Investigation of the effects of pomegranate juice against toxicity in the testes in lead-administered rats

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SUMMARY

Lead (Pb) is a heavy metal that can damage various organs, tissues, and systems. Antioxidants are used to prevent or reduce the negative effects of heavy metals. Pomegranate juice (PJ) is one of the antioxidants that alleviate the harmful effects of heavy metals. In this study, 28 Wistar albino rats were divided into four groups. These groups were determined as control (C), lead acetate (LA) (50 mL/kg per rat), PJ (1 mL), and LA + PJ. It was observed that the malondialdehyde level of the LA+PJ decreased compared to the LA. The glutathione level of the LA decreased compared to other groups. LA + PJ had higher glutathione S-transferase enzyme activity than C and lower than LA. Carboxylesterase activity increased in the LA + PJ compared to C and LA. Pb level in the LA + PJ was higher than C and lower than LA. Also, Mn levels increased in LA+PJ compared to LA. Testosterone decreased in LA but increased in PJ and LA+PJ compared to LA. LA and PJ had similar histological structures to C and PJ. It was determined that PJ had a curative effect against Pb toxicity in testicular tissue.

Key words: Antioxidants – Lead acetate – Oxidative stress – Pomegranate juice – Testes

INTRODUCTION

Population growth and industrialization result in heavy metal pollution that harms living organisms ecologically, physiologically, biologically, and economically (Li et al., 2021).

Lead (Pb) is considered one of the top ten most hazardous substances by the World Health Organization, as it can linger in the body for extended periods (Kucukler et al., 2021). Exposure to Pb can lead to renal dysfunction, liver cirrhosis, damage to the cardiovascular system, and anemia. It can cause pathological damage to the ovaries and testes, resulting in poor reproductive performance. Furthermore, lead has the potential to cause harm to cells and be toxic when it interacts with biological materials. In recent years, research has focused on using antioxidants as a preservative against the harmful effects of lead (Oyeyemi et al., 2022).

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This metal, commonly found in household products and paints, can mix with soil and air as dust or flakes, leading to pollution of water, food, and air (Jahan et al., 2021; Khodabandeh et al., 2021). Exposure in humans occurs through water, food intake, and respiration (Jahan et al., 2021; Yousef et al., 2019). Lead absorbed into soft tissues may cause dysfunction in organs including the liver, kidney, brain, testis, uterus, and ovary (Abdelhamid et al., 2020; Dumitrescu et al., 2015). In addition, it has been reported to have adverse effects on the cardiovascular, digestive, and skeletal systems (Davuljigari and Gottipolu, 2020; El-Khadragy et al., 2020). Pb poisoning usually occurs with chronic exposure. However, occupational exposure to lead-containing agents and accidental or suicidal ingestion of these agents can cause acute poisoning (Samarghandian et al., 2021).

The use of antioxidants to prevent or reduce the adverse effects of heavy metals has attracted significant interest from researchers (Ozkaya et al., 2016; Yousef et al., 2019). One of the antioxidants, Pomegranate juice (PJ) contains compounds that mitigate the harmful effects of heavy metals. PJ contains ascorbic acid and minerals Fe, Ca, Mg, Se, and Zn (Aksu et al., 2017). PJ has three times the free radical scavenging and iron-reducing capacity of both red wine and green tea (Aksu et al., 2017). The beneficial effects of pomegranate, which is widely grown in Mediterranean countries, have been known for a long time (El-Beltagi et al., 2020; Mayuoni-Kirshenbaum et al., 2013). The pomegranate plant has multiple uses in traditional medicine, with its seeds and peels treating diarrhea, flowers used for diabetes, bark and roots for ulcers, and leaves for digestive disorders (Pepe et al., 2020). Studies have shown that pomegranate consumption leads to healthier cell growth and longer life span in rats (Alsataf et al., 2021). Studies have shown that the consumption of PJ can improve the concentration of spermatids, spermatocytes, and spermatogonia, increase sperm motility and testosterone levels, and enhance sexual behaviors (Al-Mutary and Abu-Taweel, 2020; Türk et al., 2008). Literature has reported that PJ can prevent oxidative stress and boost fertility (Al-Mutary and Abu-Taweel, 2020; Alahmadi, 2020; Bououza et al., 2022). In addition, PJ contains anthocyanins delphinidin-3-glucosidase, delphinidin-3.5 glucosidase, cyanidin, pelargonidin, and ellagitannins. The antioxidant property of PJ is due to punicalagin, one of the main ellagitannins in its content (El-Beltagi et al., 2020). Adıyaman hicaz pomegranate, rich in vitamins and minerals, is a fruit grown in Turkiye.

