compared against a clinical isolate of *Fusarium solani*. The organism was isolated from a nail lesion of an immunocompetent adult male in routine fungal culture on dermasel agar. For susceptibility testing the organism was grown on two sets of dermasel agar containing, 1.0, 0.5, 0.25, 0.125, 0.062 & 0.031 mg of thymoquinone and amphotericin B/ml. It was grown on dermasel agar alone as control. The growth on 10th day of inoculation was recorded as % inhibition taking growth of control as 100%.

There was 0, 2.0, 3.0, 18.3, 59.3, and 100% inhibition of growth of *Fusarium solani* with 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg thymoquinone /ml on 10th day of incubation. At similar concentrations of amphotericin B, there was 6.2, 24.5, 31.2, 39.7, 54.4 and 72.4% inhibition of growth. There was no complete inhibition of growth at any concentration of the drug.

The study shows that at higher concentrations thymoquinone, giving a steep dose-effect relationship, more effectively inhibited the growth of a clinical isolate of *Fusarium solani* as compared to amphotericin B which gave shallow dose-effect relationship. However, amphotericin B was better at lower concentrations.

Key words: *Nigella sativa*, thymoquinone, amphotericin B, *Fusarium solani*

Introduction:

Nigella sativa called as Habbah Al-Sauda in Arabic, is commonly used as a natural remedy for many ailments over 2000 years and is frequently added to bread and pickles as a flavouring agent (Al-Kadi & Kandil, 1986). Many active principles have been isolated from *N. sativa* seed including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellidine, nigellimine-N-oxide and alpha-hedrin (Al-Dakhkhany, 1963; Ata-ur-Rahman et al., 1985; Ata-ur-Rahman & Malik, 1995; Kumara & Huat, 2001). Besides many other pharmacological effects, activity of *N.sativa*

التداخلات الدوائية لعقاري الأيسوميتاميديم و الدايمينازين مع بعض الوسائط الموضعية

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كلية الصيدلة ، جامعة الخرطوم ، السودان

الملخص:

أجرى هذا البحث لدراسة تأثيرات لمضادين من مضادات المثقيبات(الترانسوما) وهما الأيسوميتاميديم والدايمينازين على التقلصات التي أحدثها كل من السيروتونين والنورأدرينالين والهستامين في الشريان الأبهر المعزول من الجمال. وذلك لتفسير بعض أعراض السمية التي تظهر عند استعمال هذين العقارين في الجمال.

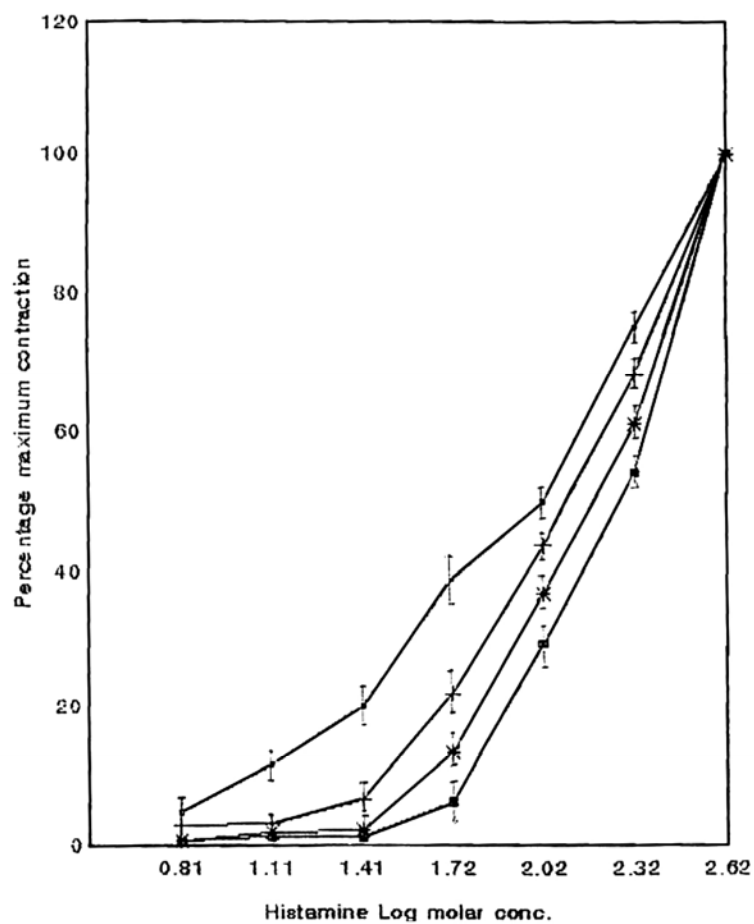
أدت إضافة كل من السيروتونين و النورأدرينالين والهستامين إلى الشريان الأبهر لأحداث تقلصات تتناسب طردياً مع جرعات المحفز.

أدت معالجة الأبهر بعقار الأيسوميتاميديم بجرعات (١,٩, ١٩, ١٩٠ مايكرومولار) إلى تثبيط التقلصات التي أحدثها كل من السيروتونين والنورأدرينالين و الجرعات الصغيرة من الهستامين ولكن زادت من التقلصات التي أحدثتها الجرعة الكبيرة من الهستامين.

أدت معالجة الأبهر بعقار دايمينازين بنفس الجرعات أعلاه إلى زيادة التقلصات التي أحدثتها كل من مادتي السيروتونين والنورأدرينالين بينما ثبتت تلك التي أحدثها الهستامين.

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—○— Histamine + Diminazene 1.9X10⁻⁶M
 * Diminazene 1.9 X 10⁻⁵M ■ Diminazene 1.9X10⁻⁴M

Fig. (7) : Effects of diminazene aceturate (Berenil) on the cumulative dose-response curves for histamine in isolated camel aorta

* Each point represents the mean of five animals

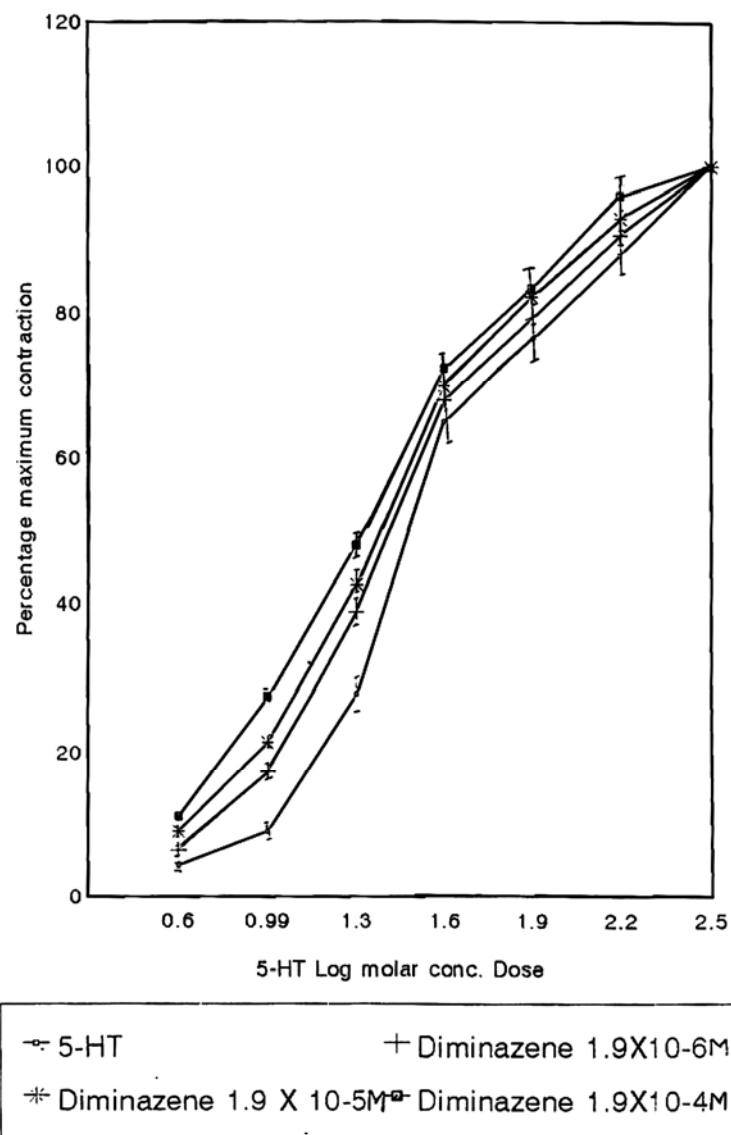


Fig. (6) : Effects of diminazene aceturate (Berenil) on the cumulative dose-response curves for 5HT in isolated camel aorta

* Each point represents the mean of five animals

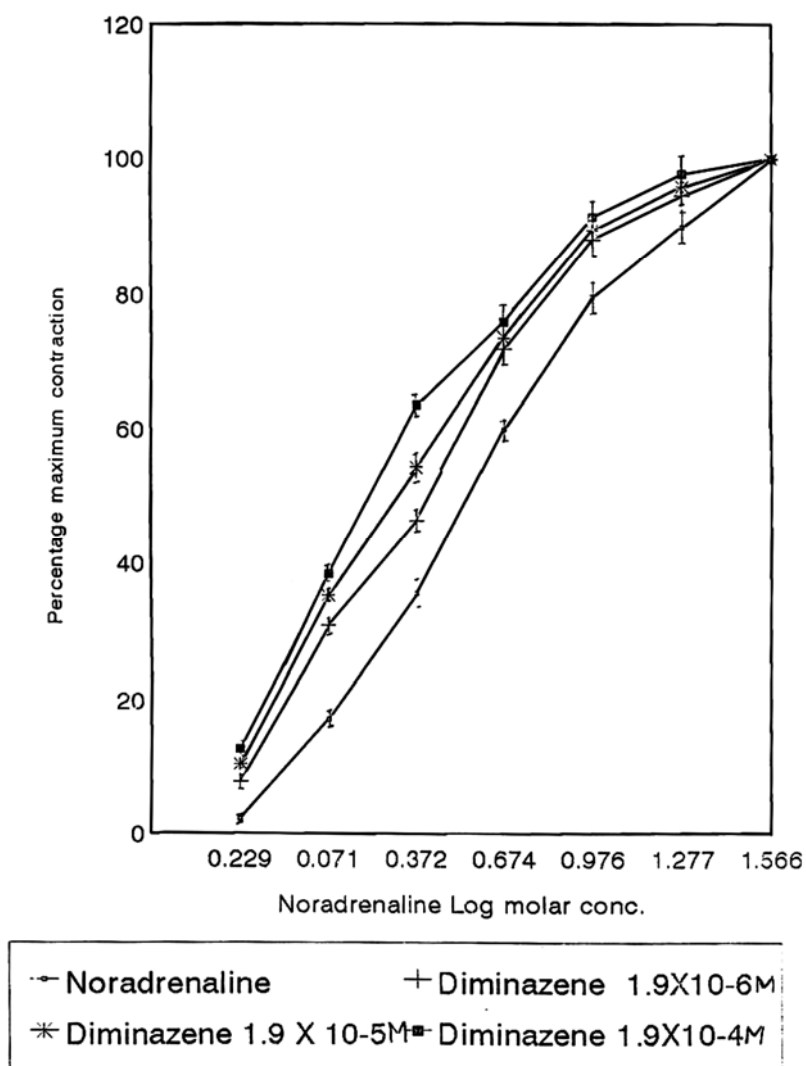


Fig. (5) : Effects of diminazene aceturate (Berenil) on the cumulative dose-response curves for noradrenaline in isolated camel aorta.

* Each point represents the mean of five animals

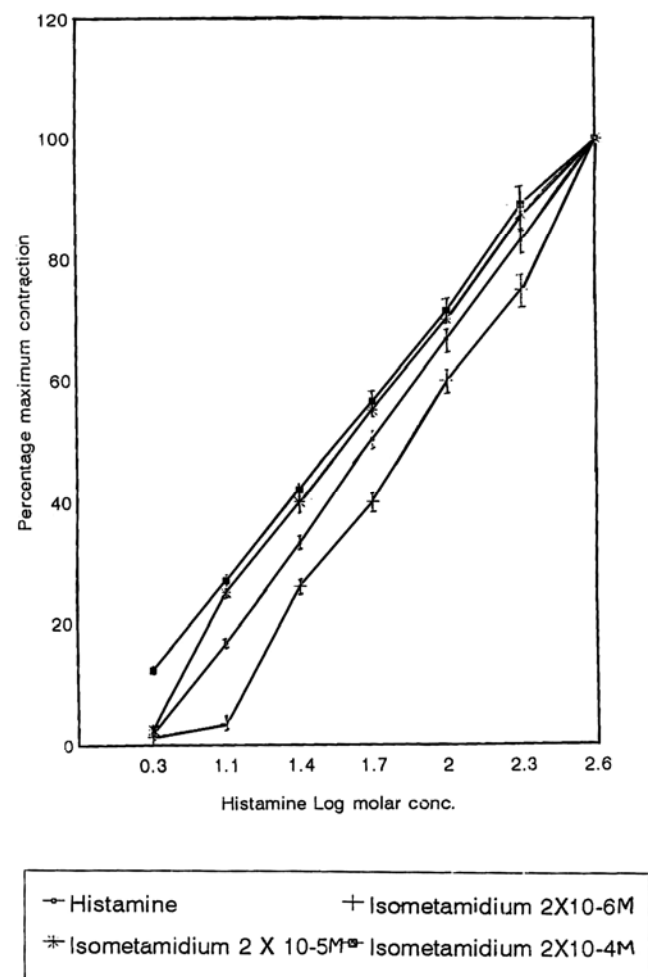


Fig. (4) : Effects of isometamidium (Samorin) on the cumulative dose-response curves for histamine in isolated camel aorta

* Each point represents the mean of five animals

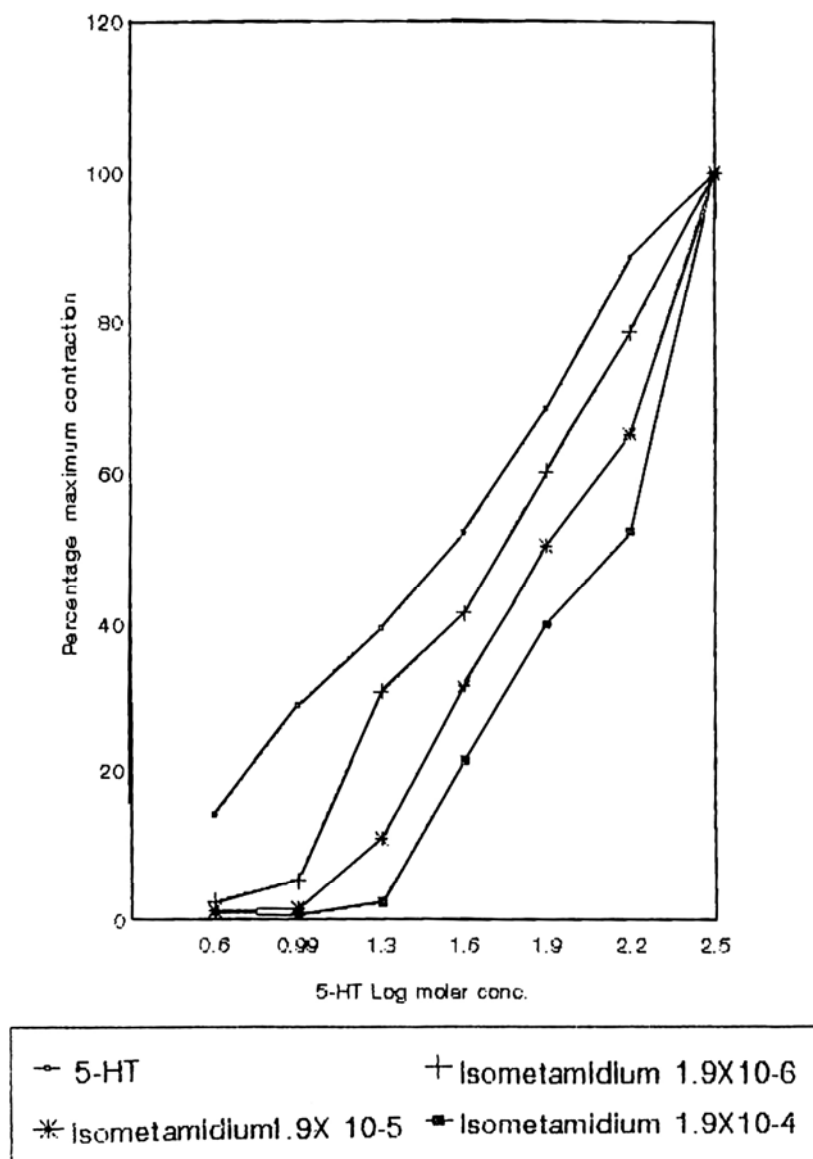


Fig. (3) : Effects of isometamidium (samorin) on the cumulative dose-response curves for 5HT in isolated camel aorta

* Each point represents the mean of five animals

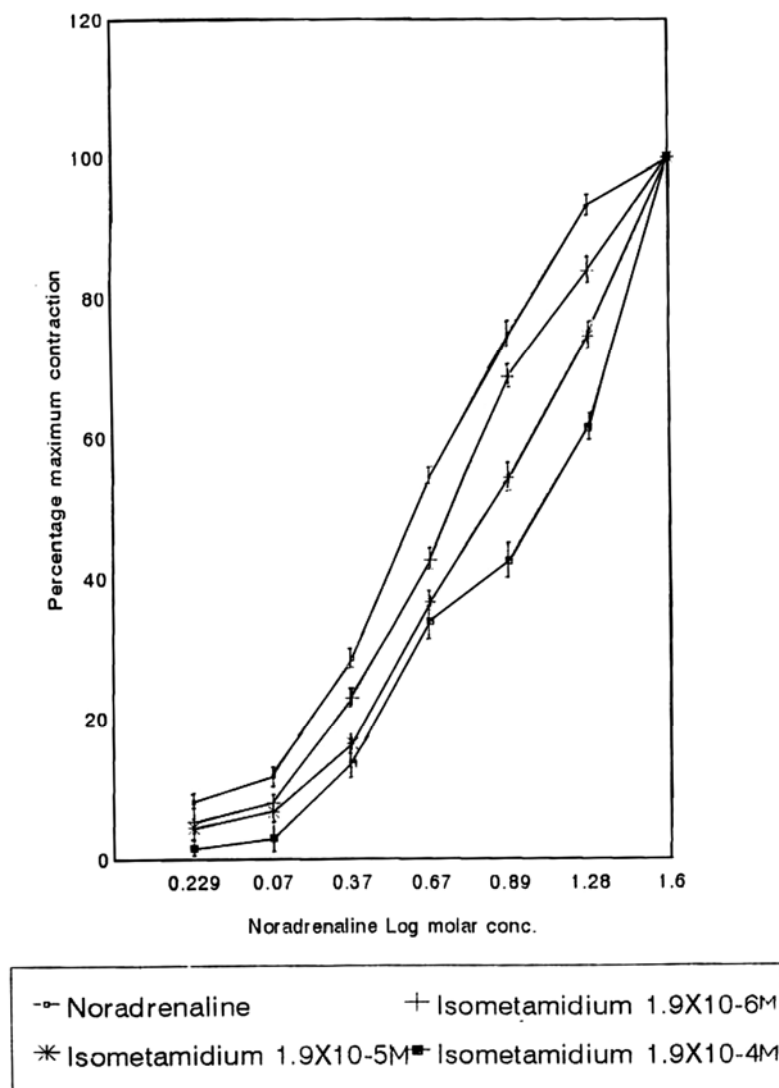


Fig. (2) : Effects of isometamidium (Samorian) on the cumulative dose-response curves for noradrenaline in isolated camel aorta

* Each point represents the mean of five animals

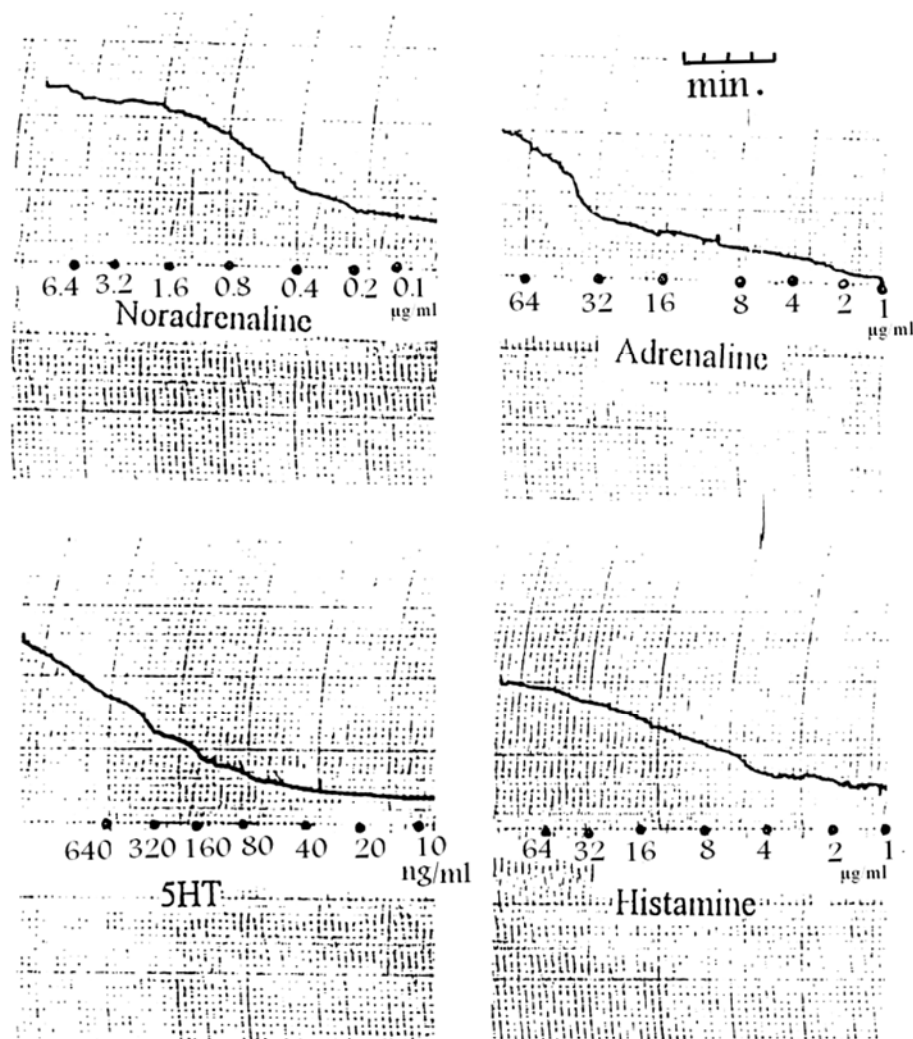


Fig. 1 : Typical in vitro responses of isolated camel artery in Krebs solution aerated with pure O_2 at 37°C . Resting tension 3g. The vessel contracted to cumulative doses of noradrenaline (0.1-6.4 $\mu\text{g/ml}$), adrenaline (1-64 $\mu\text{g/ml}$), 5HT (10-640 ng/ml), and histamine (1-64 $\mu\text{g/ml}$)

* Indicate the time points when additional compound was added to the incubation solution

However, at these concentrations, isometamidium might be acting on the mast cell located within the vascular smooth muscle to release more histamine or blocking diamine oxidase (histaminase).

