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Nano-Methoxatin Improves Ischemia-Reperfusion Injury in Torsion-Detorsion Model of Ovary

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ABSTRACT

Using a rat ovary model, protective effects of nano-methoxatin (NMXTN) were studied on ischemia-reperfusion injury. Following randomization of forty-eight healthy female Wistar rats ~250 g, the animals were subjected to eight experimental groups (n = 6): Group SHAM: Only laparotomy was performed. Group IS: Only a 3-hour ischemia was performed. Group IS/Oil: Only a 3-hour ischemia was performed and 50 µl soybean oil alone (Solvent of MXTN) was administered 30 min before cessation of ischemia. Group IS/REP: The procedure included a 3-hour ischemia followed by a 3-hour reperfusion and 50 µl soybean oil alone was administered 30 min before cessation of ischemia. Group IS/MXTN: The procedure included a 3-hour ischemia only and 50 µl (0.3 mmol/l/IP) of MXTN 30 min before cessation of ischemia. Group IS/NMXTN: The procedure included a 3-hour ischemia only and 50 µl (0. 3 mmol/l/IP) of NMXTN 30 min before cessation of ischemia. Group IS/REP/MXTN: The procedure included a 3-hour ischemia, a 3-hour reperfusion and 20 µl (0.3 mmol/l) of MXTN 30 min before cessation of ischemia. Group IS/REP/NMXTN: The procedure included a 3-hour ischemia, a 3-hour reperfusion and 20 µl (0.3 mmol/l) of NMXTN 30 min before cessation of ischemia. Significantly amended development of ischemia/reperfusion tissue injury was observed in animals treated with NMXTN compared to those of other groups (p = 0.001). Mean values of biochemical indices were significantly higher than those observed for other groups (p = 0.001). Significantly lower values of MDA were observed in IS/REP/NMXTN animals compared to those of other groups (p = 0.001). Where ovarian tissue is exposed to ischemia intraperitoneal administration of NMXTN could bear clinical benefits in diminishing ischemia-reperfusion injury.

Introduction

Long mesovarium and adnexal venous congestion, that could result in torsion of ovary and subsequently obstruction of the ovarian vessels, cause a life-threatening reduction in tissue blood flow and permanent tissue damage.[1] As much early as possible diagnostic and therapeutic measures are required to preserve ovarian functions to help preclude future infertility.[2] Once ovarian torsion is diagnosed, detorsion

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of the twisted adnexa and assessment of the tissue reperfusion is required to help prevent future infertility even in case of cyanotic tissues.[2,3]

A suggested pathogenesis of tissue injury in the course of reperfusion is buildup of the activated neutrophils that produce reactive oxygen species.[4] Lipid peroxidation in the cell is the most injurious consequence of free radicals that result in decrease in the membrane potential and subsequently, cell injury. Malondialdehyde (MDA), one of the end products of lipid peroxidation, also results in serious cell damage through induction of polymerization and cross linking in membrane components.[5] Free oxygen radicals react with DNA and form 8-hydroxyguanine (8-OHGua) that is one of the damage products of DNA.[6] In spite of the fact that generation of free oxygen radicals occurs continuously in cells, the presence of endogenous antioxidant defense systems preserves tissues from the harmful effects of free oxygen radicals.[7]

Methoxatin (MXTN) acts as an antioxidant and it has been reported that it could inhibit lipid peroxidation injury, increase thymidine incorporation into fibroblasts and increase production of growth factors. In animal models, high doses of MXTN have been reported to protect tissues against hypoxic/ischemic injury.[8]

The use of nanoparticles besides improving the solubility also provides controlled releasing. The physiologic characteristic of the peritoneal cavity which helps remove toxic metabolites from the body has been successfully exploited to provide peritoneal dialysis in end stage renal disease patients.[9] The same characteristics of the peritoneal membrane also provide a useful portal of entry in the body for several pharmacological agents. One advantage would be that the drug achieves therapeutic efficacy in the region of interest while minimizing the systemic toxicities. Intraperitoneal administration seems more effective and available where oral administration of an agent may cause difficulties. It is clear that transperitoneal absorption of the agent is far faster than oral administration.[10]

The present study was different from the other studies in the literature for using NMXTN on ischemia/reperfusion injury. Aimed to study peritoneal effects of NMXTN on ischemia/reperfusion injury, a study was designed to determine if NMXTN could in fact protect against ischemia/reperfusion induced ovarian damage. The assessments were based on histological and biochemical parameters.

