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The Role of Hesperidin in Regulating Gonadotropin and Estrogen Receptors During Ovarian Follicle Recovery After Torsion/Detorsion in Rats

Linda Mohammadzadeh Boukani[1], Maryam Sadr Ameli[2], Haleh Niktash[3], Seyedeh Sara Salami[1*], Seyed Mahdi Mirtajaddini[1], Fariba Khodaeifar[4*], Ghasem Rostami[5], Kian Keyhan[6], Amir Afshin Khaki[1]

Abstract

Objectives: This study aimed to evaluate the impact of hesperidin on the expression of follicle-stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHCGR), and estrogen receptors (ER) during follicular development in female rats following ovarian torsion/detorsion (OTD).

Methods and Materials: In this experimental study, 32 female rats were randomly divided into four groups: Group 1 (control), group 2 (OTD group, subjected to ovarian torsion/detorsion), group 3 (OTH group, torsion/detorsion + 50 mg/kg hesperidin), and group 4 (healthy group, receiving 50 mg/kg hesperidin without torsion). After 21 days of treatment, ovarian tissues were harvested for histological assessment, and mRNA levels of ER, FSHR, and LHCGR were analyzed. Serum concentrations of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estrogen were also measured.

Results: Histological analysis revealed that OTD induced structural damage, including oocyte and granulosa cell shrinkage, which was mitigated by hesperidin administration. The treated groups exhibited increased serum levels of estrogen, LH, and FSH, along with decreased testosterone levels. Additionally, hesperidin significantly upregulated the expression of ER, FSHR, and LHCGR, suggesting a positive effect on ovarian function and fertility.

Conclusions: The results indicate that hesperidin exerts protective effects on ovarian tissue following torsion/detorsion. It modulates hormonal balance and enhances the expression of key reproductive receptors (ER, FSHR, LHCGR), suggesting protective effects on ovarian function; however, further clinical studies are required before definitive conclusions can be drawn regarding its impact on fertility.

Keywords: Ovary, LHCGR, FSHR, Hesperidin, Torsion/detorsion

Introduction

Ovarian torsion is considered one of the critical emergencies in gynecology, resulting from the partial or complete twisting of the ovarian pedicle along with the fallopian tube around its axis (1). This condition results in a reduction or complete interruption of blood flow, ultimately leading to tissue ischemia. If there is a delay in diagnosis and treatment, it can lead to severe tissue damage, loss of ovarian follicles, impaired reproductive function, and eventually infertility. Currently, the primary treatment approach involves surgical detorsion and restoration of blood flow (2). However, the sudden reperfusion can paradoxically cause secondary injury through mechanisms such as oxidative stress, inflammation, and apoptosis. Therefore, increasing attention has been given to adjuvant pharmacological interventions aimed at reducing reperfusion-induced damage and promoting tissue repair (1).

The ovarian follicle is a dynamic structure whose function is governed by complex hormonal signaling pathways. Gonadotropins such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play critical roles in follicular development and maturation through their specific receptors (FSHR and LHR). In addition, estrogen and its receptors (ERα and ERβ) are essential for granulosa cell proliferation, follicular growth, and ovulation (3,4). Disruption in the expression or function of these receptors during ovarian injury can impair the folliculogenesis process, ultimately affecting fertility. Studies have demonstrated that antioxidant supplementation can significantly reduce the extent of tissue and biochemical damage associated with ischemia-reperfusion injury (1,4).

Hesperidin, a naturally occurring flavonoid abundantly

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[1]Clinical Research Development Unit of Tabriz Valiasr Hospital, Tabriz University of Medical Sciences, Tabriz, Iran. [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran. [3]Department of obstetrics and gynecology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. [4]Department of Traditional Medicine, Faculty of Traditional Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. [5]Department of Urology, Mazandaran University of Medical Sciences, Sari, Iran. [6]Andover High School, Massachusetts, USA.
*Corresponding Author: Fariba Khodaeifar, Email: dr.fkhodaiefar@gmail.com; Seyedeh Sara Salami, Email: salamiiisa25@gmail.com

Key Messages

- Hesperidin protects ovarian tissue from torsion/detorsion-induced damage through antioxidant and hormone-regulating mechanisms.
- Hesperidin enhances ovarian receptor expression and hormonal balance, suggesting potential use in fertility preservation therapies.

found in citrus fruits, has attracted considerable attention in recent years due to its potent antioxidant, anti-inflammatory, and anti-apoptotic properties (5,6). Previous research has demonstrated that hesperidin offers significant protective effects in various models of ischemia-reperfusion injury, including those affecting cardiac, hepatic, and cerebral tissues. However, little is known about its role in ovarian ischemia-reperfusion injury, particularly regarding the regulation of hormonal receptor expression during the follicular repair phase (5,7).