As shown in the results section, isometamidium (1.9, 19, 190 μ M) inhibited 5-HT and noradrenaline responses while diminazene produced potentiation. Drugs that augment or potentiate adrenergic activities act by blocking the uptake of the neurotransmitter and/or inhibiting the monoamine oxidase (MAO) which inactivates noradrenaline. It seemed that diminazene was acting in a similar manner to potentiate noradrenaline response on the isolated camel aorta.

In the camel aorta, inhibitory effects of trypanocides on 5-HT responses can be explained by their effects on the receptor. While the augmentation of the effect may be explained by inhibition of uptake and/or inhibition of the enzymes monoamine oxidase or hydroxyindole-O-methyltransferase.

In conclusion, the reported serious adverse effects of isometamidium and diminazene when these drugs were used in Dromedary Camels, could largely be ascribed to the trypanocides interactions with all the drugs employed.

Interaction of isometamidium and diminazene with selected autonomic and autacoid mediator substances:

The two trypanocides had no contractile effects on the camel isolated aorta, but had effects on the autacoid and autonomic agents used.

Effects of isometamidium:

Noradrenaline responses were markedly inhibited and the maximum response was reduced with isometamidium concentrations (1.9, 19, 190 μ M) (Fig 2).

5-HT responses were also inhibited dose-dependently, (Fig 3). At 2 μ M, histamine responses were weakly inhibited, but at 20, 200 μ M histamine responses were potentiated in a dose-dependent manner, (Fig 4).

Effects of diminazene:

At doses (1.9, 19, 190 μ M), the noradrenaline responses were shifted to the left (Fig. 5).

5-HT responses were also potentiated (Fig 6).

Histamine responses were inhibited at 119 and 190 μ M dose -dependently (Fig. 7).

Discussion:

As shown in the results section, 5-HT, noradrenaline, adrenaline and histamine contracted the isolated camel aorta in a dose-dependent fashion. This observation is consistent with previous results obtained on the pulmonary aorta and vein and the bronchial aorta of cattle; (Archambault,1977; Arowolo and Eyro, 1979, 1984).

The responses of histamine on the isolated aorta were inhibited by diminazene aceturate (1.9, 19 and 190 μ M), and isometamidium (2 μ M). The results may reflect that at these concentrations, the two trypanocides show antihistaminic properties, and the inhibitory effect on histamine might be due to the blockade of the receptors of histamine by the trypanocides.

Isometamidium (20 μ M and 200 μ M) enhanced the histamine responses on the isolated aorta. These concentrations of isometamidium are likely to be achieved *in vivo* in Dromedary Camels. Administration of isometamidium chloride (0.5 & 1mg/kg) resulted in plasma concentrations of 44.3 \pm 10.2 and 49.9 \pm 10.2 μ M respectively, half an hour after treatment (Ali and Hassan, 1986).

- Carbachol.
- Histamine acid phosphate.
- Isoprenaline.
- L-Noradrenaline.
- Serotonin creatinine sulphate
- Vasopressin.
- Diminazene aceturate. (Berenil, Hoechst)
- Isometamidium chloride. (Samorin, M&B)

4. Statistical Analysis:

Results were expressed as mean \pm SEM.

Dose-response curves were fitted using Medusa soft ware and effective median dose (ED₅₀) values were directly determined.

Results:

Susceptibility of camel isolated aorta to selected autonomic and autacoid mediator substances:

The camel aortic strips contracted dose-dependently to 5-HT, noradrenaline, adrenaline and histamine. The tissue was most sensitive to 5-HT, noradrenaline, adrenaline and least sensitive to histamine. Typical individual responses are shown in Figure (1). While carbachol (0.5-64 μ g/ml), isoprenaline (0.05 - 64 μ g/ml), angiotensin (0.01 - 0.64 μ g/ml) and vasopressin (1 IU) did not show any contraction at the doses used.

ED₅₀ values for each agonist were obtained using Medusa soft ware. Results are shown in table (1).

Table (1)

ED₅₀ values for each agonist in camel aorta strips

* Each value is the mean of five experiments.

Drug	ED ₅₀
Adrenaline	34.8 \pm 1.8 μ M
Noradrenaline	9.6 \pm 1.5 μ M
Histamine	82.7 \pm 4.2 μ M
5-HT	0.13 \pm 0.01 μ M

The aim of this work is to investigate the interaction of two trypanocides with histamine, 5-HT, adrenaline and noradrenaline responses on isolated camel aorta, in order to elucidate the mechanism of toxic actions of these trypanocidals in Dromedary Camel.

Materials and Methods:

1. Susceptibility of camel isolated aorta to selected autonomic and autacoid mediator substances:

Strips of aorta branch were obtained from freshly killed camel at a local slaughtering house. Branches of the aorta were usually transported to the laboratory in cold Krebs' solution. The tissues were then trimmed of excess fat, connective tissue and parenchyma. Rings 3 mm wide were opened to form strips, mounted in 10 ml organ bath containing Krebs' solution, and gassed with pure O₂ at 37° C. Vascular strips were allowed to equilibrate for approximately 45 min. under a 3g resting tension.

Contractions to the autacoid and autonomic drugs were recorded with isotonic transducer (T₃) connected to FC 100 coupler on Oscillograph 400MD/2(George Washington LTD, England). Cumulative doses for adrenaline, noradrenaline, 5HT, histamine, carbachol, isoprenaline, angiotensin and vasopressin were added to the organ bath and their responses were recorded.

2. Interaction of isometamidium and diminazene with selected autonomic and autacoid mediator substances:

The aorta preparation was prepared as described before.

Then the previous cumulative doses of each autacoid were re-established in the presence of each trypanocide. The trypanocide was left in contact with the tissue for 10 minutes, contractions were recorded with isotonic transducer (T₃).

Three doses of each trypanocide (1.9, 19, 190 µM) were used.

Log concentration response curves of agonists were plotted.

3. Sources of Drugs:

All drugs were of BDH analar except otherwise stated. These included:

- Adrenaline hydrogen tartrate.
- Angiotensin.

Pharmacological Interactions of Isometamidium and Diminazine With Some Mediator Substances

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Abstract:

The effects of isometamidium and diminazene upon responses of serotonin, noradrenaline and histamine were studied in isolated camel aorta to explain the mechanism of toxicity of these trypanocidal drugs in Dromedary Camel.

It was found that 5-HT, noradrenaline, adrenaline and histamine contracted the isolated camel aorta in a dose-dependent fashion. Isometamidium (1.9, 19, 190 μ M) inhibited 5-HT and noradrenaline responses while histamine responses were inhibited at low doses and potentiated at high doses. Diminazene (1.9, 19, 190 μ M) produced potentiation of 5-HT and noradrenaline responses while histamine responses were inhibited at the same doses. Isometamidium and diminazene were used at different molarities because toxicity was observed at different doses.

Key words:

Diminazene, Isometamidium, Camel aorta , Autacoids, Autonomic antagonists.

Introduction:

The use of antitrypanosomal drugs for cure of trypanosomal infection is one of the several techniques used in controlling trypanosomal infection in man and animals.

Many drugs are now used in the treatment of this infection, such as isometamidium and diminazene.

Use of isometamidium in camels at doses of 0.5 -1.0 mg/kg caused some serious adverse effects including salivation, diarrhoea, frequent urination, defecation, trembling, hind leg weakness and recumbency (Schillinger *et al.*,1985; Ali and Hassan ,1986). While diminazene at doses of 10-40 mg/kg caused hyperaesthesia, salivation, intermittent convulsions, frequent urination and sweating(Leach, 1961; Homeida *et al.*, 1981).

Toxicity in camels have been associated to either the release of or an interaction with autacoids such as serotonin and histamine and cholinergic agents by the trypanocidal drugs within the host tissue (Wien, 1993; Hawking, 1963;Goodwin and Borehan, 1966; Steck, 1971).

تأثير الكلوتريمازول على القرحة المعدية والأثنى عشرية

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الملخص :

تشمل الدراسة معرفة تأثير دواء من مشتقات الأميدازول (كلوتريمازول) على القرحة المعدية والأثنى عشرية الناتجة عن التأثير الكيميائي أو الضغوط النفسية وتأثيرها على افرازات المعدة لحامض الهيدروكلوريك. ولقد تم اختيار هذا المركب بناء على أن :

- (١) الاوميبرازول وهو من مشتقات الاميدازول كان أول دواء يرخص في السوق للاستعمال كمضاد للقرحة ويعمل على تثبيط مضخة البروتونات مباشرة.
- (٢) لتأثيرها على مستوى بعض المركبات مثل البروستاغلاندين والثرومبوكسان واليكوتراين.

أستحدثت القرحة المعدية عن طريق استخدام الايثانول والاندوميثاسين و عن طريق غمس الجرذان في الماء البارد (الضغط النفسي)، بينما تم استحداث القرحة الأثنى عشرية عن طريق استخدام مركب السيستامين تمت دراسة كمية إفراز الحمض المعدي بطريقة ربط الفم المعدي السفلي للجرذان المعالجة بالكلوتريمازول أو غير المعالجة (مجموعة ضابطة).

أوضحت نتائج الدراسة قدرة الكلوتريمازول على خفض مستوى الافرازات المعدية بشكل مطرد مع كمية الجرعة من الدواء (بنسبة تصل إلى ٨٠ %)، كما أن المعالجة المسبقة بالكلوتريمازول قللت من تكون القرحة المعدية الناتجة من الايثانول والاندوميثاسين والضغط النفسي (بنسبة تصل إلى ١٠٠ %)، كما استطاع الكلوتريمازول حماية الأثنى عشر من القرحة الناتجة بواسطة السيستامين.

من نتائج هذا لبحث أنه سلط الضوء على فعالية الكلوتريمازول ضد التقرحات المعدية والأثنى عشرية التجريبية، والتي تحتاج إلى المزيد من البحث لدراسة إمكانية استخدامها في تجارب سريرية.

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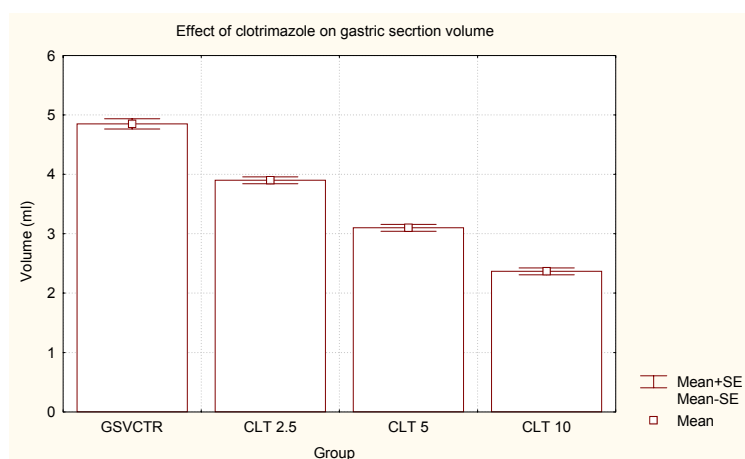


Figure 5: Effect of different doses of Clotrimazole (2.5, 5 and 10 mg/kg) on gastric acid secretion in pylorus ligated (Shay) experimental female Albino rats.

GSVCTR= control group with ligated pylorus.

CLT= test groups with ligated pylorus and treated with clotrimazole in different doses

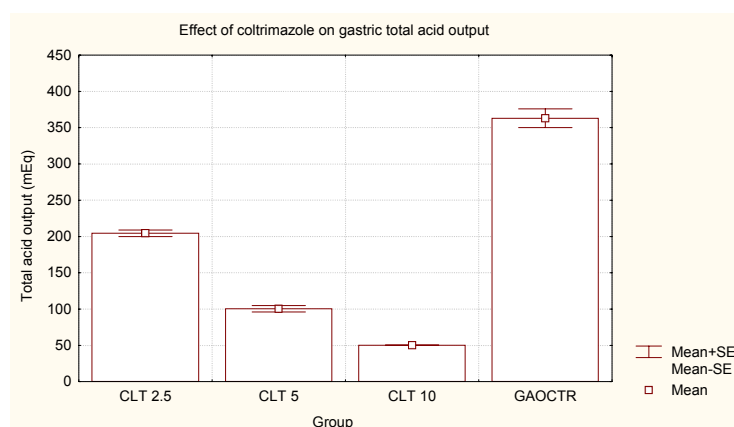


Figure 6: Effect of different doses of Clotrimazole (2.5, 5 and 10 mg/kg) on gastric total acid output in pylorus ligated (Shay) experimental female Albino rats.

GAOCTR= control group with ligated pylorus.

CLT= test groups with ligated pylorus and treated with clotrimazole in different doses.

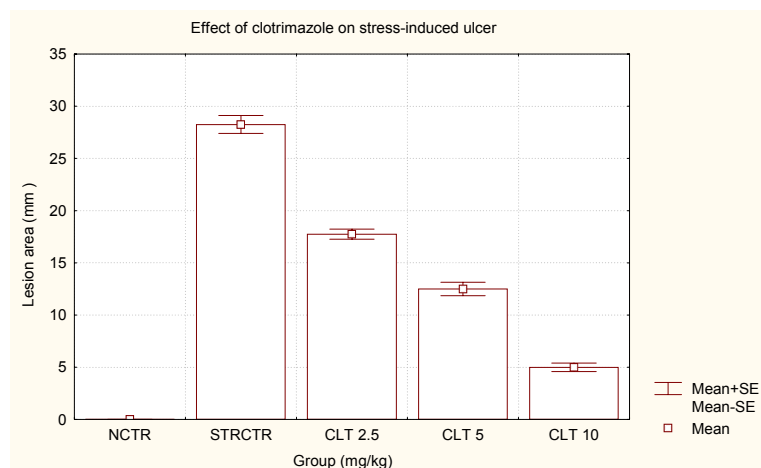


Figure3: Effect of different doses of Clotrimazole (2.5, 5 and 10 mg/kg) on stress-induced ulcer in experimental female Albino rats.

NCTR= control group without any treatment.

STRCTR= control group immersed in cold water.

CLT= test groups immersed in cold water and treated with clotrimazole in different doses

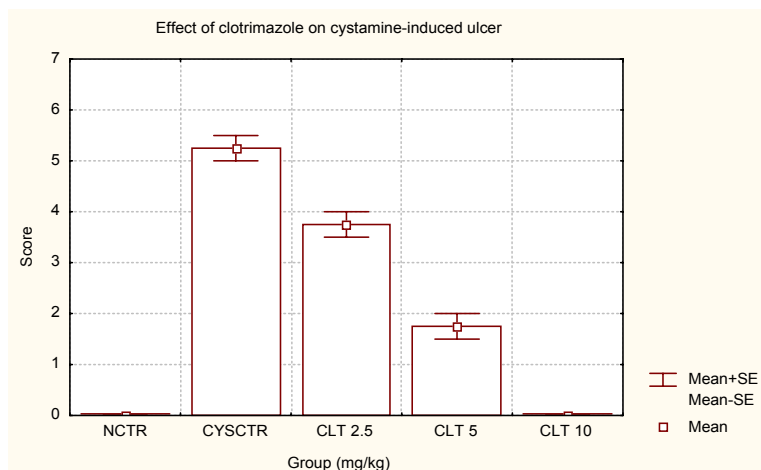


Figure4: Effect of different doses of Clotrimazole (2.5, 5 and 10 mg/kg) on cystamine-induced ulcer (400 mg/kg in 10% aqueous solution) in experimental female Albino rats.

NCTR= control group without any treatment.

CYSCTR= control group treated with cystamine only.

CLT= test groups treated with cystamine and clotrimazole in different doses

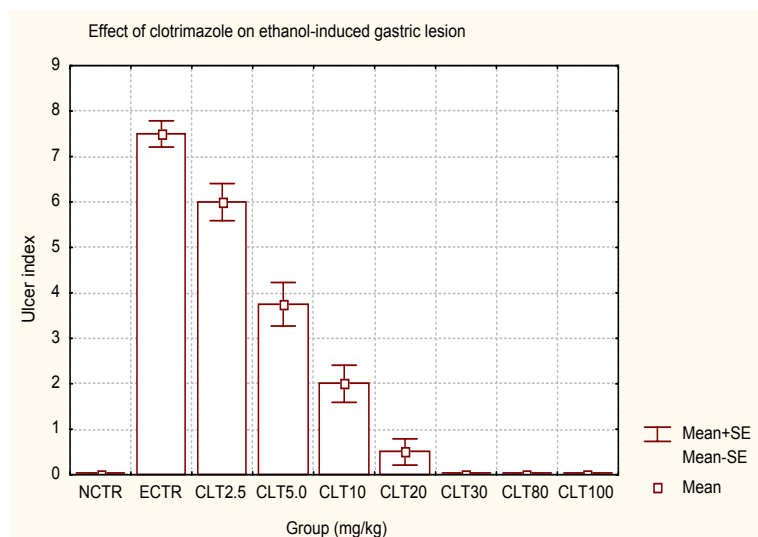


Figure1: Effect of different doses of Clotrimazole (2.5, 5, 10, 20, 30, 80 and 100 mg/kg) on ethanol-induced gastric lesions (1 ml) in experimental female Albino rats.

NCTR= control group without any treatment.

ECTR= control group treated with ethanol only.

CLT= test groups treated with ethanol and clotrimazole in different doses.

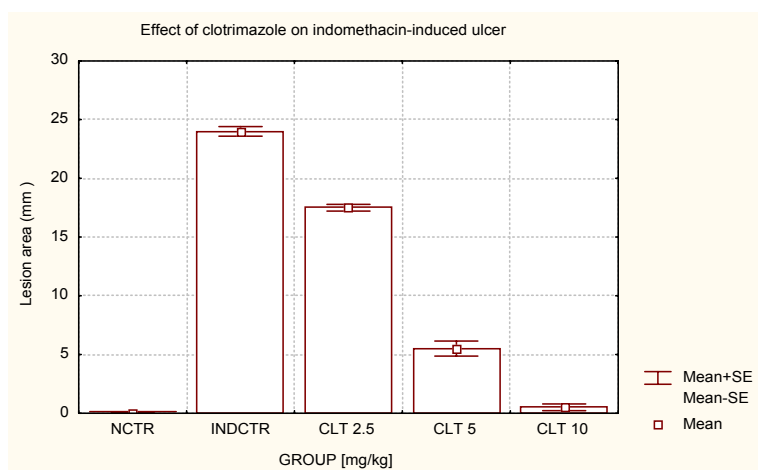


Figure2: Effect of different doses of Clotrimazole (2.5, 5 and 10 mg/kg) on indomethacin-induced ulcer (30 mg/kg body weight) in experimental female Albino rats.

NCTR= control group without any treatment.

INDCTR= control group treated with indomethacin only.

CLT= test groups treated with indomethacin and clotrimazole in different doses

synthesis by econazole [18,31]. This may explain the ability of this drugs to significantly inhibit the formation of gastric lesions induced by indomethacin.

Furthermore, the results revealed that pretreatment of animals with clotrimazole protected them against stress-induced lesions, in a dose-dependent pattern. The disturbances of gastric mucosal microcirculation [34,35], altered gastric secretion [36,37] and abnormal gastric motility [38] have been considered to be the pathogenic factors responsible for stress-induced gastric lesions. Numerous recent studies have indicated a substantial role of oxygen-derived free radicals (ODFR) [39] and leukotrienes [40] in mediating stress-induced mucosal injury. The ratio of prostacyclin to thromboxane A_2 is considered to be an important factor in the maintenance of gastric mucosal microcirculation and integrity [22]. The ability of clotrimazole to inhibit this cascade either by inhibiting (ODFR) formation [41,42] or by inhibiting formation of thromboxane and increasing formation of PGE_2 might be responsible for protecting gastric mucosa against stress-induced lesions [18,31].

Pretreatment of rats with clotrimazole significantly protected rats against cysteamine-induced duodenal ulcer, in a dose dependent pattern. The pathogenesis of cysteamine-induced duodenal lesions is far from clear. Cysteamine ulcers are considered to be associated with the hypersecretion of gastrin and hydrochloric acid and decreased mucosal resistance [43,44]]. The anti duodenal ulcer activity of clotrimazole may to a large extent be attributed to its ability to directly inhibit acid secretion by blocking H^+-K^+ ATPase [30].

Pretreatment of rats with clotrimazole produced a dose dependent decrease in the volume and acid output of gastric secretion in Shay rats. The increase in gastric acidity is considered an important contributing factor in the pathogenesis of gastric and duodenal ulcers and is often termed 'aggressive factor' [45]. The regulation of gastric acid secretion is complex; endogenous gastrin, histamine, somatostatin and cholinergic mechanisms play major roles in controlling gastric secretions [46]. These entire pathways converge on and modulate the activity of the proton pump of the parietal cells [47]. Clotrimazole possibly decreases acid secretion at the last step by blocking H^+-K^+ ATPase [30].

In conclusion, clotrimazole have gastroduodenal protective activity. Further studies are suggested to shed more light on the role of clotrimazole in the prophylaxis and/or the treatment of gastrointestinal ulcer diseases.

Cysteamine-Induced Duodenal Ulcers

All the normal control rats have shown no formation of the duodenal ulcers. Administration of cysteamine hydrochloride produced elongated lesions extending longitudinally down the duodenum. The lesion area of the rats in the cysteamine group was found to be 5.25mm² (figure 4). Pretreatment of rats with Clotrimazole at doses of 2.5 and 5 mg/kg produced statistically significant decrease in the intensity of cysteamine-induced ulcers, which was dose-dependent, complete protection of gastric mucosa was observed in the rats treated with a dose of 10mg clotrimazole /kg body weight (figure 4).

Rats Gastric Secretion and Total Acid Output in Pylorus-Ligated (Shay)

The control rats' pylorus ligated for 6h resulted in accumulation of 4.85ml of gastric secretions (pgs) and a total acid output (pao) of 363 mEq. Pretreatment of rats with 2.5 mg/kg of clotrimazole insignificantly reduced the gastric secretion volume, while the volume of gastric secretion in the rats treated with 5 and 10mg/kg of clotrimazole was statistically significant reduced (figure5). A significant total acid output, which was dose-dependent, was observed in the rats treated with 2.5, 5 and 10 mg/kg of clotrimazole (figure6).

Discussion

The results of this study indicate significantly the ability of clotrimazole to inhibit the formation of gastric ulcer in rats induced by absolute ethanol. This inhibition is dose dependent and complete protection was achieved at doses >30 mg/kg. The mucus gel adhering to the gastric mucosal surfaces protects the underlying epithelium against acid, pepsin and necrotizing agents such as absolute ethanol and indomethacin [27,28]. Ethanol causes damage of rat gastric mucosa by stasis of blood flow [29]. Therefore, it seemed likely that the gastroprotective activity of clotrimazole against the deleterious effects of ethanol could result, at least in part, from the decreasing of acid secretion [30]. This is accompanied by an increase in PGE₂ production [18, 31], which prevents the stasis of mucosal blood flow [8].

