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EFFECT OF *Nigella sativa* OIL TREATMENT ON THE SEX ORGANS AND SPERM CHARACTORS IN RATS EXPOSED TO HYDROGEN PEROXIDE

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ABSTRACT

The effect of *Nigella sativa* oil (0.8ml/kg B.W) on testis, accessory sex glands and epididymal sperm characters was studied in mature male albino rats treated with hydrogen peroxide H_2O_2(0.5% in drinking water) for 30 days orally. The results showed thatH_2O_2 treatment caused a significant decrease in the percentage of live/dead sperms 13.66% associated with a significant increase in the percentage of and morphologically abnormal sperms 39.5%.While a significant increase in the percentage of live/dead sperms in both *Nigella sativa* oil treated group and *Nigella sativa* oil joined to H_2O_2 (85%), (72.16%) , respectively accompanied with a decrease in percentage of morphologically abnormal sperms compared with H_2O_2 treated group. It is concluded that *Nigella sativa* oil possess an antioxidative actions to counter act the impairment in the epididymal sperm characters caused by H_2O_2 treatment.

INTRODUCTION

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen derived species, a highly reactive oxidizing agents, belonging to the class of free radicals (Sikka *etal.,* 1995). The most common reactive oxygen species that have potential implications in reproductive biology include superoxide (O_2) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO) radicals and the very reactive hydroxyl (OH') radicals. The nitrogen–derived free radical nitric oxide (NO') and peroxy nitrite anion (ONOO') also appear to play a significant role in reproduction.(Sikka, 1996). Excessive reactive oxygen species generation can overwhelm the protective mechanism and initiate changes in lipid and/or protein layers of sperm plasma membrane. Additionally changes in DNA can be induced (Sanocka and Kurpisz, 2004). Mammalian sperm cells have high content of specific lipidic composition of polyunsaturated fatty acids, plasmalogenes and sphingomyelins (Aitken *etal*., 1989).This unusual structure of sperm membrane is responsible for its flexibility and the functional ability of sperm cells, However, spermatozoa's lipids, are the main substrates for peroxidation, that may provoke severe functional disorder of sperm (Aitken *etal*.,1989).

On the other hand, antioxidants, in general, are compounds and reactions which dispose, scavenge and suppress the formation of ROS or oppose their reactions (Sikka, 1996). Recently, dietetical antioxidants and their roles in free radical removal and prevention of tissue, cellular lesion have garnered attention

especially for their usefulness in reproduction and management of infertility.*Nigella sativa* oil have known for their antioxidant properties in ameliorating inflammatory

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disease (EL-Dakhakhny *etal*., 2002).In addition, Mahmoud *etal*. (2002) pointed to the role of *Nigella sativa* oil against mice liver damage caused by *Schistosoma mansoni* infection by improving the immunological host system with its antioxidant effect. On the other hand, Black cumin seed oil enhances potency and have aphrodesiac action (Laver, 1984).The present study was designed to investigate the anti oxidative effect of *Nigella sativa* oil on testis, accessory sex glands, epididymal sperm characteristics in adult male rats exposed to oxidative stress induced by hydrogen peroxide.

MATERIALS AND METHODS

1.Extraction of oil from *Nigella sativa* **seeds:**The *Nigella sativa* seeds were purchased from local herb grocery in Mosul city.The seeds were ground in porcelain mortar.one Kg of the powdered seeds with appropriate amount of ethanol were heated to(80-90C˚)in the extraction apparatus (soxhelet) (Maynard, 1970). Heating and extraction continued until uncoloured solvent was collected. This distillate was extracted by steam distillation until clear water was obtained. Moisture was removed by anhydrous sodium sulphate and the resultant extract was evaporated using water bath (40C˚), this led to obtain the oil which was kept in a dark flask (Harvey and John,1998). The percentage of oil extracted was calculated by the following equation:

$$\text{Percentage of oil} = \frac{\text{oil weight}}{\text{Sample weight}} \times 100$$

The percentage of black seed oil was 12.7 %.

2. Experimental design: Twenty four male albino rats of 90-100 days of age were used, they were housed in polypropylene cages under controlled conditions of temperature(24-26C˚)and natural lighting (10 hours light/14 hours dark)food and water were supplied *ad libitum*. The animals randomly divided into four groups (6 rats/group). The first group received normal saline and served as control.The second group received 0.5%H_2O_2 (Al shaheed factory-Iraq) in drinking water(Matkovics,1977).The third group received *Nigella sativa* oil (0.8ml/kg B.W) orally by gavage needle.The fourth group received 0.5%H_2O_2 in drinking water and *Nigella sativa* oil (0.8ml/kg B.W)orally by gavage needle. At the end of thirty days experimental period, the animals were scarificed by the ether administration and their weights were recorded.The abdominal cavity was opened, Testis, Epididymis (head, body,tail),prostate and seminal vesicle were immediately removed, trimmed, blotted in folds of filter paper and weight.Total epididymal head sperm count in 1ml was determined according to Sakamoto and Hashimoto

procedure(1986).The right epididymal head was dissected into small parts in petri dish with the addition of 9.8 ml neutral formalin buffer and 0.1ml of 5% eosin stain. Sperm count was done using hemocytometeric technique. The percentage of live, dead and morphologically abnormal sperms were counted using eosin-nigrosin stained smears.Data were analyzed statistically using one-way analysis of variance.Group differences were determined using Duncan test (Steel and Torrie,1960).The level of significance was at $p < 0.05$.

