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Caffeic acid improves antioxidant capacity and moderates ovarian histopathological alterations in mice premature ovarian insufficiency model

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Abstract

Objective: Premature ovarian insufficiency (POI) is a heterogeneous condition described as the cessation of ovarian function in women below 40, often linked to oxidative stress. This study investigates the effect of caffeic acid (CA) on antioxidant capacity and histopathological changes in the ovaries of POI model mice.

Materials and methods: In this experimental study, 32 female mice were randomly divided into four groups (n=8): control, CA, POI, and POI+CA. POI was induced by daily injection of D-galactose (200 mg/kg) for six weeks. CA was administered intraperitoneally (IP) daily starting from the fifteenth day for four weeks (60 mg/kg). Finally, the mice were anesthetized, and their ovaries were collected after blood sampling. The levels of hormones (Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), and estradiol), oxidative stress markers (Total antioxidant capacity (TAC), Malondialdehyde (MDA), Glutathione peroxidase (GPx), and superoxide dismutase (SOD)), histopathological changes, and the expression of genes *Nrf2*, *SOD1*, and *Gpx1* were evaluated.

Results: The results indicated that POI led to a significant increase in FSH, LH, and MDA levels ($p<0.001$) while decreasing SOD, GPx, and TAC levels compared to the control group ($p<0.01$). Additionally, the expression of *Nrf2*, *SOD1*, and *Gpx1* genes was significantly lower in the POI group ($p<0.01$), and there was a notable reduction in the number of follicles compared to the control group ($p<0.01$). Caffeic acid treatment (POI+CA) significantly improved hormone levels, histopathological and oxidative stress factors, and related genes compared to the POI group ($p<0.01$).

Conclusion: The outcomes indicated that CA moderates the hostile effects of POI on the ovaries in mice by decreasing oxidative stress and increasing antioxidant defenses.

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Introduction

In medicine, Infertility is defined as the inability of a couple to conceive after 6 to 12 months of trying (Obeagu et al. 2023). The etiology of infertility can be attributed to various factors, with approximately 30-40% of cases related to female factors, 30-40% attributed to male factors, and 10-20% resulting from a combination of issues affecting both partners (Golmohammadi et al. 2022). Several factors contribute to female infertility, including hormonal imbalances, psychological stress, dietary habits, excessive physical exercise, pelvic infections, anovulation, poor ovarian quality, tubal damage, and endometriosis. Among these, ovarian dysfunction plays a critical role in female fertility. The ovaries are essential endocrine organs within the female reproductive system, responsible for initiating and regulating puberty as well as facilitating the menstrual cycle (Bala et al. 2021). One of the important causes of ovarian problems is premature ovarian insufficiency (POI) (Chen et al. 2021).

POI is a multifactorial disorder association with elevated FSH levels (hypergonadotropic) and reduced estrogen levels (hypogonadism) (Chen et al. 2021; Delkhosh et al. 2019). Unfortunately, the precise mechanisms underlying the pathogenesis of POI remain unclear, and diagnosis is often delayed. Factors contributing to POI include autoimmune diseases, metabolic disorders (such as galactosemia), exposure to radiotherapy and radiation, chemotherapy, infections, genetic predispositions, and iatrogenic causes. Amenorrhea or oligomenorrhea for more than four months accompanied by hot flashes and atrophy of the urogenital system are symptoms of POI (Chen et al. 2021; Li et al. 2019). Current treatment options for POI include hormone replacement therapy (HRT), oocyte donation, psychological support, and lifestyle modifications such as diet and exercise. However, it is important to note that HRT has been associated with an increased risk of breast and uterine cancer, as well as coronary heart disease in women with POI (Chon et al. 2021).

Clinical POI is a common characteristic of galactosemia, and POI eventually manifests in galactosemia-affected women (He et al. 2017; Yan et al. 2018). Prior research has demonstrated that the d-galactose (D-gal) mouse model effectively simulates the development of POI, making it a valuable tool for exploring therapeutic interventions. D-gal impairs ovarian function and leads to polycyclic ovary inflammation by promoting excessive production of reactive oxygen species (ROS) and buildup of advanced glycation end-products (AGEs)(He et al. 2017; Yan et al. 2018). Prior research has demonstrated that administration of antioxidants such as icarin (Li et al. 2019), puerarin (Chen et al. 2021), ginsenoside Rg1 (He et al. 2017), curcumin (Yan et al. 2018), and resveratrol (Li and Liu 2018) can improve the symptoms of POI in animal models.

It has been scientifically confirmed that caffeic acid has antioxidant, immuno-modulatory, anti-apoptotic, anti-inflammatory, and antibacterial effects (Birková et al. 2020). Studies have shown that caffeic acid prevents the adverse effects of polycystic ovary syndrome (PCOS) (Chiang et al. 2023), ischemia/reperfusion (Kart et al. 2009), and oxidative stress (Tokmak et al. 2015) on ovarian function. Caffeic acid performs this process through changes in the expression of genes and oxidative stress factors.

