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Nigella Sativa Oil Protects the Rat Ovary from Oxidative Injury Due to Ischemia-Reperfusion

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Corresponding Author: Zeyneb Bakacak, e-mail: zeynebbakacak@gmail.com**Source of support:** Departmental sources**Background:** The aim of this study was to evaluate the effects of *Nigella sativa* (*N. sativa*) oil (NSO) on ovarian oxidative damage following ischemia-reperfusion injury, using a rat model of ovarian torsion.**Material/Methods:** Forty-eight female albino Wistar rats were divided into six groups: (Group 1) laparotomy only; (Group 2) intraperitoneal NSO (2 ml/kg), 1 hour following laparotomy; (Group 3) 3 hours of ovarian ischemia; (Group 4) 3 hours of ovarian ischemia followed by 3 hours of reperfusion; (Group 5) 3 hours of ovarian ischemia and 2 ml/kg of NSO 1 hour before laparotomy; (Group 6) 3 hours of reperfusion after 3 hours of ovarian ischemia and 2 ml/mg of NSO 1 hour before laparotomy.**Results:** The antioxidant status, ceruloplasmin level, native thiol, total thiol, and disulfide levels of the control group (Group 1) were significantly increased compared with the ovarian ischemia-reperfusion group treated with NSO (Group 6) ($p=0.003$, $p=0.002$, $p=0.006$, $p=0.001$ and $p=0.003$, respectively); these levels in the ovarian ischemia group (Group 3) and ischemia-reperfusion group (Group 4) were statistically similar to those of the ovarian ischemia + NSO group (Group 5) and ovarian ischemia-reperfusion + NSO group (Group 6).**Conclusions:** In this preliminary rat study, administration of NSO shortly after the onset of ovarian ischemia-reperfusion injury, did not significantly reduce levels of markers of oxidative injury. Further studies are required to evaluate the ovarian changes at the tissue level, and to determine the optimum dose of NSO.**MeSH Keywords:** **Disulfides • *Nigella Sativa* • Reperfusion Injury****Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/905356>

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Background

Ovarian torsion (adnexal torsion or turbo-ovarian torsion) is a cause of acute abdominal pain in women, which requires emergency gynecological surgery [1]. Although adnexal torsion is most common in sexually mature women, it can also affect pre-pubertal and post-menopausal women [1]. Early diagnosis and treatment are important to salvage the viability of the ovary and to maintain fertility [1-3]. Organ-protective surgery is necessary to maintain ovarian function, especially in young female patients, and laparoscopy is performed in cases of ovarian torsion so that the ovary can be conserved [1-3]. However, the process of de-torsion can be followed by oxidative injury to the ovary as the ischemic ovarian tissue is reperfused with oxygenated blood, with the generation of reactive oxygen species (ROS), resulting in an inflammatory response and tissue damage, known as ischemia-reperfusion injury [1-4].

Recent studies have demonstrated that the pathogenesis of many diseases is related to the increased activity of free radicals. For this reason, antioxidants have attracted attention as a possible remedy for various aging-related diseases, cancer and coronary heart disease. Paradoxically, reperfusion injury inflicts greater damage on tissues and organs than does ischemic injury. Many studies have investigated the ability of pharmacological agents to reduce reperfusion injury in animal models of ischemia-reperfusion injury [4-8].

Nigella sativa (*N. sativa*) is a member of the Ranunculaceae family, which grows in Eastern Europe, the Mediterranean, the Middle East, and West Asia. In the U.K. and U.S. [9]. *N. sativa* is known as black cummin; in Middle Eastern countries, *N. sativa* is known as shonaiz. *N. sativa* oil (NSO) is derived from the plant seeds and has been used in the Middle East and India as a complementary and alternative medicine (CAM) for the treatment of many diseases, including cancer, asthma, rheumatic conditions, and microbial infections [9-15].

