Anti-androgenic and insulin-sensitizing actions of *Nigella sativa* oil improve polycystic ovary and associated dyslipidemia and redox disturbances

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**Background/Aim:** *Nigella sativa* oil has been shown to improve metabolic syndrome-associated dyslipidemia and oxidative stress. Effects of *N. sativa* oil on letrozole-induced polycystic ovary syndrome (PCOS)-associated hormonal, metabolic, redox, and lipid dysregulation in rats were investigated.

**Methods:** Female Sprague-Dawley rats were randomly assigned into four groups (*n* = 10 per group): control, PCOS, and PCOS treated with *N. sativa* oil (5 or 10 ml/kg/day) once daily *per oral* starting at day 7 after the commencement of letrozole (1 mg/kg) until day 56. Body weight and estrous cycle were recorded starting from day 7 and day 28, respectively, until day 56. Rats were dissected and serum and ovaries were collected for biochemical and histomorphological assessments.

**Results:** *Nigella sativa* oil (10 ml/kg/day) treatment significantly increased: number of rats undergoing regular cycle (80% vs. 20%; *p* < 0.05), average number of regular cycles (5.00 ± 0.59 vs. 0.20 ± 0.42; *p* < 0.05), appearance of corpus luteum; but reduced number of cystic follicles (7.60 ± 1.51 vs. 6.10 ± 0.19; *p* < 0.05) in PCOS rats. Body weight was also reduced by the oil in PCOS rats. However, ovarian weight, granulose, and theca cells layers showed no significant change. The oil also restored altered circulating levels of gonadotropins, steroids, and reduced fasting blood glucose. The ovarian total cholesterol, triglycerides, low-density lipoprotein-cholesterol, and malondialdehyde were markedly reduced while insulin sensitivity and ovarian high-density lipoprotein-cholesterol, superoxide dismutase, and glutathione peroxidase activities were greatly increased.

**Conclusion:** *Nigella sativa* oil improved polycystic ovarian-morphology and metabolic disorders via its anti-androgenic and insulin-sensitizing actions to ameliorate PCOS-associated dyslipidemia and redox disturbances.

**Introduction**

Polycystic ovarian syndrome (PCOS) is the commonest endocrine disorder affecting about 20% women of reproductive age around the world. It is characterized by hyperandrogenism, acyclicity, polycystic ovary and metabolic disorders [1] such as insulin resistance.
(IR), obesity, and diabetes [2]. PCOS-induced up-regulation of pro-inflammatory and oxidative stress markers increases patients’ risk of dyslipidemia, type 2 diabetes mellitus (T2DM), and cardiovascular disorders [3,4]. Dyslipidemia has been reported in most PCOS patients (obese/non-obese and young/old) [5]. Dyslipidemia has a prevalence of 70% in women with PCOS and has been linked with hyperandrogenism and IR. Hyperandrogenemia and IR affect lipid profile in PCOS via mechanisms relating to cholesterol metabolism, uptake, and efflux from peripheral cells. They also increase immune cells activation to release pro-inflammatory cytokines and enhance excessive production of oxidants. Therefore, elevated oxidative stress is commonly reported in PCOS [6].

PCOS is usually treated based on clinical symptoms and therapeutic schedule. Dyslipidemia is a foremost therapeutic schedule in overweight/obese PCOS women, thus weight loss is recommended [5]. However, the combined effect of IR, hyperandrogenemia, low metabolic rate, and disturbed appetite regulation makes weight loss difficult in PCOS [7]. Clomiphene citrate (CC), the first-line treatment of PCOS is associated with minor and major side effects apart from the subsection of PCOS women that are CC resistant. Other forms of treatment such as gonadotropin-releasing hormone (GnRH), metformin, letrozole, and tamoxifen are also associated with side effects [6].

*Nigella sativa* oil was reported to modulate lipid metabolism and redox status such as by reducing low-density lipoprotein cholesterol (LDL-C) and by increasing high-density lipoprotein (HDL-C) in IR syndrome [8]. Administration of 2.5 ml *N. sativa* oil per day in T2DM reduced total cholesterol (TC) improved glycemic control without any side effects [8,9]. The antioxidant and lipid modulating effects of *N. sativa* oil and its major active compound thymoquinone (TQ) were reported in different *in vitro* and *in vivo* disease models [10–13]. Recent studies support the hypolipidemic and redox modulating properties of *N. sativa* oil and TQ [13–17]. Here we report the beneficial effect of *N. sativa* oil in ameliorating PCOS-associated conditions (dyslipidemia and redox disturbances) via its anti-androgenic and insulin-sensitizing actions.

