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Evaluation of combined efficacy of Astragalus extract, vitamin E, and selenium on lead induced toxicity and apoptosis in rat ovaries

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Lead (Pb), a pervasive environmental pollutant, has been linked to various reproductive health issues, including infertility and hormonal imbalances. Species of *Astragalus*, renowned for their antioxidant and anti-apoptotic properties, may mitigate these adverse effects by promoting folliculogenesis and restoring hormone levels disrupted by Pb exposure. In this study, the protective potential of astragalus polysaccharides (APS) against lead induced ovarian toxicity was investigated, both when administered alone and in combination with vitamin E and selenium. The aim was to determine whether the observed protective effects are linked to the antioxidant capacity of APS, and to explore the possible contribution of its anti-apoptotic properties. Thirty-six female Wistar rats (220-250 g) were divided into treatment groups and administered lead, APS, vitamin E, and selenium for a duration of 15 days. Estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and antioxidant levels were measured at the end of the study. Additionally, ovarian tissue damage and apoptosis were assessed through immunohistochemistry. The results indicated that Pb induced reductions in hormone levels were significantly restored to near control levels in the APS treated groups. Moreover, antioxidant levels were notably elevated in these groups. Immunohistochemical analysis revealed a significant reduction in stromal and follicular degeneration in the ovaries of rats treated with APS. In conclusion, APS, both independently and in combination with vitamin E and selenium, exhibited substantial protective effects against Pb induced ovarian toxicity.

Keywords: Astragalus polysaccharides, Lead toxicity, Ovarian damage, Apoptosis, Oxidative stress

Chronic exposure to lead has been associated with various health issues, including hematological, renal, neurological, gastrointestinal, behavioural, and reproductive system disorders[1]. As a known reproductive toxicant, lead disrupts gonadal structures and functions, leading to infertility[2]. Lead impedes follicle formation, oocyte development, ovulation, and corpus luteum formation, resulting in pathological changes[3]. Studies have shown that lead exposure damages reproductive organs in both male and female animals[4,5] with females experiencing impaired fertility, preeclampsia, preterm birth, miscarriages, and infertility[3]. Lead exposure disrupts hormone production, follicular maturation, ovulation, and the ovarian cycle, ultimately leading to decreased fertility and developmental defects[4]. Pollutant-induced ovarian toxicity is primarily driven by oxidative stress and endocrine disruption[6], causing DNA damage, apoptosis, and inflammatory responses[7]. *Astragalus*, a genus of perennial plants found in temperate regions, has approximately 3,000 species[8]. Astragalus polysaccharides (APS) are known for its pharmacological properties, including anticancer, anti-ageing, antiviral, antibacterial, and antioxidant effects[9]. Selenium (Se) and vitamin E are essential nutrients that modulate antioxidant enzyme activity, influencing germ cell proliferation and apoptosis[10]. Both have been shown to mitigate oxidative damage caused by heavy metals, including lead[11].

This study aimed to evaluate whether APS, administered alone or in combination with vitamin E and selenium, enhances their protective effects against lead induced ovarian toxicity. Although previous studies have documented the antioxidant and anti-apoptotic properties of APS, evidence is limited regarding whether its protective effects in lead toxicity are mediated through these mechanisms. This study seeks to contribute to the literature by exploring both aspects of its protective role.

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Materials and Methods

In this study, APS was administered both alone and in combination with vitamin E and selenium, which are known for their protective antioxidant effects against lead toxicity[12]. The antioxidant effects of APS were thus compared with those of vitamin E and selenium, and its potential anti-apoptotic effects were also investigated. In this context, experimental groups were established to assess hormone levels, oxidative stress-related enzyme activities, as well as pathological and immunohistopathological changes associated with oxidative stress and apoptosis.

Preparation of APS membranaceus extract

A total of 100 g of APS membranaceus root and stem were ground into a fine powder and extracted with 1 L of absolute ethanol (10% w/v) by incubation in a water bath at 40 °C for 24 hours. After incubation, for deproteinization added 2% trichloroacetic acid (TCA) the mixture was filtered through a 0.22 µm membrane filter under vacuum to obtain the extract. The filtrate was subsequently concentrated using a rotary evaporator (IKA, CMF300, People′s Republic of China) to remove the ethanol. According to the Chinese Pharmacopoeia (2020), the maximum recommended dose of A. membranaceus for humans is 0.43 g/kg (equivalent to 30 g for a 70 kg individual). Wang *et al*. calculated the maximum dose for rats to be 2.7 g/kg based on the human-to-rat dose conversion formula, and accordingly, the extract was prepared as a 0.27 g/mL solution[13]. Prior to oral administration, each rat was weighed to determine the appropriate dose. The extract was then diluted with distilled water to a total volume of 5 mL and administered via an orogastric tube[14].

Experimental groups and study design

A total of 36 adult female Wistar rats (weighing 220-250 g) were used in this study. The animals were supplied by the Experimental Animal Research and Application Center of Sivas Cumhuriyet University (Sivas, Turkey). During the experimental study, the rats were kept in a controlled room with humidity 50%, temperature 25±2 °C, and 12-hour dark/light cycle with free access to water and food. This study is approved by the Sivas Cumhuriyet University, Animal Experiments Local Ethics Committee (Approval No. 537, dated April 20, 2021).

The rats were randomly assigned to six experimental groups, with each group consisting of six animals. The treatment period was standardised to 15 days for all groups. The doses of the active compounds used in the study were determined based on literature sources[15-17].

The experimental groups were as follows: Control group (saline solution, i.p.); AST (APS 5 mL/kg/day, administered by oral gavage); Lead (lead acetate) (15 mg/kg/day, i.p. for 15 days); Lead + AST; Lead + vitamin E-selenium (Vitamin E 60 mg/kg/day, Selenium 1 mg/kg/day, administered by oral gavage); Lead + vitamin E-selenium + AST.

At the end of the 15-day treatment period, the rats were euthanised by decapitation under ketamine-xylazine anaesthesia. 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

The wild plant *Astragalus microcephalus* Willd. used in this study was collected from a rural area in Sivas, Turkey. The identification of the plant specimens was conducted by Prof. Dr. H. Aşkın AKPULAT, a faculty member in the Department of Biology at Sivas Cumhuriyet University. The root portions of the collected specimens were dried and ground. Subsequently, 1 g of the plant material was extracted with 10 mL of ethanol (10:1 mL extraction solvent/g herb) for 24 hours. After extraction, the mixture was centrifuged (Nuve, NF800, Turkey) at 3075 × g for 10 minutes and then processed using an evaporator (IKA, CMF300, People's Republic of China).