Previously published studies have shown that *Nigella sativa* (*N. sativa*) oil (NSO) has been reported to have anti-inflammatory, antioxidant, immunomodulatory, anti-carcinogenic, anti-hypertensive, and anti-ischemic properties [9-15]. Some previous studies have shown that NSO has protective effects against damage induced by ischemia-reperfusion injury [9-13]. In this study, NSO was used, which included the following pharmacologically active constituents: thymoquinone (TQ), dithymoquinone (DTQ), thymol (THY), and thymohydroquinone (THQ), in the oil derived from the *N. sativa* seed. It is important to make clear that a pure extract of NSO was used in this study, as some previous studies have used ethanol extracts of NSO that include flavonoid antioxidants, quercetin, kaempferol, and luteolin [15]. The aim of this study was to investigate the effects of NSO, derived from the seeds of

N. sativa, in a rat model of ovarian torsion and ovarian ischemia-reperfusion injury.

Material and Methods

Ethical approval and animal care

Ethical committee approval for this study was received from the Experimental Animal Laboratories, Ankara, Turkey (date: 21.10.2016, No. 198). The study protocol was planned in accordance with the Guide for the Care and Use of Laboratory Animals.

Animal surgery and treatment

Forty-eight female albino Wistar rats (180-240 g) were housed in stainless steel cages under a controlled 12 hour light: dark cycle, with an ambient temperature of 24-25°C and humidity of 55-60%. The rats were fed with standard rodent chow and had access to water *ad libitum*. The anesthesia used was a combination of ketamine (Ketalar®) (Parke Davis, Eczacıbasi, Istanbul, Turkey) and xylazine (Rompun®) (Bayer AG, Leverkusen, Germany), used during the surgical procedures. The lower abdominal incision area on each rat was shaved, and a 2 cm midline incision was made through the skin.

Forty-eight female albino Wistar rats were divided into six groups: (Group 1 - Control) underwent laparotomy only; (Group 2) received intraperitoneal *Nigella sativa* (*N. sativa*) oil (NSO) (2 ml/kg), 1 hour following laparotomy; (Group 3) 3 hours of ovarian ischemia; (Group 4) 3 hours of ovarian ischemia followed by 3 hours of reperfusion; (Group 5) 3 hours of ovarian ischemia and 2 ml/kg of NSO 1 hour before laparotomy; (Group 6) 3 hours of reperfusion after 3 hours of ovarian ischemia and 2 ml/mg of NSO 1 hour before laparotomy.

The protocols were applied as follows for each group: Group 1 received only laparotomy, and the abdominal wall was closed with 3/0 silk sutures after 2 min of observation. Group 2 were treated with NSO (2 ml/kg) intraperitoneally 1 hour before laparotomy. Group 3 was the ovarian torsion group, and ovarian ischemia was applied for 3 hours by placing vessel clips under the rat ovaries after adequate peritoneal dissection. Group 4 was the de-torsion group, and 3 hours of ischemia was applied followed by 3 hours of reperfusion. Group 5 was the ovarian torsion and NSO-treated group, with ovarian ischemia applied for 3 hours by placing vessel clips under the rat ovaries after adequate peritoneal dissection; 2 ml/kg of NSO was administered 1 hour before laparotomy. Group 6 was the ovarian de-torsion and NSO-treated group, with 3 hours of ovarian ischemia applied followed by 3 hours of reperfusion and with 2 ml/kg of NSO administered 1 hour before laparotomy.

Table 1. Biochemical values in the groups of rats studied.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	p
TAS	1.3±0.1	1.8±0.1	1.2±0.2	1.2±0.2	1.3±0.2	1.2±0.1	0.003
TOS	24.1±8.5	21±9	17.4±8.1	21±13.8	29.2±20.2	22.4±16.4	0.652
Ceruloplasmin*	65.1±16.3	58.3±3.1	46.2±10.9	51.6±10.5	40.7±10.1	38.6±11.5	0.002
Catalase	250±180.5	284.4±143.5	254.7±126.1	289.5±197.2	406.4±205.8	300.6±207.7	0.789
Myeloperoxidase	22.7±43.2	20.4±46.2	24.2±51.4	3.2±2.9	6.8±3.9	10±13.1	0.660
Nativethiols*	71.2±31.2	33.4±13.7	28±11.1	35.3±17.5	34.3±8.3	23.5±8.1	0.006
Total thiols*	206.7±30.7	138.1±34.1	112.5±38.5	143±50.2	123.2±46.9	90.2±28.4	0.001
Disulphides*	67.8±7.8	52.3±10.7	42.3±13.8	53.9±18.2	44.4±20.5	33.4±14.2	0.003
Disulphide/ nativethiol ratio	112.4±51.9	167.2±36.9	159.9±37.2	168.3±66	125.5±63.2	159.3±93.4	0.240
Disulphide/total thiol ratio	33.3±5.1	38.2±2	37.8±1.7	37.8±3.2	33.4±8.5	35.6±7	0.240
Nativethiol/total thiol ratio	33.4±10.2	23.6±4.1	24.3±3.4	24.5±6.3	33.2±17	28.8±14.1	0.240