In this study, considering the critical role of gonadotropin and estrogen receptors (ERs) in ovarian function, we aim to evaluate the effect of hesperidin on the expression of these receptors during the recovery phase following torsion/detorsion in a rat model. We hypothesize that hesperidin may contribute to follicular regeneration and functional restoration of the ovary by preserving or upregulating the expression of FSHR, LHR, and ERs. The findings of this research may pave the way for the development of effective adjunctive therapies in patients with ovarian torsion and enhance their reproductive outcomes.

Materials and Methods

The research was conducted on 32 female rats, each weighing approximately 180-220 g. The rats were randomly assigned to four groups, with eight rats in each group. Animals were randomly allocated into four groups using a simple randomization method with an equal chance of selection.

- Group 1: Sham of receiving normal saline intraperitoneally daily.
- Group 2: Ovarian torsion was induced for 3 hours, followed by detorsion, and the rats were treated with normal saline for 21 days (OTD, ovarian torsion/detorsion).
- Group 3: Ovarian torsion was induced, and detorsion was performed after 3 hours. In this group, 50 mg/kg of hesperidin (dissolved in 10% DMSO and normal saline) was administered intraperitoneally to all rats 30 minutes before detorsion. Hesperidin was administered daily for 21 days (OTH) (8).
- Group 4: Hesperidin injection 50 mg/kg without induction of torsion in the ovary for 21 days (Hes).

Ovarian Torsion/Detorsion Induction

In this study, an ovarian ischemia-reperfusion injury model was established in adult female rats by inducing ovarian torsion. Animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in the supine position on a surgical platform. After disinfecting the abdominal area with povidone-iodine and alcohol, a midline incision approximately 2 cm in length was made using a scalpel. The uterus and its adnexa were gently exteriorized, and the right ovary was selected as the target for the procedure. To induce ischemia, the right ovary, along with its vascular pedicle, was rotated 720 degrees (two full turns) clockwise around its axis and fixed in the twisted position using a fine nylon suture. The abdomen was temporarily closed with sutures, and the animals were maintained under controlled conditions for 3 hours. After the ischemic period, to initiate reperfusion, the animals were re-anesthetized, the sutures were removed, and detorsion was performed by restoring the ovary to its normal anatomical position. Finally, the abdominal wall was closed in two layers (muscle and skin), and the animals were returned to their cages for subsequent treatment protocols or tissue sampling at predetermined time points. In the sham group, all surgical procedures were performed similarly, except that no torsion was induced; the ovary was merely exposed and then returned to its original position. All surgical steps were conducted in accordance with institutional guidelines for the ethical use of laboratory animals (1,4).

Ovarian Tissue Examination

Ovarian tissue samples were collected from the experimental rats and fixed in a 10% formalin solution for histological processing. The tissues were then embedded in paraffin, and 5-µm-thick sections were prepared using a microtome. Hematoxylin and eosin (H&E) staining was performed on these sections to evaluate the counts of different follicle types, including preantral, antral, Graafian follicles, atretic bodies, and corpus luteum. The stained sections were examined under a light microscope for both qualitative and quantitative analysis of follicular development (9).

Evaluation of FSHR, LHCGR, and ER Gene Expression by RT-PCR

Left ovarian tissues were utilized for RNA extraction to assess the gene expression levels of FSHR (follicle-stimulating hormone receptor), LHCGR (luteinizing hormone/choriogonadotropin receptor), and ER using the reverse transcription-polymerase chain reaction (RT-PCR) method. The following steps were performed:

Ovarian tissues were rapidly frozen in liquid nitrogen and stored at -196 °C until further processing. Total RNA was extracted from the ovarian tissues using a commercial RNA extraction kit (Thermo Scientific, Waltham, MA) according to the manufacturer's protocol. The concentration and purity of the extracted RNA

were determined using a NanoDrop spectrophotometer. Complementary DNA (cDNA) synthesis was performed using 500 ng/ml of the extracted RNA with a cDNA synthesis kit. Specific primer sequences for the FSHR, LHCGR, and ER genes, along with the housekeeping gene GAPDH for normalization, were used for RT-PCR amplification (Table 1).

