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Experimental Research Article

Chrysin's potential in ameliorating the toxic effects of Cyclophosphamide on mouse oocytes and embryos

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ABSTRACT

Introduction: The study investigates the protective effects of chrysin (CR) on the quality of oocytes and embryos in rats undergoing in vitro fertilization after treatment with cyclophosphamide (CPH).

Methods: In this study, female NMRI mice were divided into five groups: I. control group, II. sham group, III. CPH group (receiving 120 mg/kg.wk of CPH intraperitoneally (IP), IV and V: CR groups receiving 5 and 10 mg/kg. day CR for four weeks. For oocyte induction, ten units of pregnant mare serum gonadotropin were injected IP after the last injection. All mice were then sacrificed by aspiration of their oocytes for further experiments. The growth of embryos was investigated using mature oocytes in vitro.

Results: CR significantly increased the number of 2 cells and 4 cells after 24 and 48 hours compared to the CPH group. Groups treated with CR showed a significant increase in the expression level of the BMP-15 and GDF-9 genes in a dose-dependent manner compared to the CPH group.

Conclusion: In mice, CR reduced oxidative damage and oocyte cytokine levels in ovarian tissue after CPH-induced degeneration.

Keywords:
Chrysin
Cyclophosphamide
Oocyte quality
Oocyte collection

Introduction

Cancer is the out-of-control growth of body cells, and according to the World Health Organization (WHO), 70% of cancer-related deaths occur in low- and middle-income countries (Plummer et al. 2016). Organizations like the WHO and various NGOs are working to improve cancer care in developing countries through initiatives such as strengthening healthcare systems, training medical professionals, raising awareness about cancer prevention and early detection, increasing access

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to affordable cancer treatments, and supporting research into cancer prevention and treatment in low- and middle-income settings.

Ovarian cancer is one of the most dangerous and deadly diseases a woman can face in her lifetime (Jessmon et al. 2017).

It is a complex and challenging disease, and the lack of effective early detection methods makes it particularly concerning. Cancer is the fifth cause of death among women, and this issue shows the necessity of continuing research and development in this field (Siegel et al. 2017). Chemotherapy, radiation therapy, and surgery are cancer treatment options, but on the other hand, they are accompanied by side effects and unpleasantness (Shahbaz et al. 2023). Previous research has reported that chemotherapy is a risk factor for premature ovarian failure (POF), and this female disease leads to amenorrhea, metabolic abnormalities, menopause, and infertility (Chen et al. 2022; Lin et al. 2017). In POF disease, ovarian follicles decrease before the age of 40, and a small number of follicles remain in women's ovaries (Kovanci and Schutt, 2015). Cancer survival rates are improving with advances in chemotherapy, but the use of chemotherapy drugs is associated with poor and adverse therapeutic effects (Shahbaz et al. 2023). Cyclophosphamide (CPH), is an alkylating agent and the most common anticancer agent used to treat various cancers and autoimmune diseases (Athira et al. 2022).

Its widespread use highlights its effectiveness. This drug targets rapidly dividing cells, inevitably affecting both healthy tissues and cancerous ones. Understanding and managing the specific side effects effectively is crucial. CPH, like other chemotherapy drugs, can cause a range of issues, including myelosuppression, nausea and vomiting, and hair loss (Koike et al, 2018). However, CPH treatment for ovarian cancer can damage normal tissues, including causing ovarian damage and destruction of primordial follicles, with dose-dependent effects (Athira et al, 2022). Additionally, CPH's toxic effects inhibit DNA synthesis, increase reactive oxygen species (ROS), and disrupt the ovary's antioxidant mechanisms (Helsby et al. 2019).

The ongoing research efforts in cancer treatment focus on supportive care by optimizing strategies to manage side effects and improve quality of life during treatment. While CPH remains a valuable tool in ovarian cancer treatment, understanding its side effects and actively seeking ways to mitigate them is crucial (Chen et al. 2016). Open communication with healthcare professionals and exploring available options for fertility preservation and supportive care are essential for women facing this challenging situation (Meirow et al. 2001).