The choice of a 2 ml/kg dose of NSO, and the ovarian ischemia and reperfusion periods were chosen following a review of previously published studies, but we noted that there was a lack of data in the literature about the dose-related effects of NSO in animal studies [12–15]. In this study, we chose ten times the previously used daily dose for the rat, because we applied the agent only once.

From each animal, 3 ml of intra-cardiac was collected for analysis of oxidative stress markers. The total antioxidant capacity (or status) (TAC) (mmol Trolox eq/L) and the total oxidant status (TOS) ($\mu\text{mol H}_2\text{O}_2$ Eq/L) levels were evaluated using automatic and colorimetric measurement methods, as previously described [16,17]. TAC and TOS levels were measured using commercially available kits (Relassay, Turkey).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 21 (SPSS IBM, Armonk, NY, USA). Continuous variables were expressed as the mean \pm standard deviation. Kruskal-Wallis test was used for the comparison of the groups, and Mann-Whitney U test was used for posthoc analysis. A value of $p < 0.05$ was accepted as statistically significant.

Results

Nigella sativa (*N. sativa*) oil (NSO) extracted from the plant seeds were used in this study.

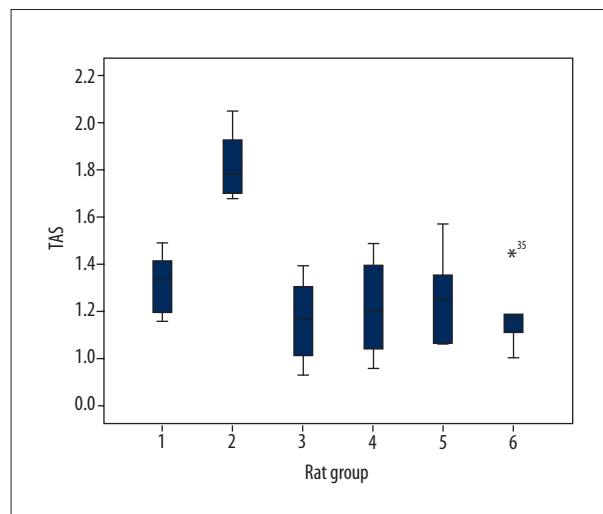


Figure 1. Total antioxidant capacity (TAC) values in the groups of rats studied.

The total antioxidant capacity (TAC) and total oxidant status (TOS), ceruloplasmin (oxygen free radical scavenger), catalase, myeloperoxidase, native thiol, total thiol, and disulfide values of the experimental groups are given in Table 1.

The TAC, ceruloplasmin, native thiol, total thiol and disulfide values of the control group (Group 1) were significantly higher than those of the ovarian ischemia-reperfusion + NSO group (Group 6) (respectively $p=0.003$, $p=0.002$, $p=0.006$, $p=0.001$ and $p=0.003$).

