

RESEARCH ARTICLE

Protective Effects of Chrysin in Rats with Ovarian Torsion

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Article ID: KVFD-2021-25357 Received: 02.01.2021 Accepted: 08.06.2021 Published Online: 12.06.2021

Abstract

The objective of this study is to assess the protective effect of chrysin (CH) against ovarian torsion-detorsion injury. Thirty-two female albino rats were divided into 4 groups as Control, Torsion/Detorsion (T/D), TD-CH, and CH. Ovarian torsion was done for 3 hours on TD and TD-CH groups and then detorsion was performed. 50 mg/kg of CH was administered to the TD-CH group via oral gavage half an hour before the detorsion. Subsequently, 50 mg/kg of CH was administered via oral gavage to TD-CH and CH groups for 14 days. At the end of the experiment, blood samples were collected and ovarian tissue were taken. SOD and GPx activities and GSH and 8-OHdG levels were studied in serum and ovarian tissue. Also, IL-18, KIM-1, NGAL, Cys-C levels were studied in serum samples. GSH levels and GPx, SOD activities in both serum and ovarian tissue were significantly lower in TD group compared to the control and TD-CH groups ($P<0.05$), whereas the 8-OHdG level was significantly higher ($P<0.05$). Treatment with CH resulted in a decrease in 8-OHdG level and an increase in GSH level, GPx and SOD activities in both serum and ovarian tissue in the TD-CH group compared to TD group ($P<0.05$). Chrysin could ameliorate ovarian injury. Essentially, this outcome is thanks to the antioxidant, anti-inflammatory, and antiapoptotic effects of chrysin. Furthermore, it also has significant effects on DNA oxidative damage.

Keywords: Ovary torsion, Chrysin, 8-OHdG, Antioxidant

Ovaryum Torsiyonu Uygulanmış Ratlarda Chrysinin Koruyucu Etkisi

Öz

Bu çalışmadaki amacımız ovaryum torsiyon detorsiyon hasarına karşı chrysinin (CH) koruyucu etkisini değerlendirmektir. Otuz iki adet dişi wistar albino rat 4 gruba ayrıldı. Grup 1: Kontrol grubu, grup 2: TD grubu; grup 3: TD-CH grubu; grup 4: CH grubu. TD ve TD-CH gruplarına 3 saat torsiyon uygulandı ve 3 saatin sonunda detorsiyon yapıldı. TD-CH grubuna detorsiyondan yarım saat önce 50 mg/kg CH oral gavaj ile verildi. Daha sonra TD-CH ve CH gruplarına 14 gün boyunca 50 mg/kg CH oral gavaj ile verildi. Deneme sonunda ratlar sakrifiye edilerek kan örnekleri ve ovaryum dokusu alındı. Serum ve ovaryum dokusunda SOD ve GPx aktiviteleri, GSH ve 8-OHdG seviyeleri, serum örneklerinde ise 8-OHdG, IL-18, KIM-1, NGAL, Cys-C seviyeleri çalışıldı. TD grubunda hem serum hem de ovaryum dokusunda, GSH seviyeleri, GPx ve SOD aktiviteleri kontrol ve TD-CH gruplarından anlamlı olarak düşükken ($P<0.05$), 8-OHdG seviyesi anlamlı olarak yüksekti ($P<0.05$). TD-CH grubunda, CH uygulaması TD grubu ile karşılaştırıldığında hem serum hem de ovaryum dokusunda 8-OHdG seviyesini anlamlı olarak düşürürken, GSH seviyesi, GPx ve SOD aktivitelerini anlamlı olarak arttırmıştır ($P<0.05$). Chrysin ovaryum hasarını düzeltebilir. Bu sonuç esas olarak chrysinin antioksidant, antiinflammatuar ve antiapoptotik etkilerinden kaynaklanmaktadır. Dahası DNA hasarı üzerinde de önemli etkileri vardır.

