

***Role of Aniseed (*Pimpinella anisum*) Oil on
Mammary Gland Performance in Rats***

A Thesis

**Submitted to the council of the College of Veterinary Medicine
at the University of Baghdad in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in Veterinary Medicine/Animal Physiology**

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2005

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DEDICATION

To

My parents who raised me

To

My brothers and sisters who stood with me

To

My teachers who taught me

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'Thanks to God for all his blessings'

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MAMMARY GLAND PERFORMANCE IN RATS**

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كفاءة الغدد اللبنية في الجرذان

أطروحة

مقدمة إلى مجلس كلية الطب البيطري في جامعة بغداد وهي
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ABSTRACT

This study was designed to determine the effects of aniseed oil administration during the last trimester of gestation on some plasma biochemical constituents and mammary gland histo-physiological changes at the first and eleventh days of lactation in rats.

The effective dose (ED_{50}) which produce 50% of maximum response of aniseed oil was determined in the pilot study, by studying the dose response curve, Fifty primiparous pregnant rats, at the 15th day of pregnancy, were randomly divided into five equal groups as control and four treated groups. Animals of the treated groups received aniseed oil orally in four gradual increasing doses, during the last week of gestation (0.02, 0.04, 0.08, 0.12 gm/kg B.w), respectively. After parturition, five animals from each group were randomly isolated and their mammary gland weight (MGwW%) was recorded. The remaining animals were allowed to suckle their litters for the first eleventh days of lactation period. During this period, daily litter weight gains (LWGs%) was calculated, then at the eleventh day of lactation litter stomach weight (LSwW%) was calculated. The results showed that (0.04gm/kg B.w) of aniseed oil produced the highest increase in MGwW% at the first day of lactation, LWGs% during the first eleven day of lactation and LSwW % at the eleventh day of lactation. By using these three parameters, the ED_{50} was calculated, which was equal to (0.043 gm/kg B.w).

The ED_{50} (0.043 gm/kg B.w) of aniseed oil was used in experiments, to evaluate mammary gland performance depending on the following parameters:

- A. Concentration of some biochemical constituents in the plasma at the first and eleventh day of lactation, including: 1.Total proteins,

triglycerides, total cholesterol and glucose. 2. Ca, P, Mg, Na ions and prolactin hormone.

B. Histological studies of the mammary tissue at the first and eleventh day of lactation, including 1. Histophysiological. 2. Histochemical of alkaline phosphatase (ALP) & acid phosphatase (ACP). 3. Transmission electron microscope examination (TEM) of mammary epithelial cell.

Twenty primiparous pregnant rats, at the 15th day of pregnancy, were randomly divided into two equal groups: control (10 rats) and treated (10 rats). Animals of treated group received aniseed oil (0.043 gm/kg B.w) orally daily during the last week of gestation. After parturition, five rats from each group were randomly isolated, and their MGwW% was recorded. Blood samples, were collected for biochemical study and Tissue samples, from mammary glands were prepared for histophysiological study, histochemical study, besides TEM examination. The remaining animals continued suckling their pups, for the first eleventh day of lactation. After that, the animals were scarified and blood and mammary tissue samples were taken for the same purposes mentioned above.

The results of biochemical study revealed that aniseed oil lead to a significant reduction at the first day of lactation in the following parameters: plasma total protein, triglycerides, cholesterol, glucose, Ca, P and Mg . While the plasma Na ion concentration increased in the treated group at the first and eleventh day of lactation, with not detectable of prolactin at the first day of lactation, but increased significantly at the eleventh day of lactation.

The histological study manifested slightly hyperplasia in expense of adipose tissue. Aniseed oil treated rats elicited greatly dilated alveoli, which were filled with milk secretion at eleventh day of lactation. The histochemical study revealed that there was a marked increase in ALP

activity at the first and eleventh day of lactation in treated rats, whereas the ACP was relatively strong at the first and eleventh day of lactation in the treated rats. TEM of treated group at the first day of lactation showed a relatively more and larger lipid droplet (LD), secretory vesicles (SV) and rough endoplasmic reticulum (RER) as compared to control. While the sections of mammary epithelial cells at eleventh day of lactation revealed a well developed RER, more LD and SV.

The second experiment was designed to investigate the site of action of aniseed oil. Fifteen female rats, at five weeks of age, were randomly divided into three equal groups, treated for two weeks as follow: group I ovariectomized (OA) rats received aniseed oil 0.043 gm/kg B.w.; group II intact (IA) rats received aniseed oil 0.043 gm/kg B.w.; group III sham operated (S) control rats received tap water. Four parameters; mammary gland weight %, uterine weight %, follicular stimulating hormone and lutenizing hormone (MGwW %, UwW %, FSH and LH levels) were employed. This investigation showed that MGwW% and UwW% were highest in IA, while the OA rats showed the highest levels of FSH and LH.

