MORPHOLOGICAL STUDIES ON THE EFFECT OF *NIGELLA SATIVA* SEEDS ON THE MAMMARY GLAND OF MATURE MALE NORWAY RAT

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Abstract

Nigella sativa seeds (NSS) are reputed traditionally and scientifically as a potent agent that promote milk secretion (galactagogue), but their mechanism of action is studied trivially. One suggestion was that these seeds are bio-transformed to sex hormones within the ovaries. Therefore, this investigation was designated to throw light on the action of these seeds in the absence of ovaries i.e. in male rats.

Thirty Norway male albino rats were used in this investigation. They were divided into experimental (n=20,fed NSS 2g /Kg body weight /day for 14 days) and control (n=10, fed placebo for 14 days). After sacrifice mammary gland and blood samples were obtained.

Experimental rats revealed a significant increase ($p<0.01$) in weight, hypertrophied mammary gland lobules with dilated ducts and rise in serum prolactin level, as compared to the controls.

The Mammogenic action of NSS is most probably conveyed via extra-ovarian pathway. *Nigella sativa* seeds have an estrogen and progesterone like action or their steroidal saponins may be biotransformed to these hormones and cause mammogenesis .

Key words: *Nigella sativa* seeds, Male rat, Mammogenesis

Introduction

Mammogenesis is defined as the development of the mammary gland to the functional state, while Lactogenesis is defined as the secretion of milk by the mammary gland, while "galacttagogue" is defined as the agent that promotes secretion and flow of milk[1].

Nigella sativa seeds (NSS) (English name=Black cumin, small fennel and Black seeds, Arabic name = Habbat -suda, Habbat Al-Baraka, Family name= Raununculaceae)[2,3] had been prescribed as a galactagogue in several parts of the world by the practitioners of traditional medicine[2,4]. Recently, it has been proved that NSS enhance mammogenesis, lactogenesis and galactop-oiesis[5,6]. However, the mechanism of action of these seeds was not investigated or worked out previously, except trivially[6]. One study sugg-ested that the galactagogual role of NSS is due to the diosgenin (steroidal saponin found in NSS), which has an oestrogen-like action but not progesterone -like action, as it increased the number of ducts of mammary glands[6]. On the other hand, another study showed that NSS increased progesterone receptors

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without any effect on oestrogen[7].
Therefore, this study was designed to throw light on the role of NSS in the absence of estrogen and progesterone (i.e.) in male mammary gland.

Materials and methods

A total of thirty Norway adult male rats (*Rattus rattus* norvegicus albinus) were used in this investigation. They were isolated in a relatively controlled environment at a temperature of 25°C ± 2°C, in the "Animal Breeding Center", College of Science / Univ. of Baghdad.

Animals were partitioned into two groups (Table-1)

Table(1): animal grouping and description

Group	No.	Age (w)	Treatment
Control	**10**	**10-12**	**3 ml D.W.**
Treated	**20**	**10-12**	**NSS suspended in 3 ml D.W.**

No. = number of animals, w = week, D.W. = distilled water

After cleaning, standa-rdizeation (in the "Dept. of Pharmacognosy" College of Pharmacy/ Univ. of Baghdad) and grinding, NSS powder was given to the experimental animals in a dose of 2g/kg body weight/day (suspended in 3 ml distilled water and administered via oro-gastric tube) for two weeks[8,9,10]. Controls, on the other hand, received 3 ml of distilled water only, under identical conditions.

All rats were sacrificed after two weeks of treatment. From each ether anaesthetized rat, two pieces of mammary glands were removed. Blood samples were obtained (via intra-cardiac puncture) from each dissected rat, to be used for prolactin immunoassay using the ELFA technique [Enzyme Linked Fluorescent Assay]- Assay was completed automatically and calculated by 30410 VIDAS prolactin kits [Bio- Meriux- France].

Tissue specimens were processed to paraffin blocks, which were sectioned into 6µm thick sections. Tissue sections were stained by haematoxyline and eosin (H&E) [11] and periodic acid Schiff (PAS) stains[11].

After staining, tissue sections were morphometrically assessed for mammogenesis and photographed. For statistical studies, student "t" test was employed using the least significant difference[12].

Results

All rats were maintaining good general health and well being during this study. Experimental rats arrayed good appetite, remarkable food intake and significant increase in weight (Table-2).

Table-2: Body weight changes in both experimental and control rats before and after treatment.

Groups	Body weight (g) before treatment M ± SD	Body weight (g) after treatment M ± SD
Control	**237.5 ± 8.3**	**249.5 ± 10.2**
Treated	**238.2 ± 9.8**	**260.8 ± 8.6***

M ± SD = mean ± standard deviation
*** = Significant results at P < 0.01**
(g) = gram

Control male rats revealed a non-active shrinked mammary gland with tiny lobules that contain few secretary tubules (figure.1), while experimental male rats manifested marked hyperplasia of the acinar and ductal epithelium (figure.2), 274.7% increase in number of nuclei / cross section of an acinus and rise in serum prolactin level (Table 3).

