Response of dietary supplementation of black seed (*Nigella sativa*) oil on hematological parameters, serum biochemistry and reproductive hormones in male rabbits

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ABSTRACT

To elucidate the therapeutic effects of Black seed (*Nigella sativa*) oil on hematological parameters and male gonadotrophins, this study was conducted on 20 male rabbits of 8-9 months of age. These experimental rabbits were divided into two groups (n=10): one was control and the other was treated with black seed oil. Black seed oil was administrated orally @ 5ml/kg b. wt/day for 60 days to the treated group in addition to their normal diet. The animals were euthanized under gaseous anesthesia at the end of the trial. Before slaughtering, the blood was collected from jugular vein into vacutainers for hematological and serological examinations. Serum was separated from blood by centrifugation for serum biochemistry analysis. One-way analysis of variance (ANOVA) was used to compare parameter means. Statistical picture showed that erythrocyte count and its indices along with leukocytic cells count were significantly (P<0.05) increased with the black seed oil treatment. Serological analysis showed significant (P<0.05) upsurge in the plasma total proteins, albumin and globulin, while the total lipids, triglycerides and cholesterol followed otherwise trend with treatment. However, black seed oil treatment showed non-significant (P>0.05) effect on serum glutamate pyruvate transaminase and glutamate oxaloacetate transaminase. Significant (P<0.05) elevation in the hormones levels (FSH, LH and testosterone) was also observed in the treated group. Keeping in view these findings, we may conclude that black seed (*Nigella sativa*) oil is beneficial and may be recommended in patients with poor blood profile and male infertility problems in humans as well as in animals.

Key words: Black seed (*Nigella sativa*) oil, rabbits, blood analysis, serum biochemistry.

INTRODUCTION

The scientists of 21st century have emphasized on the use of natural products because of their safety, as well as good bioavailability and, are seeking ways to replace synthetic medicines with natural products (Ahmad et al., 2013). Since the beginning of civilization, people have been using medicinal plants for therapeutic effects (Sogut et al., 2018). According to a survey, medicinal plants and herbs are widely used (approximately 65–80% of world's population), especially in less developed countries, for their primary health care (Calixto, 2005). Among the medicinal plants and herbs, the seeds of *Nigella sativa* (NS), also referred to as black cumin, black seed and Kalonji, has recently gained attention for its vast therapeutic properties in many acute and chronic diseases (Farah and Begum, 2003).

The Holy Prophet Muhammad (Peace Be upon Him) said:
‘Use the Nigella sativa, which is a healing for all diseases except ‘death’” (Al-Bukhari, 1976). Folklore use of BS oil and seed extract have long history in various system of traditional medicine like Unani Tibb, Siddha and Ayurveda.

Proximate analysis of NS seeds shows that there is 20-27% crude protein, 34.5-38.7% lipids and 23.5-33.2% carbohydrates (Babany et al., 1978). The chemical composition of volatile oil of NS seeds and the extracted oil consists of eight fatty acids (99.5%), and thirty-two compounds (86.7%). The important fatty acids of the fixed oil are oleic acid, linoleic acid and palmitic acid at the following percentage: 55.6, 23.4 and 12.5%, respectively. The major compounds of the volatile oil are cymene (14.8%), limonene (4.3%), transanethole (38.3%) and carvone (4.0%) as reported by Nickavar et al. (2003).

NS seeds have been minutely investigated and shown its broad spectrum therapeutic and biological properties including anti-inflammatory, analgesic, antibacterial, antidiabetic, anthelmintic, immunomodulatory and bronchodilatory (Ahmad et al., 2013; Ali et al., 2017). It also contains many hepato-renal protective proteins and anticancer constituents, hence, recommended in the treatment of many metabolic and infectious diseases as a protective and immune-booster agent (Abdel-Ghaffar et al., 2003; Gharby et al., 2015). The therapeutic properties of NS can be attributed to the presence of antioxidants, such as thymoquinone, carvencral, p-cymene and 4-terpinol. High concentrations of polyunsaturated fatty acids are found in NS oil along with phytosteracopherols and dihomolinolic acids (Ahmad et al., 2013; Sultan et al., 2014). NS supplementation in meal is the source of high protein (30%) and most of the essential amino acids are required for normal body metabolism (Aydin et al., 2008; Taha et al., 2007). Omar et al. (2002) reported that the use of diet supplemented with NS seed oil decreases the feed conversion ratio, hence improves the growth performance and immune responses, which in turn, increases the economic return of chickens. However, low doses of NS seed and its oil are beneficial than the higher toxic dose (El-Bahr, 2007).