Oxidative stress occurs because of increased reactive oxygen species (ROS) by decreasing antioxidant enzyme activities. Excessive production of ROS caused by lead acetate affects the blood-testicular barrier and leads to degeneration in testicular tissue, resulting in decreased sperm count and quality (Dolati et al., 2020; El-Khadragy et al., 2020; Ileriturk et al., 2021). Major components of the sperm cell membrane are highly susceptible to oxidative damage. Therefore, free radicals can directly damage cells at high concentrations of superoxide anion, hydrogen peroxide, and nitric oxide (Dorostghoal et al., 2020; Ozkaya et al., 2018). It has been observed that pomegranates increase testosterone levels and help spermatogenesis (Dkhil et al., 2013). In addition, it is known that pomegranate extract positively affects oxidative stress parameters in testicular tissue and prevents spermatogenic deterioration (Leiva et al., 2011).

Antioxidant molecules, alone or in combination, are used for preventive or therapeutic purposes. Studies investigating pomegranate juice's antioxidant, neuroprotective, antineoplastic, and anti-inflammatory properties have been used safely in traditional and modern medicine for centuries (Annaç et al., 2022). The objective of our study is to investigate the potential of pomegranate juice in mitigating the detrimental effects of lead acetate on testicular tissue. Our examination includes analysis of biochemical parameters such as malondialdehyde (MDA), glutathione (GSH), glutathione S-transferase (GST), carboxylesterase (Ces), element levels, and histopathological findings in rats.

MATERIALS AND METHODS

Pomegranate juice preparation

The pomegranates obtained from Adıyaman were washed, filtered, and cut in half. Then, the seeds and the whole white pulp were crushed us-

ing an electric mixer. The juice was stored at -20 °C in qies of 1 mL/kg for later use.

Chemical composition of pomegranate juice

Our researcher investigated the chemical content of a local product obtained from Adıyaman province in a study funded by the Silk Road Development Agency as part of the "My City Adıyaman" project numbered "TRC1/18/KBG/0.036" by the "Development of Urban Culture and Urban Awareness Financial Support Program". The phenolic acid content of PJ was determined to be 490.75 mg/kg, anthocyanin 137.1 mg/L, ellagic acid 175 mg/100 g, total flavonoids 63 mg/kg, and total antioxidants 1530 mg/kg by researchers (Annaç et al., 2022).

Preparation of lead acetate

500 ppm Lead acetate was dissolved in 1 liter of distilled water (Annaç et al., 2022).

Preparation of pomegranate juice

PJ is prepared with a juicer for gavage application (Annaç et al., 2022).

Animals

A total of 28 male Wistar albino rats were obtained from the Fırat University Animal Experiment Centre. All animals were housed at a temperature of 22 ± 20°C and a 12-hr light/darkness cycle. Rats were fed ad libitum with normal tap water and a standard pellet diet (purchased from Korkutelim Yem Gıda, Antalya, Turkey; % 3.15 crude fat, % 4.96 crude cellulose, crude ash % 4.91, % 0. 43 methionine, % 1.30 lysine, % 0.62 calcium, % 0.74 phosphor, 0.04 sodium, % 24.00 crude protein). The Fırat University Animal Experiments Centre Ethics Committee approved all animal procedures that followed National Institutes of Health (NIH) guidelines (2022/16).

The study groups and applications are given in Table 1.

The rats were sacrificed using ketamine hydrochloride and xylazine hydrochloride at the end of the 30-day experiment. The left testis was stored in 10% formaldehyde for histopathological and morphological analysis, while the right testis was stored at -20 °C for biochemical analysis.

Biochemical Procedures

The testes samples were first homogenized in a cooled homogenization buffer (0.1 M, pH 7.4 in potassium phosphate buffer; 0.15 M KCl, one mM EDTA, one mM DTT) at four times the total tissue weight (w/v) using a polytron homogenizer (Heidolph RZ 2021, Germany). The MDA levels in one portion of the homogenates were calculated. The leftover homogenates were put into Eppendorf tubes and centrifuged for 20 min at 16.000 g and 4 °C (Sigma Centrifuge Model 2–16K, Sigma). The supernatant was used to determine the reduced amounts of GSH, GST, and Ces.

Determination of testes MDA and reduced GSH levels

Using a microplate reader spectrophotometer equipment, MDA and decreased GSH levels were measured (Thermo TM Varioskan Flash, Thermo Scientific). The thiobarbituric acid reaction was employed to evaluate the testicular malondialdehyde levels (Buege and Aust, 1978). Wet tissue weight (nmol/mg) was used to express the results (Koksal et al., 2003). The substance's interaction with DTNB to produce a molecule that absorbs at 412 nm was used to gauge reduced GSH activity. As nmol/mg tissue, reduced GSH was expressed (Moron et al., 1979).

Table 1. The study groups and application. Four groups were formed, with seven animals in each group.

