

Effect of alcoholic extract of *Nigella sativa* on fertility in male rats

J. A. A. Al-Sa'aidi*, A. L. D. Al-Khuzai and N. F. H. Al-Zobaydi

Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Al-Qadisiya, Al-Qadisiya, Iraq
* Email: jbr20042002@yahoo.com

Abstract

An experiment was conducted to determine the effect of alcoholic extract of black seed *Nigella sativa* L. on fertility parameters in white male rats (*Rattus norvegicus*). A total of 60 mature males were divided into three equal groups. The first one (Control) intake drinking water, while the other two groups (T1 and T2) intake the extract in two doses (0.5 and 1.5 g/Kg, respectively) daily for 53 days. The results revealed that treatment with alcoholic extract of *Nigella sativa* led to significant decrease ($P < 0.01$) of excitation time in its three stages (1st mount, 1st ejaculation and 2nd trail), significant increase ($P < 0.01$) in body weight gain (g), reproductive parameters (seminiferous tubules thickness and diameters, account of spermatogonia, primary and secondary spermatocytes, spermatids, free spermatozoa, account of sertoli and Leydig cells, diameter of Leydig cells and the height of epithelial cells entirely covered epididymal caudal), hormones (testosterone and follicle stimulating hormone) as well as protein concentration, and significant decrease ($P < 0.01$) in leutinizing hormone and cholesterol concentration. It could be concluded that daily oral administration of 0.5 and 1.5 g/Kg B.W of alcoholic extract of *Nigella sativa* L. for 53 days lead to clear improvement of male rats fertility.

Keywords: *Nigella sativa* L., Male rat, Fertility.

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تأثير المستخلص الكحولي لبذور الحبة السوداء *Nigella sativa* في خصوبة ذكور الجرذان

* جبار عباس احمد الساعدي، علاوي لعبيبي داغر الخزاعي و نيران فليح حسن الزبيدي

فرع الفلسفة والأدوية، كلية الطب البيطري، جامعة القادسية، القادسية، العراق

الخلاصة

أجريت التجربة الحالية لتحديد تأثير المستخلص الكحولي لبذور الحبة السوداء *Nigella sativa* L. في خصوبة ذكور الجرذان البيض *Rattus norvegicus*. تم تقسيم 60 ذكراً بالغاً إلى ثلاث مجموعات متساوية. المجموعة الأولى (السيطرة) تناولت ماء الشرب، بينما تناولت مجموعتنا المعاملة (T1 و T2) المستخلص الكحولي لبذور الحبة السوداء بجرعتين (0.5 و 1.5 غم/كغم من وزن الجسم)، على التوالي يومياً ولمدة 53 يوماً. أظهرت النتائج أن استخدام المستخلص الكحولي لبذور الحبة السوداء أدى إلى انخفاض معنوي ($P < 0.01$) في وقت التهيج بمراحله الثلاث (الوقت المستغرق للمحاولة الأولى و القذف الأول والمحاولة الثانية)، ارتفاع معنوي ($P < 0.01$) في معدل الكسب الوزني (غم) و معايير التناسل (سمك النبيبات المنوية وأقطارها و أعداد سليفات النطف و الخلايا النطفية الأولية والثانوية و طلائع النطف و النطف الحرة و أعداد خلايا سرتولي وخلايا لايدك و أقطار خلايا لايدك و ارتفاع الخلايا الظهارية المبطنة لذيل البربخ)، إضافة إلى ارتفاع تركيز هرمون الشحمون الخصوي و الهرمون محفز الجريب و تركيز البروتين الكلي، مع حصول انخفاض معنوي ($P < 0.01$) في تركيز الهرمون اللوتيني والكولسترول. يستنتج بأن التجريب اليومي للمستخلص الكحولي من بذور الحبة السوداء *Nigella sativa* L. بالجرعتين (0.5 و 1.5 غم/كغم من وزن الجسم) لمدة 53 يوماً يؤدي إلى تحسن واضح في معايير الجهاز التناسلي لذكور الجرذان البيض والذي ينعكس إيجاباً في خصوبتها.

Introduction

Large number of medicinal plants and their constituents have been shown beneficial therapeutic potentials. Seeds of *Nigella sativa* have been employed for thousands of years as a spice and food preservative. The oil and the seed constituents, in particular thymoquinone, have shown potential medicinal properties in traditional medicine (1). The black seed used as a laxative, diuretic, treating infectious fever and local treatment for skin infections and wounds (2). Also hair full treatment, headache, ears pain, parturition diseases, toothache, digestive system disturbances, glands diseases, fraction healing, liver, spleen, and eye diseases (3).

