Short communication

iMedPub Journals
http://www.imedpub.com/

Journal of Reproductive Endocrinology & Infertility
ISSN 2476-2008

2017
Vol.2 No.1:11

DOI: 10.21767/2476-2008.100011

Thymoquinone Effect (an Active Metabolite of the Plant *Nigella sativa*) on Ischemia-Reperfusion Injury in Rat Ovary

Ozlem Gun Eryilmaz[1], Hatice Kansu-Celik[1*], Esma Sarikaya[1], Sureyya Barun[2], Aslihan Avci[3], Sule Ozel[1] and Candan Ozogul[4]

[1]Department of Reproductive Endocrinology, Zekai Tahir Burak Womens Education and Research Hospital, Ankara, Turkey

[2]Department of Pharmacology, Gazi University, Ankara, Turkey

[3]Department of Biochemistry, Ankara University, Ankara, Turkey

[4]Department of Histology and Embryology, Gazi University, Ankara, Turkey

[*]**Corresponding author:** Hatice Kansu-Celik, Department of Reproductive Endocrinology, Zekai Tahir Burak Womens Education and Research Hospital, Ankara, Turkey, Tel: 00905337865433; E-mail: h_kansu@yahoo.com

Received date: June 23, 2016; **Accepted date:** July 18, 2016; **Published date** July 20, 2016

Citation: Eryilmaz OG, Kansu-Celik H, Sarikaya E, Avci A, Ozel S, et al. (2016) Thymoquinone Effect an Active Metabolite of the Plant *Nigella sativa*) on Ischemia-Reperfusion Injury in Rat Ovary. J Reproductive Endocrinol & Infert 1: 3.

Abstract

Objectives: To determine the antioxidant effects of thymoquinone (one of the active components of the plant *Nigella sativa*) on ischemia-reperfusion injury in rat ovary.

Study design: Twenty four rats were divided into four groups: control (n=6), ischemia (n=6), ischemia (n=6), ischemia-reperfusion+thymoquinone (n=6). Ovarian torsion was surgically reperfusion+saline induced by vascular clamps. The ovary was clamped for 3 hours and 30 minutes before reperfusion 10 mg/kg thymoquinone was injected intraperitoneally. Following three hours of reperfusion, right oophorectomy was done and the rats were sacrificed.

Main outcome measures: Histological disturbances of the ovarian tissues were scored and tissue levels of catalase (CAT), malondealdehyde (MDA) and xanthine oxidase (XO) were measured.

Results: In the thymoquinone treated group, tissue CAT levels were significantly higher (p=0.02) and MDA levels were significantly lower (p=0.042). XO levels were similar among the groups. Histological examinations of thymoquinone group revealed a better preserved ovarian tissue compared to the control, ischemic and the saline treated ischemia-reperfusion groups.

Conclusion: Thymoquinone at dose of 10 mg has a potent antioxidant and tissue preserving effect against the ischemia-reperfusion injury in the rat ovary prone to torsion/detorsion.

Keywords: Ischemia/Reperfusion; Thymoquinone; Ovarian tortion; MDA

Introduction

Ovarian torsion is an emergent gynecological consultation, with a prevalence of 2.5% to 7.4% [1]. It is mostly seen in the population of young women of reproductive age, and if a quick diagnosis is not achieved, the ovary may be lost, which carries an importance related with the likely future fertility problems [2]. Excluding the irreversibly damaged and necrotic ovaries, the suggested treatment route in young, fertile women is the laparoscopic detorsion and evaluating the tissue reperfusion [3].

During reperfusion following the ischemic state, hypoxic and/or necrotic cells exposed to the new oxygen (O_2) supply produce radical oxygen species (ROS) which causes injury to the post-ischemic cells and tissues [4]. Reperfusion creates the oxidative stress that refers to an imbalance between the production and manifestation of ROS and antioxidants.

A balance is needed between ROS and the antioxidants in order to establish a healthy status for the intracellular and intercellular interactions. The mechanism in the cellular damage is mainly based on reacting with and disturbing the intracellular components. The most important of these are the lipids, proteins and nucleic acids. Reaction with the lipids in the cell membrane causes lipid peroxidation and disturbs the membrane integrity, permeability and ion transport. Free radicals act on the lysosome membrane, and disturbed organ lets the various oxidative enzymes free into the cytoplasm, at which point the main cellular destruction starts [5]. Malondealdehyde (MDA), a stable metabolite of the ROS-mediated lipid peroxidation cascade, is a highly toxic molecule and significantly increases in ischemia/reperfusion (I/R).

