Effects of oral administration of black seed (*Nigella sativa)* oil on histomorphometric dynamics of testes and testosterone profile in rabbits

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Abstract: The present study was conducted to evaluate the influence of oral administration of black seed (*Nigella sativa)* oil on histomorphometrical characteristics of testes and testosterone profile in adult rabbits. Twenty adult male rabbits aged seven months were divided into two groups: control and treated. Black seed oil was administrated orally for 60 days at 5ml/kg body weight/day on daily basis in addition to the food and water *ad lib* to the treated group. Biometric parameters of the testes were recorded immediately after their removal. Tissue samples of testes were processed with paraffin tissue preparation technique. Histometrical parameters of testes were measured with the help of automated image analysis software Image J®. Serum testosterone concentration was determined with Radioimmunoassay technique. Statistical analysis revealed significant ($P<0.05$) rise in weight, length, circumference and volume of testis in treated group than control group. The values of histometrical parameters studied viz., thickness of spermatogenic epithelium, diameter and area of seminiferous tubules, diameter of lumen of seminiferous tubules, number of spermatogenic layers of testes and serum testosterone concentration were found significantly ($P<0.05$) higher in treated group than control group. Based on the data it is conceivable that the oral administration of black seed oil has potential to stimulate testicular function in adult rabbits.

Keywords: Black seed (*Nigella sativa*) oil, rabbits, testes, testosterone level, histomorphometry.

INTRODUCTION

For the past few years, use of ethnobotanical knowledge and research has gained substantial consideration among the scientists (Heinrich, 2000). Recently, World Health Organization (WHO) has reported that about 65–80% of world's populace in developing countries is dependent principally on plants and herbs for their primary health care (Calixto, 2005).

Among these medicinal plants, the seed *Nigella sativa* Linnen is generally called as black seed or black cumin and locally called as kalonji. The Holy Prophet Mohammed (Peace Be upon Him) said: *'Use the Nigella sativa, which is a healing for all diseases except 'death" (Al-Bukhari,1976). Nigella sativa* is being used as herbal medicine for past many centuries to cure acute as well as chronic diseases (Farah *and Begum,* 2003).

Infertility is the biggest problem in couple's lives and about 30 % infertility occur due to male influence (Isidori *et al*., 2006). Most situations can inhibit spermatogenesis and decrease sperm production and quality. Many elements like chemotherapy, toxins, drug and insufficiency of vitamin intake and air pollutions may cause harmful effect on development of spermatozoa and the production of sperms (Mosher and Pratt, 1991). Many plants are widely used for intensifying the sexual desire and to relieve sexual impotence. These plants provide an increase of nutritive value for improving sexual performance and sexual desire (Yakubu *et al*., 2007 and Sumalatha *et al*., 2010).

Nigella sativa seed oil increases the reproductive efficiency in hyperlipidemic rats (Samir, 2007). Aqueous extract of *Nigella sativa* seed has increased the development of spermatozoa in male rats. *Nigella sativa* increased the interstitial cells and diameter in rats' testis. Mixture of equal quantity of *Raphanus sativus*, *Eruca sativa* and *Nigella sativa* meals improved semen physical characteristics and decreased free radicals in seminal plasma (El-Tahomi *et al*., 2010). However, there is scanty literature available concerning effects of black seed (*Nigella sativa*) oil on histomorphometrical characteristics of testis and testosterone profile in rabbits. The present study was therefore conducted to evaluate the influence of oral administration of black seed (*Nigella sativa)* oil on histomorphometric dynamics of testes and testosterone profile in adult rabbits.

MATERIALS AND METHODS

Plant materials

Black seed *(Nigella sativa)* were bought from local grain market of Faisalabad and taxonomic identification of

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seeds was confirmed by a qualified taxonomist of Institute of Horticultural Sciences, University of Agriculture Faisalabad. After identifications, the seeds were dried and oil was extracted by traditional method.

Animals

A total of 20 mature adult male rabbits aged 7 months weighing between 1-1.2 kg were bred in animal house of Faculty of Veterinary Science, University of Agriculture, Faisalabad. Animals were kept under optimal management conditions including controlled temperature, humidity, ventilation and light. Leucern (1 kg/rabbit) and wheat porridge (20 mg /kg) were given daily to each animal. Water was available *ad labitum.*

Treatment

Twenty rabbits were divided into two equal groups containing 10 rabbits each. Control group received food and water only. Treated group was orally administered black seed (*Nigella sativa)* oil 5 ml/kg body weight on daily basis for 60 days in addition to food and water *ad libitum in morning and evening.*

Collection of samples

After the completion of 60 days trial, rabbits were slaughtered with a sharp metallic knife. Two test tubes were used for collection of blood samples from each rabbits at slaughter. Serum were separated by centrifugation at 2000 rpm for 15 minute and stored at -20^{o}C till analysis. Serum was used for determination of testosterone concentration.