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RESULTS AND DISCUSSION

Treatment with H_2O_2 (0.5%) in drinking water for 30 days caused a significant ($p<0.05$) decrease in epididymal head weight (Table1), the percentage of live/dead sperms with a significant ($p<0.05$) increase in the percentage of morphologically abnormal sperms (Table2) as compared with the control group. Oral administration of *Nigella sativa* oil (0.8ml/kg B.W) for 30 days showed a significant ($p<0.05$) decrease in the percentage of morphologically abnormal sperms with an increase in the percentage of live/dead sperms (Table2) as compared with the H_2O_2-treated groups. On the other hand, the group treated with 0.5% H_2O_2 and 0.8 ml/kg B.W. *Nigella sativa* oil revealed a significant ($p<0.05$) increase in weights of the testis, epididymis (head and body) and seminal vesicle (Table1),the percentage of live/dead sperms with an associated a significant ($p<0.05$) decrease in the percentage of morphologically abnormal sperms (Table2) when compared with the group treated with H_2O_2.The results of the present study demonstrated that H_2O_2-induced oxidative stress resulted in a decrease in epididymal head weight accompanied by disturbance of sperm characters in rats. The H_2O_2-induced oxidative stress in the present study may lead to the production of high amounts of reactive oxygen species. One mechanism suggests that H_2O_2 administered orally probably increases oxygen production in the stomach which reaches the blood and increases oxygen tension in tissues causing increased production of reactive oxygen intermediates (Loven and Obrelery, 1985). These intermediates may be responsible for the decrease in the percentage of live sperms and increased percentage of dead sperms and morphologically abnormal sperms. In the male reproductive tract, oxidative stress may create aground for sperm deterioration and transitory or persistent infertility (Kurpisz, 2004). The results of the present study seem to agree with those obtained by Jassem and Yousif (2004) in rats and Aziz (2000) in mice. Mazilli *et al*. (1994) found a positive correlation between O_2 levels and sperm abnormalities and the increase in reactive oxygen species, including, Superoxide anion, caused increase in the percentage of dead, morphologically abnormalities with a decrease in percentage of live sperms in human. Peroxidation of poly unsaturated fatty acids has implicated in a wide variety of pathological conditions, infertility amongst them agree with suggestion by Atiken *et al*.(1989) that, low(physiological) levels of lipid peroxidation reflect the influence of reactive oxygen species on sperm metabolism enhancing the ability of spermatozoa to interact with zona pellucida while higher,

pathological lipid peroxidation of sperm membranes can be unbalanced oxidative stress. It has been known for along time that oxygen metabolism can be deterimental to the cells and tissue and ROS release has effects on male infertility. Sanocka *et al*.(2003)reported that the unpaired electrons make the external orbit vulnerable and imitate the effect of ionizing radiation within the tissue affecting the lipids, proteins and DNA. Lipid components located in the sperm membranes are involved in regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually in membrane fusion and so peroxidation of sperm lipids may disturb sperm functions which in extreme cases even completely inhibit spermatogenesis (Sanocka and Kurpisz,2004).Interestingly, antioxidants seem to offer new ways of conservative

therapy that is currently used in a variety of diseases such as infertility. Sikka *et al*. (1995) mentioned that there is a variety of biological and chemical antioxidants that attack ROS present in seminal fluid and, in turn help spermatozoa to comabat oxidative insult maintaining sperm motility and function. On the other hand, Gavella *et al*. (1996) reported that infertile men had lower antioxidant status level than fertile men, especially those with poor sperm motility. Oxidative stress revealed improper balance between ROS generation and scavenging activities. The scavenging potential in gonads and seminal fluid is normally maintained by adequate level of antioxidants superoxide dismutase (SOD), catalase and probably glutathione (GSH), peroxidase and reductase(Sikka *et al*., 1995).The results of the present study demonstrate the beneficial effect of the treatment with *Nigella sativa* oil as manifested by the(85%) increase in the percentage of live/dead sperms, and (0.66%)decrease in the percentage of morphologically abnormal sperms reflecting its antioxidants effect that counteract the H_2O_2 effect on sperms, also black seed oil enhances potency. It has a powerfulally in restoring sexual performance by renew virility by enhancing the production of body fluids, male hormones and rejuvenating blood circulation. *Nigella sativa* oil constituents possessed avariable antioxidant activities.

