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The significance of *Nigella sativa* in improving semen quality, and blood oxidant/antioxidant status in male New Zealand rabbit

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Black cumin or *Nigella sativa* (NS) is one of the folk medicine and prophetical remedy to a lot of body disorders. Reproductive laziness needs such potentiator. So, the aim of the present study was to potentiate the reproductive performance of bucks suffering environmental or body stress giving emphasis to the semen parameters and the blood antioxidant/oxidant status. Methods: twenty seven apparently normal bucks were divided into three equal groups. One kept as control and the other two had taken 1.5 and 3.0 ml of filtered black cumin water extract (50:350 w/v). The collected semen and blood serum were subjected to physical and chemical assays respectively. A significant ($P<0.013$) decrease in the ejaculate volume and abnormal sperm% in both Nigella water extract (NWE) compared to the control group. While, a significant increase in PH, mass and individual motility% and live sperm% were recorded in NWE groups compared to the control one. Ascorbic acid, glutathione reduced and testosterone were increased significantly for the bucks receiving 3.0 ml NWE. While, the nitric oxide and malondialdehyde were significantly decreased in the same group compared to the control. From the previous results it is concluded that 1.5 - 3.0 ml of *Nigella sativa* water extract (50g/350ml, w/v) with its antioxidants content could significantly overcome the reactive oxygen species in the male reproductive system and conclusively the whole body health of male rabbit to increase its fertility.

Keywords: Nigella extract; rabbit; semen; antioxidant.

INTRODUCTION

The prophetical medicine was a guide for a vast category of investigations especially in the Arab world. Black cumin or *Nigella sativa* (NS) is a marvelous herb with a generous historical and religious background where its seeds have been indicated as a traditional medicine for various illnesses including male sexual dysfunctions (Salem, 2005). NS seeds meal was recommended to replace 50% of soya meal protein in the ration; it has a reproductive potential for the male rabbits (El-Nattat and El-Kady, 2007). Besides, it had been proved that the addition of 1-1.4% of black cumin seeds to the ration of broiler chickens enhanced the immune responsiveness (Al-Mufarrej, 2014) ; while in turkeys, the addition of 3% to the ration would enhance immune responsiveness and suppress pathogenicity of influenza viruses (Sajid et al., 2016). Nigella seeds meal, added as a non-traditional source of plant protein, up to 12% for growing rabbits, has no harmful effects on the growth performance and blood constituents of New Zealand rabbit. Although, the increase of the addition of black cumin in rabbit ration has a

deteriorating effects on liver and kidneys (Zeweil et al., 2008). While, (El-Tohamy and El-Kady, 2007) investigations on the growing rabbit had recorded that there is a narrow limit for the addition of black seeds in the ration of growing rabbits. NS constitutes many different chemical ingredients including thymoquinone (TQ) (30-48%) (Ahmad et al., 2013), flavonoids, anthocyanins, alkaloids and essential fatty acids, particularly linoleic and oleic acid (Bashandy, 2007). Other minute components were present as nigellidine, nigellicine, nigellicimine, nigellicimine-N-oxide, alpha-hederin, saponin, vitamin B complex and vitamin E and some minerals (K, Na, Ca, P, Mg,Cu,and Fe) (Gholamnezhad et al., 2016) Thymoquinone has antibacterial, diuretic, hypotensive and immuno-potentiating activities via increasing neutrophil percentage and hence increasing the defense mechanism of the body against infection (Kanter et al., 2015). Besides, the seeds contain eight essential amino acids that improve natural immune system activity (Omar et al., 1999).

