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Effects of Interaperitoneal Administration of α-Tocopherol Acetate on Ischemia-Reperfusion Injury in Ovaries in Female Cats of Animal House Shelter Undergoing Elective Ovariohysterectomy

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ABSTRACT

Effects of interaperitoneal administration of α-tocopherol acetate on ischemia-reperfusion injury in ovaries torsion and detorsion were studied using a cat ovary model. Twenty healthy female DSH cats ~3 kg were randomized into five experimental groups (n = 5): Group SHAM: The cats underwent only celiotomy. Group Ischemia: A 3-hour ischemia only. Group I/R: A 3-hour ischemia and a 3-hour reperfusion. Group I/ATA: A 3-hour ischemia only and 10 mg/kg intraperitoneal administration (IP) of α-tocopherol 2.5 hours after induction of ischemia. Group I/R/ATA: A 3-hour ischemia, 3-hour reperfusion, and 10 mg/kg IP of α-tocopherol 2.5 hours after induction of ischemia. Animals treated with ATA showed significantly ameliorated development of ischemia and reperfusion tissue injury compared to those of other groups ($p < 0.05$). The significantly higher values of SOD, tGSH, GPO, GSHRd, and GST were observed in I/R/ATA animals compared to those of other groups ($p = 0.001$). Damage indicators (NOS, MDA, MPO, and DNA damage level) were significantly lower in I/R/ATA animals compared to those of other groups ($p = 0.001$). Intraperitoneal administration of ATA could be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia.

Introduction

Various factors such as an elongated mesovarium and congested adnexal veins may lead to the twisting of the ovary, which then causes a blockage in the blood vessels supplying the ovary. This can result in a dangerous decrease in blood flow to the tissues and lead to permanent damage.[1] Hence, it is crucial to diagnose and treat ovarian torsion at the earliest opportunity in order to safeguard ovarian functions and avert potential infertility.[2] Upon identifying ovarian torsion, the suggested approach is to untwist the affected adnexa and assess the restoration of blood flow in order to safeguard against potential infertility, even in situations where the tissues appear cyanotic.[2,3] The process of ovarian torsion-detorsion is referred to as ischemia-reperfusion injury.[4] Reperfusion of the ischemic tissue results in significantly more severe harm to the tissue compared to the harm caused by ischemia.[5] Multiple factors contribute to the damage caused by reperfusion in the cell, with the most notable being oxygen-derived free radicals that are quickly produced in the tissue following reperfusion.[6] Due to physiological or pathological modifications, there is an occurrence of oxidative impairment accompanied by alterations in favor of the oxidation mechanism.[7] The timely identification of the condition is crucial in order

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to minimize the adverse effects of ischemic and reperfusion injury, as well as their subsequent consequences, which remain unavoidable with this particular methodology. As a result, research endeavors aimed at the prevention of reperfusion injury are of utmost significance.[8]

A proposed mechanism for tissue damage during reperfusion involves the accumulation of activated neutrophils that release reactive oxygen species.[9] The most detrimental consequence of free radicals in the cell is lipid peroxidation, which leads to a decrease in membrane potential and subsequent cellular injury. Malondialdehyde (MDA), a byproduct of lipid peroxidation, also causes significant harm to cells by inducing polymerization and cross-linking of membrane components.[10] Free oxygen radicals react with DNA and shape 8-hydroxy guanine (8-OHGua) that's one of the harm items of DNA.[11]

In show disdain toward of the reality that era of free oxygen radicals happens ceaselessly in cells, the nearness of endogenous antioxidant defense frameworks jam tissues from the destructive impacts of free oxygen radicals.[12] Various specialists, anti-inflammatory and antioxidant free radical foragers have been detailed with promising advantageous impacts on anticipation of ischemic/reperfusion wounds in tissues.[13-16] Alpha-tocopherol is the most studied and bioavailable component of vitamin E and has antioxidant properties. It is widely believed that its intake helps reduce the risk of many chronic diseases related to oxidative stress.[17] Therefore, α-tocopherol is a widely used ingredient in the functional food, cosmetics, and pharmaceutical industries. However, the development of suitable dosage forms remains challenging due to its hydrophobicity and known sensitivity to oxygen and light. The present study differs from other studies in the literature regarding the use of α-tocopherol acetate (ATA) on ischemia/reperfusion injury in cats. Aiming to investigate the peritoneal effects of ATA on ischemia/reperfusion injury, a study was designed to determine whether ATA could effectively protect against ovarian injury caused by ischemia/reperfusion in cats. Evaluation was based on biochemical parameters.