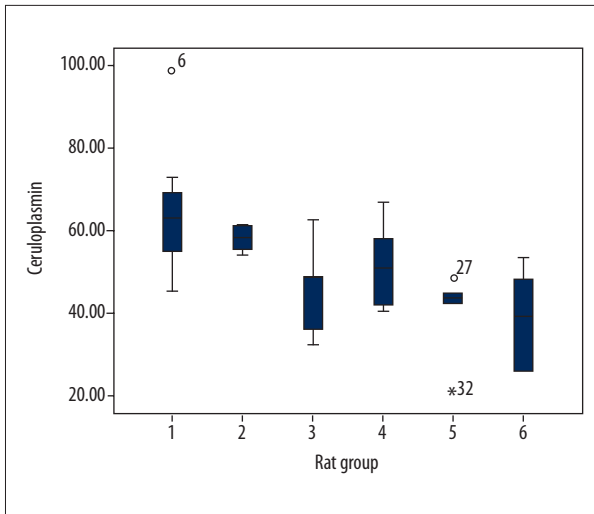


Figure 2. Ceruloplasmin (oxygen free radical scavenger) values in the groups of rats studied.

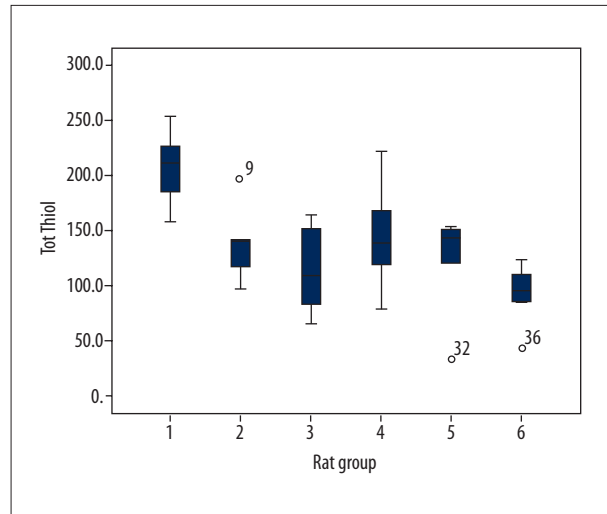


Figure 4. Total thiol (antioxidant) values in the groups of rats studied.

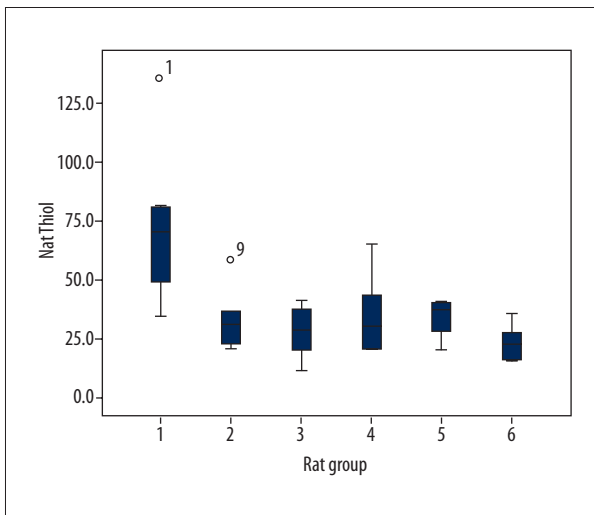


Figure 3. Native thiol (antioxidant) values in groups of rats studied.

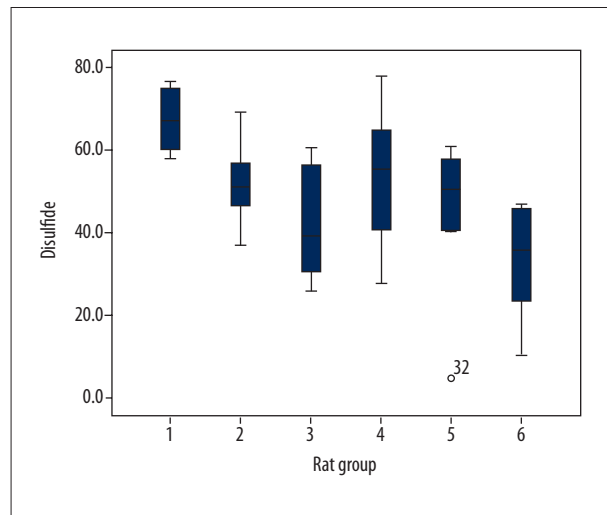


Figure 5. Disulfide activities in the groups of rats studied.