Concerning the animal fertility, NS has been noticed to improve sperm motility, viability and some other sperm parameters (Al-Sa'aidi et al., 2009 and Parandin et al., 2012).The extract of NS seeds ameliorated reproductive toxicity induced by various compounds (Abdul Rahman et al., 2013 and Elshama et al., 2013). Oral administration NS oil resulted in significant improvement in live sperm percentage and possessed a powerful antioxidant capacity that protects epididymal sperm (Tawfeek et al., 2006). Furthermore, the oil significantly enhanced sperm characteristics in normal and hyperlipidaemic rats (Bashandy, 2007). However, in another study, NS oil failed to exhibit any significant effects on sperm variables except epididymal sperm number (Mansour et al., 2013). (Kolahdooz et al., 2014) reported that oral supplementation of NS oil to infertile men with a variety of factors of infertility caused a significant improvement of semen quality parameters. (Umar et al., 2017) indicated dietary supplementation of black seed oil exhibited a stimulatory action on testicular function in adult rabbits and resulted in a significant increase in testicular weight, length, circumference and volume. They added that the feeding of NS for 50 days resulted in significant increase of biometric parameters of testes (spermatogenic epithelium thickness, seminiferous tubules diameter, lumen and number of spermatogenic layers of testes) and serum testosterone concentration.

So, the present study was designed to investigate the antioxidant activity of an aquatic extract of crumbled black seeds on sperm characteristics, sexual hormones and some related serum biochemical parameters to the fertility of male rabbits.

MATERIALS AND METHODS

Experimental animals

Twenty seven mature apparently healthy male New Zealand rabbits were adopted for 10 weeks at the animal house lab, National Research Centre, Giza-Egypt. The animals were purchased from a private farm according to their breeding and production records. All environmental conditions were adapted (temp. 28 ± 2°C, relative humidity 60±5%) with good ventilation. They received commercial rabbit pellets (120 gram/day) and fresh water *ad libitium*. All animals were comfortable with no signs of respiratory or digestive disorders.

Nigella sativa water extract (NWE)

Fifty grams of crumbled NS seeds were impregnated in 350 ml distilled water and stirred for 2 hours then stored for 48 hours at 10°C with shaking every 2 hours till the end of incubation. The whole watery content was then filtrated with gauze (0.1 pores) to remove the coarse particles.

Experimental design

27 mature New Zealand bucks were incorporated in the present study and were randomly allocated into three equal groups (n=9) according to the randomized complete block design (Oehlert, 2010). The first group was treated as control and fed on commercial rabbit ration 18% crude protein and 2600 kcal/kg. This group received 3.0 ml distilled water through mouth gavage. The second and third groups raised on the same ration as the control group and received 1.5 and 3.0 ml of NWE/animal, respectively through an oral gavage. The experimental design has been approved by the NRC Ethical Medical Committee agreement under license 17154.

Collection and evaluation of semen

Semen was collected from bucks at the end of the experimental period. Bucks were trained to mount a teaser female and ejaculated onto an artificial vagina (IMV, France) adjusted for its inner temperature at 42-45°C and lubricated its mouth with a K-gel®. The reaction time (sec) was recorded immediately when the teaser was mounted till the ejaculation sniffle and recumbence of the buck. The collected semen was evaluated for its physical characteristics through the graduated collecting

tube for its volume without gel (ml), consistency, PH and if any ejaculate contained calcium carbonate or urine, it was discarded. The microscopical parameters were estimated for mass motility and individual motility % according to (Petitjean**,** 1965). Life and dead sperm % and abnormal sperm % were counted on a slide film with the use of eosin-negrosin stain (Bamba ,1988).

Blood sampling

At the end of the experimental period, blood were collected from the middle ear vein into plain tubes and were centrifuged at 3500 rpm for 15 min. Serum were collected into clean dry sterile tubes and stored in deep fridge at -80°C till achieving the biochemical analysis.

Biochemical analysis and antioxidant status

Serum in the different groups were analyzed for nitric oxide (NO) using Griess method (Jabłońska et al., 2007) ascorbic acid (AA) (Shrivas et al., 2005), glutathione reduced (GSH) (Tipple and Rogers, 2012), lipid peroxides (LPO) using the method of Buege and Aust (Jabłońska. et al., 2007) Serum testosterone was assessed using a solid-phase enzyme immunoassay (ELISA) total testosterone commercial kit (Biosource, Testo ELISA, Belgium).

Statistical analysis

Replicates were analyzed according to the randomized complete block design using ANOVA test to compare means of the different groups. This was accomplished through (SAS, 2008) computerized program v.9.2 general linear model with further testing of significance between means at $P<0.05$ using the Duncan Multiple Range test.

