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ORIGINAL PAPER

Effect of autologous cytokine-rich serum and platelet-rich plasma administration on oxidative status, minerals and proinflammatory cytokines in brain and serum in cyclophosphamide-induced ovarian failure

Mustafa Ermiş[1] · Erol Karakaş[2] · Hanifi Erol[3] · Gökhan Akcakavak[4] · Recai Aci[5] · Furkan Ümit[6] · Özhan Karatas[7] · Gülay Çiftci[6]

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Abstract
Cyclophosphamide (CP) is one of the most commonly used chemotherapy agents and carries a high risk of ovarian damage. This study aimed to evaluate the effects of autologous cytokine-rich serum (ACRS) and platelet-rich plasma (PRP) on brain oxidative status, mineral levels, and proinflammatory cytokines in rats with CP-induced ovarian failure. A total of 42 female Wistar rats (12-weeks-old) were used in the study. Six of these rats were allocated as donors, and the remaining 36 rats were randomly distributed into six groups (n = 6 per group). Group 1 received no treatment. On the 1st and 7th days, 75 mg/kg of CP was administered intraperitoneally to Groups 4, 5, and 6. On day 1, PRP was administered intraovarianly to Groups 2 and 5, while ACRS was administered intraovarianly to Groups 3 and 6. Additionally, PRP and ACRS were administered intraperitoneally to the respective groups on 7th and 14th days.The study was terminated at the end of the 31st day. Brain tissue and blood samples were collected for biochemical analyses and ovarian tissue samples were collected for histomorphological examinations. Morphological analysis using Hematoxylin–Eosin (HE) staining and immunohistochemical evaluation for AMH, α-SMA, and IL-1β were conducted on the ovaries. Proinflammatory cytokines and insulin levels were measured using ELISA test kits. TAS/TOS levels were assessed using Relassay Diagnostic kits. Biochemical parameters and mineral levels were measured using autoanalyzer. Histopathological evaluation revealed that follicular degeneration, congestion, hemorrhage, edema, and inflammatory cell infiltration, as well as the number of atretic follicles and IL-1β immunoreactivity, were observed at the highest levels in the CP group (Group 4). In contrast, the numbers of primordial, primary, secondary, and tertiary follicles, along with AMH and α-SMA immunoreactivity levels, were found to be the lowest in this group. However, positive therapeutic effects were observed in the CP-treated groups (Groups 5 and 6). In the serum, increased levels of AST, ALT, creatinine, glucose, LDL, TOS, Ca, Fe, Mg, IL-1β, IL-1α, TNF-α, and NF-kB were detected in the CP groups (G4, G5, G6) compared to the control groups (G1, G2, and G3). In brain tissue, a decrease of total protein and total cholesterol levels were observed in the CP groups (G4, G5, G6) compared to the control groups, while increases in Na, Cl, Fe, IL-1β, IL-1α, TNF-α, and NF-kB levels were detected. In conclusion, PRP and ACRS therapies from the patient's own blood have a potential as supportive or chemopreventive strategies with reduced side effects and treatment costs.

Keywords ACRS · Brain · Cyclosphosphamide · Cytokines · Oxidative Status · PRP · Ovarian

Extended author information available on the last page of the article

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Introduction

Cyclophosphamide (CP) is an alkylating chemical agent that is commonly used in cancer treatments and as an immunosuppressive drug. However, despite its therapeutic benefits, CP is associated with significant toxicity to various organs, particularly the ovaries, which can lead to ovarian failure. Clinical studies have shown that women treated with CP may experience infertility due to varying degrees of ovarian toxicity (Sharma et al. 2020). Compared to other chemotherapeutic agents, CP has the highest tendency to accelerate ovarian failure. This tendency results from the increased sensitivity of ovarian follicles to the cytotoxic effects of CP, leading to early ovarian failure, primary ovarian insufficiency, and infertility (Almeida et al. 2022). It is stated that CP can cause severe ovarian damage and lead to early ovarian failure due to ovarian toxicity, affecting various mechanisms such as oxidative stress, inflammation, and apoptosis (Elahi et al. 2023). In addition to ovarian toxicity or early ovarian failure in female animal models (Elahi et al. 2023; Lande et al. 2017), CP also has adverse effects on other organs such as the heart, kidneys, brain, and liver (Devi et al. 2016; Cruz-Valencia et al. 2021). The hormonal disruption caused by CP negatively affects the menstrual cycle, ovulation, and reproductive health (Abogresha et al. 2021). Brain tissue is particularly sensitive to oxidative damage due to its low antioxidant capacity, high energy demand, and the presence of multiple unsaturated fatty acids (Behairy et al. 2024). Treatment with chemotherapy agents like CP is highly toxic to brain cells and causes cognitive impairments (Iqubal et al. 2019); however, studies aimed at preventing or treating the effects of CP on the brain are limited (Zarei and Shivanandappa 2016). Brain tissue is particularly sensitive to oxidative damage due to its low antioxidant capacity, high energy demand, and the presence of multiple unsaturated fatty acids (Aboubakr et al. 2021; Behairy et al. 2024). Treatment with chemotherapy agents like CP is highly toxic to brain cells and causes cognitive impairments (Iqubal et al. 2019); however, studies aimed at preventing or treating the effects of CP on the brain are limited (Zarei and Shivanandappa 2016).

The toxic metabolites of CP and the drug itself also interfere with intracellular antioxidant systems that play a key role in the detoxification of reactive oxygen species (ROS) (Tsai-Turton et al. 2007). One of the key mechanisms of CP treatment is the increased production of oxygen-free radicals such as ROS. Oxidative stress occurs when the production of free radicals exceeds the rate at which they are neutralized by antioxidant mechanisms (Luan et al. 2019; Raeeszadeh et al. 2021). Oxidative stress occurs when excess free radicals react with proteins, cell membranes, and DNA, damaging cell structures and leading to degenerative diseases. The brain and nervous system are particularly vulnerable to oxidative stress due to their limited antioxidant capacity (Shulman et al. 2004).

CP also causes inflammation as an immune response to infections, tissue damage, or chemical toxicity (Baharmi et al. 2022) and affects the release of cytokines as immunomodulatory agents (Wang et al. 2007). Antioxidants are substances that delay or prevent cellular damage and inhibit free radical processes (Young and Woodside 2001).

Platelet-Rich Plasma (PRP) is a treatment method performed with autologous platelets purified from whole blood. Platelets secrete growth factors and active metabolites that support the three phases of wound healing and tissue repair (inflammation, proliferation, remodeling) (Schug and Stannard 2011). This treatment is considered a promising approach with anti-inflammatory and tissue-healing properties, believed to improve tissue repair through various mediators stored in α-granules (Vetrano et al. 2013; Giusti et al. 2014).

Cytokines are proteins that play an important role as mediators and regulators of the immune response. Cytokines are produced in response to stimulation by infectious agents and their derived products, inflammatory mediators, and the cytokines themselves (Rahman et al. 2012).

Autologous Cytokine-Rich Serum (ACRS) is obtained from whole blood and is primarily enriched with growth factors and cytokines. This serum, obtained by exposing circulating leukocytes to activating surfaces, stimulates the de novo synthesis of anti-inflammatory cytokines and growth factors, thereby triggering the regeneration process. ACRS has been developed as a practical and relatively inexpensive treatment option by awakening the patient's own healing system (Frizziero et al. 2013; Barreto and Braun 2016; Godek 2016). This study aimed to evaluate the effects of ACRS and PRP on brain oxidative status, mineral levels, and proinflammatory cytokines in rats with CP-induced ovarian failure, as well as their impact on hormone levels, cell types, follicular development, and inflammation in the ovaries.

Materials and methods

Experimental groups

Mice and rats have been widely used as animal models in biomedical research because of their anatomical, physiological, and genetic similarities to humans (Brayda, 2013). Randomization in animal studies is critical to reduce selection bias and balance confounding factors between treatment groups. Forty-two (12-weeks-old) Wistar Albino female rats, weighing an average of 200–230 g, were used

in this study. Female rats born to the same mother were placed in housing cages at 30 days of age and cared for until they were 12 weeks old. Randomization at the animal level raises ethical concerns as it may lead to aggression when a stranger is placed in the same cage. Therefore, the study used the 'Whole Cage Randomization' method to protect welfare.The rats to be included in the experiment were housed in pre-disinfected cages of 4–5 rats each, and the treatments were applied at the same time. They had ad libitum access to food and water. During the study, a room temperature of 22 ± 2 °C, 60% humidity, and a 12/12 h light/dark cycle were maintained.

Six rats were designated as donors, while the remaining 36 were randomly assigned to six experimental groups (n = 6 per group) based on a power analysis.

The experimental groups were designated as follows:

Group 1 (Control group): No administration and treatment was applied to the rats.

Group 2 (PRP control group): Under general anesthesia, the rats' abdomens were opened, and on day 1, a single dose of 0.5 ml PRP was administered intraovarianly to the right ovaries. Subsequently, 0.5 ml of PRP was given intraperitoneally to the rats on days 7 and 14 without any surgical procedure (Ahmadian et al. 2020; Navali et al. 2022).

Group 3 (ACRS control group): Under general anesthesia, the rats' abdomens were opened, and on day 1, a single dose of 0.5 ml ACRS was administered intraovarianly to the right ovaries. Then, 0.5 ml of ACRS was given intraperitoneally to the rats on days 7 and 14 without any surgical procedure, with one week intervals between treatments (Isogai et al. 2008; Masuki et al. 2016).

Group 4 (CP group): Rats in this group were administered 75 mg/kg of CP intraperitoneally on the 1st and 7th days without any surgical procedure (Nair et al. 2020; Yeral et al. 2019).

Group 5 (CP+PRP treatment group): On day 1, 75 mg/kg CP was administered intraperitoneally, and then under general anesthesia, the rats' abdomens were opened, and a single dose of 0.5 ml of PRP was administered intraovarianly to the right ovaries (Yeral et al. 2019; Nair et al. 2020). On day 7, both CP and PRP were administered intraperitoneally. On day 14, only PRP was administered intraperitoneally. The study was terminated on day 31.

Group 6 (CP+ACRS treatment group): On day 1, 75 mg/kg of CP was administered intraperitoneally, and then under general anesthesia, the rats' abdomens were opened, and a single dose of 0.5 ml of ACRS was administered intraovarianly to the right ovaries (Yeral et al. 2019; Nair et al. 2020). On day 7, both 75 mg/kg of CP and ACRS were administered intraperitoneally. On day 14, 0.5 ml of ACRS was administered intraperitoneally.