Materials and Methods

Ethical Considerations

The animals were belonged to an animal house shelter and the authorities were aware of the procedures with written consent form. The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[18] The Ethical Committee of the Urmia University approved all the experiments. Two weeks before and during the experiments, the animals were housed in individual pet cages with an ambient temperature of (23 ± 3) °C, stable air humidity and a natural day/night cycle. The cats had free access to food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups.

Study Design and Animals

Twenty healthy female DSH cats ~3 kg were randomized into five experimental groups (n = 5): Group SHAM: The cats underwent only celiotomy. Group Ischemia: A 3-hour ischemia only. Group I/R: A 3-hour ischemia and a 3-hour reperfusion. Group I/ATA: A 3-hour ischemia only and 10 mg/kg intraperitoneal administration (IP) of α-tocopherol (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany- CAS Number: 10191-41-0) 2.5 hours after induction of ischemia. Group I/R/ATA: A 3-hour ischemia, a 3-hour reperfusion and 10 mg/kg IP of α-tocopherol 2.5 hours after induction of ischemia. The dosage was modification of a study performed by Najafpour *et al.* in 2018.[19] Animals were premedicated with intramascular injection of ketamine-acepromazine (ketamine 10%, 5 mg/kg and acepromazine 2%, 0.05 mg/kg) and induced with intravenous injection of ketamine–diazepam (ketamine 10%, 10 mg/kg and diazepam 0.5%, 0.5 mg/kg).

Following intubation the animals were kept under inhalation anesthesia with isoflurane 1.5%. After termination of the procedures, all of animals were undergone routine onariohystrectomy procedure. They were rested for two weeks in the hospital and then were delivered back to animal shelters. The right ovaries were cleaned of surrounding soft tissues and then stored in a freezer at –80 °C for biochemical assessments.

Surgical Procedure for Induction of Ischemia and Reperfusion

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 cats. 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.

Biochemical Assessments

Tissue Processing for Biochemical Assessments of Ovary. The tissue samples of ovaries were kept at - 80 °C

for 3 days, and then enzyme activities were determined in cat 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 ºC. Then the supernatants were used to determine the enzymatic activities. All assays were carried out at room temperature.

Superoxide Dismutase (SOD) Analysis. 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.[20] levels of Superoxide dismutase in the homogenates were measured colorimetrically using a commercial kit (Navand Salamat, Tehran, Iran) following the company's instructions. 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.

Nitric Oxide Synthase (tNOS) Activity. Nitric oxide synthase activity of cat ovaries was measured spectrophotometrically using the oxidation of oxyhemoglobin to methemoglobin by NO as described by others.[21] The absorption difference between 401 and 421 nm was continuously monitored with a dual wave length recording spectrophotometer at 37 °C. For the total NOS (tNOS) assay, the incubation medium contained 1.6 mmol/l oxyhemoglobin, 200 mmol/L $CaCl_2$, 1 mmol/l $MgCl_2$, 100 mmol/l L-arginine, 100 mmol/l of the reduced form of nicotinamide-adenine dinucleotide phosphate, 40 mmol/l potassium phosphate (pH = 7.2), 1 mmol/l NG-nitro-L-arginine, and 10% (vol/vol) tissue extract with 50 mmol/l L-valine to inhibit arginase.[22]

Malondialdehyde (MDA) Analysis. Concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test.[23] The cat 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 by using 1,1,3,3-tetramethoxypropane.