**Materials and Methods**

**Chemicals and reagents**

Essential oil (100%) of *N. Sativa*, extracted using cold pressing, was procured from Hemani International (Hemani International KEPZ, Karachi, Pakistan) and letrozole from Novartis Pharmaceuticals (Accord Healthcare, Middlesex, UK). The assay kits for thio-barbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), cholesterol, triglycerides (TGs), HDL-C, and LDL-C are products of Cell Biolabs, Inc. (Cell Biolabs Inc, San Diego, USA). Serum sex steroids (testosterone; T and estradiol; E2) and gonadotropins [follicle stimulating hormone (FSH) and luteinizing hormone (LH)] levels were determined using enzyme-linked immunosorbent assay (ELISA) kit procured from Monobind Inc, Lake Forest, USA. Glutathione peroxidase (GPX) assay kit is from Sigma-Aldrich, St. Louis, MO, USA. Blood glucose level was determined using Glucose Roche Diagnostic glucometer strip. Other chemicals include carboxymethylcellulose (CMC), normal saline, formalin, glucose, ethanol, diethyl ether, and picric acid. The chemical reagents were of analytical grade and purchased mainly from Sigma-Aldrich except otherwise stated.

**Animal ethical committee approval**

The study procedure and animal handling were carried out in accordance with the Guideline for Care and Use of Laboratory Animals of the University of Ilorin and were approved by the Ethics Committee (UERC/ASN/2019/1805), Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.

**Experimental animals**

Virgin female Sprague-Dawley rats (40) weighing 150.0 ± 5.0 g and aged 6 weeks old with 4–5 days regular estrus were obtained, kept, and observed for general well-being in the Animal Holding Facility of the University of Ilorin under standard housing condition (12 hours light/dark cycle at 21°C–24°C and 40%–45% humidity). They were kept in polypropylene cages and fed standard rat chow and water *ad libitum*. The animals were acclimatized for 7 days before treatment commenced.

**Experimental design**

The animals were randomly assigned into four groups of which *n* = 10 animals per group: control, PCOS, and treatment groups (*N. sativa* oil; 5 and 10 ml/kg/day). The selected doses of *N. Sativa* oil have been considered as the standard and maximum volumes of oils that could be reasonably administered to rats over a long period of time without dose/volume-related disturbances [18]. All animals were induced with PCOS, except the control group, using oral letrozole (1.0 mg/kg). Control and PCOS groups

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**N. sativa oil improves polycystic ovary**
received vehicle [10 ml/kg/day, per oral (p.o.)] and the treatment groups were given *N. sativa* oil (5 and 10 ml/kg/day, p.o.). Body weight and estrous cycle were monitored from day 7 and day 28, respectively, after the commencement of letrozole administration until the 56th day (Fig. 1).

**PCOS induction and treatments**

PCOS was induced in the experimental rats using letrozole (1 mg/kg) that was prepared in 0.5% CMC and administered to the rats, orally, once daily throughout the experimental period of 56 days. In addition, *N. sativa* oil (100%), in graded doses of 5 and 10 ml/kg/day, [18] was administered to the test groups from day 7 of the commencement of letrozole administration and 0.5% CMC p.o. was administered to the control group throughout the experimental period (Fig. 1). The study analysis was conducted and data were collected between August 2018 and March 2019.

**Estrous cycle assessment and specimen collection**

Vaginal smear cytology was carried out to determine estrous phases following standard procedure [19]. Rats from all the groups were fasted overnight and anesthetized with diethyl ether on day 57. Peripheral blood was collected through retro-orbital sinus puncture and serum was separated by centrifugation and stored at −20°C until biochemical analysis. Dissected right ovaries were weighed, fixed in 4% paraformaldehyde at 4°C overnight, and stored in 70% ethanol before histological processing while the left ovaries were homogenized in ice cold 1:4 w/v phosphate buffered saline and centrifuged at 4°C for 30 minutes at 3,000 relative centrifugal force. The supernatant collected was used for the estimation of antioxidant enzymes, protein content, and lipid profile.

**Hormonal assays**

Serum T, E2, LH, and FSH were determined using species-specific ELISA kit (Monobind Inc, Lake Forest, USA) following the manufacturer’s instructions. The absorbance in each well was read at 450 nm in a microplate reader (Biocompare, CA, USA) and compared with the standard. The gonadotropins detection limit, coefficient of variations (CVs) for inter- and intra-assays were 0.134 mIU/ml, 5.2% and 8.6%, respectively, for FSH and 0.054 mIU/ml, 7.2% and 3.1% for LH. The steroids detection limit, CVs for inter- and intra-assays were 0.058 pg/ml, 5.2% and 8.6%, respectively, for T and 8.20 pg/ml, 3.1% and 7.2% for E2. All immunoassays were performed in a single batch.