Injury, suggesting damaged cell wall integrity and permeability. Moreover, it can exacerbate I/R injury through its ability to react with DNA and proteins [6].

Another member of the oxidative stress is the enzyme xanthine oxidase (XO). Under some circumstances, it produces superoxide ion (O_2 and H_2O_2), which has a significant oxidative potential that disturbs the cell integrity [7].

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes that are the only chemicals having compensatory action on the ROS load, and their activity may prevent cell damage. Some of the enzymatic antioxidants are superoxide dismutase, catalase (CAT) and peroxiredoxins whereas non-enzymatic forms are also present like vitamin C, vitamin E, N-acetylcysteine and melatonin [8].

Prevention or minimization of the reperfusion injury in the ischemic ovaries is the most important point in the function of the post-ischemic tissue and the future fertility [9]. Few reports exist concerning ovarian reperfusion injury and its prevention. Disulfiram, vitamin E, pentoxifylline, melatonin, tadalafil, erythropoietin, dimethyl sulfoxide, atorvastatin, genistein, L-carnitine, N-acetyl cysteine, famotidine, vitamin C were found to have beneficial effects [8,10-17].

Thymoquinone (TQ, 2-isopropyl-5-methyl-1,4-benzoquinone) is one of the active components of the plant *Nigella sativa* Linn. (Ranunculaceae), which is also known as black seed or black cumin. This plant is a traditional drug used for medical purposes [18]. This herbal medication has been reported to have immune stimulatory, anti-inflammatory, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, antiparasitic, antioxidant, and anticancer effects [19-23]. The antioxidant effect of TQ has been shown on the I/R injury in various organs such as the brain, kidney, testis, and gastric mucosa [21,22,24-26].

Although several antioxidant enzymes and drugs have been used to decrease ovarian IR injury to date [10,11-13,16-18,27-29] only one study has investigated the protective effect of thymoquinone in ovarian IR injury [30]. In present study, we aimed to show the antioxidant and tissue protective effect of TQ at a lower dose of 10 mg in a rat model of ovarian I/R, which was investigated via histopathological examinations and analyses of tissue CAT, XO and MDA.

Materials and Methods

Animals and experimental design

In this study, we followed the recommendations of the Declaration of Helsinki for animal care and complied with the ''Principles of Laboratory Animal Care (National Institutes of Health [NIH] publication No. 86-23, revised 1985). The study was approved by the Gazi University Ethics Committee for Animals (Report no. B.30.2.GUN.0.05.06.00/137-12279).

A total of 24 Wistar-albino female rats were used in the present research. All of the rats were young and in cycling reproductive age, with weights ranging from 250-300 g. Animals were divided into four randomized groups as follows:

C (Control group; n=6); I (Ischemia group; n=6); I/R + saline (Ischemia/reperfusion + saline group; n=6); I/R + TQ (Ischemia/reperfusion + thymoquinone group; n=6).

The surgical procedures were performed while each rat was under anesthesia with ketamine (Eczacibasi, Istanbul, Turkey,50 mg/kg i.m.) and xylazine (9 mg/kg i.m.). Rats were placed in dorsal recumbent position, and presurgical sterilization procedures were done with skin preparation, cleaning and covering with sterile drapes. Laparotomy was performed with a mid-line 2.5 cm incision. Ovarian torsion was induced by vascular clamps, which were applied just below the right ovary in the junction area of the uterine horn [9]. At the end of the reperfusion period, ovaries were excised and samples were used for the biochemical and histological examinations. Rats were sacrificed by exsanguination through the abdominal aortic artery.

Rats in the control group were sham operated and oophorectomized (group I). In ischemia group, unilateral right ovary was clamped for 3 hours and then oophorectomy was performed (group II). In ischaemia/reperfusion+saline and ischaemia/reperfusion+thymoquinone groups (Group III and IV), right ovary was prone to 3 hour of ischemia and 1 ml saline and 10 mg/kg thymoquinone (MP Biomedicals, LLC, Illkirch, France, LOT No: 45521) that was diluted in 1 ml of saline were injected i.p., respectively 30 minutes before 3 hour of reperfusion (24). In all of the excised ovaries, a small part was fixed for histological examinations and the rest was freezed at -80°C.