Nigella sativa has hypoglycemic effect (4), and used as immunopotentiating, immunomodulating and interferon like activities (5,6), as well as its action in amelioration of reproductive performance of rams (7) and female mice (8) and it's role in correcting the CNS functions by the abilities of Thymoquinone TQ to minimize the tension of epilepsy in rats when black seed used as an anticonvulsant, muscle relaxant, analgesic and CNS depressant activity (9-11).

Most of the pharmacological activities are attributed to the presence of thymoquinone as an active component (12). Thymoquinone possess anti – oxidant effects through enhancing the oxidant scavenger system as well as its potent anti – inflammatory mediators prostaglandins and leukotriens (1).

The aim of the present study was to determine the role of *N. sativa* seed extract in improving the fertility of male rats.

Materials and methods

In the present study which carried out from (1st of December 2006 to the 1st of June 2007), 60 males and 30 females rats in the age of sexual puberty (50 – 60) days, reared in the animal house of the collage of veterinary medicine, university of Al - Qadisiya under controlled condition of temperature, light, ventilation as well as ad libitum intake of water and feed throughout the experimental period. The females were used in the measurement of excitation times, while males were assigned to three equal groups, the first (control) was drenched drinking water, while the second and third groups were drenched drinking water supplemented by (0.5 and 1.5 g\ kg B.W.) of alcoholic extract of Black seed (*N. s.*), respectively.

Measurements

Rats were group weighted initially at the 1st day of experiment, and then weighted at the end of the experiment to determine the body weight gain. The study of excitation time (1st mount, 1st ejaculation and 2nd trial), testis weight

and size, epididymal caudal sperm functions (13), (sperm concentration, sperm motility percentage, sperm grade activity, forward progressive movement, abnormal sperm morphology and sperm viability) in addition to biochemical tests of blood which included: Leutinizing Hormone (LH), Follicle Stimulation Hormone (FSH), Testosterone (T), Cholesterol and Total Protein (14) as well as the histological study for testes and epididymis caudal.

Statistical analysis

All data of the experiment were analyzed statistically using the Completely Randomized Design (CRD) (Single – Factor), (Al – Rawi and Khalaf Ala, 2000), and Duncan multiple range test was used to determine the differences among means of treatment (15).

Results

Excitation time

The results of this study revealed that the treatment with alcoholic extract of *Nigella sativa* led to a significant decrease ($P < 0.01$) of excitation time in its three stages, especially in T2 group compared with T1 and control groups (table –1).

Table (1): Effect of alcoholic extract of black seed *Nigella sativa* on the excitation time (sec.) in male rats.

Excitation time	Treatment		
	C	T1	T2
1 st mount	15.1 ± 0.959 a	10.4 ± 1.056 b	5.9 ± 0.795 c
1 st ejaculation	61.6 ± 1.648 a	46.6 ± 1.536 b	18.7 ± 0.633 c
2 nd trial	61.2 ± 1.083 a	22.0 ± 1.577 b	8.6 ± 1.368 c

Body weight gain

The results showed a significant increase ($P < 0.01$) in body weight gain (g) in T2 compared with T1 and Control groups. It also showed a significant increase ($P < 0.01$) in testis weight, and size of T2 and T1 compared with control group (table–2).

Sperms parameters

The results revealed a significant increase ($P < 0.01$) in sperm concentration, sperm motility percentage, sperm grade activity, forward progressive movement and sperm viability as well as a significant decrease in abnormal sperm percentage in treated groups compared with control group (table –3).

Table (2): Effect of alcoholic extract of black seed *Nigella sativa* on the body weight gain, testis weight and size.

Measurement	Treatment		
	C	T1	T2
Body weight gain (g)	18.08 ± 2.791 a	31.73 ± 3.387 b	103.83 ± 11.740 c
Testis weight (g)	0.559 ± 0.039 a	0.766 ± 0.028 b	1.257 ± 0.102 c
Testis size (cm ³)	1.599 ± 0.096 a	3.593 ± 0.120 b	5.202 ± 0.360 c

Table (3): Effect of alcoholic extract of black seed *Nigella sativa* on the reproductive parameters.