The polysaccharide content in the *Astragalus microcephalus* wild extract was characterised using Quadrupole-Time of Flight Liquid Chromatography/ Mass Spectrometry (Q-TOF LC/MS), following a standard analytical method[18]. The analyses were performed using an Agilent 6530 Accurate Mass Q-TOF-MS/MS system (Agilent Technologies, Santa Clara, CA, USA), coupled with an Agilent 1290 UPLC system and equipped with an electrospray ionisation (ESI) source (Fig. 1).

Biochemical analysis

Following euthanasia, blood and ovarian tissues were collected from the rats. The ovarian tissues were immediately excised under ice-cold conditions,

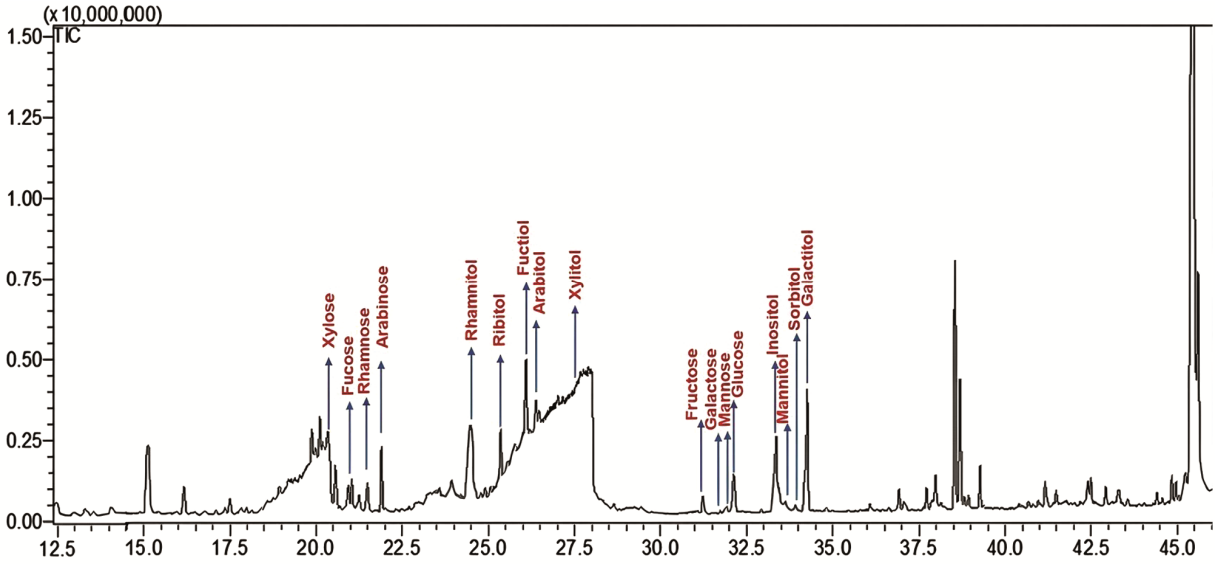

Fig. 1 — Q-TOF LC/MS analysis graphic of Astragalus extract.

carefully blotted free of blood and tissue fluids, weighed, and stored at -80°C (Antech, Eco Toch, Turkey) for subsequent analysis.

Lipid peroxidation was assessed by measuring the malondialdehyde (MDA) level, which reflects the formation of thiobarbituric acid reactive substances (TBARS). MDA was quantified based on its reaction with thiobarbituric acid, forming a colored MDA $(TBA)_2$ complex under aerobic conditions. The intensity of this complex was measured using a spectrophotometer (Perkin Elmer, USA) at 532 nm. MDA concentrations were expressed as "nmol/mL" for serum and "nmol/mg protein" for ovarian tissues. 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 commercially available enzymatic assay kit (Cayman Chemical Company, Ann Arbor, MI). The assay utilises a tetrazolium salt to detect superoxide radicals generated by xanthine oxidase and hypoxanthine. It measures the activity of all three types of SOD (Cu/Zn, Mn, and Fe SOD). One unit of SOD activity is defined as the amount of enzyme required to achieve 50% dismutation of the superoxide radical. Absorbance was measured at 440-460 nm using a plate reader (Thermo, Multiskan GO Microplate, Waltham, USA). SOD activity was expressed as "U/mL" for serum and "U/mg protein" for ovarian and liver tissues.

Glutathione peroxidase (GSH-Px) enzyme activity in blood and ovarian tissues was measured using a commercial assay kit (Cayman Chemical Company, Ann Arbor, Michigan, USA). GSH-Px is a critical antioxidant enzyme that plays an essential role in protecting cells from oxidative damage caused by reactive oxygen species (ROS).

Catalase (CAT) activity in blood and ovarian tissues was determined using the Catalase Colourimetric Activity Kit (Thermo Fisher Scientific, cat no. EIACATC, USA).

Hormone analysis

Estradiol (E2), Follicle-Stimulating hormone (FSH), and Luteinizing hormone (LH) levels were determined using the ELISA method, following the manufacturer's instructions (BT Lab, China). The specific catalogue numbers for the kits used were as follows: Rat Estradiol (E2) ELISA Kit (Cat. No: EA0011Ra), Rat Follicle-Stimulating hormone (FSH) ELISA Kit (Cat. No: EA0015Ra), and Rat Luteinizing hormone (LH) ELISA Kit (Cat. No: EA0058Ra).

Pathologic examination

Necropsies were performed on the rats, and the ovarian tissues were immersed in a 10% buffered formalin solution. The collected samples were then processed according to standard procedures and embedded in paraffin blocks. Sections of 5 µm thickness were obtained from the blocks and examined for histopathological changes under a light microscope using hematoxylin& eosin (H&E) staining. The identified histopathological findings were analysed semi-quantitatively and categorised as absent (0), mild (1), moderate (2), and severe (3).