Histopathology

The ovarian tissue samples collected from all the groups were blindly sent to the laboratory. Tissues were fixed in 10% phosphate-buffered formalin and then embedded in paraffin. The paraffin block sections were 5 µm in thickness and routinely stained with hematoxylin and eosin (H & E). The microscopic examination of the sections with regard to the degenerative changes was also done blindly.

Tissue damage scoring was assessed in the histopathological evaluation. The parameters were the congestion, hemorrhage, edema, and perivascular lymphocyte infiltration. The severity of the histological changes were scored from 0 to +3 (0=no pathological finding; 1, 2 and 3 ≤ 33%, 33-66% and >66% affected ovary, respectively) [16].

CAT activity determination

The CAT activity was assessed according to the method of Aebi [31] and was evaluated in whole ovary homogenates. The principle of the method was based on the determination of the rate constant k (s^{-1}, k) of the H_2O_2 decomposition rate at 240 nm. Tissue samples were homogenized in ice-cold isotonic 0.9% sodium chloride solution and centrifuged for 5 min at 10,000 g at 4°C. The H_2O_2 added to the assessment medium is decomposed by the catalyzing activity of the CAT enzyme, and reduction in absorbance at 240 nm is monitored with a spectrophotometer. The reduction in absorbance is directly proportional to the enzyme activity. The results were expressed as IU/mg protein.

This article is available from: 10.21767/2476-2008.100011

XO activity determination

The XO activity was assessed according to the method of Hashimoto [32]. The enzyme activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm. This method is based on the amount of uric acid produced by XO from the xanthine, added to the medium. Tissue homogenate was incubated (100 µL) for 24 h at 25°C in 2.80 mL of medium containing phosphate buffer (pH 7.5, 50 mM) and xanthine (0.067 mmol final concentration in each tube). The absorption at 293 nm of the resultant clear supernatant was measured against blank. The activity was expressed in units per gram protein (mIU/mg protein).

MDA determination

The tissue MDA levels were assessed according to the method of Dahle [33]. This method is based on the reactivity with thiobarbituric acid (TBA) at 90-100°C. The sample was mixed with 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of TBA in a boiling water bath for 30 min. After cooling, the absorbance was read at 532 nm. The results were expressed as nmol/mg protein.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences software (SPSS, version 15.0). The data were presented as median (minimum-maximum) values. The MDA, XO and CAT levels were compared with Kruskal-Wallis test, and Bonferroni-corrected Mann-Whitney U test was used for post-hoc analysis when Kruskal-Wallis test revealed a statistical significance between the four groups. A p value <0.05 was considered statistically significant.

Table 1: P values of the tissue damage scores between the groups.

Groups	p value
Group I-II	0.005
Group I-III	0.003
Group I-IV	0.075
Group II-III	0.212
Group II-IV	0.022
Group III-IV	0.004

Results

Histopathologic examination

Total tissue damage scores are shown in Figure 1. The difference between the four groups were statistically significant; p=0.001. Tissue damage was significantly higher in the ischemia and the ischemia/reperfusion+saline groups when compared with the control group; p=0.05 and p=0.03, respectively (Table 1).

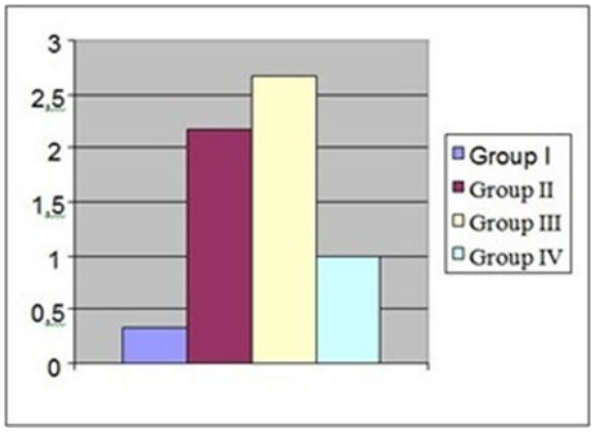

Figure 1: Tissue damage scores of the ovaries in the control (Group I), ischemia (GroupII), ischemia/reperfusion+saline (Group III) and ischemia/reperfusion +Thymoquinone (Group VI) groups.

