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Protective effects of *Astragalus microcephalus* polysaccharides combined with vitamin E and selenium against *mercury-induced* ovarian toxicity in female rats

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Abstract: **Background**: Exposure to mercury (Hg) is well-established to induce menstrual cycle disorders and disrupt sex hormone functions. *Astragalus* species, characterized by their potent antioxidant and anti-apoptotic properties, have shown potential in mitigating these adverse effects by promoting folliculogenesis and restoring hormonal balance. **Objectives**: This study aimed to evaluate the protective effects of *Astragalus microcephalus* polysaccharides (APS), both alone and in combination with vitamin E and selenium (Vit E+Se), against ovarian damage and oxidative stress induced by mercuric chloride toxicity in rats. **Methods**: Thirty-six female Wistar rats were synchronized via vaginal smear and randomly assigned to six groups: Control, Mercury (0.43 mg/kg $HgCl_2$), APS (2.7 g/kg), Vit E+Se, Mercury+APS and Mercury+Vit E+Se. Following a 15-day subacute exposure period, serum levels of estradiol, LH and FSH were measured. Oxidative stress markers and ovarian histopathology were evaluated. Apoptotic and inflammatory pathways were assessed through immunohistochemical staining of Caspase-3, AIF and NF-κB. **Results**: Hg-induced reductions in hormone levels were significantly restored to near control levels in the APS-treated groups and antioxidant levels were notably elevated in these groups, indicating mitigation of oxidative stress. Furthermore, immunohistochemical analysis revealed a significant reduction in stromal and follicular degeneration and a decrease in apoptotic activity in the ovaries of rats treated with APS. **Conclusion**: APS exerts a significant protective effect against mercury-induced ovarian toxicity. This protection is mediated through the attenuation of oxidative stress, inflammation and apoptosis, as evidenced by the modulation of Caspase-3, AIF and NF-κB pathways. The results suggest that APS, particularly in synergy with vitamin E and selenium, offers a potential therapeutic strategy for managing heavy metal-induced reproductive damage.

Keywords: Apoptosis; *Astragalus* polysaccharides; Mercury; Oxidative stress; Reproductive damage

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INTRODUCTION

Heavy metals such as iron, zinc and selenium are essential trace elements involved in physiological functions, whereas cadmium, lead and mercury are non-essential and toxic. Exposure to non-essential heavy metals may lead to oxidative stress, inflammation and organ damage, contributing to diseases like cancer, neurodegeneration and immune dysfunction (Kumar *et al.*, 2022).

Mercury (Hg) exists in three forms: elemental, inorganic and organic. Human exposure to mercury occurs primarily through the consumption of seafood and, to a lesser extent, via dental amalgams, skin whitening creams, waste batteries, broken thermometers and fluorescent bulbs. Environmental pollution is another potential source of mercury exposure, with food residues being a significant contamination factor (Bjorklund *et al.*, 2019; Yang *et al.*, 2020).

Exposure to mercury can disrupt menstrual cycles and sex hormones by targeting the hypothalamic-pituitary-gonadal (HPG) axis, as mercury can cross the blood-brain barrier and accumulate in neuroendocrine tissues. In women, mercury exposure has been shown to inhibit the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Saeed *et al*., 2025). This neuroendocrine interference directly impacts the feedback mechanisms of the HPG axis, leading to decreased levels of estrogen and progesterone, potentially resulting in dysmenorrhea, menstrual irregularities, uterine displacement, premature menopause and various forms of ovarian dysfunction (Massanyi *et al*., 2020; Balachandar *et al*., 2020). Studies using experimental animals have reported that exposure to mercury can cause infertility, stillbirth, miscarriages and congenital malformations (Massanyi *et al*., 2020; Lee *et al*., 2019; Tan *et al*., 2009).

It has been suggested that cellular damage is attributable to elevated oxidative stress and apoptosis in tissues exposed to mercury (Zulaikhah *et al.*, 2020; Morcillo *et al.*, 2016). The hypothesis that Selenium (Se) and vitamin E can mitigate the adverse effects of mercury toxicity is predicated on their capacity to reduce oxidative stress (Moniruzzaman *et al.*, 2021; Liang *et al.*, 2023).

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Astragalus species, traditionally used in herbal medicine, have been reported to exhibit antioxidative and apoptosis-regulating effects, largely attributed to their polysaccharide components. *Astragalus* polysaccharides (APS), a major bioactive constituent extracted from *Astragalus* species, have been shown to exhibit diverse pharmacological properties, including glycemic and lipid regulation, anticancer activity, anti-aging effects and immunomodulation (Li *et al.*, 2022; Liang *et al.*, 2025). While most studies have focused on *Astragalus* membranaceus, *Astragalus microcephalus* was specifically chosen for this study as it is a prominent member of the Turkish flora and its potential protective effects warrant investigation. However, the extant literature contains no study that has examined the protective effect of APS derived from this species against mercury-induced ovarian toxicity. This study aims to experimentally evaluate the protective efficacy of APS against mercury-induced oxidative damage in the ovaries, with a particular focus on its potential to regulate apoptosis.

To better contextualize the antioxidative strength of APS, vitamin E and selenium, two well-established antioxidants, were included as comparator groups. By doing so, the study seeks to clarify whether the protective effect of APS is comparable to these standard antioxidants when mitigating mercury-induced cellular damage.

MATERIALS AND METHODS

Experimental groups and study design

In the present study, 36 adult female Wistar rats (weighing between 220 and 250 grams) were obtained from Sivas Cumhuriyet University Experimental Animal Application and Research Center (Sivas, Turkey). During the experimental study, the rats were kept in a controlled room with 50% humidity, temperature of 25 ± 2 ºC and 12:12 h dark/light cycle with free access to water and food. This study was approved by the Sivas Cumhuriyet University, Animal Experiments Local Ethics Committee (Approval No. 536, dated April 20, 2021).