The testicular tissue samples were collected from each rabbit immediately after slaughter and immersed in Bouin's solution for histological studies. Samples were washed with normal saline after taking the gross anatomical measurements.

Macroscopic evaluation

Following collection, the samples were observed carefully for their gross features (i.e. color, shape and consistency) and biometric characteristics (i.e length, width, thickness, circumference and volume). Vernier's caliper was used for measurement of length, width and thickness (cm). Measuring tape was used for measurement of circumference (cm) of testes. The weight of each sample of testes were recorded by using an electrical weighing balance (Ogunlade *et al*., 2006). The volumes of testes were estimated with the help of graduated measuring cylinder by water displacement method.

Histological analysis

Testes cut in many pieces of 3-5 mm thickness were fixed in Bouin's solution. The fixed samples were processed by the paraffin tissue preparation technique and stained by the hematoxylin and eosin stains (Bancroft and Gamble, 2008).

Histometrical examination

Photomicrographs of each testis were captured using Nikon Optiphot 2 microscope at 200X. These photos were used to determine the thickness of germinal layer, diameter, area, diameter of lumen and spermatogenic cell layer of seminiferous tubules of each testes with the help of automated image analysis system Image J version 1.47v (Research Service Branch, National Institute of Mental Health, Bethesda, Maryland, USA). The volume of 10 cross section seminiferous tubule (Vst) were obtained by the formula, $Vst = \pi.h(d^2/4)$, where h represents thickness of section (5µm) and d represent the tubule diameter (µm) (Moura *et al*., 2011). Spermatogonia were counted at 400Xunder the microscope.

Hormonal analysis

For serum testosterone concentration analysis, the serum samples extracted via centrifugation (@ 1500rpm for 10 minutes) of collected blood samples were stored at -20 C. the assay was performed RIA (Radioimmunoassay) using kit (IMMUNOTEC, Marseille, France). For testosterone assay, the normal detectable concentration was 0.02ng/ml

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was used to compare the means of parameters. Least Significance Difference (LSD) test was used to compare the groups mean at 5% level of significance and Tukey's test was used to compare the group means at 1% level of significance.

RESULTS

Effects of black seed (Nigella sativa) oil on Morphometrical parameters of testes

Effects of black seed (*Nigella sativa)* oil on the mean values of morphometrical parameters including length, width, thickness, circumference and weight of testes in rabbit are given in the table 1a.The statistical results of some morphological parameters of testes (weight, length, circumference and volume) were significantly ($P<0.05$) higher in treated group as compared with control group whereas width and thickness, color, shape and consistency of testes remained unaltered.

Effects of black seed (Nigella sativa) oil on Histometrical parameters of testes

Effects of black seed (*Nigella sativa)* oil on the mean values of histometrical parameters including the thickness of germinal layer, diameter, area, diameter of lumen, spermatogenic cell layer and number of spermatogonia of seminiferous tubules of testes in rabbit are presented in the table 1b. The statistical results of histometrical parameters namely percentage area of interstitial cells in relation to seminiferous tubules, the thickness of germinal layer, diameter, area, diameter of lumen, spermatogenic

Table 1: Mean ±SEM of gross anatomical parameters of testes, histometrical parameters of seminiferous tubules in testes and testosterone level in serum of rabbits of treated group with black seed (*Nigella sativa)* oil (n=10) and control group (n=10)

Parameters	Groups	
	Treated	Control
a. Gross anatomical Parameters		
Weight (g)	1.35±0.04	1.29±0.04
Length (cm)	2.53±0.06	2.25±0.03
Width (cm)	0.87±0.02	0.86±0.02
Thickness (cm)	1.00±0.05	0.85±0.02
Circumference (cm)	3.03±0.02	2.56± 0.03
Volume (cm^3)	1.18±0.02	0.87±0.03
b. Histometrical Parameters		
Diameter of ST (µm)	133.98±3.01	104.72±3.94
Area of ST (μm^2)	1423±567	8647±642
Thickness of spermatogenic layer ST (µm)	60.47±2.23	34.58±1.47
Lumen of ST (µm)	13.72±0.68	34.28±1.57
Volume of ST (μm^3)	725±2.8	432±3.2
Percentage area of ST (%)	79.70±2.09	55.20±2.47
Percentage area of interstitial tissue (%)	20.30±2.09	43.80±2.24
No. of spermatogonia in ST	34.10±2.43	22.40±0.54
Serum Testosterone Level (ng/ml)	20.10±1.38	15.40±0.80

ST: seminiferous tubules

cell layer and number of spermatogonia of seminiferous tubules of testes were significantly ($P<0.01$) higher in treated group as compared with the control group.