Myeloperoxidase (MPO) Analysis. The activity of MPO in the total homogenate was measured according to previously described methods.[24] The sample was weighed and homogenized in 2 ml of 50 mmol/l phosphate buffer containing 0.5% hexadecyltrimethyl ammonium bromide (HDTMAB) and centrifuged at 3500 rpm for 60 min at 4 °C. The supernatant was used to determine MPO activity using 1.3 ml 4-aminoantipyrine-2% phenol (25 mM) solution. 25 mmol/l 4-aminoantipyrine–2% phenol solution and 0.0005% 1.5 ml H_2O_2 were added and equilibrated for 3–4 min. After establishing the basal rate, a sample suspension (0.2 ml) was added and mixed. Increases in absorbance at 510 nm for 4 min at 0.1-min intervals were recorded. Absorbance was measured at 412 nm.

Total Glutathione (tGSH) Analysis. The amount of GSH in the total homogenate was measured according to the previously described methods with some modifications.[25] The sample was homogenized at pH 7.5, in Tris–HCl buffer (2 ml of 50 mmol/l). The homogenate was precipitated with trichloroacetic acid (0.1 ml of 25%), and the precipitate was removed after centrifugation at 4200 rpm at 4 °C for 40 min, and the supernatant was used to measure GSH level. A total of 1500 µl of measurement buffer (200 mmol/l Tris–HCl buffer containing 0.2 mmol/L EDTA at pH = 7.5), 500 µl supernatant, 100 µl DTNB (10 mmol/l) and 7900 µl methanol were added to a tube and vortexed and incubated for 30 min in 37 °C. 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich, St. Louis, USA) was used as a chromogen; it formed a yellow-colored complex with sulfhydryl groups. The absorbance was measured at 412 nm using a spectrophotometer (Beckman DU 500, USA). The standard curve was obtained using reduced glutathione.

Glutathione Peroxidase (GPO) Analysis. GPO activity was determined according to the method of Lawrence and Burk.[26] After tissue homogenization, supernatant was used for GPO measurement. Following the addition of KH_2PO_4, EDTA, GSH, B-NADPH, NaN_3, 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.

Glutathione Reductase (GSHRd) Analysis. GR activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm according to Carlberg and Mannervik method.[27] After tissue homogenization, supernatant was used for GR measurement. After the NADPH and GSHAM addition, chronometer was on and absorbance was measured for 5 min in 30 min intervals at 340 nm spectrophotometrically.

Glutathione S-Transferase (GST) Activity. GST activity was determined by Habig and Jakoby.[28] 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.

Measurement of 8-Hydroxy-2 Deoxyguanine (8-OH Gua). Measurement of 8-hydroxy-2 deoxyguanine (8-OH Gua) was performed based on a modified method described by others.[8] Briefly, the amount of 8-OH gua and guanine (Gua) was measured using a HPLC system equipped with an electrochemical detector, HP Agilent 1100 module series and E.C.D. HP 1049 A. The amount of 8-OH gua and Gua was analyzed on a 250 4.6 mm Supelco LC-18-S reverse-phase column. The mobile phase was 50 mM potassium phosphate, pH 5.5, with acetonitrile, a 97 volume acetonitrile and a 3 volume potassium phosphate, and the flow rate was 1.0 ml/min. The detector potential was set at 0.80 V for measuring the oxidized base. Gua and 8-OH Gua (25 pmol) were used as standards. The 8-OH gua levels were expressed as the number of 8-OH gua molecules/105 Gua molecules.

Statistical Analysis

Experimental results were expressed as means± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using repeated measures and a factorial ANOVA with two between-subject factors. Bonferroni test for pairwise comparisons was used to examine the effect of time and treatments. The differences were considered significant when $p < 0.05$.

Results

Biochmical Findings

Superoxide Dismutase (SOD) Analysis. The value of SOD activity was 65.3 ± 0.37 mmol/min/mg tissue in the SHAM group. The values of SOD were decreased to 32.1 ± 0.21 and 51.4 ± 0.26 mmol/min/mg tissue in I and I/R groups, respectively. However, interaperitoneal administration of 10 mg/kg of α-tocopherol acetate inverted the trend and increased the activity of SOD to 71.3 ± 0.13 mmol/min/mg tissue in the ovarian tissue in I/R/ATA group. The value of SOD activity in I/R/ATA group was significantly higher than those of the other experimental groups (p = 0.001) (Table 1).