Although ROS is necessary for egg maturation, fertilization, fetal growth, and pregnancy to a certain level, its excessive production causes granulosa cells (GC) apoptosis and defective antioxidant defense mechanisms (Agarwal et al. 2003). The mechanism of action of CPH on ovarian damage is related to the facilitation of GC apoptosis, and the resulting oxidative stress is known as a mechanism in the destruction of ovarian follicles (Yuksel et al. 2015). It also leads to the activation of inflammation in the eggs and GC (Hou et al. 2021). Hence, we concluded that the discovery of a new approach can prevent ovarian damage caused by chemotherapy and preserve fertility and endocrine function in women (Chen et al. 2022). The attention of researchers has been directed to medicinal plants, fruits, and vegetables due to their better safety profile and antioxidant properties for the treatment of cancers and the removal of chemotherapy side effects (Shahbaz et al. 2023). One of the valuable compounds of the flavonoid family (7,5-hydroxy flavone) is chrysin (CR), which is produced from honey, propolis, and various plants (Wu et al. 2011). CR has many properties including antioxidant, anti-apoptotic, anti-inflammatory, and anti-cancer (Mantawy et al. 2019). It also limits cell growth and increases cell death through apoptosis in different cancer cells, therefore CR as a therapeutic and preventive agent shows a promising strategy (Shahbaz et al, 2023). Growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15) are two members of the beta growth factor family, which are known as essential factors involved in the regulation of ovarian function (Sanfins et al. 2018). These two genes have evolved rapidly in the mammalian clade, which indicates the importance and unique function of these two proteins in female fertility (Ahmad et al. 2018). They also affect folliculogenesis, ovulation regulation, and egg quality. These two growth factors regulate the differentiation and function of GC during follicular development (Sanfins et al. 2018).

Both GDF9 and BMP15 genes encode protein ligands of the TGF-β superfamily that can bind to various TGF receptors. BMP15 is involved in oocyte maturation and follicle development through the activation of GC. A

defect in this gene is associated with POF and may lead to ovarian dysgenesis. Also, the lack of this protein can lead to early activation of primordial follicles, possibly through the upregulation of signaling pathways. In another study, it has been reported that the absence of this protein causes a severe decrease in the proliferation of GC, which leads to the cessation of follicular growth in the pre-antral stage and hypoplasia of the ovary, resulting in complete infertility (Jiao et al. 2023). GDF-9 plays a regulatory role in ovarian function, and its reduced expression may be linked to polycystic ovary syndrome (PCOS). Additionally, mutations in GDF-9 are more commonly observed in mothers of dizygotic twins. The crucial diseases that contribute to GDF-9 are polycystic ovary syndrome and POF. GPCR pathway and Apoptosis pathways in synovial fibroblasts are the pathways related to it. Gene Ontology (GO) associated with this gene includes cytokine activity and TGF-β receptor binding. An important paralog of this gene is BMP15.

Some papers administered a single dose to cause infertility (Koike et al. 2018; Ozatik et al. 2023), while another considered a dose of 75 mg/kg to be safe for infertility induction (Meirow et al. 2001). Additionally, 120 mg/kg/week of CPH was injected over four weeks (Chen et al. 2016). New studies demonstrated that 2 mg/kg. day CR can ameliorate the harmful effects of cisplatin on ovotoxicity in the studied rats (Mentese et al. 2022). Also, CR has shown a neuroprotective effect in the toxicity caused by anticancer drugs such as Ifosfamide (CPH analog) in a dose of 25 mg/kg. day (Salimi et al. 2023). Also, CPH can produce its toxic effect in all tissues and it has been reported that these effects in testicular and ovarian tissue can be improved by using CR (Taslimi et al. 2019).

Therefore, this study aims to investigate the quality of embryos obtained from in vitro fertilization in NMRI mice treated with CPH and the protective effects of CR on the quality of oocytes in oogenesis.

Materials and Methods

All reagents and materials were provided by Sigma Chemical Company (St. Louis Mo, USA).

Animals

60 female and 20 male NMRI mice obtained from the Pasteur Institute of Iran were used as experimental samples. Mice were maintained for two weeks in a controlled environment (12 h light/dark cycle) with access to food and water. The mice were randomly divided into five groups, each containing 12 mice. Control group (no treatment), sham group (only solvent intraperitoneal injection (ipi)), CPH (120 mg/kg.wk), CR5 (5mg/kg. day CR), CR10 (10mg/kg. day CR) all for 28 consecutive days. Animals were sacrificed at the end of treatment, and ovulation was induced by PMSG (5/7U). Oocytes were collected from the oviduct by conventional method and then dissected and denuded. The maturation rate of eggs in each group was measured after 24 and 48 hours in α-MEM culture medium and checked under an inverted microscope. Fresh sperm on HTF medium with 4 mg/ml of BSA were used for fertilization of intact mice Metaphase II (MII) oocytes. After 24 and 48 hours, the samples were tested for fertilization and the number of 2 and 4-cell embryos.

