Original Article

The Impact of Chrysin on The Folliculogenesis and Ovarian Apoptosis in Ischemia-Reperfusion Injury in The Rat Model

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Abstract

Background: The ovarian Ischemia/reperfusion is one of the gynecological emergency concerns that may lead to the ovary damage and folliculogenesis. The present research aimed to evaluate the impact of the Chrysin (CH) on the ischemia-reperfusion (I/R) injury in the rat model.

Materials and Methods: In this experimental research, 48 adult female rats, 8 weeks age and 180-200 g weight, have been categorized into 6 equal groups (n=8) including one sham and 5 ovarian torsion groups (OT+CH groups) that received different treatments. Each group has been treated 30 min before detorsion with gavage of CH or normal saline for 1 week and pregnant mare serum gonadotropin (PMSG) has been injected on the day 5 for initiating folliculogenesis. Finally, bio-chemical, molecular, histopathological, apoptotic and hormonal evaluations were performed.

Results: The anti-oxidant enzyme, superoxide dismutase and glutathione peroxidase, ameliorated in the ovarian tissues of the OT+CH groups in comparison with the OT group ($P<0.001$). Moreover, the level of serum Luteinizing hormone considerably declined and estradiol level ($P<0.001$), partly enhanced in the rats treated with CH in comparison with the ones in the OT group ($P<0.05$). In addition, histopathological scores of the OT+CH groups ameliorated in comparison with the OT group scores ($P<0.05$). Furthermore, the expression *Caspase-3* and *Bax* genes were significantly increased while the expression of *Bcl-2* was notably decreased in the OT group in comparison with the sham group ($P<0.05$).

Conclusion: Here, it seems that CH is possibly beneficial for the protection of ovaries against reperfusion injury and ischemia.

Keywords: Bax/Bcl-2, Chrysin, Ischemia, Ovary, Reperfusion Injury

Citation: Mohammadi Z, Hosseinianvari SM, Ghazalian N, Fani M, Mahmudian AS, Brazvan B, Shokoohi M, Abtahi-Eivary SH, Moghimian M. The impact of chrysin on the folliculogenesis and ovarian apoptosis in ischemia-reperfusion injury in the rat model. Int J Fertil Steril. 2022; 16(4): 299-305, doi: 10.22074/IJFS.2021.540364.1200.

Introduction

Adnexal torsion refers to higher resistance or termination of blood circulation due to the ovaries' rotation around a suspensory ligament (1, 2). With regard to lower venous pressure, venous return ends as a result of torsion whereas the arterial blood flow continues regularly and edema expands in the ovarian tissues. Because of edema, supplying blood to ovaries discontinues that result in high ovarian pressure and ischemic damages (3). Then, prolonging this period leads to the necrosis as well as irreversible damages in the ovarian tissues. Notably, ovarian torsion has been considered the commonest gynecological emergency that accounts for 2.7% prevalence (4).

Nonetheless, detorsion of the twisted ovaries results in the other risk called ischemia/reperfusion (I/R) injury that has an association with the tissues neutrophil infiltration and reperfusion. The production of reactive oxygen species (ROS) enhances in the ovarian tissues due to the reperfusion procedure (5), leading to cellular damage via peroxidation of the poly-unsaturated fatty acids (6, 7).

Multiple anti-oxidants prevent oxidative injuries and inflammations in the ovarian tissue (8). In this regard, Karaçor et al. (9) found that providing Iloprost has a higher impact on the reduction of the ischemia-reperfusion (I/R) injuries in the ovarian tissues. Some studies have shown the helpful impact of administering

Received: 05/October/2021, Accepted: 07/December/2021
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Royan Institute
International Journal of Fertility and Sterility
Vol 16, No 4, October-December 2022, Pages: 299-305

Montelukast or Telmisartan that prevent the ovarian I/R injury (10, 11).

To sum up, any acceptable treatment has been not presented for curing the ovarian failure induced by I/R injury; therefore, we tried the usage of Chrysin (CH) for curing the ovarian failure in the rats.