The study was terminated at the end of the 31st day. Brain tissue and blood samples were collected for biochemical analyses and ovarian tissue samples were collected for histomorphological examinations.

Surgical procedure

The experimental animals were anesthetized under general anesthesia with xylazine (10 mg/kg body weight intraperitoneally) and ketamine (60 mg ketamine hydrochloride/kg body weight intraperitoneally). Under deep anesthesia, the animals' abdominal regions were prepared under aseptic conditions, and a 2 cm of skin incision was made along the linea alba. The subcutaneous connective tissue and abdominal muscles were incised, and the right ovary was dissected. A 0.5 ml dose of PRP/ACRS was administered intraovarianly to the right ovary, and the skin and subcutaneous tissues were then closed. Following this, 0.5 ml of PRP/ACRS was administered intraperitoneally to the rats twice, with one-week intervals, without any surgical procedure (Ahmadian et al. 2020; Zhang et al. 2024).

PRP preparation protocol

Donor rats (n = 3 for PRP preparation) were anesthetized, and blood was collected from their hearts into acid-citrated dextrose tubes (PRP tubes) for the preparation of fresh PRP. The blood was centrifuged at 1000 rpm for 10 min. Following centrifugation, three layers were formed: red blood cell layer at the bottom, buffy coat containing platelets, leukocytes, and a few erythrocytes in the middle, and platelet-poor plasma at the top. A pipette was used to aspirate the plasma fraction up to the interphase region and transfer it into another tube. From 10 ml of blood, 6 ml of PRP was obtained. The resulting PRP was administered intraovarianly under general anesthesia after opening the abdomen of the rats (Ahmadian et al. 2020; Navali et al. 2022).

ACRS preparation protocol

The commercial "RegenX Ellence" Autologous Cytokine-Rich Serum (ACRS) kit tube sets were obtained from Clinic Biotech Biotechnology Industry and Trade Limited Company, located at Kızılırmak Mah. Dumlupınar Blv. A No: 9A-158 Çankaya/Ankara. Donor rats (n = 3 for ACRS preparation) were anesthetized, and intracardiac blood was collected. For the preparation of fresh ACRS, the blood was transferred into specialized kit tubes and incubated for 3 h in a special incubator. After incubation, the blood was centrifuged at 4000 rpm for 5 min. The resulting serum was aspirated and transferred into another tube. From 10 ml of blood, 6 ml of ACRS was obtained, and the resulting ACRS

was administered intraovarianly to the rats under general anesthesia after opening their abdomens (Isogai et al. 2008; Masuki et al. 2016).

At the end of 31 days, after the rats were weighed, 10% ketasol (0.8–1.3 ml/kg) and 2% basilazine (2–5 mg/kg) were administered via intraperitoneally. The animals were decapitated after blood was drawn from their hearts. Following the decapitation, ovary and brain tissue samples were taken and the tissues were kept at −20 °C until analyzes (Ermiş and Çiftci 2024). The blood samples were kept in the laboratory for 20 min and coagulated. Then, they were centrifuged at +4 °C for 10 min at 1550 × g, and their serums were removed and divided into aliquots. Serums were stored at −20 °C until analyzes.

Histopathological examination

Ovarian tissues collected after necropsy were fixed in a 10% neutral formaldehyde solution. The tissues were then passed through graded alcohols (70, 80, 90, 100%) and xylene solutions to prepare paraffin blocks. Sections taken from the paraffin blocks were placed on slides and stained with Hematoxylin–Eosin (HE) for examination under a light microscope (Tuzcu et al. 2022). Histopathologically, follicular degeneration, congestion, hemorrhage, edema, and inflammatory cell infiltration were evaluated. The assessment was performed semi-quantitatively by a blinded pathologist based on the percentage of affected cells in each Sect. (0; none, 1–33%; mild (+), 33–66%; moderate (++), >66%; severe (+++)). Additionally, according to a previously published study, primordial, primary, secondary, tertiary, and atretic follicles in each tissue were counted and evaluated (Akcakavak et al. 2024).

Immunohistochemical examination

Sections of 4–5 µm thickness were taken from the paraffin blocks onto adhesive slides. The sections underwent paraffin extraction and rehydration processes. Immunohistochemical staining was performed using a commercial kit following the manufacturer's instructions, as reported in a previous study (Akcakavak et al. 2024). Primary antibodies used were anti-Mullerian hormone (AMH, sc-166752, Santa Cruz Biotechnology, USA, 1/300 dilution), α-SMA (Affinity biosciences, AF1032, 1/200 dilution), and IL-1β (Affinity biosciences, AF5103, 1/200 dilution). The chromogen used was 3,3'-diaminobenzidine (DAB), and counterstaining was performed with Mayer's hematoxylin. Immunohistochemical evaluation was conducted semi-quantitatively by a blinded pathologist at ×20 magnification (0; none, 1; mild, 2; moderate, 3; severe, 4; very severe) (Karaman et al. 2024; Kazak et al. 2024).

Determination of some biochemical parameters in sera

Glucose (Biosistem, lot: 11803), creatinine (Biosistem, lot: 11802), total cholesterol (Biosistem, lot: 11805), HDL (Biosistem, lot: 11557), LDL (Biosistem, lot: 11585), triglycerides (Biosistem, lot: 11,828), total protein (Biosistem, lot: 11800), albumin (Biosistem, lot: 11573), ALT (Biosistem, lot: 11832), AST (Biosistem, lot: 11830), and uric acid (Biosistem, lot: 11821) levels in serum were measured by the spectrophotometric method using specific kits on an autoanalyzer device (Biosistem A25, Spain).

Preparation of whole brain tissue homogenate

Brain tissue homogenization was prepared as specified in the ELISA test kit procedure. For this, the tissue was washed with ice-cold PBS (0.01 mol/L, pH 7.0–7.2) and then weighed. After the tissue was cut into small pieces, homogenization was performed with 5–10 ml of PBS using a homogenizer on ice. Then, the cell membrane was disrupted with an ultrasonicator once or twice in ice, and the freeze-thawing process was applied twice to further disrupt the cell. The homogenate was then centrifuged +4 °C for 5 min at 3000 × g. The supernatant was separated into aliquots and kept at −20 ˚C until analyzes (Ermiş and Çiftci 2024). The brain tissue supernatants of the rats were used to determine the levels of minerals, IL-1α, IL-1β, TNF-α, and NF-κB.

Determination of the levels of certain minerals in serum and brain tissue

The levels of P, Na, K, Ca, Mg, Cl, and Fe in serum and brain tissue supernatants were measured by the spectrophotometric method using an autoanalyzer device (A25 Random Access Analyzer, Biosystems, Spain). In this device, briefly, the sample and reagents were taken and mixed in appropriate amounts by the device, followed by optical reading at a specific time and temperature, and the relevant analyzes results were calculated and retrieved from the device [Na (Biosistem, lot: 5201), Mg (Biosistem, lot: 11797), K (Biosistem, lot: 5202), Ca (Biosistem, lot: 11570), P (Biosistem, lot: 11508), Cl (Biosistem, lot: 5207), and Fe (Biosistem, lot: 11509)].

Determination of IL-1α, IL-1β, TNF-α, and NF-κB levels in serum and brain tissue

The levels of IL-1α (Catalogue number: 201-11-0119), IL-1β (Catalogue number: 201-11-0120), TNF-α (Catalogue number: 201-11-0765), and NF-κB (Catalogue number:

201-11-5141) in serum and brain tissue supernatants were determined using rat-specific ELISA kits (Sunred Biological Technology; Shanghai, China). The procedure of the kit was followed, and OD_{450} values were calculated using Magellan Standard Tracker (V7–2) software, taking into account the OD_{450} values of the standards.

Determination of total antioxidant capacity (TAS) and total oxidant capacity (TOS) amounts in serum and brain tissue

Brain tissue supernatants and serum were tested for TAS and TOS using the TAS and TOS kits (Rel Assay Diagnostics, Gaziantep, Turkey). Spectrophotometric techniques were used to measure the change in the amount of absorption (Erel 2004, 2005). TAS method depends on the sample's antioxidants' capacity to prevent a peroxidase metmyoglobin from oxidizing ABTS (2,2′-azino-di-3-ethylbenz-thiazoline sulfonate) into $ABTS^{+}$. The TAS level was expressed as mmol Trolox equivalent/L (mmol Trolox equiv/L). TOS method relied on the oxidation of ferrous ions into ferric ions in the presence of various oxidative species in an acidic medium. Xylenol orange was used to test the amounts of ferric ions. The results showed that μmol H_2O_2 equivalent/L (μmol H_2O_2 equivalent/L). The oxidative stress index (OSI) was used to define the TOS/TAS ratio.

Statistical analyzes

SPSS statistical software for Windows (SPSS-PC, SPSS Inc., Chicago, Illinois, USA) was used for statistical analyzes. The data were analyzed using one-way analysis of variance (ANOVA) to evaluate differences between groups, with significant differences identified by Duncan's multiple range test, and Pearson correlation test was applied to assess relationships between variables. he data obtained in the study are presented as mean ± standard error.