Similarly, pretreatment of rats with clotrimazole significantly protected rats against indomethacin-induced gastric ulcers in a dose-dependent pattern. Gastropathy associated with chronic use of NSAIDs is one of the major public health problems. Although it has been proposed that a deficiency of endogenous prostaglandins and increase of thromboxane A₂ due to the inhibition of cyclooxygenase by indomethacin which is involved in these effects, the exact pathogenic mechanism remains to be elucidated [32,33]. Clotrimazole causes an increase of PGE₂ production with selective inhibition of thromboxane

Statistical Analysis

Data are presented as mean from 4 rats per group. Statistical analyses were performed using the statistical package for social sciences (SPSS) system. Differences with a p value <0.05 were considered significant. Figures are presented as mean \pm SEM by using Statistica vr.5.0 program.

Results

Ethanol -Induced Gastric Lesions

The normal control rats have shown no formation of the gastric ulcers. The treatment of rats with one-milliliter absolute ethanol produced extensive gastric lesions in the glandular mucosa of the stomach in 100% of the control animals. These lesions were characterized by multiple hemorrhagic red bands (patches) of different sizes along the axis of the glandular stomach. The ulcer index mean was found to be 7.5 in control animals one hour after ethanol administration. Pretreatment of rats with clotrimazole at doses of ≥ 30 mg/kg completely prevented the formation of gastric lesions. Pretreatment of rats with clotrimazole at the doses of 2.5, 5, 10 and 20 mg/kg produce statistically significant inhibition of the formation of gastric lesions, which was dose-dependent (figure 1).

Indomethacin-Induced Gastric Mucosal Damage

All the normal control rats in this group have shown no formation of the gastric ulcers. The administration of indomethacin resulted in production of gastric lesions mainly in the glandular stomach in 100% of the animals. The lesion area in the control group was found to be 24mm² (figure 2).

Pretreatment of rats with clotrimazole at doses of 2.5, 5 and 10 mg/kg produced statistically significant decrease in the intensity of indomethacin-induced ulcers, which was dose-dependent (figure 2).

Water-Immersion Restraint Stress Induced Gastric Lesions

All the normal control rats have shown no formation of the gastric ulcers. The rats exposed to water immersion and restrain stress showed considerable ulcerogenicity in the form of haemorrhagic mucosal lesions in the stomach. There was evidence of intraluminal bleeding in these animals. The lesion area in the control group was 28.25 mm² (figure 3).

Pretreatment of rats with Clotrimazole at doses of 2.5, 5 and 10 mg/kg produced statistically significant decrease in the intensity of water-Immersion restraint stress induced ulcers in a dose-dependent fashion (figure 3).

linear ulcer less than 10mm in length. The scores of each single lesion were then summed to determine the ulcer index which will be represented by lesion area (mm^2).

Stress-induced gastric ulcer:

One hour after clotrimazole treatment in different doses (2.5, 5 and 10 mg/kg), rats were placed in a restraint cage and immersed vertically to level of the xiphoid process in a water bath (15°C - 20°C) for 7-9 hours. Then, the animals were sacrificed using anesthetic ether. The stomachs were removed and opened along the greater curvature. After washing with saline, the gastric lesions were quantified [23]. The ulcers were scored according to the methods of Valcavi et al [22] as in the previous method.

Study of Gastric Secretion Using Pylorus Ligated (Shay) Rats method

Female Wistar Albino rats weighing 180-250 grams, approximately of the same age and fed on standard chow diet were used. They were fasted for 36 hours before experimentation. Only water was allowed ad libitum.

After 30 minutes of clotrimazole administration in different doses (2.5, 5 and 10 mg/kg), the pylorus was ligated under light ether anesthesia, care being taken not cause bleeding or to occlude blood vessels. The animals were sacrificed 6 hours after pylorus ligation [24].

The stomachs were removed, contents collected, volume measured and centrifuged. One milliliter of supernatant was titrated against 0.01N NaOH to determine the acidity using phenolphthalein as indicator and total acid output calculated [25].

Induction of Duodenal Ulcer by Cysteamine Hydrochloride

Female Wistar Albino rats weighing 180-250 grams, approximately of the same age and fed on standard chow diet were used.

Duodenal ulcers were induced by administration of two doses of cysteamine hydrochloride (400 mg/kg in 10% aqueous solution) at an interval of 4 hours according to the method described by Szabo [26]. Clotrimazole in different doses (2.5, 5 and 10 mg/kg) were administered by gavage 30 minutes before each dose of cysteamine hydrochloride and the duodenum was excised carefully and opened along the antimesenteric side. The duodenal ulcers were scored using a scale of 0 to 3 where: 0 = no ulcer; 1 = superficial mucosal erosion; 2 = deep ulcer or transmural necrosis, and 3 = perforated or penetrated ulcer. The sum of the intensity of each lesion was used as the ulcer index [25].

Female Wistar Albino rats weighing 180-250 grams, approximately of the same age and fed on standard chow diet were used. They were fasted for 36 hours before experimentation. Only water was allowed ad libitum. The animals were randomly divided into groups. The aqueous solutions of the ulcerogens and clotrimazole were freshly prepared before administration

Methods

Ethanol induced gastric ulcer: [19]

clotrimazole were given by gavage in different doses (2.5, 5, 10, 20, 30, 80 and 100 mg/kg). After 30 minutes, the animals were administered 1ml of absolute ethanol by gavage except the animals of control group. After 1 hour, the animals were sacrificed by ether anesthesia. The stomach was removed and opened along the greater curvature, washed with saline and the lesions were assessed. Patchal lesions of the stomach were scored according to the method described by Schiantarelli, Cadel et al. [20] using the following scale: 0= normal mucosa; 1= hyperemic mucosa or up to 3 small patches; 2=4-10 small patches; 3=more than 10 small or up to 3 medium-sized patches; 4= 4-6 medium-sized patches; 5=More than 6 medium-sized or up to 3 large patches; 6=4-6 large patches; 7= 7-10 large patches and 8= More than 10 large patches or extensive necrotic zone. 'Small-sized patch' was defined as up to 2mm across (maximum diameter); 'medium-sized patch' as between 2mm and 4mm across; and 'large-sized patch' as more than 4mm across.

Indomethacin-induced gastric ulcer:

Indomethacin was suspended in 1% carboxymethylcellulose in distilled water and administered by gavage at the dose of 30mg/kg body weight. Clotrimazole in different doses (2.5, 5 and 10 mg/kg) were given orally 60 minutes prior to indomethacin administration [21]. The animals were sacrificed 7-9 hours after indomethacin administration using ether anesthesia. The stomachs were removed and opened along the greater curvature. After washing with saline, the gastric lesions were quantified. The ulcers were scored according to the methods of Valcavi et al. [22] using the following scale: 10= Deep circular ulcer more than 8mm diameter; 8= Deep circular ulcer between 7 to 8 mm diameter; 7= Deep circular ulcer between 6 to 7 mm diameter; 6= Deep circular ulcer between 5 to 6 mm diameter; 5=Deep circular ulcer between 4 to 5 mm diameter; 4= Deep circular ulcer between 3 to 4 mm diameter; 3= Deep circular ulcer between 2 to 3 mm diameter; 2= Deep circular ulcer between 1 to 2 mm diameter; 1= Deep circular ulcer less than 1mm diameter; 6= Deep linear ulcer 10mm or more in length and 3= Deep

arachidonic acid metabolism via generation of free radical and other mediators, which affect the integrity of gastric mucosa [2,3,4].

The increase in gastric acidity is considered an important contributing factor in the pathogenesis of gastric and duodenal ulcers and is often termed 'aggressive factor' [5]. Prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂) are believed to have potent anti-ulcer and cytoprotective properties [6] by retarding the senescence of cells, reducing their exfoliation [7] and preventing stasis of gastric mucosal blood flow [8], induction of superoxide dismutase [9], increasing bicarbonate and mucus secretion and decrease HCl secretion, vasodilation and re-epithelization [10]. Several studies have shown that thromboxane A₂ (TXA₂) is a powerful vasoconstrictor in the stomach of the rat [11], and because of the importance of blood flow in maintaining gastric mucosal integrity [12], TXA₂ may be implicated in the pathogenesis of gastric ulceration. Leukotrienes are the principal mediators of polymorphonuclear-leukocyte-infiltration inflammatory reactions [13], indicating the involvement of leukotrienes in the genesis of cytodestruction of gastric mucosa [14]. Neutrophils have been implicated in the development of inflammation and injury in a number of tissues including the gastric mucosa [15] by releasing a number of substances that result in tissue injury including oxygen derived free radicals (ODFR) such as the superoxide anion, hydrogen peroxide, hypochlorous acid, as well as enzymes such as myeloperoxidase (MPO) and proteases [16, 17].

None of the peptic ulcer treatments is perfect, nor can alter the root causes of ulceration and all have disadvantages. Therefore the main aim of this project is to attempt to identify an imidazole agents, which may have fewer side effects and higher efficacy than currently approved benzimidazole drugs such as lansoprazole. It is known that substituted benzimidazoles inhibit gastric acid secretion by blocking H⁺ /K⁺– ATPase [58]. In addition, Clotrimazole inhibit the thromboxane A₂ formation [18]

Materials

Chemicals

Indomethacins, cysteamine hydrochloride, crboxymethylcellulose, Absolute alcohol, Diethyl ether, NaOH and HCl are purchased from Sigma chemical company (USA). Clotrimazole (Canesten®) is purchased from Bayer Company (Germany).

Animals

Effect of Clotrimazole on Chemically and Stress Induced Peptic Ulcer

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Abstract:

Clotrimazole, substitutive benzimidazole which is currently available in markets as an antifungal agent, has been studied for its ability to inhibit gastric secretion and to protect the gastric and duodenal mucosa against chemically and stress-induced ulcers. The rationale for drug selection was based on:

- 1) An imidazole compound omeprazole was the first approved and marketed antiulcer drug, which directly inhibit hydrochloric acid secretion.
- 2) Some imidazole compounds were shown to alter the levels of prostaglandins, thromboxanes and leukotrienes.

Acid secretion studies were undertaken in pylorus-ligated rats with and without clotrimazole treatment. Experimental gastric lesions were induced by water-immersion restraint stress, indomethacin and absolute ethanol in rats; whereas duodenal ulcers were produced by treatment of rats with cysteamine. The results of this study demonstrated that clotrimazole produce a dose-dependent inhibition of gastric acid secretion in rats. Pretreatment with clotrimazole significantly attenuated the formation of stress-, indomethacin- and ethanol-induced gastric lesions. Clotrimazole also protected intestinal mucosa against cysteamine-induced duodenal ulcers. In conclusion, this study demonstrated that clotrimazole possess significant antiulcer and cytoprotective activity against various experimentally induced gastroduodenal lesions. Although the effects of clotrimazole require further evaluation, the experimental observations derived from this study provide compelling evidence to justify future investigations on the clinical relevance of using such agents in clinical trials.

Keywords

Clotrimazole, Peptic ulcer, prostaglandin, thromboxanes and leukotrienes.

Introduction

Peptic ulcer disease [PUD] is a major health problem which has a tremendous economical burden on the health institutes [1]. The etiopathology of stress and chemically induced gastric and duodenal ulcer is far from clear. A number of processes have been implicated in the pathogenesis of peptic ulcer including disruption of mucosal blood supply and hypoxic disturbance of

مقارنة المعايير الدموية و البيوكيميائية في عجول الأبقار الحساوي المرباه على الحليب فقط وعلى نظام التربية التقليدي

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الملخص :

تم في هذه الدراسة قياس المتغيرات الدموية والبيوكيميائية في عجول الأبقار الحساوي المرباه على الحليب فقط أو على نظام التربية التقليدي عند عمر ٢ إلى ١٤ أسبوع. لقد تم ملاحظة انخفاض حجم خلايا الدم المتراصة والهيموجلوبين والفيبرينوجين والحديد عند عمر ٨ إلى ١٤ أسبوع في العجول المرباه على الحليب فقط مقارنة بالتي تم تربيتها على النظام التقليدي مما نتج عنه أن العجول المرباه على الحليب فقط قد تصاب بالأنيميا نسبة لعدم وجود الحديد في الحليب.

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1997) has been attributed to the intake of large quantities of milk by calves at early weeks of development, but which might result into iron deficiency. The significant decline in MCV and MCH while MCHC remained stable, was consistent with the development of microcytic normochromic anaemia in milk-fed calves (Reece, 1997), hypochromia could develop only when calves become very anaemic (Howard, 2004).

The values recorded for total WBC and percentage lymphocytes and neutrophils in all calves agreed with those previously reported for calves of a similar age (Tennant *et al.*, 1974 and Rwakishaya *et al.*, 1985). Values for eosinophils were not constant, they were sometimes higher in milk-fed and at other times higher in conventionally reared calves.

The increase in albumin-globulin ratio was expected as such value will reflect the dietary intake of protein (Payne *et al.*, 1973). Mean blood fibrinogen concentration was significantly higher when calves were first bled than at any subsequent age in milk-fed calves.

Significantly higher values of fibrinogen concentration were recorded for conventionally-reared calves compared to milk-fed calves.

The initial decrease in serum iron concentration in milk-fed calves maybe due to an increase in plasma volume associated with intake of large amount of milk diet (Reece, 1997). Such decline in iron concentration continued upto 14 weeks of age. Serum iron concentration was observed in conventionally-reared calves suggesting that serum iron decrease immediately after birth would not increase until there was an increase in iron intake. These findings also suggest that calves raised solely on milk tend to become anaemic as a result of lack of iron in their normal diet which is cow's milk.

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Table (3)
Mean (\pm SEM) serum biochemical values in milk-fed Hassawi calves (n=10) from 2 to 14 weeks of age

Age (Weeks)	2	5	8	11	14
Serum protein (g/L)	64.10 \pm 0.50	62.1 \pm 0.50	63.0 \pm 0.50	62.5 \pm 0.45	63.5 \pm 0.55
Albumin (g/L)	31.5 \pm 0.20	32.00 \pm 0.50	33.00 \pm 0.40	32.40 \pm 0.40	33.3 \pm 0.45
Globulin (g/L)	33.6 \pm 0.25	30.1 \pm 0.50	30.00 \pm 0.50	30.10 \pm 0.50	30.5 \pm 0.50
Fibrinogen (g/L)	6.40 \pm 0.30	4.2 \pm 0.30	3.59 \pm 0.35*	3.89 \pm 0.30*	3.70 \pm 0.30*
Serum iron (μ mol/L)	34.5 \pm 0.51	32.1 \pm 0.40	25.3 \pm 0.30*	18.1 \pm 0.25*	18.6 \pm 0.25*
Serum copper (μ mol/L)	12.1 \pm 0.51	12.6 \pm 0.40	12.0 \pm 0.40	12.6 \pm 0.30	12.3 \pm 0.30

Table (4)
Mean (\pm SEM) serum biochemical values in conventionally-reared Hassawi calves (n=10) from 2 to 14 weeks of age

Age (Weeks)	2	5	8	11	14
Serum protein (g/L)	64.5 \pm 0.41	61.8 \pm 0.40	64.0 \pm 0.50	62.4 \pm 0.40	63.6 \pm 0.41
Albumin (g/L)	31.0 \pm 0.50	31.0 \pm 0.50	33.0 \pm 0.40	32.4 \pm 0.46	33.2 \pm 0.40
Globulin (g/L)	33.5 \pm 0.40	30.8 \pm 0.40	31.0 \pm 0.60	30.0 \pm 0.60	30.4 \pm 0.50
Fibrinogen (g/L)	6.25 \pm 0.30	4.4 \pm 0.40	3.50 \pm 0.40*	4.6 \pm 0.40	5.05 \pm 0.50
Serum iron (μ mol/L)	34.0 \pm 1.1	31.01 \pm 1.2	25.6 \pm 1.5*	29.6 \pm 0.81	31.1 \pm 0.83
Serum copper (μ mol/L)	12.5 \pm 0.6	11.5 \pm 0.5	12.1 \pm 0.6	11.6 \pm 0.5	11.8 \pm 0.5

Discussion:

The PCV and Hb concentration of blood from milk-fed Hassawi calves were lower than those from conventionally-fed calves. Similar results were previously reported (Rwakishaya *et al.*, 1985). The decline in PCV and Hb values in postnatal period which known as physiological anaemia (Reece,

Table (2)
Mean (\pm SEM) haematological values in conventionally-reared Hassawi calves
(n=10) from 2 to 14 weeks of age

Age (Weeks)	2	5	8	11	14
PCV (4L)	0.38 \pm 0.01	0.36 \pm 0.01	0.35 \pm 0.01	0.36 \pm 0.01	0.35 \pm 0.01*
Hb (9/dL)	12.50 \pm 0.40	12.20 \pm 0.40	11.80 \pm 0.41	11.90 \pm 0.41	12.10 \pm 0.41*
RBC ($\times 10^{12}$ /L)	9.90 \pm 0.2	10.10 \pm 0.22	10.35 \pm 0.25	11.1 \pm 0.20	11.3 \pm 0.20*
MCV (fL)	38.1 \pm 0.5	35.6 \pm	33.7 \pm	33.1 \pm	33.6 \pm
MCHC (g/dL)	33.0 \pm 0.5	33.1 \pm 0.45	32.9 \pm 0.5	32.8 \pm 0.5	33.3 \pm 0.5
MCH (Pg)	12.8 \pm 0.4	11.9 \pm 0.35	11.8 \pm 0.30	12.1 \pm 0.30	12.03 \pm 0.30*
Differential % WBC Neutrophils	30.0 \pm 0.5	29.6 \pm 0.45	29.0 \pm 0.40	30.4 \pm 0.50	30.3 \pm 0.5
Lymphocytes	65.0 \pm 0.6	66.2 \pm 0.6	67.0 \pm 0.7	65.2 \pm 0.6	64.5 \pm 0.6
Eosinophils	1.4 \pm 0.20	1.2 \pm 0.20	0.8 \pm 0.010	0.6 \pm 0.10	0.6 \pm 0.10
Basophils	0.6 \pm 0.1	0.8 \pm 0.1	0.2 \pm 0.1	0.4 \pm 0.1	0.4 \pm 0.1
Monocytes	3.0 \pm 0.20	2.2 \pm 0.20	3.0 \pm 0.20	3.4 \pm 0.20	4.2 \pm 0.25

*P<0.05, significantly different from values in milk-fed calves.

The biochemical values in milk-fed Hassawi calves are given in Table 3. Total serum protein did not change with age, but albumin and globulin ratio increased significantly ($P<0.001$) between two and eight weeks of age. Mean fibrinogen concentration decreased with time ($P<0.001$). Mean serum iron but not copper also significantly ($P<0.001$) decreased with time.

Table 4 shows the biochemical values in conventionally-reared calves. Again total protein and albumin-globulin ratio did not change with age. Serum fibrinogen and iron were significantly ($P<0.001$) lower at 8 weeks of age then gradually increased ($P<0.001$) at 11 and 14 weeks of age. Values of fibrinogen and iron were significantly ($P<0.001$) greater than those in milk-fed calves at 11 and 14 weeks of age.

Statistical analysis:

Data were analyzed by one way ANOVA, using GLM procedure of SAS and Duncan's multiple range test to detect significant differences among means.

Results :

Mean haematological values in milk fed calves raised solely on milk (group 1) are given in Table 1.

Table (1)
Mean (\pm SEM) haematological values in milk-fed Hassawi calves (n=10)
from 2 to 14 weeks of age

Age (Weeks)	2	5	8	11	14
PCV (4L)	0.37 \pm 0.02	0.33 \pm 0.01	0.29 \pm 0.01*	0.29 \pm 0.01*	0.30 \pm 0.01*
Hb (g/dL)	12.31 \pm 0.50	11.30 \pm 0.30	10.31 \pm 0.30	9.50 \pm 0.01*	9.66 \pm 0.22*
RBC ($\times 10^{12}$ /L)	9.20 \pm 0.71	9.47 \pm 0.51	9.33 \pm 0.25	9.25 \pm 0.20*	9.35 \pm 0.20
MCV (fL)	37.61 \pm 0.70	36.10 \pm 0.42	33.15 \pm 0.40*	32.60 \pm 0.70*	32.30 \pm 0.70*
MCHC (g/dL)	31.8 \pm 0.30	32.60 \pm 0.31	33.60 \pm 0.32	33.50 \pm 0.31	33.30 \pm 0.30
MCH (Pg)	12.30 \pm 0.30	11.80 \pm 0.08	11.20 \pm 0.30	10.20 \pm 0.20*	10.55 \pm 0.35
Differential % WBC					
Neutrophils	31.050 \pm 2.00	30.1 \pm 2.00	33.6 \pm 1.50	32.4 \pm 2.00	30.5 \pm 1.50
Lymphocytes	65.30 \pm 1.5	66.4 \pm 1.5	60.2 \pm 1.5	61.3 \pm 1.5	65.1 \pm 2.0
Eosinophils	0.4 \pm 0.11	0.6 \pm 0.12	1.4 \pm 0.12	1.5 \pm 0.10	0.8 \pm 0.12
Basophils	0.2 \pm	0.3 \pm 0.13	0.7 \pm 0.0	0.8 \pm 0.11	0.5 \pm 0.13
Monocytes	2.9 \pm 0.22	2.6 \pm 0.20	4.1 \pm 0.20	4.0 \pm 0.22	3.1 \pm 0.24

The PCV was significantly ($P < 0.001$) decreased at 8, 11 and 14 weeks of age. Mean Hb concentration followed a similar trend. MCV and MCH also decreased significantly with age. The total and percentage WBC did not change with time.

Table 2 shows the haematological values in conventionally-reared calves. PCV and haemoglobin and other haematological values did not change with age. Comparison of table 1 and 2 shows that values of eosinophils were significantly ($P < 0.05$) higher during weeks 2 and 5 in conventionally reared calves and during week 8 and 4 in milk-fed calves. Values of PCV, Hb, RBC and MCH were significantly ($P < 0.05$) higher in conventionally-reared compared to milk-fed calves.

Materials and Methods

Animals:

Twenty healthy calves at the age of 7 days were used in the study. Calves were obtained from Hassawi herd maintained at King Faisal University Research station or purchased from local farms. The calves were divided into 2 groups.

Group1: (Milk-fed calves) Ten Calves were left to suckle their mothers at all times. Salt licks and water were made available *ad libitum*.

Group2: (Conventionally – reared calves) Ten calves were used in this group. After morning milking of cows, calves were left to suckle their mothers. Thereafter, calves were offered commercially formulated concentrate (11% protein) with alfalfa hay, salt licks and water were made available *ad libitum*. Calves were individually penned on straw. Experiments on group 1 and 2 calves were lasted for 14 weeks.

Collection of blood samples

Calves were bled from Jugular vein in three weeks intervals between 2 and 14 weeks of age. Blood was either collected into EDTA tubes for estimation of haematological components or in plain tubes for estimation of serum biochemical components.

Determination of haematological parameters:

Packed cell volume (PCV) was determined by microhaematocrit method. Haemoglobin (Hb) concentration was determined by Cyanmethaemoglobin method; red (RBC) and white (WBC) blood cells were determined by Coulter counter (model ZBI, Coulter Electronic, Hialeah, USA) Thin blood smears for differential WBC count were obtained by Haemo-Scan differential cell counter. Mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation according to the formulae of Schalm *et al.*, (1975).

Determination of biochemical parameters:

The VETTEST 8008 biochemical analyzer (Sanofi Animal Health Ltd., England) was used to determine the serum concentration of total protein, albumin, globulin and fibrinogen using specific kits.

Serum copper, and iron levels were determined using Shimadzu AA6800 Atomic Absorption spectrophotometer.