Nitric Oxide Synthase (NOS) Activity. The value of tNOS activities was increased in I and I/R groups that were significantly higher than those of SHAM group (p = 0.001). However, interaperitoneal administration of 10 mg/kg of α-tocopherol acetate inverted the trend and decreased tNOS activity in the cat's ovary. (Table 1).

Malondialdehyde (MDA) Analysis. The results of the present study showed that concentration of MDA in SHAM group was 4.6 ± 0.15 µmol/g protein in ovarian tissue. The MDA level I/R group was significantly increased to 9.7 ± 0.21 µmol/g protein (p = 0.001). Intraperitoneal administration of α-tocopherol acetate significantly decreased level of MDA in ovarian tissues of I/R/ATA animals (p = 0.001) (Table 1).

Myeloperoxidase (MPO) Analysis. The level of MPO was significantly increased in I and I/R groups (p = 0.001). Intraperitoneal administration of α-tocopherol acetate reversed the trend and significantly decreased level of MPO in ovarian tissues of I/R/ATA animals (p = 0.001) (Table 1).

Total glutathione (tGSH) Analysis. The values for tGSH levels were 9.3 ± 0.30 and 4.5 ± 0.15 nmol/g protein in SHAM and I/R animals, respectively. Intraperitoneal administration of α-tocopherol acetate significantly increased level of GSH in ovarian tissues of I/R/ATA animals (p = 0.001) (Table 1).

Glutathione Peroxidase (GPO) Analysis. The values for GPO levels were 35.5 ± 2.17 and 14.8 ± 1.12 u/g protein in SHAM and I/R animals, respectively. Intraperitoneal administration of α-tocopherol acetate significantly increased level of GPO in ovarian tissues of I/R/ATA animals (p = 0.001) (Table 1).

Glutathione Reductase (GSHRd) Analysis. The GSHRd activities in ovarian tissue in the SHAM and I/R animals were 31.3 ± 2.78 and 16.3 ± 1.17 u/g protein, respectively. Intraperitoneal administration of α-tocopherol acetate significantly increased level of GSHRd in ovarian tissues of I/R/ATA animals (p = 0.001) (Table 1).

Glutathione S-Transferase (GST) Activity. The GST activities in ovarian tissue in the SHAM and I/R animals were 18.2± 1.37 and 12.7 ± 1.88 u/g protein, respectively. Intraperitoneal administration of α-tocopherol acetate significantly increased level of GST in ovarian tissues of I/R /ATA animals (p = 0.001) (Table 1).

Measurement of 8-Hydroxy-2 Deoxyguanine (8-OH Gua). The levels of 8-OHGual/Gua, a DNA damage product, were1.3±0.11 and 2.4±0.10 pmol/L in SHAM and I/R animals, respectively. Intraperitoneal administration of α-tocopherol acetate significantly decreased level of GSHRd in ovarian tissues of I/R/ATA animals (p = 0.001) (Table 1).

Discussion

In the present study it was investigated whether intraperitoneal administration of α-tocopherol acetate was useful or not in the prevention of ovarian damage in ischemia/reperfusion conditions in cat ovaries and it was found to have beneficial effects. Biochemical assessments were performed in SHAM, ischemia, ischemia-reperfusion, ischemia-controlled plus intraperitoneal administration of α-tocopherol acetate groups. Biochemically, the activities of SOD, NOS, MDA, MPO, GSH, GPO, GSHRd, GST and a DNA damage product of 8-OHGua/Gua were assessed in the ovarian tissues of the

Table 1. Comparison of the activities of SOD, NOS, MDA, MPO, GSH, GPO, GSHRd, GST and a DNA damage product of 8-OHGua/Gua in the ovarian tissues of the animals of the all experimental groups. Data are expressed as Mean±SD.