**Evaluation of oral glucose tolerance, fasting blood glucose, and serum insulin**

Fasting blood glucose and oral glucose tolerance test (OGTT) was carried out as previously described [20]. Fasting serum insulin concentration was measured, using Accu-Bind ELISA kits procured from Monobind Inc., according to the manufacturer’s

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**Figure 1.** The experimental design. Rats were assigned into control, PCOS, and *N. sativa* oil (5 and 10 ml/kg/rat/day)-treated PCOS groups. Control received carboxymethyl cellulose (0.5% CMC p.o.), PCOS and *N. sativa* oil (5 and 10 ml/kg/rat/day)-treated PCOS groups received letrozole (1 mg/kg p.o.) prepared in 0.5% CMC throughout the experimental period. In addition, the *N. sativa* oil-treated groups were subsequently treated with *N. sativa* oil (5 and 10 ml/kg/rat/day) throughout the experimental period starting from day 7 after the commencement of letrozole. At the end of the experimental period (57th day), rats from all the groups were euthanized using diethyl ether anesthesia and blood and ovaries were collected for biochemical analysis and histopathological studies, respectively.
instructions. Quantitative Insulin Sensitivity Check Index (QUICKI) was estimated according to the following formula [21].

\[
\text{QUICKI} = \frac{1}{\log (I_0) + \log (G_o)}, \text{ where } I_0 \text{ is the fasting plasma insulin (microunits/ml) and } G_o \text{ is the fasting serum glucose (mg/dl)}.
\]

**Evaluation of ovarian lipid profile**

Ovarian TGs, TC, HDL-C, and LDL-C concentrations were estimated using commercially available assay kits (ERBA Diagnostics, USA) following the manufacturer’s instruction on a fully automated analyzer based on spectrophotometric principle.

**Determination of ovarian antioxidant enzymes activity**

CAT activity was assayed according to the previously described rapid spectrophotometric method of Cohen et al. [22] and the enzyme activity was calculated according to Aeubi [23]. The enzyme activities were normalized with the protein content of each sample. CAT activity was calculated as units per milligram of protein. SOD activity was measured according to the method previously described [24] which is based on the ability of SOD to scavenge superoxide anion radical and inhibits the auto-oxidation of pyrogallol. The absorbance was measured kinetically at 420 nm, 25°C for 3 minutes. SOD activity was calculated as U/mg of protein, with 1U defined as the amount that inhibited the rate of pyrogallol autoxidation by 50%. GPX activity assay kit (Sigma-Aldrich, St. Louis, MO, USA) was used for measuring the GPX activity. It is based on the oxidation of reduced glutathione by GPX coupled to the disappearance of nicotinamide adenine dinucleotide phosphate reduced (NADPH) by glutathione reductase. GPX activity was calculated as units per gram of protein, with 1U GPX causing the formation of 1.0 µmol of nicotinamide adenine dinucleotide phosphate (NADPH) from NADPH per minute.

**Lipid peroxidation (LP) assay**

Measurement of LP was carried out based on the TBARS method as described by Buege and Aust [25]. LP in the form of malondialdehyde (MDA) equivalent was expressed as nmol/mg protein in the sample.

**Hematoxylin and eosin (H&E) staining**

Ovarian tissues, fixed in 4% paraformaldehyde, were processed following the standard protocol. The tissues were sectioned (5 µm), mounted onto Superfrost Plus slides (Fisher Scientific), and stained with H&E following the standard protocol for histology. Histological examination was performed using a Nikon light microscope (Nikon, Tokyo, Japan) with an in-built camera attached to a monitor. Assessment was blind to the experimental design and performed on three sections from each group.

**Statistical analysis**

Using data from previously published literature [26], the sample size was estimated in the NCSS 2007 software (NCSS Statistical Software). Normalcy test was performed on the data and those that were not normally distributed were transformed before analysis using a log transformation. The statistical package, i.e., IBM SPSS Version 23 (SPSS Inc., Chicago, IL), was used for data analysis. Briefly, values presented in Tables and charts are means ± standard error of mean. Statistical differences were tested by One-way-analysis of variance with post hoc test using Fisher’s least significant difference Multiple-Comparison Test. Analysis was considered statistically significant at \( p < 0.05 \).