Total tissue damage scores of the ischemia/reperfusion+TQ group were significantly lower than the ischemia and the ischemia/reperfusion+saline groups; p=0.022 and p=0.004, respectively (Table 1). Score between the control and the ischemia/reperfusion+TQ groups was insignificant; p=0.075 (Table 1).

Histologic examination of the ovarian sections revealed the followings:

Group I, control group: The results showed normal-in-appearance ovaries (Figure 2).

Group II, ischemia group: Moderate congestion, hemorrhage and perivascular lymphocyte infiltration were observed (Figure 2).

Group III, ischemia/reperfusion group+saline: Severe hemorrhage together with severe edema and perivascular lymphocyte infiltration were seen (Figure 2). Developing follicles were disrupted together with granulosa cell desquamations. Vascularization in the ovarian medulla was increased.

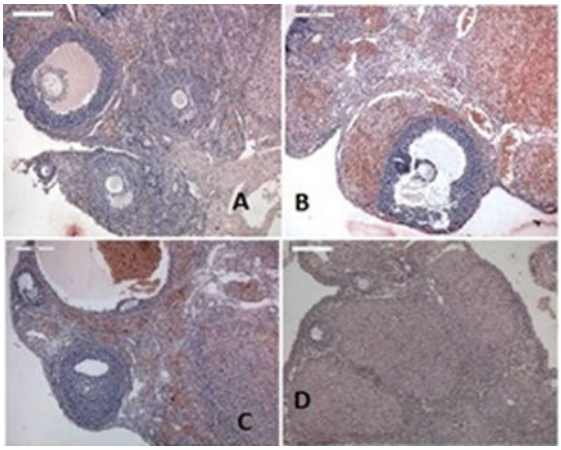

Figure 2: Histopathologic examination of the ovaries from group I (A) had a normal appearance. Group II (B) showed moderate congestion, hemorrhage and perivascular lymphocyte infiltration. Ovarian sections after ischemia and reperfusion in group III (C) showed severe hemorrhage together with severe edema and perivascular lymphocyte infiltration. Developing follicles were disrupted together with granulosa cell desquamations. Hemorrhage, edema and perivascular lymphocyte infiltration significantly reduced by intraperitoneal administration of thymoquinone in group IV (D). Hematoxylin and eosin.

Group IV, ischemia/reperfusion+thymoquinone: Negligible hemorrhage and congestion were seen (Figure 2). The histological appearances of the ovaries of Group I/R+TQ were close to the control group.

Biochemical analysis

Table 2: The levels of the ovarian tissue MDA, XO and Catalase activity in the control, ischemia, I/R and I/R+Thymoquinone (TQ) groups. Values are expressed as Median (Minimum- Maximum) *Group 4 is significantly different from all other groups.

	Catalase (IU/mg protein)	MDA (nmol/mg protein)	XO (mIU/mg protein)
Control group (n=6)	184.08 (156.30-314 .06)	9.48 (7.22-10.27)	1,67 (1.43-2.46)
Ischemia group (n=6)	365.23 (157.35-470 .23)	84.04 (5.07-165.75)	1.36 (0.59-2.92)
Ischemia/reperfusion group (n=6)	291.28 (206.93-767 .83)	84.04 (5.07-165.75)	2.70 (1.54-3.30)
Ischemia/reperfusion+TQ group (n=6)	993.04 (755.36-1234,79)*	30.62 (15.87-149.41)	1.99 (1.20-2.35)
P value	0.02	0.042	0.165

Table 2 shows the comparison of CAT, XO and MDA levels of the ovaries between the groups. XO levels of the groups I, II, III and IV were 1.67 (1.43-2.46); 1.36 (0.59-2.92); 2.70 (1.54-3.30) and 1.99 (1.20-2.35), respectively and the difference was insignificant (p=0.165). CAT levels were significantly higher in group IV compared to groups II and III; 993.04 (755.36-1234,79) *vs.* 365.23 (157.35-470.23) and 291.28 (206.93-767.83), respectively (p=0.02) and MDA levels were significantly lower in group IV compared to groups II and III 30.62 (15.87-149.41) *vs.* 84.04 (5.07-165.75) and 84.04 (5.07-165.75), respectively (p=0.042).