Anahtar sözcükler: Ovaryum torsiyon, Chrysin, 8-OHdG, Antioksidant

INTRODUCTION

Ovarian torsion, which is defined as the twisting of the ovary and vascular stem around the axis of the suspensory

ligament, accounts for 3% of all gynecological emergencies ^[1]. Albeit ovarian torsion occurs in all women and primarily in women of reproductive age ^[2]. Depending on the degree of torsion, venous return to the ovarian tissue decreases, and

How to cite this article?

Dirik D, Kömüroğlu AU, Koşal V, Başbuğan Y, Özdek U, Kolusarı P, Keleş ÖF: Protective effects of chrysin in rats with ovarian torsion. *Kafkas Univ Vet Fak Derg*, 27 (4): 417-424, 2021.
DOI: 10.9775/kvfd.2021.25357

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subsequently, stromal edema and internal bleeding occur. If arterial blood flow stops, ischemic and necrotic processes begin in the tissue. Early diagnosis and management is indispensable to preserve ovarian function^[3]. Due to non-specific symptoms such as vomiting, nausea, and pelvic pain, a delay is experienced in diagnosis almost always. Diagnostic difficulty in ovarian torsion leads to loss of ovarian tissue and function^[4]. Ovarian torsion/detorsion (T/D) or ischemia/reperfusion (I/R) is a pathophysiological incident in which histological damage, which is associated with decreased perfusion following the lack of oxygen in the ovarian, occurs^[5].

It releases reperfusion in the tissue following ischemia, and subsequent reactive oxygen species^[4]. As a result of the reperfusion process, an excessive amount of molecular oxygen supplementation occurs in the ovary tissue. These reactive oxygen species (ROS) attack the cell membrane through the peroxidation of polyunsaturated fatty acids and lead to cellular damage^[6].

Thus, oxidation has devastating effects on the ovarian tissue^[5]. In healthy state, the ROS level is kept under control by antioxidants such as glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD). However, in the event of oxidative stress, uncontrolled production of ROS damages biomolecules such as proteins, DNA, RNA and lipids, and cellular structure^[7]. It has been revealed that I/R causes DNA strand breaks, oxidative DNA damage, and mutations^[8]. 8-hydroxy-2' deoxyguanosine (8-OHdG) is a marker used to assess oxidative DNA damage and is a risk factor for various diseases^[9].

Several antioxidants have been found to be effective in preventing oxidative damage and inflammation in ovarian tissue, which is exposed to I/R injury^[10]. Chrysin is a natural flavonoid, found in honey, propolis, and various plant extracts. Antioxidant, anti-inflammatory, and anti-diabetogenic effects of chrysin have been reported in numerous studies^[11,12]. It has been also revealed that it has protective effects in testicular/I/R injury^[13].

Kidney injury molecule-1 (KIM-1), cystatin C (Cys-C), and Neutrophil gelatinase-associated lipocalin (NGAL) have emerged as a sensitive marker in the early diagnosis of glomerular damage^[14]. KIM-1 is a type 1 transmembrane glycoprotein and is significantly upregulated from proximal tubular cells following renal stress such as ischemia or nephrotoxicity^[15]. Neutrophil gelatinase-associated lipocalin (NGAL) is a stress protein released from damaged tubular cells following various damaging stimuli, and it is known as one of the promising biomarkers of acute kidney injury^[16]. Cys-C is eliminated only by the kidneys, and early-stage renal lesions might lead to a change in serum Cys-C level^[14].

Interleukin-18 (IL-18) is the proinflammatory cytokine of the IL-1 superfamily and mediates the proinflammatory response and ischemic proximal tubular damage^[17]. Chrysin

alleviates renal impairment and morphological anomalies caused by ischemic reperfusion injury. Chrysin suppresses tubular apoptosis and inflammation in renal I/R injury^[18].

Thus, to reveal these effects, we aimed in this study to investigate the effects of CH on oxidant and oxidant enzymes, in the event of ovarian T/D.

MATERIAL AND METHODS

Ethical Statement

This study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (YUHAD-YEK, Date: 17/06.2020; Decision number: 2020/06-15).