**Results**

*Nigella sativa* oil restored cyclicity and improved ovarian histomorphology in PCOS rats

Control rats exhibited a normal estrous cycle of 4–5 days, ~6.75 ± 0.33 average number of complete cycles in 28 days, and all the rats (10 of 10; 100%) cycles normally (Fig. 2A–C). *Nigella sativa* oil (10 ml/kg/rat/day, p.o.) improved estrous cycles, increased significantly number of complete cycles in 28 days (5.00 ± 0.59 vs. 0.20 ± 0.42; \( p < 0.05 \)), and the number of rats (8 of 10; 80%, \( p < 0.05 \)) undergoing regular cycle when compared to the PCOS group (Fig. 2A–C). Ovarian histomorphology also showed no cytoarchitectural abnormalities in the control rats (Fig. 2D). The granulose cell layer remains markedly diminished without significant improvement in the theca interna layer but there was an increase appearance of corpus luteum in the ovary of *N. sativa* oil-treated rats (Fig. 2D). PCOS rats were completely acyclic and remain static at "pseudo diestrus" phase with significant (\( p < 0.05 \)) decrease in the average number of complete cycles in 28 days (0.20 ± 0.42 vs. 6.75 ± 0.33; \( p < 0.05 \)) and most of the PCOS rats (8 of 10; 80%) failed to cycle (\( p < 0.05 \)) (Fig. 2A–C). The ovary-to-body weight ratio (%) showed no significant difference (\( p > 0.05 \)) among the control (0.12 ± 0.03), PCOS (0.08 ± 0.08), and the treated groups (*N. sativa* oil 5 ml/kg/rat/day; 0.10 ± 0.02 and *N. sativa* oil 10 ml/kg/rat/day; 0.10 ± 0.01).
However, PCOS rats exhibited significantly increased number of cystic follicle orientated in the periphery of the ovary (7.60 ± 1.71 vs. 0.00 ± 0.00; \( p < 0.05 \)) and enlarged cystic follicular area characterized by a thickened theca interna cell layer and a diminished granulosa cell layer without any obvious corpus lutea in PCOS rats compared with controls. Nigella sativa oil (5 ml/kg/rat/day and 10 ml/kg/rat/day) reduced the number of cystic follicle significantly when compared to PCOS (6.50 ± 0.18 and 6.10 ± 0.19 vs. 7.60 ± 1.51; \( p < 0.05 \)) (Fig. 2F).

**Nigella sativa oil reduced elevated body weight in PCOS rats**

PCOS group showed significant (\( p < 0.05 \)) increase in body weight when compared to all other groups. Body weight of N. sativa oil treated and the reference groups were significantly (\( p < 0.05 \)) higher compared to controls but remain similar throughout the experiment (Fig. 3).

**Nigella sativa oil altered circulating levels of gonadotropins and sex steroids in PCOS rats**

The serum levels of T, LH, and FSH increased and E2 decreased significantly (\( p < 0.05 \)) in the PCOS group when compared to control. Treatment with N. sativa oil (5 or 10 ml/kg/rat/day) significantly (\( p < 0.05 \)) altered serum levels of E2 (increased) and LH (decreased). However, treatment with N. sativa oil (10 ml/kg/rat/day) reduced serum T and
FSH levels significantly ($p < 0.05$) when compared with PCOS rats. Notably, serum levels of E2 and LH in the treated groups showed no significant ($p > 0.05$) change when compared with controls. T and LH levels remain significantly ($p < 0.05$) increased when compared with control and decreased when compared with PCOS rats (Table 1).

**Nigella sativa oil improved letrozole-induced PCOS associated metabolic disturbances**

PCOS group exhibited significant ($p < 0.05$) increase in serum insulin and fasting blood glucose levels with associated significant ($p < 0.05$) decrease in insulin sensitivity when compared with the control group. Nigella sativa oil significantly ($p < 0.05$) reduced fasting blood glucose and serum insulin levels associated with significant ($p < 0.05$) increase in insulin sensitivity when compared with the PCOS group. Fasting serum insulin and blood glucose levels in N. sativa oil-treated group remain significantly ($p > 0.05$) elevated when compared with control and decreased when compared with PCOS rats.

Table 1. Effect of 7 weeks administration of N. sativa oil on serum levels of LH, FSH, E2, and T in letrozole (1 mg/kg)-induced PCOS rats.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>E2 (pg/ml)</th>
<th>T (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.50 ± 1.84</td>
<td>0.90 ± 0.06</td>
<td>16.52 ± 2.11</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>PCOS</td>
<td>9.00 ± 0.50*</td>
<td>1.27 ± 0.07*</td>
<td>12.23 ± 0.21</td>
<td>0.98 ± 0.22*</td>
</tr>
<tr>
<td>N. sativa oil (5 ml/kg)</td>
<td>6.00 ± 0.52*</td>
<td>1.17 ± 0.05*</td>
<td>15.10 ± 1.55</td>
<td>0.62 ± 0.14*</td>
</tr>
<tr>
<td>N. sativa oil (10 ml/kg)</td>
<td>6.00 ± 0.02*</td>
<td>1.01 ± 0.03*</td>
<td>15.66 ± 0.72</td>
<td>0.48 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± standard errors (n = 10). LH = luteinizing hormone; FSH = follicle stimulating hormone; E2 = estrogen; T = testosterone.

