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Effect of black seed *Nigella sativa* (L.) seed extract on reproductive organs of male albino rats

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Abstract :

This study aimed to evaluate the probable effect of *Nigella sativa* L. seed extract (NSSE) on reproductive organs of male albino rats .The extract was administrated orally for 30 days at (0.1, 0.2, 0.3, 0.4 ml/100 gm of body weight (b.wt.) .day^{-1}) to adult male rats in addition to control group .Body and reproductive organs weight (testes, epididymis, seminal vesicle and prostate) for control and treated rats were measured. Histological study were performed in testes and epididymis to calculate the thickness of germinal layer, diameter of seminiferouse tubules and diameter of lumen of seminiferouse tubules in testes and thickness of epithelial layer, diameter of seminiferouse tubules and diameter of lumen of seminiferouse tubules in epididymis .Also the testes used to calculate the number of spermatogenic cell spermatogonia, primary and secondary spermatocyte ,spermatids , sperms and sterol cells after treatment. Results of experiment indicate that the extract decreased significantly ($p>0.05$) the body weight in all groups in contrast with control group ,while the weight of testes, seminal vesicle and prostate were not changed , the weight of epididymis increased significantly ($p<0.05$) in concentration of 0.4 ml /100 gm b.wt. .In testes, the diameter of seminiferous tubules and the diameter of its lumen showed no significant changes when compared with control group, while the thickness of germinal layer of seminiferous tubules increased significantly ($p<0.05$) in concentration of 0.4 ml /100 gm b.wt..In epididymis the diameter of seminiferouse tubules and the diameter of lumen of seminiferouse tubules not changed, but there was significant changes between other groups ,while the thickness of epithelial layer which lying the tubules decreased significantly ($p>0.05$).The volume density of spermatogonia in concentration of 0.4 ml /100 gm b.wt. and secondary spermatocyte in concentration of 0.1 ,0.3 ,0.4 ml / 100 gm b.wt.increased significantly ($p<0.05$).

الخلاصة :

هدفت الدراسة الحالية إلى تقييم تأثير مستخلص بذور نبات الحبة السوداء في الأعضاء التناسلية لذكور الجرذان البيض البالغة. جرع المستخلص فمويا"لذكور الجرذان البيض البالغة لمدة ٣٠ يوما" بالتراكيز (0.1 ،0.2،0.3 ،0.4 مل/100 غم من وزن الجسم.يوم$^{-1}$) بالإضافة إلى مجموعة السيطرة.تم حساب وزن الجسم والأعضاء التناسلية(الخصى والبربخ والحويصلة المنوية والبروستات) للحيوانات المعاملة والسيطرة. أجريت الدراسة النسيجية للخصى والبربخ لحساب سمك الطبقة الجرثومية وقطر الأنابيب ناقلة المني وقطر تجاويفها في الخصية وكذلك قياس سمك الطبقة الظهارية وقطر الأنابيب ناقلة المني وقطر تجاويفها في البربخ .كما تم حساب أعداد خلايا الانطاف (سليفة الخلايا النطفية والخلايا النطفية الأولية والثانوية والأرومة النطفية وخلايا النطف) وكذلك حساب أعداد خلايا سرتولي في الخصية . دلت نتائج الدراسة إن مستخلص بذور نبات الحبة السوداء أدى إلى خفض وزن الجسم بشكل معنوي ($p<0.05$) في جميع التراكيز مقارنة مع مجموعة السيطرة ،بينما لم تتأثر أوزان الخصى والحوصلة المنوية والبروستات،وازداد وزن البربخ معنويا" ($p<0.05$) في التركيز 0.4 مل /100 غم من وزن الجسم . في الخصى لم يتأثر قطر الأنابيب ناقلة المني وقطر تجاويفها ، بينما ازداد معنويا" ($p<0.05$) سمك الطبقة الجرثومية للأنابيب ناقلة المني في التركيز 0.4 مل /100 غم من وزن الجسم .في البربخ لم تتأثر معنويا" $p<0.05$) أقطار الأنابيب ناقلة المني وأقطار تجاويفها مقارنة مع معاملة السيطرة في حين انخفض معنويا" ($p<0.05$) سمك الطبقة الطلائية المبطنة لتلك الأنابيب في التراكيز 0.1 و0.3 و0.4 مل /100 غم من وزن الجسم ، وازدادت معنويا" ($p<0.05$) أعداد سليفة الخلايا النطفية في التركيز 0.4 مل /100 غم من وزن الجسم والخلايا النطفية الثانوية في التراكيز 0.1 و 0.3 و0.4 مل /100 غم من وزن الجسم.