In Vitro Fertilization Process

Oocyte Collection

The amount of 7.5 IU of PMSG (Introit Corporation, Canada) was injected intraperitoneally into the mice. Five hours after the last dose of CR, they were treated in five experimental groups. Oviducts were dissected under a stereomicroscope with two insulin syringes and placed in an Embryocul-MHRM medium without BSA. In Embryocul-MHRM medium containing 15% BSA, cumulus-oocyte complexes (COCS) were removed by Pasteur pipette and incubated at 37°C and CO2 %5 until the start of sperm preparation.

Sperm Preparation and fertilization

Environment Embryocul-MHRM was placed in a test tube and incubated at 37°C and 5% CO2 for 1 hour on male mice sacrificed for the experiment. We saw that the tail was separated from both sides and chopped with small scissors. Sperms were added to 50 µL Embryocul-MHRM medium drops containing ten oocytes after swimming to 37°C under 6% CO2 in humidified air for 4-6 hours. The second transfer of oocytes to 30 µL of culture medium (Embryocul-MHRM medium containing 15% BSA) was performed.

The Assessment of Embryo Development

Two stereomicroscopes (motic SMZ 168; Hong Kong) were used to observe the development of embryos after

TABLE 1: qPCR primer sequences

Genes		Primers Sequence	Tm	Amplicon	PCR program
GapDH	Forward	AAGAGGGATGCTGCCCTTAC	59.4	120 bp	Denaturation: 94 °C Annealing: 57 °C Extention: 72 °C
	Reverse	ATACGGCCAAATCCGTTCAC	58.6		
BMP15	Forward	CAAGGGAGAACCGCACGATTG	61.9	110 bp	Denaturation: 95 °C Annealing: 59.5 °C Extention: 72 °C
	Reverse	AGGAAAGTCCAGGGTCTGTACATG	61.8		
GDF9	Forward	AGCAACCAGGTGACAGGAC	59.5	120 bp	Denaturation: 94 °C Annealing: 57.5 °C Extention: 72 °C
	Reverse	AGAGGCAGAGTTGTTCAGAGTG	60.0		

TABLE 2: Zygote 2 and 4 cell count after fertilization and IVF

	24h			48h		
Group	**Degeneration (%)**	**2 Cells (%)**	**4 Cells (%)**	**Degeneration (%)**	**2 Cells (%)**	**4 Cells (%)**
Control	30.77	69.23	0	14.81	21.20	64.00
Sham	32.10	67.90	0	16.80	21.07	62.13
CPH	47.00	53.00	0	67.00	19.00	48.00
CR5	42.30	57.70	0	19.00	23.10	57.90
CR10	37.30	62.70	0	15.30	24.50	60.20

IVF in 1, 2, 3, and 4 days.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

A small kit ®Rneasy (Qiagen, Hilden, GmbH, Germany) was used to extract RNA from ovarian tissue. Total RNA was quantified and quality checked twice in triplicate using a NanoDrop TM 2000 spectrophotometer (Thermo Fisher Scientific, Inc). Then, RNAs were reverse transcribed using the Prime-Script RT reagent kit (Takara Corporation, Kyoto, Japan). We analyzed quantitative RT-PCR using RealQ Pluse 2X MasterMix Green-Without RoxTM Amplicon (stenhuggervej, Denmark). To determine the expression level of BMP15, GFDF-9, and GAPDH (Table 1), specific primers were obtained from Macrogen (Macrogen Co, Seoul, Korea). A real-time PCR system was used for cDNA amplification (Roche Applied Science, CA, USA). Gene expression data were normalized using GAPDH. The relative expression of target genes was analyzed using method 2. Table 1 lists the forward and reverse primer sequences.

Measurement of oxidative and nitrosative stress markers

Malondialdehyde (MDA), nitric oxide (NO), and catalase (CAT) were determined to investigate the effects of CR on oxidant-antioxidant balance in ovarian tissues (Bargi et al, 2017).

Statistical Analysis

For all statistical analysis, SPSS16 was used. Average and standard deviation were calculated. The mean of fragmented oocytes, fertilized oocytes, 2-cell, 4-cell, and 8-cell embryos, morula, and blastocysts between groups were compared by the Mann-Whitney test. We measured the outcomes of oxidative stress using one-way ANOVAs and LSD analysis. Differences are significant at $P<0.05$ level.

Results

Effects of CR on descriptive indicators of IVF

Maturation of oocytes in IVM in the CR group does-dependently (CR5: 42.30%, CR10: 37.30%, after 24h; CR5: 19.00%, CR10: 15.30% after 48h) have a

TABLE 2: Outcomes of IVM oocytes

	Control Count (%)	Sham	CPH	CR5	CR10
GV	14 (8.86)	24 (15)	36 (18.75)	36 (20.93)	34 (17.00)
MI	46 (29.11)	38 (23.75)	54 (28.13)	46 (26.74)	64 (32.00)
MII	78 (49.37)	70 (43.75)	38 (19.79)	42 (24.42)	64 (32.00)
D	20 (12.66)	28 (17.50)	64 (33.33)	48 (27.91)	38 (19.00)

lower degeneration rate than the CPH group (47.00% after 24h and 67.00% after 48h) (Table 2).