*Mean values were significantly different compared with control rats at $p < 0.05$.

#Mean values were significantly different compared with PCOS rats at $p < 0.05$.

![Figure 4](image-url)  
**Figure 4.** Serum insulin, insulin sensitivity, fasting blood glucose, and OGTT in letrozole-induced PCOS rats. A, Serum insulin concentrations. B, Insulin sensitivity. C, Fasting blood glucose concentrations. D, OGTT curve. Values are means ± standard errors (n = 10). * Mean value was significantly different compared with normal control ($p < 0.05$). # Mean value was significantly different compared with the PCOS group ($p < 0.05$).
90 minutes after oral glucose load (2 g/kg) was administered to the PCOS and N. sativa oil (5 ml/kg/rat/day)-treated groups (Fig. 4D). *Nigella sativa* oil (10 ml/kg/rat/day) enhanced glucose tolerance by lowering the blood glucose concentration at all points along the OGTT curve without a significant difference when compared to controls. *Nigella sativa* oil (5 ml/kg/rat/day) lowered the blood glucose concentration significantly ($p < 0.05$) only at the 90 minutes point of the OGTT curve (Fig. 4D).

**Nigella sativa oil improved ovarian lipid profile in letrozole-induced PCOS rats**

The PCOS ovarian tissue lipid profile showed a significant ($p < 0.05$) increase in TC, TG, LDL-C, and decrease HDL-C when compared with control (Table 2). *Nigella sativa* oil (5 ml/kg/rat/day) reduced TG and increased HDL-C significantly ($p < 0.05$) when compared with the PCOS group. The high dose of *N. sativa* oil (10 ml/kg/rat/day) significantly ($p < 0.05$) reduced ovarian TC, TG, LDL-C, and increased HDL-C when compared with the PCOS group. Notably, *N. sativa* oil (10 ml/kg) further reduced LDL-C more significantly ($p < 0.05$) when compared with control (Table 2).

**Nigella sativa oil enhanced ovarian antioxidant status in letrozole-induced PCOS rats**

PCOS group exhibited significant ($p < 0.05$) decrease in ovarian CAT, SOD, and GPX activities and an increase in LP (MDA) when compared to the control group (Fig. 5). Treatment with *N. sativa* oil significantly ($p < 0.05$) increased the ovarian SOD and GPX activities in a dose-dependent manner but CAT activities remain comparable with the PCOS group. Lipid peroxidation as assessed by the MDA equivalent was significantly ($p < 0.05$) reduced by the high dose of *N. sativa* oil (10 ml/kg/rat/day) only when compared to the PCOS group (Fig. 5).

**Discussion**

Nutritional and pharmacological benefits of *N. sativa* oil are commonly reported in conditions relating to pre-diabetes, IR, and T2D [14,17]. These conditions along with chronic systemic inflammation, disturbed redox status, and dyslipidemia are common co-morbidities of PCOS [6,27]. Therefore, we hypothesized that metabolic disorders relating to lipid profile and redox status that was previously shown to be modulated by *N. sativa* oil in T2D could also be ameliorated in PCOS. Till date, the modulatory properties of *N. sativa* oil on PCOS-associated dyslipidemia and redox disturbances remain unclear. Hence, investigation on the therapeutic schedule to improve lipid profile and redox status in PCOS using *N. sativa* oil could pave way for the use of this dietary oil as a supplemental functional food against PCOS-associated disorders, especially in the overweight/obese women.

Cystic ovary, acyclicity, altered ovarian thecal interna, and granulose cell layer which constitute core features of PCOS were all observed in the letrozole-induced PCOS rats except the ovary weight which showed no significant change (Fig. 1A–F). Daily administration of *N. sativa* oil (10 ml/kg/rat/day) restored uterine cyclicity in most of the animals, reduced ovarian follicular cyst, and enhanced corpus luteum appearance in a manner comparable to the control except in few details such as theca interna which is thinner in the control group. Previous studies have variously shown that letrozole, an alpha aromatase inhibitor, induced most of the endocrine and ovarian features of PCOS. Exposure to letrozole induced PCOS with striking morphological similarities to human PCOS, including a thickened theca cell layer, anovulation, and increased ovarian weight and size along with increased body weight [17,20].