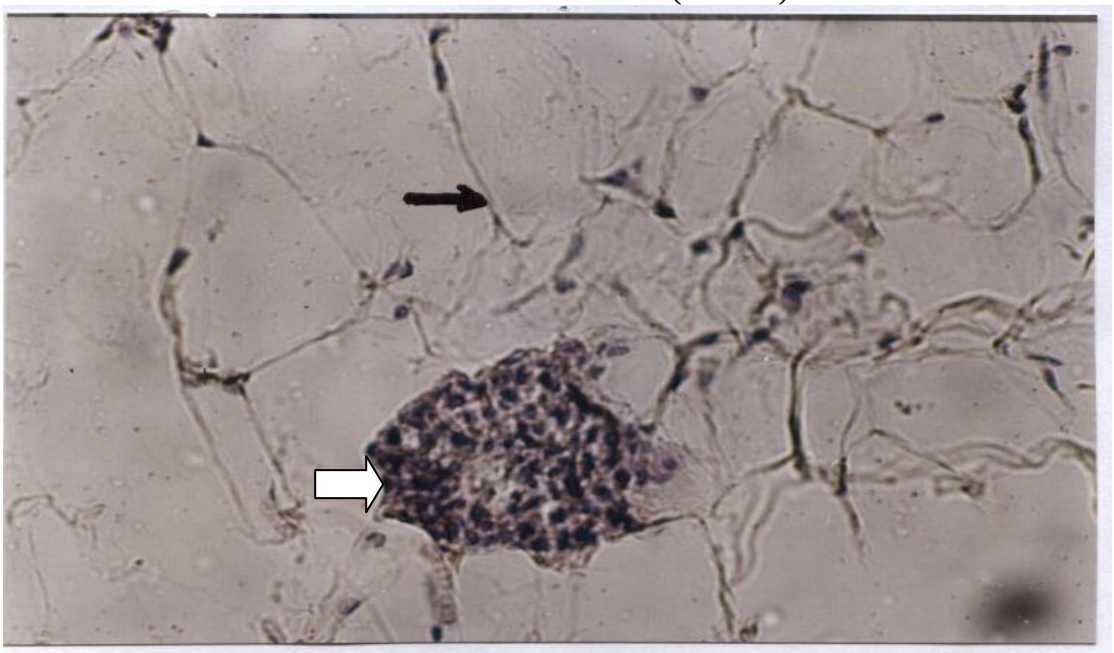

Figure (1): H&E stained section of control male mammary gland, showing very small lobule(white arrow) within huge area of adipose tissue (black arrow). (x40)