Effects of Black seed (Nigella sativa) Oil on Testosterone profile: The statistical results shown testosterone level were significantly ($P<0.01$) greater in treated group (20.10±1.38 ng/ml) as compared with the control group (15.40±0.80 ng/ml) of rabbits. (table 1)

DISCUSSION

To our knowledge, this is the first ever report to evaluate a more comprehensive effect of black seed *(Nigella sativa)* oil on the histomorphometric dynamics of the testes and testosterone profile in rabbits. Thus, the results are compared in general with other species. Selection of rabbits as model animals was done due to factors like easy handling and maintenance. They live well at any temperature and are cost effective from research point of view. Moreover, rabbits are sexually active all over the year.

Nigella sativa has been used by humans as a spice and medicine for hundreds of years. The seeds of *Nigella sativa* contain fixed and essential oils, saponin, alkaloids and proteins. The seeds of *Nigella sativa* have very less degree of toxicity (Ali and Blunden, 2003). The effect of the *Nigella sativa* seed on the male reproductive system could be due to fatty acid contents (Palmitic acid 12.5%, Linoleic acid 55.6%, and Oleic acid 23.4%) as determined by Zanouny *et al.,* 2013. However, Al-Ali *et al.,* 2008 and Gökce *et al. (*2010) have reported that thymoquinone is the major component of *Nigella sativa* which has major effect on the testicular parameters. In this connection it is important to note that Ayan *et al.,* (2015) has recently reported that thymoquinone has antioxidant activity and decreases superoxide dismutase, glutathione peroxidase and malondialdehyde levels in testicular tissues. This plays a protective role against oxidative injury of testis in rats.

Daily oral administration of *Nigella sativa* seed oil for 60 days significantly increased weight, circumference, volume, length, width and thickness of testes in treated group as compared with those of control group. These findings are in line with those in rats (Muhammad *et al.,* 2009; Mansour *et al.,* 2013), different breeds of sheep lambs (Salhab *et al.,* 2001*,* Ozturk *et al*., 2002, Hamdon, 2005, Zanouny *et al.,* 2013 and Kassab, 2007), cattle and buffaloes (Abu-Elawa, 1995).

Among the histological parameters, mean thickness of spermatogenic layer and diameter of seminiferous tubules in testes of treated group were significantly higher as compared with those of control group. Similar findings have been reported in rats (Al-Taee, 2008, Mohammad, 2009 and Al-Saaidi, 2009)

Oral administration of black seed *(Nigella sativa)* oil influenced positively histomorphometric parameters of testes by increasing the area, volume, luminal diameter of seminiferous tubules, and percentage area of interstitial tissue in relation to seminiferous tubules of testes. Numbers of spermatogonia were also significantly higher in treated group as compared with the control group. This

increased number of spermatogonia is supported by previous reports in rats (Mohammad, 2009 and Al-Saaidi *et al*., 2009). Light microscopic studies have revealed that treated group had an increased number of spermatocytes and spermatids relative to the control group in rats. The rapid increase in number of spermatocyte and spermatids could be due to stimulation of cell division between these cells (Ashraf *et al.,* 2013). Recently Mansour *et al.,* (2013) have reported that daily oral administration of *Nigella sativa* oil and pomegranates extract for 6 weeks produced a significant increase in epididymal sperm concentration and sperm motility accompanied with decreased abnormal sperm concentration. They have related that with decreased lipid peroxidation in wistar male rats. They further attributed improvements observed in sperm quality to prevention of excessive generation of free radicals, produced by antioxidant property of *Nigella sativa* oil.

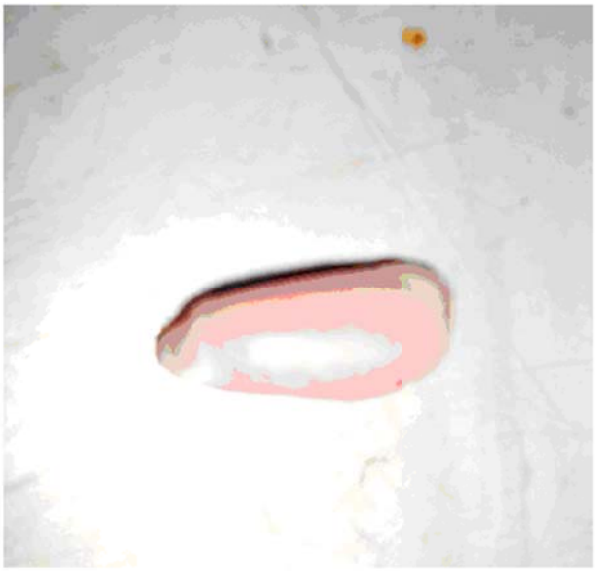

Fig. 1: Photograph of rabbit's testes of treated group with black seed (*Nigella sativa*) oil. Treated group organs are larger than control group.

