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The effects of *Nigella sativa* oil administration on some physiological and histological values of reproductive aspects of rats

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Summary

The goal of this study to investigate the effects of *Nigella sativa* (Ns) oil on reproductive values, some hematological parameters serum biochemical characteristics, some sexual hormones concentration and histological changes of treated and normal male reproductive organs. The experiment (1) dealt with 20 males and 20 female rats at 21 days of age, 10 rats of each sex were giving orally Ns oil at the rate of 1ml/kg/day for 30 days and the others left as a control group. Insignificant changes were occurred in hematological parameters except the white blood cells (WBCs), were significantly increased in treated groups ($P<0.05$). The treated groups showed significant increases in total protein and significant decreases in total cholesterol liver enzymes markedly increased in treated rats Significant increases in the levels of LH, FSH and testosterone for males and LH, FSH, estrogen and progesterone for females were recorded. The experiment (2) dealt with effects of Ns oil on 20 adult males and 20 adult female rats that were given same dose of Ns oil for 30 days. There were significant increase in litter size and weight of rats born in treated groups. The experiment (3) dealt with the effects of Ns oil on castrated males fifteen adult rats were divided equally into 3 groups, two groups were castrated while, the third group was left as a control group; one castrated group was treated with same dose of Ns oil for 30 days, there were significant increases in serum testosterone concentration and weight of accessory glands in treated group. Histological changes in the accessory glands of treated groups were evident.

In conclusion, the administration of 1ml/kg/day of Ns oil stimulated the secretion of sexual hormones that led to improve protein synthesis of hepatic enzymes, white blood cells count and decrease the serum cholesterol concentration in blood

Keywords: Black seed, Reproductive Rat, Hematology, Biochemistry, Histology

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تأثير تجريع زيت الحبة السوداء في بعض القيم الفسلجية والتكاثرية والنسجية للجرذان

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الخلاصة

أجري هذا البحث بهدف دراسة تأثير زيت الحبة السوداء في فعالية التكاثر و بعض الجوانب الدمية والبايوكيميائية والهرمونات الجنسية وبعض تراكيب الأنسجة في الجرذان السوية شملت الدراسة ثلاثة تجارب. التجربة اللأولى أجريت على 20 ذكر و 20 أنثى من الجرذان بعمر 21 يوم أعطيت زيت الحبة السوداء عن طريق الفم الى 10 ذكور و 10 أناث و بجرعة 1مل/كغم/يوميا ولمدة 30 يوما أظهرت النتائج عدم وجود فروقات معنوية في المقاييس الدمية عدا أرتفاع في عدد خلايا الدم البيض في المجاميع المعالجة أرتفع تركيز البروتين الكلي في مصل الدم معنويا و أنخفض تركيزالكوليسترول الكلي فيه و أرتفعت تراكيز الأنزيمات الكبدية في المجاميع المعالجة مع زيادة معنوية في هرمون (LHوFSH) و هرمون الشحمون الخصوي في الذكور و كذلك هرمون (LH , FSH) وهرمون الأستروجين و البروجستيرون في الأناث أما التجربة الثانية فقد شملت 20 ذكر بالغ و 20 انثى بالغة أعطيت زيت الحبة الى 10 ذكور و10 أناث بنفس الجرعة و المدة المذكورة في التجربة الأولى أظهرت النتائج زيادة معنوية في عدد الجرذان المولودة و خصوبة الأناث وفي التجربة الثالثة تم دراسة تأثير زيت الحبة السوداء على الذكور المخصية و تضمنت التجربة 15 ذكرا بالغ وقسمت الى ثلاثة مجاميع متساوية أجريت عملية الخصي للمجموعة الأولى و الثانية و تركت الثالثة بدون خصي (سيطرة) أعطي الزيت لأحد المجاميع المخصية بنفس الجرعة والمدة في التجربة الأولى لوحظ وجود زيادة معنوية في تركيز هرمون الشحمون الخصوي ووزن الغدد اللأحقة مع تغيرات في المقاطع النسجية في المجموعة المعالجة.

يستنتج من من هذه الدراسة أن لزيت الحبة السوداء بجرعة 1مل /كغم/يوم لمدة 30 يوما تأثير أيجابي في زيادة أفراز الهرمونات الجنسية التي بدورها أدت الى تحسين تمثيل البروتين وكذلك الأنزيمات الكبدية وخلايا الدم البيض وخفض تركيز الكوليسترول في مصل الدم.