In this work, we assessed the protective impact of caffeic acid on histological changes and oxidative stress markers in the mice ovaries with POI induced by D-gal.

Materials and Methods

Animals and experimental design

In this experimental study, 32 female mice (Balb/c, 6-7 weeks) were prepared from the Royan Institute in Tehran, Iran, and housed under controlled conditions (20-25° C, 12-hr darkness/light cycle and 40–70% humidity) in the animal house of

Ardabil University of Medical Sciences (ARUMS), Ardabil, Iran. Water and food were provided *ad libitum* during the study. All procedures were carried out according to the approval of the ethics committee of ARUMS (IR.ARUMS.AEC.1400.018) for work with animals and laboratory methods. During the acclimatization period, the normal functioning of the reproductive system was assessed by monitoring the estrous cycle. Then, the female mice were randomly divided into the four following groups (n=8): 1) control (cont), 2) caffeic acid (CA), 3) premature ovarian insufficiency (POI) and caffeic acid (PO + CAI) group. POI was induced by daily D-gal injection (200 mg/kg body weight, Sigma-Aldrich) subcutaneously for a duration of six weeks (He et al. 2017). In this way, from the beginning of the study, D-gal was injected in POI and POI+CA groups, and at the same time, the estrous cycle of the mice was controlled for 2 weeks (to ensure the induction of POI). From the third week (the 15^{th} day), CA (60 mg/kg body weight, Sigma-Aldrich) was injected intraperitoneal (IP) daily for 4 weeks (Khoshdel et al. 2022) in CA and POI+CA groups.

Estrous cycle assessment

Vaginal smear analysis is a widely employed technique for monitoring the stages of the estrous cycle (Ajayi and Akhigbe 2020). In this procedure, the tail of the mouse is gently lifted, and vaginal cells are rinsed with 100 µl of saline using a sampler. The cells are collected by pipetting 4-5 times, transferred onto a glass slide, and a smear is prepared. After air-drying, the smear is stained with 0.1% crystal violet to facilitate the identification of the estrous cycle stages: proestrus, metestrus, estrus, and diestrus (Ajayi and Akhigbe 2020).

Sampling

One day following the final injection, the fasting mice were weighed and anesthetized with IP injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). Subsequent to cardiac blood sampling, the ovaries were immediately excised. The ovaries were weighed, and one ovary was fixed in 10% buffered formalin for histopathological examination. The other ovary was stored in liquid nitrogen for subsequent analysis of *Nrf2*, *SOD1*, and *Gpx1* gene expression, as well as for the measurement of oxidative stress parameters, including total antioxidant capacity (TAC), malondialdehyde (MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD).

Hormonal assay

The blood sample of mice (in the diestrus stage), after clotting at room temperature, was centrifuged (10 min/900 g) and serum was separated. Finally, the levels of Follicle-Stimulating Hormone, Luteinizing Hormone, and estradiol (E2) hormones were measured by the ELISA method using Pishtaz Teb company (Iran) kits based on the kit protocol.

Oxidative stress factors assessment

The lipid peroxidation level of the ovarian tissue was assayed based on the MDA level. Briefly, ovarian tissue was homogenized in 1 ml of 1.5% Potassium Chloride (KCl) and MDA levels were measured based on the thiobarbituric acid reaction (TBARS). Also, the ovarian tissue SOD, GPx and TAC activity was evaluated with ZellBio kits (Germany) pursuant to the manufacturer's protocol (Bahrami et al. 2024).

Real-time PCR

RNA isolation and qPCR experiments were done pursuant to the previous studies' method (Panahizadeh et al. 2023; Rezaie et al. 2023). First, total RNA was collected from the ovarian tissue by using the Trizol solution (Biobasic, Canada). Second, the total concentration of RNA was measured by a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, USA). A260/A230 and A260/A280 ratios were acquired for determining the purity and quantity of the RNA preparation. After that, the RNA was reverse transcribed into cDNA by a cDNA synthesis kit (SMOBIO: RP1300) pursuant to the manufacturer's protocol. Further, cDNA was exploited for the RT-PCR. The primers designed by oligo7 software, were synthesized by Gen Fan avaran (Tehran, Iran) and are listed in Table 1. *GAPDH* was determined as a reference gene. RT-qPCR data were analyzed using the threshold cycle (Ct) values and the $2^{-\Delta\Delta Ct}$ method.