The objective of the present study was to evaluate the hematobiochemical influence of oral administration of black seed (N. sativa) oil on hematology and biochemical parameters in adult rabbits.

MATERIALS AND METHODS

Animals

A total of twenty healthy adult male rabbits weighing between 1000-1200 g and aged between 8-9 months were subjected to this study. The animals were aclimatized under optimal environment conditions for temperature, humidity, ventilation and light for two weeks in the animal house of Faculty of Veterinary Science, University of Agriculture, Faisalabad. Lucerne@ 1 kg/rabbit/day and wheat porridge @ 20 g /kg/day were given daily to each animal along with water ad libitum.

Plant materials

NS seedwere bought from local grain market of Faisalabad and taxonomic identification of seeds was confirmed by a qualified taxonomist of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. After identifications, the seeds were dried (air dry) and oil was extracted using traditional method.

Method for extraction of oil

The black seeds were cleaned and foreign materials were removed. Seeds were ground using mill stones and then, placed into steel rolls. High oil content of the seed were mechanically pressed in expellers and thereafter preheated. It was then added into heated conditioners. The oil content of seed were fed into one end of a cylinder where a power-driven worm conveyor expelled oil contents of seed material to the other end of the cylinder. The pressure was exerted on the seed and through this process oil was squeezed out.

The separation of oil from seeds was done through solvent extraction method. The pre-processed black seeds were treated with solvent in a multistage counter current process until the remaining oil content were reduced to the lowest level. Through the distillation process, mixture of oil and solvent were separated and the solvent again recycled into the solvent extraction process and the crude black seed oil was stored and ready for refining (Rubin et al., 1979).

Research design

The animals were randomly divided into two groups A and B with 10 biological replicates in each group. Group A was considered as control which received food and water only. Group B was treated with NS oil orally @ 5 ml/kg body weight on daily basis for 60 days in addition to the diet of Group A.

Collection of samples

The rabbits were slaughtered with the help of a sharp knife after gaseous anesthesia at the end of trial (60 days). Before slaughtering, blood was collected from jugular vein into two test tubes, one with and the other without EDTA, for hematology and serum analysis, respectively.

For serum separation, the blood was allowed to clot and then centrifuged at 3000 rpm for 5 min. The serum was
Table 1: Mean ±SEM values of hematological parameters at the end of experiment (60 days) in control (Group A) and NS oil (@ 5ml/kg/day) treated group (Group B).

<table>
<thead>
<tr>
<th>Hematological parameter</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Erythrocyte Count (x10^6/ul)</td>
<td>5.54±0.11</td>
<td>6.32±0.19*</td>
</tr>
<tr>
<td>Total Leukocyte Count (x10^9/l)</td>
<td>5.74±0.11</td>
<td>6.70±0.15*</td>
</tr>
<tr>
<td>Hb level (g/dl)</td>
<td>12.1±0.13</td>
<td>12.5±0.05*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>36.86±0.61</td>
<td>38.52±0.55*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.38±0.16</td>
<td>41.2±0.15^</td>
</tr>
<tr>
<td>MCV (um^3)</td>
<td>57.64±0.11</td>
<td>56.46±0.08^</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.52±0.31</td>
<td>20.28±0.19*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>19.52±0.31</td>
<td>20.28±0.19*</td>
</tr>
</tbody>
</table>

*: Indicate a significance (P<0.05) difference (increase) between treated and control group.

^: Indicate a significance (P<0.05) difference (decrease) between treated and control group.

separated and stored at -20°C for biochemical analysis.