Materials and Methods

Experimental Information

All chemicals used were of analytical grade, used as received without any further purification and obtained from Sigma-Aldrich.

Animal Grouping

Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of (23 ± 3) °C, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups. The present study was designed and modified based on a method described by others.[11]

Forty-eight female Wistar rats ~250 g were randomized into five experimental groups (n = 6): Group SHAM: Only laparotomy was performed. Group IS: Only a 3-hour ischemia was performed. Group IS/Oil: Only a 3-hour ischemia was performed and 50 µl soybean oil alone (Solvent of MXTN) was administered 30 min before cessation of ischemia. Group IS/REP: The procedure included a 3-hour ischemia followed by a 3-hour reperfusion and 50 µl soybean oil alone was administered 30 min before cessation of ischemia. Group IS/MXTN: The procedure included a 3-hour ischemia only and 50 µl (0.3 mmol/l/IP) of MXTN 30 min before cessation of ischemia. Group IS/NMXTN: The procedure included a 3-hour ischemia only and 50 µl (0.3 mmol/l/IP) of NMXTN 30 min before cessation of ischemia. Group IS/REP/MXTN: The procedure included a 3-hour ischemia, a 3-hour reperfusion and 20 µl (0.3 mmol/l) of MXTN 30 min before cessation of ischemia. Group IS/REP/NMXTN: The procedure included a 3-hour ischemia, a 3-hour reperfusion and 20 µl (0.3 mmol/l) of NMXTN 30 min before cessation of ischemia.

The right ovaries were transferred to a 10% formaldehyde solution for histopathological assessments and the left ovaries were cleaned of surrounding soft tissues and then stored in a freezer at –80 °C for biochemical assessments.

Ethical Consideration

The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[8] This work involved the use of experimental animals and procedures that

did not differ from established internationally recognised high standards ('best practice') of veterinary clinical care for the individual animals. The study was registered under registration code# 61535-15/8/2019 in Ethical Committee of Islamic Azad University, Urmia Branch, Iran.

Surgry

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[12] The ethical Committee of the Urmia University of Medical Sciences approved all the experiments. A longitudinal midline incision was made in the lower abdomen and the uterine horns and adnexa were exposed. For induction of ischemia, a vascular clamp was applied on vessels of the ovaries in rats. After a 3-hour period of ischemia, both ovaries were surgically dissected out for histopathological and biochemical assessments. For induction of ischemia/reperfusion, both ovaries underwent ischemia the same way and at the end of a 3-hour period, the vascular clamps were chosen, removed and a 3-hour reperfusion was continued. Then, the ovaries were dissected out for histopathological and biochemical assessments.

Histology

Ovaries were fixed in 10% buffered formalin for 24 hours. The tissue samples were then processed and embedded in paraffin. A 5-µm semi-thin section was prepared and embedded in paraffin. The samples were then dewaxed, rehydrated and stained routinely with Hematoxylin and Eosin method. The sections were then observed under a light microscope.