Table (1): Effect of 30 days oral administration of hydrogen peroxide H_2O_2 (0.5% in drinking water) and *Nigella sativa* oil (0.8ml/ kg B.W) on weights of (body, testis , epididymis (head, body tail)and male accessory sex glands in rats.Numbers of animals 6 rats/group. Values are expressed as mean ± SE. Values with different letters are significantly different at $P< 0.05$.

Treatment	Control	H_2O_2	*Nigella sativa* oil	*Nigella sativa* oil +H_2O_2
Body weight(g) (Pre-treated)	86.5±6.687 a	107.5±3.64 b	110.83±2.6 b	121.33±7.2 b
Body weight (g) (Post-treated)	133.66±7.7 a	152.5±7.76 a	136.16±3.6 b	142.6±4.24 b
Testis weight (mg/100g B.W)	467.8±49 ab	340.2±48.2 b	426.7±48.1 ab	523.316±53.5 a

Epididymis weight (mg/100g B.W)	Head	75.46±9.02 a	31.88±2.5 b	31.03±6.13 b	77.69±8.46 a
	Body	15.168±139 b	19.45±2.634 b	19.516±3.092 b	53.745±4.352 a
	Tail	63.109±2.7 ab	51.966±3.709 ab	76.783±3.389 a	43.385±15.78 b
Prostate weight (mg/100g B.W)		136.8±12.2 b	142.06±23.4 b	310.6±45.37 a	134.8±12.89 b
Seminal vesicle weight (mg/100gB.W)		29.53±1.72 b	27.9±5.73 b	40.96±5.22 ab	50.07±6.05 a

It inhibited the non enzymatic peroxidation in ox brain phospholipidliposomes (Houghton *et al*.,1995). Nearly similar results were observed by Rady *et al.* (1997) in chicken glutathione redox system. Morever, Burits,(2000)reported that the essential oil of black cumin seeds and components thymoquinone and carvacrol

have respectable radical scavenging properties, which may give explain the increase in the percentage of live/dead sperms with decrease in the morphologically abnormal sperms by *Nigella sativa* oil treatment that counteract the H_2O_2 effect in the present study. It is concluded from this study. That *Nigella sativa* oil have a protective and antioxidant effect on testicular, accessory sex gland functions in animals exposed to oxidative stress induced by hydrogen peroxide and whether the anti-oxidative action induced by *Nigella sativa* oil as a results of a direct action or a results of indirect effects, e.g including antioxidative enzymes cascade, is unknown and should be subjected for further investigation.

Table (2): Effect of 30 days oral administration of hydrogen peroxide (H_2O_2)(0.5% in drinking water) and *Nigella sativa* oil(0.8ml/kgB.W) on epididymal sperm characters in rats. Numbers of animals 6 rats/group.Values are expressed as mean ± SE.Values with different letters are significantly different at $P< 0.05$.

Treatment	Percentage of live/dead sperms	Percentage of morphologically abnormal sperms	Epididymal headSperms$\times 10^6$ (Sperm/ml)
Control	9405± 1.3345 a	0.333±0.333 c	$1.19\times10^6 \pm 0.054\times10^6$ a
H_2O_2	13.6667±2.073 d	39.5±2.6173 c	$0.76\times10^6 \pm 0.066\times10^6$ a
Nigella sativa oil	85±1.4606 b	0.6667±0.6667 c	$2.6\times10^6 \pm 1.384\times10^6$ a
Nigella sativa oil + H_2O_2	72.1667±1.815 c	22.3333±0.9189 b	$3.75\times10^6 \pm 1.579\times10^6$ a

تأثير المعاملة بزيت الحبة السوداء على الخصى والغدد الجنسية اللاحقة وخصائص نطف البربخ في الجرذان البالغة المعرضة للكرب التأكسدي المستحدث ببيروكسيد الهيدروجين

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الخلاصة

دراسة تأثير اعطاء زيت الحبة السوداء(٠ مل/ كغم وزن الجسم) ولمدة ٣٠ يوما على الخصى والغدد الجنسية اللاحقة وخصائص نطف البربخ في ذكور الجرذان البالغة المعرضة للكرب التأكسدي المستحدث ببيروكسيد الهيدروجين (٠% في ماء الشرب) لمدة ٣٠ يوما اظهرت النتائج ان الكرب التأكسدي قد سبب انخفاضا في النسبة المئوية للنطف الحية/الميتة ٦٣ ١٣% مع زيادة النسبة المئوية للنطف المشوهة ٩٣% .اما المعاملة بزيت الحبة السوداء فقد سبب انخفاضا في النسبة المئوية للنطف المشوهة ٦٣ ٠ % مع زيادة في النسبة المئوية للنطف الحية/الميتة ٨٥% مقارنة بالحيوانات المعاملة ببيروكسيد الهيدروجين(٠% في ماء الشرب) .وقد استنتج من الدراسة ان لزيت الحبة السوداء خصائص مضادة للاكسدة تغلبت على التغييرات الحاصلة في خصائص نطف البربخ عند المعاملة ببيروكسيد الهيدروجين .

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