Results

Histopathological and immunohistochemical findings

The control groups (Group 1, Group 2, Group 3) exhibited normal histological appearance and normal follicular morphology (Fig. 1A–C). In the experimental groups (Group 4, Group 5 and Group 6), follicular degeneration, congestion, hemorrhage, edema, and inflammatory cell infiltrations were observed (Fig. 1D–F). Histopathological evaluation showed that the levels were the most severe in the CP group (Group 4), and that there was a positive healing effect in the CP treated groups (Groups 5 and 6), with Group-6 (CP+ACSR) being more effective (Table 1). The statistical scores for follicular counts across groups are provided

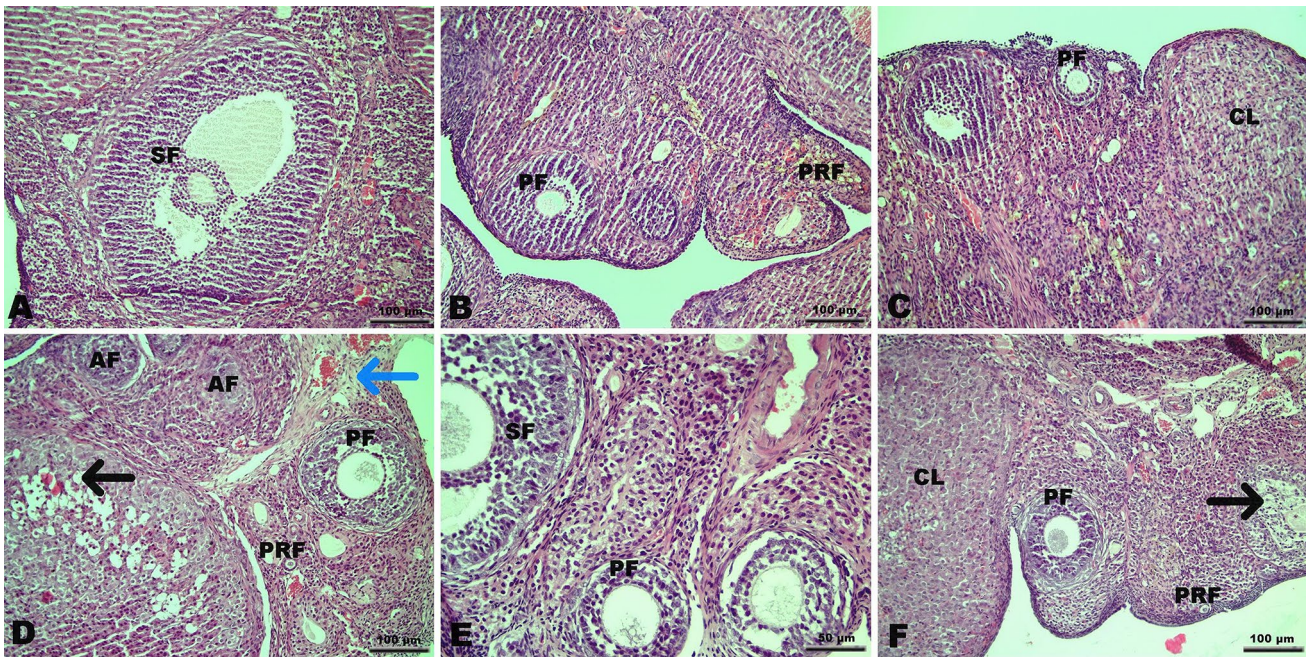


Fig. 1 Histopathological examination of experimental groups. Hematoxylin–Eosin: H-E; Control group ovaries showed normal histology. Group 4–6, showed a decrease in the number of follicles and histopathological lesions. **A** control (Group 1); **B** PRP control (Group 2); **C** ACSR control (Group 3); **D** CP (Group 4); **E** CP+PRP (together protective, Group 5); **F** CP+ACSR (Group 6); PRF: primordial follicle; PF: primary follicle; SF: secondary follicle; AF: atresic follicle; CL: corpus luteum; black arrow: degeneration; blue arrow: edema

Table 1 Histopathological evaluation among groups (Mean ± SE)

Histopathological lesion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Follicular degeneration	0.17 ± 0.17^{c}	0.33 ± 0.21^{c}	0.33 ± 0.21^{c}	2.67 ± 0.21^{a}	2.33 ± 0.21^{a}	2.00 ± 0.21^{b}
Congestion	0.50 ± 0.22^{c}	0.50 ± 0.22^{c}	0.33 ± 0.21^{c}	2.67 ± 0.21^{a}	2.17 ± 0.30^{ab}	1.17 ± 0.17^{b}
Hemorrhage	0.00 ± 0.00^{c}	0.17 ± 0.17^{c}	0.17 ± 0.17^{c}	2.83 ± 0.17^{a}	2.17 ± 0.30^{ab}	1.50 ± 0.22^{b}
Edema	0.00 ± 0.00^{d}	0.17 ± 0.17^{d}	0.17 ± 0.17^{d}	2.83 ± 0.21^{a}	2.00 ± 0.26^{b}	1.17 ± 0.17^{c}
Inflammatory cell infiltration	0.17 ± 0.17^{d}	0.67 ± 0.21^{d}	0.50 ± 0.22^{d}	2.50 ± 0.17^{a}	2.00 ± 0.26^{ab}	1.33 ± 0.21^{c}

$^{a-d}$ Letters in the same row indicate statistical significance between groups ($p < 0.001$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP + PRP; Group 6: CP + ACSR)

Table 2 Evaluation of follicle counts among groups (Mean ± SE)

Follicle	Group -1	Group -2	Group -3	Group -4	Group -5	Group -6
Primordial Follicle	42.67 ± 0.42^{a}	41.00 ± 0.51^{b}	40.17 ± 0.40^{b}	21.33 ± 0.67^{e}	24.67 ± 0.33^{d}	28.67 ± 0.42^{c}
Primary Follicle	22.17 ± 0.60^{a}	21.83 ± 0.30^{a}	21.83 ± 0.60^{a}	10.17 ± 0.60^{d}	13.50 ± 0.34^{c}	15.00 ± 0.26^{b}
Secondary Follicle	18.67 ± 0.42^{a}	18.17 ± 0.60^{a}	17.17 ± 0.47^{ab}	7.00 ± 0.30^{d}	9.00 ± 0.25^{e}	11.33 ± 0.33^{c}
Tertiary Follicle	16.67 ± 0.55^{a}	16.17 ± 0.80^{a}	15.17 ± 0.60^{a}	5.17 ± 0.48^{d}	7.83 ± 0.40^{c}	10.00 ± 0.58^{b}
Atresic Follicle	6.67 ± 0.42^{b}	7.50 ± 0.67^{b}	8.50 ± 0.76^{b}	25.33 ± 0.83^{a}	22.50 ± 0.50^{c}	20.33 ± 0.61^{d}

$^{a-e}$ Letters in the same row indicate statistical significance between groups ($p < 0.001$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP + PRP; Group 6: CP + ACSR)

Table 3 Immunohistochemical expressions of AMH, α-SMA and IL-1β among groups (Mean ± SE)

Primers	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6
AMH	2.50 ± 0.22^{ab}	2.67 ± 0.21^{a}	2.67 ± 0.21^{a}	1.33 ± 0.21^{c}	1.67 ± 0.21^{c}	2.00 ± 0.25^{b}
α-SMA	2.50 ± 0.22^{a}	2.33 ± 0.21^{a}	2.33 ± 0.21^{a}	1.33 ± 0.17^{c}	1.83 ± 0.17^{bc}	2.00 ± 0.21^{b}
IL-1β	0.33 ± 0.21^{c}	0.17 ± 0.17^{c}	0.33 ± 0.21^{c}	2.67 ± 0.21^{a}	1.67 ± 0.21^{b}	1.17 ± 0.17^{b}

$^{a-c}$ Letters in the same row indicate statistical significance between groups ($p < 0.001$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP + PRP; Group 6: CP + ACSR)

in Table 2. In Group 4, compared to the control groups, there was a significant decrease in the number of primordial, primary, secondary, and tertiary follicles, while a significant increase in atretic follicles was observed. Group 5, and Group 6 showed positive effects on follicle count compared to Group 4, with Group 6 showing the most prominent effectiveness (Table 2).

Immunohistochemical scores are presented in Table 3. In the AMH immunohistochemical staining, the control groups (Group 1, Group 2, Group 3) showed moderate to strong immunoreactivity and had the highest statistical scores. Group 5, and Group 6 showed increased statistical scores compared to Group 4, with the highest statistical scores observed in Group 6 (Fig. 2). In α-SMA immunohistochemical staining, the control groups (Group 1, Group 2, Group 3) exhibited moderate to strong immunoreactivity. In Group 4, immunoreactivity was reduced compared to the control groups. Group 5, and Group 6 increased α-SMA immunoreactivity, and the highest statistical scores were found in Group 6 (Fig. 3). In IL-1β immunohistochemical staining, the control groups (Group 1, Group 2, Group 3) showed mild or no immunoreactivity. Group 4 showed an increase in immunoreactivity compared to the control groups. Group 5, and Group 6 reduced IL-1β immunoreactivity, with the lowest scores found in Group 6 (Fig. 4).

Levels of some routine biochemical parameters in serum

Blood samples collected at the conclusion of the experiment (31 days) were used for all measurements. The levels of Ure, Cre, UA, Glu, AST, ALT, and insulin in serum are presented in Table 4.

AST enzyme levels were found to be higher in the CP-treated groups (Groups 4, 5, and 6) compared to the control groups (Groups 1, 2, and 3) ($P < 0.05$). However, no significant difference was observed within the control groups (Groups 1, 2, and 3) or within the CP-treated groups (Groups 4, 5, and 6) ($P > 0.05$). ALT enzyme activity levels were found to be higher in the CP-treated groups (Groups 4, 5, and 6) compared to the control groups. It was observed that in the control groups, Group 1 had the lowest ALT levels, while Groups 2 and 3 had significantly higher levels ($P < 0.05$). In the CP-treated groups, Group 2 had the highest ALT levels, while levels in Group 5 decreased ($P < 0.05$), and in Group 6, they did not significantly change ($P > 0.05$).

The urea level was highest in Group 1, and this difference was significant compared to Groups 2, 4, 5, and 6 ($P < 0.05$). In the CP-treated groups, Group 4 had the lowest urea level, while the levels in the treated groups (Groups 5 and 6) began to increase, although the difference was not significant ($P > 0.05$). The creatinine levels were significantly