Introduction

Nigella sativa (Ns) is a herbaceous plant which is known throughout the world by different names such as black seed, black cumin and black caraway. The black seed oil is reported to be beneficial due to its content of a hundred components such as aromatic oils trace elements and vitamins (1). Since Nigella sativa oil contains high percentage of unsaturated fatty acids such as linoloic acid it has a positive influence reproductive function (2). Bashandy (3) showed that NS oil improved the fertility index in normal and hyper lipidemic male rats. The aim of the present study was to investigate the effect of Ns oil on some hematological biochemical values and its effect on reproductive function.

Materials and Methods

The present study was conducted on the male and female rats (*Rattus norvegicus*). The design of study based on three experiments: experiment (1) included 20 males and 20 females rats were divided randomly into two groups (10) treatment and (10) control for each sex of 21 days old as that recorded by Zaoui (4). Ns oil was given orally according to body weight for a period of 30 consecutive days at the rate of 1ml/kg/day. Treated group was given oil for 30 days, once a day by using stainless steel gavages needle.

Experiment (2) included 40 rats (20 males and 20 females) divided into four groups, each group consisted of (5) males and (5) females Group (1) was given Ns oil to males and females rats Group(2) was given Ns oil only to males rats group (3) was given Ns oil only to females rats while the group(4) control Percentage of fertility, number, sex ratio and weight of newborn at (21) days of age were recorded. Experiment (3) fifteen males were divided randomly into three equal groups.

Group 1 (Control; C) rats were given control diet group2 castrated rats were given control diet, group 3 castrated rats were given control diet and Ns oil according to body weight at the rate of 1ml/kg/day for 30 consecutive days. Hormonal assay involved testosterone, estrogen, progesterone, LH and FSH levels determinated by (ELISA) using kits of BioCheck Canada. Blood picture of RBC, Hb, PCV and WBC were determined by automated hematology analyzer (5). Biochemical analysis of total serum cholesterol, total protein, Aspartate aminotranfarase (AST), Alanine aminotranferase (ALT), and alkaline phosphatase (ALP) were determined by using kits of BioLab France.

Histological section of tissue samples were sectioned and processed according to (6) and stained according to standard methods (7).

Statistical analysis were done by Program of Statistical Analysis System (8). The differences between means at level of probability (0.05) for all of each factor were tested using Duncans Multiple Range test (9).

Results

The results revealed insignificant differences recorded in RBC's count hemoglobin concentration (Hb) and packwd cell volume (PCV) after 30 days of treatment compared to C groups(Table 1), while there was significant elevation in WBC's count of treated group. There were significant increases in the levels of AST, ALT and ALP (Table2), and total protein in the treated groups in compare with the control groups while there was decreased (P<005) of total cholesterol concentration in the treated groups. Hormonal activities of LH, FSH, progesterone, estrogen and testosterone were showed significant increase in the treated groups' (Table3 and 4). In the second experiment there were significant increases in the number of rats born in group 4. There was No effect of treatment on sex ratio, while the weight of born rats increased significantly in group 4. In the experiment 3 (Table 5) the testosterone concentration and weight of accessory glands in C group have increased in comparison with castrated groups. In the castrated treated group the mention of parameters were higher than castrated non treated group (Table5).

Histological section of the preputial glands of castrated no treated male rats showed an increased in connective tissues and hemolysis (plate1) over the control group In castrated treat group there was a decrease in connective tissue (Plate2). Seminal vesicle of castrated non treated rats (Plate3) show atrophy of epithelial cells lack of mucus and large follicles in comparison with the control

group that show mucus in the lumen with normal epithelial cells. In castrated treated group rats seminal vesicle (Plate4) shown elongated nucleus and better appearance and less atrophied The different sizes of vacuoles and degeneration of epithelial cells are seen in castrated non treated group (Plate5) while the vacuolation and degeneration is less in castrated treated groups (Plate6) comparison with the control group that show tubuloalveolar glands lined by simple epithelial cells.