For immunohistochemical inspection sections of 5 µm, placed on polylysine-coated slides, were subjected to a series of treatments including xylene and alcohol washes, followed by PBS washing. The slides were then incubated in 3% H_2O_2 for 10 minutes to block endogenous peroxidase activity. Antigen retrieval was performed using an antigen retrieval solution, applied for two sessions of 5 minutes each at 500 watts. After applying a protein blocking solution, the tissues were washed with PBS and incubated overnight at +4°C with primary antibodies: 8-OHdG (Santa Cruz, Cat. No. sc-66036, dilution 1:200), Caspase 3 (Biorbyte, Cat. No. Orb382909, dilution 1:100), and Cytochrome C (Abcam, Cat. No. ab110325, dilution 1:300). For secondary detection, the large volume detection system: anti-polyvalent, HRP (Thermo Fisher, Catalog No: TP-125-HL) was used according to the manufacturer′s instructions. DAB (3,3′-Diaminobenzidine) was used as the chromogen. After counterstaining with Mayer′s hematoxylin, the slides were mounted with entellan and examined under a light microscope. The immunopositivity in the ovarian tissues was evaluated semi-quantitatively, categorised as absent (0), mild (1), moderate (2), severe (3) and very severe (4).

Each pathological evaluation was numerically scored to enable statistical analysis, and the results were statistically interpreted. All histopathological evaluations were conducted in a double-blind manner.

Statistical analysis

MDA, SOD, CAT, GSH-Px, and hormone levels were expressed as mean ± standard error and statistically analysed using one-way analysis of variance (ANOVA), followed by Duncan′s multiple range test. All analyses were performed using SPSS software (version 23). The Kruskal-Wallis test was used to assess the statistical significance of differences in histopathological findings. To assess statistical power, post-hoc multiple comparisons were conducted using the Mann-Whitney U test with Bonferroni correction. A *P*-value of less than 0.05 was considered statistically significant.

Results

LC/MS analysis results

In this study, a Q-TOF LC/MS system was used for analysis. The analysis identified 17 distinct monosaccharides and alditol derivatives (Fig. 1). The retention times observed were consistent with the specific chemical properties of polysaccharides and alditols. These findings are in agreement with similar studies reported in the literature.

Hormone level findings

Estradiol, Luteinizing hormone (LH), and Follicle-Stimulating hormone (FSH) levels were measured in the serum samples obtained from the rats included in the study. Significant decreases in hormone levels were observed in all groups treated with Pb (P<0.05). In the treatment groups, hormone levels increased significantly (P<0.05) and approached control levels (Fig. 2).

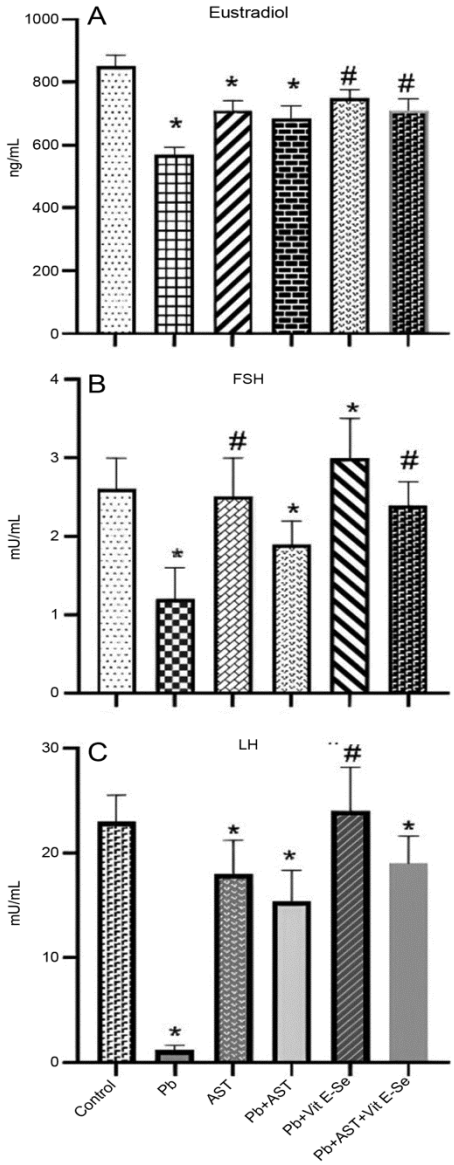

Fig. 2 — Serum hormone levels. (A) Estradiol, (B) Follicle-stimulating hormone, (C) Luteinizing hormone levels were measured in the serum. ($^{*,\#}P$<0.05).

Biochemical parameter findings

In the lead exposed group, MDA enzyme levels were found to be elevated compared to the control group, while antioxidant enzyme levels (SOD, CAT, GSH-Px) were reduced. In contrast, in all groups treated with Astragalus, enzyme levels approached those measured in the control group. The values of MDA, SOD, CAT, and GSH-Px in ovarian tissue and blood samples obtained in the study are presented in Table 1.

Pathologic inspection results

Pathological examinations were performed separately to assess tissue damage, oxidative stress, and apoptosis-related parameters. A statistically significant difference was observed among the groups ($P< 0.05$; Table 2). The ovarian tissues from the control and AST groups displayed a normal histological appearance. The histopathological analysis revealed stromal tissue degeneration in the interstitial regions, as well as follicular degeneration. In the Pb group, stromal degeneration was observed at a severe level, while it was moderate in the Pb+AST and Pb+vit E-Se groups, and mild in the Pb+vit E-Se+AST group. Follicular degeneration was severe in the Pb group, moderate in the Pb+AST and Pb+vit E-Se groups, and mild in the Pb+vit E-Se+AST group. In the degenerating follicles, the normal arrangement of granulosa cells was disrupted, and their structural integrity was compromised (Fig. 3).

Statistically significant differences were observed between the groups concerning the immunoreactivity of 8-OhDG, caspase 3, and cytochrome C (Table 3, $P<0.05$). In 8-OhDG staining, mild immunopositivity was noted in the control and AST groups. In contrast, severe immunopositivity was detected in the Pb and Pb+AST groups, moderate immunopositivity in the Pb+vit E-Se group, and mild immunopositivity in the Pb+vit E-Se+AST group (Fig. 4).