It is well known that CH has been proposed as one of the natural flavonoids in the propolis, honey as well as several other plant extracts. Many investigations confirmed anti-inflammatory, anti-diabetogenic and anti-oxidant effects of CH (12-14). Researchers confirmed the advantages of the anti-oxidants for I/R injury in the brain, heart and ovary. As far as we know, there are no reports about the CH impact of the I/R injury in the course of folliculogenesis. Therefore, we aimed at the evaluation of the impacts of CH on experimental I/R ovaries injuries in the rat model.

Materials and Methods

Ethical considerations

The Ethical Committee of the Gonabad University of Medical Sciences (Khorasan Razavi, Iran) verified this experimental research (IR.GMU.REC.1398.134).

Experimental design

Totally, 48 adult female Wistar rats with a weight of 230 ± 10 g and 8 weeks age have been stored at a constant temperature of 25 ± 2°C at 30 to 70% humidity with 12-h light/12-h dark cycle in the animal room with free access to food and water.

Then, they were classified into 6 equal groups (n=8):

1. Sham group: A longitudinal cutting (2.5 cm) was considered in the mid-line of lower abdomen and 5/0 nylon (SUPA medical divice, Iran) sutures were used to close the incision.
2. Torsion/detorsion group (OT): The left ovaries were chosen to induce torsion for 3 hours. After that, 30 min prior to the detorsion operation, the normal saline (Samen pharmacutical Co, Iran) was administered intra-peritoneally.
3. Torsion/detorsion/Chrysin group (OT+CH30): As well as OT group, we induced the ovarian torsion, but the 30 mg/kg CH 30 minutes prior to the detorsion operation was administered.
4. Torsion/detorsion/Chrysin group (OT+CH50): Like OT group, we induced the ovarian torsion, but the 50 mg/kg CH 30 minutes prior to the detorsion operation was administered.
5. CH groups (CH30): This group did not receive any operation; however, each rat received 30 mg/kg CH (Cat No: 480-40-0, Sigma Aldrich, Germany).
6. CH groups (CH50): This group did not receive any operation; however, each rat received 50 mg/kg CH.

Then, each group has been treated. The treatment was started 30 minutes before detorsion and continued for once daily for 1 week, with gavage CH or normal saline and PMSG has been injected on day 5 for initiating folliculogenesis. At the end, the animals have been sacrificed and the ovary and blood samples have been gathered.

Surgical procedure

Following the acclimatization period, we used Xylazine (10 mg/kg) and Ketamine (50 mg/kg) (Sigma Aldrich, Germany) to anesthetize the rats. After that, we made a longitudinal cutting (2.5 cm) in the central area of the lower abdomen and a small peritoneal cutting has been made, which revealed the left uterine horns and adnexa. Then, the left ovary has been rotated 720° in a clockwise direction around its axis and fixed to the abdominal wall with 6/0 nylon for avoiding its detorsion. Next, 5/0 nylon sutures have been used to close the incision, which kept torsion for 3 hours. Moreover, 30 minutes prior to the opening of the ovarian torsion, CH extract has been injected intraperitoneally. With the completion of ischemic period that lasted 3 hours, the ovaries' twisting opened and a week has been allowed to the ovaries' reperfusion. By ending this operation, we injected Buprenorphine (0.02 mg/kg, Exir, Iran) as the analgesics and at the end of a week, the animals of each group have been were anesthetized by Xylazine and Ketamine. Afterwards, the blood specimens have been drawn out from the heart in order to assess the metabolic changes. In addition, the blood samples have been centrifuged at 4000 rpm for 5 minutes and serum has been separated. Next, all the serum samples have been added into 3 micro-tubes (500 µL, Shimi Tajhiz, Iran) and transported to freezer at a temperature of -70°C till the experiment time. Finally, we removed the ovarian tissues for evaluating the genes expression and histological changes (15).