Table 1. Primers used for RT-PCR analysis

Gene	GenBank Accession Number	Primer sequences (5´ to 3´)	Product size (bp)
Nrf2	NM_010902.5	F: GCC CAC ATT CCC AAA CAA GA	**61**
		R: TCT CTG CCA AAA GCT GCA TAC	
SOD 1	NM_011434.2	F: AGC GGT GAA CCA GTT GTG TT	**150**
		R: CGG GCC ACC ATG TTT CTT AG	
GPx 1	NM_001329528.1	F: AGT GCG AAG TGA ATG GTG AGA	**127**
		R: CAC ACC GGA GAC CAA ATG ATG	
GAPDH	NM_008084.3	F: ATG GTG AAG GTC GGT GTG AA	**114**
		R: GAG GTC AAT GAA GGG GTC GT	

Histopathological assessment

At room temperature, ovarian tissue was fixed in a 10% buffered formalin for 72 hr. After tissue processing, it was embedded in paraffin, sectioned into 5-µm slices and stained with Hematoxylin and eosin (H&E). Ultimately, histopathological changes were studied using a light microscope. For this purpose, the number of follicles (primary, secondary, and antral follicles), and corpora lutea were evaluated in every fifth section according to the techniques described in an earlier study (He et al. 2017). Counting was done clockwise and oocyte nucleus observation in each follicle was considered a count index (He et al. 2017; Razavi et al. 2017).

Statistical analysis

All statistical analyses were performed using SPSS version 16 software, and all values are expressed as Mean ± SD. In this way, after confirming the data's normality using the Kolmogorov-Smirnov test and homogenizing the variances using the post hoc Tukey test, the ONE-WAY ANOVA test was performed. The results with $p \leq 0.05$ were considered statistically significant.

Results

Effect of POI and CA on the weight of body and ovary

The results indicate that there was no statistically significant difference in body and ovarian weights (Table 2) between the POI group and the control group ($p>0.05$). Additionally, the administration of CA to the POI group (POI+CA) did not lead to a significant change in body or ovarian weights compared to the POI group ($p=0.21$). However, when comparing the control group with the CA group, a statistically significant decrease in body weight was observed in the CA group during both the third and sixth weeks of treatment ($p<0.01$).

The effect of POI and CA on the level of hormones

The results (Table 2) of the study demonstrate that mice with POI exhibited significantly elevated levels of FSH and LH, while E2 levels were notably decreased when compared to the control group ($p<0.001$). In contrast, treatment with CA in mice resulted in a significant reduction of FSH and LH levels, along with an increase in E2 levels in the POI+CA group relative to the POI group ($p<0.01$). Furthermore, no statistically significant differences in sex hormone concentrations were observed between the control group and the CA group ($p=0.36$).

Table 2. Effect of POI and caffeic acid on body/ovary weight and hormonal assay

Parameters	**Cont**	**CA**	**POI**	**POI+CA**
Body weight at the beginning of the experiment (g)	26.71± 1.25	24.25.54±	26.5±2.2	24.87±1.45
Body weight in the third week (g)	29.28±1.49	26.5±1.69[c]	27.87±2.16	27.25±1.83
Body weight at the end of the experiment (g)	33.00±2.16	27.37±2.13[c]	31.00±2.26	28.87±1.64
Ovary weight at the end of the experiment (mg)	26.1±0.60	27.4±0.54	24.1±0.27	25.1±0.51
FSH (ng/ml)	95.75± 1.3	96.12 ± 1.17	138.25 ± 1.61[a]	122.62± 1.58[b]
LH (ng/ml)	12.87 ± 0.63	11.25 ± 0.59	19.75 ± 0.75[a]	16.5± 0.8[b]
Estradiol (E2) (pg/ml)	9.37±0.41	9.33±0.33	5.00±0.26 [a]	6.37±0.32[b]

Data are mean ± SD. a show a significant difference between the Cont and POI groups; b shows a significant difference between the POI + CA and POI groups and c shows a significant difference between the Cont and CA groups ($p<0.05$).

The effect of POI and CA on the oxidative stress markers in the ovarian tissue

The study findings (Figure 1) indicate a statistically significant increase in MDA levels in the ovarian tissue of the POI group when compared to the control group ($p<0.001$). However, the administration of CA in the POI + CA group led to a significant reduction in MDA levels compared to the POI group ($p<0.001$). Additionally, there was no statistically significant difference in MDA concentrations between the control group and the CA group ($p=0.95$). A significant decrease in the mean activity of GPx and SOD in ovarian tissue was observed in the POI group compared to the control group ($p<0.001$). Although the administration of CA in the treated group (POI + CA) resulted in an increase in the mean activity of GPx ($p=0.66$) and SOD ($p=0.07$) in ovarian tissue, these changes were not statistically significant compared to the POI group. Furthermore, no statistically significant differences were found when comparing the activities of GPx and SOD between the control group and the CA treated group ($p=0.99$). Also, the results demonstrated that POI significantly reduces the TAC of ovarian tissue when compared to the control group ($p<0.001$). The administration of CA to the treated group significantly inhibited the decline in TAC relative to the POI group ($p<0.05$). Additionally, no statistically significant difference was found between the control and the CA groups in oxidative stress factors ($p>0.05$).