Indices	Group SHAM	Group I	Group I/R	Group I/ATA	Group I/R/ATA
SOD (mmol/min/mg)	65.3 ± 0.37	32.1 ± 0.21	51.4 ± 0.26	43.9 ± 0.67	71.3 ± 0.13*
NOS (nmol/min/mg)	3.7 ± 0.13	3.5 ± 0.10	3.5 ± 0.40	3.7 ± 0.17	3.0 ± 0.15*
MDA (µmol/g protein)	4.6 ± 0.15	11.7 ± 0.76	9.7 ± 0.21	8.3 ± 0.52	5.3 ± 13*
MPO (U/g protein)	6.3 ± 0.15	15.7 ± 0.35	13.5 ± 0.20	11.1 ± 0.10	7.0 ± 0.13*
tGSH (nmol/g protein)	9.3 ± 0.30	2.5 ± 0.15	4.5 ± 0.15	6.7 ± 0.27	8.7 ± 0.17*
GPO (U/g protein)	35.5 ± 2.17	10.5 ± 2.56	14.8 ± 1.12	15.7 ± 1.97	25.2 ± 2.15*
GSHRd (U/g protein)	31.3 ± 2.78	9.7 ± 1.73	16.3 ± 1.17	21.8 ± 1.44	27.9 ± 2.88*
GST (U/g protein)	18.2 ± 1.37	8.7 ± 1.29	12.7 ± 1.88	13.9 ± 1.15	16.3 ± 1.11*
8-OHGua/Gua (pmol/L)	1.3 ± 0.11	2.5 ± 0.17	2.4± 0.10	1.5 ± 0.13	1.3 ± 0.10*

I: Ischemia, I/R: Ischemia-reperfusion, I/ATA: Ischemia plus intraperitoneal administration of α-tocopherol acetate, I/R/ATA: Ischemia plus reperfusion plus intraperitoneal administration of α-tocopherol loaded nanoparticles SOD: Superoxide dismutase, NOS: Nitric oxide synthase, MDA: Malondialdehyde, MPO: Myeloperoxidase, tGSH: Total glutathione,GPO: Glutathione peroxidase, GSHRd: Glutathione reductase, GST: Glutathione S-transferase and 8-OHGua/Gua: 8-hydroxy-2 deoxyguanine. * P<0.0 vs. groups I, I/R and I/ATA.

animals of the all experimental groups. Ischemia, ischemia-reperfusion and intraperitoneal α-tocopherol acetate applied to tissues were analyzed histopathologically. In the present study, levels of SOD in ovarian tissue were assessed and compared in all the experimental groups. The SOD activity in SHAM and I/R/ATA showed no significance difference. 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 [36,37]. Our results showed that in the I/R/ATA animals, SOD was increased compared to those in I , I/R and I/ATA groups and intraperitoneal administration of α-tocopherol acetate, secured ovarian tissue against ischemia-reperfusion injury.

It has been demonstrated that hypoxia causes iNOSs that play an important damaging role in I/R injury.[29] iNOS is increased after cellular stimulation via cytokines in macrophages, neutrophils, and microglia and may also contribute to late-stage tissue injury.[30] The iNOS is derived primarily from the polymorphonuclear neutrophilic leukocytes during reperfusion and down-regulation of iNOS could limit cell injury caused by hypoxia.[31] Findings of the present study showed that the iNOS levels in I and I/R groups ovarian tissues were increased compared to those of the SHAM animals. Down-regulation of iNOS could limit cell injury caused by hypoxia. Our results showed that in the I/R/ATA animals, iNOS was down-regulated compared to those in I/R group. Thus, interaperitoneal administration of 10 mg/kg α-tocopherol acetate protected ovarian tissue against ischemia-reperfusion injury more than 100 mg α-tocopherol.

MDA levels in the present study were found to be much lower in the I/R/ATA animals compared to those in other experimental groups. This could protect the tissues against ischemia-reperfusion injury in α-tocopherol acetate treated animals.

It has been demonstrated that MPO activity is increased in ischemia-reperfusion induced ovarian tissue.[32] This finding was in agreement with results of the present study. MPO activity was suppressed in α-tocopherol acetate treated animals of our study.