RESULTSAND DISCUSSION

Data revealed that the gulp down of 1.5 and 3.0 ml NWE to the treated bucks didn't differ significantly the reaction time compared to the control group. However, the two doses increased significantly ($P<0.0057 \sim 0.0003$) the PH, Mass motility, Individual motility%, Live sperm% when compared to the control. On the other hand, they decreased significantly ($P<0.0130 \sim 0.0001$) the volume and the abnormal sperms % compared to the control group (Table, 1).

The precise means of improving fertility by NS is not fully understood.Under ordinary conditions, the reactive oxygen species (ROS) are balanced by antioxidant systems of seminal plasma (Lamirande and Gagnon 1999), but with disturbances in these balance by diminution of the antioxidant activity or over production of ROS in semen for any cause, elevated concentrations of oxidative stress would be injurious to semen quality (Schulte et al., 2010 and Aitken and Krausz, 2001). Mature sperm cell is a very sensitive target cell to oxidative stress (OS) as it hasn't enough cytoplasmic defenses (Garg and Garg, 2011). NS contains high levels of antioxidants (Dragland et al., 2003). Its phytochemical analysis showed the presence of volatile oil, fixed oil, nigellin, melanthin and arabic acid, carvene, carvone and cymene (Nadkarni, 1976), thymohydroquinone (El-Fatatry,1975) and thymoquinone (Houghton et al.,1995)

The presence of different alkaloids (nigellidine, nigellimine and nigellicine) has also been shown in NS seeds (Atta-ur-Rahman et al., 1985 ,1992 and 1995).The beneficial effects of black seeds are due to their protection against cellular damage caused by oxidative stress. This is through their antioxidant components which have been noticed to improve spermatogenesis and steroidogenesis (Menezo et al., 2014a). In the present study, it was declared that oral administration of NWE in a dose of 1.5 and 3.0 ml /animal for 7 weeks (experimental period) caused a significant increase in most fertility parameters especially in higher dose. There was significant increase in PH, mass motility, individual motility, live sperm and abnormal sperm% when compared to the control.

On the other hand, the reaction time was apparently decreased on receiving the high level of NWE coinciding the significant increase in testosterone. This is in regard to the valuable properties of black seed owing to their shield protector against cellular damage caused by oxidative stress. Following a rise in free radicals, DNA damage, lipid peroxidation, protein and biomembrane damage in sperm may occur (Aitken et al., 2014 and Agarwal et al., 2014) .A valid argument claimed that the NS ingredients with antioxidant properties can transfer electrons to oxidizing agents and inhibit free radical production and sperm damage (Adedara et al., 2014). This has been noticed to improve spermatogenesis and steroidogenesis (Menezo et al., 2014b). In case of another oxidative stress, it was proved that NS, in hyperlipidemic rats, could ameliorate fertility (Bashandy, 2007).

Table 1.The oral supplementation effect of *Nigella sativa* water extract (NWE) on the semen quality in New Zealand bucks.

Parameters	Control	1.5 ml NWE	3.0 ml NWE	F-cal	P>F
Reaction time (sec)	23.86 ± 1.87[a]	21.43 ± 3.03[a]	20.71 ± 3.85[a]	0.30	0.7471
Volume (ml)	0.57 ± 0.04[a]	0.41 ± 0.03[b]	0.47 ± 0.02[b]	5.58	0.0130
PH	7.59 ± 0.15[b]	8.27 ± 0.06[a]	8.19 ± 0.11[a]	11.19	0.0007
Mass motility (score 0-9)	6.29 ± 0.42[b]	7.57 ± 0.48[a]	8.29 ± 0.18[a]	6.97	0.0057
Individual motility (%)	65.00 ± 1.54[b]	80.00 ± 3.62[a]	80.00 ± 3.09[a]	9.00	0.0020
Live sperm (%)	89.62 ± 0.70[b]	92.80 ± 0.81[a]	94.20 ± 0.66[a]	10.95	0.0003
Abnormal sperm (%)	8.23 ± 0.44[a]	4.80 ± 0.44[b]	4.20 ± 0.39[b]	27.18	<0.0001

Data are represented in Mean ± Std.Err.
Different superscript (a, b…etc) are significantly different between means using Duncan multiple range test (P<0.05).