C	Rats were given distilled water for 30 days.
PJ	Rats were given PJ via 1 mL/kg gavage every two days for 30 days.
LA	Rats were given an average of 50 mL/kg LA from a stock solution per animal every day for 30 days.
LA+PJ	Rats were given 50 mL/kg LA from stock solution daily and 1 mL PJ every two days for 30 days.

Determination of testes trace element levels

Pb, Fe, Mn, Zn, and Cu were measured by Agilent 7700 x ICP-MS in rat testes tissues. Spex Certiprep Multi-element calibration standard was used to prepare an external calibration solution.

Measurement of testes GST activity

The substrate used to test GST activity was a solution containing 20 mM of 1-chloro-2.4- dinitrobenzene (CDNB), which was initially produced in 96% ethanol. In the reaction, reductive glutathione (0.002 M) served as the cofactor (Habig et al., 1974). Briefly, the microplate wells were filled with ten microliters of supernatant, 100 microliters of phosphate buffer (0.1 M, pH 6.5), 100 microliters of the GSH mixture, and ten microliters of CDNB. These were inserted into the microplate reader system, and the change in absorbance was monitored for two min at 25°C while the light was at 344 nm. As nmol/min/mg protein, specific GST activity was determined.

Determination of testicular total Ces activity

Spectrophotometric methods adapted to the microplate reader system (ThermoTM Varioskan Flash-Thermo Fisher Scientific, Vantaa, Finland) were used to measure the total Ces activity. The (p-nitrophenolacetate) PNPA used for the substrate was prepared in 26 mM ethanol (96%). Differences in absorbance were recorded at 405 nm for 2 min at 25 °C.

Determination of total testis protein

250 µl of Bradford reagent and 5 µl of diluted supernatant (1:4) were applied to each microplate well to determine the total protein concentration in the supernatant samples (Bradford, 1976). After 15 min of incubation, the absorbance was measured at 595 nm. The protein concentration was determined using measurements of bovine serum albumin (BSA) standard solutions (0-1.4 mg BSA/mL) to create a calibration curve. Calculated protein values were used to determine specific activity levels of the enzyme under study (Lowry et al., 1946).

Determination of testis Pb concentrations

Testis Pb levels were measured at Adıyaman University Central Research Laboratory. Lead concentration was determined in the testis using NexION 350 inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, MA, USA)

Determination of testosterone levels

Serum testosterone analyses were performed on the Beckman coulter Dxl 800 device at the Central Biochemistry Laboratory of İnönü University Turgut Özal Medical Center.

Histopathological Evaluation

The standard histological tissue follow-up process involved dehydrating and polishing the testicular tissues once fixation was complete. The tissues were turned into paraffin blocks after the follow-up procedure. For histological analysis, sections of 5-µm thickness were cut from paraffin blocks. The acquired sections were deparaffinized, dyed with Masson trichrome and H&E, and then mounted. Using a Carl Zeiss brand Axiocam ERc5 type digital camera microscope, the sections were examined histopathologically, and photos were taken for morphometric analysis.

Serial sections of 10-micrometer thickness were obtained by applying 1/20 sampling from testicular tissues for analysis. In addition, seminiferous tubule area, seminiferous epithelial thickness, and connective tissue sheath measurements were made on the photographs taken from the sections. For these measurements, “closed polygon calculation” and “correct calculation” tools were selected in the Image j software program.

Statistical analysis

SPSS 22.0 program was used for statistical calculations. Results were tabulated as mean ± SEM. Differences were considered significant when $P \leq 0.05$. For the statistical evaluation One way ANOVA was used. Also, the Turkey-HSD test was used to classify the significant groups.

Table 2. Testis biochemical parameters in C, LA, PJ, and LA +PJ treated groups.

Parameters	C	LA	PJ	LA +PJ
MDA (nmol/mg protein)	15,3±0,75	34,8±2,32c	17,1±0,70z	19,6±1,04z
GSH (nmol/mg protein)	49,8±3,25	25,3±2,15c	52,8±6,25z	44,8±4,29y
GST (nmol/min/mg protein)	81,4±4,32	138,2±2,83c	101,2±4,27az	115,9±2,80cy
Ces (nmol/min/mg protein)	1,60±0,13	0,54±0,02c	0,65±0,03c	1,84±0,04bz

Values are expressed as means ± SEM; n=7 for each treatment group.
Comparison with group C. a: p <0.05, b: p <0.01, c: p <0.001
Comparison with group LA. x: p <0.05, y: p <0.01, z: p <0.001

RESULTS

MDA, reduced GSH, GST, and Ces levels in the testes

Testicular tissue biochemical parameter levels are given in Table 2.