At the beginning of the study, the estrous cycles of the rats were monitored via vaginal smear analysis to ensure physiological synchronization; and based on the presence of cornified epithelial cells, animals identified as being in the estrous phase were randomly assigned into six experimental groups, each consisting of six rats. The duration of the treatment period was standardized to 15 days for all groups. The dose of mercury chloride (15 µg/kg/day, i.p. for 15 consecutive days) was selected based on previously published studies that established its effectiveness in inducing ovarian toxicity in rats (Jafari Khorchani *et al*., 2021; Wang *et al*., 2021; Li *et al*., 2022; Kumar *et al*., 2022). This 15-day treatment period was specifically designed to encompass approximately three to four complete estrous cycles in rats, thereby modeling a subacute exposure scenario rather than an acute intoxication or chronic low-dose exposure (Davis *et al*., 2001; El-Desoky *et al*., 2013; Reagan-Shaw *et al*., 2008; Shaikh *et al*., 2026). This duration is sufficient to observe the disruptive effects of mercury on the hypothalamic-pituitary-ovarian axis and the subsequent protective intervention of APS over multiple reproductive cycles. Although mercury is not routinely encountered as a pharmaceutical agent, such exposure levels may occur under specific environmental or occupational conditions. Therefore, the selected dose provides a toxicologically relevant and translational framework for assessing the protective effects of therapeutic agents against mercury-induced ovarian damage.

The experimental groups (n=6 per group) were organized as follows for a 15-day treatment period:

1. Control group: Received physiological saline solution (i.p.).
2. *Astragalus* (APS) group: Received *Astragalus* polysaccharides (2.7 g/kg/day, oral gavage).
3. Hg group: Received mercury chloride (15 µg/kg/day, i.p.).
4. Hg + APS group: Received mercury chloride (15 µg/kg/day, i.p.) and APS (2.7 g/kg/day, oral gavage).
5. Hg + Vit E-Se group: Received mercury chloride (15 µg/kg/day, i.p.) and vitamin E-selenium (Vit E: 60 mg/kg/day; Se: 1 mg/kg/day, oral gavage).
6. Hg + Vit E-Se + APS group: Received mercury chloride (15 µg/kg/day, i.p.) along with Vit E-Se and APS at the aforementioned doses.

At the end of the 15-day treatment period, the rats were euthanized by decapitation under ketamine-xylazine anesthesia. Blood samples were collected and centrifuged and the sera were separated for hormonal and biochemical analyses. The sera were stored at -20°C. The ovaries were excised, with one ovary designated for biochemical analyses and the other fixed in 10% formaldehyde solution for histopathological examination.

Plant extraction and Q-TOF LC/MS analysis

In this study, *Astragalus microcephalus* specimens were sourced from a rural locality in Sivas, Turkey and botanically verified by Prof. Dr. H. Aşkın Akpulat from the Department of Biology at Sivas Cumhuriyet University. After collection, 100 grams of dried root and stem parts were finely powdered and subjected to extraction using 1 liter of ethanolic solvent (10% w/v) in a water bath maintained at 40 °C for 24 hours, resulting in a crude plant extract. Following extraction, 2% trichloroacetic acid (TCA) was added to the plant extract to remove proteins and thereby isolate the polysaccharide fraction. After hydrolysis of the polysaccharides using 2M trifluoroacetic acid (TFA) at 120°C for 2 hours, the mixture was subsequently filtered under vacuum using a 0.22 µm membrane filter and the resulting filtrate was concentrated with a rotary evaporator (IKA, CMF300, People's Republic of China) to eliminate residual ethanol.

The dosage of APS (2.7 g/kg) was determined based on the maximum human intake recommended by the Chinese Pharmacopoeia (2020) and converted using the body surface area (BSA) normalization method. First, the Km factor (body weight/body surface area) was identified as 37 for humans and 6.0 for rats. The human-to-rat Km ratio was calculated as 6.16 (approximately 6.2) by dividing the human Km by the rat Km (37 / 6.0). Accordingly, the human equivalent dose (HED) of 0.43 g/kg (equivalent to 30 g for a 70 kg individual) was multiplied by this ratio (0.43 x 6.2), yielding a target rat dose of approximately 2.7 g/kg (Wang *et al*., 2023; Liu *et al*., 2024). The polysaccharide composition of the *A. microcephalus* extract was determined through Quadrupole-Time of Flight Liquid Chromatography/Mass Spectrometry (Agilent 6530 Q-TOF LC/MS, USA), using standardized analytical protocols (Yang *et al.*, 2023) (Table 1).

Biochemical analysis

The ovarian tissues were immediately excised under ice-cold conditions, carefully blotted to remove excess blood and tissue fluids, weighed and stored at -80°C (Antech, Eco Toch, Turkey) for subsequent analysis. Ovarian tissues were weighed and homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) using a glass-teflon homogenizer at 4°C (Allsheng Bioprep-6, PRC). The homogenate was then centrifuged at 3.000 rpm for 15 minutes and the supernatant was collected for biochemical analysis and determination of protein concentrations using the Bradford method. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels, an indicator of thiobarbituric acid reactive substances (TBARS) formation. MDA was quantified based on its reaction with thiobarbituric acid (TBA) to form a colored MDA–$(TBA)_2$ complex under aerobic conditions and the absorbance was measured spectrophotometrically (Perkin Elmer, USA) at 532 nm. Absorbance values were compared with a series of standard solutions of 1,1,3,3-tetraethoxypropane (TEP). Superoxide dismutase (SOD) activity was determined using a commercial enzymatic assay kit (Cayman Chemical Company, Ann Arbor, MI), with absorbance measured at 440–460 nm using a plate reader (Thermo, Multiskan GO Microplate, Waltham, USA). Glutathione peroxidase (GSH-Px) activity was measured using a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) and catalase (CAT) activity was determined using the Catalase Colorimetric Activity Kit (Thermo-Fisher Scientific, cat. no. EIACATC, USA). All biochemical analyses were performed both in serum and ovarian tissues homogenate and all measurements were conducted in triplicate to ensure accuracy and reproducibility.