Table 2.The effect of *Nigella sativa* water extract (NWE) on the serum oxidant/antioxidants indicators and testosterone level of male rabbit.

	Nitric oxide (nmol/ mL)	Ascorbic acid (mg/L)	Reduced Glutathione (mg/dL)	Malondi-aldehyde (nmol/ mL)	Testosterone (ng/ml)
Control	22.02 ± 0.97[a]	3.79 ± 0.05[b]	10.84 ± 0.81[a]	0.96 ± 0.03[a]	3.79 ± 0.17[a]
1.5 ml NWE	24.56 ± 1.95[a]	2.87 ± 0.00[c]	3.13 ± 0.06[c]	1.03 ± 0.05[a]	4.58 ± 0.16[a]
3.0 ml NWE	15.89 ± 0.87[b]	5.47 ± 0.14[a]	6.22 ± 1.54[b]	0.92 ± 0.08[a]	3.85 ± 0.66[a]
f-cal.	10.82	243.48	14.85	0.95	1.18
Sig.	0.0004	0.0001	0.0001	0.4013	0.3244

Data are represented in Mean ± Std.Err.
Different superscripts (a, b, …) indicate significant difference between means using Duncan multiple range test at P<0.05.

Table 2 showed that the nitric oxide was significantly (P<0.0004) decreased when bucks received a dose of 3.0 ml black cumin water extract compared to the bucks in the control and those received 1.5 ml of the extract. On the other hand, the ascorbic acid showed a significant (P<0.0001) increase, in case of using 3.0 ml Nigella extracts, compared to the control. The reduced glutathione was significantly (P<0.0001) increased in both treated groups with black cumin extract (1.5 and 3.0 ml) compared to the control. LPO represented by the malondialdehyde (MDA) was decreased significantly (P<0.0022) compared to the control while, the testosterone was significantly (P<0.0001) increased in both NWE groups compared to the control one.

Phytochemical analysis indicated the rich presence of unsaturated fatty acids (Linoleic acid 55.6%, Oleic acid 23.4%, Palmitic acid 12.5%, Stearic acid 3.4% and else) in NS seeds (Nickavar et al., 2003) which play a role in maintaining the sperm membrane in an intact status due to their antioxidative role. Also, thymoquinone one of the major content of NS has an antioxidant protective effect that may repress the expression of cyclooxygenase-2 enzyme and lipid peroxidation (Al Wafai, 2013) this may improve tissue and serum testosterone and testis tissue through the reduction of MDA levels and increasing antioxidant indicators (including glutathione profile and other antioxidant enzymes) while it increases the semen quality parameters (Zohra et al., 2012). In vitro, (Mahmood et al., 2003) had studied the effect of NS on the NO production by murine macrophages and they reported that the nigella water extract had an inhibitory effect on the production of NO. In the present study, the negative correlation between NO + LPO in one hand and semen characteristics, ascorbic acid, glutathione reduced and testosterone on the other one agreed with the later concept of (Zohra et al., 2012). Finally, the low ROS especially NO participates with a positive role in sperm capacitation which in turn increases the sperm fertilizing capacity (Garg and Garg, 2011).

CONCLUSION

1.5 - 3.0 ml of *Nigella sativa* water extract

(50g/350ml, w/v) with its antioxidant content could significantly overcome the reactive oxygen species in the male reproductive system and conclusively the whole body health of male rabbit to increase its fertility.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

W.El-Nattat, W. Khalifa and G. El Sisy designed and performed the experiments and also wrote the manuscript. W. Khalifa performed the Nigella extraction. W.El-Nattat and G. El Sisy performed the semen collection and examining the semen characteristics. G.Abusinaa, A. Abo El-Maaty and N. Maghraby performed the animal treatment and biochemical analysis.. W.El-Nattat performed data analysis. All authors share experiment design and reviewed the manuscript. All authors read and approved the final version.

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