Tissue fixation, samples preparation, and histopathological evaluation

Following the ovariectomy process, we fixed the ovaries and put the samples in 10% formalin (Cat No: 1.1150, Notron, Iran) for 72 hours. After dehydration, we put them in paraffin (Cat No; CellWax, UK) and procured histological slides with 5 µm cutting by microtome. Then, hematoxylin-eosin (H&E) has been used to stain the samples (16). Moreover, for histo-metrical and histological investigations, the tissue sections of all ovaries have been examined from the cortex to medulla in a spiral clockwise direction. In the next step, we counted the number of pre-antral, antral and graafian follicles, as well as corpora lutea in each slide, and compared them with various groups for the respective analyses (10). The sections were observed by a microscope (BX63 , Olympus, Tokyo, Japan) and magnification x400.

Apoptotic cell detection

TUNEL staining, the ovarian tissues were monitored to assay apoptotic cells. According to this method, the samples have been fixed in formalin 10% for 1 week and dehydrated by ascending degrees of alcohol (Razi, Iran). Then, Xylene has been used to clear the samples

and finally we blocked them in paraffin. In the next step, paraffin blocks have been incised into sections of 5 µm thickness and the slides have been transferred to Poly-L-Lysine slides (Sigma Aldrich, Germany). Afterwards, we deparaffinized the tissue sections and act with regard to the common histological procedures. It should be mentioned that we put the samples in 3% hydrogen peroxide solution (Chemicaliran, Iran) in ethanol (Razi , Iran) for 15 minutes for blocking the tissue internal peroxidase. The samples were washed, then proteinase K (Boehringer Mannheim, Germany) was used and the samples were incubated at the room temperature for 20 minutes. Then samples were rewashed and incubated with the reaction solution of the TUNEL staining kit (C10619, TermoFisher, Germany). Following, slides were incubated with a diaminobenzidine (DAB) solution (34002, TermoFisher, Germany) at room temperature for 15 minutes and then hematoxylin (TermoFisher, Germany) phosphate buffered saline (PBS) (6) staining was performed for all slides. At the end, those cells with brown nucleus have been assessed as the TUNEL-positive cells (17).

RNA extraction

The Favor Prep Blood/Cultured Cell Total RNA Mini Kit (FABRK000, Favorgen, Taiwan) has been used to isolate the total RNA. While preparing the RNA extraction, we added 5-10 mg of the rat ovaries into 800 µl of lysis buffer (FABRK000, Favorgen, Taiwan). Upon the homogenization, we transported the solution and added 200 µl of Chloroform (CX1055-6, EMD Millipore, Germany). Then, centrifuge has been performed at 4ºC, 12000 rpm, and 10 minutes. Following the centrifugation, the three-phase process has been formed and we transferred the upper phase to a new 1.5 ml micro-tube and added 200 µl ethanol (70%) (Razi, Iran), mixed completely by vortexing for 30 seconds and transported cautiously to an RNA binding pure link spin column (FABRK000, Favorgen, Taiwan). In the next step, we washed the buffer to remove the impurities and eluted the extracted total RNA in 50 µl of RNase-free water stored at -80ºC. Afterwards, Nanodrop Epoch 2 microplate spectro-photometer (model No. UV-1100, Biotech, USA) has been applied for quantitative evaluation of 260/280 and 260/230 ratio of absorbance values so that a 260/280 ratio of 2.0 and a 260/230 ratio in ranges between 2.0 and 2.2 have been chosen as pure for RNA. Finally, %1.5 agarose gel electrophoresis was utilized for assessing the samples' integrity.

cDNA synthesis

According to the research design, kit of cDNA synthesis (YT4500, Yekta Tajhiz Azma®, Iran) was used to convert the total RNA (> 500 ng) to cDNA. Then, 500 ng of total RNA was employed for the first-strand cDNA synthesis in a total volume of 20 µl based on the manufacturer's manual. Upon the centrifugation, we incubated the tubes at 70°C for 5 minutes. In addition, for oligo (dT), the tubes were incubated at 42°C for 60 minutes and the reaction was ended by heating at 70°C for 5 minutes. It is notable that for every reaction set, one RNA sample was procured without RevertAidTM M-MuLV reverse transcriptase (YT4500, Yekta Tajhiz Azma®, Iran) (RT reaction) for providing a negative control in the consequent PCRs. Finally, the RT reaction product was maintained at -20°C for less than a week. However, in order to enjoy a longer storage, the samples were transferred to -70°C.