Caspase 3 immunopositivity was absent in the control and AST groups, while it was significantly high in the Pb group, severe in the Pb+vit E-Se group,

Table 2 — Hematoxylin & Eosin (H&E) staining findings

Groups	Stromal degeneration	Follicular degeneration
Control	0.16± 0.40[a]	0.33± 0.51[a]
AST	0.16± 0.40[a]	0.16± 0.40[a]
Pb	2.66± 0.51[b]	2.83± 0.40[b]
Pb+AST	1.66± 0.51[c]	2.16± 0.40[c]
Pb+vit E-Se	1.44± 0.40[c]	2.00± 000[c]
Pb+vit E-Se+AST	1.00± 0.00[d]	1.16± 0.40[d]

[a,b,c,d]Different letters in the same column represent differences between groups. ($P<0.05$)

Table 3 — Immunohistochemical staining findings

Groups	8-OhDG	Caspase 3	Cytochrome C
Control	1.16± 0.40[a]	0.33± 0.51[a]	1.00± 0.40[a]
AST	1.00± 0.00[a]	0.33± 0.51[a]	1.33± 0.51[a]
Pb	2.83± 0.40[b]	3.83± 0.40[b]	3.66± 0.51[b]
Pb+AST	2.26± 0.51[b]	2.14± 0.40[c]	2.23± 0.40[c]
Pb+vit E-Se	1.83± 0.40[c]	1.5± 0.00[d]	2.23± 0.40[c]
Pb+vit E-Se+AST	1.16± 0.40[a]	1.00± 0.00[a]	1.66± 0.51[a]

[a,b,c,d]Different letters in the same column represent differences between groups. ($P<0.05$)

Table 1 — Blood and ovarium biochemical parameters of rats given Pb

Groups	MDA (nmol/protein)		SOD (U/mg protein)		CAT (U/mg protein)		GSH-Px (nmol/mg protein)	
	Blood	Ovarium	Blood	Ovarium	Blood	Ovarium	Blood	Ovarium
Control	6.4±1.2[a]	3.5±0.6[a]	4.2±0.8[a]	1.8±0.4[a]	7.6±1.0[a]	1.5±0.3[a]	92±6.5[a]	62±5.5[a]
Pb	15.8±2.8[a]	8.2±1.2[a]	1.8±0.4[a]	0.5±0.2[a]	2.8±0.4[a]	0.4±0[a]	39±4.5[a]	26±6.2[a]
AST	8.4±1.5[b]	4.4±0.7[b]	3.5±0.3[b]	1.1±0.2[b]	6.4±0.7[b]	1.0±0.4[b]	76±5.4[a]	53±4.2[b]
Pb+AST	11.2±1.5[c]	5.2±0.4[c]	2.8±0.5[c]	0.8±0.3[b]	4.8±0.5[c]	1.2±0.3[c]	59±7.5[b]	44±4.1[c]
Pb+vit E-Se	7.2±1.2[b]	3.8±0.6[c]	3.0±0.4[c]	1.5±0.3[c]	5.4±0.4[b]	1.2±0.4[d]	69±4.8[c]	54±6.2[d]
Pb+AST+vit E-Se	7.0±1.1[d]	4.0±0.3[d]	3.9±0.6[d]	1.6±0.5[d]	6.2±0.6[d]	1.3±0.3[d]	83±5.9[d]	58±5.1[d]

[a,b,c,d]Different letters in the same column represent differences between groups. ($P<0.05$)

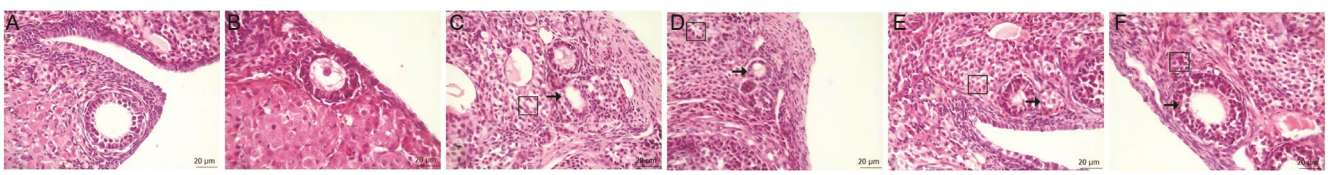

Fig. 3 — Histopathological examination. (A) Control group with normal histological appearance, (B) AST group, (C) Pb group-exhibiting severe degeneration, (D) Pb+AST group-showing moderate degeneration, (E) Pb+vit E-Se group with moderate degeneration, (F) Pb+vit E-Se+AST group demonstrating mild degeneration. Stromal degeneration (□), follicular degeneration (→).

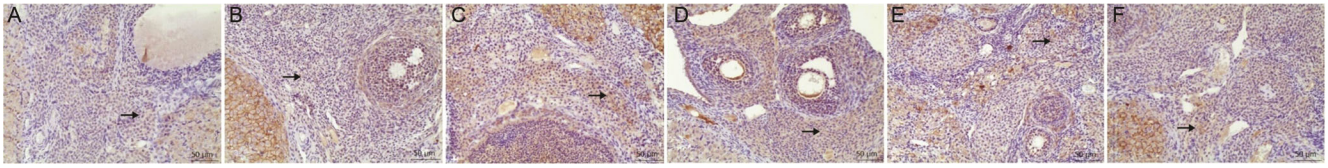

Fig. 4 — 8-OhDG immunohistochemical assessment. (A) Control group and (B) AST group. Mild levels of immunopositivity, (C) Pb group and (D) Pb+AST group. Severe levels of immunopositivity, (E) Pb+vit E-Se group. Moderate levels (F) Pb+vit E-Se+AST group. Mild levels of 8-OhDG immunopositivity in stromal cells (→).

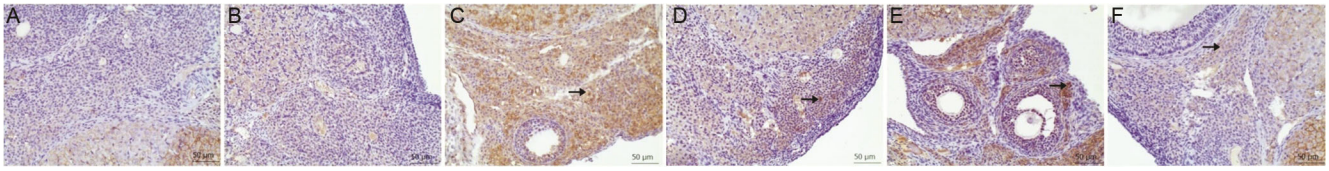

Fig. 5 — Caspase 3 IHC examination. (A) Control group and (B) AST group. Immunonegative, (C) Pb group. Very severe levels (D) Pb+AST group. Moderate levels (E) Pb+vit E-Se group. Moderate levels (F) Pb+vit E-Se+AST group. Mild levels of Caspase 3 immunopositivity in stromal cells (→).