Reaching 2 and 4 cell embryos after 24 and 48 hours in the groups CR5 and CR10 were increased compared to the CPH group (Table 2). After 24 hours, 57.70% of the CR5 group and 62.70% of the CR10 group reached the two-cell stage, but 53.00% of the CPH group reached the 2-cell stage (Table 2). After 48 hours, 57.90% of CR5 and 60.20% of CR10 reached the four-cell stage, but 48% of the CPH group reached the 4-cell stage (Table 2).

Effects of CR on descriptive indicators of IVM

In a total of 79 female control mice oocytes, on average, about 12.66% of degenerated immature oocytes were arrested. On average, 8.86% remained in the germinal vesicle (GV) stage and showed no sign of meiosis. On average, 29.11% progressed and stopped on the musical stage. 49.37% went to stage 2 and matured (Table 3).

96 CPH oocytes from female mice arrested an average of 33.33% of degenerated immature oocytes. On average, 18.75% remained in the GV stage and showed no signs of meiosis initiation. On average, 28.13% progressed to the musical stage and stopped. On average, 19.79% went to stage 2 and matured (Table 3).

In a total of 96 CR5 female mice eggs, on average, about 27.91% of immature oocytes were depleted. On average, 20.93% remained in the GV stage and showed no signs of meiosis. On average, 26.74% progressed to the stage of meiosis one and stopped. On average, 24.42% went to stage 2 and matured (Table 3).

109 oocytes from CR10 female mice retained an average of 19.00% of degenerated immature oocytes. On average, 17.00% remained at the GV stage and showed no signs of meiosis initiation. On moderate, 32.00% advanced to the musical stage and stopped. On average, 32.00% progressed to stage 2 and matured (Table 3).

Effects of CR on Morphology of follicles

CR administration to mice significantly increased the number of primary and secondary follicles in different doses. CPH causes the destruction of follicles (Fig. 1A). Compared to the control group, the CPH group showed a reduction of primary and secondary follicles. CR5 and CR10 groups showed an increase in primary and secondary follicles compared to the CPH group (Fig. 1B).

Effects of CR on MDA concentrations

The animals in the CPH group had higher MDA than the control and sham groups (Fig. 2B; $P<0.04$). Administration of CR5 and CR10 decreased MDA compared to CPH group ($0.03>P$ for CR10; Fig.2B). The effect of the highest dose compared to the lowest dose was more significant for improving MDA concentration, but the CR5 dose had no significant effect ($P>0.03$).