Animal and Experimental Design

Thirty-two non-pregnant female Wistar albino rats, aged between 8 to 10 weeks, weighing between 150 and 200 g were used in the study. Animals were kept in polypropylene cages at 21°C with a 12-h light and 12-h dark cycle. Food and water were provided to the animals via *ad libitum* intake.

Rats were divided into 4 groups, with 8 rats in each group.

Control group (C group): No treatment was performed on the rats in this group.

Torsion/Detorsion (TD) group: Torsion was performed on ovaries of the rats in this group for 3 h, and subsequently, detorsion was performed.

Torsion/Detorsion-Chrysin (TD-CH) group: Torsion was performed on ovaries of the rats in this group for 3 h, and then 50 mg/kg of Chrysin was administered via oral gavage for 14 days^[2].

Chrysin (CH) group: 50 mg/kg of Chrysin was administered to this group via oral gavage for 14 days^[2].

The rats were sacrificed following the 14-day study.

Surgical Procedure

All surgical interventions were performed in a sterile setting and under appropriate laboratory conditions. Ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg) were administered intraperitoneal for anesthesia. The abdomen was entered through a 2 cm longitudinal incision in the lower abdominal region of the rats. The vascular clip was applied approximately 1 cm above and below the left ovary, and reperfusion was achieved via relaparotomy 3 h later^[19]. Half an hour before reperfusion, 50 mg/kg of chrysin was administered via oral gavage, and they were kept at 4°C for 15 min to coagulate. Subsequently, they were centrifuged at 3000 RPM for 15 min. Serums were stored at -20°C until the study day. The ovary was removed meticulously and stored at -20°C for biochemical assessment.

Collection of Samples

At the end of the study, in order to assess biochemical and pathological parameters, blood and ovary samples were taken 24 h after the administration of the last dose. Blood samples were taken into tubes without anticoagulant by cardiac route.

Biochemical Analysis

Following the surgical procedure, ovary tissue was removed and used for biochemical analysis. For biochemical analysis, ovary tissue was homogenized. The obtained supernatant and serum were stored at -20°C until they were studied. Ovary tissue SOD (Catalog No: SG-20188; Sinogeneclon Co., Hangzhou, China), GPx (Catalog No: SG-20976; Sinogeneclon Co., Hangzhou, China), 8-OHdG (Catalog No: YLA0061RA, YL Biotech Co. Ltd. Shamghai, China) and GSH (Catalog No: SG-20391; Sinogeneclon Co., Hangzhou, China), serum IL-18 (Catalog No: SG-20281, Sinogeneclon Co., Hangzhou, China), 8-OHdG (Catalog No: YLA0061RA, YL Biotech Co. Ltd. Shamghai, China), GSH (Catalog No: SG-20391; Sinogeneclon Co., Hangzhou, China), GPx (Catalog No: SG-20976; Sinogeneclon Co., Hangzhou, China), SOD (Catalog No: SG 20188; Sinogeneclon Co., Hangzhou, China), KIM-1 (Catalog No: SG 20751; Sinogeneclon Co., Hangzhou, China), NGAL (Catalog No: SG-20801; Sinogeneclon Co., Hangzhou, China), Cys-c (Catalog No: SG-20197; Sinogeneclon Co., Hangzhou, China) levels were measured using ELISA kits via following the instructions of the manufacturer.

Histopathological Examination

Necropsy of rats was performed at the end of the trial. Ovarian tissue samples were taken and fixed in 10% buffered formaldehyde solution. The routine follow-ups of the tissues were performed. They were embedded in paraffin blocks and 4 µm sections were taken with a microtome. The sections were stained with hematoxylin-eosin (H&E), examined under a light microscope (Nikon 80i-DS-RI2), and photographed.

Statistical Analysis

The software of SPSS 20.0 (SPSS for Windows Chicago, IL, USA) was used for statistical analysis. All data was presented

as mean and standard deviation. The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed or not. One-way ANOVA was used for comparison of biochemical parameters between groups, and different groups were compared with post hoc Tukey's test. The results were considered statistically significant at $P < 0.05$.