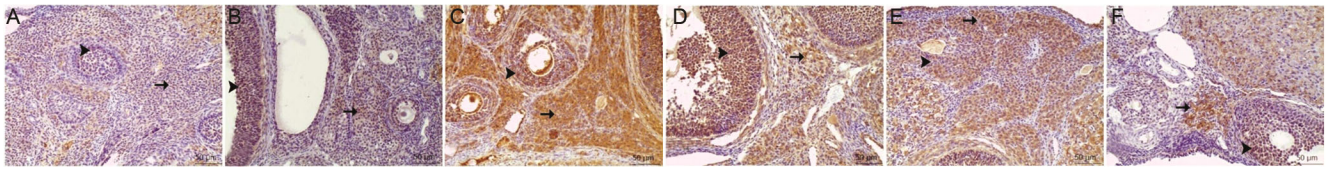

Fig. 6 — Cytochrome C immunohistochemical assessment. (A) Control group and (B) AST group. Mild levels (C) Pb group. Very severe levels, (D) Pb+AST group and (E) Pb+vit E-Se group. Moderate levels of cytochrome C immunopositivity in stromal cells (→) and granulosa cells (➤).

moderate in the Pb+AST group, and mild in the Pb+vit E-Se+AST group (Fig. 5). Immunopositivity for both 8-OhDG and caspase 3 was localised primarily in the stromal cells (Fig. 4 & 5).

In cytochrome C immunohistochemical staining, the most intense positivity was predominantly observed in the stromal cells and granulosa cells within the follicles. The Pb group exhibited a very high level of positivity, which was severe in the Pb+AST and Pb+vit E-Se groups, and moderate in the Pb+vit E-Se+AST group. Mild levels of cytochrome C immunopositivity were noted in the control and AST groups (Fig. 6).

Discussion

Exposure to toxic metals, particularly lead (Pb), poses significant risks to reproductive health. With the rise of industrial activities, the exposure to heavy metals, including lead, has become a growing concern[19]. Lead is widely used in many industrial sectors, and its accumulation in the environment contributes to a variety of health issues, including infertility, particularly in females[4,20].

Lead pollution has raised widespread concerns regarding its impact on reproductive systems across both animal and human populations. Despite efforts such as the banning of lead-based paints and gasoline, lead remains pervasive in the environment due to its non-degradable nature[21]. This persistent exposure can disrupt reproductive functions by interfering with hormonal balance and causing pathological changes in reproductive organs[22,23]. Previous research has explored various treatments for lead poisoning, including chelation with ethylene-diamine-tetraacetic acid (EDTA)[24,25]. While supplementation with vitamin E has shown promise alongside traditional chelation therapies, the potential of plant extracts like *Astragalus* species in mitigating lead toxicity remains underexplored. APS are known to contain active compounds with various beneficial properties, such as antioxidant, anti-inflammatory, and immune-regulatory effects[26,27]. This study investigates the protective effects of APS, both alone and in combination with vitamin E and selenium, against lead-induced toxicity. Lead exposure in female rats has been linked to severe ovarian damage, including congestive and degenerative changes[22]. In both experimental animals and humans, exposure to lead and other toxic metals has been associated with reproductive disorders, including disruptions to menstrual cycles, ovulation, and fertility[22,23,28]. Lead exposure interferes with the hypothalamic-pituitary axis, suppressing the production of critical hormones such as Follicle-

Stimulating hormone (FSH) and Luteinizing hormone (LH), which are essential for normal reproductive function[24,29,30]. Follicle-Stimulating hormone (FSH) stimulates the maturation of oocytes, and an increase in Luteinizing hormone (LH) occurs when oocytes mature in the ovaries. LH, secreted by the pituitary gland, stimulates the release of the oocyte through the rupture of the follicle, a process known as ovulation[31]. The follicle then becomes functional, converting into a corpus luteum, which secretes progesterone for embryo implantation in the uterus[32]. In cases of lead-like metal toxicity, the reduction in estrogen levels inhibits trophoblast formation and decreases progesterone levels, leading to decreased synthesis of LH and FSH[4,33].

In our study shown as Fig. 2, it was observed that lead administration resulted in decreased levels of Estradiol, FSH, and LH, while significant recovery was noted in both the APS treated groups and those receiving the vitamin E + selenium combination. The increase in hormone levels can be attributed to the strong antioxidant effects of APS, vitamin E, and selenium, which reduce tissue damage and may enhance the production of FSH and LH, thereby promoting follicular development and increasing estrogen levels.

Significant increases in IL-6, TNF-α, MDA, nitric oxide, caspase-3, NRF2, NF-κB, and HMOX-1 levels in the ovaries of rats following lead exposure, alongside significant decreases in antioxidants[34]. Lead exposure can lead to strong oxidative stress and an increase in reactive oxygen species (ROS)[35,36]. Antioxidants support the female reproductive system positively by preventing the formation of reactive oxygen species, scavenging existing free radicals, and facilitating the repair of damaged cellular structures triggered by ROS[3,7,37]. In our study, as shown in Table 1, MDA levels increased significantly in rats treated with lead acetate, while CAT, SOD, and GSH activities decreased markedly. However, the administration of AST extract restored these activities to near-control levels. Pb poisoning has been shown to significantly reduce oocyte maturation and fertilization *in vivo*, and to decrease catalase, glutathione peroxidase, and total superoxide dismutase activities in mouse ovaries[6]. Pb exposure primarily exerts cytotoxic effects on folliculogenesis through oxidative stress, subsequently inducing other damage pathways. Therefore, adding exogenous antioxidants can be effective in mitigating the toxic effects of Pb on cells or tissues. For instance, oral administration of vitamins C and E significantly reduced blood Pb concentrations and alleviated oxidative stress in the brain of rats, improving liver damage[38]. Furthermore, the intake of vitamins C and E has been shown to regulate the interaction of thiamine, reducing blood Pb levels and its accumulation in the kidneys and bones during both simultaneous and post-exposure Pb treatment[39-41].

Results of our study indicated that while MDA levels increased in both blood and ovarian tissues of rats exposed to lead, significant decreases were observed in antioxidant parameters such as SOD, CAT, and GSH-Px. In all treatment groups, there were significant changes compared to the lead-exposed group, indicating that the oxidative stress induced by lead was alleviated by ASP, vitamin E, and selenium.