Hematological analysis

Hematological analysis of parameters including total RBCs count (TEC), total WBC count (TLC), hemoglobin level (Hb), mean corpuscular volume (MCV), Hematocrit (HCT) level, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) was performed using hematological analyzer (Abacus Junior Vet®) to elucidate the effect of oral administration of NS oil on rabbits.

Serum biochemistry

Serum biochemistry parameters, including concentration of total proteins, albumin, triglyceride, serum glutamate oxaloacetate transaminase (SGOT) total lipids, total cholesterol and serum glutamate pyruvate transaminase (SGPT), were measured with the help of commercial diagnostic kits (Combination, Pasteur Lap.®). However, concentration of globulin was obtained by calculating the difference. All biochemical parameters were determined using spectrophotometer (Spectronic 21 DUSA).

Hormonal analysis

For serum testosterone, FSH and LH concentration analysis, the stored serum samples were used. The serum was run on commercially available Radioimmunoassay (RIA) kits (IMMUNOTEC, Marseille, France). For testosterone assay, the normal detectable concentration was 0.02 ng/ml.

Statistical analysis

To compare the means of the parameters, one-way analysis of variance (ANOVA) was performed. The 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 hematological parameters

The results obtained after the statistical analysis of the hematological parameters including TEC, TLC, Hb, PCV, HCT, MCV, MCH and MCHC of blood in rabbits are shown in Table 1. All the studied hematological parameters were found to significantly (P<0.05) increased in treated animals (Group B) as compared with the control animals (Group A) except the MCV which showed the otherwise trend in results.

Effects of black seed (Nigella sativa) oil on serum biochemistry

The effect of NS oil on different serological parameters including concentration of total proteins, albumin, globulin, SGOT, SGPT and total lipids, triglyceride and total cholesterol of serum in rabbits are shown in Table 2. The mean values of total protein, albumin and globulin, were measured to be significantly (P<0.05) higher in NS oil treated group. Some serological parameters such as total lipids, triglycerides and total cholesterol were significantly (P<0.05) decreased by the NS oil treatment, while SGOT and SGPT concentrations remained uneffected by NS treatment (P<0.05).

Effects of black seed (Nigella sativa) oil on reproductive hormonal profile

The effects of black seed (N. sativa) oil on the mean values
Table 2: Mean ±SEM values of serum biochemistry at the end of experiment (60 days) in control (Group A) and NS oil (@ 5ml/kg/day) treated group (Group B).

<table>
<thead>
<tr>
<th>Serum biochemistry</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Total proteins (g/100ml)</td>
<td>8.17±0.01</td>
</tr>
<tr>
<td>Albumin (g/100ml)</td>
<td>3.56±0.01</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.55±0.02</td>
</tr>
<tr>
<td>SGOT (mu/mol)</td>
<td>28.58±0.02</td>
</tr>
<tr>
<td>SGPT (mu/mol)</td>
<td>52.30±0.02</td>
</tr>
<tr>
<td>Total lipids (mg/dl)</td>
<td>312±13.04</td>
</tr>
<tr>
<td>Triglyceroids (mmol/L)</td>
<td>1.66±0.010</td>
</tr>
<tr>
<td>Total cholestrol (mg/100ml)</td>
<td>100.54±0.31</td>
</tr>
</tbody>
</table>

*: Indicate a significance (P<0.05) difference (increase) between treated and control group.
^: Indicate a significance (P<0.05) difference (decrease) between treated and control group.

Table 3: Mean ±SEM values of reproductive hormonal profile at the end of experiment (60 days) in control (Group A) and NS oil (@ 5ml/kg/day) treated group (Group B).

<table>
<thead>
<tr>
<th>Hormonal Profile</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>333.54±3.43</td>
</tr>
<tr>
<td>FSH (mU/ml)</td>
<td>0.664±0.02</td>
</tr>
<tr>
<td>LH (mU/ml)</td>
<td>0.554±0.015</td>
</tr>
</tbody>
</table>

*: Indicate a significance (P<0.05) difference (increase) between treated and control group.

of reproductive hormonal levels including testosterone, FSH and LH of serum in rabbits are given in the Table 3. A significant (P<0.05) upsurge of serum concentrations of testosterone, FSH and LH was observed in NS oil treated rabbits (Table 3).