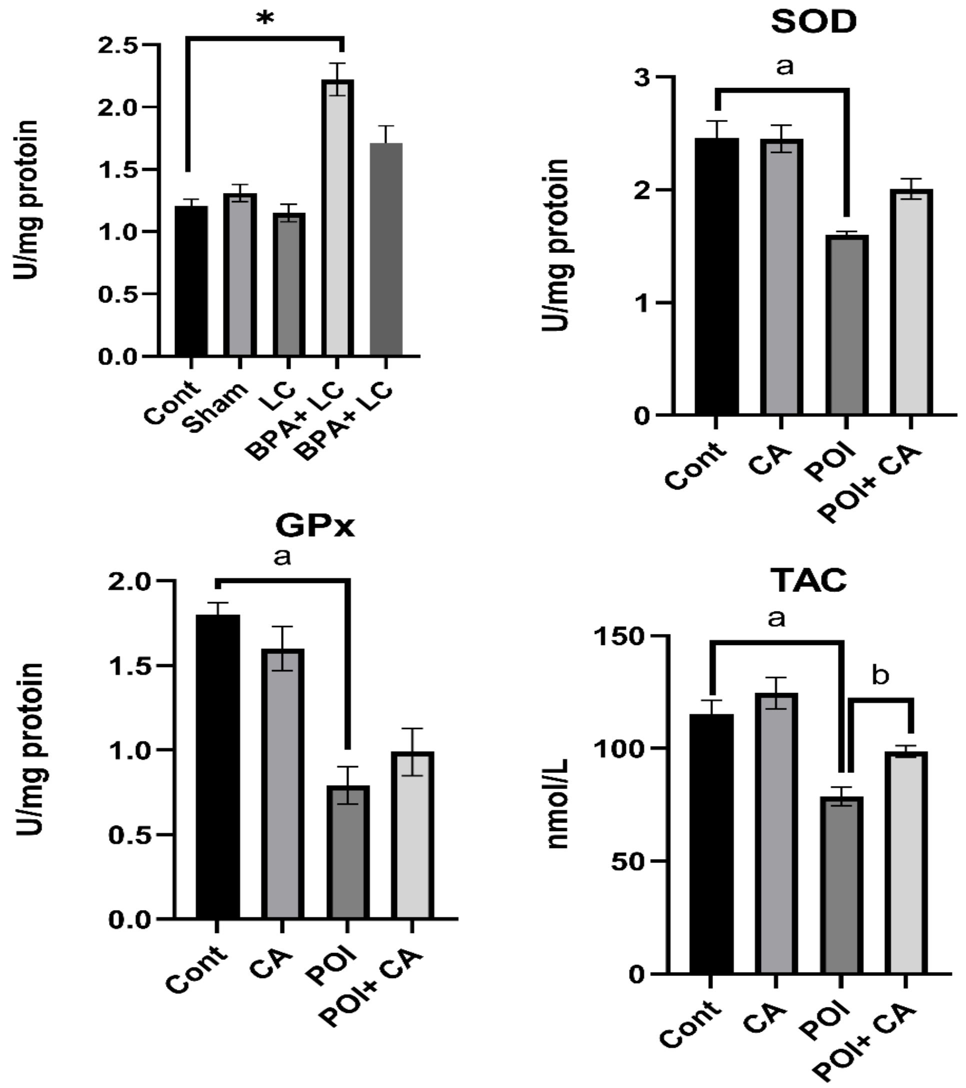

Figure 1. Comparing the level of MDA, GPx, SOD and TAC in ovarian tissue. Data are mean ± SD. a show a significant difference between the Cont and POI groups; b shows a significant difference between the POI + CA and POI groups (p<0.05).

The effect of POI and CA on the expression of *Nrf2, SOD1* and *GPx1* genes

The findings (Figure 2) indicate that POI has a substantial impact on the expression of *Nrf2* and *GPx1* genes compared to the control group (p<0.001). However, the administration of CA to mice in the POI + CA group successfully mitigated this decline in gene expression induced by POI (p<0.001). In addition, the analysis of the CA group and the control group showed that the administration of CA markedly upregulated the expression of the *Nrf2* gene with a statistically significant difference (p<0.001). Furthermore, there was no notable disparity regarding the *GPx1* gene between these two groups (p=0.09).

Although the data indicated a decrease in *SOD1* gene expression in the POI group compared to the control group, this decrease was not statistically significant (p=0.12). Treatment of mice in the POI + CA group significantly increased *SOD1* gene expression compared to the POI group (p<0.01). In addition, the comparison between the CA group and the control group revealed that the administration of CA substantially elevated the expression of the *SOD1* gene in contrast to the control group (p<0.001).