Real-time polymerase chain reaction

The real- time polymerase chain reaction (PCR) was performed in a total volume of 20 µl consisting of Primer (0.4 µM), BioFact™ 2X Real-Time PCR Smart mix Syber green (BioFact, Korea), cDNA (20 ng/µl) and nuclease-free water (model No. 7498 ABI, USA). These primers were designed by the Perlprimer 1.1.20 software (Table 1). All reactions were done in triplicate. Also, *β-actin* was chosen as an endogenous housekeeping gene. Therefore, 45 thermal cycles were run as follows: 5 minutes at 95ºC, 45 cycles, 95ºC for 15 seconds, and 61ºC for 1 minutes. Then, Delta CT values were computed using the *β-actin* CT values via $2^{-\Delta\Delta CT}$ method where ΔCt refers to the difference(s) between CT-value of the target genes and CT-value of *β-actin* (1).

Table 1: Primers for quantitative real-time reverse transcription-polymerase chain reaction

Gene	Oligomer sequence (5'-3')	Amplicon size (bp)
β-actin	F: GTCGTGCTTGCCATTCAG R: GGTATCTTCTTTCCATTCTTCAGTAG	309
Bax	F: TTTGCTACAGGGTTTCATCCAG R: GTTGTCCAGTTCATCGCC	145
Bcl2	F: TGTGGATGACTGACTACCTGAACC R: CAGCCAGGAGAAATCAAACAGAGG	122
Caspase3	F: GTGGAACTGACGATGATATGGC R: CGCAAAGTGACTGGATGAACC	135

Evaluation of oxidative stress markers

The serum level of oxidative stress markers was measured according to the procedures described in details in our previous study (5).

Measurement of luteinizing hormone and estrogen level

The serum level of the Estrogen hormones was determined by the Demeditec Diagnostics kit (E-FS-E117, Germany). And, the serum luteinizing hormone (LH) level was assayed by ELISA kit (CSB-E12654r. Cusabio: China) (18).

Statistical analysis

SPSS version 20 (IBM, USA) was used to perform statistical analyses. The Kolmogorov-Smirnov test was employed to determine normal distribution of data. It is notable that all data have been written as the mean ± standard (mean ± SE) error. Also, one-way analysis of variance (ANOVA) as well as Tukey post-hoc test was run to comparing Oxidative Stress values and histopathological variables. Here, the statistical significance level was considered $P<0.05$.

Results

Histological assay

The number of follicles, antral, Graafian as well as pre-antral follicles were evaluated and its comparison between the OT group and the sham group showed a significant increase (P<0.001). We observed a significant reduction (P<0.001) in the corpus luteum in the OT group in comparison with the sham group. The number of Graafian and pre-antral follicles were decreased significantly in the OT group in comparison with the sham group (P<0.001, P=0.002). Comparing the number of preantral follicles, we observed a significant decline in the OT group and the OT+CH group (P<0.001) and a significant increase in the number of corpus luteum in the OT group (P<0.001, Table 2, Fig.1).

Table 2: The count of follicles in study groups

Group	Preantral follicles	Antral follicles	Graafian follicles	Atretic bodies
Sham	16.1± 0.22	4.3 ± 0.26	5.5 ± 0.18	2 ± 0
OT	4.6 ± 0.26***	2.5 ± 0.18***	2.7 ± 0.16***	5.6 ± 0.18***
OT+CH30	6.5 ± 0.18###	2.6 ± 0.18	3.3 ± 0.18	3.1 ± 0.12###
OT+CH50	7.7 ± 0.16###	2.6 ± 0.18	3.3 ± 0.18	3.1 ± 0.12###
CH30	14.1 ± 0.12	4.3 ± 0.18	4.1 ± 0.22***	2 ± 0
CH50	14.7 ± 0.16	4 ± 0.18	4.3 ± 0.18**	2 ± 0

Data are presented as mean ± SD. OT; Ovarian torsion, CH; Chrycin, *; Significant difference with sham groups, #; Significant difference with OT group, **; P=0.002, ***; P<0.001, and ###; P<0.001.