Real-time quantitative PCR (qPCR) reactions were set up in a 48-well plate using SYBR Green PCR master mix, with cDNA as the template and gene-specific primers.

qPCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System with the following cycling conditions: an initial denaturation step at 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 58 °C for 30 seconds, and extension at 72°C for 30 seconds. A final melting curve analysis was performed.

The relative gene expression levels were calculated using the Pfaffl method and expressed as ratios of $2^{-\Delta CT}$ target): $2^{-\Delta CT}$ reference), where ΔCT represents the difference in threshold cycle values between the target gene and the reference gene (GAPDH) (4,10).

Evaluation of Estrogen, Testosterone, FSH, LH, and Levels in Serum Samples

Blood samples were collected from the experimental rats, and serum was isolated for hormone analysis. The concentrations of estrogen and testosterone in the serum were quantified using enzyme-linked immunosorbent assay (ELISA) kits from Demeditec Diagnostics (Germany). Additionally, the concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in serum were evaluated using ELISA kits from Zelbio (Italy), following the manufacturer's instructions. Optical density readings were obtained using a microplate reader, and hormone concentrations were calculated based on standard curves generated with known hormone concentrations (4,10).

Statistical Analysis

Histological analysis, gene expression studies, and hormone assays were evaluated using appropriate statistical methods, such as analysis of variance (ANOVA), with post hoc tests applied when necessary. A significance level of $P < 0.05$ was considered statistically meaningful. Results were expressed as mean ± standard deviation (SD) or as specified in the respective analyses. Data normality was assessed using the Shapiro–Wilk test, and Tukey's post hoc test was applied following ANOVA.

Results

Histological Result

The count of preantral, antral, and Graafian follicles presented a notable decrease in the ovarian torsion (OTD) group compared to the sham group. Additionally, the count of atretic bodies was significantly enhanced in the OTD group ($P=0.001$). Substantial variances were also observed between the OTD group and the treatment group in terms of the number of follicles and atretic bodies ($P=0.001$).

In the experimental groups receiving hesperidin treatment, the numbers of preantral, antral, and Graafian follicles were significantly higher than the OTD group ($P=0.001$). Additionally, the therapeutic groups exhibited a marked reduction in the count of atretic bodies compared to the OTD group ($P=0.001$) (Table 2 and Figure 1).

Table 1. Primer Sequences Used for Quantitative Real-Time PCR Analysis of FSHR, LHCGR, ERα, and GAPDH Genes

Gene	Primer direction	Sequence (5' → 3')	Product size (bp)
FSHR	Forward	AGTGACTCCTGTGCTGGACA	150
	Reverse	GTCCAGGAGTTGGAGACAGC	
LHCGR	Forward	CTGCTGCTGAGGATGTGGTT	142
	Reverse	GGGAAGGTTGTTGGTGATGG	
ERα	Forward	ACCCTGAAGTCTGTCCTGGA	138
	Reverse	TGGTGCTTCAACATTCTCCCT	
GAPDH	Forward	AGACAGCCGCATCTTCTTGT	120
	Reverse	CTTGCCGTGGGTAGAGTCAT	

Table 2. Histological Assessment of Ovarian Follicles in Different Experimental Groups

Group	Preantral follicles	Antral follicles	Graafian follicles	Atretic bodies
Sham	7.4±1.2	6.0±0.65	5.0±1.4	1.0±0.74
OTD	1.5±0.25*	1.2±0.20*	0.75±0.050*	6.8±0.92*
OTD+ hesperidin	4.3±0.48*#	3.50±0.35*#	3.6±0.50*#	2.2±0.89*
Hesperidin	7.8±1.2#	7.00±0.60#	6.0±0.80#	0.7±0.50#

Data are presented as mean ± standard deviation (SD). A significant reduction in the number of healthy follicles and an increase in atretic bodies were observed in the OTD group compared to the sham group. Hesperidin treatment improved follicular morphology and reduced atresia (*P<0.05 vs. Sham; #P<0.05 vs. OTD).