Comparative Study of Haematological and Blood Biochemical Components in Milk-Fed and Conventionally-reared Hassawi Breed Calves

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Abstract :

Haematological and blood biochemical values of milk-fed and conventionally reared Hassawi calves were recorded between 2 and 14 weeks of age. Packed cell volume (PCV) and Haemoglobin (Hb), fibrinogen and iron concentrations declined between 8 and 14 weeks of age in milk-fed but not in conventionally reared calves. Results suggest that Hassawi calves raised solely on milk tend to become anaemic as a result of lack of iron in their normal diet.

Introduction:

Hassawi cattle breed is a mix of *Bos indicus* and *Bos Taurus*. The cattle are raised in the Eastern region of Saudi Arabia by farming families in mixed farming system (Mohammed and AL-Shami, 2003). The breed numbers are declining very fast from 10449 head in 1986 to an estimated maximum of 4500 head at present (Ministry of Agriculture Year Book, 1998). The breed had never been described in the literature (Smith, 1980; Mason 1996). Phenotypic characterization of the breed has been given recently (Mohammed, 1997).

The concentrations of blood components are often measured during the course of disease, either as an aid to diagnosis or to investigate the effects of the disease on metabolic processes. To enable valid use of blood values to be made of such measurements, it is necessary to take into account the factors that can modify these values during normal metabolism. Among the physiological factors that were reported to influence blood composition of calves was the breed (Figueredo et al., 1983; Rwakishaya et al., 1985). Previous investigators have found differences between blood values of various breeds of calves and between crossbred calves and their parent breeds (Figueredo et al., 1983).

The objective of this study was to compare haematological and blood biochemical components of milk – fed versus conventionally – reared Hassawi breed calves.

متغيرات تجلط الدم في صغار الجمال (السلييل) وأمهاتها

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الملخص :

تمت دراسة متغيرات تجلط الدم في عدد ٦٢ من صغار الجمال (السلييل) وأمهاتها أثناء وقت الولادة. وقد أظهرت الدراسة إطالة مدة تجلط الدم وفترة النزف وتجلط الدم النشط ومدة البروثرومبين ومدة البروثرومبين النشط ومدة الريتاليز مع عدم وجود تغير واضح في معدل نواتج تحطم الفبرين في صغار الجمال (السلييل) مقارنة مع أمهاتها.

تشير هذه النتائج إلى ضعف ميكانيكية تجلط الدم في صغار الجمال (السلييل) والتي يمكن اعتبارها صفة فسيولوجية في هذا العمر المبكر.

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may vary within the same species or from species to another (Taylor et al., 2000) .

Statistically significant differences were observed between mean values for neonates and their dams in coagulation variables. Similarly the coagulation profiles of newborn calves, kittens, pups, guinea pig, rabbits and prigs exhibit marked differences from those of the comparable adult animals. In each of these species, reduced levels of prothrombin and related clotting factors were found in relation to adult (Gentry and Downie 1984).

Prolongation of prothrombin time, PTT and ACT in the neonates is attributed to deficiency of the clotting factors operating in intrinsic pathway of clotting system. This in turn is due to immediate synthetic capabilities of the liver rather than vitamin K deficiency (Hathaway and Bonnar, 1980; Forestier *et al.*, 1985). Prothrombin time is a primary measure of extrinsic and common pathways of coagulation, where as PTT is a measure of intrinsic and common pathways, activated coagulation time will be prolonged when there are difference in activity of factors V11, 1X, prothrombin or fibrinogen (Bateman *et al.*, 1999), the ACT test is a simple, inexpensive and rapid haemostatic test for disorders involving intrinsic or common pathway of coagulation.

Reptilase time was significantly prolonged in neonates in this study. Such prolongation in the absence of significantly elevated FDP, is indicative of a defective of fibrin polymerization (Beck, 1982). This is not unexpected since fetal fibrinogen is qualitatively different from adult fibrinogen.

The relative deficiency in the various components of the haemostatic mechanism established in this study occurs in otherwise healthy symptom – free neonates. Therefore these alterations should be considered as physiological properties (Buchanan, 1978). Indeed, none of 28 neonates had any clinical evidence of defective haemostasis. The reference values obtained would be used in interpreting veterinary laboratory results and in monitoring the effect of therapeutic interventions in various hemorrhagic and thrombotic disorders of the neonate.

Acknowledgements:

The author thanks the Deanship of Scientific Research for financial support.

Results :

The results of coagulant variables of 62 she-camels and their neonates (weighing 30-37 Kg) are given below (table 1). The mean values of platelet count, TBT, ACT, CT, PT, PTT, RT and FDP in neonate camels were significantly different from their dams. Platelet count of neonates were significantly lower ($P < 0.05$) than the values of their dams. on the other hand, The results indicated that ACT, TBT, CT, PT, PTT, RT and FDP values of neonate camels were significantly higher than the observed values of their dams.

Table (1)
The level of different anticoagulant parameters

Coagulant variable	Neonates	Dams
Platelet count (10^5 cells/ μ l)	$0.9 \pm 0.05^*$	1.6 ± 0.06
TBT (min)	$6.2 \pm 0.6^*$	4.3 ± 0.5
ACT (sec)	$200 \pm 20^*$	160 ± 20
CT (min)	$6.2 \pm 0.6^*$	4.01 ± 0.5
PT (sec)	$16.6 \pm 03^*$	8.2 ± 2
PTT (sec)	$70 \pm 9^*$	40 ± 5
RT (sec)	$21 \pm 3^*$	12 ± 2
FDP (μ g/ml)	$15.3 \pm 2.1^*$	$14.3 \pm 2.3^*$

Mean \pm SD of platelet count, clotting time (CT), Template bleeding time (TBT), activated coagulation time (ACT), Prothrombin time (PT) fibrin degradation products (FDP), reptilase time (RT), activated partial thromboplastin time (PTT) and fibrin degradation products in neonatal camels and their dams (N=62), *P < 0.05.

Discussion:

The overall coagulation profile in the she camel is comparable to the cow (Feldman *et al.*, 2000). Comparatively, clotting times of domestic animals are longer in the following order: cat; dog and pig; horse and sheep; cow and camel; and finally birds and chickens (Gentry and Downie, 1984; Swenson and Reece, 1996). Platelets count is a direct measure of primary haemostasis as they aggregate to form a plug that stops bleeding. The TBT is an indirect measure of primary haemostasis and is dependant on the number of circulating platelet (Kopp *et al.*, 1985). The value of TBT of 4.3 minutes reported here

birth simultaneously (before feeding colostrum) by venipuncture of Jugular vein.

Platelet counts: Platelet Counts were performed on sodium citrate – anticoagulant blood using the automated hematology analyzer (Baker 9010 hematology analyzer Biocmmunochen, Allentown USA).

Template bleeding Time (TBT): TBT was measured by a template bleeding device (Surgicutt International, Technidyne Corp. Edison, NJ, UA), Blood from incision on skin was collected periodically onto filter paper. The TBT was measured from the discharge of the device until bleeding had stopped.

Clotting variables: blood clotting time (CT) was determined by capillary tube method (Schalm *et al.*, 1975) in fresh blood taken from tip of the ear. Fresh blood obtained from Jugular vein was also used for determination of clotting variables (Feldman *et al.*, 2000).

For determination of the activated clotting time (ACT), prothrombin time (PT) partial thromboplastin time (PTT), and fibrin degradation products (FDP). For ACT assays, blood was aspirated and placed in 20-ml syringes and quickly injected into 2 warmed (37° C) evacuated tubes containing diatomaceous earth (Sigma, UK), tubes were mixed by gentle inversion and incubated at 37° C for 1 minute. Tubes then were removed from the water bath, rocked gently, and returned to the water bath. The ACT was recorded as mean time to initial clotting in each tube. The PT was determined by addition of 0.2 ml of warmed rabbit thromboplastin reagent (Sigma, UK), to 0.1 ml of warmed (37° C) plasma (Sodium citrate) and measurement of the interval until clot detection, using a fibrometer (Simplastin, Organon Teknika corp, Durhan, USA). The PTT was determined as follows: 0.1 ml of sample plasma was added to 0.1 ml of warmed action-activated cephaloplastin reagent, incubated for 3 minutes at 37° C, and mixed with 0.1 ml of warmed CaCl₂ solution, and the interval until clot detection was measured. For each camel and the mean value calculated. Control values for PT and PTT were established, using human plasma (sodium citrate).

The fibrin degradation products (FDP) and reptilase time (RT) were measured by a modification of Laurell's technique (Laurell 1965) using commercial kits (Murex Biotech Limited, Kent, UK) A Kruskal – wallis statistical test was used to determine whether the parameter varied significantly by age. Values of $P < 0.05$ were considered significant.

Coagulation Variables in Camel Neonates and Their Dams

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Abstract:

A wide range of haemostatic variables were studied in a number of 62 neonate camels and their dams at the time of delivery. The results indicated significant prolongation of the coagulation time, template bleeding time, activated coagulation time, prothrombin time, activated partial thromboplastin time, reptilase time, and a reduction in platelets count with no change in fibrin degradation products was observed in neonates in comparison with their dams. This indicates a significant diminution in coagulation mechanism in neonate which is considered to be of physiological nature at this early age.

Key words: coagulation, camel, blood.

Introduction :

Several studies on the camel have shown that hematological parameters exhibit considerable variations at different periods of life (Elias and Yagil 1984, Hussein *et al.*, 1992, Bay *et al.*, 2000).

Furthermore, environmental and nutritional factors could affect haematological parameters (Evans *et al.* 1999). In addition to that, normal reference levels for haemostatic variables are needed for both maternal and neonatal blood in the presence of a normal uneventful delivery. So that disorders of haemostasis can be detected.

Disease, nutritional or environmental agents may activate the clotting system (Brown, 1975), after haemostasis through their effects on platelet or endothelial function (primary haemostasis), or through activation of secondary haemostasis or inhibition of fibrinolysis (Taylor *et al.*, 2000). Such agents are considered as risk factors for fetal loss (Tibary and Annousi, 1997). The purpose of this study is to determine the normal values of various blood coagulant variables in neonate camels and their dams at the time of delivery.

Materials and methods

The present experiment was conducted on 62 (34 female camels of 4-7 years old) (*Camelus dromedaries*) and their newborns at day 1 postpartum (28 camels). The animals were maintained by individual farmers and kept in open pens. Blood samples were collected from each camel and neonatal calves at

التعرف على نشاط الإفرازات الخلوية في أنموذج الفأر المعالج بنبات سم الفأر

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**معهد الأبحاث ، دولة الكويت

الملخص :

يعتبر نبات سم الفأر من الأعشاب الطبية المهمة في تحفيز جهاز المناعة . و قد تم دراسة الإفرازات الخلوية التالية في أنموذج الفأر ، IL-2, IL-4, IL-10, IL-12, IFN- γ , و GM-CSF. لقد تبين ارتفاع هذه الإفرازات في خلايا طحال الفأر المعالج و المستثارة بمادة الفايتهيموجلاتين (PHA) ما عدا الإفراز الخلوي GM-CSF . و قد تبين ارتفاع الإفرازات الخلوية في الأيام 5 , 10 , 20 , و 30 . و قد تم فحص التأثير المناعي لنبات سم الفأر على إنتاج الإفرازات الخلوية بأسلوب آخر من خلال تقنية تكثير البصمة الوراثية (PCR). و قد اثبت الفحص فرق واضح بين البصمة الوراثية للإفرازات الخلوية في الأيام 5 و 10. حيث تم رصد نشاط البصمة الوراثية للإفرازات الخلوية في اليوم 10 للخلايا المستثارة بالفايتهيموجلاتين و غير المستثارة. أظهر العشب تأثيرا واضحا في زيادة إنتاج الإفرازات الخلوية لخلايا طحال الفأر.

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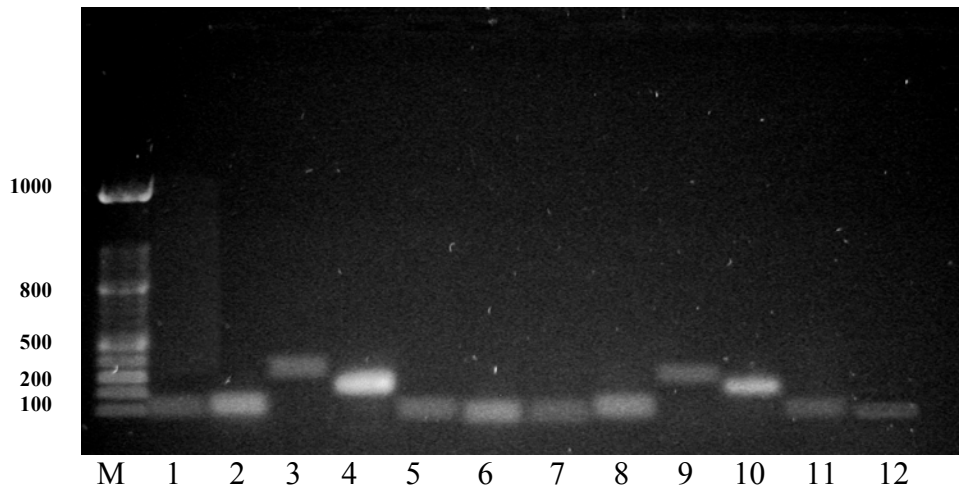


Figure.4 Effect of WS on mice cytokines gene expression using RT-PCR after 15 days of treatment: M= 100 bp marker, (WS+PHA treatment) 1= IL-4, 2= IL-10, 3= IL-12, 4= INF- γ , 5= GM-CSF and 6= IL-2. (WS treatment only) 7= IL-4, 8= IL-10, 9= IL-12, 10= INF- γ , 11= GM-CSF and 12= IL-2.

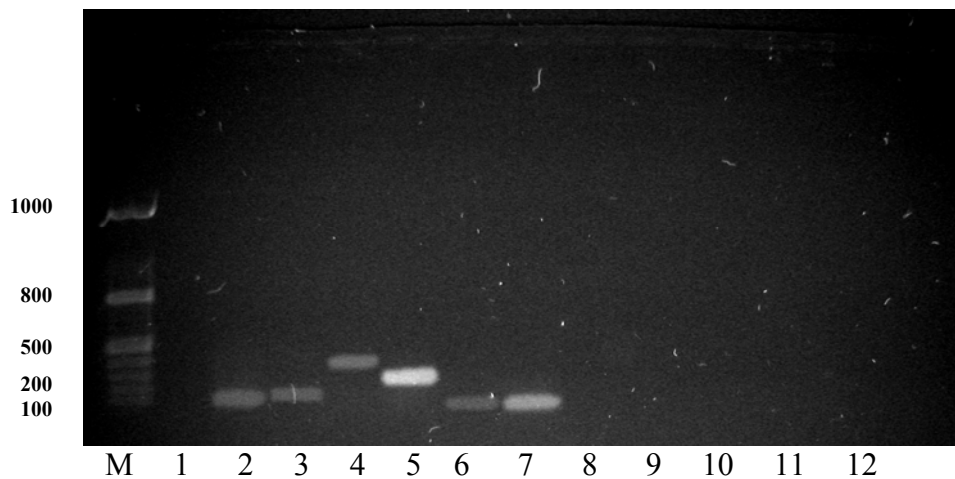


Figure.5: Effect of WS on mice cytokines gene expression using RT-PCR after 5 days of treatment: M= 100 base pair (bp) marker, (WS+PHA treatment) 1= IL-4, 2= IL-10, 3= IL-12, 4= INF- γ , 5= GM-CSF and 6= IL-2. No bands were detected for the cells treated with WS only.

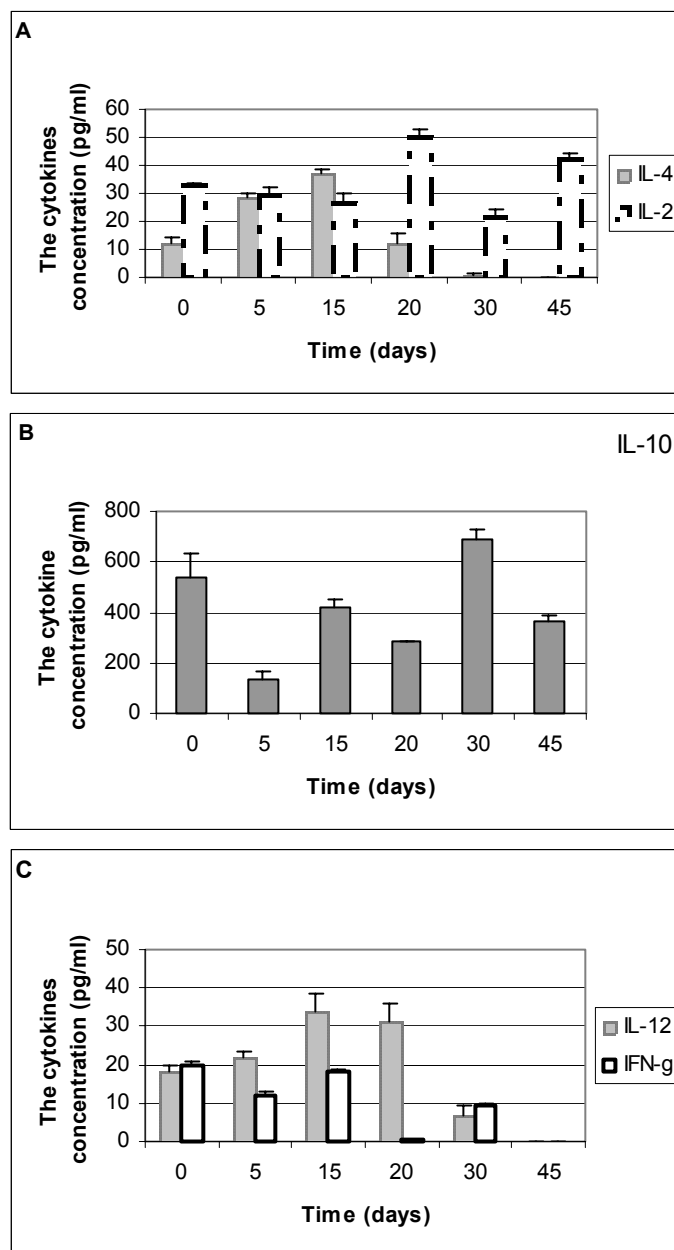


Fig.3: The level of the cytokines monitored by ELISA in spleen cells pulsed with PHA. The level of cytokines IL-2, IL-4, IL-10, IL-12 and IFN- γ of control and mice model treated with *Withania somnifera*. The plate A depicts the level of IL-2 and IL-4, B indicates the level of IL-10 and C refers to the level of IL-12 and IFN- γ .

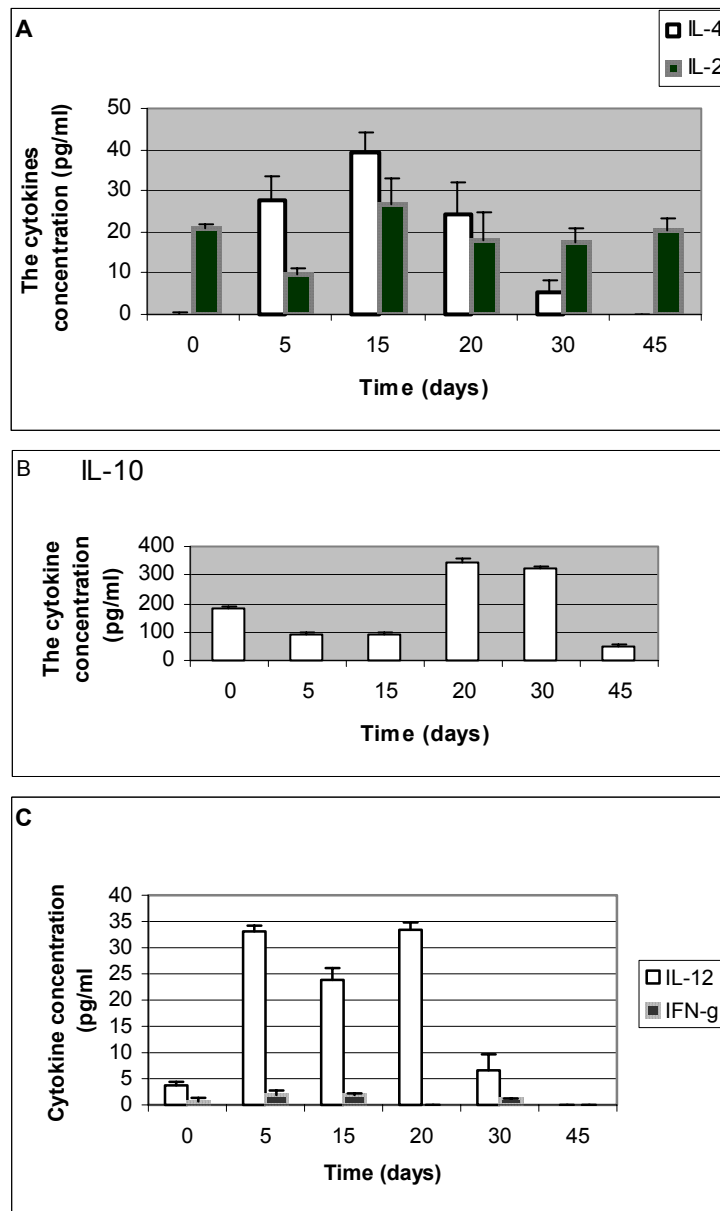


Fig.2: The level of the cytokines monitored by ELISA in spleen cells not pulsed with PHA. The level of cytokines IL-2, IL-4, IL-10, IL-12 and IFN- γ of control and mice model treated with *Withania somnifera*. The plate A depicts the level of IL-2 and IL-4, B indicates the level of IL-10 and C refers to the level of IL-12 and IFN- γ .

Table (3) : The one-factor analysis of variance of the hematological pictures of mice treated with WS

	control		W5 [§]		W15 [§]		W20 [§]		W30 [§]		W45 [§]		P value
	M* [§] ±St [§]		M ±St		M±St		M±St		M±St		M±St		
WBC	6.367	±1.503	6.275	±0.585	6.5	±1.63	5.22	±1.4	8.13	±1.49	6.91	±1.69	0.11
RBC	7.207	±0.554	6.854	±1.255	6.4	±0.35	6.95	±0.96	7.92	±0.88	6.92	±1.20	0.82
HGB	11.50	±0.886	10.20	±1.69	9.0	±0.66	10.46	±1.81	12.36	±1.74	9.02	±1.65	0.61
HCT	33.80	±2.246	30.74	±4.50	27.97	±2.35	32.36	±4.02	37.18	±4.83	32.07	±5.43	0.88

*, Mean

§ Standard deviation

§ 5, 15, 20, 30, 45 referred to the time intervals.

The cytokines activity in mice model treated with *Withania somnifera*
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Table (2) : Duncean multiple range test for means of cytokines levels at different time points.