There is no "safe" level of Pb exposure. Low levels of Pb accumulation in mouse ovaries could disrupt folliculogenesis by increasing the number of less primitive and atretic antral follicles[3]. Histopathological changes in ovaries may occur as a result of lead exposure[31]. Reports have documented pathological features such as widespread edema, necrosis in ovarian follicles, and denudation at various stages in ovarian follicles following lead acetate intoxication[2]. In study, it was shown that increasing the dose of lead acetate from 0.5 mg/kg to 60 mg/kg caused morphological changes in the cortex and medulla of rat ovaries[2]. Experimental studies have reported effects such as oocyte damage, corpus luteum disintegration, reduced fertility, and edema and necrosis in follicles following acute and chronic lead exposure[42].

In our study, histopathological examination of ovarian tissues revealed stromal and follicular degeneration in the lead-exposed group, while these degenerative changes were reduced in the treatment groups (Fig. 3 & Table 2).

Oxidative stress can lead to DNA damage, resulting in the production of various byproducts. One of the key markers 8-OHdG, which is generated through the oxidation of guanine[43,44]. This modification occurs when hydroxyl radicals interact with DNA, causing the formation of 8-hydroxyguanine, which is then converted to 8-OHdG. As a result, 8-OHdG serves as an important biomarker for detecting oxidative DNA damage[43]. In our study, a statistically significant increase in 8-OHdG immunoreactivity was observed in all groups exposed to lead. However, significant decreases in

immunoreactivity were noted in the groups treated exclusively with ASP and those administered vitamin E combined with selenium (Fig. 4).

Caspases are a group of proteases that play a pivotal role in programmed cell death, or apoptosis[45]. These enzymes are synthesised as inactive precursors and are activated through proteolytic cleavage. Once activated, caspases degrade proteins that are involved in DNA repair, promoting the activation of endonucleases, which are responsible for DNA degradation[46]. Apoptosis acts as a cellular safeguard, ensuring the removal of cells with damaged DNA. In our study, an increase in caspase 3 levels was observed in all groups exposed to lead. In contrast, significant reductions in caspase 3 levels were noted in the groups treated with ASP alone and those receiving a combination of vitamin E and selenium (Fig. 5).

Cytochrome C is primarily localised in the mitochondrial intermembrane space, but under certain pathological or stress-induced conditions, its distribution can extend to the cytosol, nucleus, and extracellular space[47]. Within the mitochondria, cytochrome C functions as an electron carrier in the electron transport chain, contributing to adenosine triphosphate (ATP) synthesis, regulating cardiolipin peroxidation, and modulating the dynamics of reactive oxygen species[48]. Under cellular stress, cytochrome C can be released into the cytosol, where it triggers caspase-dependent apoptotic cell death[49,50]. In this study, an increase in cytochrome C levels was observed in all groups exposed to lead. In contrast, significant reductions in cytochrome C levels were recorded in the groups treated with APS, vitamin E, and selenium (Fig. 6). The correlation between the 8-OHdG and caspase-3 results and the cytochrome C findings in our study suggests that tissue damage is associated with apoptosis induced by oxidative stress.

Immunohistochemical examination of tissues, a statistically significant increase in immunoreactivity for 8-hydroxy-2′-deoxyguanosine (8-OHdG), caspase-3, and cytochrome C was detected in all groups exposed to lead. Significant reductions were observed in the groups treated solely with ASP and those receiving vitamin E + selenium (Table 3).

Conclusion

In conclusion, the findings of our study demonstrate that APS exhibits strong protective effects against lead-induced ovarian toxicity, both when administered alone and in combination with vitamin E and selenium. In all groups exposed to lead, we observed a reduction in hormone levels, an increase in oxidative enzyme levels, and a decrease in antioxidative enzyme levels. However, in the groups treated with APS extract, vitamin E, and selenium, hormone and antioxidant enzyme levels approached those of the control group. These results suggest that APS exerts its protective effects by mitigating oxidative stress in the ovaries.

Furthermore, histopathological and immune-histochemical evaluations revealed stromal and follicular damage in the ovaries of lead exposed animals. While damage to the stroma and follicles was more pronounced in the lead exposed groups, treatment with APS resulted in outcomes comparable to those observed in the control group. To explore the underlying cellular mechanisms, we assessed oxidative DNA damage using 8-OHdG and examined apoptosis through the markers caspase 3 and cytochrome C. Immunohistochemical analysis revealed significant increases in 8-OHdG, caspase 3, and cytochrome C levels in the lead exposed groups. In contrast, all these markers returned to near control group levels in the APS treated groups. These findings suggest that oxidative stress leads to DNA damage and apoptosis in lead-induced ovarian toxicity, and that APS mitigates these effects by reducing oxidative damage and suppressing apoptosis.

The results obtained from our study are consistent with previous studies in the literature and further support our findings. Based on the findings of this study, it can be concluded that the administration of APS, both alone and in combination with vitamin E and selenium, provides a substantial protective effect against ovarian toxicity induced by lead exposure. This protective effect is likely attributable to the antioxidant properties of these substances, which reduce apoptosis and tissue damage.

This study has several limitations that should be considered when interpreting the findings. First, the relatively small sample size may limit the generalizability of the results. Second, the short duration of treatment restricts the ability to assess long term effects of the interventions. Lastly, as this study was conducted on an animal model, extrapolating the results to humans should be approached with caution due to physiological differences between species. Despite these limitations, the findings provide valuable preliminary insights and underscore the need for further long-term and clinical studies to validate the protective effects observed.

Ethical statement

All experimental procedures applied in this study were examined by Sivas Cumhuriyet University Experimental Animal Research Ethics Committee and approved on 20.04.2021 with the number 537.

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Conflicts of interest

The authors declare no conflict of interest.

References

1 Mandal GC, Mandal A & Chakraborty A, The toxic effect of lead on human health: A review. *Human Biology and Public Health,* 3 (2022) 1.

2 Osowski A, Fedoniuk L, Bilyk Y, Fedchyshyn O, Sas M, Kramar S, Lomakina Y, Fik V, Chorniy S & Wojtkiewicz J, Lead exposure assessment and its impact on the structural organization and morphological peculiarities of rat ovaries. *Toxics,* 11 (2023) 769.

3 Ogunlade B, Gbotolorun SC & Ogunlade AA, D-ribose-L-cysteine modulates lead acetate-induced hematobiochemical alterations, hormonal imbalance, and ovarian toxicity in adult female Wistar rats. *Drug and Chemical Toxicology,* 45 (2022) 1606.