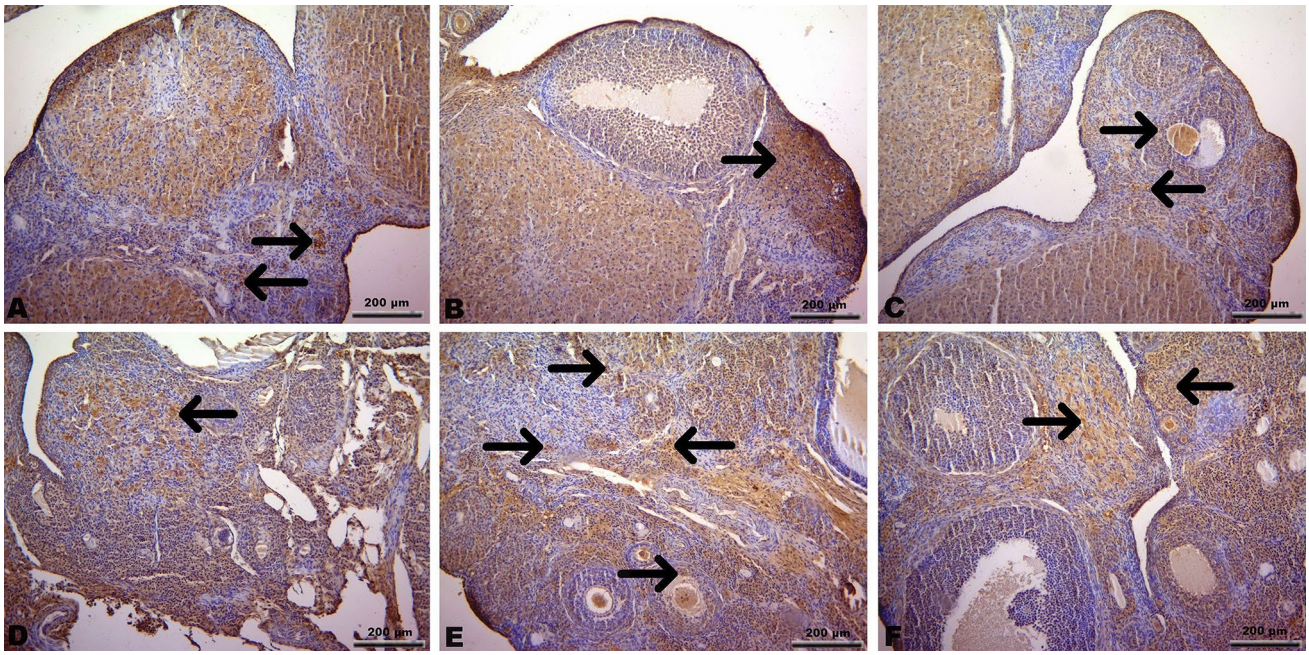


Fig. 2 The appearance of AMH immunoreactivity among groups, IHC staining (DAB was used as chromogen). **A** severe AMH immunoreactivity appearance in group control (Group 1); **B** appearance of severe AMH immunoreactivity in group PRP control (Group 2); **C** appearance of moderate AMH immunoreactivity in group ACRS control (Group 3); **D** appearance of mild AMH immunoreactivity in group CP (Group 4); **E**–**F** appearance of moderate AMH immunoreactivity in groups CP+PRP (Group 5) and CP+ACRS (Group 6), respectively

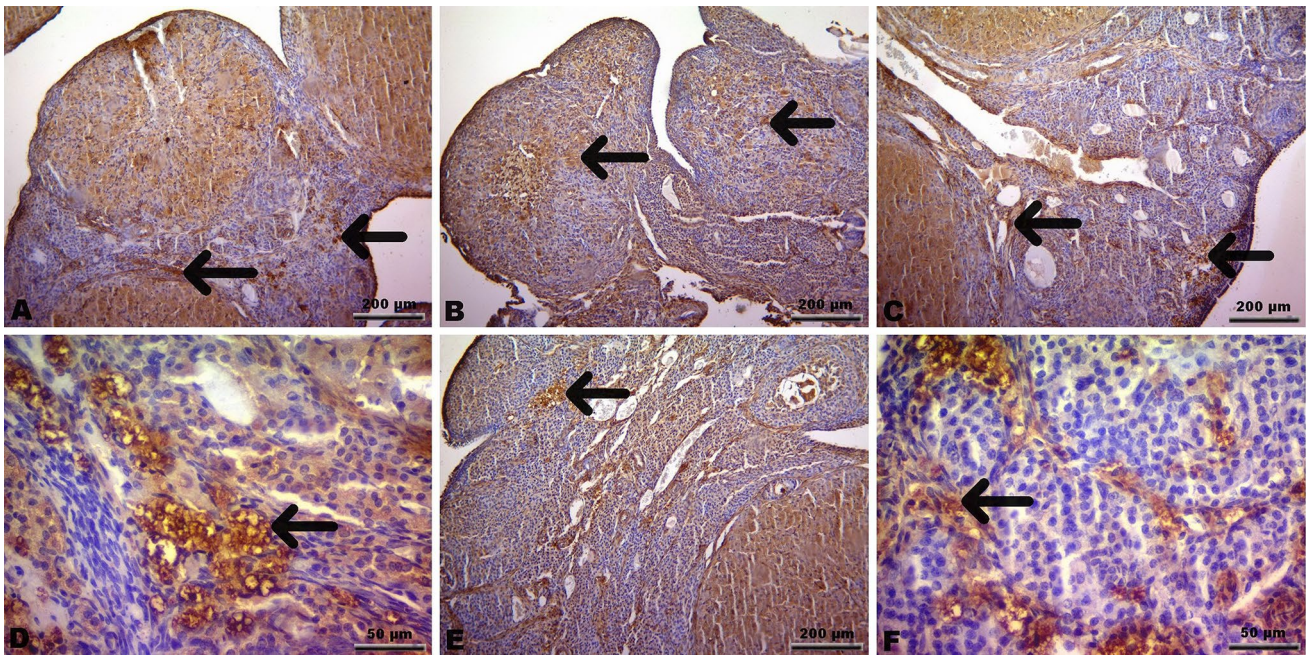


Fig. 3 The appearance of α-SMA immunoreactivity between groups, IHC staining (DAB was used as chromogen). **A** appearance of moderate α-SMA immunoreactivity in control (Group 1); **B** appearance of severe α-SMA immunoreactivity in group PRP control (Group 2); **C** appearance of moderate α-SMA immunoreactivity in group ACRS control (Group 3); **D** appearance of mild α-SMA immunoreactivity in group CP (Group 4); **E**–**F** appearance of mild α-SMA immunoreactivity in groups CP+PRP (Group 5) and CP+ACRS (Group 6), respectively

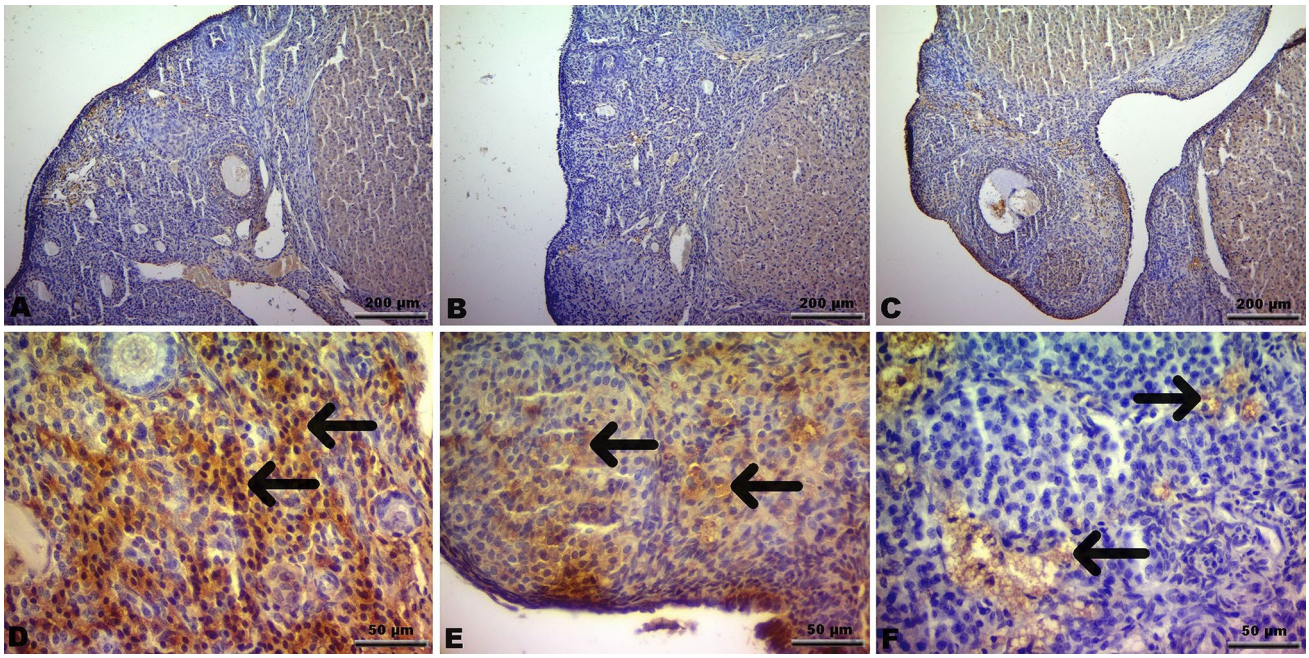


Fig. 4 The appearance of IL-1β immunoreactivity between groups, IHC staining (DAB was used as chromogen). **A**–**B**–**C** Appearance of mild IL-1β immunoreactivity in groups control (Group 1); PRP control (Group 2) and ACRS control (Group 3), respectively; **D** severe IL-1β immunoreactivity appearance in group CP (Group 4); **E** moderately severe immunoreactivity view in group CP+PRP (Group 5); **F** mild severe immunoreactivity appearance in group CP+ACRS (Group 6)

Table 4 Urea, creatinine (Cre), uric acid (UA), glucose (Glu), AST, ALT and insulin levels in serum

	Urea (mg/dL)	Cre (mg/dL)	UA (mg/dL)	Glu (mg/dL)	Insulin (IU/L)	AST (U/L)	ALT (U/L)
Group 1	43,93±1,6 [a]	0,29±0,04 [ab]	1,1±0,09	151,75±6,4 [a]	2,54±0,06	79,33±6,5 [a]	34,8±4 [a]
Group 2	36,71±1,7 [b]	0,25±0,03 [ac]	1,15±0,1	175,03±0,3 [ab]	2,39±0,1	80,83±6,4 [a]	49,33±3,7 [b]
Group 3	39,21±2,1 [ab]	0,23±0,01 [c]	1,25±0,2	176,75±11,2 [b]	2,34±0,1	90±9,7 [a]	50,83±1,1 [b]
Group 4	34,58±0,9 [b]	0,33±0,02 [d]	1,23±0,1	267,35±13,2 [ab]	2,38±0,1	135,16±8,5 [b]	70,83±8,4 [c]
Group 5	36,45±1,8 [b]	0,32±0,02 [bd]	0,83±0,05	345,45±15,06 [c]	2,49±0,1	142±8,6 [b]	54,33±2 [b]
Group 6	37,17±1,9 [b]	0,34±0,03 [d]	1,07±0,1	343,82±8,1 [c]	2,59±0,1	155±14,3 [b]	59±5,3 [bc]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

Table 5 Total protein (TP), albumin (Alb), Glo/Alb and globulin (Glo) levels of the groups

	TP (g/L)	Alb (g/L)	Glo (g/L)	Glo/Alb
Group 1	63,83±1,5 [a]	34,11±0,2 [ab]	29,71±1,4 [a]	0,86±0,04 [a]
Group 2	62±0,7 [a]	35,5±0,4 [b]	26,5±0,7 [bc]	0,74±0,02 [b]
Group 3	57,83±1,2 [b]	32,73±0,9 [a]	25,1±0,3 [c]	0,76±0,01 [b]
Group 4	54,66±1,08 [bc]	25,93±0,5 [c]	28,73±0,9 [ab]	0,79±0,02 [ab]
Group 5	52±1,4 [c]	30,36±0,7 [d]	21,63±0,7 [d]	0,7±0,01 [b]
Group 6	55,75±1,8 [bc]	29,95±1,6 [d]	25,8±0,4 [c]	0,86±0,04 [a]

[a,b,c,d] Differences between groups indicated with different letters in the are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

higher in the CP-treated groups compared to the control groups ($P<0.05$). However, no significant difference was observed between the CP-treated groups ($P>0.05$).