IL-2 (Mean± SE)	IL-4 (Mean± SE)	IL-10 (Mean ±SE)	IL-12 (Mean± SE)	IFN- γ (Mean± SE)
[§] W20 49.88±3.021 ^{aY}	W15C 39.369±4.649 ^a	W30 664.6±2.54 ^a	W15 35.85±4.099 ^a	P 20.233±0.35 ^a
W45 42.293±1.845 ^{bY}	W15 36.9±0.75 ^a	P 488.48±102.797 ^b	W5C 33.70±0.915 ^a	W15 16.50±0.00 ^{ab}
^{*p} 33.067±0.377 ^{cY}	W5 27.633±1.206 ^b	W15 409.1±1.378 ^{bc}	W20C 32.55±0.05 ^a	W5 12.223±0.315 ^{ac}
W5 27.787±1.738 ^{cdY}	W5C 26.967±3.743 ^b	W45 379.05±10.775 ^{bc}	W20 32.45±4.05 ^a	W30 7.737±0.735 ^c
^F W15C 26.81±5.343 ^{cdY}	W20C 25.68±6.028 ^b	W20C 338.45±13.741 ^{bee}	W15C 25.00±1.837 ^b	W15C 1.933±0.00 ^d
W15 25.24±3.283 ^{cdeY}	W20 12.1±3.419 ^c	W30C 318.9±2.54 ^{ce}	W5 20.95±1.556 ^b	W5C 1.467±0.495 ^d
W30 21.723±2.292 ^{deY}	P 11.92±1.195 ^c	W20 283.4±3.811 ^{cdef}	P 16.20±2.404 ^c	W30C 1.20±0.00 ^d
^o C 20.9±0.735 ^{deY}	W30C 8.355±2.045 ^{cd}	C 188.05±8.66 ^{cfig}	W30C 6.55±2.925 ^d	C 0.76±0.705 ^d
W45C 20.343±2.271 ^{deY}	W30 0.497±0.745 ^d	W5 149.9±13.4 ^{fg}	W30 5.45±2.155 ^d	W20 0.296±0.00 ^d
W20C 18.147±5.342 ^{efY}	W45 0.168±0.02 ^d	W5C 96.8±9.09 ^g	C 3.75±0.45 ^d	W20C -
W30C 17.27±3.413 ^{efY}	W45C 0.155±0.007 ^d	W15C 91.45±4.41 ^g	W45 -	W45 -
W5C 9.903±1.389 ^{fY}	C 0.093±0.162 ^d	W45C 46.35±5.023 ^g	W45C -	W45C -

^Y For each mean values with a superscript in common are not significantly different from each other with level significance 5% over all comparisons.

^{*} Control cells pulsed with PHA

^o Control cells not pulsed with PHA

^F Cells of treated mice but not treated with PHA

[§] Cells of treated mice treated with PHA

5. 15, 20, 30, 45 referred to the time intervals.

Table (1)

Oligonucleotides Sequences of upstream (sense) and downstream (antisense) primers of mouse Cytokines

Gene	Primers	Size of expected cDNA (bp)
IL-2	Sense 5'-GAAGATGAACTTGGACCTCTGC -3' Antisense 5'-CAGAGTTGCTGACTCATCATCG -3'	167
IL-4	Sense 5'-TATTGATGGGTCTCAGCCCC-3' Antisense 5'-GAGTCCCTTTTTTCTGTGACCTGG-3'	140
IL-10	Sense 5'-TAGAAGTGATGCCCCAGG -3' Antisense 5'-TCATTCTTCACCTGCTCCACTGC -3'	159
IL-12p40	Sense 5'-TCCGGAGTAATTTGGTGCTTCACA -3' Antisense 5'-ACTGTACAACCGCAGTAATACGG -3'	396
IFN- γ	Sense 5'- AGCGGCTGACTGAACTCAGATTGTAG -3' Antisense 5'- GTCAGAGTTTTTCAGCTGTATAGGG -3'	244
GM-CSF	Sense 5'-TGCTCTGGAGAACGAAAAGAACG -3' Antisense 5'-ATTGAGTTTGGTGAGGTTGCCCC -3'	132



Figure.1 *Withania somnifera* leaves and fruit as they appear in their natural habitat.

proinflammatory cytokines, interleukin-1, interleukin-6 and TNF- α , in parallel to the cytokines in this study seems important.

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significant increase in IL-4, IL-10 and IL-12, whereas, in the Pulsed cells IL-2, IL-4, IL10 and IL-12 were significant. The increase in the cytokines activity in the treated mice was in accordance with other reported studies (Davis and Kutton, 1999; Dhuley, 1997). Davis and Kutton (1999) reported an enhancement of IL-2, IFN- γ and GM-CSF in the treated Balb/c mice. Therefore, the enhancement in the level of all studied cytokines can be envisaged in view of the reports on the potentiation of WS of innate immunity, cell mediated immunity and antibody production (Davis and Kutton, 2000, 2002, Agarwal, *et al.*, 1999). The continuous significant elevation in the level of IL-4, IL10 and IL-12 to day 20 and IL-10 to the day 30 indicates that the WS has a wide scale of immunoregulatory effect on the Th1 and Th2 cytokines. Furthermore, the gene expression of all cytokines on day 15 even that of the non pulsed cells endorse the mitogenic activity of this plant that was reported elsewhere (Devi *et al.*, 1996, Ziauddin *et al.*, 1996).

A distinct significant difference in cytokines activity between pulsed and non-pulsed cells indicates the imunopotentiating influence of this plant in enhancing the stimulatory response of the cells to the mitogen PHA. Nevertheless, failure in detection of any significant increase in the IFN- γ level within time is questionable. Interferon- γ is considered one of the important cytokines in enhancement of innate and cell mediated immunity (Gray, 1994). Although, the level of IFN- γ indicated a significant difference between control cells that were pulsed with PHA and the same cells that were non-pulsed, its level showed no significance at all time points in the treated cells. The failure in detection of any significant increase in IFN- γ level could be due to the immunoregulatory effect of IL-10 which has shown significant elevation.

The blood picture of mice treated with WS indicated no significant differences from that of control animals. It seems that the hematological analyses in this study suffered a certain set back. The non-significant changes in blood picture clearly contradict the reports that indicated the vast changes in the blood pictures brought about by WS treatment (Davis and Kutton, 2000; Agarwal, *et al.*, 1999). Elevation in the cytokines synthesis indicated by the ELISA and supported by their distinct gene expression should be associated with obvious physiological activity of the white blood cells as well as significant increase in their count. Hence, despite this unsatisfactory result of the blood picture analysis, the overall results of cytokines activity that was measured by ELISA and RT-PCR substantiate the immunostimulatory activity of this plant. However, further analysis on the effect of this plant on the

indicated significant rise on day 5, 15 and 20, whereas; IL-10 indicated a significant rise on day 20. On the other hand, cytokines monitored in cells pulsed with PHA showed that IL-2 level was significantly increased on day 20 and 45, whereas, IL-4 was increased significantly on day 5 and 15. Interleukin-10 was significant on day 30. The level of IL-10 continued to increase until day 30. On day 5, 15 and 20, IL-12 was significantly increased (Table-2).

The cytokines gene expression.

The analysis of cytokines gene expression with RT-PCR indicated the gene expression of all cytokines on day 15 (Fig.4), which was clearly distinct from that was observed with control and day 5 (Fig.5). Cytokines of the control cells did not indicate any signals of gene expression. The analyses of cytokines gene expression on day 5 revealed that only cDNA of cytokines of cells that were pulsed with PHA were detectable. However, cytokines cDNA from both groups of cells, pulsed and non-pulsed, were detectable on day 15. The gene expression of GM-CSF was detectable despite its undetectable level by ELISA.

Although, examination of cytokines gene expression with RT-PCR is a qualitative method, a broad comparison with the cytokines level measured by ELISA (quantitative method) could exhibit a clearer picture of the cytokines activity. The absence of a distinct cytokines gene expression on day 5 of cells not pulsed with PHA correlates with the steady increase of most of the cytokines at the same period (Fig.2). On day 15, however, both PHA treated and non treated cells indicated obvious expression of all of the cytokines genes (Fig. 4), which correlates with the significant increase of most of the cytokines at this period (Table-2).

The blood pictures of mice treated with WS.

Complete hematological study on mice that were treated with WS revealed no significant difference from the control mice. The results are summarized in table-3.

Discussion:

Withania somnifera is an important herb in the Ayurvedic medical system. The immunostimulatory and anti-inflammatory properties of WS were studied extensively (Devi *et al.*, 1996; Dhuly, 1998; Owais *et al.*, 2005, Ziauddin *et al.*, 1996). The treated mice with WS expressed a significant cytokines activity in this study. The effect of WS on the IL-4, IL-10 and IL-12 is addressed for the first time. The statistical analysis indicated a significant increase in the cytokines activity with time. Non-pulsed cells from treated mice indicated

GeneAmp® PCR system 2400 Thermocycler (Applied BioSystems, USA) as follow, initial step for one minute and 45 seconds at 95 °C and then 35 cycles of amplification in which the melting was at 95 °C for 15 seconds and the anneal-extension at 60 °C for 30 seconds. Finally the reaction was kept at 72 °C for 7 minutes before it cooled down at 5 °C.

Agarose gel electrophoresis:

The products of RT-PCR were run on 1% agarose gels (Sigma chemical Co, Spain) dissolved in 1x tris-acetate EDTA (TAE) buffer (PH8) (50X TAE buffer is made of 40 mM Tris-acetate, 1 mM EDTA). The gel was fixed in the horizontal gel electrophoresis apparatus and run in 1L 1X TAE buffer containing 25 µl of 0.5µg/ml Ethidium bromide. The samples and the 100bp ladder marker (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) were Loaded with 30% glycerol, and run at 90V for 90min. The gel was then visualized with ultra-violet illuminator (Fisher Scientific, USA) and photographed by the C-4040 digital camera (Olympus, Japan).

The blood picture of mice treated with WS:

The analysis of blood picture, cells and proteins of control and treated mice, carried out at the hematology laboratory of King Fahad general hospital at Hofuf. The blood cells were counted in the coulter machine (Cell-Dyn-3700, USA).

The statistical analysis :

The data were analyzed with two-factor analysis of variance followed by Duncan multiple range test.

Results:

The cytokines levels in the WS treated-spleen cells of pulsed or not-pulsed with PHA.

The levels of cytokines concentration measured by ELISA test are summarized in Fig.2 and Fig.3. The GM-CSF failed to show any detectable levels. The two-factor analysis of variance of cytokines that were monitored in cells of treated mice (cells were either pulsed with PHA or grown in medium free of PHA) indicated a significant difference with time and response to the mitogen [IL-2 ($P>0.006$), IL-4 ($P>5E-11$), IL-10 ($P>0.0002$), IL-12 ($P>2E-10$)]. The results of the tow-factor analysis of variance were analyzed further by the Duncan multiple analyses (Table-2). The analyses revealed that the cytokine of spleen cells of treated mice but not pulsed with PHA, IL-2 and IFN- γ showed no significance difference with time. However, IL-4 and IL-12

was used to measure the level of the cytokines according to manufacturer direction.

Briefly, 50 µl of the assay diluent, were added to each well, then 50 µl of standard control and samples were added. After 2 hrs incubation, a 100 µl of conjugate were added to each well after thorough washing and incubated for overnight. After thorough washing, a 100 µl of the substrate solution was added to each well and incubated for 30 minutes. Finally a 100 µl of the stop solution was added to each well and the optical density was measured using ELISA reader with dual wavelength 450 and 570 nm (Thermo Labsystem, Finland).

The cytokines gene expression with RT-PCR.:

The Cytokine gene expression of IL-2, IL-4, IL-10, IL-12, IFN-γ and GM-CSF were examined with RT-PCR. The test was performed as follows:

RNA extraction :

Total RNA (toRNA) was extracted from the spleen cells that were incubated with or without the mitogen. The extraction was performed using total RNA extraction kit (Qiagen Ltd, Valencia, CA, USA). The procedures were carried out according to the manufacturer's directions.

RT-PCR:

Approximately 1µl of toRNA was reverse transcribed to Complementary DNA (cDNA) using 20 µl reverse transcription reaction mixture containing a final concentration of 5 mM MgCl₂, 1X of a 10 X PCR buffer II , 2.5µM Random Hexamers, 1 mM of each of dGTP, dATP, dTTP and dCTP, 1U/µl RNase inhibitor , and finally 2.5 U/µl reverse transcriptase(GeneAmp® RNA PCR kits, Perkin Elmer Applied Biosystems, Branchburg, NJ, USA). The mixture was incubated at 42°C for 15 minutes heated to 99°C for 5 minutes and maintained at 5°C for 5 minutes Using GeneAmp® PCR system 2400 (Applied BioSystems, USA).

PCR amplification:

An 80 µl of PCR mixture was prepared for each cytokine containing a final concentration of 2mM MgCl₂ , 1X of a 10X PCR buffer II, and 2.5U/100 µl of *Thermus aquiticus* DNA polymerase (Ampli Taq® DNA polymerase) and approximately 40-45 pM of upstream and downstream primers (Proligo, USA) (Table-1) of each cytokine which were as those reported elsewhere [IL-2, IL-4, IL-10, and GM-CSF by (Bullock and Jahnson, 1996) IL-12p40 and IFN- γ (Nomura *et al*, 2002)]. PCR mixture of 100 µl was then amplified using

percolation for 24 hours at room temperature. The extract was centrifuged at 1000 rpm for 10 minutes. The solvent of supernatant evaporated at 40°C under vacuum condition. The residue was dissolved in drinking water at concentration of 1/20 of the LD50 (940 mg/100 g. body weight, Abdel-Magied *et al.*, 2001). Water adopted ad-libitum to the mice along the experimental period.

Mice:

A total of 60 Wister albino male mice used were housed in hygienic fiber glass cages. Animals were maintained in 6 groups of 10 mice each. Mice were apparently healthy and weighed 25 –30g. They were fed on commercial pellets, (Grain Silos and Flour mills Organization – Riyadh). The mice were allowed free access to treated drinking water and feed. Throughout the experimental study, all animals were observed daily for any signs. Three groups of total of 6 groups were treated by the drinking water containing 1/20 LD50 ethyl alcohol extract of WS. The other 3 groups were considered as control by drinking tap water only. The total period of the experiment was continued for 45 days. At each time point, 5 days, 15 days, 20 days, 30 days and 45 days, five mice of each of the two groups of the animal, one treated and one control, were killed by cervical dislocation and the spleens were removed aseptically. The extra 10 mice were kept as a reserve with the control groups.

Preparation of spleen cells:

Spleen cells of each group were harvested by squeezing the cells out by repeated injection of Dubelcco's modified Eagle's medium in the spleen. The cells were then washed once with the medium. The number of spleen cells were adjusted to a final concentration of 9×10^6 cells/ml in growth medium RPMI-1640 (Sigma) containing 5% foetal calf serum, (Sigma) antibiotics and antimycotic (final concentration of 10 unit/ml penicillin G Sodium, 10 µg/ml streptomycin sulfate and 0.025 µg/ ml amphotericin B as fungizon) (Sigma Technology, USA). The cells then either incubated with final concentration of 10-12 µg/ml of phytohaemagglutinin (PHA) (Sigma) or without the presence of the mitogen at 37°C in the presence of 5% CO₂ for 48 hours. Control cells were also incubated in medium with and without PHA.

Monitoring the cytokines level with ELISA :

The cytokines, IL-2, IL-4, IL-10, IL-12, IFN-γ and GM-CSF levels were monitored using Quantkine mouse cytokines immunoassay kit (R&D Systems, USA). The harvested RPMI-1640 medium of spleen cells of each time points

bacteriological activity against *Aspergillus fumigants* and *Salmonellosis* (Dhuley, 1998; Owais *et al.*, 2005). The therapeutical and immunostimulatory activity of WS was studied widely in mice model (Agarwal *et al.*, 1999; Aphale *et al.*, 1998; Davis and Kutton, 1999; Dhuley, 1997).

Treatment of Balb/c mice with 20 mg/dose/animal of WS root extract resulted with significant increase in white blood cells (WBC), bone marrow cellularity and enhanced the circulating anti-sheep red blood cells (SRBCs) antibodies. Treated mice expressed an enhancement in the phagocytosis of peritoneal macrophages and inhibited delayed type hypersensitivity (Davis and Kutton, 2000). Agarwal *et al.* (1999) reversed the immunosuppressive effect of cyclophosphamide in mice by treatment with WS. The treated mice also revealed significant increase in the hemagglutinating and hemolytic anti-sheep SRBCs antibodies. The lymphocytes cytotoxicity against thymoma cells was enhanced in vivo and in vitro after treatment with WS (Davis and Kutton, 2002). The study on the immunostimulatory effect of WS was taken further by measuring the cytokines in mice model (Davis and Kutton, 1999; Dhuley, 1997). Cytokines are crucial mediators in the regulation of immune responses and haemopoiesis (Nicola, 1994). Cytokines, interleukin-2 (IL-2) interferon- γ (IFN- γ) and granulocyte monocytes-colony stimulating factor (GM-CSF) were increased significantly in mice that were treated with powdered root of WS. The level of these cytokines reversed when these animals received cyclophosphamide (Davis and Kutton, 1999). On the other hand, macrophages from mice that were suffered eminent suppression from the treatment with carcinogen Ochratoxin-A, indicated a significant increase in interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α) and chemotaxis when treated with WS (Dhuley, 1999).

This study is aimed to examine the immunostimulatory activity of WS in mice model by measuring wider range of cytokines, IL-2, IL-4, interleukin-10 (IL-10), interleukin-12 (IL-12), IFN- γ and GM-CSF by enzyme linked immunosorbent assay (ELISA) and their gene expression was examined using reverse transcriptase-polymerase chain reaction (RT-PCR).

Materials and Methods

Plant:

The aerial parts of Ws (leaves, stem & seeds) (Fig.1) were freshly collected from the local areas in the Al-Ahssa farms. Approximately one kg of dried plant material were grounded and extracted with 80% ethanol by shaking and

The Cytokines Activity in Mice Model Treated with *Withania somnifera*

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Abstract

Withania somnifera (WS) is one of the important herbs in the Ayurvedic and diagnosis medical systems. Mice were administered WS extracts in the dose of 1/20 of LD50 in drinking water. The influence of *Withania somnifera* on the cytokines, IL-2, IL-4, IL-10, IL-12, IFN- γ and GM-CSF in the spleen cells of mice model indicated a significant increase in their level. GM-CSF failed to show any detectable level in control and at all time periods. Mice cells pulsed with PHA and non-pulsed cells expressed a significant increase in their levels as measured by ELISA on day 5, 15, 20, and 30. The immunostimulatory effect of *Withania somnifera* was revealed further with RT-PCR which indicated a distinct difference between controls, day 5 and day 15. At day 15 genes of all cytokines were expressed in the cells pulsed with PHA and in the non-pulsed cells. The plant exerted clear influence on the cytokines activity measured in the spleen cells of treated mice.

Keywords: *Withania somnifera*, cytokines, mice, IL-10

Introduction:

Withania somnifera (WS) is one of the important herbs in the Ayurvedic and diagnosis medical systems. *Withania somnifera* is also known as ashwagandha, Indian ginseng and winter cherry. Ashwagandha is a member of Solanaceae family that is widely grown in Africa, the Mediterranean and India. The roots of this plant are considered the major portion that has the therapeutic use. The major active ingredients in WS are groups of alkaloids (isopelltierine, araferine) and steroidal lactones (withanolides, withaferins). There are 12 alkaloids, 35 withanolides and several sitoindosides (anonymous, 2004; Lakshmikumaran, 2000).

Ashwagandha or WS has been shown to have an overwhelming use as liver tonic, anti-inflammatory and in treatment of anxiety, cognitive neurological disorders and Parkinson's disease (no author, 2004). The plant is also found useful in enhancing the cancer radiotherapy (Devi *et al.*, 1996) and has

تواجد الديدان الخيطية في أمعاء الدجاج البلدي في محافظة جدة المملكة العربية السعودية

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الملخص :

تم في هذا البحث دراسة وصف وانتشار ثلاثة أنواع من الديدان الخيطية لأول مرة في مدينة جدة المملكة العربية السعودية. جمعت هذه الديدان من أمعاء ١٩ دجاجة من مجموع ٣٢ من الدجاج البلدي المعروف (*Gallus gallus domesticus*) المتواجد في أماكن متفرقة من ضواحي مدينة جدة مثل: الخمرة ووادي غران ووادي أم جبلين حيث بلغت نسبة الإصابة ٦٠٪. ونتج عن هذا الوصف التعرف على الأنواع التالية:

Ascaridia galli من عائلة (Ascaridiidae)، *Subulura brumpti* من عائلة (Subuluroidae) و *Capillaria caudinflata* من عائلة (Capillariidae). كما يعتبر النوع الأخير تسجيل نادر الانتشار في منطقة الشرق الأوسط.

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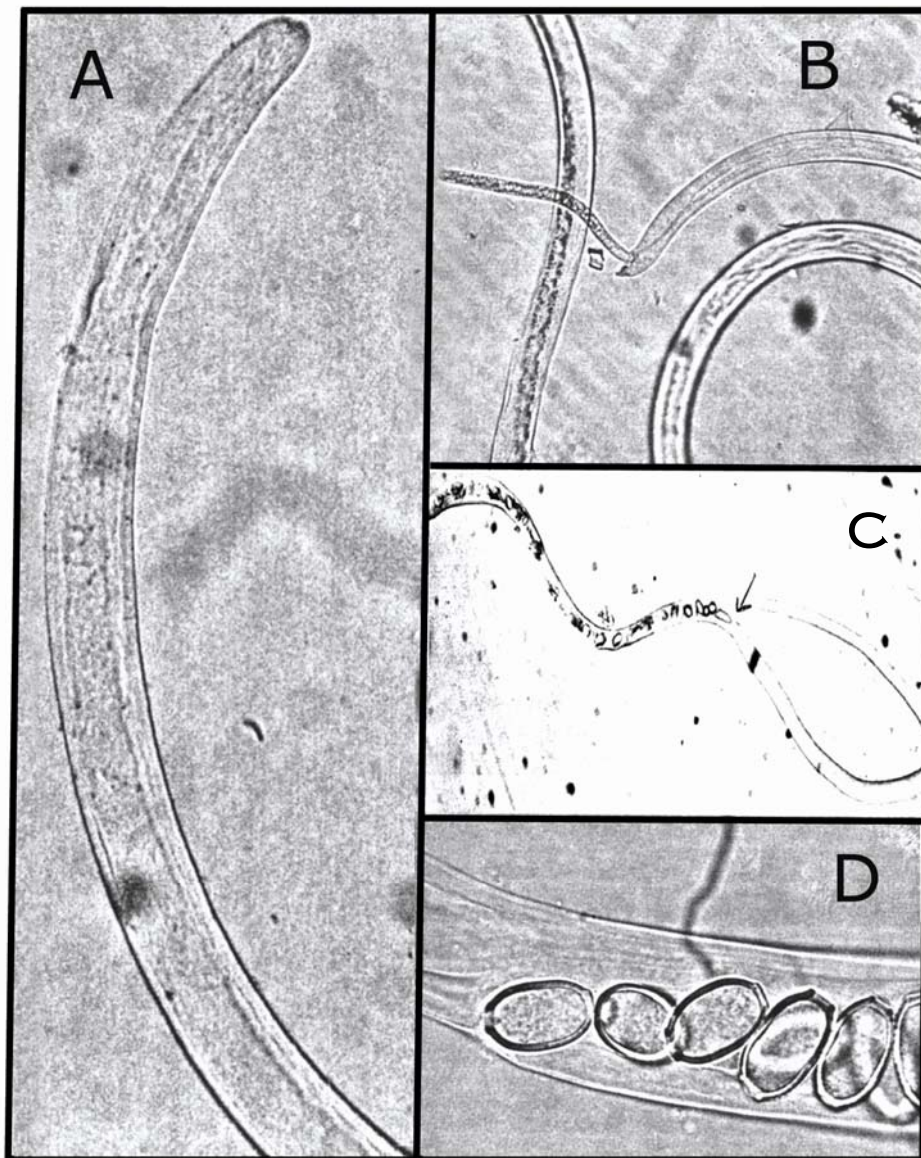


Fig 3 *Capillaria caudinflata*: A. The posterior end of the female. B. The posterior end of the male with single spicule. C. Vulva pointed with arrow. D. The eggs inside the female.