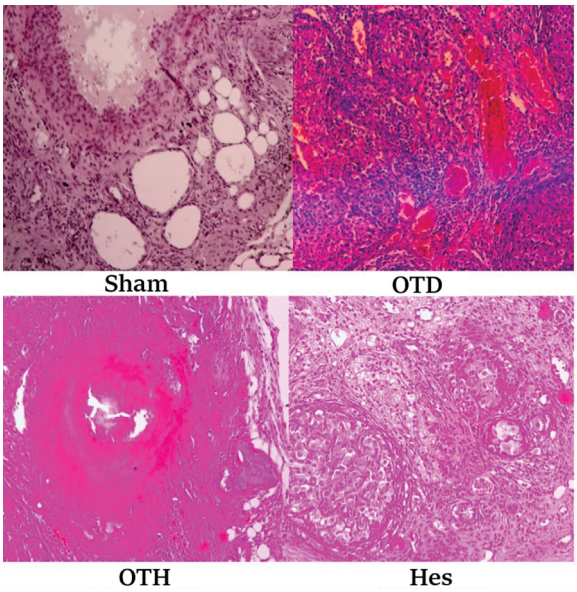

Figure 1. Representative Histological Sections of Ovarian Tissue Stained With H&E in Different Experimental Groups. The Sham group exhibits healthy follicles, whereas the OTD group displays notable degeneration and an increase in atretic bodies. Hesperidin-treated groups reveal improved follicular structure.

The Gene Expression of LHCGR, FSHR, and ER

The FSH receptor genes (FSHR) were reduced in the OTD group compared to the Sham group. In contrast, groups treated with hesperidin exhibited higher FSHR gene expression ratios compared to the OTD group ($P<0.05$). Similarly, the gene expression of LHCGR, which is related to the LH hormone receptor, was meaningfully diminished in the OTD group. However, it was upregulated in groups receiving 50 mg/kg of hesperidin compared to the OTD group ($P<0.05$).

Furthermore, the induction of ovarian torsion resulted in the downregulation of ER gene expression, as observed in the sham group ($P<0.05$). In contrast, the hesperidin-treated groups demonstrated an upregulation in ER gene expression (Figures 2-4).

Hormonal Calculation Results

The levels of LH and FSH, as gonadotropin hormones, were elevated in the OTD group compared to the Sham group ($P<0.05$). In contrast, the levels of these hormones in the hesperidin-treated group were lower than those in the OTD group ($P<0.05$). The serum concentration of estrogen was significantly declined in the OTD group compared to the sham group ($P<0.05$); however, it was notably higher in the therapeutic groups than in the OTD group ($P<0.05$).

Conversely, the concentration of testosterone was meaningfully higher in the OTD group compared to the sham group ($P<0.05$). The level of testosterone, however, meaningfully diminished in the group treated with hesperidin when associated with the OTD group ($P<0.05$) (Table 3).

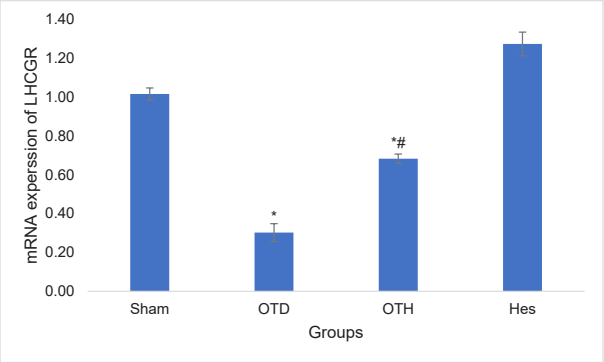

Figure 2. Relative Gene Expression Levels of LHCGR in Ovarian Tissues of Different Groups Measured by Real-Time RT-PCR. *Hesperidin treatment significantly upregulated LHCGR expression compared to the OTD group ($P<0.05$). *$P < 0.05$ vs. Sham; ♯$P < 0.05$ vs. OTD.

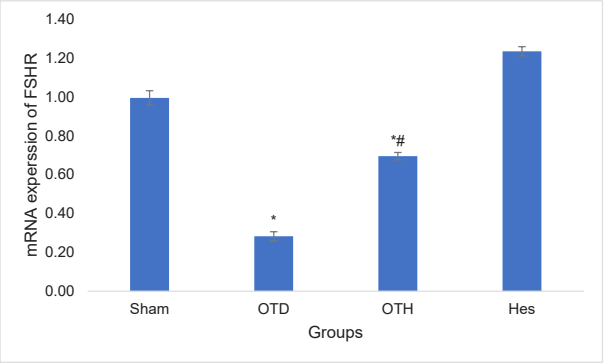

Figure 3. Relative Gene Expression Levels of FSHR in Ovarian Tissues Across Experimental Groups. *A significant reduction in the OTD group was observed, which was reversed with hesperidin treatment ($P<0.05$). *$P < 0.05$ vs. Sham; ♯$P < 0.05$ vs. OTD.