Parameter	Treatment		
	C	T1	T2
Total sperm concentration (million/ml of seminal fluid)	87.7 ± 1.459 a	105.5 ± 2.829 b	141.6 ± 3.809 c
Motile sperm concentration (%)	59.85 ± 1.176 a	83.15 ± 1.183 b	90.15 ± 0.434 c
Percentage of progressive movement (%)	20.66 ± 0.487 a	43.11 ± 0.893 b	53.48 ± 0.722 c
Effective movement degree (degree)	2.40 ± 0.106 a	3.15 ± 0.080 b	3.570 ± 0.055 c
Percentage of abnormal sperm (%)	28.54 ± 0.621 a	21.33 ± 1.234 b	16.36 ± 1.115 c
Sperm viability (percentage of alive sperm/dead sperm) (%)	70.2 ± 1.756 a	89.5 ± 1.166 b	94.7 ± 0.650 c

Biochemical parameters of serum

The results showed a significant increase ($P < 0.01$) of testosterone and leutinizing hormone and a significant decrease ($P < 0.01$) of follicle stimulating hormone in treatment groups compared with control. As well as a significant decrease ($P < 0.01$) of cholesterol concentration and a significant increase ($P < 0.01$) of total protein concentration in blood serum of treated animals compared with control (table -4).

Histological Study

The histological study of the testicular (Fig 1-3) – epididymal caudal (Fig 4-6) of experimental animals, otherwise treated animals showed a significant increase ($P < 0.01$) in diameter and wall thickness of the seminiferous tubules, account of spermatogonia, primary and secondary spermatocytes, spermatids and free spermatozoa in the seminiferous tubule lumen. In addition, there was a significant increase ($P < 0.01$) in number of sertoli cells, Leydig cells and nuclear diameter of Leydig cells. As well as a significant increase ($P < 0.01$) in the height of the

epithelial cells which entirely covered the epididymal caudal in treated groups compared with control (table-5).

Table (4): Effect of alcoholic extract of black seed *Nigella sativa* on the biochemical parameters in serum.

Parameter	Treatment		
	C	T1	T2
Testosterone ng/ml	0.451 ± 0.016 a	2.122 ± 0.055 b	2.481 ± 0.031 c
FSH mIU/ml	1.361 ± 0.020 a	0.589 ± 0.013 b	0.541 ± 0.017 c
LH mIU/ml	1.438 ± 0.031 a	1.868 ± 0.017 b	1.988 ± 0.016 b
Cholesterol mg/dl	75.28 ± 3.253 a	55.1 ± 2.279 b	48.77 ± 1.143 b
Total Protein g/dl	6.59 ± 0.199 a	7.44 ± 0.217 b	8.66 ± 0.125 c

Table (5): Effect of alcoholic extract of black seed *Nigella sativa* on the testicular – epididymal caudal histological parameters.

Parameter	Treatment		
	C	T1	T2
Seminiferous tubule diameter (µm)	213.986 ± 2.743 a	246.321 ± 3.855 b	275.086 ± 3.080 c
Seminiferous tubule thickness (µm)	55.841 ± 0.467 a	65.399 ± 1.021 b	74.828 ± 0.981 c
Account of spermatogonia per tubular section (cell)	54.9 ± 1.422 a	80.34 ± 1.638 b	99.82 ± 3.287 c
Account of primary spermatocytes per tubular section (cell)	31 ± 0.636 a	36.46 ± 0.688 b	43.12 ± 0.688 c
Account of secondary spermatocytes per tubular section (cell)	25.46 ± 0.683 a	30.82 ± 0.495 b	36.64 ± 0.532 c
Account of spermatids per tubular section (cell)	126.04 ± 1.045 a	147.92 ± 1.334 b	171.62 ± 1.272 c
Account of free spermatozoa per tubular section (cell)	23.02 ± 1.159 a	48.32 ± 1.050 b	62.44 ± 1.599 c
Account of sertoli cells per tubular section (cell)	18.3 ± 0.611 a	25.48 ± 0.520 b	28.48 ± 0.518 b
Account of Leydig cells per field (cell)	18.46 ± 0.600 a	21.56 ± 0.689 b	21.08 ± 0.629 b
Diameter of Leydig cells nuclei (µm)	5.444 ± 0.039 a	5.860 ± 0.009 b	5.928 ± 0.010 b
Height of epithelial cells entirely covered epididymal caudal (µm)	20.698 ± 0.174 a	27.128 ± 0.297 b	29.599 ± 0.395 b



Figure (1): section of seminiferous tubules from Control rat testis, showed the normal spermatogenic stages (H & E X 500).