RESULTS

Ovarian tissue SOD and GPx activities and GSH and 8-OHdG levels are presented in *Table 1*. It was determined that in TD Group, whereas ovarian tissue GPx and SOD activity and GSH levels were significantly lower compared to TD-CH and the control group, the 8-OHdG level was significantly higher ($P < 0.05$). Whereas the TD-CH group ovarian tissue GSH and 8-OHdG levels were significantly higher than the control group, SOD activity was found to be lower ($P < 0.05$). The ovarian tissue GPx activity was similar in the control group and TD-CH groups ($P > 0.05$).

It was found out that serum 8-OHdG and IL-18 levels of TD group were significantly higher compared to the control, TD-CH, and CH groups ($P < 0.05$) (*Table 2*). Serum GSH level, GPx, and SOD activities were significantly lower in TD group compared to TD-CH, control, and CH groups ($P < 0.05$). Serum KIM-1 level was determined to be significantly lower in CH group compared to the other groups ($P < 0.05$). It was found out that serum NGAL level was significantly higher in TD group compared to C, TD-CH, and CH groups ($P < 0.05$). It was determined that serum CYC-C level was significantly higher in TD and TD-CH groups compared to C and CH groups ($P < 0.05$).

As a result of the examinations, a normal histological appearance was observed in the ovary tissues of the control group (*Fig. 1-A,B*). However, venous congestion and interstitial edema in the cortex of the ovary, capillary hyperemia in the secondary follicle, degenerative-necrotic changes in the luteal cells in the corpus luteum, and accumulation of hemosiderin pigment were detected in TD group (*Fig. 1-C,D,E*). Besides, a lesser venous congestion, degenerative and necrotic cells in the corpus luteum, and accumulation of hemosiderin pigment in macrophages were detected in ovarian tissues in D-CH group compared

Table 1. Ovary tissue SOD, GPx, activities and GSH and 8-OHdG levels

| Parameters | Grops | | | |
|----------------|--------------------------|-------------------------|--------------------------|-------------------------|
| | Control | TD | TD-CH | CH |
| 8-OHdG (ng/mL) | 2.01±0.02 ^c | 2.5±0.07 ^a | 2.31±0.05 ^b | 1.73±0.9 ^d |
| GPx (IU/mL) | 27.28±1.4 ^a | 24.83±1.1 ^b | 28.39±0.8 ^a | 27.17±0.1 ^a |
| SOD (ng/mL) | 204.54±12.3 ^a | 150.54±6.5 ^c | 179.11±10.7 ^b | 168.8±7.2 ^b |
| GSH (ng/mL) | 123.03±4.9 ^b | 100.11±3.2 ^c | 148.83±4.8 ^a | 128.04±8.5 ^b |

^{a,b,c,d} Values within a row with different superscripts differ significantly at $P < 0.05$; TD: Torsion/Detorsion; TD-CH: Torsion/Detorsion-Chrysin; CH: Chrysin; GPx: Glutathione peroxidase; SOD: superoxide dismutase; GSH: Glutathione