Biochemistry

The tissue samples of ovaries were kept at -80 °C for 3 days, and then enzyme activities were determined in rat ovary tissues. The ovary tissues were ground with liquid nitrogen in a mortar. One half gram was weighed for each group and then treated with 4.5 ml of an appropriate buffer. This mixture was homogenized on ice with use of an ultra-turrax homogenizer (IKA, Werke, Germany) for 15 minutes. Homogenates were filtered and centrifuged by using a refrigerator centrifuge at 4 °C. Then the supernatants were used to determine the enzymatic activities. All assays were carried out at room temperature. Superoxide dismutase estimation was based on the generation of superoxide radicals produced by xanthine and the xanthine oxidase system, which reacts with nitroblue tetrazolium to form formazan dye.[13] Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction and is expressed as millimoles per minute per milligram of tissue. Concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test.[14] To evaluate SOD, the rat ovaries were rinsed with cold saline. The corpus mucosa was scraped, weighed, and homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added to a solution containing 2-thiobarbiturate (1.5 ml of 8 g/l), acetic acid (1.5 ml of 200 g/l), sodium lauryl sulfate (0.2 ml of 80 g/l), and distilled water (0.3 ml). The mixture was incubated at 98°C for 1 hr. n-butanol:pyridine 5 ml (ratio:15:l) was then added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The standard curve was obtained using 1,1,3,3-tetramethoxypropane.[14] GPO activity was determined according to the method of Lawrence and Burk.[15] After tissue homogenization, supernatant was used for GPO measurement. Following the addition of KH2PO4, EDTA, GSH, B-NADPH, NaN3, and GR, the mixture was incubated. As soon H_2O_2 was added the chronometer was turned on and the absorbance at 340 nm was recorded for 5 min every 15 sec. GST activity was determined by Habig and Jakoby.[16] Enzyme activity was determined in a 4-ml cuvette containing 30 mM GSH, 30 mM 1-chloro-2,6-dinitrobenzene, 0.1 M PBS (pH = 6.5), and tissue homogenate at 340 nm using a spectrophotometer.

Statistical Analysis

Experimental results were expressed as means ± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). The Mann Whitney-U test was adopted to analyze non-parametric variables of histology scores. Parametric variables of biochemistry values were calculated using one-way ANOVA. The differences were considered significant when $p < 0.05$.

Results

Histology

The histologic design of the ovarian tissue in the SHAM animals was normal. Ovarian tissues in the

ischemia group showed condensed hemorrhage and severe vascular congestion in many of the cells. The tissues in the IS/REP group showed histopathological changes of condensed hemorrhage, infiltration of inflammatory cells. Polymorphonuclear leukocytes (neutrophils) were dominant cell types. In IS/NMXTN group general histologic and cellular structures of the tissues were not normal in appearance, however, mild vascular congestion and edema were observed. In IS/REP/NMXTN group only a slightly mild hemorrhage was around ovarian follicles. The general histologic structure of the ovarian tissue in this group was normal and no important pathologic findings in the structural level were observed except for only a slightly mild inflammation, vascular congestion and edema (Figure 1).

Biochemistry

The values of superoxide dismutase (SOD) were decreased in IS, IS/oil and IS/REP groups. However, intraperitoneal administration of NMXTN inverted the trend and increased the activity of SOD in the ovarian tissue in NMXTN treated group. The value of SOD activity in IS/REP/NMXTN group was significantly higher than those of the other experimental groups (p = 0.001). The MDA levels in IS/REP and IS/Oil groups were significantly increased (p = 0.001). Intraperitoneal administration of NMXTN significantly decreased level of MDA in ovarian tissues of NMXTN treated animals (p = 0.001). Intraperitoneal administration of NMXTN significantly increased level of GPO in ovarian tissues of NMXTN treated animals (p < 0.05) (Table 1). Intraperitoneal administration of NMXTN significantly increased level of GST in ovarian tissues of NMXTN treated animals (p = 0.001) (Figure 2).

Discussion

The present study it was investigated whether intraperitoneal administration of NMXTN was useful or not in the prevention of ovarian damage in ischemia/reperfusion conditions in rat ovaries and it was found to have beneficial effects. Histopathological and biochemical assessments were performed in SHAM, ischemia, ischemia-reperfusion, ischemia-controlled plus intraperitoneal administration of NMXTN groups. Ischemia, ischemia-reperfusion and intraperitoneal NMXTN applied to tissues were analyzed histologically. Results showed that oxidative stress level followed a

Table 1. Average size and number of the molecules. The data are expressed as mean ± SD.