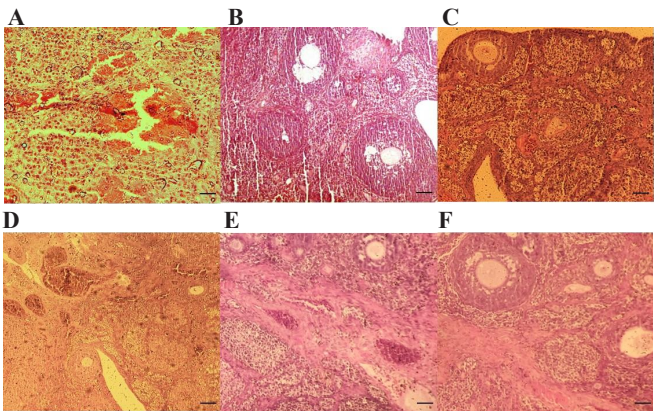

Fig.1: Histological findings. **A.** Sham group. **B.** Ovarian torsion/detorsion. **C.** Three hours' ovarian torsion and receiving 30 mg/kg of Chrysin. **D.** Three hours' ovarian torsion and receiving 50 mg/kg of Chrysin. **E.** Healthy rats receiving 30 mg/kg of Chrysin. **F.** Healthy rats receiving 50 mg/kg of Chrysin (scale bar: 20 µm).

Apoptosis index

The count of TUNEL positive cells in the TD group in comparison with the sham group was higher significantly (Fig.2). Apoptosis index in the pre antral, antral and grafian follicles were enhanced in the OT group in compression with the sham group. Also, the apoptosis index of ovarian tissue cells and follicles of our treated groups with various doses of CH was significantly in comparison with the TD group.

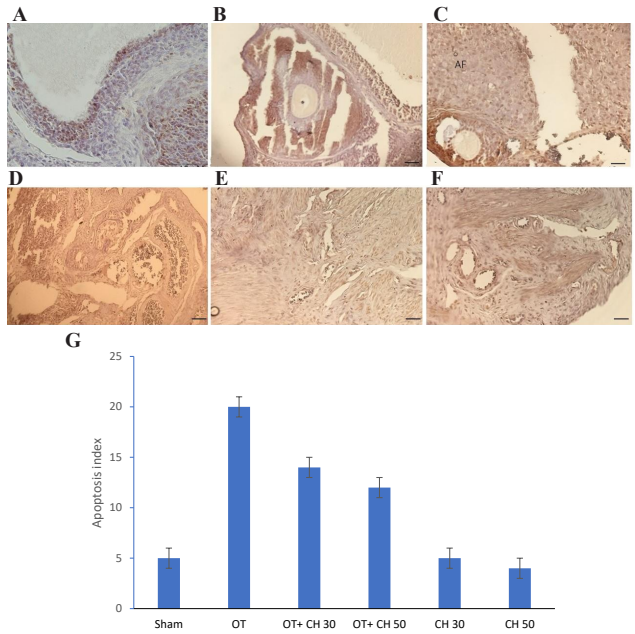

Fig.2: Apoptotic cells of ovarian tissue. **A.** Sham group. **B.** Ovarian torsion/detorsion. **C.** Three hours' ovarian torsion and receiving 30 mg/kg of Chrysin. **D.** Three hours' ovarian torsion and receiving 50 mg/kg of Chrysin. **E.** Healthy rats receiving 30 mg/kg of Chrysin. **F.** Healthy rats receiving 50 mg/kg of Chrysin. **G.** Apoptotic index of ovarian tissue in study groups.

Expression of *Bax*, *Bcl-2*, and *Caspase-3*

Outputs obtained by the *Bax* gene expression indicated a significant reduction in the sham group in comparison with the OT+CH groups, OT+CH30 group and OT+CH50 group (P<0.001, Table 3). Moreover, comparing the OT group with OT+CH groups showed a significant reduction of Bax gene in OT+CH50 group (P<0.001); however, any differences were not seen in the OT+CH30 group.