Present study revealed that serum testosterone concentration was found significantly higher in treated as compared with the control rabbits. These findings comparable to previous observations in rats (Al-Saaidi, 2009: Parandin, 2012 and Mansour *et al.,* 2013). The increase in the testosterone level in oil treated group may be due to effect of black seed (*Nigella sativa*) oil on 17β hydroxysteroids dehydrogenase enzyme which is important for production of testosterone (Gromadzka *et al.,* 2002). Ali and Blunden 2003 have reported that biological activity of *Nigella sativa* is due to thymoquinone, the major component of the black seed oil. Recent study (Ayan *et al.,* 2015) suggests that thymoquinone has a protective function for injured testicular tissues due to its antioxidant properties. Furthermore, the increased testicular size leads to an increase in the no. of interstitial/leydig cell, which leads to increased level of serum testosterone (Preston *et al*., 2012).

Fig. 2: Photograph of rabbits' testes of control group (smaller than control group organs).

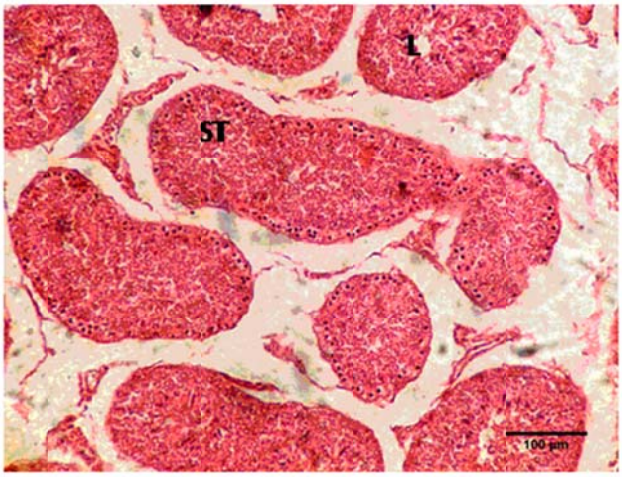

Fig. 3: Photomicrograph of testes of treated group: Seminiferous tubules presented larger luminal diameter (L), increased thickness of spermatogenic layers decrease interstitial tissue (IT) and higher number of spermatogonia. Hematoxylin and eosin (H&E) 200X

All these findings support the fact that use of the black seed (*Nigella sativa)* extract has a positive effect on the spermatogenetic activity in animals (Al-Helali, *et al.,* 2002 and Al-Mayali *et al.,* 2007).

CONCLUSION

Nigella sativa has been used in humans as a spice and medicine for hundreds of years, and its use in human is undoubtedly safe. Oral administration of black seed oil influences positively the weight and macro- and microscopic parameters of testes in rabbits along with an

elevation in the testosterone level which has clear cut impact on the stimulation of spermatogenic activity. Thus, oral administration of *Nigella sativa* may be considered as a therapeutic support to cure the male infertility in mammalian species.

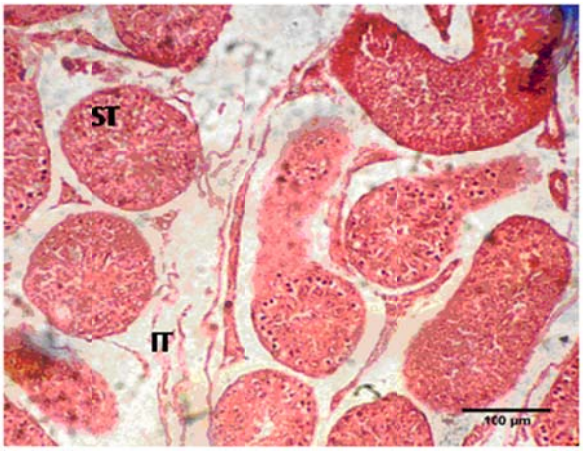

Fig. 4: Photomicrograph of testes of control group showing seminiferous tubules activity of rabbits., with smaller diameter and area of seminiferous tubules (ST), larger lumens (L), thickness of spermatogenic layer of seminiferous tubules and maximum interstitial tissue (IT) and less number of spermatogonia. Hematoxylin and eosin (H&E) 200X

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