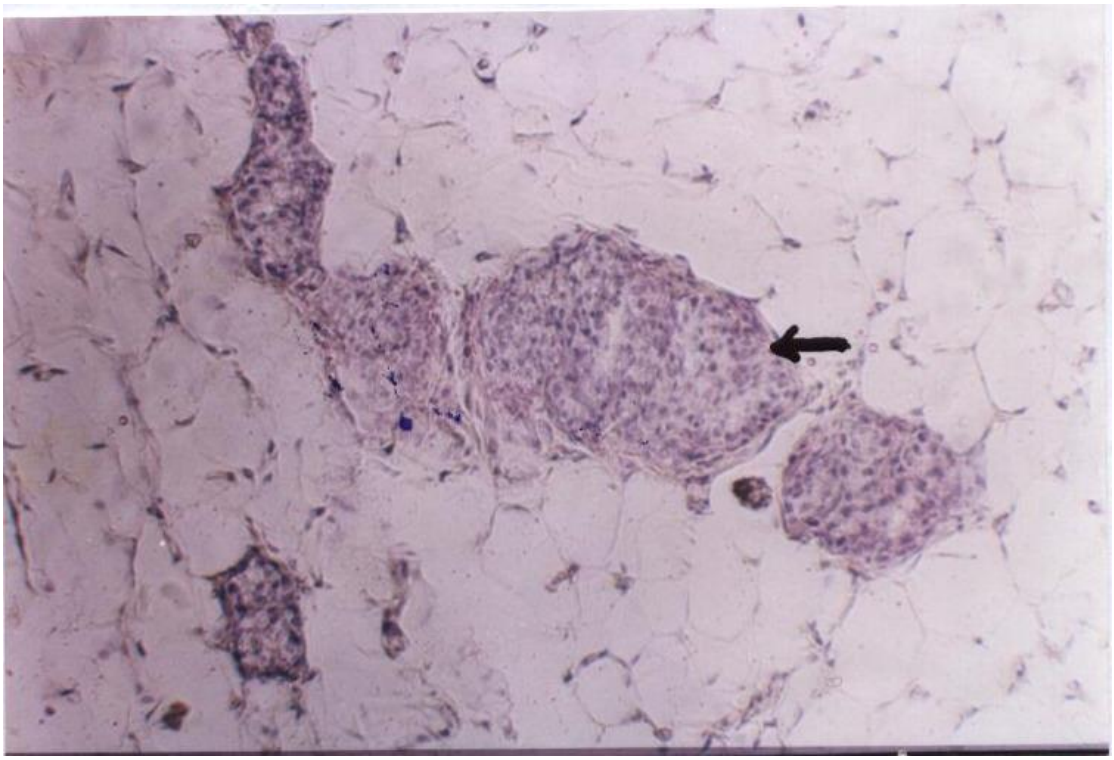

Figure (2): H&E stained section of experimental mammary, revealing dilated lobule (black arrow) on the account of adipose tissue. (x40)

Table-3: serum prolactin level at the day of sacrifice.

Groups	Serum prolactin level (ng/ml) M ± SD
Control	3.66 ± 0.11
Treated	6.3 ± 0.18*

M ± SD = mean ± standard deviation
*** = significant at P< 0.01**
(ng/ml) = nanogram/ milliliter

Discussion

All NSS treated rats, in this study, were disclosing good general health and remarkable increase in appetite, which can explain the significant increase in their body weight. This outcome coincides with NSS hypoglycaemic action [13,14,15,16,17,18] (which may be responsible for this increase in appetite and weight gain).

Histologically , the features of inactive mammary gland of controls and low serum prolactin level are similar to normal male mammary gland described in textbooks[18,19].

On the other hand, the obvious increase in the lobule size of the experimentals' mammary glands, significant rise in serum prolactin level and 274.7% hyperplasia of acinar cells prove that NSS have mammotrophic and possibly lactogenic activity in male mammary gland, (i.e.) these seeds can induce mammogenesis and possibly lactogenesis in the absence of ovary and its hormones (estrogen and progesterone).

It has been found that estrogen enhances both ductal development of the mammary gland and stimulates prolactin secret-ion from acidophils of pituitary gland[19]. Therefore, the estrogen – like action that had been elicited in this investigation is most probably due to the steroidal saponin (diosgenin) component of NSS[6].

Anyhow , the hyperac-tivity of the experimental male mammary gland excludes the ovarian pathway in biotrans-formation of diosgenin to sex hormones, but the high serum prolactin level prevails a central pathway. Such study has not been worked out previously (Medline and internet reviewed).

Future studies should be directed towards further hormonal immunoassay (for estrogen, progesterone, insulin and thyroxin), pituitary gland (acidophils) histology, estrogen and prolactin receptors on the acinar epithelium and the effect of long term treatment of NSS.

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دراسات شكليائية لتأثير بذور الحبة السوداء على الغدد اللبنية لذكر الجرذ النرويجي الناضج

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الخلاصة:

لبذور الحبة السوداء سمعة تقليدية و علمية كمدرة للحليب، لكن آلية عملها لم تدرس إلا نادرا. أشارت بعض الدراسات في هذا المجال إلى إن هذه البذور تتحول في المبايض إلى هرمونات جنسية. و لبيان صحة هذه الادعاءات صمم هذا البحث لدراسة مدى فعالية هذه البذور بغياب المبايض(اى في الذكور).

تضمن البحث دراسة ثلاثون جرذ نرويجي ابيض ذكر. قسمت هذه الحيوانات إلى قسمين: حيوانات تجريبية (عددها = 20 جرذ، أعطيت مسحوق بذور الحبة السوداء لمدة 14 يوما) و حيوانات السيطرة (عددها = 10 جرذ، أعطيت الماء المقطر لمدة 14 يوما). بعد التضحية بالحيوانات، تم دراسة الغدد اللبنية نسجيا و دراسة الدم كيميائيا.

أظهرت الحيوانات التجريبية زيادة معنوية إحصائيا في أوزانها، كما بينت زيادة في حجم فصيصات الغدد اللبنية و توسع في قنواتها و كذلك أشارت إلى زيادة في مستوى هرمون البرولاكتين في الدم مقارنة مع مجموعة السيطرة.

نستنتج من هذه الدراسة بان النمو الحاصل في الغدد اللبنية عند اخذ بذور الحبة السوداء لا يمر من خلال تحولها في المبايض و إنما بطريقة أخرى ربما تكون من خلال المراكز العليا و هذا ما يحتاج إلى دراسة أخرى في المستقبل.