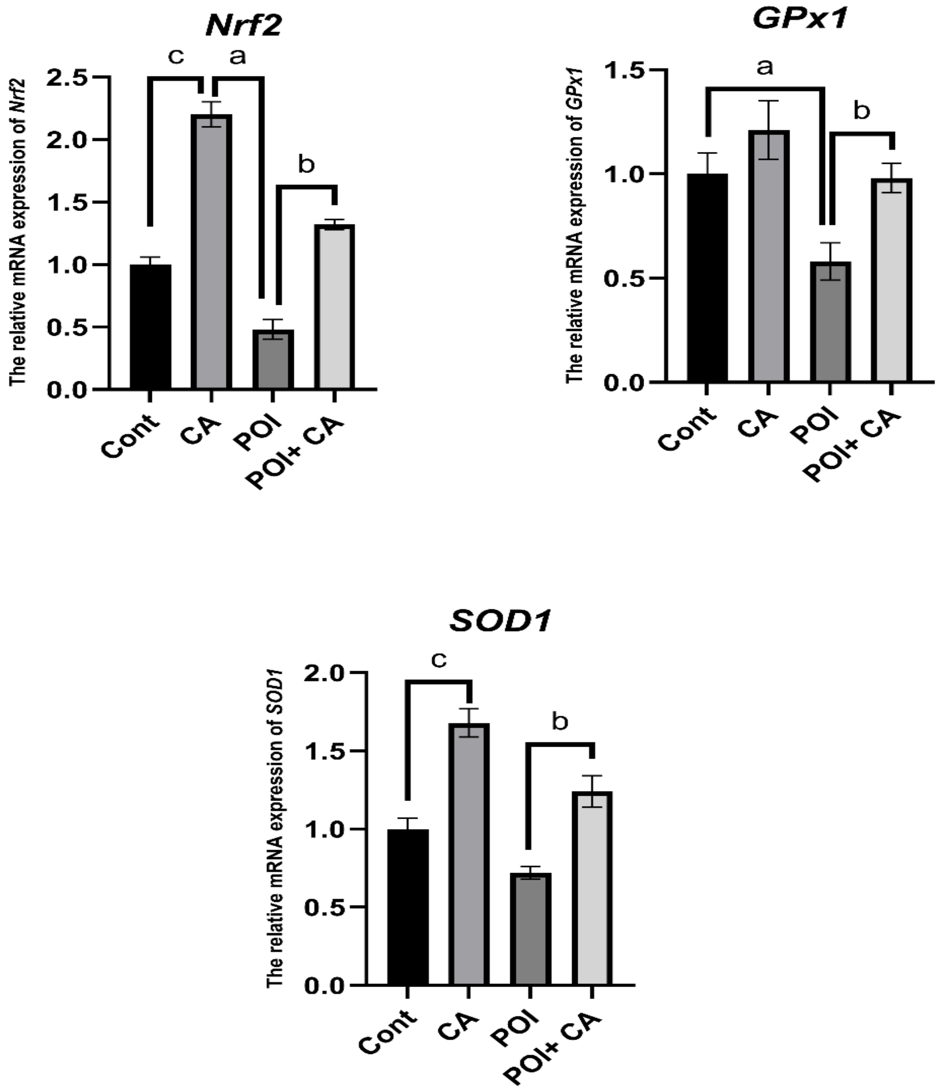

Figure 2. Comparing the expression of *Nrf2*, *SOD1* and *GPx1* genes in ovarian tissue. Data are mean ± SD. a show a significant difference between the Cont and CA groups; b shows a significant difference between the Cont and POI groups; c shows a significant difference between the POI + CA and POI groups ($p<0.05$).

The effect of POI and CA on the histopathology of ovarian tissue

Histological examinations revealed that the ovaries, along with the structure of follicles and oocytes, appeared normal in both the control and CA groups. In contrast, the primary ovarian insufficiency (POI) group exhibited tissue damage in granulosa cells and oocytes, characterized by atretic follicles, and in some cases, a lack of zona pellucida formation. The POI + CA group displayed less tissue damage compared to the POI group (Figure 3). Additionally, the study indicated a significant reduction in the number of primary, secondary, and antral follicles, as well as corpus luteum (an indicator of ovulation), in the POI group compared to the control group ($p<0.01$). However, the administration of CA in the POI + CA group effectively mitigated the decrease in the number of primary follicles when compared to the POI group ($p<0.01$). While CA treatment did not prevent the decline in secondary and antral follicles relative to the POI group, these changes were not statistically significant (Figure 4). Furthermore, no statistically significant differences were observed between the control and CA groups in the number of follicles and corpus lutea ($p=0.48$).