GSH is an antioxidant used to measure oxidative stress. Reperfusion after ischemia is reported to cause severe damage to ovarian tissue and suppress the GSH levels.[33] GSH plays a role in the protection of the cell against oxidative stress and toxic compounds as well as the metabolic processing of many endogenous compounds like estrogen, prostaglandin, and leukotrienes.[33] GSH, as an antioxidant, reacts with peroxides and free radicals and converts them into harmless products and subsequently protects the cells against the potential oxidative damage of free radicals. These findings were in agreement with our results. We found that oxidative stress was minimized and the severe damage due to sudden reperfusion was prevented in α-tocopherol acetate treated animals. GPO activity is significantly reduced in tissues undergoing oxidative stress-related conditions like ischemia-reperfusion injury.[34] 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.[35] In the present study a significant decrease in GPO activity was observed in ovarian tissues of I/R/ATA animals.

GSH is oxidized during the detoxification of hydrogen peroxide radical. GSHRd is a NADPH-dependent enzyme that converts oxidized glutathione to reduced glutathione.[36] GSHRd is reported to show higher activity in healthy tissue and in parallel with tissue damage ita activity is decreased.[37] In our study activity of GSHRd was

significantly increased in α-tocopherol acetate treated animals compared to those of I and I/R groups.

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.[38] Activity of GST has been reported to be suppressed in oxidative tissue injury induced by ischemia.[38] Consistently, our findings showed that GST activity in ovarian tissue of α-tocopherol acetate treated animals was significantly lower than those in I and I/R groups.

DNA molecules are damaged if free radicals are in a close proximity to the DNA molecules.[39] Hydroxyl radical reacts very easily with deoxyribose and the bases and causes DNA damage through extracting hydrogen from nucleic acids or reacting with double bonds.[40] 8-OH Gua is considered an important marker of DNA oxidation.[41]). Our findings showed that the ovarian tissues of I and I/R animals had higher levels of 8-OHGua than those of the SHAM animals. However, our results showed that there was no significant difference between SHAM and α-tocopherol acetate treated animals regarding the levels of DNA damage.

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.[42] [56].

Vitamin E has been reported as a useful agent both for the prevention and treatment of I/R injury in many organs. The administration of vitamin E has reported to reduce the generation of reactive oxygen species (ROS), monocyte adhesion, phosphorylation of c- Jun N-terminal kinase (JNK), p38 MAP kinase, and signal transducer and activator of transcription (STAT)-3 in TNF-a-stimulated cells.[43] It has also been documented that the administration of vitamin E prior to the conservative surgery (detorsion) provides a significant decrease for the oxidative stress markers in the ovarian tissues.[43] The comparison between the oxidative status and antioxidative status is clear enough to suggest that the administration of vitamin E, as reported previously, leads to a decrease in the oxidative stress and an increase in the antioxidation.[44]

The findings of the present study showed that α-tocopherol acetate in very low concentrations, 10 mg/kg α-tocopherol gave rise to significant improvements.

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.[45] Interaperitoneal 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.[45] It seems time saving is very important in emergency conditions like ovarian torsion.

In conclusion, biochemical analyses indicated that intraperitoneal administration of α-tocopherol acetate could be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia. Regarding the transperitoneal absorption of the α-tocopherol acetate 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 10 mg/kg α-tocopherol acetate could improve ischemia-reperfusion injury in ovarian tissue exposed to ischemia. Thus, dose-response studies should be conducted for α-tocopherol acetate to determine its maximal efficacy in minimizing ischemia-reperfusion injury in ovarian tissue.