Average size of the molecules (nm)	**77 ± 3**
Average number of NMTXN	37000 ± 9000
Stability of NMTXN	> 15 days

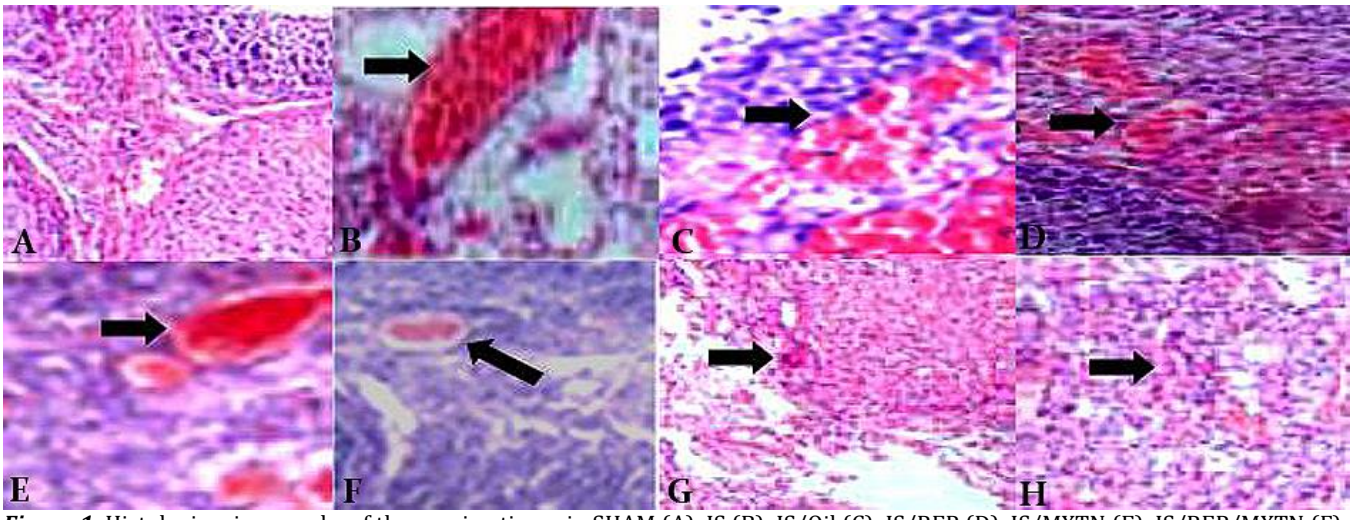

Figure 1. Histologic micrographs of the ovarian tissue in SHAM (A), IS (B), IS/Oil (C), IS/REP (D), IS/MXTN (E), IS/REP/MXTN (F), IS/NMXTN (G), IS/REP/NMXTN (H) groups. (A) Shows normal secondary follicles with compact stroma between them. (B) Many follicles at different stages of development are observed. Edematous ovarian stroma and multiple dilated congested blood vessels with some areas of hemorrhage are shown. (C) Edematous ovarian stroma and dilated congested blood vessels with some areas of hemorrhage are shown. (D) Ovarian follicles at different stages of development with edema in the stroma and hemorrhage are observed. (E), Edema in the stroma and hemorrhage are observed (F) There is edematous ovarian stroma and few dilated congested blood vessels with some areas of hemorrhage. (G) Many follicles at different stages of development are seen. There is edematous ovarian stroma and few dilated congested blood vessels with some areas of hemorrhage. (H) Normally appearing ovarian tissue with preserved healthy follicles at different stages of development with slight edema or hemorrhage is observed.