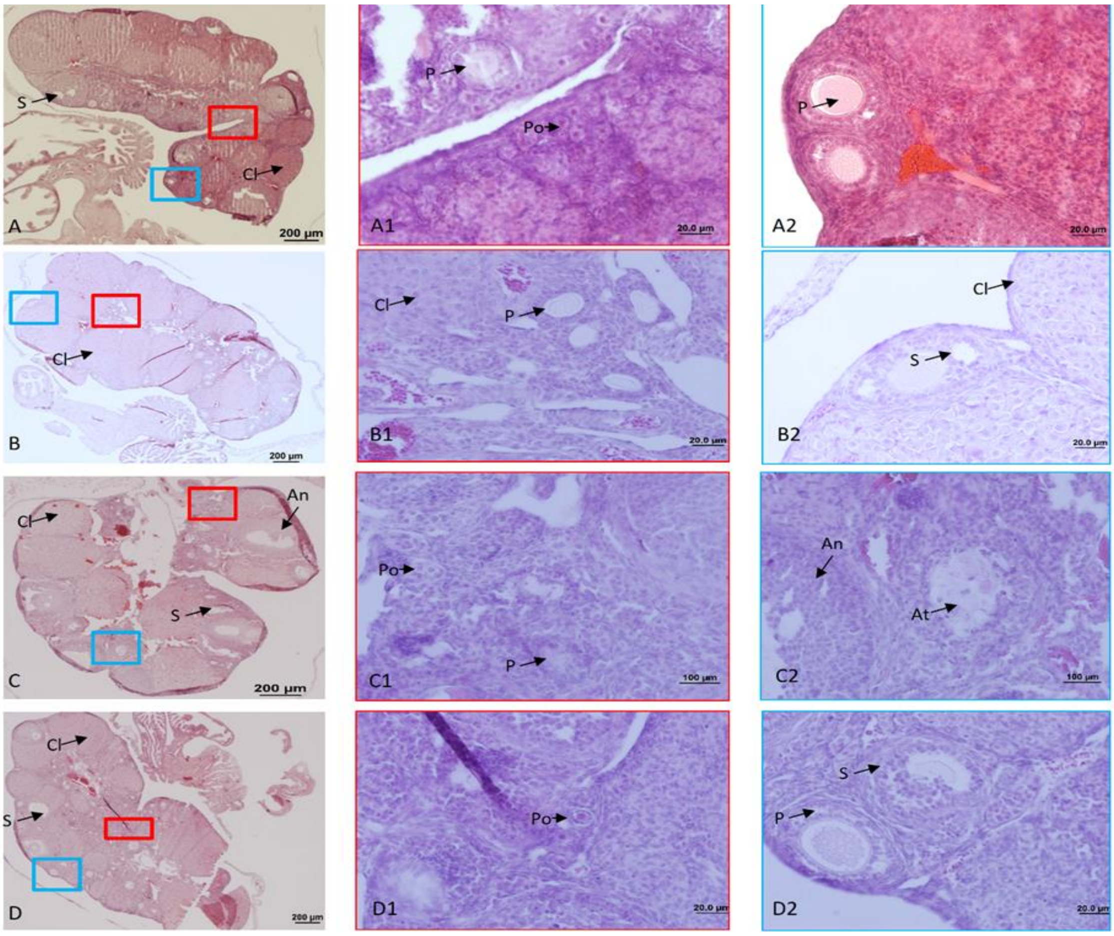

Figure 3. Light microscopy of ovarian tissue in different groups (10X and 40X). Representative photographs of H&E staining in cont group (A), caffeic acid group (B), POI group (C), and POI + CA group (D). Type of ovarian follicles including Primordial follicles (Po), Primary follicle (P), Antral follicle (An), Atretic follicle (At), and corpus luteum (Cl) can be seen in the figures. POI decreased the number and diameter of follicles. The treatment of mice with caffeic acid decreased the adverse effects of POI on these parameters.