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Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Oelsner G, Shashar D. Adnexal torsion. *Clinical Obstetrics and Gynecology*. 2006; 49: 459-463. doi: 10.1097/00003081-200609000-00006
2. Geimanaite L, Trainavicius K. Ovarian torsion in children: management and outcomes. *Journal of Pediatric Surgery.* 2013; 48: 1946-1953. doi: 10.1016/j.jpedsurg.2013.04.026
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. doi: 10.1016/j.jpedsurg.2005.01.008
4. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. *Journal of Pathology*. 2000; 190: 255-266. doi: 10.1002/(SICI)1096-9896(200002)190:3<255::AID-PATH526>3.0.CO;2-6
5. Zimmerman BJ, Granger DN. Reperfusion injury. *Surgical Clinics of North America.* 1992; 72: 65-83. doi: 10.1016/s0039-6109(16)45628-8

6. Nakagiri A, Sunamoto M, Takeuchi K. Evidence for the involvement of NADPH oxidase in ischemia/reperfusion-induced gastric damage via angiotensin II *Journal of Physiology and Pharmacology.* 2010; 61: 171-179.
7. Javanmardi S, Khordadmehr M. Benidipine reduces ischemia/reperfusion injury following testicular torsion/detorsion in rats. *Iranian Journal of Veterinary Surg*ery. 2017; 12: 21-27. doi: 10.22034/ivsa.2017.97394.1128
8. Ingec M, Isaoglu U, Yilmaz M, Calik M, Polat B, Alp HH, Kurt A, Gundogdu C, Suleyman H. Prevention of ischemia-reperfusion injury in rat ovarian with the on-off method. *Journal of Physiology and Pharmacology*. 2011; 62: 575-582.
9. Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB,Boveris A. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia- reperfusion injury. *Molecular Aspects of Medicine.* 2004; 25: 199-210. doi: 10.1016/j.mam.2004.02.020
10. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. *Journal of Lipid Research*. 1998; 39: 1529-1542.
11. 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.
12. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90: 7915-7922. doi: 10.1073/pnas.90.17.7915
13. 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. doi: 10.1016/j.fertnstert.2010.08.017
14. Mogilner JG, Lurie M, CoranAG, Nativ O, Shiloni E, Sukhotnik I. Effect of diclofenac on germ cell apoptosis following testicular ischemia-reperfusion injury in a rat. *Pediatric Surgery International*. 2006; 22: 99-105. doi: 10.1007/s00383-005-1580-9
15. Halici Z, Karaca M, Keles ON, Borekci B, Odabasoglu F, Suleyman H, Cadirci E, Bayir Y, Unal B. Protective effects of amlodipine on ischemiareperfusion injury of rat ovary: biochemical and histopathologic evaluation. *Fertility and Sterility.* 2008; 90: 2408-2415. doi: 10.1016/j.fertnstert.2007.10.007
16. Anderson AM, Mitchell MS, Mohan RS. Isolation of curcumin from turmeric. *Journal of Chemical Education*. 2000; 77: 359–360. doi: 10.1021/ed077p359
17. Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, Schuch W, Sheehy PJA, Wagner KH. Vitamin E. *Journal of the Science of Food and Agriculture*. 2000; 80: 913-938. doi: 10.1002/(SICI)1097-0010(20000515)80:7<913::AID-JSFA600>3.0.CO;2-3
18. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain.* 1983; 16: 109-110. doi: 10.1016/0304-3959(83)90201-4
19. Najafpour A, Azizizadeh H. interaperitoneal administration of αlpha-tocopherol loaded nanoparticles improves ischemia-reperfusion injury in rat ovaries torsion and detorsion model. *Bulletin of Emergency and Trauma.* 2018; 6(3): 207-216. doi: 10.29252/beat-060304
20. Sun Y, Larry WO, Ying L. A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry.* 1988; 34: 497-500.
21. Feelisch M, Noack EA. Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *European Journal of Pharmacology*. 1987; 139: 19-30. doi: 10.1016/0014-2999(87)90493-6
22. Knowles RG, Merrett M, Salter M, Moncada S. Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat. *Biochemical Journal*. 1990; 270: 833-836. doi: 10.1042/bj2700833
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry.* 1979; 95: 351-358. doi: 10.1016/0003-2697(79)90738-3
24. Wei H, Frenkel K. In vivo formation of oxidized DNA bases in tumor promoter-treated mouse skin. *Cancer Research.* 1991; 51: 4443-4449.
25. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*. 1968; 25: 192-205. doi: 10.1016/0003-2697(68)90092-4
26. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. 1976. *Biochemical And Biophysical Research Communications*. 2012; 425: 503-509. doi: 10.1016/j.bbrc.2012.08.016
27. Carlberg I, Mannervik B. Glutathione reductase. *Methods in Enzymology*. 1985; 113: 484-490. doi: 10.1016/s0076-6879(85)13062-4
28. Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. *Methods in Enzymology.* 1981; 77: 398-405. doi: 10.1016/s0076-6879(81)77053-8
29. Chatterjee PK, Patel NS, Kvale EO, Cuzzocrea S, Brown PA, Stewart KN, Mota-Filipe H, Thiemermann C. Inhibition of inducible nitric oxide synthase reduces renal ischemia/reperfusion injury. *Kidney International*. 2002; 61: 862-871. doi: 10.1046/j.1523-1755.2002.00234.x
30. Jeddi S, Zaman J, Zadeh-Vakili A, Zarkesh M, Ghasemi A. Involvement of inducible nitric oxide synthase in the loss of cardioprotection by ischemic postconditioning in hypothyroid rats. *Gene* 2016; 580: 169-176. doi: 10.1016/j.gene.2016.01.014
31. Ferdinandy P, Schulz R. Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia-reperfusion injury and preconditioning. *British Journal of Pharmacology*. 2003; 138: 532-543. doi: 10.1038/sj.bjp.0705080
32. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacology and Therapeutics*. 1991; 51: 155-194. doi: 10.1016/0163-7258(91)90076-x
33. Celebi F, Akbas A, Saglam MB. Effect of sertraline in indomethacin-induced gastric mucosal damage. *Asian Journal of Chemistry.* 2012; 24: 1966-1970.
34. 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. doi: 10.1016/j.cbi.2005.12.009
35. Sharma H, Zhang X, Dwivedi C.The effect of ghee (clarified butter) on serum lipid levels and microsomal lipid peroxidation. *AYU* 2010; 31: 134-140. doi: 10.4103/0974-8520.72361
36. Polat B, Suleyman H, Alp HH. Adaptation of rat gastric tissue against indomethacin toxicity. *Chemico-Biological Interactions.* 2010; 186: 82-89. doi: 10.1016/j.cbi.2010.03.041
37. Shi HY, Li ZH, Zhang YX, Chen L, Xiang DY, Zhang YF. Two pear glutathione S-transferases genes are regulated during fruit development and involved in response to salicylic acid, auxin, and glucose signaling. *PLoS One*. 2014; 9: e89926. doi: 10.1371/journal.pone.0089926
38. 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. doi: 10.1016/j.phymed.2012.07.004