Hormone analysis

Levels of estradiol (E2), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured using the enzyme-linked immunosorbent assay (ELISA) method, following the manufacturer's instructions (BT Lab, China). The specific catalog numbers for the kits used were as follows: Rat (E2) ELISA Kit (Cat. No: EA0011Ra), Rat (FSH) ELISA Kit (Cat. No: EA0015Ra) and Rat (LH) ELISA Kit (Cat. No: EA0058Ra).

Pathologic examination

Necropsies were performed on the rats and the ovarian tissues were subsequently immersed in a 10% buffered formalin solution. Sections of 5 µm thickness were cut from the paraffin blocks and examined under a light microscope following hematoxylin-eosin (H and E) staining. Histopathological findings were evaluated semi-quantitatively using a four-point scoring system based on the severity and extent of tissue damage. In this system, "absent (0)" indicated no observable pathological changes; "mild (1)" referred to minimal and focal histological alterations such as slight congestion or localized edema; "moderate (2)" represented more pronounced but not widespread changes, including moderate follicular degeneration, inflammatory infiltration, or vascular congestion; and "severe (3)" denoted extensive and diffuse pathological findings such as widespread follicular atresia, severe stromal edema, hemorrhage or marked inflammatory infiltration. These criteria were adapted from previously published scoring methods in reproductive toxicology studies.

For immunohistochemical analysis, tissue slides were incubated overnight at 4 °C with the following primary antibodies: Caspase 3 (Biorbyte, Cat. No. Orb382909, dilution 1:100), apoptosis inducing factor (AIF) (Avivasysbio, Cat. No. AVARP02013, dilution 1:150) and Nuclear Factor kappa B (NF-κB) (Abcam, Cat. No. ab7971, dilution 1:150). The secondary antibody, Large Volume Detection System: anti-Polyvalent, HRP (Thermo Fisher, Cat. No. TP-125-HL), was applied according to the manufacturer's instructions. 3,3′-Diaminobenzidine (DAB) was used as the chromogen and slides were subsequently counterstained with Mayer's Hematoxylin. Immunopositivity in ovarian tissues was evaluated semi-quantitatively using a five-point scoring system. In this system, "absent (0)" indicated no detectable immunoreactivity; "mild (1)" represented weak and focal staining; "moderate (2)" indicated clearly visible but not extensive staining; "strong (3)" denoted widespread and intense staining; and "very strong (4)" corresponded to diffuse and intense immunoreactivity throughout the tissue.

Each histopathological assessment was assigned a numerical score to facilitate quantitative analysis and the resulting data were subjected to statistical evaluation. All evaluations were performed in a double-blind fashion to ensure objectivity and eliminate observer bias.

Table 1: Q-TOF LC/MS analysis of *Astragalus microcephalus* extract polysaccharides (APS).

Compound name	Retention time (min)	Mass-to-charge ratio (%)	Molecular formula	Molecular weight (g/mol)	Abundance of identified compounds
Xylose	20.6276	94.24	$C_5H_{10}O_5$	150.13	2.7 x 10^8
Fucose	20.9169	95.49	$C_6H_{12}O_5$	164.16	1.1 x 10^8
Rhamnose	21.1359	91.11	$C_6H_{12}O_5$	164.16	1.0 x 10^8
Arabinose	21.3250	97.78	$C_5H_{10}O_5$	150.13	2.2 x 10^8
Rhamnitol	24.7287	94.11	$C_6H_{14}O_5$	166.17	3.0 x 10^8
Ribitol	24.9974	99.04	$C_5H_{12}O_5$	152.14	2.9 x 10^8
Fucitol	25.2425	96.83	$C_6H_{14}O_5$	166.17	0.5 x 10^8
Arabitol	25.5479	94.77	$C_5H_{12}O_5$	152.14	3.2 x 10^8
Xylitol	26.2143	92.16	$C_5H_{12}O_5$	152.15	4.5 x 10^8
Fructose	30.5792	95.96	$C_6H_{12}O_6$	180.16	0.7 x 10^8
Galactose	31.0901	96.21	$C_6H_{12}O_6$	180.16	0.2 x 10^8
Mannose	31.3008	98.35	$C_6H_{12}O_6$	180.16	0.5 x 10^8
Glucose	31.4711	94.18	$C_6H_{12}O_6$	180.16	1.2 x 10^8
Inositol	32.4216	96.16	$C_6H_{12}O_6$	180.16	2.6 x 10^8
Mannitol	33.2026	96.60	$C_6H_{14}O_6$	182.17	0.5 x 10^8
Sorbitol	33.3700	95.51	$C_6H_{14}O_6$	182.17	0.3 x 10^8
Galactitol	33.7630	96.89	$C_6H_{14}O_6$	182.17	3.5 x 10^8

Abundance of Identified Compounds refers to the relative peak area percentage (%) of each compound identified via Q-TOF LC/MS analysis.

Statistical analysis

All statistical analyses were performed with SPSS software (version 23). Levels of MDA, SOD, CAT, GSH-Px and hormones were expressed as mean ± standard error (SE) and analyzed using one-way ANOVA, followed by Duncan's post hoc test for multiple comparisons. Differences in histopathological scores among groups were assessed using the Kruskal–Wallis test. Where significant differences were identified, pairwise comparisons were conducted using the Mann–Whitney U test with Bonferroni correction to reduce the likelihood of false-positive results. A p-value of less than 0.05 was considered statistically significant.