4 Qu J, Niu H, Wang J, Wang Q & Li Y, Potential mechanism of lead poisoning to the growth and development of ovarian follicle. *Toxicology,* 457 (2021) 152810.

5 Zhou L, Wang S, Cao L, Ren X, Li Y, Shao J & Xu L, Lead acetate induces apoptosis in Leydig cells by activating PPARγ/caspase-3/PARP pathway. *International journal of environmental health research,* 31 (2021) 34.

6 Jiang X, Xing X, Zhang Y, Zhang C, Wu Y, Chen Y, Meng R, Jia H, Cheng Y & Zhang Y, Lead exposure activates the Nrf2/Keap1 pathway, aggravates oxidative stress, and induces reproductive damage in female mice. *Ecotoxicology and environmental safety,* 207 (2021) 111231.

7 Hong W, Liu Y, Liang J, Jiang C, Yu M, Sun W, Huang B, Dong N, Kang L & Tang Y, Molecular mechanisms of selenium mitigating lead toxicity in chickens via mitochondrial pathway: Selenoproteins, oxidative stress, HSPs, and apoptosis. *Toxics,* 11 (2023) 734.

8 Salehi B, Carneiro JNP, Rocha JE, Coutinho HDM, Morais Braga MFB, Sharifi-Rad J, Semwal P, Painuli S, Moujir LM & de Zarate Machado V, APS species: Insights on its chemical composition toward pharmacological applications. *Phytotherapy Research,* 35 (2021) 2445.

9 Abd Elkader H-TAE, Essawy AE & Al-Shami AS, APS species: Phytochemistry, biological actions and molecular mechanisms underlying their potential neuroprotective effects on neurological diseases. *Phytochemistry,* 202 (2022) 113293.

10 Jahan M, Islam M, Gautam M & Bhuiyan M, Lead acetate induced toxicities and antitoxic effect of Vitamin E and selenium in mice. *Bangladesh Journal of Veterinary Medicine (BJVM),* 19 (2021) 75.

11 Kapper C, Stelzl P, Oppelt P, Ganhör C, Gyunesh AA, Arbeithuber B & Rezk-Füreder M, The Impact of Minerals on Female Fertility: A Systematic Review. *Nutrients,* 16 (2024) 4068.

12 Sentkowska A & Pyrzyńska K, The influence of synthesis conditions on the antioxidant activity of selenium nanoparticles. *Molecules,* 27 (2022) 2486.

13 Wang Z, Borjigin G, Zhang M, Yang C, Wang Z & Kuang H, Simultaneous determination and pharmacokinetics study of three triterpenoid saponins in rat plasma by ultra-high-performance liquid chromatography tandem mass-spectrometry after oral administration of APS Membranaceus leaf extract. *Journal of Separation Science,* 46 (2023) 2300282.

14 Liu M, Di YM, May B, Zhang AL, Zhang L, Chen J, Wang R, Liu X & Xue CC, Renal protective effects and mechanisms of APS membranaceus for diabetic kidney disease in animal models: An updated systematic review and meta-analysis. *Phytomedicine,* (2024) 155646.

15 Tatli Seven P, Iflazoglu Mutlu S, Seven I, Arkali G, Ozer Kaya S & Kanmaz OE, Protective role of yeast beta-glucan on lead acetate-induced hepatic and reproductive toxicity in rats. *Environmental Science and Pollution Research,* 28 (2021) 53668.

16 Jafari Khorchani M, Samare-Najaf M, Abbasi A, Vakili S & Zal F, Effects of quercetin, vitamin E, and estrogen on Metabolic-Related factors in uterus and serum of ovariectomized rat models. *Gynecological Endocrinology,* 37 (2021) 764.

17 Wang S, Hou L, Wang M, Feng R, Lin X, Pan S, Zhao Q & Huang H, Selenium-alleviated testicular toxicity by modulating inflammation, heat shock response, and autophagy under oxidative stress in lead-treated chickens. *Biological Trace Element Research,* (2021) 1.

18 Yang M, Li J, Zhao C, Xiao H, Fang X & Zheng J, LC-Q-TOF-MS/MS detection of food flavonoids: Principle, methodology, and applications. *Critical reviews in Food Science and Nutrition,* 63 (2023) 3750.

19 Raj K & Das AP, Lead pollution: Impact on environment and human health and approach for a sustainable solution. *Environmental Chemistry and Ecotoxicology,* 5 (2023) 79.

20 Mukherjee AG, Wanjari UR, Renu K, Vellingiri B & Gopalakrishnan AV, Heavy metal and metalloid-induced reproductive toxicity. *Environmental Toxicology and Pharmacology,* 92 (2022) 103859.

21 Elayaperumal S, Sivamani Y, Bhattacharya D, Lahiri D & Nag M, Eco-Friendly Biosurfactant Solutions for Petroleum Hydrocarbon Cleanup in Aquatic Ecosystems. *Sustainable Chemistry for the Environment,* (2025) 100207.

22 Hassan MM, Chughtai AH, Ali M, Yasin I, Rehman UU, Haider K, Ullah H, Batool S & Abdullah M, Heavy Metal Toxicity in Human Health: Mechanisms, Impacts, and Mitigation Strategies. *Dialogue Social Science Review (DSSR),* 2 (2024) 533.

23 Ramakrishnan R & Nagella P, The accumulation, antioxidant defences, and secondary metabolite production in common sage (Salvia officinalis L.) under lead toxicity. *Medicinal Plants - International Journal of Phytomedicines and Related Industries,* 16 (2024) 519.

24 Jomova K, Alomar SY, Nepovimova E, Kuca K & Valko M, Heavy metals: Toxicity and human health effects. *Archives of Toxicology,* (2024) 1.

25 Xie Y-H, Song H-X, Peng J-C, Li S-J, Ou S-Y, Aschner M & Jiang Y-M, Treatment of Manganese and Lead Poisoning with Sodium Para-aminosalicylic Acid: A Contemporary Update. *Toxicology Letters,* (2024).

26 Satarug S, C. Gobe G, A. Vesey D & Phelps KR, Cadmium and lead exposure, nephrotoxicity, and mortality. *Toxics,* 8 (2020) 86.

27 Colak S, Comlekcioglu N, Aygan A, Kocabas YZ & Comlekcioglu U, Phytochemical Properties and Bioactive Potential of Various APS spp. from Turkey. *Food Bioscience,* (2025) 105901.