The MDA levels of group C and group PJ showed no statistically significant difference in our investigation. It was found that the MDA level was higher in the LA group than in the control group (p<0.001). When compared to the LA group, the MDA level in the LA+PJ group reduced (p<0.001). The GSH level of the LA group decreased compared to other groups (p<0.001; p<0.01**).** In addition, GSH levels increased in the PJ and LA+PJ groups compared to the LA group **(**p<0.001; p<0.01) (Table 2).

When compared to the C group, it was seen that the LA group's level of GST enzyme activity increased (p<0.001). It was shown that the PJ group's level of GST enzyme activity increased in comparison to the C group (p<0.05) and decreased compared to the LA group (p<0.001). Additionally, the LA + PJ group's level of GST enzyme activity increased in comparison to the C group (p<0.001) and decreased compared to the LA group (p<0.01). Comparing the LA + PJ group to the C and LA groups, it was found that the Ces enzyme activity level increased in the LA + PJ group (p<0.01; p<0.001) (Table 2).

Findings on the testis elements analysis

Testicular tissue element concentration levels are given in Table 3. In our investigation, it was found that the LA group's Pb level increased in comparison to the control group (p<0.001). The PJ group was at a lower level than the LA group (p<0.001). It was discovered that the LA + PJ group's Pb level was lower than that of the LA group and higher than that of the C group (p<0.001) (Table 3).

The LA showed lower levels of Mn compared to the C group (p<0.01; p<0.05). It was observed that the PJ and LA + PJ group's Mn level increased relative to the LA group (Table 3).

Findings on the serum testosterone levels

Serum testosterone levels are given in Table 4. In this study, serum testosterone levels were found to be lower in the LA group compared to

Table 3. Elements' concentrations in the rat testis tissues.

Parameters	C	LA	PJ	LA +PJ
LA (ppb)	27,33±3,28	138,01±3,39c	26,96±1,39z	60,42±6,52cz
Fe	24077,80±1432,44	26346,98±1255,81	22797,85±850,75	22548,51±853,18
Mn	329,43±4,82	275,99±13,23b	312,99±5,22x	315,20±9,81x
Zn	19218,71±5610,33	18993,39±304,89	23906±222,85	22790,89±634,07
Cu	1888,02±185,05	1705,28±150,02	1820,15±205,02	1945,17±285,02

Values are expressed as means ± SEM; n=7 for each treatment group.
Comparison with group C. a: p <0.05, b: p <0.01, c: p <0.001
Comparison with group LA. x: p <0.05, y: p <0.01, z: p <0.001

the control group ($p<0.05$). In contrast to the LA group, testosterone levels were found to be higher in the PJ and LA+PJ groups ($p<0.01$) (Table 4).

Table 4. Testicular biochemical parameters: testosterone levels.

Parameters	Testosterone Level
C	6.54±0.67
LA	4.16±0.25a
PJ	7.27±0.39y
LA+PJ	8.16±0.71z

Values are expressed as means ± SEM; n=7 for each treatment group.
Comparison with group C. a: $p<0.05$, b: $p<0.01$, c: $p<0.001$
Comparison with group LA. x: $p<0.05$, y: $p<0.01$, z: $p<0.001$

Histologic findings of testis tissue

In the histopathological evaluation of testicular tissue, it was determined that the seminiferous tubule, seminiferous epithelial cells, and interstitial connective tissue in the C and PJ groups were histologically normal. No pathology was found in the evaluation (Figs. 1, 1a-c and 2a-c). When compared to the C and PJ groups, the histopathological evaluation of the LA group showed significant deterioration. Degeneration of the seminiferous epithelium, irregular shapes of the seminiferous tubules, and edema in the interstitial connective tissue were observed (Fig. 1, 3a-c). In the examination of the LA+PJ group, it was determined that the histological structure was similar to that of the C and PJ groups. It was observed that the smoothness of the seminiferous tubule shapes was preserved, and the edema in the interstitial connective tissue decreased. Degeneration findings were observed in the seminiferous epithelium (Fig. 1, 4a-c).