Table 3: The Bax, *Bcl-2*, and *Caspase-3* genes experssion in study groups

Group	*Bax*	*Bcl-2*	*Caspase-3*
Sham	0.20 ± 0.02	1 ± 0.03	0.25 ± 0.03
OT	1 ± 0.04***	014 ± 0.01***	1 ± 0.02***
OT+CH30	0.42 ± 0.06###	0.45 ± 0.04	0.45 ± 0.06###
OT+CH50	0.35 ± 0.08###	0.45 ± 0.07	0.40 ± 0.04###
CH30	018 ± 0.03***	0.92 ± 0.05	0.22 ± 0.02
CH50	016 ± 0.01***	0.95 ± 0.04	0.18 ± 0.03

Data are presented as mean ± SD. The asterisk sign (*) represents a significant difference between the OT and sham groups and (#) indicates a significant difference between OT+CH0.5 and OT group. OT; Ovarian torsion, CH; Chrycin, ***; P<0.001, and ###; P<0.001.

We observed no significant difference expression of Bcl-2 in the control group in comparison with the OT group; while, CH30 and 50 groups showed a significant decline (P<0.001). Also, we considered a significant reduction of Bcl-2 expression in the OT+CH 50 group (P<0.001); although, the OT+CH30 group showed no changes.

The *Casp3* gene expression indicated a significant decline in the sham in comparison with the OT+CH groups, OT+CH 30 group and OT+CH 50 group (P<0.001). Also, we observed a significant reduction of *Casp3* expression in the OT+CH 50 group (P<0.001); although, the OT+CH 30 group showed no changes.

Biochemical results

Serum level of malondialdehyde

The malondialdehyde (MDA) level of serum significantly enhanced in the sham group in comparison with the OT group (P<0.001). Moreover, treatment with CH diminished the MDA level in the treated groups in comparison with the OT group (P<0.001, P=0.001, Fig.3A).

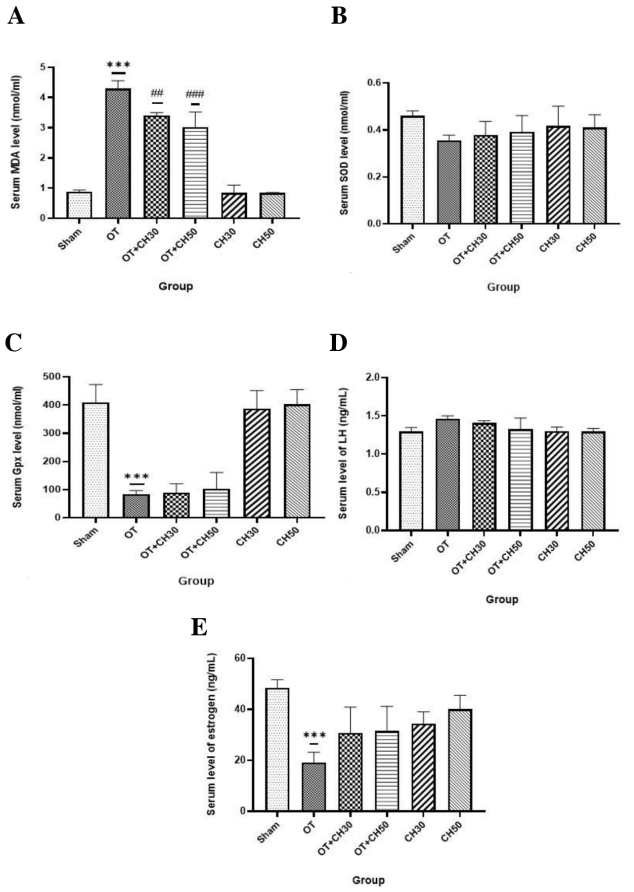

Fig.3: Biochemical and hormonal results. Comparison of the serum level of **A.** MDA, **B.** SOD, **C.** GPx, **D.** LH, and **E.** Estrogen in various groups. MDA; Malondialdehyde, SOD; Superoxide dismutase, GPx; Glutathione peroxidase, and LH; Luteinizing hormone.