RESULTS

LC/MS analysis results

In the present study, a Q-TOF LC/MS system was used to analyze the samples. This analysis enabled the identification of a total of 17 different monosaccharides and alditol derivatives (Table 1).

Hormone level results

The serum samples obtained from the rats included in the present study were analyzed for estradiol, LH and FSH levels. A significant decrease in hormone levels was observed in all groups treated with Hg ($p<0.05$). In the *treatment* groups, hormone levels increased significantly ($p<0.05$) and approached control levels (Fig. 1). In summary, figure 1 illustrates that while mercury significantly suppressed serum reproductive hormones, APS *treatment* effectively restored estradiol, LH and FSH levels toward their physiological baseline.

Biochemical parameter results

In the Hg-exposed group, serum MDA levels were significantly elevated, while the activities of antioxidant enzymes (SOD, CAT, GSH-Px) were markedly decreased compared to the control group. Similarly, ovarian tissue homogenates from the Hg-exposed rats showed increased MDA concentrations and reduced antioxidant enzyme activities. Conversely, in all APS-treated groups, serum and ovarian tissue enzyme activities and MDA levels showed a trend toward normalization, approaching the values observed in the control group (Table 2). The data in table 2 demonstrate that APS and Vitamin E-Se *treatment*s significantly alleviated mercury-induced oxidative stress by enhancing antioxidant enzyme activities and reducing lipid peroxidation.

Pathologic examination findings

Tissue samples were examined to assess the extent of tissue damage, oxidative stress and apoptosis-related changes. Statistically significant differences were found among the groups (Table 3). As summarized in table 3, APS administration notably preserved the follicular reserve and prevented the reduction in follicle counts typically caused by mercury toxicity. Ovarian tissues from the control and APS groups showed normal histological features. In contrast, histopathological analysis revealed stromal and follicular degeneration in the mercury (Hg) group. Stromal degeneration was severe in the Hg group, moderate in the Hg + APS and Hg + Vit E-Se groups and mild in the Hg + Vit E-Se + APS group. Similarly, follicular degeneration was most pronounced in the Hg group, moderate in the Hg + APS and Hg + Vit E-Se groups and mild in the Hg + Vit E-Se + APS group.

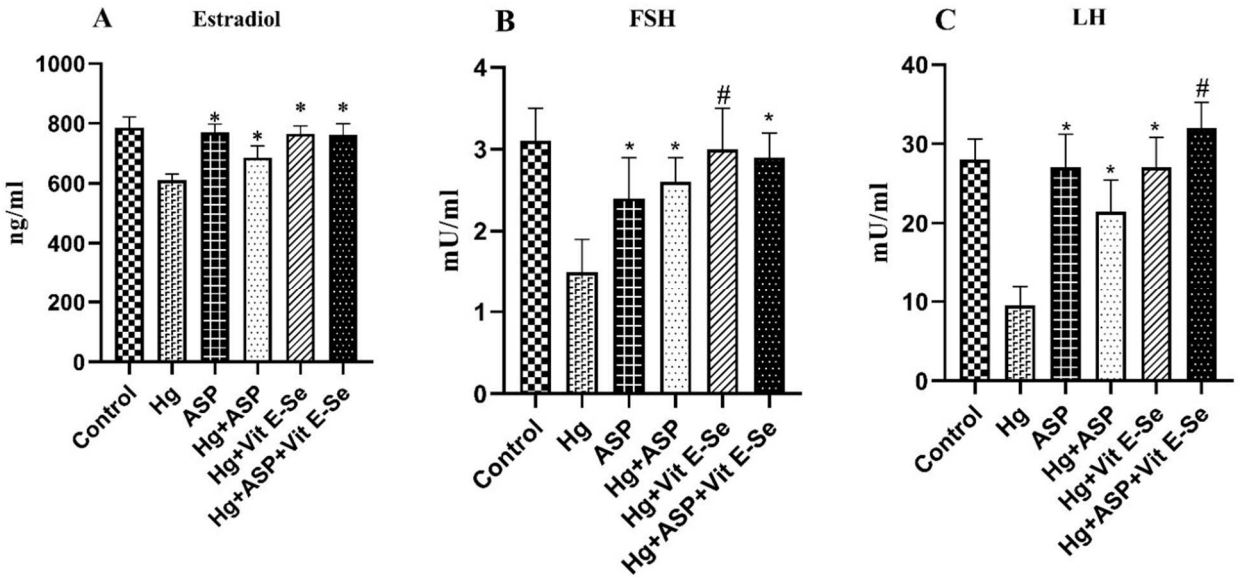

Fig. 1: Serum hormone levels

Serum hormone levels; (A) E2; (B) FSH and (C) LH levels. Different symbols (# and *) indicates a significant difference between groups in each bar graphs (*,# P<0.05 ANOVA) The asterisks indicates statistical difference between the Hg group and *treatment* groups. Each group consisted of 6 animals (n = 6).

Table 2: Serum and ovarian tissues homogenate biochemical parameters of rats given Hg.

Groups	MDA mean±SD		SOD mean±SD		CAT mean±SD		GSH-Px mean±SD	
	Serum nmol/ml	OTH nmol/ml/ mg protein	Serum IU	OTH IU/mg protein	Serum IU	OTH IU/mg protein	Serum IU	OTH IU/mg protein
Control	7.5±1.4	4.5±0.6	5.2±0.8	2.0±0.4	8.7±1.0	1.8±0.2	112±7	72±5
Hg	18.2±2.8	11.4±2	1.5±0.2	0.6±0.2	2.4±0.4	0.6±0.1	42±5	35±6
APS	10.2±1	6.5±1.2	2.5±0.4	1.6±0.2	7.0±0.7	1.5±0.4	92±6	58±3
Hg+APS	15.5±0.8	7.2±0.8	3.8±0.5	1.4±0.3	5.6±0.5	1.6±0.2	63±4	56±4
Hg+Vit E-Se	8.5±1.4	5.2±0.5	4.2±0.6	1.8±0.5	7.2±0.4	1.6±0.4	95±8	63±7
Hg+APS+ Vit E-Se	9.5±1.2	6.0±0.7	4.9±0.6	1.8±0.6	6.8±0.5	1.5±0.5	103±7	65±5

OTH: ovarian tissue homogenate, different letters in the same column represent differences between groups p<0.05. Each group consisted of 6 animals (n = 6).