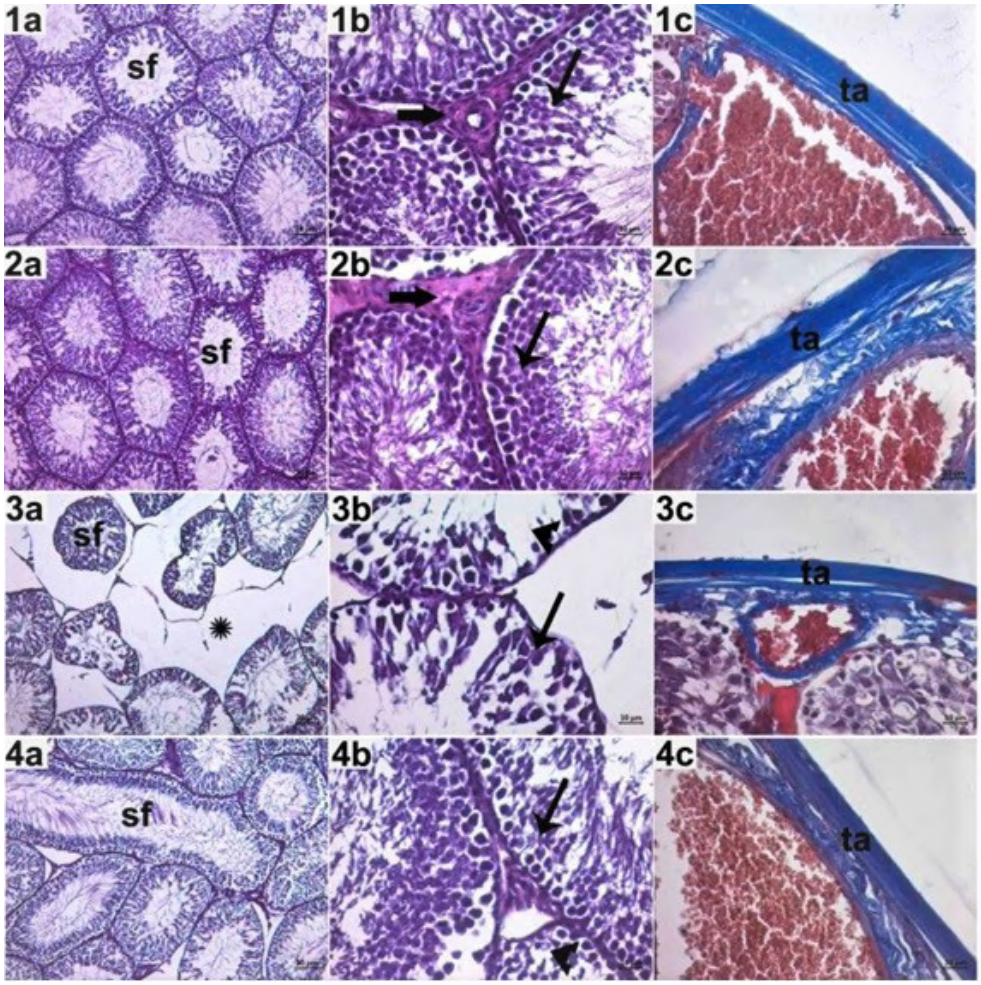

Fig. 1.- (**1a-c**): Images of testicular tissue of the C Group. (**2a-c**): Images of testicular tissue of the PJ Group. (**3a-c**): Images of testicular tissue of the LA group. (**4a-c**): Images of testicular tissue of the LA + PJ group. (1a, 2a, 3a and 4a; x10 objective magnification, 1b-c, 2b-c, 3b-c and 4b-c, x40 objective magnification. a and b: H&E staining, c: Masson trichrome staining). SF; seminiferous tubule, ta; connective tissue sheath, thick arrow; interstitial connective tissue area, thin arrow; seminiferous epithelium, arrowhead; seminiferous epithelial degeneration, star; edema in the interstitial connective tissue area.

Table 5. Histomorphometric analysis results of testicular tissue.

Groups	Tubule Area (mm^2)	Tubule Epithelial Thickness (μm)	Sheath thickness (μm)
C	385 ± 28,23	9235 ± 401,56	19761,83 ± 1451,42
LA	219,16 ± 16,81 *	6387,83 ± 405,03 *	12709,83 ± 925,97 *
PJ	373 ± 39,77	9600 ± 226,10	17227,66 ± 891,79
PJ+LA	338,16 ± 21,38	8136,33 ± 283,40	13982,83 ± 771,81

* Values are expressed as means ± SE; n=7 for each treatment group.
Statistical significance compared to the LA group: *: p<0.01

Histomorphometric findings

Histomorphometric findings are given in Table 5. The measurements revealed that the lead acetate group had a statistically significant decrease in seminiferous tubule area, seminiferous epithelial thickness, and connective tissue thickness when in comparison to the other groups ($p < 0.01$) (Table 5).

DISCUSSION

Pb is one substance that harms the male reproductive system in humans and animals. Pb causes testicular dysfunction and infertility. It also causes a decrease in body weight (Al-Megrin et al., 2019). Our study aimed to find a new way of treating Pb toxicity in rats by administering PJ.

Álvarez-Cervantes et al. (2021) investigated the effect of PJ in their study and found no negative harm to pomegranate juice. In their study, the tissues and serum of rats were found to have normal biochemical and hematological parameters. Al-Olayan et al. (2014) found normal histopathological findings in testicular tissue in the treatment group, in which they gave PJ. In our study, normal histological findings were observed in the PJ group (Fig. 1). Thus it was concluded that PJ has no toxic effect.