Introduction

The use of herbal medicine increases every day and still finds a wide use worldwide, traditional herbs have more acceptance than prescription drugs .in many cultures ,because it was mostly attributed to being safer than drugs, also patients believe that by using this type of medication there is no need for a physician (Heber, 2003).Black cumin,one of these alternatives plant belonging to the family Ranunculaceae, grows as a small herb and is cultivated throughout Indian and Western Asia and the tropical region of the world for its seeds (Satyavata and Gupta,1987).The seeds contain 40% fixed oil,asaponin (melantine) and up to 1.4 % volatile oil .Black cumin contains a yellow brown volatile oil with unpleasant odors. Its possesses carvone (45-60%),d-limonene and cymene. A carbonyl compound, Nigellone ($C_{18}H_{22}O_4$) has been isolated from the seeds, it is supposed to be effective in cough and bronchial asthma. It contains amyristic acid (0.26%), palmitic acid(6.31%), stearic acid (2.45%), oleic acid (44.45%), and linoleic acid (35.99%). Bitter principle (nigellin), tannins, resins, proteins, redusing sugar (glucose), Arabic acids and other alcohol-soluble organic acids have also been isolated from it (Chakravarty,1976). The seed contains alkaloids nigellicin, nigellidin, quananzoline, steroid α-spinasterol, campsterolcholesterol, stigmasterol and flavonoids of trailing quercetin-3-glucoside (Merfort, 1997).The seeds of *N. sativa* are considered as antidiabetic, hepatoprotective, antihyperlipidemic, anti-inflammatory carminative, stimulant, diuretic, emmenagogue, galactogogue, and are used in the treatment of mild cases of ouerperal fever (Mashhadian and Rakhshadeh, 2005).

Materials and Methods

Plant material

oil extract of *N.sativa* L. seeds was bought from local market .

Animals

Male albino rats aged between (8-10)weeks were obtained from the Animal House , Collage of Science, University of Babylon. The rats were housed in wire mess cages under standard condition with 12 hr light and 12 hr dark cycle during the whole period of experiment . Food and tap water provided *ad libitum*. The animals were divided into five experimental groups of 4 rats per group. The daily dose of (NSSE) administrated orally to each treated animals every morning for 30 days.

Group 1: rats treated with distilled water.

Group 2: rats treated with 0.1 ml.100 gm (b.wt.) /day.

Group 3: rats treated with 0.2 ml.100 gm (b.wt.) /day.

Group 4: rats treated with 0.3 ml.100 gm (b.wt.) /day.

Group 5: rats treated with 0.4 ml.100 gm (b.wt.) /day.

Body weight

Initial and final body weight of the animals were recorded.

Reproductive organ weights

24h after the last dosing of the respective treatment , all animals were sacrificed, the testes, epididymis, seminal vesicle and prostate were removed, cleaned from adherent tissues, drying by filter paper and weighted.

Histological study

The testes and epididymis were subjected to fixation in 10% formaldehyde solution, dehydration in ethanol, embedded in paraffin wax, sectioned on 5µ and stained with haematoxylin and eosin (Lutffy and AL-Hajj, 1984). Then the slides examined under light microscope (10x) to measured vertical and horizontal diameter of seminiferous tubules, thickness of germinal layer, height of epithelial layer and diameter of lumen in testes and epididymis and then calculate the number of spermatogonia, primary and secondary spermatocytes, spermatids, sperms and sertoli cells in testes (Weibal, 1979).

Statistical analysis

Statistical analysis of the obtained data was performed according to Snedcor and Cochran (1980).

Results :

Body and organ weights

The oral administration of (NSSE) at all dose levels decreased body weight significantly($p<0.05$) in comparison with control group, the weight of testes, seminal vesicle and prostate were not changed, while the weight of epididymis increased significantly ($p<0.05$) Table-1.