Table 3: Hematoxylin-Eosin staining findings.

Groups	Stromal degeneration mean	Follicular degeneration mean
Control	0.33	0.16
Hg	3.66	2.66
APS	0.33	0.16
Hg+APS	2.16	2.23
Hg+Vit E-Se	2.12	1.26
Hg+APS+ Vit E-Se	1.16	0.33

Different letters in the same column represent differences between groups p<0.05. Each group consisted of 6 animals (n = 6).

In degenerated follicles, the normal arrangement of granulosa cells was disrupted and their structural integrity was lost (Fig. 2). Immunohistochemical staining showed statistically significant differences in Caspase-3, AIF and *NF-κB* immunoreactivity between the groups (Table 4). Overall, table 4 shows that the increase in apoptotic markers and structural degeneration induced by mercury was significantly minimized in groups receiving APS and combined therapies. Increased levels of Caspase-3, AIF and *NF-κB* were detected in all mercury-exposed groups, whereas marked reductions were observed in the APS-treated group and in those receiving combined vitamin E and selenium treatment.

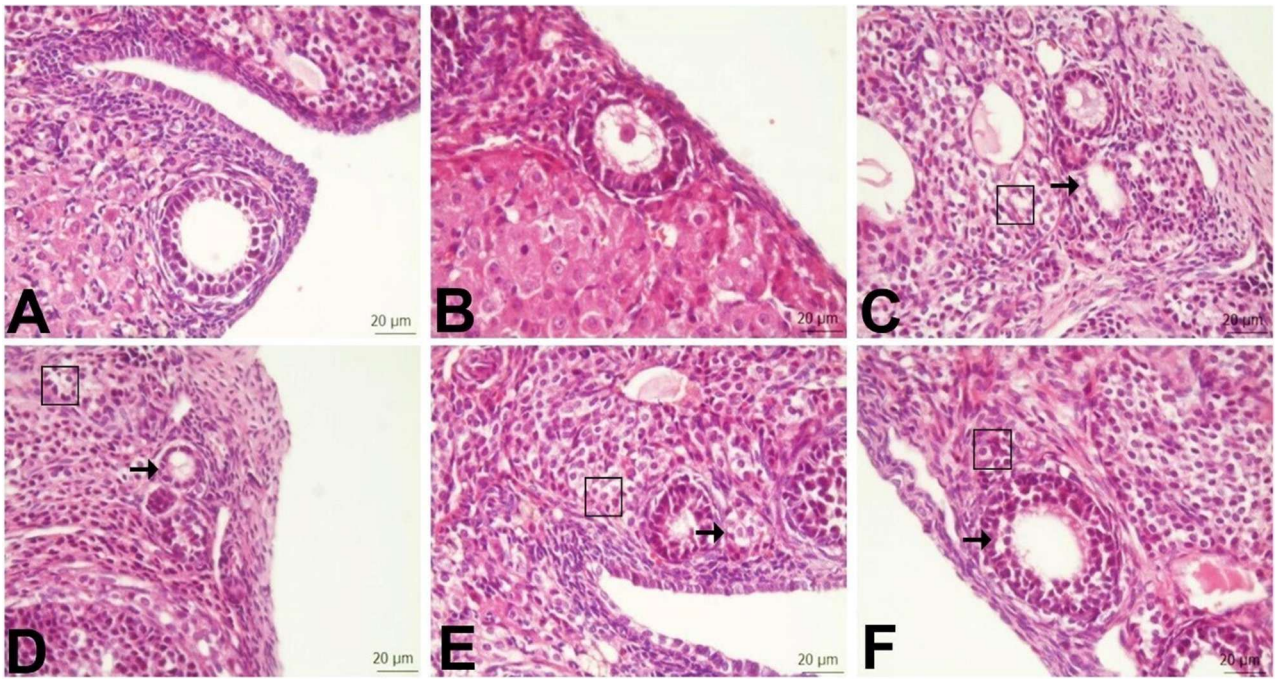

Fig. 2: Ovarian histopathological *examination.*
Histopathological examination; (A) Control group with normal histological appearance; (B) APS group; (C) Hg group, exhibiting severe degeneration; (D) Hg+APS group, showing moderate degeneration; (E) Hg+Vit E-Se group, with moderate degeneration; (F) Hg+Vit E-Se+APS group, demonstrating mild degeneration. Stromal degeneration (ϒ), follicular degeneration (→), H and E. Each group consisted of 6 animals (n = 6).

Table 4: Immunohistochemical staining findings.

Groups	Caspase-3 mean	AIF mean	*NF-κB* mean
Control	1.00	0.16	0.33
Hg	3.83	2.83	2.66
APS	0.83	0.16	0.16
Hg+APS	2.23	1.16	1.63
Hg+Vit E-Se	2.00	0.83	0.83
Hg+APS+ Vit E-Se	1.66	1.00	0.72

Different letters in the same column represent differences between groups $p<0.05$. n = 6 per group

DISCUSSION

Toxic metals, including mercury (Hg), are widely recognized as endocrine-disrupting chemicals (EDCs) due to their ability to interfere with hormone receptors, mimicking or blocking hormonal activity (Liu *et al.*, 2023). In this study, exposure to Hg induced severe ovarian damage, oxidative stress and hormonal imbalances in rats. Consistent with prior research, Hg exposure led to degenerative changes in ovarian tissues (Hassan *et al.*, 2024; Scheuhammer *et al.*, 2007), hormonal disruptions affecting reproductive function (Bjørklund *et al.*, 2019; Henriques *et al.*, 2019) and pathological conditions in multiple organs (Yang *et al.*, 2020; Patel *et al.*, 2022).