Sections of prostate gland in control group is shown normal epithelial cells with mucus in lumen. The atrophy in secretary cells is evident in castrated non treated groups (Plate7) while that of the castrated treated rats (Plate8) showing the highest of secretary cells and more fluid in the lumen

Discussion

In present experiments the RBC's count, Hb and PCV were insignificant differentiated and the resulted are agreed with the finding of (10). The observed significant elevation in WBC"s count in this study may be due to active materials known as nigllone thymoquinone and thymohydroquinone in Ns oil (11). The rises of hematological parameters of male rather than female rats is attributed to androgen hormone. The decrease in total cholesterol concentration in treated groups due to the administration of Ns oil which is considered as an atheroscleroti agents due to the presence of essential fatty acid which can prevent fat induced hyperlipemia and inhibit the key enzyme in cholesterol synthesis (12). The higher levels of cholesterol concentration in females are attributated to the ability female rats to store high level of cholesterol in their adrenals for the use in pregnancy (13). There is a significant increase in total protein level in treated groups which indicate a stimulation effect of the oil on metabolic processes involved in protein synthesis, Meral (14) have reported that Ns increases thyroxin hormone that in turn increases growth hormone secretion affecting on total proteins synthesis in males are due to increased testosterone as an anabolic agent toward the promoting the protein synthesis. The marked elevated levels of AST, ALT and ALP in Ns administrated rats are due to physiological process (15). The Ns oil treatment led to significant increase in LH and FSH levels which may be due to the direct effect of oil on hypothalamus which in turn increases Gonadotropic Releasing Hormone (GnRH), furthermore fatty acids can stimulate GnRH-dependent pathways that initiate changes in gonads function (16). The positive increased effect of estrogen and progesterone concentration in treated groups is maybe attributed to the contents of the Ns oil especially thymoquinone that enter in building of cholesterol which is important source of cholesterol esters that may have a role in estrogen and progesterone synthesis (17). The increase in testosterone level in treated groups may be due to the effects of Ns oil to stimulate the activity of 17ß-hydroxysteriod dehydragenase the most important key enzyme in the testosterone synthesis pathway (18).

In the second experiment the increase in the number of rats born could be due to oral administration of Ns oil to female rats which mated with treated male rats (group 4) which in turn stimulate the secretion of testosterone hormone (19), as mentioned earlier. Moreover, significant increase in weight of born rats may be due to the effect of Ns oil that shows marked of action in the rats (20).
In the third experiment the higher significant values of testosterone and higher weights of accessory glands in control compared to castrated two groups is due to the presence of androgen there was also a significant increase in testosterone concentration and weight of accessory glands in castrated treated group compared to castrated non treated group due to the administration of Ns as mentioned earlier Histological section showed that there are effects of Ns oil on accessory sex glands in castrated rats when compared with castrated non treated rats, this result may be due to the hormonal effects such as (19).

Table 1 Effects of *Nigella sativa* oil on RBC count hemoglobin and packed cell volume in male and female rats

Factors	No	Means ± SE		
		RBC ($x 10^6$ /µl)	Hemoglobin (gm / dl)	PCV%
Treatment				
C	**16**	**646±028 a**	**1423±049 a**	**4076±196 a**
T	**16**	**675±025 a**	**1426±031 a**	**4130±138 a**
Sex:				
Female (F)	**16**	**601±020 b**	**1363±039 b**	**3736±120 b**
Male (M)	**16**	**720±024 a**	**1487±036 a**	**4469±158 a**
Interaction:				
C X F	**8**	**557±027 b**	**1300±054 b**	**3438±161 c**
T X F	**8**	**646±021 a**	**1425±051 ab**	**4035±103 b**
C X M	**8**	**735±021 a**	**1546±053 a**	**4714±149 a**
T X M	**8**	**705±045 a**	**1428±040 ab**	**4225±261 ab**

Means having different letters within each factor/column differ significantly (P<005) according to Duncan test

Table 2 Effects of *Nigella sativa* oil on Alkaline phosphatase, AST and ALT in rats

Factors	No	Means ± SE		
		Alkaline phosphatase (IU/L)	AST (IU/L)	ALT (IU/L)
Treatment				
C	**16**	**3904±0261 b**	**56913±2156 b**	**35101±1914 b**
T	**16**	**4897±0044 a**	**79031±1521 a**	**65561±2934 a**
Sex:				
Female (F)	**16**	**3811±0237 b**	**65026±4134 b**	**47580±5465 a**
Male (M)	**16**	**4990±0020 a**	**70919±2240 a**	**53083±3511 a**
Interaction:				
C X F	**8**	**2893±0009 d**	**49659±1465 c**	**29931±1857 c**
T X F	**8**	**4729±0017 c**	**80392±1908 a**	**65229±5962 a**
C X M	**8**	**4916±0013 b**	**64167±1655 b**	**40272±2148 b**
T X M	**8**	**5065±0005 c**	**77671±2397 a**	**65894±1153 a**

Means having different letters within each factor/column differ significantly (P<005) according to Duncan test