Kolawole et al. (2014) stated in their study that Pb-administered Wistar albino male rats had a deterioration in sperm count and motility, and, histopathologically, there were significant changes in testicular tubules and tubule walls. According to Sudjarwo et al. (2017) reported that Pb reduces sperm count and motility and causes altered histopathological findings (testicular damage, necrosis of seminiferous tubules, and spermatid) in testicular tissue. In their study, Ibrahim et al. (2021) found deterioration in testicular tissue, a decrease in sperm cell count, and a decline in spermatogenesis in general in rats to which Pb was administered. In comparison to the other groups, rats given Pb had a statistically significant decrease in seminiferous tubule area, seminiferous epithelial thickness, and connective tissue thickness.

Aksu et al. (2017) studied forty adults male Sprague Dawley rats. Pb application to the rat group resulted in histopathological changes, including seminiferous tubule atrophy, intertubular edema, tubule wall thinning, and decreased sperm motility. They stated that the PJ they used in the treatment group repaired the atrophy of the seminal tubules and the thinning of the tubule wall. They have also shown in their studies that it increases sperm motility. Our research showed signs of degeneration in the seminiferous epithelium, irregular shapes of the seminiferous tubules, and edema in the interstitial connective tissue in the LA group. After examining the LA+PJ groups, it was concluded that the testes' histological structure was similar to that of the C and PJ groups. The LA+PJ group demonstrated preserved smoothness of the seminiferous tubule shapes and a reduction in interstitial connective tissue edema. Degenerative findings were also observed in the seminiferous epithelium. Our study confirms the positive and restorative effects of PJ, with histopathological findings that are consistent with Aksu et al. (2017).

Owolabi et al. (2014) and Asadpour et al. (2013) reported that testicular toxicity of lead increases MDA levels and lipid peroxidation that cause tissue damage, and decreases the activity of antioxidant defense mechanisms to prevent excessive free radical formation (Asadpour et al., 2013;

Owolabı et al., 2014; Sudjarwo and Sudjarwo, 2017). In their study on testicular tissue, Aksu et al. (2017) found that PJ significantly decreased MDA levels in Pb exposure. In our investigation, similarly, the MDA level decreased in the LA+PJ group compared to the LA group. Also, in their study, it was observed that the GSH level was significantly reduced in all tissues when comparing the group receiving lead alone with the control group. However, the levels of GSH were found to significantly increase in the PJ treated groups compared to the LA and control groups. In our study, the GSH level of the LA group decreased compared to the other groups. In addition, it was significantly increased in rats given PJ compared to the LA group.

In our study, the LA group had higher levels of GST enzyme activity compared to the C group, whereas the PJ group had higher levels compared to the C group, but lower levels compared to the LA group. In addition, the LA+PJ group's GST enzyme activity was shown to be higher than in the C group, while it was lower in the LA group. The role of GST in GSH metabolism is crucial. Lead-induced inhibition of GST activity can lead to a decrease in GSH levels (Wang et al., 2013). When the activity of the Ces enzyme was measured, it was found to decrease in the LA group in comparison to the C group. However, it increased in the PJ and LA+PJ groups. In addition, Ces level increased in the LA+PJ group compared to the LA group. The role of Ces enzymes in metabolizing drugs is crucial, as it can greatly impact their effectiveness in the body's tissues (Xing et al., 2010). Ces is thought to be involved in testosterone biosynthesis and protect testicular cells from the effects of harmful and toxic agents (Özkaya et al., 2021). The results of our research on Ces demonstrate that the detoxification process of testicular tissue is affected by exposure to Pb, whereas the management of the toxic outcomes arising from this exposure is attributed to PJ.

Aksu et al. (2017) stated in their study that PJ reduced lead accumulation in testicular tissue. Furthermore, it was observed that the diminished concentration of Cu in the testes of the LA group was elevated in the groups where PJ was administered. Fe level increased in the LA group but fell to the control level in the PJ-treated groups. The amount of Zn in the PJ-applied groups was even higher than in the control group. The findings of our study exhibited similarities to those of Aksu et al.; however, the levels of Fe, Cu, and Zn in our research did not yield statistically significant results compared to other groups. The observed variance could potentially be attributed to the Adıyaman pomegranate, which contains a distinct composition compared to the pomegranate utilized in the study conducted by Aksu et al. (2017). The amount of Mn was also examined in our study, and a significant decrease was found in the LA group compared to the C group. Furthermore, when compared to the LA group, the PJ and LA+PJ groups showed a significant increase. Mn functions as an antioxidant, shielding sperm from harm caused by free radicals. Moreover, it plays a crucial role as a coenzyme in the production of sex hormones including progesterone, estrogen, and testosterone (Qureshi and Abbas, 2013). The levels of Mn might have decreased due to damage to the testicles caused by LA, while PJ contributed to an increase in this level.