Figure 1 shows that the TAC values of the control group (Group 1) and the NSO only group (Group 2) were significantly increased compared with the TAC values of the remaining groups ($p=0.003$, for both). However, the TAC values of Groups 3 and 4 were statistically similar to the TAC values of the remaining groups.

Figure 2 shows that the ceruloplasmin values of the control group (Group 1) and the NSO only group (Group 2) were significantly increased compared with the ceruloplasmin values of the remaining groups ($p=0.002$, for both). However, there were no significant differences between the ceruloplasmin values of Groups 3 and 4 compared with the other groups.

Figure 3 shows that the native thiol values of the control group (Group 1) and the NSO only group (Group 2) were significantly increased compared with the native thiol values of the remaining groups ($p=0.006$, for both). However, Groups 3 and 4 had statistically similar native thiol values with those of the remaining groups. There was a significant difference between Groups 5 and 6 in the levels of native thiol ($p=0.037$).

Figure 4 shows that the total thiol values of the control group (Group 1) and the NSO only group (Group 2) were significantly increased compared with the total thiol values of the remaining groups ($p=0.001$, for both). However, there were no significant differences in total thiol values of Groups 3 and 4 compared with the other groups. There was no statistical difference between Groups 5 and 6 in the levels of total thiol.

Figure 5 shows that the disulfide activities of the control group (Group 1) and the NSO only group (Group 2) were significantly increased compared with the disulfide activities of the remaining groups ($p=0.003$, for both). However, there was no statistical difference between Groups 3 and 4 for disulfide activity.

Discussion

In this study, we examined the protective effects of *Nigella sativa* (*N. sativa*) oil (NSO) against oxidative damage induced by ovarian ischemia-reperfusion injury in a rat model. The results of this study showed that the NSO dose was not effective in reducing oxidative stress-related biochemical damage in ovarian ischemia-reperfusion injury, but antioxidant effects, that may indicate reduced oxidative stress and early ischemia-reperfusion ovarian damage, were demonstrated. However, no morphological analysis of the ovarian tissues was done for each group of rats in this preliminary study.

The torsion of ovarian vascular pedicle over its axis results in decreased arterial blood flow and obstruction of venous and lymphatic drainage, resulting in ovarian ischemia [1–3]. Ovarian ischemia is characterized by an increase in ovary size, ovarian hemorrhage, and venous congestion [1–3]. Adnexal torsion reduces blood flow, which results in an increase in lactic acid, hypoxanthine, and lipid peroxide levels, which can be measured in the blood [18,19]. Although de-torsion is usually applied as a treatment for adnexal torsion, it is followed by neutrophil infiltration activation and an increase in free oxygen radicals and cytokines, such as nitric oxide and tumor necrosis factor- α [18–22]. Oxidative stress is defined as the imbalance between free oxygen radical production and the production of antioxidants, which inactivate these free oxygen radicals [23–25].

Following ovarian de-torsion, the oxygen concentrations in the ovarian tissue increase as a result of vascular reperfusion. Increased numbers of oxygen molecules react with hypoxanthine and xanthine, triggering the production of ROS and subsequently leading to tissue damage [23–25]. The elevated production of free oxygen radicals enhances the damage in ischemic tissues by increasing the peroxidation of cell membrane and mitochondrial lipids, leading to endothelial cell damage, cytokine production, and antioxidant enzyme inactivation [23–25]. This process is called ischemia-reperfusion injury, which can cause more tissue damage ischemia [23–25]. It has previously been proposed that the use of antioxidant pharmacological agents before or during reperfusion might protect against ovarian ischemia-reperfusion injury [23–25].

In this study, the total antioxidant capacity (TAC) value was significantly increased in the rat group without ovarian ischemia-reperfusion injury, treated with NSO before laparotomy

(Group 2), a finding which is supported by findings from a previous study [24]. However, there were no significant between-group differences in the total oxidant status (TOS) values. The measurement of TAC and TOS values was adopted for this study because it was possible to record these values simultaneously within a short period of time, and at a lower cost [27,28].