It was determined that glucose levels were higher in the CP-treated groups compared to the control groups. Additionally, after CP administration, higher glucose levels were observed in the treated Group 5 and Group 6 compared to Group 4 ($P<0.05$). No significant difference was observed in insulin levels between the groups ($P>0.05$).

Total protein and albumin levels were found to be lower in CP-administered groups than in control groups. It was also observed that the globulin level in the control groups was higher in Group 1 than in Groups 2 and 3 ($P<0.05$). In the CP-treated groups, Group 4 had the highest globulin level, followed by Groups 6 and 5 ($P<0.05$) (Table 5). Total cholesterol level was highest in Group 1 of the control groups, followed by Groups 2 and 3. Among the

Table 6 Total cholesterol (TC), triglyceride (TG), HDL, and LDL levels in serum of the groups

	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Group 1	82,1±2,7 [a]	99±7,3	48,5±2,1	10,7±0,6 [a]
Group 2	77,85±2,7 [ab]	122,11±21,2	47,16±1,4	10,43±0,4 [a]
Group 3	72,23±1,7 [b]	113,53±9,1	44,16±0,4	9,68±0,2 [a]
Group 4	90,28±1,4 [c]	124,86±15,2	41,83±4,6	16,8±0,9 [b]
Group 5	71,92±4,2 [b]	119,85±10,1	47,5±2,5	10,26±0,9 [a]
Group 6	80,1±3,2 [ab]	89,12±9,1	51,75±1,7	13,92±1,1 [c]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

CP-treated groups, the highest total cholesterol level was observed in Group 4, while Group 5 showed the lowest level ($P<0.05$). HDL levels were lowest in Group 4 ($P>0.05$), whereas LDL levels were significantly elevated in Group 4 ($P<0.05$) (Table 6). The serum Na levels were slightly higher in Group 1, Group 2, and Group 3 compared to the CP-treated groups, but this difference was not statistically significant ($P>0.05$). The K levels were lowest in Group 4 ($P<0.05$), and no significant differences were observed among the other groups (Group 1, 2, 3, 5, and 6) ($P>0.05$). The Cl levels were lowest in Group 4 and highest in Group 5 ($P<0.05$). The *P* levels were lowest in Group 4 and highest in Group 6 ($P<0.05$). The Mg levels were highest in Group 4, and there were significant differences between Group 4 and Groups 3, 5, and 6 ($P<0.05$). It was determined that the Fe level in serum was lowest in Group 1 of the control groups, with Group 2 showing an increase ($P>0.05$) and Group 3 showing a significant increase ($P<0.05$). In the CP-treated groups (Groups 4, 5, and 6), Group 4 had the highest level, and Group 6 had the lowest, but this difference was not significant ($P>0.05$) (Table 7).

A significant positive correlation was found between serum Na levels and both Cl and Ca levels (r=0.442**, r=0.427*). K levels also showed a positive correlation with Cl and *P* levels (r=0.345*, r=0.633**, respectively). Additionally, Cl levels positively correlated with phosphorus (r=0.411**) but showed a negative correlation with an unspecified parameter (r=–0.366*) (Table 8).

The levels of IL-1β, IL-1α, TNF-α, and NF-kB were highest in Group 4. In comparison, Groups 5 and 6 exhibited lower levels, with Group 6 showing the most pronounced reduction (Table 9). TNF-α levels showed a positive correlation with IL-1β, IL-1α, NF-κB, and creatinine (r=0.430**, r=0.348*, r=0.410*, r=0.504**), In contrast, a negative correlation was observed between K and both urea and creatinine levels (r=−0.486**, r=−0.373*). AST and ALT levels were positively correlated (r=0.628). Further correlations revealed a positive relationship between Cl and both Na and K (r=0.442**, r=0.345*), as well as between K and *P* (r=0.633**). Additionally, TG levels were positively correlated with NF-κB (r=0.366*), and LDL levels were positively correlated with IL-1β, TNF-α, creatinine, and total cholesterol (r=0.398*, r=0.478**, r=0.596**, and r=0.795**, respectively) (Tables 10 and 11).

Table 7 Mineral levels in the serum of the groups (Na, K, Cl, P, Ca, Fe, and Mg)

	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	P (mg/dL)	Ca (mg/dL)	Mg (mg/dL)	Fe (µg/dL)
Group 1	139,16±0,4	5,56±0,2 [a]	102±0,5 [ab]	5,95±0,3 [ab]	10,35±0,1 [ab]	2,33±0,07 [ab]	118,03±5,3 [a]
Group 2	140,5±0,4	5,46±0,2 [a]	103,16±0,4 [bc]	7,1±0,4 [c]	10,4±0,08 [b]	2,33±0,03 [ab]	137,53±4,3 [ab]
Group 3	139,16±0,6	5,39±0,1 [a]	102,5±0,3 [abc]	7,01±0,4 [c]	10,5±0,1 [b]	2,11±0,06 [c]	147,21±7,9 [b]
Group 4	138±1,7	4,47±0,1 [b]	101,33±0,6 [a]	5,58±0,2 [a]	10,63±0,2 [b]	2,46±0,05 [b]	143,43±10,6 [b]
Group 5	138,66±0,4	5,34±0,2 [a]	104±0,2 [c]	6,68±0,2 [bc]	9,55±0,2 [c]	2,21±0,06 [ac]	142,58±6 [b]
Group 6	137,75±0,2	5,33±0,1 [a]	102,25±0,7 [ab]	7,52±0,2 [c]	9,72±0,3 [ac]	2,17±0,02 [ac]	134,85±7,7 [ab]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

Table 8 Correlation of mineral levels in the serum of the groups (Na, K, Cl, P, Ca, Fe, and Mg)

	Na	K	Cl	P	Ca	Mg	Fe
Na	1	0,8	0,442**	0,123	0,427*	−0,003	0,181
K		1	0,345*	0,633**	−0,114	−0,126	−0,122
Cl			1	0,411*	−0,366*	−0,178	0,262
P				1	−0,059	−0,173	0,17
Ca					1	0,393*	0,077
Mg						1	−0,103
Fe							1

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level

Table 9 IL-1β, IL-1α, TNF-α, and NF-kB levels in the serum of the groups

	IL-1β (pg/L)	IL-1α (ng/L)	TNF-α (ng/L)	NF-Kb (ng/ml)
Group 1	1680,06 ± 42,24 [a]	651,75 ± 30,59 [ab]	174,06 ± 2,6 [a]	2,19 ± 0,1 [ab]
Group 2	1678,43 ± 59,42 [a]	687,6 ± 23,91 [ab]	185,83 ± 3,05 [a]	2,29 ± 0,1 [ab]
Group 3	1678,45 ± 75,9 [a]	651,36 ± 32,51 [ab]	177,27 ± 2,7 [a]	2,11 ± 0,1 [ab]
Group 4	1921,25 ± 90,36 [b]	724,97 ± 30,91 [a]	215,44 ± 6,8 [b]	2,57 ± 0,1 [a]
Group 5	1692,68 ± 28,06 [a]	676,03 ± 21,01 [ab]	200,46 ± 0,4 [c]	2,4 ± 0,7 [ab]
Group 6	1667,2 ± 41,99 [a]	612,91 ± 32,79 [b]	179,77 ± 4,5 [a]	2,02 ± 0,7 [b]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP + PRP; Group 6: CP + ACSR)

In brain tissue, it was observed that Na levels were higher in the CP-treated groups compared to the control groups. K levels, however, were significantly higher in Group 2 and Group 3 compared to the other groups in brain tissue ($P<0.05$). Cl levels were higher in the CP-treated groups compared to the control groups in brain tissue ($P<0.05$). Among the control groups, Group 1 had the highest and Group 3 had the lowest Cl levels ($P<0.05$), with no significant difference observed between the CP-treated groups ($P>0.05$). Ca levels were found to be decreased in the CP-treated groups, but this change was not significant ($P>0.05$). Mg levels were highest in Group 3 and Group 2 ($P<0.05$), with no significant difference observed between the other groups ($P>0.05$).

In brain tissue, it was determined that the lowest Fe level was in Group 1, while the highest was in Group 4 ($P<0.05$). No significant difference was observed among the control groups regarding Fe levels ($P>0.05$). However, in the CP-treated groups, Group 4 had the highest Fe level, while levels decreased in Group 5 and 6 ($P<0.05$) (Table 12).

In brain tissue, it was observed that the levels of urea, TC, and TP were higher in the control groups compared to the CP-treated groups. It was also determined that the TC levels were significantly higher in the control groups compared to the CP-treated groups ($P<0.05$) (Table 13).

It was observed that the levels of IL-1β, IL-1α, TNF-α, and NF-kB in the brain tissue were lower in the control groups compared to the CP-treated groups. Among the CP-treated groups, Group 4 had the highest levels of IL-1β, IL-1α, TNF-α, and NF-kB. In the CP-treated and treated groups (Group 5 and Group 6), a decrease in IL-1β, IL-1α, TNF-α, and NF-kB levels was observed. This decrease was found to be significant for IL-1β, IL-1α, and NF-kB parameters ($P<0.05$) (Table 14).