**Table 2.** Effect of 7 weeks administration of *N. sativa* oil on ovarian lipid profiles in letrozole (1 mg/kg)-induced PCOS rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>HDL-C (mmol/l)</th>
<th>LDL-C (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.56 ± 0.15</td>
<td>0.29 ± 0.06</td>
<td>0.52 ± 0.03</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>PCOS</td>
<td>3.25 ± 0.20</td>
<td>0.52 ± 0.12</td>
<td>0.33 ± 0.02</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td><em>N. sativa</em> oil (5 ml/kg)</td>
<td>3.31 ± 0.44</td>
<td>0.37 ± 0.03*</td>
<td>0.54 ± 0.03*</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td><em>N. sativa</em> oil (10 ml/kg)</td>
<td>2.54 ± 0.24*</td>
<td>0.30 ± 0.03*</td>
<td>0.60 ± 0.04*</td>
<td>0.15 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± standard errors ($n = 10$). HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; TG triglycerides.

*Mean values were significantly different compared with control rats at $p < 0.05$.

*Mean values were significantly different compared with PCOS rats at $p < 0.05$. 
Different approaches such as; prenatal and postnatal treatment with androgens, estrogen, letrozole, and antiprogestin, exposure to constant light, and genetic modification have all yielded varying characteristic features of PCOS [17,18]. However, the postnatal letrozole model, used in this study, was shown to consistently produce the cardinal features of human PCOS, i.e., hyperandrogenism, acyclicity, polycystic ovary, and metabolic disorders. More importantly, the model exhibits metabolic derangement including IR, hyperglycemia, hyperlipidemia, oxidative stress, and features of metabolic syndrome [26,28]. These features develop as a pathologic manifestation of hyperandrogenemia, a key factor in the etiology of PCOS. Letrozole inhibits non-steroidal aromatase enzymes to prevent the conversion of testosterone and androstenedione to estradiol and estrone, respectively. These alter sex steroid hormone levels and their feedback on the hypothalamic–pituitary–gonadal (HPG) axis to distort the pulsatile secretion of GnRH from the hypothalamus—a key to establishing and maintaining normal gonadal function. Consequently, letrozole causes hormonal dysregulation, circulating and intra-ovarian hyperandrogenism, the appearance of polycystic ovarian morphology, follicular atresia, and thickened theca cell layer [29]. Letrozole model is superior in the studying of ovarian features of human-like PCOS and as such selected in the present study.

Till date, there is a limited report on the beneficial effect of \textit{N. sativa} oil in PCOS. However, Arif et al. [30] recently employed in vitro and in vivo model systems to investigate the effect of TQ on ovarian morphology and function. They found that pre-treatment with TQ in a PCOS rat model [sc. inj. of

\textbf{Figure 5.} Ovarian antioxidant enzymes activities and LP product MDA in letrozole-induced PCOS rat treated with \textit{N. sativa} oil. A, Ovarian tissue CAT activities. B, Ovarian tissue SOD activities. C, Ovarian tissue GPX activities. D, Ovarian tissue MDA content. Values are means ± standard errors (\(n = 10\)). * Mean value was significantly different compared with normal control (\(p < 0.05\)). # Mean value was significantly different compared with the PCOS group (\(p < 0.05\)).
pregnant mare serum gonadotrophin (PMSG) 20 IU followed by human chorionic gonadotropin 20 IU] significantly reduced cysts formation, increased ovulation rate, and normalize ovarian levels of key factors that influence follicular maturation. TQ is the most abundant volatile phenolic compound which has been consistently implicated for pharmacological actions of *N. sativa* oil, especially the widely reported anti-inflammatory, antioxidant, and immunomodulatory actions [30]. *Nigella sativa* oil has been shown to be a reservoir for the major phenolic compounds in *N. sativa*. Using supercritical 

Critical CO₂ extraction at different conditions and hydrodistillation, a total of 47 different compounds were detected in *N. sativa* oil with TQ constituting the most abundant (33.12%–38.41%) in all factions obtained. Dithymoquinone, thymohydroquinone, and thymol were also found in an appreciable amount in *N. sativa* oil which suggests their contribution to the therapeutic and nutritional benefits of *N. sativa* oil [31].