Effects of CR on NO concentrations

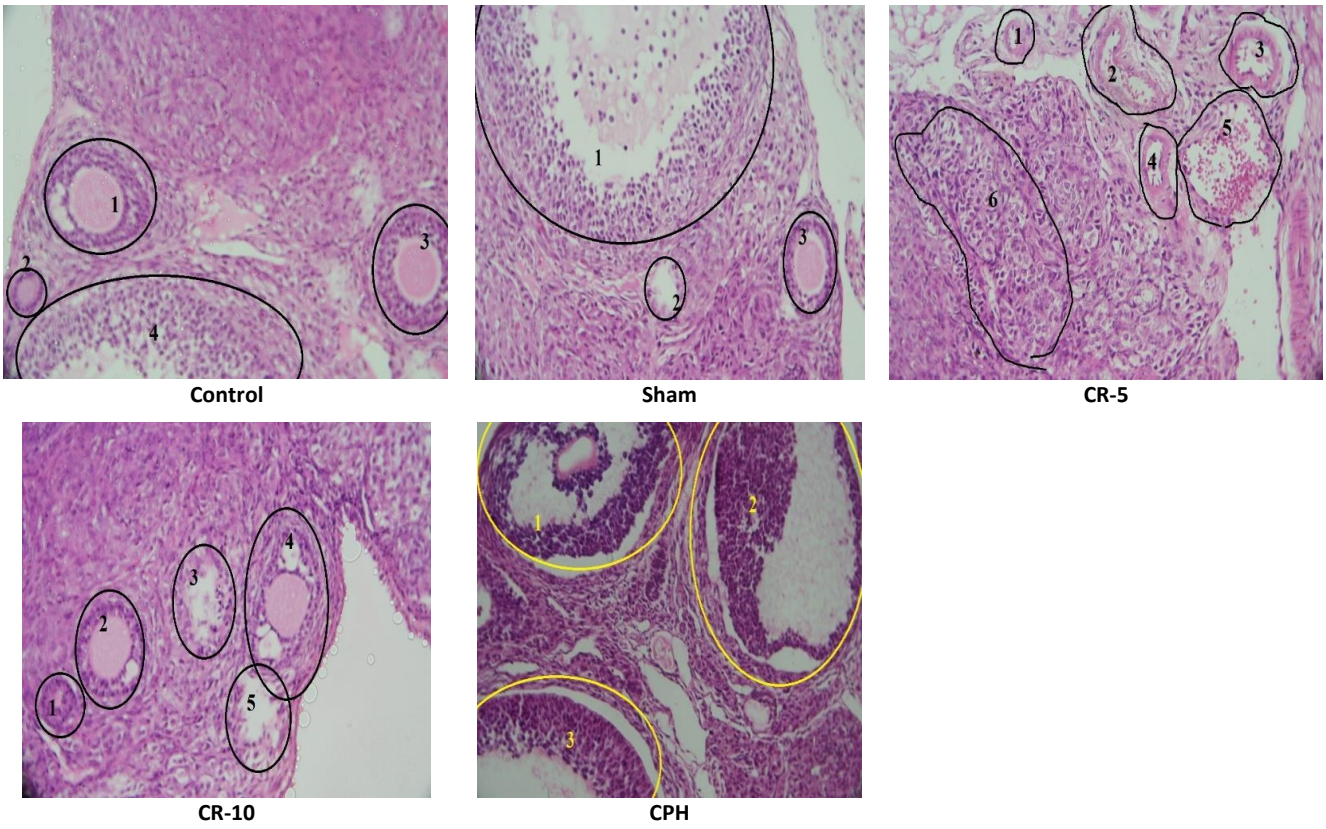
Effects of CR on NO concentrations in the group treated with CPH, NO metabolites were higher than the control and sham groups ($P < 0.01$), but the treatment with CR5 and CR10 doses reduced the NO metabolites ($P < 0.05$ for CR10; Fig. 2B). There are not many different doses of CR (Fig. 2B).

Effects of CR on CAT activity

Compared to the control and sham, administration of CPH decreases ($P < 0.01$). Compared to the CPH group, treatment with 5 and 10 mg/kg of CR improved CAT, but 5 mg/kg of CR did not cause changes in catalase content ($P < 0.05$ for CR10; Fig. 2C). In animals treated with 10 mg/kg CR, CAT was higher than animals treated with 5 mg/kg, while it was not statistically different.

Effects of CR on Gene Expression

Administration of CR increases the expression level of BMP-15 (Fig. 3A) and GFDF-9 (Fig. 3B) compared