In conclusion, aniseed oil act as slightly mammogenic and highly lactogenic, besides, it induces mammogenesis, lactogenesis and galactopoieses, most probably by its indirect action on the mammary gland.

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LIST OF ABBREVIATIONS

Acid phosphatase	ACP
Alkaline phosphatase	ALP
Body weight	B.w
Calcium	Ca
Chloride	Cl
Deoxyribonucleic acid	DNA
Eosin	E
Feed back inhibition of lactation	FIL
Follicular Stimulating Hormone	FSH
Gonadotrpın Releasing Hormone	GnRH
Hematoxyline	H
Intact +Aniseed oil	IA
Lipid Droplet	LD
Litter body weight	L B.wt
Litter's Stomach Weight to body weight	LSwW%
Litter Weight Gains	LWGs%
Lutenizing hormone	LH
Magnesium	Mg
Mammary Gland weight to body weight	MGwW%
Ovariectomized +Aniseed oil	OA
Phosphorus	P
Ribonucleic acid	RNA
Rough Endoplasmic Reticule	RER
Secretory Vesicles	SV
Sham operated (control)	S
Smooth Endoplasmic Reticule	SER
Transmission Electron Microscope	TEM
Uterine weight to body weight	UwW%

الخلاصة

استهدفت هذه الدراسة تحديد بعض التأثيرات التي يمكن أن يؤديها تناول زيت بذور نبات الينسون، في الثلث الأخير من فترة الحمل، في بعض المعايير الكيموحيوية لبلازما الدم وأنسجة الغدد اللبنية في اليوم الأول والحادي عشر من الرضاعة بوصفها مؤشراً لكفاءتها في تصنيع وإفراز اللبن في إناث الجرذان.

تم استخراج الجرعة المؤثرة المؤدية إلى نصف الاستجابة القسوى (ED_{50}) بدراسة منحنى الجرعة- الاستجابة استخدمت (50) أنثى حامل في اليوم الخامس عشر من حملها وقسمت عشوائياً إلى خمس مجموعات متساوية العدد الأولى السيطرة: تناولت حيواناتها ماء الشرب طيلة فترة التجربة، أما المجموعات المعالجة الأربعة الأخرى، فقد تناولت زيت بذور نبات الينسون في الثلث الأخير من مدة الحمل وبأربع جرعات تصاعديّة التركيز، حيث تناولت الأولى مقدار نصف جرعة شعبيّة واحدة (0.02 غم/كغم من وزن الجسم)، والثانية تناولت جرعة شعبيّة واحدة (0.04 غم/كغم من وزن الجسم)، والثالثة ضعف الجرعة الشعبيّة (0.08 غم/كغم من وزن الجسم)، أما الرابعة فقد تناولت ثلاثة أضعاف الجرعة الشعبيّة (0.12 غم/كغم من وزن الجسم). بعد الولادة مباشرة، تم عزل خمس أمهات من كل مجموعة عشوائياً وسجلت النسبة المئوية لعددها اللبنية أما الأمهات المتبقية فقد سجلت أوزان مواليدها ثم حسبت نسب الزيادات الوزنيّة اليومية للمواليد ولغاية اليوم الحادي عشر من الرضاعة، ثم حسب وزن المعدة في اليوم نفسه. أظهرت نتائج هذه التجربة تفوق معدلات نسب أوزان الغدد اللبنية في اليوم الأول من الرضاعة ونسب الزيادات الوزنيّة للمواليد أثناء فترة الرضاعة و أوزان المعدة في اليوم الحادي عشر من الرضاعة لحيوانات المجموعة التي تناولت جرعة شعبيّة واحدة (0.04 غم/كغم من وزن الجسم) بالمقارنة مع معدلات المجموعات الأربعة الأخرى. كما لوحظت علاقة خطية وثيقة بين الجرعة المتصاعدة من الزيت ونسب أوزان الغدد اللبنية من جهة وبينها وبين نسب الزيادات الوزنيّة اليومية و نسب أوزان المعدة من جهة أخرى. ومن هذه المعايير الثلاثة تم استخراج قيمة الجرعة المؤثرة من زيت بذور نبات الينسون فقد بلغت جرعة شعبيّة واحدة و تعادل (0.043 غم/كغم من وزن الجسم).