Table 3 Effects of *Ns* oil on LH and FSH in male and female rats

Factors	No	Means ± SE	
		LH (mlU/ml)	FSH (mlU/ml)
Treatment			
C	14	1599 ± 0157 b	1822 ± 0071 b
T	14	6958 ± 0429 a	7812 ± 0453 a
Sex:			
Female (F)	14	4966 ± 0866 a	5548 ± 1008 a
Male (M)	14	3591 ± 0701 b	4086 ± 0702 b
Interaction:			
C X F	7	1952 ± 0205 c	1974 ± 0043 c
T X F	7	7980 ± 0420 a	9121 ± 0377 a
C X M	7	1245 ± 0153 c	1671 ± 0111 c
T X M	7	5937 ± 0521 b	6502 ± 0420 b

Means having different letters within each factor/column differ significantly (P<005) according to Duncan test

Table 4 Effects of *N. sativa* oil on Estrogen Progesterone and Testosterone in rats

Factors	No	Means ± SE		
		Estrogen (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
Treatment				
C	8	1943 ± 0029 b	4770 ± 0215 b	0398 ± 0006 b
T	8	2475 ± 0033 a	47040 ± 4305 a	0704 ± 0017 a

Table 5 Effects of *Nigella sativa* oil on Testosterone in castrated male rats

Factors	No	Means ± SE
		Testosterone (ng/ml)
Treatment:		
Con	5	0731 ± 0008 a
Cas	5	0183 ± 0002 c
Cas and Trt	5	0229 ± 0007 b

Table 6 Effects of *N. sativa* oil on accessory glands weight in castrated male rats

Factors	No	Means ± SE
		Accessory glands (g)
Treatment:		
Con	5	1216 ± 0021 a
Cas	5	0614 ± 0039 c
Cas and Trt	5	0870 ± 0057 b

Means having different letters within each factor/column differ significantly (P<005) according to Duncan test

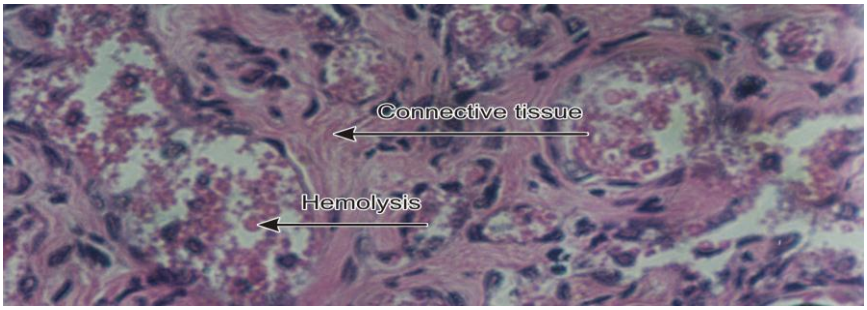

Figure – 1: Cross section from preputial gland in castrated rat showing increase in connective tissue and scattered hemorrhage (H&E stain, X40).

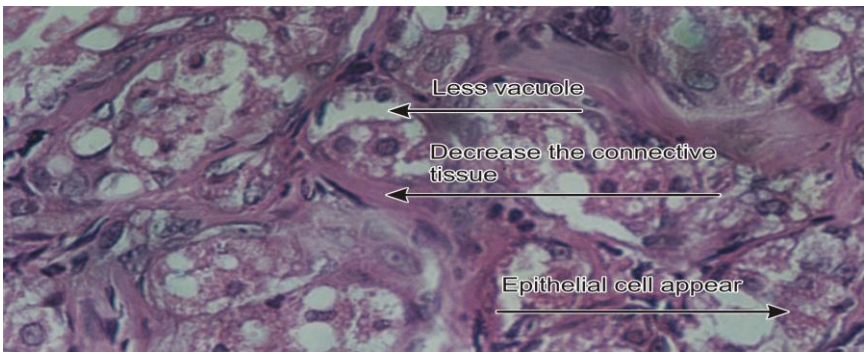

Figure – 2: Cross section from preputial gland in castrated rat showing decrease in connective tissue (H&E stain, X40).