Serum level of superoxide dismutase

The level of superoxide dismutase (SOD) serum in the OT group indicated a decline in comparison with the sham group. In addition, treating with CH enhanced this level in treated groups, but not significant (Fig.3B).

Serum level of glutathione peroxidase

According to our analysis, glutathione peroxidase (GPx) serum level remarkably diminished in the OT group in comparison with the sham group (P<0.001). Moreover, treatment with CH enhanced the GPx level in treated groups, but it was not significant (Fig.3C).

Serum level of luteinizing hormone

The serum level of LH in the OT group demonstrated an enhancement in comparison with the sham group and treatment with CH declined the level of this hormone in treated groups but it was not significant (Fig.3D).

Serum estrogen level

The level of serum estrogen declined in the OT groups in comparison with the sham group (P<0.001). Moreover, we observed no significant enhancement between the OT group and treatment groups (OT+CH) (Fig.3E).

Discussion

In the present study, we have evaluated the CH effect on the ovary in a rat model of ovarian I/R injury. We found that I/R injury considerably diminishes the ovarian reserve while is related to the great oxidative stress. This amount of oxidative stress contributes to the hormonal changes, and enhances histological damages. On the contrary, oxidative stress variables, histopathological changes and ovarian reserve markers partly improved in the animals receiving CH following the I/R injury. An ovarian detorsion without oophorectomy may protect the ovarian functions; however, prophylactic measures would be necessary against I/R injury following the procedures. There is not enough information about the cellular damages following I/R injury. For this reason, knowledge of I/R injury mechanism can present a robust platform for new treatment options as well as prevention of injuries. Halestrap et al. (19) proposed the oxygen free radical generation as one of the essential mechanisms involving in the injuries of the post-ischemic tissues and cells.

The ovarian Ischemia/reperfusion led to the generation of the pro-inflammatory cytokines caused by the inflammatory cells could stimulate migration and adhesion of the circulating neutrophils to the endothelial cells as well as ROS production that raised the neutrophil infiltration and caused the ischemic injuries. Moreover, ROS and the respective poisonous products cause DNA damages and lipid peroxidation in the mitochondrial and cellular membranes, impair ion channels, and cause cells damages and even death. In addition, the cells' damages induced by the lengthy I/R injuries could cause autophagy, apoptosis, necroptosis and necrosis (20). It is notable that moderate I/R injury can result in the dysfunction of the cells through autophagy and actuate recovery systems to be survived. Severe damages can induce the cell death through necrotic or apoptotic pathways. However, in the human physiology, anti-oxidants and ROS preserve equilibrium. Furthermore, levels of antioxidant agents in the ischemic cells would be essential to eliminate the detrimental impact of ROS. The ROS production is enhanced as a result of the decreased concentration of anti-oxidative agents in the ischemic tissues (21). In this regard, numerous investigations emphasized the drug agents of the anti-oxidant and/or anti-inflammatory impacts in the ovarian I/R injuries preventing via animal models (22, 23). According to our data, this research is the first application of the CH in the I/R injury treatment thorough folliculogenesis period in the ovary. The mechanisms of anti-inflammatory and anti-oxidative impacts of CH are not fully known. Some studies showed

that the CH anti-oxidative features suppress the inducible nitric oxide synthase and cyclooxygenase-2 expression. This inhibits the pro-inflammatory nuclear factor kappa B (NF-κB) activities that proposes anti-inflammatory impacts of CH (24, 25).

Our outputs verified anti-oxidative effect as well as anti-inflammatory impacts of the CH via examining their protecting impacts against the I/R injury in the ovary of rats like the reduction of lipid peroxidation, improvement of histo-pathological scores and enhancement of anti-oxidant activities.

It is well known that MDA is an end product of the lipid peroxidation and the enhanced level of MDA reflects OS. On the contrary, greater activity of GPx and SOD indicated the tissues cure following the oxidative damages (26).