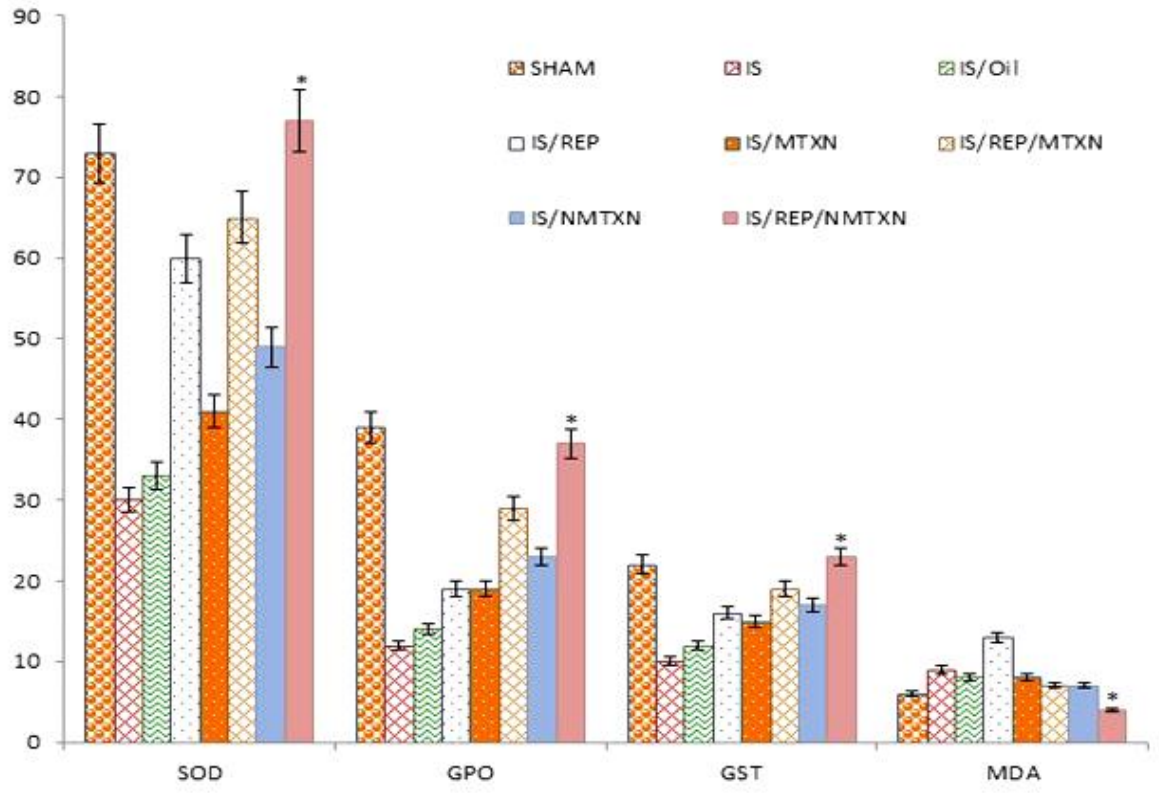

Figure 2. Bar graph shows comparison of the activities of SOD (mmol/min/mg), GPO (U/g protein), GST (U/g protein) and MDA (µmol/g protein) in the ovarian tissues of the animals of the all experimental groups. Data are expressed as Mean ± SD.
*P<0.05 vs. IS, IS/Oil, IS/REP, IS/MXTN, IS/REP/MXTN and IS/NMXTN groups.

parallelism with the tissue damage. Edema, vascular congestion, hemorrhages, and leukocyte infiltration have been used as histopathological parameters in the evaluation of the condition of the cell.[17] Edema, vascular congestion, hemorrhage, and leukocyte infiltration in the NMXTN treated animals were milder.

In the present study, levels of SOD in ovarian tissue were assessed and compared in all the experimental groups. SOD is an antioxidant enzyme that catalyzes the conversion of superoxide free radical into hydrogen peroxide and molecular oxygen. SOD and endogenous antioxidant enzymes neutralize free radicals and protect tissues from the harmful effects of free radicals and active oxygen species Arosio.[18] Our results showed that in the NMXTN treated animals, SOD was increased compared to those in other groups and intraperitoneal administration of NMXTN, secured ovarian tissue against ischemia-reperfusion injury. MDA is a lipid peroxidation product and occurs as a result of the peroxidation of fatty acids that contain three or more double bonds. MDA causes cross-linking of membrane components and leads to negative consequences like changes in ion permeability and enzyme activity via affecting the ion exchange through the cell membranes.[19,20] MDA levels in the present study were found to be much lower in the NMXTN treated animals compared to those in other experimental groups. This could protect the tissues against ischemia-reperfusion injury in NMXTN treated animals. GPO activity is significantly reduced in tissues undergoing oxidative stress-related conditions like ischemia-reperfusion injury.[21] GPO detoxifies the hydrogen peroxide radical that forms in the cell by converting it to water and prevents the formation of more toxic products from hydrogen peroxide radical.[22] In the present study a significant decrease in GPO activity was observed in ovarian tissues of NMXTN treated animals. GST binds foreign substances to the –SH group of cysteine in glutathione, neutralizes the electrophilic regions and protects the cells from the harmful effects of foreign substance regions.[23] Activity of GST has been reported to be suppressed in oxidative tissue injury induced by

ischemia.[23] Consistently, our findings showed that GST activity in ovarian tissue of NMXTN treated animals was significantly lower than those in other group.