Table 2. Serum biochemical parameters of the groups

| Parameters | Grops | | | |
|----------------|-------------------------|-------------------------|--------------------------|-------------------------|
| | Control | TD | TD-CH | CH |
| 8-OHdG (ng/mL) | 1.63±0.1 ^d | 2.09±0.07 ^a | 1.87±0.06 ^b | 1.77±0.06 ^c |
| GPx (IU/mL) | 29.25±1.4 ^a | 18.93±1.2 ^d | 21.52±0.8 ^c | 22.92±0.1 ^b |
| SOD (ng/mL) | 252.43±9.0 ^a | 226.31±5.9 ^b | 251.74±5.7 ^a | 242.33±6.4 ^b |
| GSH (ng/mL) | 125.49±5.0 ^b | 95.16±4.7 ^c | 135.87±8.3 ^a | 119.10±4.1 ^b |
| IL-18 (pg/mL) | 121.31±7.1 ^b | 141.35±7.2 ^a | 108.35±3.8 ^{bc} | 99.26±29.0 ^c |
| KIM-1 (pg/mL) | 87.36±3.0 ^a | 87.65±2.5 ^a | 88.53±3.6 ^a | 86.20±5.22 ^b |
| NGAL (ng/mL) | 1.43±0.07 ^b | 1.32±0.05 ^c | 1.52±0.05 ^a | 1.51±0.07 ^a |
| CYC-C (ng/mL) | 16.15±1.1 ^b | 18.87±0.4 ^a | 18.85±0.4 ^a | 13.95±0.6 ^c |

^{a,b,c,d} Values within a row with different superscripts differ significantly at $P < 0.05$; **TD**: Torsion/Detorsion; **DT-CH**: Torsion/Detorsion-Chrysin; **CH**: Chrysin; **GPx**: Glutathione peroxidase; **GSH**: Glutathione; **SOD**: superoxide dismutase; **IL-18**: interleukin-18; **KIM-1**: Kidney Injury molecule; **NGAL**: Neutrophil gelatinase-associated lipocalin; **CYC-C**: Cystatin-C.