Yildiz et al. used an extract of *N. sativa* as an *in vitro* antioxidant in a model of renal ischemia-reperfusion injury and reported that the TAC values increased significantly, whereas the TOS values decreased significantly after the administration of the *N. sativa* extracts [26]. These previously published findings are in contrast to our study findings, as the administration of NSO after the establishment of ovarian ischemia-reperfusion injury had no significant effect on TAC and TOS values in this study. However, the TAC value was significantly increased in rats that had received NSO before the establishment of ovarian ischemia-reperfusion injury. We believe that this finding may be explained by the differences in the administration time and dose of NSO in the present study.

Previously published studies in the literature have shown that the effects of *N. sativa* appear to be linked to the administration time and dose. With regard to optimum doses and durations of NSO, Havakhah et al. administered 150 mg/kg or 300 mg/kg of *N. sativa* 1 hour before the establishment of renal ischemia-reperfusion injury [29]. Terzi et al. administered 0.2 ml/kg of NSO intraperitoneally in animal models of intestinal and hepatic ischemia-reperfusion injury [30]. Based on these previously published findings, in the present study, we chose 2 ml/kg of NSO, administered intraperitoneally for the study of NSO in ovarian ischemia-reperfusion injury. The findings from the present study, combined with those from previous studies, do indicate that the administration of NSO before ovarian ischemia-reperfusion injury may be more effective than its administration after the establishment of ovarian ischemia-reperfusion injury.

Methanolic extracts and volatile oil fractions from NSO have been shown to inhibit lipidemic oxidative stress in rats [29,30]. Therefore, it has been proposed that a methanolic extract of NSO could be used as an antioxidant hypolipidemic agent in the treatment or prevention of diseases associated with the production of free radicals [29,30]. Further investigation of literature also provided evidence for the protective effects of NSO on ischemia-reperfusion injury within the renal, gastric, intestinal, cardiac and cerebral tissues in rat models [29–34]. These protective effects can be attributed to a decrease in lipid peroxidation and an increase in glutathione and antioxidant enzymes via superoxide dismutase and catalase activities [35].

Thiols are organic molecules containing sulfhydryl (-SH) groups, which have critical roles in the prevention of oxidative stress. Thiol groups of sulfur-containing proteins (e.g., cysteine and

methionine) are the primary targets of ROS. ROS oxidize thiol groups to form reversible disulfide bonds. The formation of such bonds is the primary indicator of free radical-induced protein oxidation.

Thiols and disulfides have important roles in the stabilization of proteins, in addition to the regulation of protein and enzyme functions, and the thiol: disulfide ratio participates in antioxidant defense, detoxification and apoptosis [35,36]. Abnormal thiol: disulfide ratios are involved in the pathogenesis of several chronic conditions, including diabetes mellitus, cardiovascular disorders, malignancy, rheumatoid arthritis, and chronic renal insufficiency [36,37]. In 2014, Erel and Neşelioğlu developed a method that could be used to measure thiol levels and disulfide activity, to assign the thiol and disulfide homeostasis level as an indicator of oxidative stress [38]. In the present study, the levels of native thiols, total thiols, and disulfide activity were significantly increased in Group 1 due to an increase in the thiol pool. Although the disulfide: native thiol ratio was reduced in Group 1, this decrease was statistically insignificant, indicating a move of the thiol and disulfide equilibrium towards native thiols. Under oxidative stress, an increase in disulfides and a decrease in thiols are expected [36–38]. In this study, the decrease in the thiol levels of the ovarian ischemia-reperfusion injury groups suggests that antioxidant defense mechanisms may be weakened.

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There were several limitations of this study. This study was preliminary and of short duration, with a small number of animals in each study group. The lack of clear antioxidant effects of NSO in the ovarian ischemia-reperfusion injury groups could have been due to insufficient dose and duration of NSO, or an inappropriate administration route. The lack of current consensus on the optimal dose and optimal duration of treatment for NSO may be addressed in future studies.

Conclusions

In this preliminary study in a rat animal model, administration of *Nigella sativa* (*N. sativa*) oil (NSO) shortly after the onset of ovarian ischemia-reperfusion injury did not significantly reduce the levels of markers of oxidative injury. Further studies are required to evaluate the ovarian changes at the tissue level, and to determine the optimum dose and duration of treatment for NSO.

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