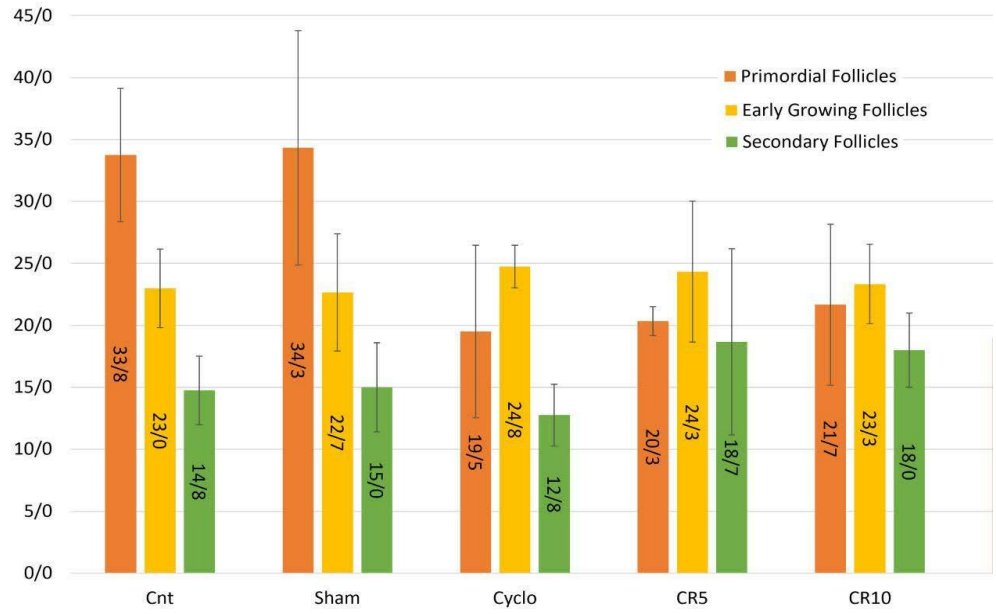


FIGURE 1. Effects of two doses of CR on different stages of follicle maturation in ovaries removed from adult female NMR mice after administration of CPH. (A): Photomicrograph of the ovarian section,
Control: 1-Secondary follicle where the antrum is not yet integrated, 2. The primary follicle should be seen at greater magnification, 3- Secondary follicle,
4. Antral follicle
Sham: 1-Antral follicle, 2- Vein
CR-5: 1 & 2- secondary follicles with oocytes form 2 small (white corner between granulosa), 3- Vein
CR-10: 1- The secondary follicle, the left half of which has been removed during tissue fixation, pink oocyte
2 & 4- Secondary follicle, the nucleus of the oocyte, is not seen
3 & 5- Large antral follicles in which coronary radiators are found regularly around the oocyte. The nucleus of the oocyte is not seen.
CPH: 1, 2, and 3 large antral follicles, the oocyte is found in follicle 1.
(B): Percent of different stages of follicle maturation in other groups.

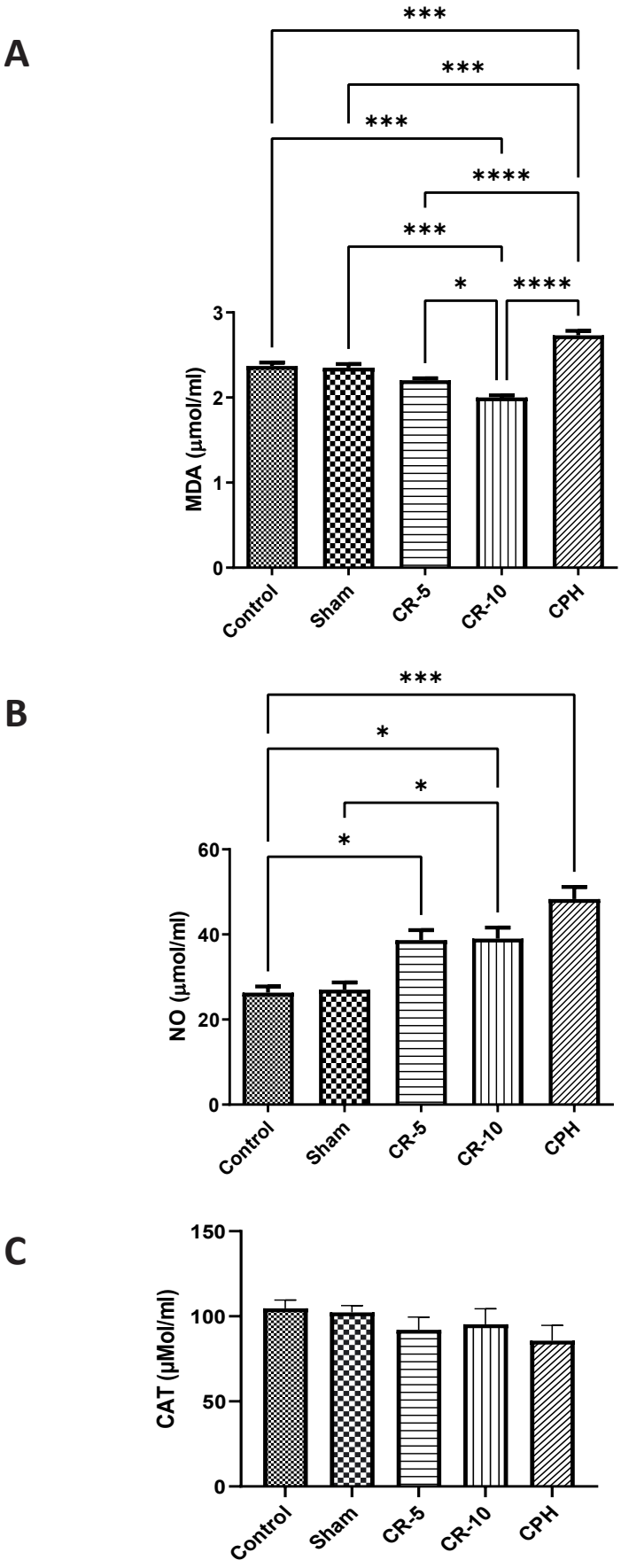


FIGURE 2. Effects of two doses of CR on oxidative and nitrosative balance after administration of CPH. (A): MDA as an oxidative marker, (B) NO, as a nitrosative marker, and (C): CAT, as an anti-oxidant marker.