Table 10 Correlation of IL-1β, IL-1α, TNF-α, NF-κb, Na, K, Cl, Ure, Cre, TC, AST, ALT, TAS, TOS and TG levels in the serum of the groups

	IL-1β	IL-1α	TNF-α	NF-κB	Na	K	Cl	Ure	Cre	TC	TG	AST	ALT	TAS	TOS
IL-1β	1	0,5	0,4**	0,3	−0,2	−0,4*	−0,1	−0,3*	0,1	0,2	0,07	0,2	0,3	−0,3	0,3*
IL-1α		1	0,3*	0,2	0,2	−0,4**	−0,03	−0,03	0,002	0,2	0,3	0,1	0,1	−0,1	0,06
TNF-α			1	0,4*	−0,2	−0,4**	−0,2	−0,3*	0,5**	0,3	0,3	0,6**	0,6**	−0,6**	0,5**
NF-κb				1	0,0	−0,2	−0,1	−0,1	0,2	0,2	0,3*	0,2	0,2	−0,1	0,3*
Na					1	0,08	0,4**	0,02	−0,1	−0,05	0,03	−0,3	−0,4**	0,2	−0,06
K						1	0,3*	0,4*	−0,1	−0,4*	−0,1	−0,4*	−0,5**	0,4**	−0,4**
Cl							1	−0,1	−0,002	−0,4**	−0,2	−0,2	−0,4**	0,06	−0,2
Ure								1	−0,1	0,07	0,08	−0,2	−0,2	0,3*	−0,3*
Cre									1	0,4*	−0,1	0,6**	0,3	−0,5**	0,6**
TC										1	0,1	0,2	0,3	−0,1	0,5**
TG											1	0,1	0,2	−0,2	0,1
AST												1	0,6**	−0,7**	0,5**
ALT													1	0,5**	0,4**
TAS														1	−0,5**
TOS															1

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level

Table 11 Correlation between the parameters in the serum of the groups

	P	LDL	Ca	Alb	UA	HDL	TP	Glu	Mg	Glo	Glo/Alb
IL-1β	−0,221	0,398*	0,08	0,234	0,081	0,001	0,17	−0,021	0,171	0,148	0,078
IL-1α	−0,346*	0,204	0,333	0,435*	0,334	−0,003	0,413*	0,087	0,092	−0,115	0,3
TNF-α	−0,231	0,478**	−0,011	0,208	0,468**	0,175	0,018	0,523**	0,398*	−0,021	−0,147
NF- κb	−0,275	0,117	0,198	0,239	0,268	0,103	0,081	0,074	0,266	−0,154	−0,071
Na	0,123	−0,215	0,427*	0,263	0,02	0,261	0,3	−0,267	−0,003	−0,6**	0,263
K	0,633**	−0,455**	−0,114	−0,179	−0,283	0,057	−0,24	−0,253	−0,126	−0,096	−0,237
Cl	0,411	−0,301	−,366*	−0,388	−0,147	−0,151	−0,445**	0,139	−0,178	−0,342*	−0,392*
Ure	−0,003	−0,236	0,179	0,181	−0,147	0,12	0,172	−0,327	0,117	0,111	0,125
Cre	0,054	0,596**	−0,071	−0,059	0,292	0,378*	−0,011	0,637**	0,343*	−0,252	0,032
TC	−0,313	0,795**	0,454**	0,541**	0,357*	0,513**	0,604**	0,03	0,699**	0,099	0,617**
TG	−0,128	−0,139	0,303	0,340*	0,13	0,105	0,155	0,039	0,046	−0,254	−0,037
P	1	−0,202	−0,059	−0,159	−0,19	0,15	−0,317	0,071	−0,173	−0,231	−0,378*
LDL		1	0,195	0,266	0,492**	0,334	0,34*	0,307	0,553**	0,159	0,325
Ca			1	0,819**	0,394*	0,262	0,783**	−0,432*	0,393*	−0,065	0,573**
Alb				1	0,395*	0,135	0,852**	−0,462**	0,604**	0,147	0,532**
UA					1	0,005	0,379*	0,17	0,523**	−0,033	0,279
HDL						1	0,184	0,184	0,305	−0,425*	0,187
TP							1	−0,51**	517**	0,095	0,897*
Glu								1	−0,07	−0,273	−0,433*
Mg									1	0,099	0,325
Glo										1	0,03
Glo/Alb											1

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level

Table 12 Mineral levels in the entire brain tissue of the groups (Na, K, Cl, P, Ca, Fe, and Mg)

	Na	K	Cl	P	Ca	Mg	Fe
	(mmol/g)	(mmol/g)	(mmol/g)	(mg/g)	(mg/g)	(mg/g)	(µg/g)
Group 1	140,33±1,1 [ab]	12,37±0,8 [a]	123,5±0,8 [a]	35,28±0,6	0,68±0,1 [a]	1,1±0,1 [a]	15,5±2,6 [a]
Group 2	137,66±0,7 [a]	14,64±0,5 [b]	122,66±0,5 [ab]	37±0,7	0,58±0,03 [ab]	1,38±0,08 [bc]	22,05±2,7 [a]
Group 3	133,5±2,4 [c]	14,72±0,6 [b]	120±1,7 [b]	36,61±0,7	0,6±0,03 [ab]	1,48±0,09 [c]	22,48±3,5 [a]
Group 4	143,66±0,4 [cd]	12,34±0,2 [a]	127,5±0,2 [c]	35,86±0,3	0,6±0,06 [ab]	1,15±0,03 [a]	32,4±3,9 [b]
Group 5	144,16±0,5 [d]	12,15±0,2 [a]	128,33±0,2 [c]	36,11±0,3	0,55±0,3 [ab]	1,11±0,04 [a]	20,03±0,09 [a]
Group 6	143,5±0,5 [cd]	12,25±0,3 [a]	128,2±0,3 [c]	35,88±0,3	0,48±0,02 [b]	1,16±0,04 [ab]	19,95±2,2 [a]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

Table 13 The levels of total cholesterol (TC), triglycerides (TG), urea, uric acid (UA), and total protein (TP) in the brain tissue of the groups

	Ure (mg/g)	TC (mg/g)	TG (mg/g)	UA (mg/g)	TP (g/g)
Group 1	6±0,5 [ab]	4±0,6 [a]	31,21±2,7	0,1±0,01 [a]	2,66±0,03 [abc]
Group 2	7,4±0,6 [b]	5,95±1,2 [a]	41,18±3,4	0,15±0,03 [a]	3,16±0,3 [bc]
Group 3	7,5±0,6 [b]	5±0,6 [a]	39,65±4,9	0,16±0,04 [a]	3,5±0,4 [c]
Group 4	6±0,2 [ab]	1,53±0,2 [b]	35,21±1,5	0,13±0,02 [a]	2,16±0,1 [a]
Group 5	5,45±0,3 [a]	1,31±0,2 [b]	31,93±1,5	0,15±0,02 [a]	2,33±0,2 [ab]
Group 6	5,86±0,4 [a]	1,91±0,09 [b]	32,71±2,4	0,23±0,02 [b]	2,3±0,1 [ab]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$) (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

Table 14 The levels of IL-1β, IL-1α, TNF-α, and NF-kB in the brain tissue of the groups

	IL-1β (pg/g)	IL-1α (ng/g)	TNF- α (ng/g)	NF-κB (ng/g)
Group 1	1748,67±32,9 [a]	614,84±14,8 [a]	229,13±13,2 [a]	2,36±0,03 [ab]
Group 2	1770,68±64,8 [a]	653,5±2,52 [ab]	253,52±0,6 [a]	2,33±0,03 [a]
Group 3	1816,19±10,4 [ab]	674,84±10 [b]	251,19±1,6 [a]	2,39±0,06 [ab]
Group 4	2108,2±20,2 [c]	777,86±24,5 [d]	298,87±0,1 [b]	2,75±0,05 [c]
Group 5	1943,01±75,4 [b]	750,87±14,1 [cd]	293,07±0,2 [b]	2,49±0,04 [b]
Group 6	1866,34±26,7 [ab]	720±14,1 [c]	283,4±0,5 [b]	2,44±0,04 [ab]

[a,b,c,d] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

In brain tissue, it was determined that the levels of IL-1β showed a positive correlation with the levels of IL-1α, TNF-α, NF-κB, Na, and Cl (r=0,631**, r=0,517**, r=0,702**, r=0,371*, and r=0,462**). Additionally, a negative correlation was observed between TG and TP levels (r=−0,405* and r=−0,354*) (Table 15).

In brain tissue and serum, TAS levels were found to be higher in the control groups compared to the CP-treated groups ($P<0.05$), although the differences were not significant within the control or treated groups ($P>0.05$) (Table 16). In contrast, TOS levels were lower in the control groups compared to the CP-treated groups in both brain tissue and serum ($P<0.05$). Among the CP-treated groups, Group 4 had the highest levels, while Group 5 had the lowest ($P<0.05$). A significant negative correlation was found between the TAS and TOS levels in the groups (r=−0,556**) (Figs. 5 and 6).

Discussion

CP has the ability to induce multi-organ toxicity (Al-Salih et al. 2020). CP can cause severe ovarian damage and lead to early ovarian insufficiency by affecting mechanisms such as oxidative stress, inflammation, and apoptosis (Elahi et al. 2023). Chemotherapy agents like CP are toxic to brain cells and can cause cognitive disorders (Iqubal et al. 2019); however, studies on preventing or treating the effects of CP on the brain are limited (Zarei and Shivanandappa 2016). The high concentrations of hormones, macrophages, neutrophils, cytokines, and growth factors in PRP can contribute to the regulation of tissue healing, regeneration, anabolism, differentiation, proliferation, angiogenesis, and inflammation (Borrione et al. 2010). PRP therapy has been reported to be applied in neuroinflammatory central nervous system models such as vascular dementia (Bayat et al. 2020), multiple sclerosis (Borhani-Haghighi and Mohamadi 2019), Alzheimer's disease (Anitua et al. 2014), stroke (Hayon et al. 2013), and Parkinson's disease (Anitua et al. 2015). These treatments have reduced inflammatory responses and shown neuroprotective effects by supporting neuronal survival. PRP, made from autologous platelets, is a promising therapeutic approach thought to enhance tissue repair and demonstrate anti-inflammatory effects through mediators in α-granules (Vetrano et al. 2013; Giusti et al. 2014). This study aims to evaluate the therapeutic effects of ACRS or PRP on oxidative status, mineral levels, and pro-inflammatory cytokines in the brain and serum of rats with CP-induced ovarian failure.