Discussion

The antioxidant effect of TQ was demonstrated in various studies. In a hypercholesterolemic rat model, TQ increased the antioxidant enzyme gluthatione peroxidase and decreased the levels of thiobarbituric acid reactive substances. These effects counteracted the formation of atherosclerosis [34]. In another study TQ-administration reduced tissue MDA levels in the ischemic rat brain and prevented lipid peroxidation and cell death. The antioxidant effect of TQ was examined in an in vitro study and microsomal lipid peroxidation was inhibited via the potent superoxide anion scavenger action of TQ [19]. Doxorubicin induced cardiotoxicity was also prevented by TQ action via inhibiting lipid peroxidation and scavanging superoxide radicals.

In this present study, TQ significantly reduced the histological damage and the oxidative stress in the rat ovaries following I/R. Related to the histological damage after I/R injury, TQ-administered ovarian tissues were close to the control ovaries. Minimal hemorrhage, lesser edema and minimal perivascular inflammatory cells demonstrated the tissue protective effect of TQ.

Related with the antioxidant action of TQ, tissue MDA levels were significantly lower in TQ-administered rat ovaries. Since MDA is an oxidant chemical resulting in cell membrane lipolysis leading to cell death, decreased MDA levels due to TQ effect protected the ovarian cells from membrane damage. XO being one of the enzymes producing toxic oxidative metabolites was decreased in the TQ-administered ovaries even if it was not significant.

With respect to the antioxidant enzyme CAT, increased levels in the I/R ovaries showed that TQ is fairly effective in stabilizing the antioxidant levels in the protection of tissues from oxidative stress during reperfusion. The enzyme CAT was thought to be decreased because of its consumption along the I/R pathways, but detection of still higher levels even after these chemical pathways of the reperfusion demonstrated how effective TQ was as an antioxidant.

A recent study by Ural et al. has evaluated the antioxidant effects of TQ at doses of 20 and 40 mg and investigated the biochemical, histopathological, and immuno-histochemical changes in ovarian torsion model [30]. Similar to our results, they showed that TQ reduced ischemic and I/R-induced injury by increasing CAT level and by decreasing the MDA and reduced the tissue damage.

This article is available from: 10.21767/2476-2008.100011

In conclusion, the results of the biochemical and histopathological parameters in our study suggest that administration of TQ even at the dose of 10 mg has beneficial effects in the prevention of I/R injury in the rat ovary prone to torsion/detorsion. Even in this smaller dose, TQ administration has a potent antioxidant action.