Histological study

In testes, diameter of seminiferous tubules showed no significant changes , the lumen of seminiferous tubules decreased significantly($p<0.05$) ,while the thick of germinal layer increased significantly($p<0.05$) .In epididymis ,the diameter of seminiferouse tubules and the diameter of lumen increased significantly($p<0.05$) ,while the thickness of epithelial layer which lying the this tubules decreased significantly($p<0.05$) Table-2.

The volume density of spermatogonia, secondary spermatocyte and sperm increased significantly ($p<0.05$) Table-3.

Discussion

A number of natural ,synthetic ,and environmental chemicals including steroids and related products were reported to possess androgenic and/or anti-androgenic activities (Ahmed *et. al*.,2005).In present study the reduction in body weight may be due to the bitter tastes of the extract which may be significantly($p<0.05$) reduce the intake of food in compared with control group, to that green tea suppressing the appetite, decreasing fat absorption, acting on neurotransmitters that modulate feelings of hunger and satiety, or increasing thermogenesis (production of body heat, which burns calories(Chantre and Lairon,2002). similar results have been observed with *Cissus sicyoides* extract (Pepato *et al* ., 2003) which reduced significantly body weight of rats, also (Viana *et al*.,2004) obtained that the aqueous extract of *Cissus sicyoides* decreased significantly body weight of rats when treated with 100,200 mg /kg of plant extract , while the weight of testes ,seminal vesicle and prostate were not changed in present experiment, Gupta *et al*.(2004) found that the methanolic extract of *Albizzia lebbek* L. pods decreased the weight of testes ,seminal vesicle ,epididymis and ventral prostate. The significant increase in epididymis weight may be due to the androgenic activities, which may reside in its steroid components(McNeil *et.al*.,2003).It was possible that testosterone levels alone caused the increase in epididymis weight, therefore androgenic activities of the *N.sativa* seed extract was induced the pituitary gonadotrophins secretion and release. Gonadal steroid was the most potent positive feedback for pituitary gonadotrophins secretion and release(Schwarts and McCormack,1972). The observed increase in mean thickness of germinal layer in testes and the number of spermatogonia and secondary spermatocytes may be due to the androgenic effect of seed extract ,or the extract may be effect indirectly on hormones of hypothalamus gland, which induced the growth of germinal and epithelial layer of seminiferouse tubules in testes and epididymis and then increase the number of sprmatogenic cell (Dixit and Gupta;1982).The most important hormones is testosterone ,which was necessary for the development ,growth and normal function of testes and male accessory reproductive glands(Prins *et.al*.,1991) High serum testosterone levels have been reported to positively affect the structure, weight and function of the testes, epididymis and prostate gland (Setty *et.al*.,1977).Amajor reproductive role for testosterone in development of the sperm cell and maintenance of normal testosterone levels was essential for this development(Jehan *et al*.,1973) or induced the growth of reproductive organs and

accessory reproductive glands in vertebrates ,which contribute in evolution of sperm physiology (Sorenesen,1979),therefore the role of testosterone in spermatogenesis was induced the evolution of spermatogonia from germinal layer of seminiferouse tubules, and also necessary in generation and differentiation of spermatogenic cell during the coordination between T and FSH (Guyton and Hall,2001).In this respect Ahmed *et . al*. (2005) found that the *Zingiber officinale* extract increase the mean diameter of seminiferouse tubules. Hetta *et. al*.(2005) found that the *Hyphaene thebaica* fruit extract increased significantly spermatogenesis after 15 and 30 days of treatment, and Al-Maamori (2006) found that alcoholic extract of *Alpina galangal* increased body and testes weight, diameter of seminiferous tubules, percentage of spermatogonia, spermatocyte, spermatids and sertoli cell.

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Table (1): Effect of different concentration of black seed *Nigella sativa* L. seed extract on mean of weight of body and genital organs of white male albino rats after treatment for 30 days.