28 Karimian M, Ghadiri M, Poormoosavi SM & Najafzadehvarzi H, Protective effects of resveratrol on the expression of catalase, glutathione peroxidase, and superoxide dismutase genes in the ovary and their activity in the serum of rats exposed to lead acetate: An experimental study. *International Journal of Reproductive BioMedicine,* 22 (2025) 883.

29 Onitsha EN & Okutu JB, Influence of Vitamin E and Selenium on Reproductive Hormones and Lipid Peroxidation Levels in Lead-induced Toxicity in Female Wistar Rats. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT),* 15 (2021) 01.

30 Rami Y, Ebrahimpour K, Maghami M, Shoshtari-Yeganeh B & Kelishadi R, The association between heavy metals exposure and sex hormones: a systematic review on current evidence. *Biological Trace Element Research,* (2022) 1.

31 Trujillo-Vázquez S, Gaona-Domínguez S, Villeda-González J, Librado-Osorio R, Luna-López A, Bonilla-González E, Valencia-Quintana P & Gómez-Olivares J, Oxidative stress in the ovaries of mice chronically exposed to a low lead concentration: A generational approach. *Reproductive Toxicology,* 115 (2023) 40.

32 Shan D, Wen X, Guan X, Fang H, Liu Y, Qin M, Wang H, Xu J, Lv J & Zhao J, Pubertal lead exposure affects ovary development, folliculogenesis and steroidogenesis by activation of IRE1α-JNK signaling pathway in rat. *Ecotoxicology and Environmental Safety,* 257 (2023) 114919.

33 Wang X, Ding N, Harlow SD, Randolph Jr JF, Mukherjee B, Gold EB & Park SK, Exposure to heavy metals and hormone levels in midlife women: the Study of Women's Health Across the Nation (SWAN). *Environmental Pollution,* 317 (2023) 120740.

34 Eddie-Amadi BF, Ezejiofor AN, Orish CN & Orisakwe OE, Zn and Se abrogate heavy metal mixture induced ovarian and thyroid oxido-inflammatory effects mediated by activation of NRF2-HMOX-1 in female albino rats. *Current Research in Toxicology,* 4 (2023) 100098.

35 Shi L, Wang X, Duan Y, Li K & Ren Y, Antagonistic effects of selenium on lead-induced oxidative stress and apoptosis of Leydig cells in sheep. *Theriogenology,* 185 (2022) 43.

36 Xu J, Zhao M, Ge X, Liu X, Wei L, Li A, Mei Y, Yin G, Wu J & Xu Q, Association Between Metal Exposure and Mitochondrial DNA Parameters and the Potential Mediation Effect of Oxidative Stress. *Exposure and Health,* (2024) 1.

37 Takalani NB, Monageng EM, Mohlala K, Monsees TK, Henkel R & Opuwari CS, Role of oxidative stress in male infertility. *Reproduction and Fertility,* 4 (2023).

38 Mesalam NM, Ibrahim MA, Mousa MR & Said NM, Selenium and vitamin E ameliorate lead acetate-induced hepatotoxicity in rats via suppression of oxidative stress, mRNA of heat shock proteins, and NF-kB production. *Journal of Trace Elements in Medicine and Biology,* 79 (2023) 127256.

39 Shalan MG, Mitigating lead acetate-induced histopathologic and physiologic disorders in rats receiving vitamin C and glutathione supplement. *Heliyon,* (2024).

40 Raeeszadeh M, Zobeiri A, Khademi N & Mortazavi P, Nephroprotective Effect of Methanolic Broccoli (Brassica olaracea, var. Italica) Extract on Oxidative-lead Damage in Mice Kidney: Biochemical Parameters and Phatological changes. *Indian Journal of Experimental Biology (IJEB),* 59 (2021) 626.

41 El-Fahla NA, Mohallal ME, Gad El-Hak HN, Dessouki AA & Abdelrazek H, Effect of lead toxicity on the kidney of Nile tilapia: amelioration by Beta-MOS®. *Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry,* 14 (2022) 31.

42 Dutta S, Gorain B, Choudhury H, Roychoudhury S & Sengupta P, Environmental and occupational exposure of metals and female reproductive health. *Environmental Science and Pollution Research,* 29 (2022) 62067.

43 Sable H & Singh V, 8-OHdG: A Biomarker for Lead and Cadmium Toxicity. *International Journal of Medical Toxicology & Legal Medicine,* 26 (2023) 11.

44 Liao G, Weng X, Wang F, Yu YHK, Arrandale VH, Chan AH-s, Lu S & Tse LA, Urinary metals and their associations with DNA oxidative damage among e-waste recycling workers in Hong Kong. *Ecotoxicology and Environmental Safety,* 284 (2024) 116872.

45 Maher AM, Elsanosy GA, Ghareeb DA, Elblehi SS & Saleh SR, Correction: 10-Hydroxy Decanoic Acid and Zinc Oxide Nanoparticles Retrieve Nrf2/HO-1 and Caspase-3/Bax/Bcl-2 Signaling in Lead-Induced Testicular Toxicity. *Biological Trace Element Research,* (2024) 1.

46 Xie S, Yin M, Xiang M, Shao L, Zhang N, Shi L, Zhang J & Yu G, Lead (Pb) Induces Osteotoxicity Through the Activation of Mutually Reinforced ER Stress and ROS in MC3T3-E1 Cells. *Biological Trace Element Research,* (2024) 1.

47 Povea-Cabello S, Brischigliaro M & Fernández-Vizarra E, Emerging mechanisms in the redox regulation of mitochondrial cytochrome c oxidase assembly and function. *Biochemical Society Transactions,* 52 (2024) 873.

48 Zhou Z, Arroum T, Luo X, Kang R, Lee YJ, Tang D, Hüttemann M & Song X, Diverse functions of cytochrome c in cell death and disease. *Cell Death & Differentiation,* 31 (2024) 387.

49 Korotkov SM, Mitochondrial oxidative stress is the general reason for apoptosis induced by different-valence heavy metals in cells and mitochondria. *International Journal of Molecular Sciences,* 24 (2023) 14459.

50 Sun Q, Li Y, Shi L, Hussain R, Mehmood K, Tang Z & Zhang H, Heavy metals induced mitochondrial dysfunction in animals: Molecular mechanism of toxicity. *Toxicology,* 469 (2022) 153136