AL-Megrin et al. (2020) stated in their study that Pb significantly reduces testosterone levels. Al Olayan et al. (2014) found an increase in testosterone levels in rats given PJ against toxicity in their study. In our study, the administration of Pb resulted in a decline in testosterone levels, whereas the consumption of PJ led to an increase in testosterone levels. The analysis shows that PJ has the potential to heal due to its positive impact on hormone levels.

Pb is a heavy metal that can harm tissues. Our histological data reveals that Adıyaman-PJ can help treat Pb-induced toxicity in testicular tissue. Moreover, PJ can effectively increase the reduced testosterone level caused by Pb exposure. Therefore, using PJ in alternative medicine to treat toxicity would be beneficial. Increasing testosterone levels can be a preferred option to treat infertility. Our research has examined the impact of PJ on the male reproductive system in rats. However, more studies are required to determine the potential effects and adverse impacts of PJ on the female reproductive system. It is important to note that our study was conducted on rats, and therefore, future research may focus on the effects of PJ on the human reproductive system.

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AUTHORS' CONTRIBUTIONS

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REFERENCES

ABDELHAMID FM, MAHGOUB HA, ATEYA AI (2020) Ameliorative effect of curcumin against lead acetate–induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. *Environ Sci Pollut Res,* 27(10): 10950-10965.

AKSU DS, SAGLAM YS, YILDIRIM S, AKSU T (2017) Effect of pomegranate (Punica granatum L.) juice on kidney, liver, heart and testis histopathological changes, and the tissues lipid peroxidation and antioxidant status in lead acetate-treated rats. *Cell Mol Biol,* 63(10): 33-42.

AL-MEGRIN WA, ALKHURIJI AF, YOUSEF AOS, METWALLY DM, HABOTTA OA, KASSAB RB, ABDEL MONEIM AE, EL-KHADRAGY MF (2019) Antagonistic efficacy of luteolin against lead acetate exposure-associated with hepatotoxicity is mediated via antioxidant, anti-inflammatory, and anti-apoptotic activities. *Antioxidants,* 9(1): 10.

AL-MEGRIN WA, ALOMAR S, ALKHURIJI AF, METWALLY DM, MOHAMED SK, KASSAB RB, ABDEL MONEIM AE, EL-KHADRAGY MF (2020) Luteolin protects against testicular injury induced by lead acetate by activating the Nrf2/HO-1 pathway. *IUBMB Life,* 72(8): 1787-1798.

AL-MUTARY MG, ABU-TAWEEL GM (2020) Effects of pomegranate juice on the sexual behavior, fertility and protective activity against aluminum exposure in male mice. *J King Saud Univ-Sci,* 32(6): 2688-2695.

AL-OLAYAN EM, EL-KHADRAGY MF, METWALLY DM, ABDEL MONEIM AE (2014) Protective effects of pomegranate (Punica granatum) juice on testes against carbon tetrachloride intoxication in rats. *BMC Complement Altern Med*, 14(1): 1-9.

ALAHMADI BA (2020) Effect of herbal medicine on fertility potential in experimental animals-An update review. *Mater Socio-Med*, 32(2): 140.

ALSATAF S, BAŞYIĞIT B, KARAASLAN M (2021) Multivariate analyses of the antioxidant, antidiabetic, antimicrobial activity of pomegranate tissues with respect to pomegranate juice. *Waste Biomass Valori,* 12(11): 5909-5921.

ÁLVAREZ-CERVANTES P, IZQUIERDO-VEGA JA, MORÁN-LEÓN J, GUERRERO-SOLANO JA, GARCÍA-PÉREZ BE, CANCINO-DÍAZ JC, BELEFANT-MILLER H, BETANZOS-CABRERA G (2021) Subacute and subchronic toxicity of microencapsulated pomegranate juice in rats and mice. *Toxicol Res,* 10(2): 312-324.

ANNAÇ E, UÇKUN M, ÖZKAYA A, YOLOĞLU E, PEKMEZ H, BULMUŞ Ö, AYDIN A (2022) The protective effects of pomegranate juice on lead acetate-induced neurotoxicity in the male rat: A histomorphometric and biochemical study. *J Food Biochem,* 46(4): e13881.

ASADPOUR R, SHAHBAZFAR A, KIANIFARD D, AZARI M, ZABOLI N (2013) Comparison of the protective effects of garlic (Allium sativum L) extract, vitamin E and N acetyl cystein on testis structure and sperm quality in rats treated with lead acetate. *Revue Med Vet,* 164(1): 27-41.