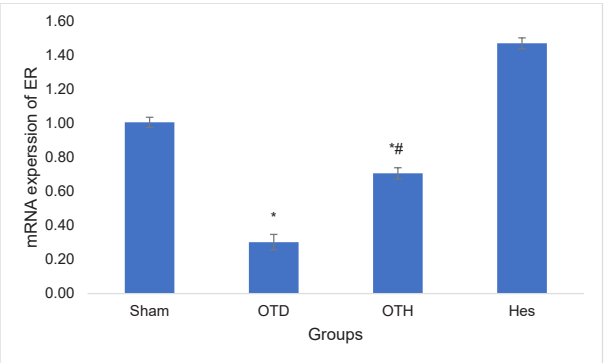

Figure 4. Relative Gene Expression Levels of Estrogen Receptor (ERα) in Ovarian Tissues. *ERα expression decreased in the OTD group and was restored in hesperidin-treated rats ($P<0.05$). *$P < 0.05$ vs. Sham; ♯$P < 0.05$ vs. OTD.

Discussion

Ovarian torsion-induced ovarian oxidative stress is a significant concern for female patients due to its potential to impair ovarian function and fertility (11,12). Ovarian torsion, a common emergency agent, has been associated with follicular depletion, disrupted hormone signaling,

Table 3. Serum Hormone Levels of Estrogen, Testosterone, FSH, and LH in Experimental Groups

Group	Estrogen ± SD	Testosterone ± SD	FSH ± SD	LH ± SD
Sham	4.35±2.15	0.70±0.10	3.12±0.12	2.15±0.27
OTD	1.45±2.40*	2.65±0.15*	6.55±0.15*	4.20±0.20*
OTD+ hesperidin	2.70±1.80*#	1.25±0.12*#	2.6±0.22*#	2.65±0.12*#
Hesperidin	5.15±1.75	0.55±0.08	3.06±0.25	2.05±0.15

Data are presented as mean ± standard deviation (SD). Ovarian torsion/detorsion (OTD) significantly disrupted hormonal balance, which was partially restored by hesperidin (*$P < 0.05$ vs. control; #$P < 0.05$ vs. OTD group).

and ovarian dysfunction (13,14). In this study, we investigated the protective effects of hesperidin, a natural flavonoid compound, against torsion/detorsion-induced ovarian damage at cellular and molecular levels in female rats.

Our findings indicate that OTD resulted in a notable reduction in the number of preantral, antral, and Graafian follicles, alongside an increased count of atretic bodies. These histopathological changes reflect ovarian torsion-induced follicular depletion and atresia, indicative of impaired follicular development and ovarian damage (15). Follicular atresia is characterized by the degeneration of ovarian follicles due to apoptosis and follicular cell death, ultimately leading to diminished ovarian reserve and compromised reproductive potential (16,17).

Follicular atresia is characterized by the degeneration of ovarian follicles due to apoptosis and follicular cell death, ultimately leading to diminished ovarian reserve and compromised reproductive potential (8,18). Many studies have been conducted in this field, showing that ovarian torsion and subsequent detorsion cause damage to ovarian tissue and follicular atresia, which results in a decrease in the number of follicles (1,19,20). Among them, Soltani et al. showed in a study that three hours of ovarian torsion and subsequent detorsion caused a decrease in the number of normal follicles and an increase in follicular atresia and ovarian damage (19).

At the molecular level, our study investigated the expression of key reproductive hormone receptors, including FSHR, LHCGR, and ER in ovarian tissues. We observed downregulation of FSHR, LHCGR, and ER gene expression in the OTD group, indicating impaired responsiveness to gonadotropins and estrogen signaling. This decreased expression of these receptors contributes to the disrupted follicular development and ovulatory dysfunction observed in individuals with OTD (4,21). Baradaran Bagheri et al reported in a study that ovarian torsion led to downregulation in mRNA expression of LHCGR, ER, and FSHR (4).

In contrast, treatment with hesperidin enhanced the reduction of mRNA expression of FSHR, LHCGR, and ER genes induced by OTD. Hesperidin supplementation resulted in higher expression levels of these receptors, suggesting a restoration of ovarian responsiveness to FSH, LH, and estrogen. The upregulation of hormone receptor expression in response to hesperidin likely contributes to the observed improvement in follicular development and ovarian function (18,22). Shoorei et al show in a study that hesperidin can improve the growth and development of follicles (23).