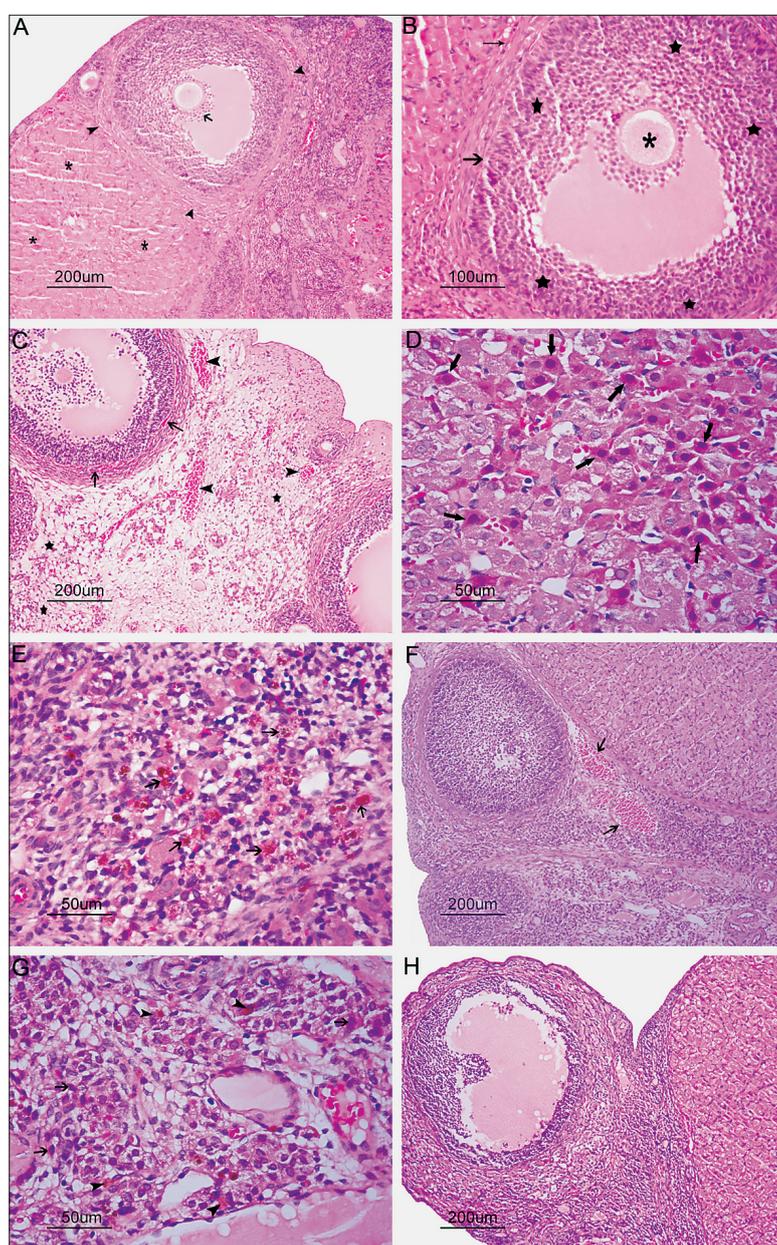


Fig 1. Histopathological assessment of the rat ovaries among the groups. **Control group (Group I):** (A) Secondary follicle (†), corpus luteum (*) and cumulus oophorus cells (→); (B) Primary oocyte (*), granulated cells (★), theca interna (▶), and theca externa (') in the ovary with histopathologically normal appearance. **TD group (Group II):** (C) Venous congestion (†) and interstitial edema (★) in the cortex of the ovary. Capillary hyperemia in the theca interna and externa in the secondary follicle (→), (D) Degenerative and necrotic changes in the luteal cells in the corpus luteum (→), (E) Accumulation of hemosiderin pigment in macrophages in the corpus luteum (→). **TD-CH group (Group III):** (F) Less venous congestion (→) is seen compared to Group II, (G) Lesser degenerative and necrotic cells (→) and hemosiderin pigment accumulation in macrophages (†) are seen in the corpus luteum compared to Group II. **CH group (Group IV):** (H) Normal histological appearance of the follicle and corpus luteum is seen in the ovary; H&E

to TD group (Fig. 1-F,G). The normal histological appearance of the ovaries was observed in CH group similar to the control group (Fig. 1-H). As a result of all findings, it was concluded that the pathological findings significantly decreased in the ovaries of the rats which were administered with Chrysin by oral route following the torsion-detorsion procedure.

DISCUSSION

Ischemia-reperfusion injury is explained by the hypothesis that the accumulation of neutrophils and platelets occurs around the inflammation site due to activated complement and other inflammatory components. This accumulation of inflammatory cells increases the production of reactive oxygen species. Moreover, glycolysis, increased lactic acid concentration, and accumulation of intracellular Ca lower the intracellular pH and ends up with acidosis. This results in an increase in the intracytoplasmic lysosomal enzymes, which leads to injury in protein and cell membranes [4]. Antioxidant and oxidant balance is disturbed in the event of ischemia-reperfusion injury. Hence, it has been suggested that it might be beneficial to use antioxidant pharmacological agents during or before reperfusion to prevent I/R injury [20].

Ovarian detorsion without ovariectomy may preserve ovarian function, yet prophylactic measures against subsequent I/R injury are required. Thus, animal models have focused on antioxidant and anti-inflammatory pharmacological agents for protecting the ovary in the event of I/R injury [2,21]. One of these agents is Chrysin (CH). The antioxidative property of Chrysin has been attributed to the inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression, and the inhibition of nuclear factor kappa B activity has been suggested to be CH antioxidative and anti-inflammatory [22]. It has been demonstrated that CH protects rat ovaries from I/R injury through improving histopathological scores, increasing antioxidative activity, and decreasing lipid peroxidation [2].

Glutathione is an abundant non-thiol protein that plays a key role in the coordination of antioxidant defense mechanisms. GSH acts as a substrate for several enzymes containing glutathione peroxidase and can capture reactive oxygen species directly. Decreased GSH level in tissue not only deteriorates cellular defense but also ends up with increased oxidative damage [23]. It has been revealed that Chrysin could increase GSH concentration by upregulating the gene transcription of glutamate-cysteine ligase (GCL), which catalyzes the rate-limiting step in glutathione synthesis, via ERK2/Nrf2 signaling [24]. In line with our study, Oral et al. [25] demonstrated in their study that the GSH level of the ovary tissue decreased significantly in torsion group compared with the control groups. It has been revealed that the decrease in GSH level following detorsion in rats with ovarian torsion might be stemming

from the consumption during oxidative stress [26]. In the current study, while GSH level of ovarian tissue in TD group was determined to be significantly lower compared to the control group, CH administration caused an increase in GSH level.