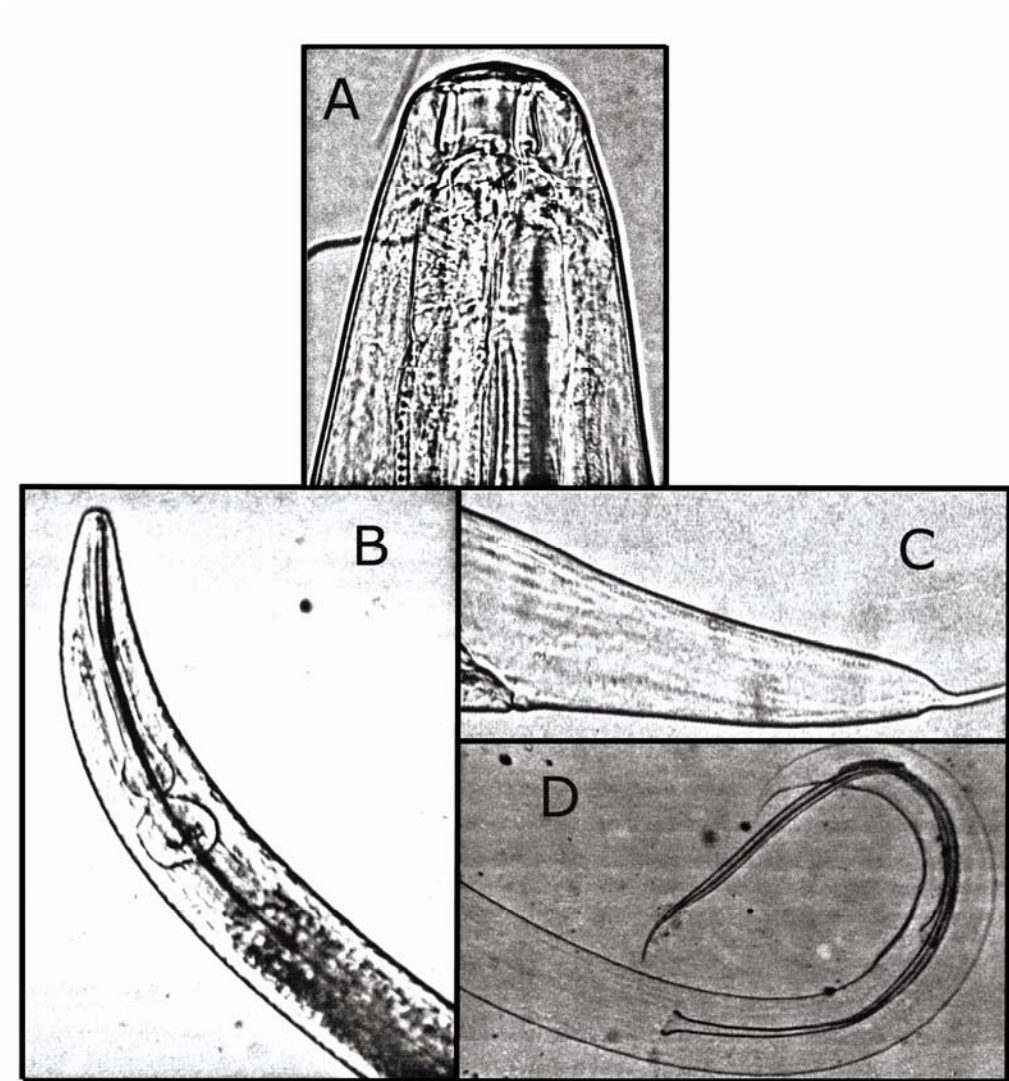


Fig. 2 *Subulura brumpti*: A. view of the anterior end showing the buccal capsule with teeth at the base. B. the anterior portion showing the esophagus with balb. C. The posterior end of the female. D. The lateral curved alae at the posterior end of the male showing the pre-cloacal sucker and spicules.

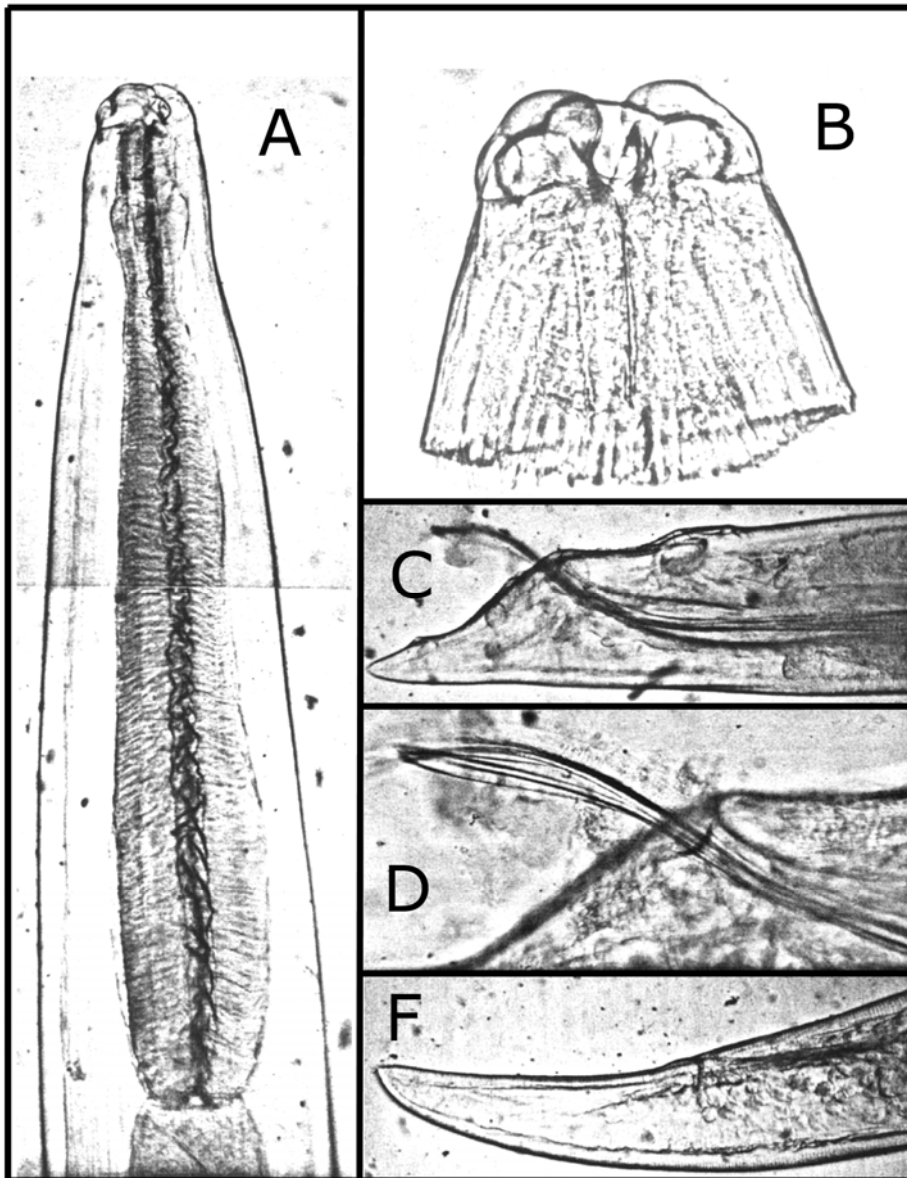


Fig. 1 *Ascaridia galli*: A. ventral view of the anterior portion showing the esophagus. B. Ventral view of the mouth showing the three lips. C & D Lateral view of posterior tail of male showing the alae and the spicules. E The posterior end of the female.

Table (1)
The prevalence and intensity of the nematodes
species in local (Baladi) breed chicken

The voucher species	No. of infected chicken	%	Number of worms in each chicken			Total
			M		F	
<i>Ascaridia galli</i>	11	34.4	1	+	2	3
			3	+	8	11
			2	+	6	8
			6	+	7	13
			1	+	3	4
			5	+	5	10
			2	+	4	6
			1	+	1	2
			0	+	1	1
			0	+	2	2
			3	+	3	6
			24	+	42	66
<i>Subulura brumpt</i>	4	12.5	13	+	32	45
			6	+	16	22
			5	+	11	16
			9	+	15	24
			33	+	74	107
<i>Capillaria caudinflata</i>	4	12.5	16	+	20	36
			11	+	22	33
			3	+	12	15
			4	+	11	15
			34	+	70	104

Much has been written regarding diseases occur in domestic, farm-raised poultry. Only two of many diseases are notifiable and subject to governmental control; the avian influenza and Newcastle disease (ND) (Kaleta 1997). ND is a limiting factor for increasing poultry production in many tropical countries, where frequent reports indicate vaccination failures. In Denmark (Horning et al (2003) investigated the influence of Nematodes species *Ascaridia galli* on vaccinated chickens against ND virus under village conditions.

Vaccinating poultry naturally infected with helminth parasites or infected with *Ascaridia galli* post- vaccination against ND has proven to produce a significantly lower immunity against ND than in poultry which is parasite-free (Glukhor 1984).

These three present species are parasites of major clinical importance for poultry. Their records in Saudi Arabia therefore very important from veterinary point of view. The loss of chickens weight and general weakness could affect the production losses and death. Due to the great value of poultry production in the Kingdom of Saudi Arabia. Further study is needed.

2. *Subulura brumpti* (Lopez-Neyra,1922) (Family: Subuluridae)
Prevalence: in 4 out of 32 (Table 1)
Diagnostic characters: small nematodes, the male 9.26 mm female 11.38 mm long. Buccal capsule is small with three minute teeth. The esophagus has small swelling posteriorly, followed by a deep constriction and then a spherical bulb. The tail of the male is provided with large lateral alae and is curved ventrally. The pre-anal sucker is an elongate slit. There are number of pairs of small caudal papillae. The spicules are equal and alate. The vulva is situated just anterior to the middle of the body. (Fig.2)
3. *Capillaria caudinflata* (Molin,1858). (Family: Capillariidae).
Prevalence: in 4 out of 32 (Table 1)
Diagnostic characters: small nematodes, the male 9.2 mm female 15.8 mm long. Body is in two parts, the anterior part is longer and thinner (thread-like) which has slender (tube-like) esophagus without bulb. The esophagus is shorter in male 3.1 mm than female 5.8 mm, lips absent but has one spicule. Anus toward the end of the posterior part. The vulva is behind the esophagus-intestinal junction. The posterior end is bluntly rounded and dull. Eggs are oval in shape with two opercula, one at each end. (Fig.3).

Discussion:

The most dreadful and debilitating parasitic diseases of domestic animal, specially chickens and other poultry animals caused by nematodes species. In the present study one of the nematodes species, *Ascaridia galli* has a cosmopolitan distribution as a parasite of poultry. Its prevalence was 34.4% which represent a double of each of the two other species 12.5% for *Subulura brumpti*, and 12.5% for *Capillaria caudinflata*. Of the specimens examined, 19 chickens had parasites, whereas the remaining 13 had none, which gave an infection rate of 60% (Table 1). Although this ratio very much corresponds with the survey in Thailand (Ehlers-Bhodigen 1985) and with incidences of intestinal parasite in Pakistan (Hayat and Hayat 1983), it was not as high as in Kenya (Irungu et al, 2004) and in Ethiopia (Eshetu et al, 2001), whereas in Ghana West Africa all the examined chickens (100%) were infected with gastro-intestinal helminthes (Poulsen et al, 2000). In the mean time, the intensity records of *Subulura brumpti* and *Capillaria caudinflata* were much higher than *Ascaridia galli* in each infected chickens (Table 1). The relationship between worms sex (male and female) and their intensity was not significant.

were found in the intestinal tract of indigenous poultry in parts of Kenya (Irungu et.al, 2004). Barus and Sonin (1980), described two species of the genus, *Subulura*; *S. skrjabini* and *S. brumpti* parasitizing *Corturnix* (*Galliformes*) in India. A total of 18 species were detected from 100 chickens in Ghana to determine the prevalence and distribution of intestinal helminthes (Poulsen et.al 2000). El-Khawed et.al (1977) identified and exhibited 87% of the hens with mono–and polyinfections, of helminthes including *S. brumti* in Khartoum, Sudan.

To my knowledge, these nematodes have not been recorded from the local (Baladi) chicken in Jeddah area.

Materials and Methods:

A total of 32 female local (Baladi) (*Gallus gallus domesticus*) breed chickens were purchased from Jeddah market during the fall and winter of 2004. They were originated from Jeddah district and its rural area such as, El-Khomrah, Ghran and Om-Jabalain villages. On return to laboratory, the birds were sacrificed and the intestine (small & large) tracts were removed, slit and incubated in warm normal saline (35-40 °C) for two hours. Recoverd nematodes were placed in distilled water for few minutes to wash and relax, then counted using a dissecting microscope. Male and female worms were separated, fixed immediately in 70% ethanol and retained for staining processes. Using standard techniques, worm specimens were dehydrated, stained, cleared in lactophenol solution (Schmidt 1992) and mounted in Berlese's media (Humason 1979). They were diagnosed and identified by using Schmidt's (1992), Soulsby's (1982) and Andreson's (1992) schematic key of nematodes families, genera and species.

Results:

Nematodes infections were found in the small and large (caecum) intestines of 19 female local (Baladi) chicken *Gallus gallus domesticus* out of 32 chickens. Three nematode species were identified. Their systematic scheme and diagnostic characters are as follow:-

1. *Ascaridia galli* (Schrank, 1788), Family: Ascaridiidae.

Prevalence: in 11 out of 32 (Table 1)

Diagnostic characters: large nematodes, the male 9.3 cm female 12.0 cm long. Three large lips, esophagus 3.3 cm. The tale of the male has small alae and bears numbers of cloacal papillae (5-10 pairs). There is circular cloacal sucker (pre-anal suckers) and two equal spicules (Fig.1).

The Occurrence of Nematodes in the Intestine of Local (Baladi) Chicken (*Gallus gallus domesticus*) in Jeddah Province – Saudi Arabia

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Abstract:

Thirty-Two local (Baladi) breed of chicken (*Gallus gallus domesticus*), from rural area of Jeddah were found to be infected with nematodes. Three species of nematodes were recorded from small and large intestine namely, *Ascaridia galli* (Ascaridiidae), *Subulura brumpti* (Subuluroidae), and *Capillaria caudinflata* (Capillariidae).

Key words: *Ascaridia galli*, *Subulura brumpti*, *Capillaria caudinflata*, *Gallus gallus domesticus*, Fowls, Jeddah.

Introduction :

Nematodes of poultry infection are widely distributed in different parts of the world, and numerous research has been existed to prevent the mortality of poultry from parasitic diseases. The poultry nematodes fauna in Western of Saudi Arabia is neglectfully studied. Abu-Zinada (1993) reported the first record and prevalence of two nematodes species, *Heterakis gallinarum* and *Subulura suctoria* from the ceecal of Guinea fowl (*Numedia meleagries*). In 1998, the above author added one species of the family Diplotriaeidae (*Diplotriaeina divergens*), infecting dead mynah birds (*Gracula sp.*) obtained from Jeddah market. In the Eastern region of Saudi Arabia, Alsagabi (1998) recorded, three nematodes species from white leghorn chicken namely; *Ascaridia galli*, *Heterakis gallinarum* and *Subulura suctoria*. In Riyadh Al-mathal (1990) noticed that the ratio of infection with nematodes in pigeons was less than with Cestodes infection (17.6 % - 26.2 %). However, she found only one species *Ascaridia columbae* in pigeons and three others in fowl (same as in Alsagabi 1998 above). The first record of *Subulura brumpti* in Egypt was in 1949 (Fahmy,1952). Eshetu (2001), described five species of nematodes obtained from rural scavenging chickens in Addis Ababa, Ethiopia. Many different species of helminthes including *Ascaridia galli* and *Capillaria sp.*

محاولات إحداث التبويض المتعدد من أجل نقل الأجنة في الإبل

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الملخص :

تمثل هذه الدراسة محاولة ناجحة لإحداث التبويض المتعدد وتجميع الأجنة في المملكة العربية السعودية. تم تحفيز التبويض المتعدد في ٦ نوق بالغة غير حوامل، بحقن ٣٠٠٠ وحدة دولية من هرمون الفرس المشيمائي المحفز للمناسل عند اليوم العاشر من حقن هرمون البروجستيرون (الذي تم حقنه لمدة عشرة أيام متوالية). تم متابعة نمو جريبات المبيض عن طريق جهاز الموجات فوق الصوتية حتى وصلت الحجم المناسب للتبويض (قطر ١٣ - ١٩ مم). عندها تم تلقيح النوق مرتين بينهما ١٢ ساعة باستخدام جمل عالي الخصوبة. ثم حقنت الحيوانات بعد أول تلقيح، باستخدام ٣٠٠٠ وحدة دولية من الهرمون الأدمي المشيمائي المحفز للمناسل. تم تجميع الأجنة بالطريقة غير الجراحية في اليوم السابع والسابع والنصف من التلقيح. وضحت مظاهر الشياخ على كل النوق بعد ٨ - ١٠ أيام من حقن هرمون الفرس المشيمائي المحفز للمناسل. استجابت ٤ نوق من ٦ للتبويض المتعدد. بلغ متوسط عدد الأجسام الصفراء للنوق الأربع 4.8 ± 8.75 ، وتراوح بين ٦ - ١٦ جسم أصفر. بلغ معدل التبويض لهذه النوق ٩٧,٢٢%. وقد تم جمع ٣ أجنة، واحد من كل ناقة. خلصت الدراسة أنه على الرغم من النتائج المشجعة في مجال إحداث التبويض المتعدد ومعدل التبويض، إلا إن معدل تجميع الأجنة ما زال في حاجة إلى دراسات مستقبلية لتحقيق نجاح مشابه للتبويض المتعدد.

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1,4 and 11 embryos). Skidmore *et al.* (1992), in their early trials, failed to collect embryos in 63% of the treated donors. Similarly, Vyas (1998) failed to recover any embryo from superstimulated Indian camels using 3000 IU eCG. On the other hand, the recent results obtained by Tinson *et al.* (2000) indicate that the embryo recovery rate is improved to give 5.6 and 7.4 embryos per donor for camels superovulated by eCG and FSH, respectively.

The variability in embryo recovery rates can be ascribed to the type of superstimulatory treatment used (Purohit, 1999) and the delayed oviductal transport or asynchronous ovulations (Mc Kinnon *et al.*, 1994). The latter assumption is supported by occurrence of pregnancy in many donors despite flushing once or even twice, 24 hours interval (Mc Kinnon *et al.*, 1994).

In conclusion, despite of the promising results obtained in this study concerning the average ovulations per donor (8.75 CLs) and ovulation rate (97.22%), embryo recovery rate needs more efforts to reach a similar success.

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Discussion:

The current investigation represents a successful attempt of superstimulation and embryo recovery in the dromedary camel in Saudi Arabia.

Despite the use of eCG in the present investigation, which is known to have low superstimulatory effect in comparison to FSH (purohit, 1999), the obtained results herein are considered truly promising. The mean number of ovulations obtained in this study (8.75 CLs) is much higher than the means of 4.6 to 5.7 CLs reported by several authors, used the same hormone (Anouasi and Ali, 1990; Ismail *et al.*, 1993; Mc Kinnon *et al.*, 1994; Vyas, 1998).

In this aspect, the higher superovulatory response observed here can be attributed to the absence of palpable follicles at the time eCG treatment (Tibary and Anouassi, 1997; Skidmore, 2000) This can be related to the higher progesterone level predominating at this time following the period of progesterone treatment which precedes the eCG administration. Jensen *et al.* (1982), Donaldson (1985) and Callesen *et al.* (1988) emphasized that higher levels of progesterone at the commencement of superstimulation is favorable in terms of ovarian response and embryo quality.

The results of the current study demonstrate that about 67% of the camels respond to the superstimulatory treatment. Similar results have been reported by Cooper *et al.* (1992), Mckinnon and Tinson (1992), Skidmore *et al.* (1992) and Vyas (1998). Skidmore (2000) stated that one of the most important problems in superstimulation of the camel is the high incidence of non-responsive females (Approximately 20-30 %) which fails to produce follicles.

The most striking result in the current investigation is the high percentage of ovulation (97.22%); only one out of 36 follicles failed to ovulate. This Indicates that the regimen used here, which allows mating the female camels twice, 12 hours apart with injection of 3000 IU hCG after the first mating, can successfully induce ovulation in the camel. Skidmore (2000) reported that in order to achieve a good ovulation rate, donors must be monitored by ultrasonography throughout the superstimulation treatment period and bred when the follicles reach a size between 13 and 16 mm in diameter.

The low embryo recovery rate, observed here has been reported by many authors. Cooper *et al.* (1990,1992), using FSH for superstimulation in the camel, obtained good superstimulation but poor embryo recovery of 1.5 embryo/donor (3 out of 11 donors responded to superstimulation and yielded



Fig. 2 Ovaries of camel number 1 containing 7 corpora lutea.



Fig. 3: A collapsed hatched blastocyst (right) and expanded hatched blastocyst (left).



Fig. 1 Ovaries of camel number 4 containing 16 corpora lutea.

Table (1)

Superstimulatory response and embryo recovery of the camels

Number of treated camels	6
Number of camels experienced estrus (%)	6 (100 %)
Interval from eCG treatment to mating	8-10 days
Number of camels responded to superstimulation (%)	4 (66.7 %)
Average number of ovulations in responded camels	8.75 \pm 4.8 CL
Range of ovulations	6-16 CL
Ovulation rate	97.22 %
Day of embryo recovery	7 to 7.5
Number of recovered embryos	3
Embryo recovery rate	75%
Stage of embryo development	Hatched blastocyst

Results :**Induction of Oestrus:**

Table (1) shows that all camels experienced oestrus as the result of the eCG treatment at the end of progesterone priming period. The interval from eCG treatment to oestrus, based on the signs of sexual receptivity and size of the ovarian follicles, ranged from 8-10 days.

Superstimulatory response:

Four of the six camels used responded to the superstimulatory treatment (66.70%), by developing more than 2 corpora lutea (Table 1). However, the other two camels (No. 2 and 6) did not respond to the superstimulation regimen and showed 1 and 0 CL, respectively.

The mean number of ovulations (CLs) given by the camels responded to superstimulation was 8.75 ± 4.80 . the highest number of ovulations (16 CLs) was given by camel No. 4 (Fig. 1). However, the least number of ovulations (6 CLs) was given by camels Nos. 3 and 5. Meanwhile, camel No. 1 produced 7 CLs (Fig. 2).

The number of anovulatory follicles among the camels responded to superstimulation was one, as estimated by rectal palpation, and ultrasonography, and confirmed by laparotomy.

Among the camel responded to superstimulatory treatment, the percentage of ovulation was accounted to be 97.22% (Table 1).