Melekoglu et al. (27) determined the effect of 50 mg/kg/day CH on the prevention of ovaries I/R injury. They demonstrated significant GPx and SOD activities increase and considerable reduction of MDA content in the CH treatment group following the I/R injuries.

Also, we found that CH influences beneficially the ovarian restoration following of I/R injuries and enhances the level of E2. Tsai et al. (28) addressed the evaluation of stem cells impact against damages to the rats' ovarian reserve. They reported a considerable increase of the serum LH and decrease of estradiol (E2) in the ovaries of I/R animals; however, treating the stem cells restored the impacts.

We observed that ovaries damages through an ovarian torsion and found that torsion-detorsion declines number of the follicles in each stage such as antral, graafian and preantral whereas numbers of the atretic bodies enhance. Put differently, CH therapy resulted in better histological signs. Also, other investigations confirmed the reduced number of follicles in each of these stages by ovarian torsion-detorsion. Therefore, they used the anti-oxidants to cure the disease ameliorated the condition (15, 29).

Nikoletopoulou et al. (30) introduced apoptosis as a one of the prominent mechanisms of the modulated mortality, which happens due to the cells damages or external stresses as well as in the course of morphogenesis and normal development. Multiple pathways mediate apoptosis. It seems two main, non-excluding, caspase-dependent pathways of apoptosis occur (31). Therefore, in the first pathway, extrinsic or receptor-mediated pathway, an external stimulus or signal is translated into an internal death signal. T-cell inactivation of the immune system is one of the examples where Fas receptor ligation starts a proteolytic cascade that results in cell death. However, in the second pathway, the upstream effector proteins such as Bax activates intrinsic pathway that is caspase machinery. Anyway, it is largely hypothesized that Bax and Bcl-2 apply antagonistic impacts (32).

The cells survival and death signals are related to apoptosis in the cells that are induced and integrated by the proteins of the Bcl-2 family as the anti-apoptotic proteins. On the contrary, higher expression of the Bax, a proapoptotic protein, mediates the great apoptosis. Sun et al. (33) determined the impacts of dexmedetomidine on the intestine I/R. They found that the level of Bax and caspase-3 considerable increases in the ischemia group and Bcl-2 decline in tissues with the completion of treatment but reverse outputs observed in the treatment group.

Ayan et al. (34) observed the protecting impact of Thymoquinone against the testicular torsion and demonstrated an apoptosis increase due to ischemia. Moreover, as an anti-oxidant, Thymoquinone remarkably decreased the damages.

In the present study, we detected that, the Bax expression level as well as a labeling index, caspase-3, that declines considerably in the ovary tissue of IR group, and the Bcl-2 expression diminishes to some extent. Some studies reported, that progesterone suppresses apoptosis and the uterine glandular cells exhibit maximum apoptotic index at the estrus and lower apoptotic index at diestrus and metestrus (35, 36). Such a change could result from the estrous cycle (metestrus) of rats or ischemia duration. Therefore, it is necessary to do additional investigations in this regard.

Conclusion

Based on the findings, as one of the hydro-alcoholic sources, CH can modulate sexual hormone level, partly, protect ovarian tissues against the oxidative stress (OS) and tissue injuries, and diminish apoptosis induced by the ovarian torsion/detorsion.

Acknowledgments

We appreciate the Deputy of Research and Technology Student Research Committee of Gonabad University of Medical Sciences, Khorasan Razavi, Iran and Clinical Research Development Unit of The Tabriz Valiasr Hospital, Azarbaijan Sharghi, Iran for their supports. A special grant has been given to the present manuscript from Deputy of Research and Technology and Student Research Committee of Gonabad University of Medical Sciences Khorasan Razavi, Iran. It is declared that there are not any conflicts of interest.

Authors' Contribution

Z.M., M.M., A.S.M.; Contributed to study conception and design. M.Sh., Z.M., S.M.H.-A., N.G., M.F., B.B.; Contributed to all experimental work, data analysis, and data interpretation. M.M., S.-H.A.-E.; Supervisor and biochemical analyses. M.Sh.; Drafted the manuscript. M.Sh., M.M., A.S.M.; Manuscript reviser. All authors read and approved the final manuscript.

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