39. Milligan JR, Aguilera JA, Nguyen TT, Ward JF, Kow YW, He B, Cunningham RP. Yield of DNA strand breaks after base oxidation of plasmid DNA. *Radiation Research.* 1999; 151: 334-342.
40. Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: an enemy within. *Trends in Genetics.* 1993; 9: 246-249. doi: 10.1016/0168-9525(93)90089-z
41. Aksoy AN. Ovarian ischemia-reperfusion injury: a brief review. *SM Journal of Gynecology and Obstetrics*. 2015; 1: 1008-1111.
42. Srivastava G, Mehta JL. Currying the heart: curcumin and cardioprotection. *Journal of Cardiovascular Pharmacology and Therapeutics.* 2009; 14: 22e7. doi: 10.1177/1074248408329608
43. Uguralp S, Bay Karabulut A, Mizrak B. Effects of pentoxifylline and vitamin E on the bilateral ovary after experimental ovarian ischemia. *Uropean Journal of Pediatric Surgery*. 2005; 15: 107-113. doi: 10.1055/s-2004-821256
44. Nugent D, Newton H, Gallivan L, Gosden RG. Protective effect of vitamin E on ischamia-reperfusion injury in ovarian grafts. *Journal of Reproduction and Fertility*. 1998; 114: 341-346. doi: 10.1530/jrf.0.1140341
45. Turner PV, Brabb T, Pekow C, Vasbinder MA. 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.