Embryo recovery and evaluation:

Trials for embryo recovery were carried out at day 7 to 7.5 post mating (0= day of oestrus). One embryo from each of camels Nos. 1, 4 and 5 was recovered. No embryos were recovered from camel No. 2.

The embryo recovery rate was 75%. The collected embryos were at hatched blastocyst stage (Fig. 3).

2. Superstimulation

Each female camel received a daily intramuscular injection of 100 mg progesterone powder (Sigma, U.S.A.) prepared in 2 ml sesame oil for 10 consecutive days. At the last day of progesterone treatment, the animals were intramuscularly injected with 3000 IU eCG hormone (Folligon; Intervet, Holland).

3. Mating and ovulation

The development of ovarian follicles was monitored by ultrasound array scanner. Scanning was daily performed for all female camels, starting 4 days from the commencement of the superstimulatory treatments until the majority of follicles were considered sufficiently mature (1.3-1.9cm in diameter). Mating of the females with one of two fertile male camels was allowed twice 12 hours interval. Each female camel received 3000 IU hCG (Chorulon; Intervet, Holland) just after the first mating. Ovulation was confirmed by scanning.

4. Embryo recovery and evaluation

Embryo recovery, by the interrupted-syringe method, was carried out according to the technique described by Skidmore *et al.* (1992). The camel was restrained in the sitting position and given an epidural analgesia at the sacro-coccygeal vertebral space (10 ml 2% Lidocaine HCL; Lido-kel 02, Kelolab, Englad). The animal was sedated with a single intravenous injection of 3 ml Xylazine (Seton 2% Laboratories, Claire, Spain). The tail was wrapped and tied up aside. Rectal faeces was removed and the perinial region was cleaned. The process of embryo recovery, using camel collection catheter (IMV technologies, France) and an embryo filter (EmCon filter, Immuno System Inc., Wisconsin, U.S.A.), was conducted according to the method described by Skidmore (2000). Collected embryos were further evaluated under low and high power research microscope. Ovulation rate was expressed as:

$$\frac{\text{Number of Cls} \times 100}{\text{Number of Cls} + \text{number of follicles}}$$

Whereas the embryo recover rate was expressed according to Mckinnon and Tinson (1992) as:

$$\frac{\text{Number of recovered embryos per attempts} \times 100}{\text{Number of collection attempts}}$$

and Ali, 1990; Skidmore *et al.*, 1992; Mckinnon and Tinson, 1992). Another method for superstimulation in camel is the use of 1-3 mg ovine FSH (follicle stimulating hormone) in a split dose regime over 3-6 days (Cooper, *et al.*, 1990, 1992; Skidmore, *et al.*, 1992; Mckinnon and Tinson, 1992). The latter investigators collected more embryos from donors stimulated with FSH than with eCG. The gonadotrophin treatments were performed just before or after the removal of the progesterone releasing intravaginal device (PRID), (Skidmore, *et al.*, 1992; Cooper, *et al.*, 1992), or on the last day of the progesterone therapy (Mckinnon and Tinson, 1992). Donor camels were mated once or twice 12 hours apart or artificially inseminated. Ovulation was enhanced with human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH), (Cooper, *et al.*, 1992; Mckinnon and Tinson, 1992). The superstimulatory response to the exogenous gonadotrophin therapy varied tremendously between individual donors according to the age and reproductive characteristics of the donors, the selected hormone therapy, and the time or season of the treatment (Anouassi and Ali, 1990; Skidmore, *et al.*, 1992). However, embryo recovery per donor camel varies from 0-30 with current average 6 per donor (Tinson *et al.*, 1998).

Flushing of embryos from donor camels has been tried on day 6 or 7 post mating (Anouassi and Ali, 1990; Cooper, *et al.*, 1990; 1992). The embryos recovered on day 7 from the first mating ranged from compact morula to expanded blastocysts (Skidmore *et al.*, 1992; Ismail *et al.*, 1993). Embryo recovery has been performed in the camel using either a two-way or a three-way catheters during standing or sitting positions (Cooper, *et al.*, 1990; Skidmore, *et al.*, 1992).

The success rate of the mentioned techniques for camel superstimulation and embryo recovery are still far behind what had been accomplished in the other farm animals. Therefore, this study was designed to investigate the efficiency of eCG in inducing superstimulation and embryo recovery in the dromedary camel.

Materials and Methods

1. Camels

Six mature, non-pregnant and non-lactating female camels were used in the present study. They were 8 to 14 years old. The camels were kept in open yard and fed on barley (2Kg/head/day). Rhodes grass hay and water were provided *ad libitum*.

Superovulation Trials for Embryo Transfer in the Camel (*Camelus Dromedarius*)

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Abstract:

The current investigation represents a successful attempt of superstimulation and embryo recovery in the dromedary camel in Saudi Arabia. Six mature non-pregnant female camels were used in this study. Camels were superstimulated by injecting 3000 IU equine chorionic gonadotropin (eCG) at the end of 10 days progesterone treatment. The development of ovarian follicles was monitored by ultrasound array scanner until the majority of follicles were considered sufficiently mature (1.3-1.9cm in diameter). Mating of the female camels with fertile male camel was allowed twice, 12 hours apart. Each female camel received 3000 IU human chorionic gonadotropin (hCG) just after the first mating. Embryo recovery, by the interrupted-syringe method, was carried out at day 7 to 7.5 post mating. All camels experienced oestrus 8 to 10 days post eCG administration. Four out of six camels responded to the superstimulatory treatment (66.70%). The mean number of ovulations (corpora lutea, CLs) produced by the camels responded to superstimulation was 8.75 ± 4.80 , with a range of 6 to 16 CLs. Among these camels, the percentage of ovulation was 97.22%. Three embryos, at hatched blastocyst stage, were collected from the four responded camels, one from each camel. In conclusion, despite the promising results of the current study concerning the superstimulatory response and ovulation rate, the embryo recovery rate needs more research to achieve similar success to superovulation.

Key Words: Superstimulation, superovulation, Embryo transfer, embryo recovery, Dromedary camel.

Introduction:

The number of offsprings likely to be produced by a prestigious female camel in her relatively short breeding life is inadequate to provide a good distribution of the desired genetic material (Musa *et al.*, 1993). Also the restricted breeding season and the camel's long gestation period justify the use of embryo transfer for increasing the reproductive efficiency in this species (Skidmore, *et al.*, 1992; Al-Eknah, 2000, 2001).

Stimulation of ovulation and induction of superstimulation in the donors are considered formidable challenge in camel embryo transfer technique (Mckinnon and Tinson, 1992). equine chorionic gonadotropin (eCG) has been successfully used in camels at various doses ranging between 1500 and 6000 IU to stimulate the ovaries for the production of multiple follicles (Anouassi

قائمة الحشرات الزائرة للورود التي جمعت في بايهيار - بيل في منطقة مادارببور في بنغلاديش على بعض النباتات المزهرة

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الملخص :

تقع بايهيار - بيل في نظام بيئي سهل مسطح في الجزء الجنوبي من بنغلاديش، غني بالزهور والطيور بما فيها أنواع مختلفة من الحشرات الزائرة والملقحة للورود، جُمعت في الدراسة الحالية الأنواع التالية من المنطقة تحت الدراسة، والتي تشمل ما يلي : لاسيوغلوسيوم، ستينونوميا، نوميا، ميفاشيل، نومادا، اميفلا، تيتروлонيا، خيلوكوبا، سيراتينا، بيثيتس، وآبيس. بالإضافة إلى هذا، تم اياض جمع أنواع من الدبابير مثل : كريسيس، سوليا، ديلتا، يومينر، بوليتس، كاليبيون، تاكيتس، ستيزور، فيلانتوس، وسيرسيريس، بالإضافة إلى أنواع من الذباب والحشرات الأخرى من الورود.

Table (2) : Insects collected during different visits from Baghiar-beel.

Bee/ wasp Taxa	Visits					
	First 19 Dec. 99	Second 23 Jan.2000	Third 5-6 March 2000	Fourth 4-5 April 2000	Fifth 1 May 2000	Sixth 12 June 2000
Bees						
HALICTIDAE <i>Lasioglossum</i> sp.	×	×	✓	✓	×	×
HALICTIDAE <i>Lasioglossum</i> (<i>Ctenonomia</i>) sp.	×	×	×	✓	×	×
HALICTIDAE <i>Nomia</i> sp.	×	×	×	✓	×	×
MEGACHILIDAE <i>Megachile</i> sp.	✓	×	×	✓	×	×
ANTHOPHORIDAE <i>Nomada</i> sp.	×	×	×	✓	×	×
ANTHOPHORIDAE <i>Amegilla</i> sp.	×	×	×	✓	×	×
ANTHOPHORIDAE <i>Tetralonia</i> sp.	×	×	×	✓	✓	×
ANTHOPHORIDAE <i>Xylocopa iridipennis</i>	×	×	×	×	✓	×
ANTHOPHORIDAE <i>Xylocopa bryarum</i> (Fab)	×	×	×	×	✓	×
ANTHOPHORIDAE <i>Xylocopa</i> sp.	✓	✓	✓	✓	✓	×
ANTHOPHORIDAE <i>Ceratina</i> sp.	×	×	✓	×	×	×
ANTHOPHORIDAE <i>Pithitis</i> sp.	×	×	✓	✓	✓	×
APIDAE <i>Apis dorsata</i>	✓	✓	✓	✓	✓	×
APIDAE <i>Apis cerana</i>	✓	✓	✓	✓	×	×
Wasps						
CHRYSIDIDAE <i>Chrysis</i> sp.	×	×	×	✓	✓	×
POMPILIDAE	×	×	×	✓	×	×
SCOLIIDAE <i>Scolia</i> sp.	×	×	×	×	✓	×
EUMENIDAE <i>Delta</i> <i>conica</i> (Fabricius)	×	×	×	×	✓	×
EUMENIDAE <i>Eumenes</i> sp.	×	×	×	✓	✓	×
VESPIDAE Polistinae <i>Polistes</i> (<i>Gyrostoma</i>) sp.	×	×	×	×	✓	×
SPHECIDAE <i>Chalybion</i> sp.	×	×	×	✓	×	×
SPHECIDAE <i>Tachytes</i> sp.	×	×	×	✓	×	×
NYSSONIDAE Stizinae <i>Stizus biclipeatus</i> (Christ)	×	×	×	✓	×	×
PHILANTHIDAE <i>Philanthus</i> sp.	×	×	×	✓	✓	×
PHILANTHIDAE <i>Cerceris</i> sp.	×	×	×	×	✓	×
Others						
Flies and other insects	×	×	×	✓	×	✓

Table (1)
Flowering plants and flower-visiting insects collected in Baghiar-beel.

Visits	Flowering Plants	Flower-visiting Insects
1 st (19 December 1999)	<i>Brassica juncea</i> , <i>Brassica napus</i> , <i>Lathyrus sativus</i> , <i>Vigna mungo</i>	<i>Apis dorsata</i> , <i>Apis cerana</i> , <i>Xylocopa</i> sp., <i>Megachile</i> sp.
2 nd (23 January 2000)	<i>Brassica juncea</i> , <i>Brassica napus</i> , <i>Lablab purpureus</i> , <i>Lathyrus sativus</i> , <i>Vigna mungo</i>	<i>Apis dorsata</i> , <i>Apis cerana</i> , <i>Xylocopa</i> sp.
3 rd (5-6 March 2000)	<i>Guizotia abyssinica</i> , <i>Litchi chinensis</i> , <i>Mangifera indica</i> , <i>Musa paradisiaca</i> , <i>Musa sapientum</i> , <i>Phoenix sylvestris</i> (collected sap)	<i>Apis dorsata</i> , <i>Apis cerana</i> , <i>Xylocopa</i> sp., <i>Ceratina</i> sp., <i>Pithitis</i> sp., <i>Lasioglossum</i> sp.
4 th (4-5 April 2000)	<i>Calotropis procera</i> , <i>Ipomoea fistulosa</i>	<i>Apis dorsata</i> , <i>Apis cerana</i> , <i>Xylocopa</i> sp., <i>Pithitis</i> sp., <i>Nomia</i> sp., <i>Lasioglossum</i> sp., <i>Megachile</i> sp., <i>Nomada</i> sp., <i>Ptenonomia</i> sp., <i>Tetralonia</i> sp., <i>Amegilla</i> sp., Chrysididae, Sphecidae (<i>Chalybion</i> sp., <i>Tachytes</i> sp.) Pompilidae, Eumenidae (<i>Eumenis</i> sp.), <i>Philanthus</i> sp., *Flies and other insects
5 th (1 May 2000)	<i>Sesamum indicum</i>	<i>Apis dorsata</i> , <i>Xylocopa</i> sp., <i>Xylocopa iridipennis</i> , <i>Xylocopa bryarum</i> (Fab), <i>Pithitis</i> sp., <i>Tetralonia</i> sp., Scoliidae (<i>Scolia</i> sp.), Eumenidae, Chrysididae (<i>Chrysis</i> sp.), <i>Philanthus</i> sp. <i>Cerceris</i> sp., <i>Stizus</i> <i>biclipeatus</i> (Christ), <i>Polistes</i> (<i>Gyrostoma</i>) sp., <i>Delta</i> <i>conica</i> (Fabricius)
6 th (12 June 2000)	<i>Acacia</i> sp., <i>Ipomoea fistulosa</i> , <i>Tamarindus indica</i>	*Flies and other insects

* Flies and other insects were not identified.

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Third visit During the third visit the bees collected were *A. dorsata*, *A. cerana*, *Xylocopa* sp., *Lasioglossum* sp., *Ceratina* sp. and *Pithitis* sp.

Fourth visit There was a totally different scenario during this visit than the past. Most of the bee colonies of *A. dorsata* had left the area, where very small sized 2-3 only remained. There were only few workers of *A. dorsata* and *A. cerana* in the foraging sites, on the contrary, the number of wasps was increased.

Fifth visit Large number of giant bees, large carpenter bees, other wild bees and wasps had been collected during this visit. Presence of *Xylocopa* sp. was highest this time than the previous visits. Number of giant bees was also high compared to the last visit. Numerous individuals of scolid wasp were collected for the first time during this visit.

Sixth visit The presence of bees and wasps were a few. The flower visitors collected were flies and other insects. All were collected from the small wild plants.

Conclusion:

The study on the relationship between flowering plants and flower-visiting insects is of great importance for Bangladesh so as to develop its nature and economy. From a part of the present study it was understood that the knowledge of the local inhabitants on insects was very poor. If they understood the benefits, they might have protected the insects for the improvement of nature and agriculture. The food source for bees during the off-season drops to a very low level; however, it would be possible to improve food availability in the following ways:

- By protecting source of wild plants.
- By making local people aware in favor of constructing homestead flower gardens.

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were shakful, bonrundhani, telkutch, staji kata and lohagara kata). In the *Guizotia abyssinica* field had numerous bees, wasps and flies. At the visiting time alternative source of food for the flower-visiting insects were searched. It was found that they visited *Phoenix sylvestris* (for sap), *Litchi chinensis*, *Musa paradisiaca*, *M. sapientum* and *Mangifera indica* plants for their foods.

Fourth visit During the fourth visit there was merely no flowering plant in the fields (just plowed dry land only), as well as in the homesteads. Only few roadsides wild plants had some flowers, bees were collecting nectar and/ pollen from there. Plants found mainly were, dholkalmi (*Ipomoea fistulosa*), akando (*Calotropis procera*) and some other wild plants.

Fifth visit Field crops again started growing in many places (jute, rice, sesame, etc.). Some of the sesame (*Sesamum indicum*) fields had full-grown plantations. The major source of flowers in the study area was sesame and a few other wild plants (the Bangla name of the wild plants even was not known). There were numerous wasps visiting sesame field, which might be collecting nectar. All the bees other than *Xylocopa* sp. had been found to enter into the flowers of sesame for nectar. In radhuni large number of flower-visiting insects was found. The presence of flowers in the homesteads, as well as in the wild habitats was very meager. Rainfall was frequent; monsoon water started increasing in the canals and rivers.

Sixth visit From the sixth visit severe scarcity of floral resources was found in the area. Only some wild plants had flowers over which many flies were hovering. Among the wild plants dotkura, koira, hatishura, bituraj and jhamti were prevalent, where also recorded the presence of babla (*Acacia* sp.) and *Tamarindus indica*. The canals, rivers, ponds, and low lands were full of seasonal water. Weather condition was too bad due to heavy rainfall. All the specimens were collected from the above-mentioned wild plants. Most of the flowers were small in size, except for *Ipomoea fistulosa*.

Flower-visiting insects:

First visit Most of the bees were collected from the mustard fields (Table 1). Some were also collected from the homesteads of various parts of the locality. The specimens collected were *A. dorsata*, *A. cerana*, *Xylocopa* sp. and *Megachile* sp.

Second visit Many of *Apis* bees were found in the fields of mustard and bean plants (Table 1). Only two individuals of *Xylocopa* sp. were collected from the *Lablab purpureus*. Bees were also collected from wild flowers. During this visit 16 hives of *A. dorsata* and 2 of *A. cerana* were found throughout the studied area in natural condition.

and homestead garden plants. The wild plants were also a good source of pollen and nectar to the flower-visiting insects, though their number was low. When the number of the flowering plants was high the presence of bees was also high that implies a relation between plants and its visitors. It was found clearly that the density of flower-visiting insects was high during the time of mustard blooming season with a peak from December to January; of course the number of species was quite limited.

The wasps that were found for longer time was *Chrysis* sp., *Eumenes* sp. and *Philanthus* sp. The most promising wasp was *Scolia* sp. Though it was found for shorter period of time, but the number of individuals was high. Yamazaki & Kato (2003) reported scolid wasp as a good pollinator of some plants in the grassland ecosystem as body is covered with long hairs. The seasonal variation of flower-visiting insects was shown in Table-2.

Plant pollinator mutualism is a key interaction in a terrestrial ecosystem (Procter *et al.*, 1996). In Bangladesh so far there has not been held any study on the plant pollinator interaction, which was held in many other countries including Japan. Plant pollinator interactions in Japan have been described at various vegetation types: alpine meadows (Yumoto, 1986), sub alpine forests and meadows (Kato *et al.*, 1993), temperate deciduous forests (Kato *et al.*, 1990; Inoue *et al.* 1990; Kakutani *et al.*, 1990), temperate lowland marsh (Kato and Miura, 1996) and temperate evergreen forests (Yumoto, 1987; 1988). In another studies, researchers here reported that the anthophilous insect communities varied in Japan throughout its varied vegetation types (Fukuda *et al.*, 1973; Sakagami and Fukuda, 1973; Go'ukon, 1992; Matsuura *et al.*, 1974; Ikudome, 1978; Ikudome, 1992; Takahashi, 1990; Kato *et al.*, 1999). However, series of studies are necessary on the plant-pollinator interaction in Bangladesh to assess the contribution on the conservation of both organisms, as well as to get maximum benefits for humankind.

Flowering phenology:

First visit Mustards (*Brassica juncea* and *B. napus*) and beans (khesari- *Lathyrus sativus*, kalai- *Vigna mungo*) started growing in large quantity in the cultivating fields (Table 1). Only a few fields had flowering crops and most of those had a few inches tall plants. In the homesteads also had relatively small source of flowers, which was found during this visit.

Second visit Mustard plants had blossomed almost in full size. In the homesteads found numerous long bean flowers (*Lablab purpureus*). All the areas were found full of plantation with different types of field crops. Large numbers of insect visitors were collected from *Brassica juncea*, *B. napus*, *Lathyrus sativus* and *Vigna mungo* fields.

Third visit Mustard flowers had already been finished. Only few guji (*Guizotia abyssinica*) field had presence of flowers, rest all were wild plants (some of those

flowering phenology that are found at the flood-plain ecosystem in Baghiar-beel, Madaripur, Bangladesh. This is the first report followed by which lists of flower-visiting insects will be prepared gradually throughout successive studies all over the country. Along with this also the relationship between plants and flower-visiting insects will be investigated, what has a role in the conservation and co-evolution of both organisms.

Methodology:

The Baghiar-beel stands between two districts, Madaripur (Amgram and Kadambari union) and Gopalganj (Kalabari union). The total area of the beel is about 4000 hectares. During the present study a total number of 10 villages were surveyed mainly from Amgram union. As the area is in a flood-plain ecosystem thus, the lowlands remain submerged under water for about five months especially during the monsoon. Among the crops grown in the area some mention worthy ones are paddy, pulses and oil seeds (mustards). All the people of the Baghiar-beel are totally dependent on the natural resources of this area, which is not seen today as before.

Bees, wasps, flies and other insects were collected by sweeping insect nets on flowering plants. The names of plants were recorded (Table 1), incase of unknown scientific names, indigenous names were recorded. The dates of collecting specimens were also mentioned in Table 1. All the flower visitors were identified through comparison with the reference specimens by using a stereomicroscope. Some of the unidentified specimens are kept for sending to the respective taxonomist for identification. The study was conducted from the month of December (1999) to June (2000), where flower-visiting insects were collected through six successive visits. The collections were continued almost from early morning to the evening (6 am- 6 pm).

The vegetations found in the Baghiar-beel area are lowland type. As mentioned above the area is subject to be submerged for a considerable time under the water, therefore, the vegetations here is highly specialized with its ecosystem.

Results and Discussions:

The bees that were found for longer period of time were *A. dorsata*, *A. cerana*, *Xylocopa* sp. and *Lasioglossum* sp., which covered the months from December, 1999 to May, 2000. Rest of the bees was found for short period of time in the area (Table 1). Wasps were found for shorter period of time, which was available mostly during the months of April and May, while numbers of bees were decreasing. This situation hasten as the monsoon proceeded in the area.

The honeybees were found in higher number from January to April, after that their density decreased, as they have migrated to other places. The number of wild plants was scarce in the area; therefore, the bees mainly dependent on the cultivated crops

List of Flower-visiting Insects Collected in Baghiar-beel, Madaripur, Bangladesh on Some Flowering Plants

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Abstract:

Baghiar-beel is situated in the flood-plain ecosystem at southern part of Bangladesh, which is endowed with rich flora and fauna including various types of flower-visiting pollinating insects. During the present study the bees of the following genera were collected from the studied area, which include *Lasioglossum*, *Ctenonomia*, *Nomia*, *Megachile*, *Nomada*, *Amegilla*, *Tetralonia*, *Xylocopa*, *Ceratina*, *Pithitis*, and *Apis*. In addition, there also collected many genera of wasps i.e., *Chrysis*, *Scolia*, *Delta*, *Eumenes*, *Polistes*, *Chalybion*, *Tachytes*, *Stizus*, *Philanthus* and *Cerceris*, as well as flies and other insects from the flowers.

Key words:

Flower-visiting insects, Flowering plants, Bees, Wasps, Madaripur, Bangladesh.

Introduction:

The study of insects is necessary to know their life history, habit and habitat, as well as their role in the ecosystem. Bees are insects of highly economic importance, which feed on pollen and nectar that are natural plant in origin. Study on the relationship between flowering plants and the flower-visiting insects are of great importance to conserve terrestrial ecosystem by conserving plant pollinator mutualism. There are 16,325 described species of bees out of ca. 30,000 global ones (Michener, 2000). Among these only few are social bees and honey producing, the rest are solitary or nonsocial bees most of which are involved in the cross pollination of various plant species. In Bangladesh more than 6 species of social bees exist, e.g. *Apis dorsata*, *A. cerana*, *A. florea*, *Trigona fuscobaltata*, *Bombus eximius* and *B. montivagus* (Bhuiya and Miah, 1990; Alam, 1967). Definitely they have very important role in the ecosystem as a natural pollinators of various plant species.