Hormonal dysregulation, a cardinal feature of PCOS, is well preserved in the letrozole model. Serum levels of T, LH, and FSH were markedly increased while E2 decreased in the PCOS rats. Treatment with *N. sativa* oil reduced the elevated serum levels of T, LH, FSH, and increased E2 to values comparable to control. Elevated T and LH levels were the main hormonal disorders commonly associated with human PCOS. However, elevated T, LH, FSH, and reduced E2 have been reported in the letrozole-induced PCOS model which concur with the current study [28]. Few reports have observed no change in the E2 and FSH levels; however, almost all related studies have reported hyperandrogenemia (elevated level of T) in the letrozole model of PCOS. The small variation in the previous and present study could be linked with the type of animal used, dose, duration, nature and form of the drug (letrozole) used for the PCOS induction [26,32]. Caldwell et al. [32], used two implantable pelleted continuous release letrozole (for PCOS induction in 21 days old female mice) which release as low as ~40–400 µg letrozole daily over a prolonged period of 90 days [26,32]. Treatment with *N. sativa* oil markedly reduced FBG and restored insulin level, insulin sensitivity, and glucose tolerance. These beneficial effects of *N. sativa* oil are commonly reported in T2D [36,37]. Enhanced insulin sensitivity and compensatory decreased insulin level ameliorate disorders relating to glucose metabolism and insulin-mediated testosterone actions. These may not be unconnected with *N. sativa* oil-induced activation of insulin receptors to improve tissue insulin sensitivity and increase peripheral utilization of glucose [38,39]. Though, further studies may be required to confirm the insulin-sensitizing actions of *N. sativa* oil in PCOS.

It has been shown that the letrozole model of PCOS develops dyslipidemia and disrupted redox balance similar to human PCOS [35,40,41]. Dyslipidemia associated with letrozole-induced PCOS manifests with elevated TC, TG, LDL-C, and reduced HDL-C or at least one of them in other studies. Studies that have reported no change in lipid profile after letrozole administration used either low dose or continuous-release models that have been previously explained [26,32]. Treatment with *N. sativa* oil improved the lipid profile by reducing the serum levels of TC, TG, LDL-C, and increasing HDL-C in PCOS rats. Hypolipidemic potential of *N. sativa* oil in metabolic syndrome has been reported in different studies [9,10,12,15]. There is no report on the hypolipidemic potential of *N. sativa* oil in the PCOS condition. This effect of *N. sativa* has been linked with the TQ via mechanisms involving regulation of hydroxymethylglutaryl-co-enzyme A reductase (HMG-CoA reductase), Apo-A1,
Apo-B100, and low-density lipoprotein-receptor (LDL-R) genes to control cholesterol synthesis in the liver [42,43]. Others have also linked the hypolipidemic potential of *N. sativa* to enhance lipid metabolism due to the antioxidative action of TQ [11,12] and clofibrate actions of the nigellamin content [44].

Redox balance, as evaluated by endogenous antioxidant enzyme activities and LP products (MDA), showed that letrozole-induced PCOS exhibited marked reduction of endogenous antioxidant enzyme activities with increased level of MDA. *Nigella sativa* oil treatment significantly increased the serum SOD and GPX activities and reduced LP in PCOS rat. Excessive production of oxidants which overwhelm the endogenous antioxidant defense system gives rise to oxidative stress. Oxidative stress has been implicated in the pathogenesis of many diseases linked with inflammation [45] and PCOS is an oxidative stress/chronic systemic inflammatory disorder [46]. *Nigella sativa* oil known for its radical scavenging activity and anti-inflammatory efficacy [47,48], the antioxidant activity in PCOS-associated oxidative stress is expected. It has been shown that both the oil and derived TQ inhibit eicosanoid generation in leukocytes and non-enzymatic LP *in vitro* and none of the essential oil and the bioactive compounds (TQ, carvacrol, t-anethole, or 4-terpineol) produced prooxidant activities in the site-specific assays [49]. The oil possessed a strong antioxidant effect that cannot be achieved by adding up the activities of the few bioactive components. *Nigella sativa* oil considerably attenuated oxidative stress in the rat brain by up-regulating the activities of antioxidant enzymes to prevent pentylentetrazol-kindled seizures better than Valproate [50]. It was also shown through *in vivo* and *in vitro* cell culture that *N. sativa* oil fractions rich in TQ are most potent in terms of antioxidant capacity and prevented toluene-induced loss of glutathione, an endogenous antioxidant, in cells and hepatic tissue [51]. Recently, Sultan et al. [13,14] showed that the fixed and essential oils of *N. sativa* enhanced serum antioxidant capacity by modulating hepatic antioxidant enzymes and boosting of immunity in streptozotocin-induced diabetic rats. Therefore, the use of *N. sativa* oil could be a potential approach in ameliorating PCOS-related hyperandrogenism, dysmenorrhea, distorted folliculogenesis, and metabolic disorder as can be exacerbated by altered redox status and chronic systemic inflammation.