CP induces apoptosis, inhibits angiogenesis, and leads to ovarian atrophy, reducing primordial follicles, oocytes, and granulosa cells (Soleimani et al. 2011). Oxidative stress

Table 15 The correlation relationships between the parameters in the brain tissue of the groups

	IL-1β	IL-1α	TNF- α	NF-κB	Na	K	Cl	Üre	TC	TG	P	Ca	UA	TP	Fe	Mg
IL-1β	1	0,6**	0,5**	0,7**	0,3*	−0,3	0,4**	−0,2	−0,4*	−0,1	−0,04	−0,04	0,08	−0,3*	0,3	−0,2
IL-1α		1	0,6**	0,5**	0,4**	−0,3	0,5**	−0,2	−0,5**	−0,1	0,008	−0,2	0,1	−0,3*	0,2	−0,1
TNF- α			1	0,6**	0,5**	−0,2	0,4**	−0,3	−0,5**	−0,2	−0,1	−0,1	0,1	−0,5**	0,3*	−0,3*
NF-κB				1	0,5**	−0,3*	0,4*	−0,2	−0,4**	−0,1	−0,2	−0,04	0,005	−0,5**	0,4*	−0,4*
Na					1	−0,5**	0,7**	−0,4**	−0,6**	−0,3	−0,2	−0,07	0,07	−0,6**	0,1	−0,6**
K						1	−0,5**	0,9**	0,7**	0,8**	0,7**	0,04	0,3*	0,6**	0,1	0,8**
Cl							1	−0,4**	−0,6**	−0,3	−0,1	−0,09	0,07	−0,4**	0,2	−0,4**
Üre								1	0,8**	0,9**	0,7**	0,1	0,3*	0,6**	0,05	0,8**
TC									1	0,6**	0,6**	0,2	0,2	0,7**	−0,1	0,7**
TG										1	0,7**	0,08	0,4**	0,5**	0,1	0,7**
P											1	0,05	0,5**	0,7**	0,07	0,8**
Ca												1	−0,2	0,1	0,1	0,09
UA													1	0,2	−0,1	0,2
TP														1	−0,006	0,8**
Fe															1	0,1
Mg																1

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level

Table 16 The levels of TAS (B-TAS), TOS (B-TOS) in brain tissue, and TAS (S-TAS), TOS (S-TOS) in serum of the groups

	B-TAS (mmol/g)	B-TOS (µmol/g)	S-TAS (mmol/L)	S-TOS (µmol/L)
Group 1	2,46±0,02 [a]	7,25±0,02 [a]	2,84±0,1 [a]	36,47±5 [a]
Group 2	2,52±0,008 [a]	7,72±0,008 [a]	2,96±0,04 [a]	38,76±2,2 [a]
Group 3	2,55±0,03 [a]	7,94±0,03 [a]	2,9±0,1 [a]	32,43±5 [a]
Group 4	1,32±0,05 [b]	7,94±0,03 [a]	2,15±0,04 [b]	83,31±8,2 [b]
Group 5	1,33±0,009 [b]	12,4±0,009 [bc]	2,19±0,02 [b]	42,15±2,2 [a]
Group 6	1,34±0,05 [b]	11,36±0,055 [c]	2,31±0,01 [b]	59,4±3,1 [c]

[a,b,c] Differences between groups indicated with different letters in the same column are significant ($P<0.05$). (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR)

is associated with reduced fertility in animal and in vitro models. In women, the imbalance between pro-oxidants and antioxidants can lead to reproductive disorders such as endometriosis, preeclampsia, PCOS, and unexplained infertility (Agarwal et al. 2012). The hormonal disruption caused by CP negatively affects the menstrual cycle, ovulation, and reproductive health (Abogresha et al. 2021). Control groups (Group-1, Group-2, Group-3) had normal histological structure, while experimental groups (Group-4, Group-5, Group-6) showed follicular degeneration, congestion, hemorrhage, edema and inflammatory cell infiltration. Group-6 showed less histopathological damage and more effective reduction of lesions compared to Group 4. A significant decrease in follicles and an increase in atretic follicles were observed in Group 4. While follicle numbers improved in Group 5 and 6, Group 6 showed the greatest effect. In immunohistochemical evaluations, strong AMH and α-SMA reactivity was observed in control groups, while a decrease was observed in Group 4. Reactivity increased in Group 5 and Group 6, and the highest score was obtained in Group 6. While IL-1β reactivity was low in control groups, it increased in Group 4 and decreased in Group 5 and 6, with the lowest score being seen in Group 6. Histopathological studies have shown that PRP treatments improved both the quantity and quality of follicles in the ovaries of mice compared to the control group, with fewer atretic follicles in the PRP-treated group (Ahmadian et al. 2020). The anticancer effects of CP are associated with phosphoramid mustard, while its toxic side effects are related to acrolein (Alarcon 2012). Acrolein creates oxidative stress in tissues by negatively affecting biochemical reactions. The reproductive toxicity of CP has been documented in both humans and experimental animals (Ghosh et al. 2002). Therefore, effective therapeutic methods are needed to address reproductive toxicity.

CP induces oxidative stress by increasing the production of oxygen-free radicals. This condition arises by promoting the production of free radicals (Jeelani et al. 2017). CP triggers inflammation as part of the immune response to infection, tissue damage, or chemical toxicity, and increases the expression of cytokines. It has also been reported to enhance programmed cell death by activating IFN-α and IFN-γ (Raeeszadeh et al. 2022). In our study, it was observed that the levels of IL-1β, IL-1α, TNF-α, and NF-kB were highest in Group 4. Groups 5 and 6 showed a decrease in levels

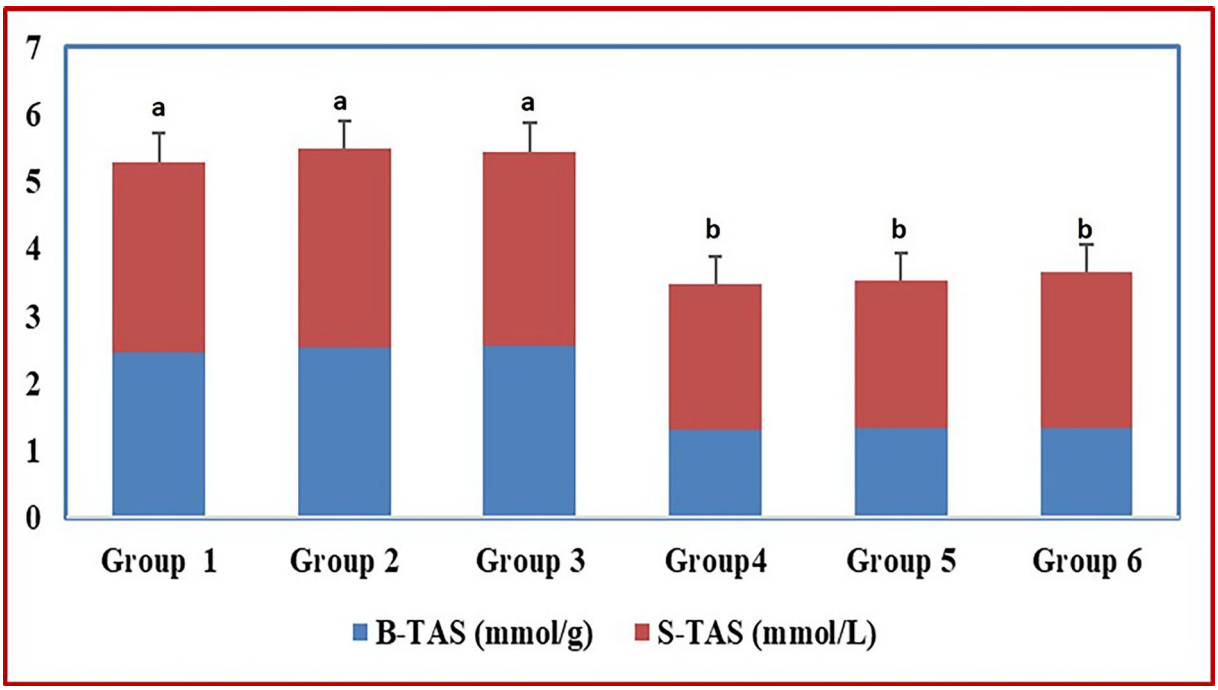


Fig. 5 TAS levels in brain tissue (B-TAS) and serum (S-TAS) of the groups. (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR), [a,b] <0.05 level

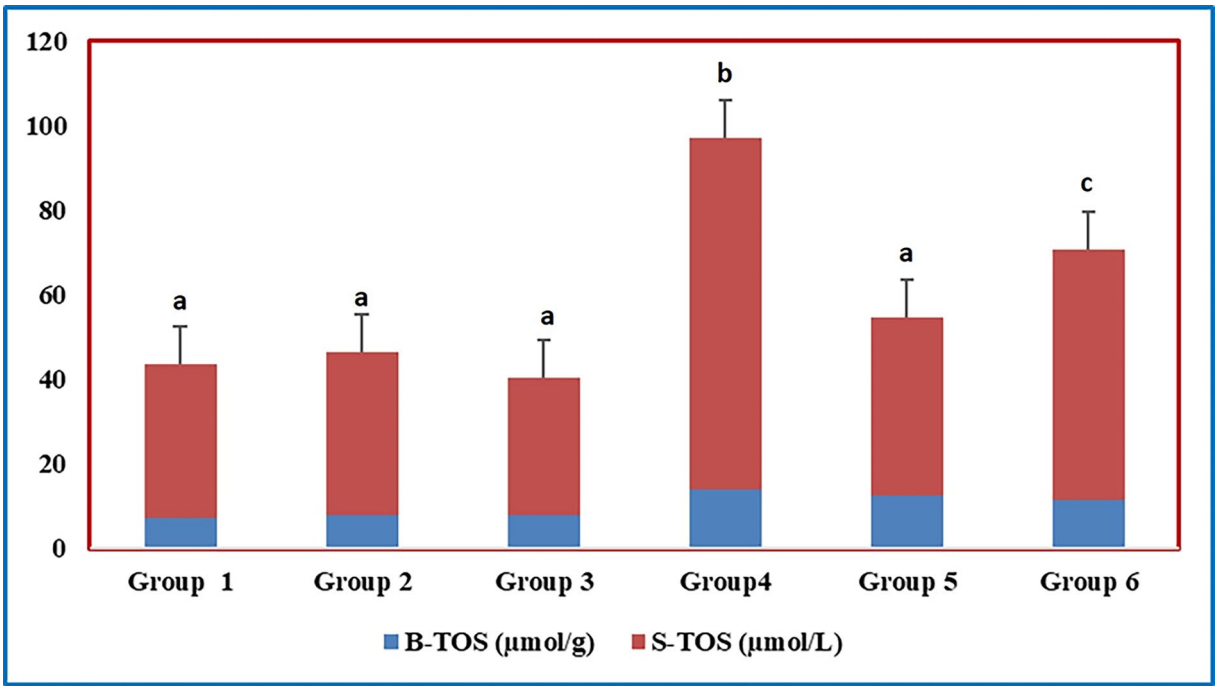


Fig. 6 TOS levels in brain tissue (B-TOS) and serum (S-TOS) of the groups. (Group 1: Control; Group 2: PRP control; Group 3: ACSR control; Group 4: CP; Group 5: CP+PRP; Group 6: CP+ACSR), $^{a,b}<0.05$ level