There are many studies in the literature about the improvement of ischemia reperfusion injury. Studies demonstrated that the agents with antioxidant or anti-inflammatory activities may be beneficial in reducing ovarian ischemia reperfusion injury. Also, studies revealed the beneficial effect of controlled reperfusion in the prevention of ovarian tissue damage. Although there are many studies in the literature; ischemia/reperfusion damage continues to be a serious problem clinically. Essentially, early diagnosis and treatment of ovarian torsion plays an important role to provide urgent protection against life-threatening complications from ischemia and to prevent future infertility.[24]

It has been indicated that NMXTN is non-toxic to mitochondria and is an effective antioxidant. $MXTNH_2$ appears to be produced (via reduction) from NMXTN when in a buffer in the presence of glutathione and this process is known to use the semiquinone (MXTNH) as an intermediate exposure to oxygen either by ambient atmosphere or by singlet oxygen readily oxidizes $MXTNH_2$ back into NMXTN. This suggests that glutathione is capable of recycling NMXTN as an antioxidant. [25]

Substances are administered by a wide variety of routes. A key factor determining the route selected is whether the agent is being administered for a local or systemic (either enteral or parenteral effect. Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism, which occurs commonly with orally administered chemicals and therapeutics.[26] Intraperitoneal administration seems more effective and available where oral administration of an agent may cause difficulties. It is clear that trans-peritoneal absorption of the agent is far faster than oral administration.[26] It seems time saving is very important in emergency conditions like ovarian torsion.

In conclusion, histopathologic results obtained from all the experimental groups were consistent with the results of the biochemical analyses indicating that intraperitoneal administration of NMXTN could be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia. Regarding the trans-peritoneal absorption of the NMXTN that is far faster than its oral administration, it could be considered in clinical practice where that ovarian torsion is the case and ovarian functions must be resumed as early as possible to preserve and prevent future infertility. The present study demonstrated that intraperitoneal administration of NMXTN could improve ischemia-reperfusion injury in ovarian tissue exposed to ischemia.

As the ovary is known to have a tolerance of ischemia and ability to recover in humans, what the delayed appearance and biochemical results would be some days after the ischemic episode needs further long-term studies. Furthermore, to reach at the optimal dose and optimal timing for maximal effect of the nanoparticles on ischemia reperfusion injury following ovary detorsion, dose-response studies at various timing in administration of NMXTN are remained to be performed.

Conflict of Interest

There were no conflicts of interest to declare.

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References

1. Melcer Y, Maymon R, Pekar-Zlotin M, Vaknin Z, Pansky M, Smorgick N. Does she have adnexal torsion? Prediction of adnexal torsion in reproductive age women. *Archives of Gynecology and Obstetrics.* 2018; 297: 685-690.
2. Balci O, Energin H, Görkemli H, Acar A. Management of Adnexal Torsion: A 13-Year Experience in Single Tertiary Center. *Journal of Laparoendoscopic and Advanced Surgical Techniques, Part A.* 2019; 29: 293-297.
3. Celik A, Ergün O, Aldemir H, Ozcan C, Ozok G, Erdener A, Balýk E. Long-term results of conservative management of adnexal torsion in children. *Journal of Pediatric Surgery*. 2005; 40: 704-708.
4. Resapu P, Rao Gundabattula S, Bharathi Bayyarapu V, Pochiraju M, Surampudi K, Dasari S. Adnexal torsion in symptomatic women: a single-centre retrospective study of diagnosis and management. *Journal of the Institute of Obstetrics and Gynaecology*. 2019; 39: 349-354.
5. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. *Journal of Lipid Research*. 1998; 39: 1529-1542.
6. Huang HY, Helzlsouer KJ, Appel LJ. The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. *Cancer Epidemiology, Biomarkers and Prevention*. 2000; 9: 647-652.

7. Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T, Zuo L. Role of ROS and Nutritional Antioxidants in Human Diseases. *Frontiers in Physiology*. 2018; 17(9): 477.
8. Hategekimana J, Masamba KG, Ma J, Zhong F. Encapsulation of vitamin E: effect of physicochemical properties of wall material on retention and stability. *Carbohydrate Polymers*. 2015; 124: 172-179.
9. Cortés-Sanabria L, Paredes-Ceseña CA, Herrera-Llamas RM, Cruz-Bueno Y, Soto-Molina H, Pazarín L, Cortés M, Martínez-Ramírez HR. Comparison of cost-utility between automated peritoneal dialysis and continuous ambulatory peritoneal dialysis. *Archives of Medical Research*. 2013; 44: 655-661.
10. Chaudhary K, Haddadin S, Nistala R, Papageorgio C. Intraperitoneal drug therapy: an advantage. *Current Clinical Pharmacology*. 2010; 5: 82-88.
11. Oral A, Odabasoglu F, Halici Z, Keles ON, Unal B, Coskun AK, Kilic C, Surer I, Salman AB. Protective effects of montelukast on ischemia-reperfusion injury in rat ovaries subjected to torsion and detorsion: biochemical and histopathologic evaluation. *Fertility and Sterility*. 2011; 95: 1360-1366.
12. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*. 1983; 16: 109-110.
13. Sun Y, Larry WO, Ying L. A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*. 1988; 34: 497-500.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. 1979; 95: 351-358.
15. Wei H, Frenkel K. *In vivo* formation of oxidized DNA bases in tumor promoter-treated mouse skin. *Cancer Research*. 1991; 51: 4443-4449.
16. Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. *Methods in Enzymology*. 1981; 77: 398-405.
17. Tolba MF, Omar HA, Azab SS, Khalifa AE, Abdel-Naim AB, Abdel-Rahman SZ. Caffeic acid phenethyl ester: a review of its antioxidant activity, protective effects against ischemia-reperfusion injury and drug adverse reactions. *Critical Reviews in Food Science and Nutrition*. 2016; 56: 2183-2190.
18. Fan C, Chen Q, Ren J, Yang X, Ru J, Zhang H, Yang X. Notoginsenoside R1 suppresses inflammatory signaling and rescues renal ischemia-reperfusion injury in experimental rats. *Medical Science Monitor*. 2020; 26: e920442.
19. Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications*. 2005; 338: 668-676.
20. Ximenes VF, Paino IM, Faria-Oliveira OM, Fonseca LM, Brunetti IL. Indole ring oxidation by activated leukocytes prevents the production of hypochlorous acid. *Brazilian Journal of Medical and Biological Research*. 2005; 38: 1575-1583.
21. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*. 2006; 160: 1-40.
22. Behroozi-Lak T, Zarei L, Moloody-Tapeh M, Farhad N, Mohammadi R. Protective effects of intraperitoneal administration of nimodipine on ischemia-reperfusion injury in ovaries: Histological and biochemical assessments in a rat model. *Journal of Pediatric Surgery*. 2017; 52: 602-608.
23. Mansoorali KP, Prakash T, Kotresha D, Prabhu K, Rama Rao N. Cerebroprotective effect of *Eclipta alba* against global model of cerebral ischemia induced oxidative stress in rats. *Phytomedicine*. 2012; 19: 1108-1116.
24. Jain A, Rani V. Mode of treatment governs curcumin response on doxorubicin-induced toxicity in cardiomyoblasts. *Molecular and Cellular Biochemistry*. 2018; 442: 81-96.
25. Mukai K. Antioxidant activity of foods: development of singlet oxygen absorption capacity (SOAC) assay method. *Journal of Nutritional Science and Vitaminology.* 2019; 65:2 85-302.
26. Turner PV, Brabb T, Pekow C, et al. Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science*. 2011; 50: 600-613.