Furthermore, our study examined the impact of OTD and hesperidin on serum hormone levels, including FSH, LH, estrogen, and testosterone. OTD significantly increased the levels of FSH and LH in plasma, reflecting impaired pituitary-ovarian axis function and decreased gonadotropin receptors (24,25). Given that our results at the molecular level showed that ovarian torsion causes a decrease in the expression of gonadotropin hormone receptor genes, this result could be consistent with an increase in the levels of these hormones in the blood. Additionally, OTD led to decreased estrogen levels and elevated testosterone levels, indicative of disrupted hormone balance and ovarian steroidogenesis (24, 26). Tafrishi Nejad et al revealed in a study that ovarian torsion followed by detorsion led to an increase in testosterone and a decrease in estrogen level (27).

Treatment with hesperidin restored serum hormone levels towards normal physiological ranges (28). Hesperidin supplementation resulted in balance in FSH and LH concentrations, reflecting improved pituitary function and gonadotropin secretion (5,29). Moreover, hesperidin normalized estrogen levels and attenuated testosterone elevation, indicating a restoration of ovarian steroidogenesis and hormone balance (5). These findings highlight the beneficial effects of hesperidin in preserving endocrine function and hormonal homeostasis in the context of ischemia-induced ovarian damage. A study by Shokoohi et al stated that hesperidin, as an antioxidant supplement, can prevent tissue damage caused by oxidative stress (5). Various studies have shown that antioxidant compounds can improve ovarian function, the folliculogenesis process, and hormonal balance (1,3,4,20).

The protective mechanisms of hesperidin against ischemia/reperfusion-induced ovarian injury are likely multifaceted, primarily involving its potent antioxidant and anti-inflammatory properties (23,30). Hesperidin acts as a scavenger of reactive oxygen species (ROS) and inhibits oxidative stress-mediated damage in ovarian tissues. By reducing oxidative stress and inflammation, hesperidin helps preserve follicular integrity and enhances hormone receptor expression, thereby promoting follicular development and overall ovarian function (30,31).

Moreover, hesperidin may modulate signaling pathways involved in folliculogenesis and hormone regulation, leading to enhanced follicular growth and hormone responsiveness (8). The upregulation of FSHR, LHCGR, and ER gene expression in response to hesperidin underscores its regulatory effects on reproductive hormone pathways. It supports its role in mitigating ischemic-related reproductive complications. These results suggest that hesperidin could be a promising therapeutic agent for protecting ovarian health in female patients undergoing ovarian ischemia/reperfusion (4, 32).

Conclusions

Our study provides valuable insights into the protective effects of hesperidin against OTD-induced damage in female rats. Hesperidin supplementation preserved ovarian follicular count, restored hormone receptor expression, and normalized serum hormone levels, highlighting its potential as a therapeutic agent for preserving ovarian function and fertility in women undergoing ischemia/reperfusion. Further investigations into the molecular mechanisms underlying hesperidin's protective actions are warranted. Such research could lead to the development of targeted interventions aimed at mitigating oxidative stress-related reproductive complications and improving the quality of life for females with ovarian torsion. These findings suggest that incorporating hesperidin into treatment regimens could be beneficial for those at risk of ovarian damage due to ischemia/reperfusion.

Authors' Contribution

Conceptualization: Fariba Khodaeifar, Amir Afshin Khaki.
Data curation: Linda Mohammadzadeh Boukani, Seyed Mahdi Mirtajaddini, Seyedeh Sara Salami.
Formal analysis: Kian Keyhan, Maryam Sadr Ameli, Haleh Niktash.
Funding acquisition: Seyed Mahdi Mirtajaddini.
Investigation: Seyedeh Sara Salami, Seyed Mahdi Mirtajaddini, Ghasem Rostami, Kian Keyhan.
Methodology: Fariba Khodaeifar, Amir Afshin Khaki.
Project administration: Fariba Khodaeifar, Amir Afshin Khaki.
Resources: Fariba Khodaeifar, Amir Afshin Khaki.
Software: Ghasem Rostami, Kian Keyhan.
Supervision: Fariba Khodaeifar, Amir Afshin Khaki.
Validation: Fariba Khodaeifar, Amir Afshin Khaki.
Visualization: Fariba Khodaeifar, Amir Afshin Khaki.
Writing–original draft: Ghasem Rostami,Seyed Mahdi Mirtajaddini.
Writing–review & editing: Seyedeh Sara Salami, Seyed Mahdi Mirtajaddini, Ghasem Rostami.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

This investigational study was approved by the Ethical Committee of Tabriz University of Medical Science (ethical code: IR.TBZMED.VCR.REC.1398.095).

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