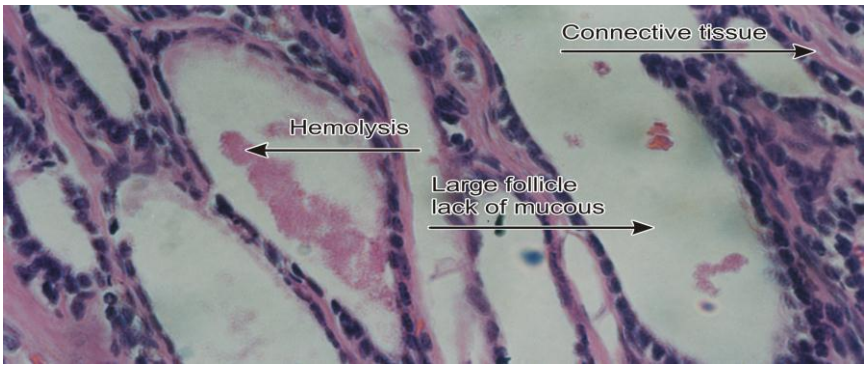

Figure – 3: Cross section from seminal vesicle of castrated rat showing atrophy of epithelial cell and lack of mucus in the lumen (H&E stain, X40).

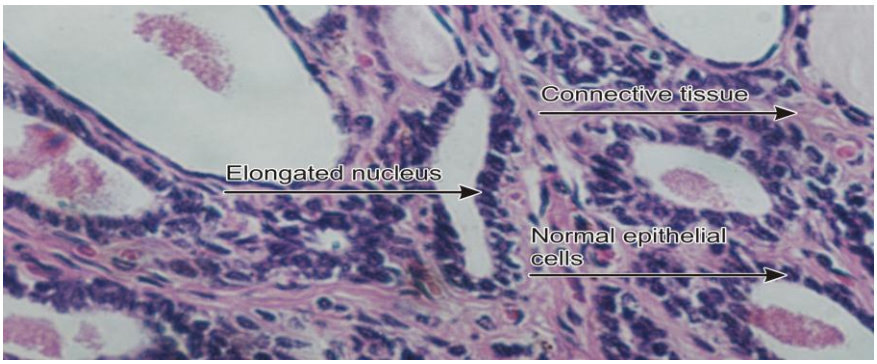

Figure – 4: Cross section from seminal vesicle of castrated treated rat showing elongated nucleus with mucus in the lumen (H&E stain, X40).

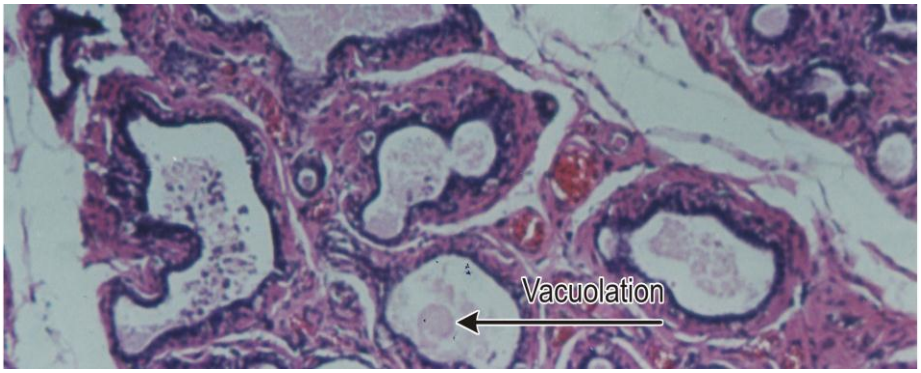

Figure – 5: Cross section from prostate of castrated treated rat showing vacuolation and atrophy in the secretory cells (H&E stain, X10).

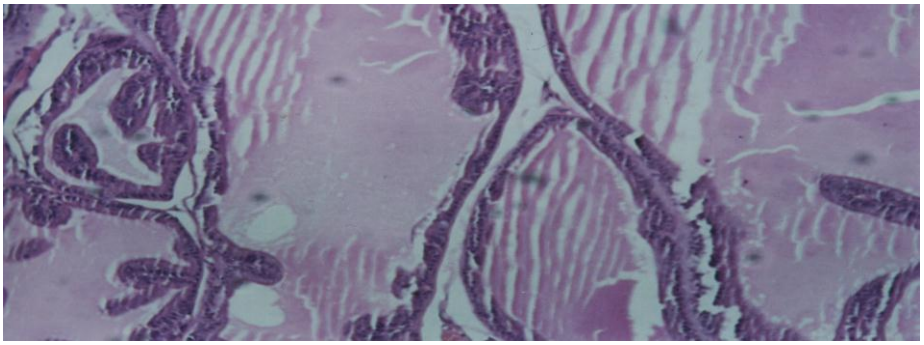

Figure – 6: Cross section from prostate of castrated treated rat showing the highest of secretory cells and more fluid in the lumen (H&E stain, X10).

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