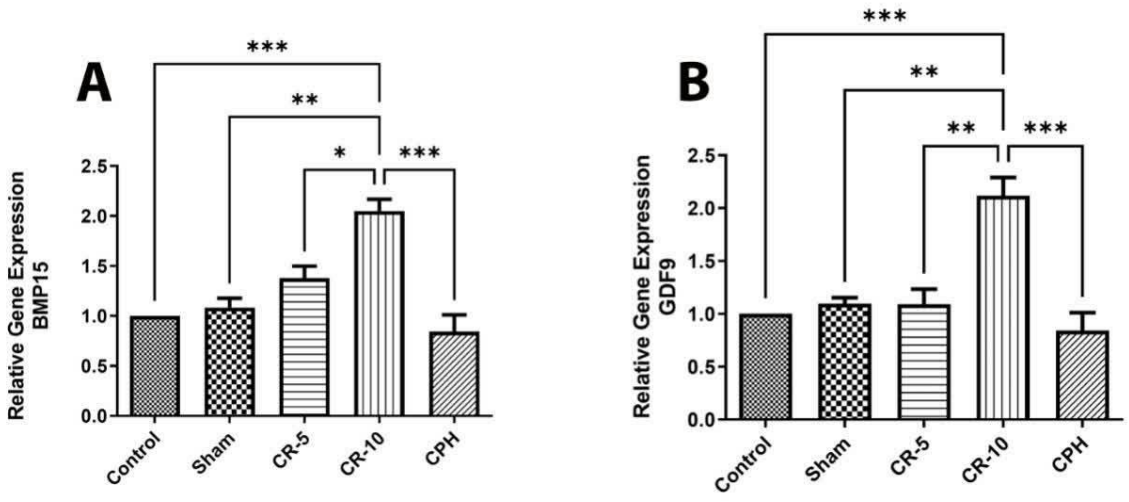


FIGURE 3. Effects of two doses of CR on gene expression secreted by the oocytes into the ovarian follicles after administration of CPH. (A): BMP15 and (B) GFDF-9.

to the CPH group ($p<0.001$). The expression of BMP-15 and GFDF-9 in the CPH group was decreased compared to the control group. There was no statistical difference between CR5 and CR10.

Discussion

Ovaries are a pair of glands in the female reproductive system in which many primordial follicles rest. An ovarian follicle is a spherical sac filled with fluid that contains an egg. After entering the growing follicular pool, follicles mature, ovulate, or undergo atresia. As a result, the fertility of a female individual depends on the initial follicular reserve. A set of follicles begins to grow in each reproductive cycle, and after the reserve is exhausted, the individual enters the reproductive senescence stage.

This study's purpose is to investigate the effects of chrysin on different stages of embryonic development and egg maturation. After 24 hours, the oocyte analysis showed that the treated groups had more lost oocytes than the control and sham groups. Group CR5 lost 42.30% of eggs, CR10 lost 37.30% of eggs and the CPH group lost 47% of eggs. CR does not negatively affect oocytes in the GV stage, as shown by the increase in the number of oocytes in the GV stage. MI-stage oocytes that resumed meiosis and reached maturity were more numerous than other groups. The eggs of the CR10 group reached the MI phase in 30.28% compared to the control and CPH groups in 29.11%. Also, the number of oocytes in the MII stage was reduced in the presence of CR5 and CR10 compared to the control group. Regarding the effect of CR on sperm and egg fertilization, CR reduced the number of 2-cell embryos produced after 24 hours in the treatment group. After 24 hours, CR5 and CR10 embryos were 57.70% and 62.70%, respectively. Also, 53% of the participants in the CPH group reached the 2-cell stage. After 48 hours, a significant decrease in the number of 2-cell embryos was observed. 57.90% CR5 and 60. 20% of CR10 had the highest percentage of embryos that reached the 4-cell stage, while 48% of embryos in the CPH group came to the 4-cell stage. Based on the results obtained from the present study, CR significantly increases oocyte maturation, fertilization rate, and growth of 2 and 4-cell embryos compared to CPH.

Preserving fertility is very important in the life of cancer patients after recovery (Mantawy et al. 2019). Severe reduction of primary follicle reserve following treatment with CPH limits the period of natural pregnancy and causes POF and infertility in women (Meirow et al. 2001). In this study, the administration of CPH led to the destruction and reduction of primary and secondary follicles.

Similarly, premature infertility has been observed in animals in which primordial follicles are reduced by exposure to induced radiation (Guigon et al. 2003). Treatment of mice with CPH causes loss of primordial follicles (Athira et al. 2020). There is a very effective strategy to preserve women's fertility that has less burden on the human body (Jang et al. 2017). According to previous studies, oxidative stress can reduce follicles

and eggs (Wei et al. 2016).