From the foregoing, the biological action pathway is proposed (Fig. 6) thus, *N. sativa* oil, a proven insulin-sensitizing agent [8,9,15,52,53], could have caused marked decrease intra-ovarian and serum androgen level in the letrozole-induced PCOS rats by enhancing insulin sensitivity and suppressing compensatory hyperinsulinemia (HI) via insulin-mediated inhibition of androgen production and androgen receptor signaling. These, ultimately suppress hyperandrogenism-induced polycystic ovarian morphology and restore estradiol level thereby improve estrogen-mediated negative feedback mechanism on the HPG axis. This action of *N. sativa* oil on insulin-mediated androgen-androgen receptor signaling may be more complex and cannot be the only pathway for the *N. sativa* oil induced amelioration of PCOS features in the rat. However, this is one of the biological pathways through which *N. sativa* oil might work to improve PCOS-associated metabolic derangement while improving androgen-mediated altered ovarian features in PCOS rat.

In addition, suppression of androgen-mediated changes and enhanced estrogen level, as observed in this study following treatment with *N. sativa* oil, improves estrogen receptor-mediated activation of LDL-R activity [54]. These might be responsible for the improved lipid profile in PCOS rat treated with *N. sativa* oil. Lower serum testosterone could also down-regulate scavenger receptor B1 and hepatic lipase (HL) to reduce hepatocytes’ and steroidogenic cells’ uptake of HDL-C and cholesterol eflux from peripheral cells [6]. Studies have also shown that the oil and TQ-rich fractions of *N. sativa* improved dyslipidemia of metabolic syndrome by modulating the activity of HMG-CoA [47].

Moreover, anti-androgenic action and enhanced insulin sensitivity by *N. sativa* oil could have inhibited activation of inflammatory cells limiting respiratory burst and excessive production of oxidants in mononuclear and leukocytic cells (LCs) specifically and other body cells in general [6]. *Nigella sativa* oil-mediated up-regulation of antioxidant enzymes activities and inhibition of LP can confer protection on inflammatory/immune cells and other body cells to prevent oxidative stress state in the PCOS rats. Hyperandrogenemia and IR were shown to independently or jointly increase the activation of inflammatory cells to enhance oxidants production [6].

Finally, by enhancing the insulin sensitivity and suppression of androgen-mediated alterations in the ovary of PCOS rats, *N. sativa* oil improved polycystic ovarian-morphology, circulating levels of gonadotropins and sex steroids, metabolic disorders relating to lipid metabolism and redox balance.
Figure 6. Proposed mechanisms of actions of *N. sativa* oil in PCOS. *Nigella sativa* oil interferes with letrozole-induced hyper-androgenemia to reduce circulating levels of testosterone (T) and increase circulating levels of estrogen (E) by alleviating letrozole-mediated inhibition of aromatase enzyme in the ovary. These modulatory properties of *N. sativa* oil restored estrous cyclicity and improved ovarian polycystic morphology. The increased level of E by *N. sativa* oil treatment restored the pulsatile release of GnRH by exerting the negative feedback effect on the hypothalamus. This improved LH and FSH hormones release to correct altered steroidogenesis in the PCOS ovary. *Nigella sativa* oil directly mediates LDL-R and HL expressions and indirectly enhances E-mediated upregulation of LDL-R and HL to ameliorate PCOS related dyslipidemia. Again, *N. sativa* oil directly and via anti-androgenic actions enhances insulin sensitivity to improve compensatory HI and correct PCOS-related altered glucose metabolism. The oil is also thought to suppress HI- and hyper-androgenemic-activations of mononuclear cells (MNCs) and polymorphonuclear LCs to control redox balance and LP via the upregulation of antioxidant enzymes' activities. All together these contribute to improve polycystic ovary, PCOS-associated dyslipidemia, and redox disturbances. ▲ = increase; ▼ = decrease; → = positive influence; —→ = feedback inhibition. MDA = malondialdehyde; NSO = *N. sativa* oil; LZ = letrozole.
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Conflict of interest

None.

Author’s contribution

Abdulrazaq Bidemi Nafiu conceived, designed, participated in the animal experiments and drafting of the manuscript. Suliat Alimi, Abdussalam Babalola, Ayodele Temitope Ogunlade, Fatima Dobarako Muhammad, Abdur Raheem Adesola Idowu Abioye, and Abdul musawwir Alli oluwafuyi participated in the animal experiments, data analysis, scientific advice and revised the manuscript. Lukman Aboyeji Oyewole, Olugbenga Akinola, Joseph Olajide Olayemi, Abdulbasit Amin, Wahab Imam Abdulmajeed, Ibrahim Musa carried out some biochemical assays and contributed to the writing. Mohammad Tariqur Rahman contributed with technical support, scientific advice and revised the manuscript.

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N. sativa oil improves polycystic ovary