Mercury disrupts the hypothalamic-pituitary-ovarian (HPO) axis, reducing the secretion of FSH and LH, which are essential for oocyte maturation, ovulation and progesterone production (Lee *et al*., 2019; Balachandar *et al*., 2020; Trujillo-Vázquez *et al*., 2023; Shan *et al*., 2023). These disruptions can impair follicular development and endometrial receptivity (Henriques *et al*., 2019; Massanyi *et al*., 2020). In this study, serum levels of estradiol, FSH and LH were significantly decreased in Hg-exposed rats (Fig. 1), reflecting suppression of the HPG axis. Treatment with APS, alone or in combination with vitamin E and selenium, effectively restored hormone levels toward normal. This restoration is likely linked to the potent antioxidative and anti-inflammatory activities of these agents; specifically, by scavenging reactive oxygen species (ROS) and mitigating oxidative insult to the granulosa cells and the corpus luteum, APS helps preserve the structural integrity of the ovarian follicles. This protection prevents the mercury-induced impairment of steroidogenic pathways, thereby enhancing endocrine signaling and maintaining physiological hormone secretion.

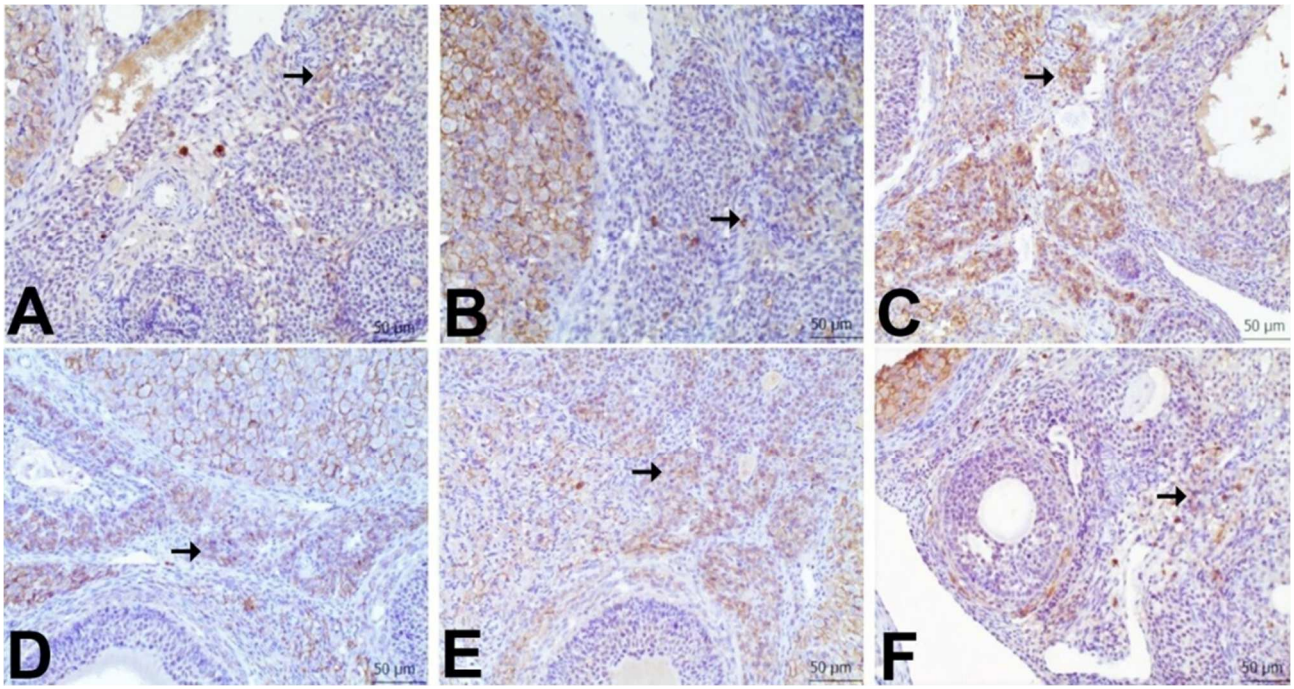

Fig. 3: Caspase-3 immunohistochemistry.
Caspase 3 Immunohistochemistry; (A) Control group and (B) APS group exhibited mild immunopositivity. The (C) Hg group demonstrated very strong immunopositivity. (D) Hg+APS group and (E) Hg+Vit E-Se group exhibited strong immunopositivity. (F) Hg+Vit E-Se+APS group exhibited moderate immunopositivity in stromal cells for Caspase 3 (arrow), as determined by IHC. Each group consisted of 6 animals (n = 6).