CR, from the flavonoid family, can be extracted from many plants and protects against inflammatory response, tumors, and oxidative stress (Mantawy et al. 2019). In addition, CR has been confirmed to exert protective effects against radiation-induced ovarian damage. In a study, they reported that CR activity increased SOD and GSH-PX, but decreased MDA levels (Li X et al. 2022). In this study, the administration of CR to the oocyte decreased MDA and NO, which indicates that the antioxidant defense system of the oocyte was improved. Previous studies have reported that CR significantly increased ovarian AMH and induced follicular growth and preservation of primordial follicles (Li et al. 2022). In the present study, administration of CR increased primary and secondary follicles. The apoptosis process has an essential effect on ovarian damage caused by radiation (Aktas et al. 2012). Also, many studies have shown that ectopic inflammation can alter the normal function of the ovarian follicle and cause infertility (Boots and Jungheim. 2015). In contrast, CR administration effectively abrogated the inflammatory responses. CR can improve radiation-induced ovarian damage by suppressing inflammation and apoptosis (Mantawy et al. 2019). BMP-15 and GDF-9 are two groups of the TGF-β family known as fertility markers in humans and are significantly involved in all stages of egg development and embryo quality.

Several studies have described the pathologic process of primary ovarian failure based on two members, BMP-15 and GDF-9 (Kuang et al. 2014). In POF patients, mutations in BMP-15 genes decreased the cooperation of BMP-15 and GDF-9, which indicates the importance of these two heterodimers in ovarian function (Sanfins et al. 2018). These two genes are necessary for the development of the initial and final stages of the follicle, and the effect of mutation on GDF-9 and BMP-15 leads to cellular and molecular abnormalities and defects in translation and secretion. These genes play an essential role in growth, ovulation, and fertilization by activating autocrine and paracrine mechanisms during follicular development (Sanfins et al. 2018).

In our research, in the CPH group, the expression level of BMP-15 and GDF-9 decreased, but, the expression level of BMP-15 and GDF-9 increased after the administration of CR. In our study, treatment with CR reduces MDA and NO metabolites and improves the cut compared to the CPH group. According to Dimer et al.'s study, CR improves ovarian toxicity caused by fluorouracil by reducing oxidative stress, and inflammation and increasing antioxidant status. This study shows that CR is a therapeutic compound that is useful for lowering ovarian damage caused by fluorouracil (Dimer et al. 2023). Temel et al.'s studies showed that CPH inhibits the activity of GR, GST, and 6PGD enzymes, which are essential enzymes of PPP and intracellular thiol metabolism. Still, in contrast to CR, it regulates enzyme activities. This regulatory effect helps prevent chemotherapy damage caused by CPH (Temal et al. 2021). In the study of Li et al., CR inhibited oxidative stress in mice induced with D-gal, and this inhibitory effect is through the suppression of ROS (Li et al. 2022). Based on the studies of Melekoglu et al. in mice that were damaged by I/R, by receiving CR, tissue oxidative stress, ovarian reserve markers, and histopathological changes were significantly improved (Melekoglu et al. 2018). In the study of Khoo et al., it was shown that CR interdicts proliferation, and impels apoptosis in most cancer cells, and the mechanism of CR's effect works through the activation of caspase and the inactivation of Akt signaling. (Khoo et al. 2010). Samarghandian et al.'s data showed that CR causes cytotoxicity and apoptosis in human prostate cancer cell lines (Samarghandian et al. 2011). In the studies of Amini et al., they concluded that bee venom and CR are effective in killing ovarian cancer cells resistant to chemotherapy through the activation of intrinsic apoptosis (Amini et al. 2015). According to Shao et al.'s findings, CR causes growth inhibitory and pro-apoptotic effects on A549 lung cancer cells (Shao et al. 2012). In a study conducted by Brechbuhl and her colleagues, it was shown that CR can sensitize multidrug resistant lung cancer cells to chemotherapy by increasing glutathione flow (Brechbuhl et al. 2012).

Conclusion

Based on our studies and the obtained results, CR in the 2 and 4-cell stages improves the reduction of fertilization, maturation, and fetal growth caused by the administration of CPH through increased growth factors affecting the ovaries and the balance of oxidative stress.

Acknowledgments

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Study Approval Statement

The study approval committee is the Northern Research Center, Pasteur Institute of Iran, Amol, Iran. Decision reference number A/P-3765/2.

Statement of Ethics

This is to inform you that the submitted proposal under the title of Chrysin's potential in ameliorating the toxic effects of Cyclophosphamide on mouse oocytes and embryos, was discussed in the meeting dated 29/04/2020 and it was approved to be implemented in this research institute.

Ethics Review Board

The authorities of Pasteur Amol Institute have read and approved this protocol with reference number 3765/2.

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