References

1. Hibbard LT (1985) Adnexal tortion. Am J Obstet Gynecol 152: 456-461.
2. Nichols DH, Julian PJ (1985) Tortion of the adnexa. Clin Obstet Gynecol 28: 375-380.
3. Oelsner G, Shashar D (2006) Adnexal tortion. Clin Obtet Gynecol 49: 459-463.
4. Grace PA (1994) Ischemia-reperfusion injury. Br J Surg 81: 637-647.
5. Carden DL, Granger DN (2000) Pathophysiology of ischemia-reperfusion injury. J Pathol 190: 255-266.
6. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 15: 316-328.
7. Hille R, Nishino T (1995) Flavoprotein structure and mechanism Xanthine oxidase and xanthine dehydrogenase. FASEB J 9: 995-1003.
8. Sağsöz N, Kisa U, Apan A (2002) Ischaemia-reperfusion injury of rat ovary and the effects of vitamin C, mannitol and verapamil. Hum Reprod 17: 2972-2976.
9. Uguralp S, Bay Karabulut A, Mizrak B (2005) Effects of pentoxifilyne and Vit E on the bilateral ovary after experimental ovarian ischemia. Eu J Pediatr Surg 15:107-113.
10. Turkoz Y, Celik O, Hascalik S, Cigremis Y, Hascalik M, et al. (2004) Melatonin reduces tortion-detortion injury in rat ovary: biochemical and histopathological evaluation. J Pineal Res 37: 137-141.
11. Yazici G, Erdem O, Cimen B, Arslan M, Tasdelen B (2007) Genistein attenuates postischemic ovarian injury in a rat adnexal torsion-detorsion model. Fertil Steril 87: 391-396.
12. Usta U, Inan M, Erbas H, Aydogdu N, Oz Puyan F, (2008) Tissue damage in rat ovaries subjected to torsion and detorsion: effects of L-carnitine and N-acetyl cysteine. Pediatr Surg Int 24:567-573.
13. Arikan DC, Bakan V, Kurutas EB, Sayar H, Coskun A (2010) Protective effect of tadalafil on ischemia/reperfusion injury of rat ovary. J Pediatr Surg 45: 2203-2209.
14. Cadirci E, Oral A, Odabasoqlu F, Kilic C, Coskun K, et al. (2010) Atorvastatin reduces tissue damage in rat ovaries subjected to torsion and detorsion: biochemical and histopathologic evaluation. Naunyn Schmiedebergs Arch Pharmacol 381: 455-466.
15. Ergun Y, Koc A, Dolapcioqlu K, Akaydin Y, Dogruer G, et al. (2010) The protective effect of erythropoietin and dimethylsulfoxide on ischemia-reperfusion injury in rat ovary. Eur J Obstet Gynecol Reprod Biol 152: 186-190.
16. Kurt A, Isaoqlu U, Yilmaz M, Calik M, Polat B, et al. (2011) Biochemical and histological investigation of famotidine effect on postischemic reperfusion injury in the rat ovary.J Pediatr Surg 46: 1817-1823.
17. Isaoglu U, Yilmaz M, Calik M, Polat B, Bakan E, et al. (2012) Biochemical and histopathological investigation of the protective effect of disulfiram in ischemia-induced ovary damage. Gynecol Endocrinol 28:143-147.
18. Ali BH, Blunden G (2003) Pharmacological and toxicological properties of Nigella sativa. Phytother Res 17: 299–305.
19. Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH (2003) Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol 26: 87-98.
20. Hosseinzadeh H, Paravardeh S, Asi MN, Sadeghnia HR, Ziaee T (2007) Effect of thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine 14: 621-627.
21. Fouda AM, Daba MH, Dahab GM, Sharaf El-Din OA (2008). Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. Basic Clin Pharmacol Toxicol 103: 109-118.
22. Khalife KH, Lupidi G (2008) Reduction of hypervalent states of myoglobin and hemoglobin to their ferrous forms by thymoquinone: The role of GSH, NADH and NADPH. Biochim Biophys Acta 1780: 627-37.
23. Alenzi, FQ, El-Bolkiny Yel-S, Salem ML (2010) Protective effects of Nigella sativa oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. Br J Biomed Sci 67: 20-28.
24. El-Abhar HS, Abdallah DM, Saleh S (2003) Gastroprotective activity of Nigella sativa oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. J Ethnopharmacol 84: 251-258.
25. Al-Majed AA, Al-Omar FA, Nagi MN (2006) Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol 543: 40-47.
26. Gökçe A, Oktar S, Koch A, Gonenci R, Yalcinkaya F, et al. (2010) Protective effect of thymoquinone in experimental testicular torsion. Urol Int 85: 461-465.
27. Ingec M, Calik M, Gundogdu C, Kurt A, Yilmaz M, et al. (2012) Biological and histopathological investigations of moclobemide on injured ovarian tissue following induction of ischemia-reperfusion in rats. Int J Fertil Steril 6: 19-26.
28. Yapca OE, Turan MI, Cetin N, Borekci B, Gul MA (2013) Use of thiamine pyrophosphate to prevent infertility developing in rats undergoing unilateral ovariectomy and with ischemia reperfusion induced in the contralateral ovary. Eur J Obstet Gynecol Reprod Biol 170: 521-525.
29. Dokuyucu R, Karateke A, Gokce H, Kurt RK, Ozcan O, et al (2014) Antioxidant effect of erdosteine and lipoic acid in ovarian ischemia-reperfusion injury. Eur J Obstet Gynecol Reprod Biol 183: 23-27.
30. Mete Ural Ü, Bayoğlu Tekin Y, Şehitoğlu İ, Kalkan Y, Cumhur Cüre M, Biochemical, Histopathological and Immunohistochemical Evaluation of the Protective and Therapeutic Effects of Thymoquinone against Ischemia and Ischemia/Reperfusion Injury in the Rat Ovary. Gynecol Obstet Invest 81: 47-53.
31. Aebi H (2005) Catalase. In: Bergmeyer HU, editors. Methods of Enzymatic Analysis. New York: Academic Press. p. 673-677.
32. Hashimato S (1974) A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. Anal Biochem 62: 425-435.

33. Dahle LK, Hilleg, Holman RT (1962) The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys 98: 253-261.

34. Nader MA, el-Agamy DS, Suddek GM (2010) Protective Effects of Propolis and Thymoquinone. on Development of Atherosclerosis in Cholesterol-Fed Rabbits. Arch Pharm Res 33: 637-643.

This article is available from: 10.21767/2476-2008.100011