The increase in GSH level and increase in SOD, CAT, and GPx activities indicate tissue healing after oxidative damage [27]. It has been revealed that 75 mg/kg/day CH administration protects against focal cerebral I/R injury effectively; also, SOD, GPx activities, and GSH levels significantly increased in the treatment group upon the administration of CH following the I/R injury [28]. It has been revealed that compared to I/R group a significant increase was detected in SOD activity in I/R-CH group, which were administered with a single dose of 50 mg/kg CH [2].

The antioxidant property of GSH has been attributed to its ability to increase antioxidant enzyme activities as well as its powerful feature of free radical capture [23,29]. In the current study, while GSH level of ovarian tissue in TD group was found to be significantly lower compared to the control group, CH administration caused an increase in GSH level.

Enzymatic antioxidants play a crucial role in protecting cells from oxidative damage [30]. SOD is the most significant enzyme that catalyzes superoxide radicals ($O_2^{\cdot-}$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). GPX is an enzyme that catalyzes H_2O_2 and lipid peroxides into water [30]. It has been shown in the ovarian torsion studies that SOD and GPX activities decreased in serum [5,31] and ovarian tissue [31]. In the current study, GPx and SOD activities in ovarian tissue and serum were determined to be significantly lower in TD group compared to C, CH, and TD-CH groups. CH administration caused decreasing SOD and GPx activities to increase. This mechanism of action is considered to stem from the hydroxyl groups in the 5th and 7th positions of the CH molecule, which directly eliminates free radicals [32]. It has also been determined that CH inhibits oxidative stress indirectly through regulating antioxidant enzyme activities [33]. It has been revealed in various studies that CH medication protects tissues against oxidative stress and induces an increase in antioxidant enzyme activities [13,34].

Reactive oxygen species cause deformation of DNA-protein bonds and changes in bases [35]. Biologically, I/R has been found to induce DNA strand breaks, oxidative DNA damage, and mutations [36,37]. 8-OHdG is one of the most stable DNA bases and is a well-established biomarker of oxidative damage in DNA [35]. 8-OHdG has been shown to be immunoreactive in ovarian I/R injury [8]. It has been revealed that ovarian tissue concentration of 8-OHdG significantly reduced in rats treated with 3-h torsion and 24-h detorsion compared to the control group [35]. Eken et al. [38] revealed in their study that ovary 8-OHdG concentration increased significantly in T/D group compared

to the control group. In the current study, serum and ovarian tissue 8-OHdG levels were determined to be significantly higher in TD group compared to TD-CH and CH groups. CH treatment significantly decreased both serum and ovarian 8-OHdG levels. CH antioxidant activity might reduce tissue damage, leading to a decrease in 8-OHdG level.

IL-18 is a pro-inflammatory cytokine and is also known as the interferon-gamma inducing factor. IL-18 is crucial in supporting host defense [39]. IL-18 is involved in the pathophysiology of various inflammatory diseases including I/R injury, transplant rejection, and autoimmune diseases [40]. It has been revealed that IL-18 whose expression is stimulated in cardiomyocytes by ROS could play a crucial role in myocardial I/R injury [41]. Administration of IL-18 binding proteins to human cardiomyocytes improves cardiac function [42], and has been shown to play a role in the cardiac inflammatory response to I/R injury in mice [40]. It was found out that IL-18 knockout mice with simulated kidney I/R injury were highly protected against I/R injury, and tubular damage was reduced [43]. CH displays various biological effects on the immune system. CH suppresses the inflammatory response and displays an anti-inflammatory response [44]. Pro-inflammatory cytokines are modulators of host responses in trauma and immune response; hence, the anti-inflammatory response serves to reduce inflammation and promote healing while acting to exacerbate the disease [44]. CH with a double bond at C2-3 and a hydrogen group at R3 could inhibit pro-inflammatory cytokines [45]. In the current study, serum IL-18 level was found to be significantly higher in TD group compared to TD-CH, CH, and control groups. CH administration caused serum IL-18 level to decrease. The decrease in serum IL-18 level upon the CH administration might be due to the inhibition of CH proinflammatory cytokines.