Extract concentration ml/100 gm of b.wt.	Body weight(gm)			Mean of increasing %	Organ weights (gm/100 gm b.wt.)	
	Before treatment	After treatment		Testes	epididymis	Seminal vesicle	prostate
	□ X ± SE	□ X ± SE		X ± SE	X ± SE	X ± SE	X ± SE
0	91.50	190.13	a 104.177±16.742	a 0.559±2.333	a 0.1957±0.057	a 0.265±0.151	a 0.2015±0.122
0.1	76.05	131.751	b 73.236±1.171	a 0.569±4.976	a 0.2012±0.123	a 0.289±0.111	a 0.963±0.4603
0.2	82	116.631	c 52.153±4.96	a 0.578±3.262	ab 0.2168±0.12	a 0.311±0.122	a 0.1799±0.077
0.3	99.850	148.198	c 48.289±2.946	a 0.596±2.569	ab 0.2280±0.13	a 0.370±0.133	a 0.1819±0.064
0.4	14.4	151.867	c 40.491±3.541	a 0.6479±1.448	b 0.2572±0.132	a 0.397±0.154	a 0.1821±0.033
Significant level			*	N.S.	*	N.S.	N.S.

X±SE: Mean± Standard error.
*: Significant effect under ($p<0.05$).
N.S.: Not significant effect.
Different symbols mean significant effect.

Table (2): Effect of different concentrations of black seed *Nigella sativa* L. seed extract on diameter of seminiferous tubules, thick of germinal layer, thick of epithelial layer and diameter of lumen in testes and epididymis of white male albino rats after treatment for 30 days.

Extract concentration ml/100 gm of b.wt.	**diameter of lumen in epididymis (µm)**	**thick of epithelial layer in epididymis (µm)**	**diameter of seminiferous tubules in epididymis (µm)**	**diameter of lumen in testes (µm)**	**thick of germinal layer in testes (µm)**	**diameter of seminiferous tubules in testes (µm)**
	X ± SE	**X ± SE**	**X ± SE**	**X ± SE**	**X ± SE**	**X ± SE**
0	a 11.86±0.266	a 3.43±0.441	a 11.3 ±1.322	a 11.35±0 .202	a 8.1±0. 288	a 282.416± 2.027
0.1	ab 10.60±0.322	b 2.06±3.055	ab 15.5 ±0.122	ab 12.83±0 .202	a 8.43±0 .819	a 283.166± 0.881
0.2	abc 11.63±0.272	ab 3.09±0.305	a 17.3 ±0.471	ab 12.65±0 .464	ab 8.03±0 .145	a 283.433± 0.333
0.3	ac 13.8±1.140	b 2.36±0.384	a 17.56 ±0.328	a 11.43±1 .918	ab 8.30±0 .288	a 284.233± 0.333
0.4	ad 14.58±1.528	b 2.43±0.819	b 19.08 ±0.656	ac 8.90±1. 234	b 8.00±0 .638	a 286.501± 0.441
Significant level	*	*	*	*	*	N.S.

X±SE: Mean± Standard error.

*: Significant effect under ($p<0.05$).

N.S.: Not significant effect.

Different symbols mean significant effect.

Table (3): Effect of different concentrations of black seed *Nigella sativa* L. seed extract on volume density (%) of spermatogenic cell in seminiferous tubules in testes of white male albino rats after treatment for 30 days.

Volume density % / Extract concentration ml /100 gm of b.wt.	Spermatogonia	Primary spermatocyte	Secondary spermatocyte	Spermatids	Sperms	Sertoli cell
	□X ± SE	□X ± SE	□ X ± SE	□ X ± SE	□ X ± SE	X ± SE
0	a 6.798±0.898	a 5.263±0.374	a 5.824±0.408	a 17.543±1.772	a 12.499±1.392	a 18.419±0.293
0.1	ab 7.455±0.292	a 5.446±0.146	b 7.017±0.253	a 17.712±1.394	ab 15.35±1.79	a 16.227±0.507
0.2	ab 7.456±0.292	a 5.701±0.253	ab 6.359±0.637	a 17.763±0.438	a 11.841±0.773	a 18.641±1.708
0.3	a 6.359±0.146	a 5.043±1.141	c 10.526±0.254	a 13.596±0.637	ab 16.228±2.025	a 19.736±0.292
0.4	b 8.114±0.153	a 5.043±0.146	bd 7.763±0.584	a 16.008±1.635	ac 9.649±0.386	a 19.517±1.647
Significant level	*	N.S.	*	N.S.	*	N.S.