To produce honey in many countries mainly two species of honeybees are used. One is *A. mellifera*, and the other is *A. cerana*. *A. mellifera* is European in origin and *A. cerana* is Asian in origin. Both of them produce honey commercially. Recently, their use in the field of crop pollination is a matter of growing demand and high prospect. The present paper is a preliminary report on the flower-visiting insects and

تصنيف أوتوماتيكي للنصوص باستخدام تقنية ال "ن-غرامس"

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الملخص :

هذه الورقة تتناول التصنيف الأوتوماتيكي للنصوص والذي يعتمد على الإرشاد في اختيار التصنيف الملائم بناءً على عدد من جزئيات الكلمات المحددة مسبقاً. الطريقة المقترحة في هذه الورقة تعتمد على التمثيل الشعاعي للوثيقة أو النص بناءً على جزئيات الكلمات (ن غرامس) وليس على الكلمات. وقد استخدم المعامل من ٢ حرف إلى ٥ حروف لكل صنف ليتم احتساب جزئيات كل صنف بناءً على عدد مرات تكرار كل جزئية في الوثيقة أو النص. يتم بعدها إنتاج جزئيات كل صنف ومن ثم تقلص عدد هذه الجزئيات باستخدام القانون الإحصائي (كاي ٢). جميع التصنيفات المرشحة تعطى أوزان نسبية باستخدام مقياس (تي أف آي دي أف) ومن ثم يحتسب الفارق بين كل صنف وآخر باستخدام طريقة (الكوساين).

أخيراً تضمنت الورقة نتائج تجارب أجريت على مدونات تحتوي على نصوص جمعت من وكالة رويترز ونصوص جمعت من مجموعات اخبارية ، لتقييم مدى قوة وفعالية الطريقة المقترحة. وقد استخدم في التقييم دالة تجمع بين الدقة في التصنيف وإمكانية إعادة الاستعمال ، حيث أظهرت النتائج أن الطريقة المقترحة حققت أداء جيد في تصنيف النصوص.

الكلمات الأساسية: تصنيف النصوص، "ن- غرامس"، قانون ال "كاي χ^2 "، طريقة "كوساين"، *TFIDF*، "روينر ٢١٥٧٨"، مجموعة "نيوز ٢٠".

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3. The approach is more powerful on the corpus 20Newsgroups than on the corpus Reuters. On this point, we find the conclusions of many authors on the disadvantages of the corpus Reuters. Because the corpus Reuters comprises some very close categories which makes their discrimination more difficult. To show that, we calculated the Cosine distance between the profiles of the categories for the two corpora. The results show that the grain, corn and wheat categories are very close in the corpus Reuters, while the ten categories of the corpus 20Newsgroup are well discriminated.

Conclusion:

The approach suggested in this article is different from several ones existing in the literature, it uses a vectorial representation based on the n-grams; an approach which has the advantage of being independent of the language in which the documents are transcribed. We made experiments on two corpora that best illustrate the interest of this approach in improving performance in text categorization. Further works may emphasize on how to take advantage of this method of text categorization in information Retrieval and data mining [Benn2005].

When $\beta > 1$, the precision plays a more significant role than the recall and measurement F_β support the classifiers with a good precision. Inversely, when $\beta < 1$, the recall is more preferred. When there is not a priori a value $\beta = 1$ is used.

Results:

In the whole of our experiments, we tried to evaluate the method on the two corpora Reuters21578 and 20 newsgroup while showing the influence of several parameters on the results.

Table (4) shows the results obtained for $n=2,3,4,5$ and $n=2+3+4+5$ while taking for each value of N , various values of K

Table (4)

N	2		3		4		5		2+3+4+5	
Corpus	Reuters	news	Reuters	news	Reuters	news	Reuters	news	Reuters	news
K=100	0.447	0.386	0.649	0.705	0.698	0.769	0.709	0.786	0.689	0.747
K=200	0.451	0.394	0.648	0.726	0.705	0.791	0.707	0.805	0.701	0.779
K=400	0.451	0.394	0.652	0.734	0.702	0.817	0.704	0.824	0.706	0.804
K=600	0.451	0.394	0.654	0.736	0.699	0.825	0.703	0.830	0.706	0.821
K=800	0.451	0.394	0.653	0.736	0.698	0.829	0.703	0.835	0.706	0.830
K=1200	0.451	0.394	0.653	0.736	0.698	0.829	0.703	0.835	0.698	0.834

The results presented in the table affirm several prepositions. Indeed, we can note that:

1. for the value of N :
 - 1.1- The best performances were obtained with the quint-grams ($n=5$).
 - 1.2- While decreasing the size of the window (the value of n) the performances deteriorate more and more.
 - 1.3- The bi-grams gives the worst results ,and are the closest to the random.
 - 1.4- Combining the n -grams ($2+3+4+5$) didn't bring a considerable improvement.
2. for the value of K :
 - 2.1- By increasing the value of K (vector size), the performances increase, then is stabilized for a value ranging between 600 and 800.

Measurements of performance :

Currently, deciding what measure decide if a categorization is correct or not is in itself an issue. The evaluation of a categorization is thus made empirically on two criteria which are most significant, the effectiveness which measures the calculating time and the memory size, and the accuracy of prediction which measures if the categorization carried out is correct or not. In our experiments, the accuracy of prediction is the criterion which imports us more. Measurement the most used to measure the accuracy of a prediction is the couple precision and recall developed initially for IR (Information Retrieval).

¹ <http://www.daviddlewis.com/resources/testcollections/Reuters21578/>

¹ <http://www.ai.mit.edu/~jrennie/20Newsgroups>

Définition1: Recall and Precision:

$$\pi_i = \frac{VP_i}{VP_i + FP_i}$$

$$\rho_i = \frac{VP_i}{VP_i + FN_i}$$

With VP_i FP_i FN_i respectively defining the well classified texts, the texts assigned by error as well as the texts omitted by the classifier (for a category i).

To evaluate a categorization, one cannot measure only the recall or the precision because these two measurements do not have any significance one without the other.

To take into account at the same time the recall and the precision, the formula F_β s used most of the time.

Definition 2: F_β

$$F_\beta = \frac{(\beta^2 + 1) \cdot \pi_i \cdot \rho_i}{\beta^2 \pi_i + \rho_i}$$

Table (2)

Word distribution of the document Reuters-21578

	Category	Learning set	Test set
1	earn	2877	1087
2	acq	1650	719
3	money-fx	538	179
4	grain	433	149
5	crude	389	189
6	trade	369	118
7	interest	347	131
8	wheat	212	71
9	ship	197	89
10	corn	182	56

- the corpus 20Newsgroup:

It is a corpus developed in CMU which consists of 20 000 electronic messages of 20 newsgroup (1000 by group). Within the framework of our experiments, we took only 10 categories out of the 20 categories present. The 1000 documents of each category are divided into a learning set and a test set. Table 3 shows the distribution of the documents.

Table (3)

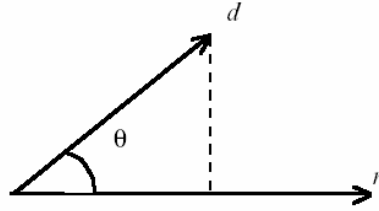
Word distribution of the document 20Newsgroups.

	Category	Learning set	Test set
1	alt.atheism	666	350
2	misc.forsale	677	333
3	rec.autos	670	333
4	rec.motorcycles	667	333
5	rec.sport.baseball	666	333
6	sci.electronics	675	333
7	sci.med	667	333
8	soc.religion.christian	664	333
9	talk.politics.mideast	670	333
10	sci.crypt	667	333

$$Cosine(d, r) = \frac{\sum_{w \in d \cap r} TFIDF_{w,d} \cdot TFIDF_{w,r}}{\sqrt{(\sum_{w \in d} TFIDF_{w,d}^2) \cdot \sum_{w \in r} TFIDF_{w,r}^2}}$$

with: w a n-grams, d the document to be categorized, r the profile of a category, $TFIDF_{w,d}$ the weight of the n-grams w in the document d and $TFIDF_{w,r}$ that of w in category r .

The figure presents a geometrical view of this distance.



Experiments :

Data preparation :

For the experiments, we used the two most used corpora in this field : the Reuters-21578 and the 20Newsgroup corpora.

- the corpus Reuters -21578:

The Reuters-21578 corpus is a set of financial dispatches emitted during the year 1987 by the Reuters agency, in English, and available free on the Web¹. This corpus is very often used for evaluation in publications, as in [Sha98] comparing their AdaBoost *algorithm* with the formula of Rocchio, or in [Joa98], [Seb2002], and [Dum98] evaluating the performances of the machines with vectors supports. [Yan99] also used this corpus to compare various algorithms (machines with vectors supports, networks of neurons, decision trees, networks Bayesians). This corpus is composed of a certain number of categories each one comprising a learning set and a testing set.

In our experiments, we used only the 10 categories the most represented within the version "ModApte" of this corpus. Table 2 shows the distribution of the documents on the two set (learning set and test set).

$$\chi_{ij}^2 = \frac{(N_{ij} - \frac{N_i X N_j}{N})^2}{\frac{N_i X N_j}{N}} \cdot \text{sign}(N_{ij} - \frac{N_i X N_j}{N})$$

With:

N_{ij} Number of occurrence of the n-grams i in the category j .

N_i Number of occurrence of the n-grams i in all the learning corpus.

N_j Number of occurrence of all the n-grams in the category j .

N Number of occurrence of all the n-grams in all the learning corpus.

The profiles of each category will thus contain K first n-grams. The influence of the value of the parameter K is studied in the experimentation part.

Classification:

In this stage, it is a question of calculating a distance between the profile of a document to be categorized and the profiles of the categories. To do this, it is first of all necessary to balance the n-grams constituting the profiles of the categories [Bekk2002]. There exist many measurements to balance the vectors, the most used is measurement are TFIDFs.

$$TFIDF(w, d) = TF_{w,d} \cdot IDF_{w,d} = TF_{w,d} \{(\log_2 \frac{N}{DF_w}) - 1\}$$

With:

$TF_{w,d}$ Number of occurrence of the n-grams W in the profile d .

DF_w Number of profiles containing the n-grams w .

There are several methods to calculate the distance between two vectors, the most used in this field and the method of the Cosine.

An approach of categorization based on the n-grams

The majority of the approaches of classification are centred on pre-linguistics processing such as the deletion of the blank words, the lemmatisation and the stemming. These pre-processing require a preliminary knowledge of the language in which are transcribed the documents. In other words, these approaches suffer from a strong dependency towards the language of the documents, which limit their applications.

The approach studied in this article is an approach based on the n-grams, an approach which has the advantage of being independent towards the language of the documents, and operates without any linguistic pre-processing.

[Yang,99], [Yang97].

5.1- Generation of the n-grams

At this stage, it is a matter of representing each category in the form of a vector whose each descriptor represents a n-gram, with each n-gram in the vector we associate his number of occurrence in the category.

	eact	stup				udil		
100	215	465	310	10	524	21		

The example of the figure presents a vector of a category in which the quadri-gram " eact ", " stup ", " udi " are repeated on the latter with respectively 100,215,524 occurrences.

Selection of the characteristic n-grams

In this second stage, it is a matter of generating a profile for each category, A profile of a category contains all the n-grams which characterizes it, this compared to the other categories. There are several methods to discriminate the classes, we chose to use the law of χ^2 multivariate to discriminate the categories.

The stages of the algorithm are to detail as follows:

Firstly, a matrix $\chi^2 [i,j]$ occurrences of the n-grams i in the category j should be built. Thereafter it is necessary to calculate the value $\chi^2 [i,j]$ which represents the independence between the n-grams i and the category j then sort the table in the decreasing order.

the term t_K is present in the documents of the class c_i . The stages are described in algorithm 1.

In all our experiments, we chose to use multivariate χ^2 method because :

- It is supervised since it is based on the information brought by the categories.
- It is multivariate because it evaluates on an overall basis the role of a term compared to the others.
- It takes account of the interaction terms/classes because it makes it possible to choose, for each category, the terms which contribute more to their discrimination.

Algorithm:

Input: $C = \{c_1, c_2, c_3, \dots, c_c\}$ // the list of the categories preset.

Train corpus//texts for learning for each category.

n.// size of the window of the n-grams.

k // size of the profiles.

Test corpus // texts for testing for each category;

Begin

For(i=0; i<c; i++)

{ generate the n-grams using the texts for learning of category i;

Compute the number of frequency of each n-grams; }

Build table N_{ij} of occurrence of the n-grams j in category i;

Compute the value χ^2 representing independence between the n-grams j and category i;

Sort the table in a decreasing order ;

For(i=0; i<c; i++)

{ Profil[i]=Vector containing K first n-grams table $\chi^2_{i,}$;

}// End of the learning phase

For(i=0; i<c; i++)

{ For each $d_i \in$ Test corpus do

{ generate the n-grams and build the vector of the document d_i

Compute the distance cosine between the document vector and the profiles of the categories;

associate the document d_i to the category to which its profiles is the closest. }

}

End

- Algorithm of categorization by using the n-grams -

Considering the importance of these advantages, the n-grams are used in several fields.

4. the law of χ^2

If the n-grams offer several advantages, the number of the generated n-grams disadvantage their uses in the field of categorization of texts. To minimize this problem, we used the law of χ^2 like a means of reduction of the number of generated n-grams [Fern2000], [Bekk2002]

The statistics of χ^2 measure the variation with independence between a descriptor T and a topic C . There exist two alternatives for this measurement, the first measures independence in term of absence/presence of a descriptor in the documents associated with a topic; Calculation requires to build the table of contingency (2×2) for each descriptor T of the corpus and each class C (table 1).

Table (1)
Table of contingency for descriptors of the corpus.

	term t_k present	term t_k absent	
term c_i present	a	c	$a+c$
term c_i absent	b	d	$b+d$
	$a+b$	$c+d$	$N=a+b+c+d$

The statistics of χ^2 can be put in the form

$$\chi_{uni}^2(t_k c_i) = \frac{N(ad - cb)^2}{(a + c)(b + d)(a + d)(c + d)}$$

This first measurement called *univariate* is used for the selection of the descriptors in [Zhang01], [Cav94], and [Yan97].

The second alternative called multivariate, is a supervised method allowing the selection of terms while taking into account, not only their frequencies in each class, but also the interaction of the terms between-them and the interactions terms/classes [He2000], [Jal2002].

The idea consists in using the contributions of the cells (t, c) to the χ^2 associated to the global cross table, where NR_{ki} is the number of times where

used in this field is the vectorial model. The second stage is that of the training in which we try to find a mathematical model able to represent, for then comparing the semantics of the texts. All the methods of training resulting from the artificial learning (AL) community can be applied to text categorization applications. The third stage is that of the classification [YMi2005] in which we assign a text to a category based on the model found in the preceding stage and which is the stage of learning]. A last stage is necessary to evaluate the performances of the system. To measure the accuracy of prediction of the system, various measures are used in the continuation of this article.

N-Grams :

A n-gram is a sequence of n consecutive characters. The set of the n-grams that can be generated is the results obtained by moving a window of n boxes on the body of text. This displacement is done by stages, a stage corresponds to a character. In our work, we used several lengths for the n-grams ($n=1,2,\dots, 5$). we replaced the space character by the character " - ". For example, the text " you and you " gives the following n-grams:

- Bi-grams: yo,ou,u-,a,an,nd,d,...etc.
- Tri-grams:you,ou-,u-a,-an,and,nd,... etc.
- Quadri-grams : you-,ou-a,u-an,-and,... etc.

The n-grams have several advantages:

- *Automatic capture of the roots of the most frequent words.*
- *Independence towards the document language.* Contrary to other techniques which require the use of specific dictionaries ((feminine-masculine; singular-plural; conjugations; etc.) for each language. Moreover, with the n-grams, we do not need preliminary segmentation of the text in words; this is interesting for the processing of languages in which the borders between words are not strongly marked, like Chinese for example.
- *Tolerances with the spelling mistakes and the deformations* For example, it is possible that the word " chapter " is written like " clapter ". A system based on the words will have difficulties to recognize the word " chapter " since the word is badly spelled. On the other hand, a system based on the n-grams is able to take into account the others n-grams (parts) like " apte ", " pter ", etc

case, it is interesting to represent the documents and the classes using the same formalism and the one used generally is a vector space.

In this article, we will be interested in categorization, i.e. supervised classification and more particularly to show the influence of the n-grams method of presentation of the documents on the results of the latter.

The article is organized in the following way. In section 2, we present problems of the categorization of texts. Section 3 presents the approach suggested with all the stages. So as to show the effectiveness of our approach, we describe in the section 4 some experiments carried out on corpora of evaluation (Reuters21578, 20 news groups) as well as a reading of these results.

Categorization of texts :

Text categorization (T.C) is the process which consists in assigning one or more categories among a preset list to a document. The manual realization of this task is extremely expensive in term of time because, it is necessary to attentively read each document to be able to decide.

In other words, the categorization of text consists in seeking a working joint (model of prediction) between a whole of texts and a whole of categories (labels, classes), one of several well-known techniques in information retrieval. [MaN2004]

Sebastiani, F defines the T.C as being the process which consists in seeking a working joint between a set of texts and a set of categories (labels, classes). Formally, the categorization of text consists in associating a Boolean value to each pair (D, C) in $D \times C$ where D is the set of the texts and C is the set of the categories.

The value T (Truth) is then associated to the couple (D, C) if the text D belongs to the class C while the value F (False) will be associated to it in the contrary case. The goal of the categorization of text is thus to build a procedure (model, classifier) $F: D \times C \rightarrow \{V, F\}$ which associates one or more labels (categories) a document D such as the decision given by this procedure " *coincides as much as possible* " with the function $E: D \times C \rightarrow \{V, F\}$ the true function which turns over for each vector D a value C [Seb2002].

The design of a system of text categorization comprises several stages. Firstly it is necessary to choose a model of representation of the documents and categories that is exploitable by the machine, the model most usually

N-grams in Texts Categorization

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Abstract:

This paper deals with automatic classification of documents; this is performed by a supervised classification since it operates on a set of preset classes. The suggested approach is original since it is based on a vector representation of the documents centred not on the words but on the n-grams of characters for n varying from 2 to 5.

Considering the significant number of the n-grams generated for each class, we used in our work the law of χ^2 to reduce the number of the characteristic n-grams of each class. The weighting of the vectors was done by using the measurement of the TFIDF, and for the calculation of the distance between two vectors, we used the method of the Cosine. The experiments were done on two well-known corpora in the community of categorization, the Reuter 21578 and the 20Newsgroups. Evaluation of the approach was performed by using a function combining both precision and recall.

The results obtained show that the technique of the n-grams is very effective in the field of the categorization of texts.

Key words: Text categorization, n-grams, the law of χ^2 , method of the Cosine, TFIDF, Reuters21578, 20 Newsgroups.

Introduction :

Automatic Text categorization is gaining popularity with the growing interest and usage of text data available as well on the world wide web as within enterprises and because, the manual realization of this classification is extremely expensive in term of time because of the appalling growth of the number of the numerical documents available. We distinguish in the field from automatic classification two types of approaches supervised classification and not supervised classification. These two methods differ on the way in which the classes are generated. In the case of not supervised classification, the groups of documents (categories) are generated automatically by the machine, while they are, in the supervised approach, defined by an expert. In this last

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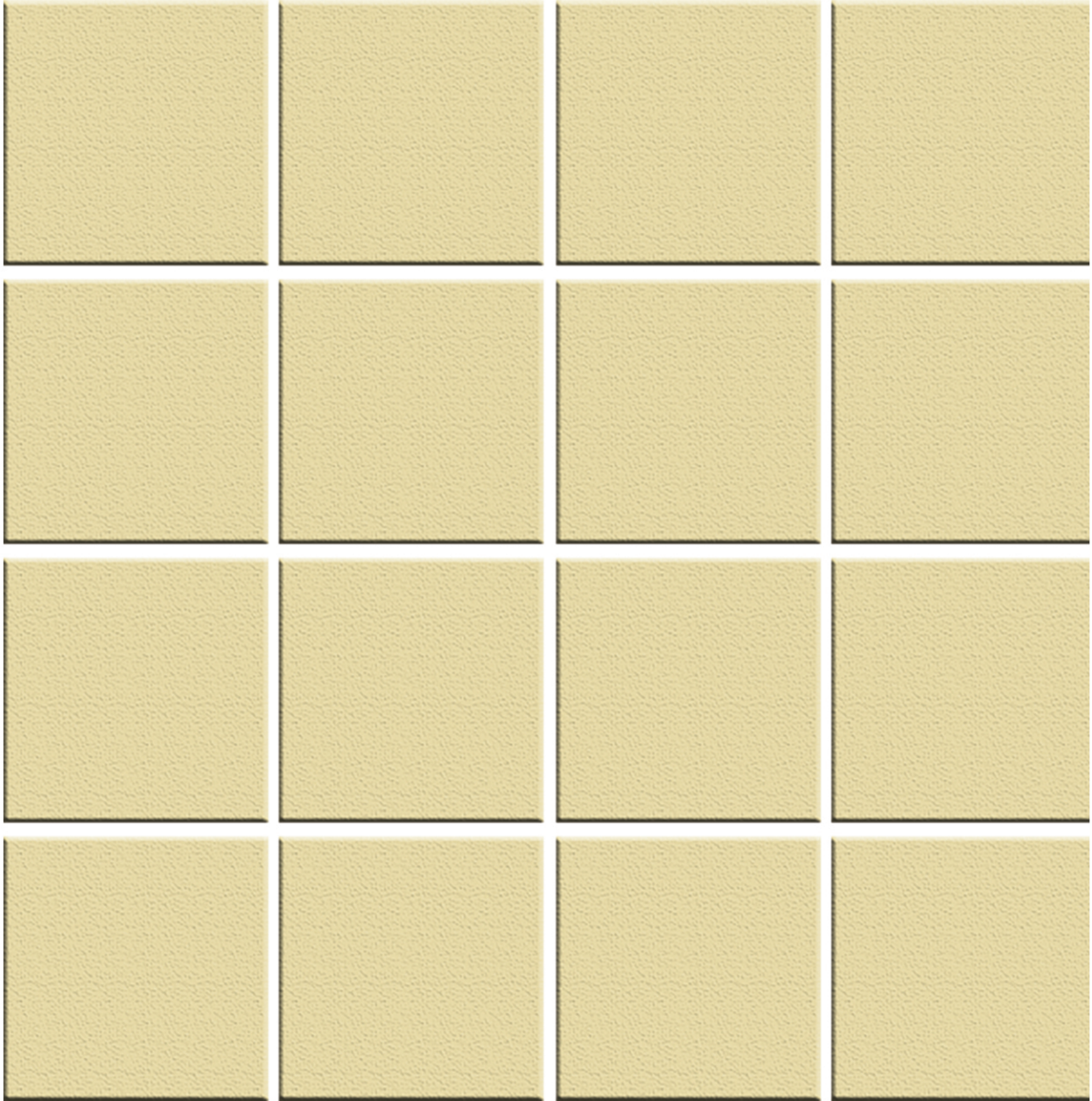
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