أجريت التجربة الأولى لدراسة بعض المعايير الكيموحيوية و بعض التغيرات النسيجية التي بواسطتها يتم تقييم فعالية الغدد اللبنية عند استخدام جرعة (ED_{50}) من الزيت (0.043 غم/كغم من وزن الجسم) في الثلث الأخير من فترة الحمل. فقد تضمنت المعايير الكيموحيوية قياس كل من: تركيز البروتين الكلي، كلوبولين، الكلسيريديات الثلاثية، الكولسترول والكلوكوز؛ بعض أيونات بلازما الدم مثل الكالسيوم، الفسفور، المغنيسيوم والصوديوم؛ وهرمون البرولاكتين. أما

الدراسة النسجية فقد شملت التغيرات الفسلجية النسجية إضافة إلى الكيمياء النسجية للأنزيمات الفسفتاز القاعدي والحامضي والدراسة النسجية الفوقية لأنسجة الغدد اللبنية في اليومين الأول والحادي عشر من الرضاعة . استخدمت (20) أنثى حامل في اليوم الخامس عشر من حملها وقسمت عشوائياً إلى مجموعتين ضمت الأولى (السيطرة) عشر إناث حامله وضمت الثانية (المعالجة) عشر إناث حامله تناولت زيت بذور نبات الينسون (0.043 غم/كغم من وزن الجسم) في الثلث الأخير من مدة الحمل.

بعد الولادة مباشرة ، تم عزل خمس أمهات من كل مجموعة عشوائياً وسجلت أوزانها والأوزان الرطبة لعددها اللبنية أخذت منها نماذج الدم والأنسجة للأغراض المذكورة أعلاه . أما الأمهات المتبقية من مجموعة السيطرة والمعالجة فقد سجلت أوزان مواليدها يومياً ولغاية اليوم الحادي عشر من الرضاعة بعدها تم تخدير الحيوانات واخذ نماذج الدم والأنسجة.

أظهرت نتائج هذه التجربة بان زيت بذور نبات الينسون أدى إلى انخفاض معنوي ($p < 0.05$) في تركيز كل من : البروتين الكلي، الدهون الثلاثية، الكولسترول والكلوكوز وتركيز بعض أيونات بلازما الدم الكالسيوم والفسفور والمغنيسيوم أما أيون الصوديوم فقد شهد ارتفاعاً معنوياً في اليوم الأول من الرضاعة مع عدم ظهور مستوى تركيز هرمون البرولاكتين ، في حين شهد اليوم الحادي عشر من الرضاعة زيادة في كل المعايير المذكورة أعلاه . وفيما يخص الدراسة النسجية التي أظهرت فرط تنسج وتوسع بسيط في الأسناخ مع زيادة في فعالية الأنزيم الفوسفاتاز الحامضي مع زيادة بسيطة في حجم وعدد القطيرات الدهنية والحوصلات الإفرازية في اليوم الأول من الرضاعة، أما الانزيم الفسفتاز القاعدي فقد اظهر فعالية عالية في كلا اليومين الأول والحادي عشر من الرضاعة . في حين شهد اليوم الحادي عشر من الرضاعة زيادة في كل التغيرات النسجية المذكورة.

استهدفت التجربة الثانية دراسة آلية عمل زيت بذور نبات الينسون بوجود وغياب تأثير المبايض، هذ تتلخص هذه التجربة اختبار فيما لو كان عمل الينسون مباشرة على الغدد اللبنية أم على المحور النخامية-تحت المهاد. استخدمت في هذه التجربة (15) أنثى في عمر خمسة أسابيع قسمت عشوائياً إلى ثلاثة مجاميع متساوية وعوملت لمدة أسبوعين على النحو التالي : المجموعة الأولى: اجري لها عملية إزالة المبايض وعولجت بالزيت بجرعة (0.043 غم/كغم من وزن الجسم). المجموعة الثانية: سليمة وتناولت الزيت بنفس الجرعة و المجموعة الثالثة : اجري لها عملية فتح وخياطة البطن فقط وتناولت ماء الشرب الاعتيادي واعتبرت كمجموعة سيطرة . تم استخدام أربعة مؤشرات هي وزن الغدد اللبنية ووزن الرحم، قياس مستوى الهرمون المحفز للجريب والهرمون اللوتيني لتحقيق هدف هذه التجربة. أظهرت نتائج هذه التجربة إن الحيوانات

السليمة والتي تناولت الزيت شهدت أعلى وزن للغدد اللبنية والرحم في حين بينت الحيوانات مزالة المبايض أعلى تركيز لقياس الهرموني. إن عدم تحفيز الغدد اللبنية والرحم بعد العلاج بالزيت في الحيوانات مزالة المبايض مع ارتفاع تركيز الهرموني يشير إلى فعل الزيت الغير المباشر على الغدد اللبنية والرحم وإنما يعمل على المحور تحت المهاد .

نستنتج من هذه الدراسة، بأن زيت بذور نبات الينسون عمل على زيادة كفاءة الغدد اللبنية في استخلاص المواد الأولية المكونة لللبن من الدم في اليوم الأول من الرضاعة إضافة إلى تصنيعها داخل الغدد وانه حث على التكوين الثديي واللبنني على الأرجح بمفعول غير مباشر عليها.