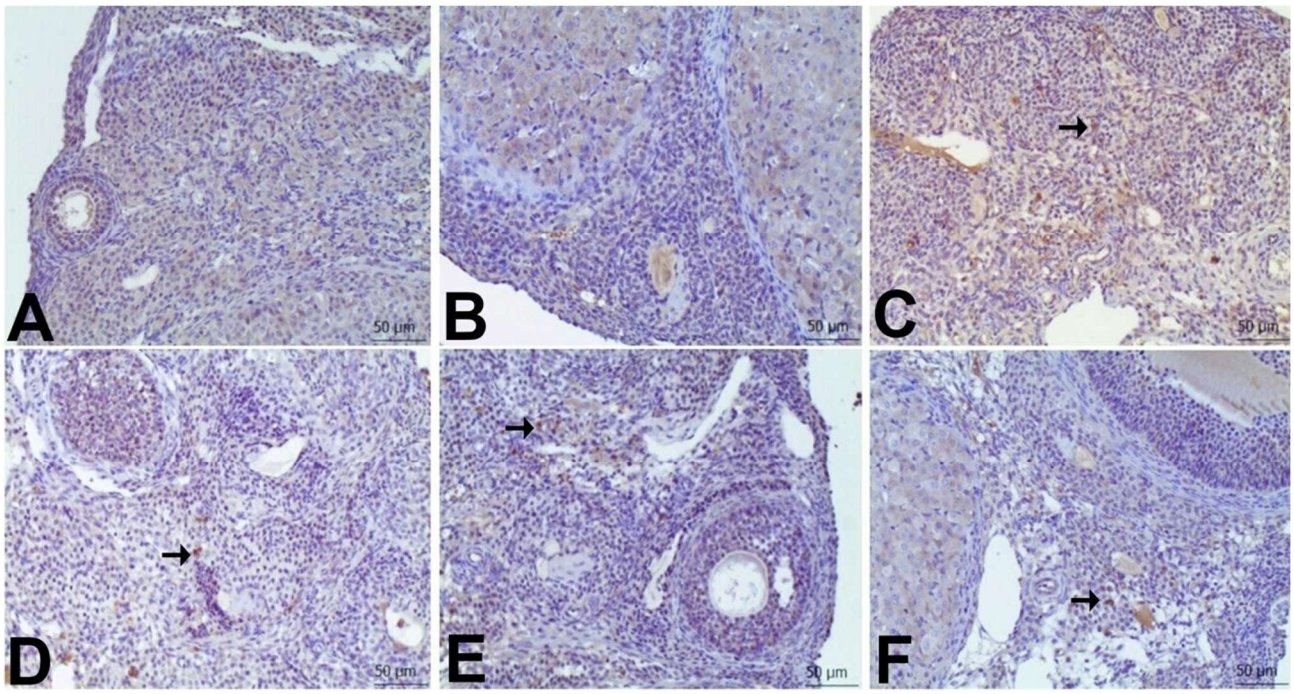

Fig. 4: AIF immunohistochemistry.
AIF Immunohistochemistry; The (A) control group and; (B) APS group exhibited immunonegative results, while the; (C) Hg group demonstrated severe immunopositivity; The (D) Hg+APS group; (E) Hg+Vit E-Se group; and (F) Hg+Vit; (E) Se+APS group exhibited mild immunopositivity in stromal cells for AIF (arrow), as indicated by IHC analysis. Each group consisted of 6 animals (n = 6).

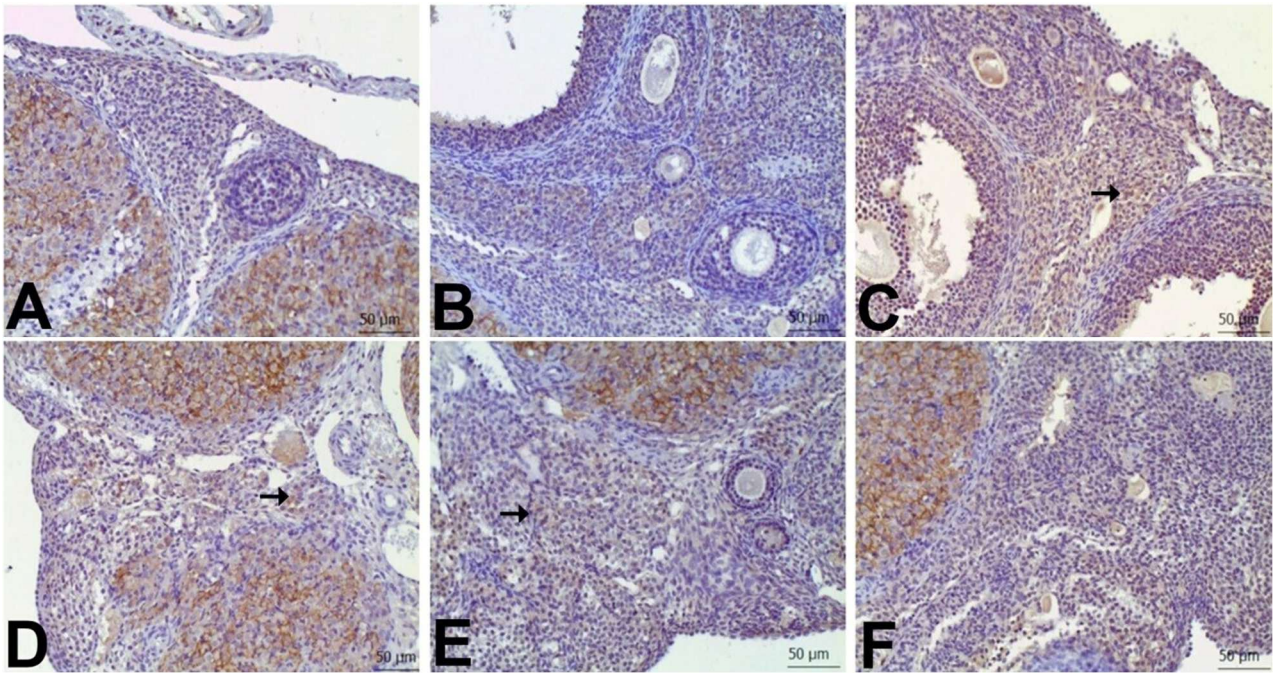

Fig. 5: *NF-κB* immunohistochemistry
NF-κB immunohistochemistry; Group (A) (control group) and Group (B) (APS group) exhibited immunonegativity. Groups (C) (Hg group) and (D) (Hg+APS group) demonstrated severe immunopositivity. Group (E) (Hg+Vit E-Se group] exhibited mild immunopositivity in stromal cells for *NF-κB* (indicated by an arrow). Group (F) (Hg+Vit E-Se+APS group) was immunonegative, as confirmed by immunohistochemistry (IHC). Each group consisted of 6 animals (n = 6).