**DISCUSSION**

NS (*N. sativa*) is extensively used worldwide as a remedy in many disorders including blood cancer and autoimmune diseases. The supplementation of NS in the meal has a diverse range of hematological effects. Some studies reported therapeutic while other showed the toxic effects of NS. In the present study, we tried to explore the hematobiochemical effects of NS oil on rabbits. The rabbits were divided into two groups: control and treated (with NS oil) groups.

In this trial, a significant (P<0.05) elevation was seen in erythrocyte count and its indices (Hb, PCV, Hematocrit, MCV, MCH) as compared with the control group, while Al-Nazawi and El-Bahr (2012) and Juma and Abdulrahman (2011) reported non-significant effect of NS seed on these parameters in rats. This difference in erythrocyte synthesis may be attributed to the dose of the NS seeds. It is also possible that there may be an ingredient in activated form in the NS oil which is not activated in NS seed. Therefore, dose rate and activity of the ingredient influenced positively the erythrocytes synthesis in our study.

When NS is administered into a diseased individual, it leads to an increase in leukocytic count that indicates it has an immune-boosting effect (Aftab et al., 2013). This finding is in accordance with the finding of the present study where the treated group showed a significant increase (P<0.05) in leukocytic count than the control group. The immune-modulatory effect of NS oil is partially mediated by thymoquinone, an antioxidant present in NS (Mohammed and Al-Suwaiegh, 2016). The bioactive compound of NS, thymoquinone, has both antioxidant and prooxidant property but latter one is only observed in cancerous cells. The immune modulation by this compound is due to the suppression of many cytokines involved in disease progression and selective prohibition of many disease related proteasomes (Khan et al., 2017).

A significant improvement in the total protein, albumin and globulin by NS oil supplementation in the treated rabbits as compared with the controlled group are in agreement with the finding of Khattab et al. (2011) on buffalo offspring. The increased concentration of aforementioned parameters can be due to the improved hepatic function by NS oil (Tousson et al., 2011). The significant decrease in the triglyceroids and total cholesterol was observed in this study which is in line with the findings of Daghash et al. (1999). However, Khattab et
al. (2011) found that NS administration in buffalo offspring caused significant increase in plasma cholesterol. The biological ingredients such as unsaturated fatty acids along with thymoquinone disturb the synthesis of cholesterol by liver and decrease the intestinal uptake of cholesterol. There was a non-significant alteration in the liver enzymes (SGOT and SGPT) concentrations, indicating that NS oil has non-toxic effects on liver. The liver enzymes, such as SGOT and SGPT are present normally in the hepatocytes and to some extent in the cardiac myocytes. Any kind of injury to these cells results in the significant augment in serum levels of these enzymes. Dollah et al. (2013) reported similar trend of liver enzymes in NS treated rats and concluded that high dose of NS (100 times than the normal) did not cause any hepatotoxic effect.

A significant rise in the reproductive hormones including pituitary gonadotrophins (FSH and LH) and testosterone level in our study is similar to the findings of Zanouny et al. (2013) in lambs when they were supplemented by NS. The chemical constituents that enhance the reproductive parameters in NS seed are different types of fatty acids such as linoleic acid, palmitic acid and oleic acid. Thymoquinone and these fatty acids confront the oxidative stress by decreasing superoxide dismutase and glutathione peroxidase in testicular compartments (Zanouny et al., 2013). These active ingredients of NS may have positively enhanced the pituitary gonadotrophins which ultimately stimulate the Leydig cells for testosterone secretion. The NS seed also improves the testicular cellular content (Umer et al., 2017) which may be associated with the elevated reproductive hormonal profile in treated rabbits.

Conclusion

Traditionally NS is used as a medicinal herb to cure many diseases in the form of dietary supplementation. This study showed that it has no toxic effects on liver and hematological parameters. However, upregulation in male reproductive hormones was observed and as such, it can be concluded that the consumption of NS as a dietary supplement may be recommended in male infertility problems in humans, as well as animals without altering the liver functions.

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