Neutrophil Gelatinase-Associated Lipocalin (NGAL) induces epithelial protection and epithelial development following ischemia [46]. It has been demonstrated that NGAL expression increases following renal ischemia [47]. NGAL is commonly found in numerous biological fluids and various cell types in humans. It serves to protect against bacterial infections in normal tissue and to modulate the oxidative system [48]. Kidney Injury Molecule-1 (KIM-1) has been suggested to play a significant role in removing damaged epithelial cells and dead cells via phagocytosis [46]. It has been revealed that ovarian tissue and serum NGAL levels in ovarian torsion are higher in T/D group compared to the control group, but this elevation is not significant [49]. It has been reported that the concentrations of KIM-1 and NGAL in urine are increased compared to the control group in the event of kidney I/R injury [50]. In the current study, serum KIM-1 level in TD group was akin to the control and TD-CH groups. Serum NGAL level was determined to be significantly higher in TD group compared to C, TD-CH, and CH groups. Serum Cyc-C level was found to be significantly higher in TD and TD-CH groups compared to C and CH groups. It

has been revealed that NGAL uses the BCL2/BAX signaling pathway in renal tubular epithelial cell apoptosis [51]. It has been demonstrated that NGAL is reduced in apoptotic tubular cells, and this renoprotective effect is thanks to the inhibition effect of caspase-3 activation [52]. Thus, the fact that the serum NGAL level in the presented study was significantly lower in TD group compared to the other groups may be due to ovarian torsion increasing apoptosis in the kidney, and it can be stated that CH in TD-CH group has a protective effect on the kidney, since the serum NGAL level is significantly higher than TD group.

In TD group, venous congestion and interstitial edema in the ovarium cortex, capillary hyperemia in the secondary follicle, and degenerative necrotic cells in luteal cells in the corpus luteum were detected when we evaluated the histopathologic findings and compared them with the control group. In the TD-CH group, lesser venous congestion, lesser degenerative and necrotic cells in the corpus luteum were detected in ovarium tissues compared to TD group. Turkoz et al. [26] found that vascular congestion, hemorrhage, and edema in the ovarium tissue increased in the torsion detorsion group compared to the control group. When the I/R process occurs, inflammatory cells cause an increase in free oxygen radicals that lead to tissue damage by the mechanism of inflammation [53]. Compression of ovarian vessels due to stromal edema and ovarian enlargement prevents lymphatic and venous outflow first and subsequently arterial inflow. Ovarian arterial pressure is then blocked due to stromal pressure, resulting in infarction and necrosis in addition to microscopic bleeding in the ovarium tissue [21]. Consistent with our study, Hortu et al. [19] revealed a higher incidence of histopathological scars such as vascular congestion and hemorrhage and increased cellular damage in the torsion group compared to the control group. It has been demonstrated that Chrysin improves histological changes such as vascular congestion, hemorrhage, edema, and inflammatory cell infiltration in ovarium tissue, which underwent ovarian torsion [2]. In the current study, chrysin treatment significantly reduced the pathological findings in the ovaries. Anti-inflammatory and antioxidant properties of Chrysin could prevent the effects of I/R injury

In conclusion, it is considered in this study that CH administration could be effective in reducing ovarian injury during the TD procedure. The antioxidant and anti-inflammatory properties of CH could protect the ovary and lower injury during torsion-detorsion. Furthermore, CH has considerable effects on oxidative DNA damage.

FINANCIAL SUPPORT

None.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

DD, AUK, UO, VK, YB, PK and OFK experimental Study, AUK and UO laboratory study, OFK pathological examination, AUK statistic, DD, AUK, UO, VK, YB, PK and OFK literature search and article writing.

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