Mercury-induced oxidative stress is primarily driven by excessive generation of reactive oxygen species (ROS), which target membrane lipids, mitochondrial DNA and intracellular proteins (Zulaikhah *et al.*, 2020; Morcillo *et al.*, 2016). ROS damage cellular structures and compromise reproductive function, whereas antioxidants counteract these effects by scavenging free radicals and repairing oxidative damage (Liang *et al.*, 2023; Morcillo *et al.*, 2016). This oxidative assault can initiate mitochondrial dysfunction, particularly through damage to the inner mitochondrial membrane, leading to loss of membrane potential, cytochrome C release and activation of apoptotic cascades. Lipid peroxidation, as reflected by elevated malondialdehyde (MDA) levels, was significantly increased in both serum and ovarian tissue homogenates of Hg-treated rats. Concurrently, activities of endogenous antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were markedly reduced, indicating compromised antioxidant defenses (Table 2). These data collectively support the hypothesis that oxidative stress is a central mediator of Hg-induced ovarian toxicity. The reversal of these changes following APS, vitamin E and Se *treatment* highlights the therapeutic role of antioxidants in reestablishing redox homeostasis.

Histopathological findings in Hg-exposed ovaries revealed stromal and follicular degeneration, edema, necrosis and cell denudation (Patel *et al.*, 2022; Kumar *et al.*, 2022; Massanyi *et al.*, 2020). These morphological disruptions are closely linked with programmed cell death pathways. In this study, a significant upregulation of apoptosis-associated proteins, including caspase-3 and apoptosis-inducing factor (AIF), was observed in the ovarian tissues of Hg-exposed rats (Figs. 3 and 4).

Both molecules play key roles in intrinsic apoptosis, with AIF translocating from mitochondria to the nucleus to induce chromatin condensation and caspase-3 executing downstream proteolytic cleavage (Maher *et al.*, 2024; Luo *et al.*, 2021; Madungwe *et al.*, 2018). Antioxidant interventions markedly reduced the expression of these markers, suggesting an anti-apoptotic effect that preserves cellular integrity.

In addition, inflammation appears to be an important secondary response to mercury toxicity. NF-κB, a transcription factor that regulates inflammatory gene expression, was elevated in Hg-treated rats (Fig. 5). This may represent either a direct activation by Hg or a consequence of oxidative stress-driven signaling. Interestingly, the literature reports conflicting roles for NF-κB in Hg exposure; some studies indicate suppression, while others report activation depending on co-exposure with other metals or stimuli (Dieguez-Acuna *et al.*, 2004; Alexandrov *et al.*, 2018). In the present study, treatments, particularly the APS combination, attenuated NF-κB expression, indicating reduced inflammation and possibly feedback inhibition of ROS production and apoptosis.

The most pronounced protective effect was observed in the group treated with the combination of APS and Vitamin E-Se. This superior efficacy can be attributed to a synergistic mechanism; while APS directly modulates antioxidant enzyme expression and provides anti-apoptotic signaling, vitamin E and selenium act as potent chain-breaking antioxidants and essential co-factors for glutathione peroxidase, respectively. By targeting different pathways of the mercury-induced oxidative cascade, the combination therapy provides a more comprehensive defense system, effectively neutralizing reactive oxygen species and preserving cellular integrity more efficiently than monotherapies.

CONCLUSION

In conclusion, these results indicate that APS exhibits robust protective properties against Hg-induced ovarian toxicity. These effects are mediated through multiple, interconnected mechanisms: reduction of oxidative stress, suppression of apoptotic signaling, modulation of inflammatory responses and restoration of hormonal balance. Improvements in histopathology and biochemical markers reinforce the therapeutic potential of APS, especially when used synergistically with vitamin E and Selenium.

Nonetheless, this study is not without limitations. The sample size was relatively small and the treatment period short, which may limit the generalizability of the results and prevent assessment of long-term reproductive outcomes. Furthermore, species differences between rodents and humans necessitate cautious extrapolation of findings. Despite these limitations, our data provide compelling preclinical evidence supporting the use of APS-based antioxidant strategies to counteract mercury-induced reproductive toxicity and pave the way for future clinical research.

The findings of this study offer significant potential clinical implications, suggesting that APS, particularly when combined with traditional antioxidants, could serve as a supportive therapeutic strategy to mitigate ovarian damage in populations at high risk of heavy metal exposure. However, translating these animal-based results to human clinical practice requires further investigation. Future research should focus on comprehensive dose-response studies to identify the optimal therapeutic window and long-term human clinical trials to evaluate the safety and efficacy of APS in environmental toxicity scenarios.

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Author's contributions
Begüm Kurt: Conceptualization, methodology and clinical supervision; Mahmut Sahin: Project administration, experimental design, data curation and writing – original draft; Alper S. Kumru: Investigation, formal analysis and software; Haki Kara: Validation, resources, supervision and funding acquisition; Mustafa Özkaraca: Pathological and immunohistochemical analysis, data visualization and review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability statement
The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
This study *was* approved by the Sivas Cumhuriyet University, Animal Experiments Local Ethics Committee (Approval No. 536, dated April 20, 2021). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare no conflict of interest.

Consent to publication
The authors confirm that the submitted manuscript is original, has not been previously published in any language or format and is not under review elsewhere. Written, informed consent for publication was obtained from all individuals whose identifiable data or images are included. Original signed consent forms have been retained and will be provided to the editors upon request.

Supplementary data
https://pjps.pk/uploads/2026/08/SUP1787918274.pdf

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