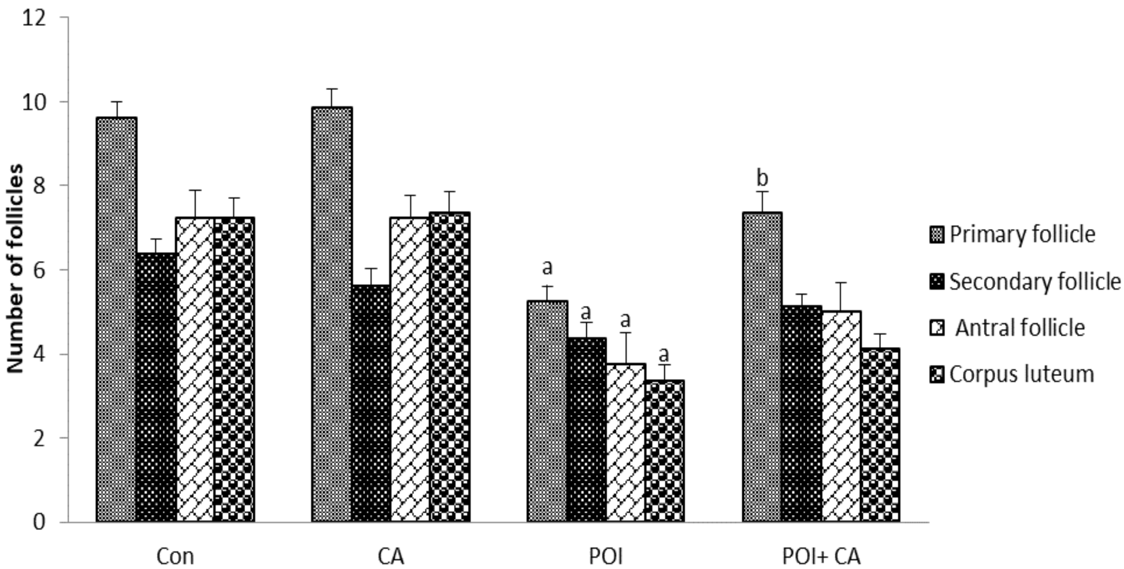

Figure 4. Comparing the number of follicles and corpus luteum in ovarian tissue. Data are mean ± SD. a shows a significant difference between the Cont and POI groups; b shows a significant difference between the POI + CA and POI groups ($p<0.05$).

Discussion

The results of the current study indicate that D-gal induced POI leads to disturbances in sex hormone levels, oxidative stress markers, antioxidant gene expression, and ovarian tissue structure in mice. Conversely, the administration of CA in the POI + CA group mitigated the adverse effects of POI on these factors.

According to the findings of this research, POI decreased the both body and ovarian weight as compared to the control group. However, these changes were not statistically significant. Prior studies by Yan et al. (2018) and Li, et al. (2019) similarly demonstrated that D-gal induced POI results in reductions in body and ovarian weights compared to control groups (Li et al. 2019; Yan et al. 2018). Disorders of female sex hormones and the degradation of ovarian tissue may contribute to the observed reduction in ovarian weight (Stuenkel and Gompel 2023). In this context, the findings indicated a significant elevation in the concentration of FSH and LH and a reduction in E2 hormones in the POI group. In support of our results, Yan et al. (2018) and Ellibishy et al. (2024) reported that POI causes disturbances in the level of FSH, LH, and E2 hormones (Ellibishy et al. 2024; Yan et al. 2018). They have reported that D-gal directly reduces FSH bioactivity and inhibits the production of E2 from granulosa cells by causing oxidative stress and galactose toxicity. Disorder of the hypothalamus-pituitary-ovary axis is a significant etiology of POI (Stuenkel and Gompel 2023). In this scenario, FSH levels increase while inhibin B, which regulates sex hormone levels, decreases. Elevated FSH stimulates follicle development; however, disruptions in folliculogenesis can lead to diminished ovarian reserve and ultimately result in POI (Ellibishy et al. 2024; Yan et al. 2018). On the other hand, histological studies demonstrated that POI decreased the quantity of follicles and corpus luteum. In line with our study, Chen et al. (2021) have shown that POI reduces the quantity of follicles (Chen et al. 2021). A decrease in the number of follicles indicates a loss of ovarian reserve and induction of POI. Additionally, the decrease in the number of corpora lutea—an indicator of ovulation—suggests a disruption in the folliculogenesis process.

Prior research has demonstrated that elevated oxidative stress and reduced antioxidant enzyme activity contribute to the development of POI (Tokmak et al. 2015). In this context, the findings of our investigation indicated an elevation in the MDA (lipid peroxidation index) level and a decrease in SOD and GPx activities, as well as TAC concentration, in the POI group.. Additionally, the findings demonstrated a decrease in the expression of antioxidant genes, such as *Nrf2*, *SOD1* and *Gpx1* reduced in this group, which indicates the induction of oxidative stress. This result is consistent with the previous studies that reported POI increased MDA levels and decreased antioxidant enzyme activity (Chen et al. 2021; Ellibishy et al. 2024; Li and Liu 2018; Li et al. 2019; Razavi et al. 2017). Research has established that ROS production is a normal physiological process essential for cellular viability and signaling pathways (Shokoohi et al. 2019; Zhang et al. 2016). However, excessive ROS production can damage the normal function of the cell (Jomova et al. 2023). D-gal-induced oxidative stress through ROS formation and AGEs accumulation destroys oocyte and granulosa cells by damaging the cell membrane, nucleus, and DNA (He et al. 2017; Yan et al. 2018). Cell membrane damage is associated with an increase in MDA and a decrease in the level of antioxidant enzymes, which reduces the potential of the cells antioxidant defense (Yousefi et al. 2019). Furthermore, DNA damage is associated with changes in the expression of various genes, such as antioxidant genes (*Nrf2*, *SOD*, and *Gpx*), which also leads to a decrease in the level of antioxidant enzymes (SOD, GPx, and TAC) (Timar et al. 2022; Zhang et al. 2022).