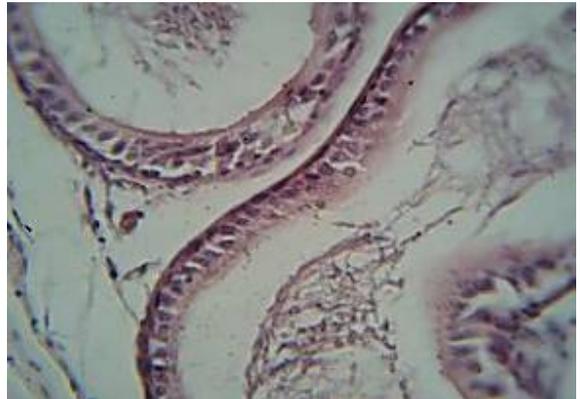


Figure (4): section of epididymal caudal from control group rat, showed the concentration of mature sperms account (H & E X 500).

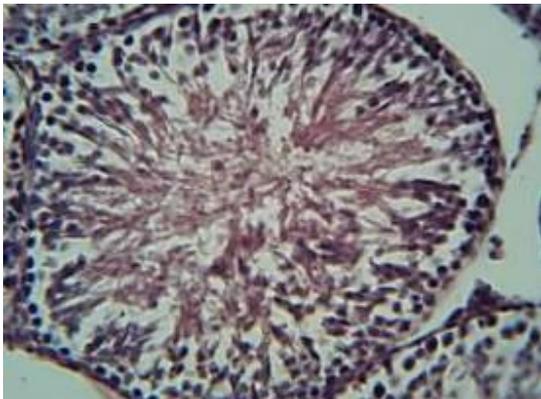


Figure (2): section of seminiferous tubules from T1 rat testis, showed the improvement in spermatogenic stages (H & E X 500).



Figure (5): section of epididymal caudal from T1 group rat, showed the higher concentration of mature sperms account (H & E X 500).



Figure (3): section of seminiferous tubules from T2 rat testis, showed the high improvement in spermatogenic stages (H & E X 500).

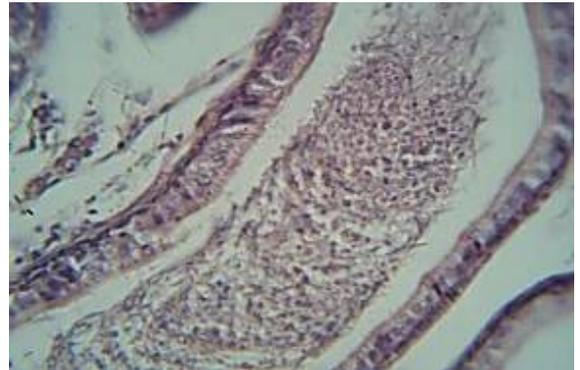


Figure (6): section of epididymal caudal from T2 group rat, showed the highest concentration of mature sperms account (H & E X 500).

Discussion

The results of current study showed that oral administration of 0.5 and 1.5 g/kg B.W of alcoholic extract of *Nigella sativa* for 53 days lead to clear improvement of male rat fertility. This was observed through increased testosterone which play an important role in increasing sexual desire (Libido) (16), also associated with an increase in body weight gain as what (17) found when added black seed to broiler's diet, and that may be due to presents a lot of primary nutritional factors in the black seed especially the amino acids like glutamic acid (18), in addition to another essential amino acids like methionin and lysine (19).

Also this study showed an increase in weight and size of the testis and epididymal caudal sperm parameters, which agree with (20) who used alcoholic extract of black seed with cadmium chloride, and his results showed an increase in testis and epididymis weight when compared with the group used cadmium chloride only. This increase may be due to the black seeds constituents of proteins, vitamins like A, B and C, in addition to presence of important minerals like zinc, copper and magnesium (21-23). The increase in testis weight of treatment group due to the increment of testosterone, or to the increase in the activity of hypothalamus – pituitary – testis axis.

The cause of testosterone hormone increment may be due to the effect of black seed on the main enzymes which affect the metabolism and steroid secretion in the testis.

The increase in sperm concentration was due in part to the increase in testosterone and FSH levels in testicular tissue, since these two hormones were responsible for spermatocytogenesis and spermiogenesis in seminiferous tubules, while testosterone is responsible for epididymal function in maturation of sperms (24).

The results of this study was in agree with (25) who reported that black seed contain alkaloids and phenols which stimulate the secretion of FSH and Testosterone.

This study registered a significant decrease in cholesterol level which may in part due to the depletion of cholesterol in steroidogenesis which showed an increase as the result revealed. This result was in agree with (26-29).

Al – Gaby (18) mentioned that the use of black seed led to increase in serum protein concentration exactly like the results of this study.

The obvious improvement in the histological study of spermatogenesis in this study agreed with Al – Helali (30) and Al – Mayali (20) results who mentioned that the use of black seed causes a clear improvement of spermatogenesis in the animals treated with aquaus and alcoholic extracts of black seeds.

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