compared to Group 4, with Group 6 showing a greater decrease. In brain tissue, the levels of IL-1β, IL-1α, TNF-α, and NF-κB were higher in the CP-treated groups compared to the control groups. In addition to triggering oxidative stress, the inflammation induced by CP leads to an increase in the protein expression of IL-1β, IL-1α, TNF-α, and NF-κB in both brain tissue and serum. These findings suggest the activation of the NF-κB/TNF-α pathway. The activation of NF-κB in response to oxidative stress facilitates the stimulation of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, leading to tissue damage in rats exposed to CP, which is consistent with previous studies (Mahmoud and Al Dera 2015; Said et al. 2016; Song et al. 2016; El-Kholy et al. 2017; El-Sheikh et al. 2017). CP has a neurotoxic effect primarily due to its ability to generate reactive oxygen species within cells (Singh et al. 2019; Ibrahim et al. 2021). Understanding the mechanisms behind the cognitive disorders and neurotoxic effects of chemotherapy drugs may help prevent these adverse effects (Alhowail et al. 2021). Several studies have highlighted the role of antioxidants in improving the harmful effects of CP on different organs, including the hippocampus (Bhattacharjee et al. 2014; Akomolafe et al. 2020; Hamza et al. 2020; Sekeres et al. 2021; Al-Allaf et al. 2022). It was determined that the TAS levels in brain tissue and serum were higher in the control groups ($P>0.05$) compared to the CP-treated groups ($P>0.05$) ($P<0.05$). In brain tissue and serum, it was determined that the TOS levels were lower in the control groups ($P>0.05$) compared to the CP-treated groups ($P<0.05$). Among the CP-treated groups, Group 4 had the highest levels, followed by Group 5, which had the lowest levels ($P<0.05$). A significant negative correlation (r=−0.556**) was found between the TAS and TOS levels in the groups. It was determined that the application of PRP or ACRS to CP-treated rats had a positive effect by increasing the TAS levels and decreasing the TOS levels. Previous studies have also reported a significant increase in oxidative stress and a decrease in TAC levels in CP-treated rats (Fouad et al. 2016; Maleki et al. 2024). Chemotherapeutic drugs work by killing both healthy and cancer cells in the body, which is one of their most severe side effects (Suman and Kaiser 2006). Additionally, immunosuppressive cytotoxic drugs such as CP can cause various side effects on the immune system (Moran 2000). The primary function of the liver is to detoxify xenobiotics and toxins (Mitra et al. 1998). The toxic metabolites formed after CP administration, acrolein and phosphoramide mustard, cause liver toxicity in animals (Senthilkumar et al. 2006b; Arundel and Lewis 2007). In our study, the levels of AST and ALT, which indicate liver health, were found to be higher in the CP-treated groups compared to the control groups. However, no significant difference was observed in AST enzyme activity between the CP-treated groups ($P>0.05$). For ALT enzyme activity, it was observed that Group 1 in the control groups had the lowest levels, while Groups 2 and 3 had significantly higher levels ($P<0.05$). In the CP-treated groups, the highest ALT enzyme activity was observed in

Group 2, while it decreased in Group 5 ($P<0.05$) and Group 6 ($P>0.05$). The increase in serum ALT and AST enzyme levels associated with CP-induced hepatotoxicity indicates that the release of cytosolic enzymes into the bloodstream results from cell damage and the loss of hepatocyte membrane integrity (Germoush et al. 2014). Previous studies have also shown that intraperitoneal injection of CP leads to an increase in enzymes and metabolites, which are serum biomarkers for liver function (Senthilkumar et al. 2006a; Hashem et al. 2020; Maleki et al. 2024).

Proteins include albumin and globulin; albumin is synthesized in the liver (Sandnes et al. 1988). Globulin consists of α1, α2, β, and γ globulin fractions, which are considered the source of immunologically active proteins found in the blood (Pagana et al. 2019). Total protein and albumin levels were found to be higher in the control groups compared to the CP-treated groups. Globulin levels, on the other hand, were observed to be higher in Group 1 of the control groups compared to Groups 2 and 3 ($P<0.05$). Among the CP-treated groups, Group 4 had the highest globulin levels, followed by Groups 6 and 5 ($P<0.05$). A decrease in serum albumin and globulin levels may indicate conditions such as kidney issues, chronic infections, inflammation, and cirrhosis (Soeters et al. 2019). It has been reported that CP treatments reduce total protein, albumin, and globulin levels (Doustimotlagh et al. 2020; Yahya et al. 2022; Maleki et al. 2024). This suggests potential hepatocellular damage following chemotherapy (King and Perry 2001). The antioxidant role of albumin in scavenging reactive oxygen species may have contributed to a significant reduction in these levels. A decrease in albumin levels is commonly associated with liver diseases and can be linked to changes in protein and amino acid metabolism (Ncibi et al. 2008). Glucose levels were found to be higher in the CP-treated groups compared to the control groups. Additionally, after CP administration, glucose levels in Group 5 and Group 6 were higher than in Group 4 ($P<0.05$).

The detection and diagnosis of acute kidney injury typically rely on serum creatinine and urea levels, with creatinine being a waste product of energy metabolism in muscles (Djousse et al. 2001). Urea levels were found to be highest in Group 1, with significant differences observed compared to Groups 2, 4, 5, and 6 ($P<0.05$). In the CP-treated groups, Group 4 had the lowest urea levels, while levels increased in the treated groups, but the differences were not statistically significant ($P>0.05$). Creatinine levels were significantly higher in the CP-treated groups compared to the control groups ($P<0.05$). However, no significant differences were found between the CP-treated groups ($P>0.05$). Chemotherapy-induced kidney dysfunction can involve damage to renal vessels or structures, hemolytic uremic syndrome, and prerenal perfusion deficiencies (Kintzel 2001; Lameire et al. 2011). The reduced blood urea caused by CP may be related to protein catabolism (Yanardag and Sacan 2007).

Electrolyte imbalance is one of the side effects of CP, and several studies have reported changes in electrolyte balance during stress conditions. For instance, stress caused by high environmental temperatures in animals has been shown to lead to decreases in the concentrations of Ca, K, P, and Na, while Cl concentrations increase (Schneider et al. 1988). Hyponatremia is a common and serious electrolyte disturbance observed in cancer patients. However, chemotherapy itself, along with factors such as chemotherapy-induced vomiting and excessive hydration, can increase the risk of developing hyponatremia (Baker et al. 2014). This study investigates the effects of CP on serum and brain tissue levels of electrolytes and minerals in different treatment groups. In the serum, Na levels were slightly higher in the control groups compared to the CP-treated groups, though the difference was not statistically significant ($P>0.05$). K levels were significantly lower in Group 4 ($P<0.05$), while Cl levels were highest in Group 5 and lowest in Group 4 ($P<0.05$). *P* levels were lowest in Group 4 and highest in Group 6 ($P<0.05$), while Mg levels were significantly higher in Group 4 ($P<0.05$). Fe levels were lowest in Group 1, with significant increases in Group 3 ($P<0.05$). In CP-treated groups, Fe levels were highest in Group 4 and lowest in Group 6, but the difference was not significant ($P>0.05$). In brain tissue, Na levels were lower in the control groups compared to the CP-treated groups. K levels were significantly higher in Groups 2 and 3 ($P<0.05$), while Cl levels were lower in the control groups than in the CP-treated groups ($P<0.05$). Ca levels decreased in CP-treated groups, though not significantly ($P>0.05$). Mg levels were highest in Groups 2 and 3 ($P<0.05$). Fe levels were lowest in Group 1 and highest in Group 4 ($P<0.05$), while CP treatment reduced Fe levels in Groups 5 and 6 ($P<0.05$). In the study, it was reported that there was a decrease in K and Na+concentrations in the groups with CP application, while there was a decrease in P, Mg, Cl and Ca levels (Dağ et al. 2018).

Conclusion

This study demonstrates that PRP or ACRS treatment following CP administration leads to significant changes in mineral and electrolyte levels in serum and brain tissue, accompanied by antioxidant and anti-inflammatory effects. These changes contribute to improved follicular health, protection against ovarian damage, and tissue repair. Understanding the biochemical effects of chemotherapy and their modulation through autologous serum therapies is valuable. Considering that PRP and ACRS are derived from the patient's own blood, these therapies are promising in

reducing side effects and treatment costs and have potential as supportive or chemopreventive strategies.

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Author contributions M.E., E.K., and H.E. conducted the experiments, designed the study, and supported the research. G.A. and O.K. performed the histopathological and immunohistochemical analyses. G.C. managed the project, performed the statistical analysis, interpreted the results, and revised the manuscript. R.A. and F.Ü. prepared the samples and conducted the analyses. All authors read and approved the final draft.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interest The authors declare no competing interests.

Ethical approval The study was conducted with the permission of Erciyes University Animal Experiments Local Ethics Committee (numbered 2024/22).

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Authors and Affiliations

Mustafa Ermiş[1] · Erol Karakaş[2] · Hanifi Erol[3] · Gökhan Akcakavak[4] · Recai Aci[5] · Furkan Ümit[6] · Özhan Karatas[7] · Gülay Çiftci[6]

✉ Gülay Çiftci
gciftci@omu.edu.tr

[1] Erciyes University Experimental Research Application and Research Center, Kayseri, Turkey

[2] Op. Dr. Erol Karakaş Clinic, Kayseri, Turkey

[3] Department of Surgery, Faculty of Veterinary Medicine, University of Erciyes, Kayseri, Turkey

[4] Faculty of Veterinary Medicine, Department of Pathology, Aksaray University, Aksaray, Turkey

[5] Aydın Adnan Menderes University, Söke Vocational School of Health Services, Aydın, Turkey

[6] Department of Biochemistry, Faculty of Veterinary Medicine, University of Ondokuz Mayis, 55220 Atakum, Samsun, Turkey

[7] Faculty of Veterinary Medicine, Department of Pathology, Sivas Cumhuriyet University, Sivas, Turkey