Prior research has shown that the injection of antioxidants can mitigate the symptoms of POI in animal models (Li and Liu 2018). In this context, Li, et al have shown that icariin exhibits antioxidant properties that can enhance ovarian weight and increase sex hormone levels in POI (Li et al. 2019). Furthermore, the findings of the current investigation indicate that the administration of CA to mice leads to a reduction in the concentrations of FSH and LH, and an elevation in E2 hormones in the POI+CA group. Furthermore, Chiang et al (2023) have shown that CA can improve reproductive parameters in PCOS by modulating sex hormone levels (Chiang et al. 2023). The ovarian microscopic structure was observed to parallel the sex hormones. Yan et al. found that treating POI mice with curcumin improved ovarian tissue structure and increased the quantity of follicles (Yan et al. 2018). Administration of ginsenoside Rg1 to POI model mice resulted in decreased tissue damage and increased follicle and corpora lutea numbers (He et al. 2017).

Chiang et al. demonstrated that CA treatment inhibits the decline in follicle and corpora lutea numbers in PCOS by modulating apoptosis and oxidative stress (Chiang et al. 2023). In line with previous study, our results indicated that follicle numbers, as well as the number of corpora lutea improved in the POI+CA group. These results display the CA capacity to protect the ovary in POI. This protective role may be attributed to its antioxidant potential which mitigates damage associated with POI by reducing oxidative stress and enhancing hormone secretion (Chiang et al. 2023). Lipid peroxidation index (MDA) is a crucial parameter for studying the extent of oxidative stress. The findings indicated that the injection of CA reduces the concentration of MDA in the POI+CA group as compared to the POI group. Görkem et al. investigated the effects of CA phenethyl ester on MDA levels in both blood and ovarian tissue of rats suffering from ischemia-reperfusion injury, demonstrating similar protective effects (Görkem et al. 2017). Conversely, the findings indicated that the administration of CA enhances the concentration of antioxidant components such as SOD, GPx, and TAC. In addition, Kart et al. found that CA phenethyl ester enhanced the levels of antioxidant factors in the ovaries of rabbits with ischemia-reperfusion-induced damage (Kart et al. 2009). These findings collectively underscore the antioxidant effects of CA, which contribute to improved hormone levels and reduced tissue damage through the alleviation of oxidative stress. Alterations in the levels of antioxidant factors may stem from the regulation of genes associated with antioxidant defense.
Prior investigations have demonstrated that the injection of antioxidants enhances the concentration of antioxidant enzymes by augmenting the expression of the *Nrf2* gene. Zhang and colleagues (2022) demonstrated that the injection of daphnetin improved conditions of POI via regulating the *Nrf2* signaling pathway (Zhang et al. 2022). Furthermore, Yan et al. have shown that curcumin alleviated POI-induced disorders via *Nrf2/HO-1* and *PI3K/Akt* signaling pathways regulation (Yan et al. 2018). Also, our results showed that CA increased the expression of *Nrf2*, *SOD*, and *Gpx* genes in the POI+CA group. In this regard, CA methyl ester was shown by Park et al. to decrease the inflammatory response generated by lipopolysaccharide in human umbilical vein endothelial cells via activating *Nrf2* (Park et al. 2023). *Nrf2* plays a pivotal role in enhancing intracellular antioxidant levels and detoxification enzyme activity through the transcriptional upregulation of multiple antioxidant response element (ARE)-containing genes (Ma 2013).

In conclusion, CA appears to mitigate D-gal induced ovarian injury and may serve a protective role against POI through multiple mechanisms. These include the regulation of genes associated with antioxidant activity, enhancement of antioxidant enzyme function, modulation of sex hormone levels, and reduction of oxidative stress. These findings suggest that CA as an antioxidant, may represent a promising protective agent in the context of POI.

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Conflicts of interest

The authors had no competing interests.

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Ethical Considerations

The ethics committee of Ardabil University of Medical Science (IR.ARUMS.AEC.1400.018) approved this study.

Code of Ethics

IR.ARUMS.AEC.1400.018

Authors' Contributions

Supervision and Fund acquisition: H.K, R.S, M.G.G Conceptualization, and methodology: H.K, R.S, R.J; Data collection: R.J, R.S, Data analysis: R.S, H.K; Writing-original draft: H.K, R.S, M.G.G, R.J Review and editing: H.K, R.S, M.G.G, R.J.

Abbreviations

CA**:** caffeic acid, D-gal: D-galactose, FSH: Follicle-Stimulating Hormone, GPx: Glutathione peroxidase, LH: Luteinizing Hormone, Nrf2: Nuclear factor erythroid 2-related factor 2, MDA: Malondialdehyde, POI: Premature ovarian insufficiency, SOD: superoxide dismutase, TAC: Total antioxidant capacity.

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