BOUOUZA F, LEILA M, HALOUI M, AJABI N, ABDENOUR C (2022) Effects of pomegranate juice consumption on the fertility and protective activity against a fungicide exposure in male wistar rats. *Uttar Pradesh J Zool*, 20-27.

BRADFORD MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem,* 72(1-2): 248-254.

BUEGE JA, AUST SD (1978) Microsomal lipid peroxidation. *Methods Enzymol,* 52: 302-310.

DAVULJIGARI CB, GOTTIPOLU RR (2020) Late-life cardiac injury in rats following early life exposure to lead: reversal effect of nutrient metal mixture. *Cardiovasc Toxicol,* 20(3): 249-260.

DKHIL MA, AL-QURAISHY S, MONEIM AEA (2013) Effect of pomegranate (Punica granatum L.) juice and methanolic peel extract on testis of male rats. *Pak J Zool,* 45(5).

DOLATI P, KHODABANDEH Z, ZAMIRI MJ, JAMHIRI I, MEHRABANI D (2020) The effect of lead acetate and quercetin on the tight and gap junctions in the mouse testis. *Biol Trace Elem Res,* 198(2): 535-543.

DOROSTGHOAL M, SEYYEDNEJAD SM, NEJAD MNT (2020) Cichorium intybus L. extract ameliorates testicular oxidative stress induced by lead acetate in male rats. *Clin Exp Reprod Med*, 47(3): 161.

DUMITRESCU E, CHIURCIU V, MUSELIN F, POPESCU R, BREZOVAN D, CRISTINA RT (2015) Effects of long-term exposure of female rats to low levels of lead: ovary and uterus histological architecture changes. *Turkish J Biol,* 39(2): 284-289.

EL-BELTAGI HS, DHAWI F, ASHOUSH IS, RAMADAN K (2020) Antioxidant, anti-cancer and ameliorative activities of Spirulina platensis and pomegranate juice against hepatic damage induced by CCl4. *Not Bot Horti Agrobot Cluj-Napoca*, 48(4): 1941.

EL-KHADRAGY M, AL-MEGRIN WA, ALSADHAN NA, METWALLY DM, EL-HENNAMY RE, SALEM FEH, KASSAB RB, ABDEL MONEIM AE (2020) Impact of coenzyme Q10 administration on lead acetate-induced testicular damage in rats. *Oxid Med Cell Longev*, 2020: 4981386.

HABIG WH, PABST MJ, JAKOBY WB (1974) Glutathione S-Transferases. *J Biol Chem,* 249(22): 7130-7139.

IBRAHIM IA, SHALABY AA, ABD ELAZIZ RT, BAHR HI (2021) Chlorella vulgaris or Spirulina platensis mitigate lead acetate-induced testicular oxidative stress and apoptosis with regard to androgen receptor expression in rats. *Environ Sci Pollut Res,* 28(29): 39126-39138.

ILERITURK M, BENZER F, AKSU EH, YILDIRIM S, KANDEMIR FM, DOGAN T, DORTBUDAK MB, GENC A (2021) Chrysin protects against testicular toxicity caused by lead acetate in rats with its antioxidant, anti-inflammatory, and antiapoptotic properties. *J Food Biochem,* 45(2): e13593.

JAHAN M, ISLAM M, GAUTAM M, BHUIYAN M (2021) Lead acetate induced toxicities and antitoxic effect of Vitamin E and selenium in mice. *Bangladesh J Vet Med (BJVM),* 19(1): 75-85.

KHODABANDEH Z, DOLATI P, ZAMIRI MJ, MEHRABANI D, BORDBAR H, ALAEE S, JAMHIRI I, AZARPIRA N (2021) Protective effect of quercetin on testis structure and apoptosis against lead acetate toxicity: an stereological study. *Biol Trace Elem Res*, 199(9): 3371-3381.

KOKSAL I, USTA M, ORHAN I, ABBASOGLU S, KADIOGLU A (2003) Potential role of reactive oxygen species on testicular pathology associated with infertility. *Asian J Androl,* 5(2): 95-100.

KOLAWOLE T, DAPPER D, OJEKA S (2014) Ameliorative effects of the methanolic extract of the rind of citrullus lanatus on lead acetate induced toxicity on semen parameters and reproductive hormones of male albino wistar rats. *Eur J Med Plants,* 4(9): 1125.

KUCUKLER S, BENZER F, YILDIRIM S, GUR C, KANDEMIR FM, BENGU AS, AYNA A, CAGLAYAN C, DORTBUDAK MB (2021) Protective effects of chrysin against oxidative stress and inflammation induced by lead acetate in rat kidneys: a biochemical and histopathological approach. *Biol Trace Elem Res,* 199(4): 1501-1514.

LEIVA KP, RUBIO J, PERALTA F, GONZALES GF (2011) Effect of Punica granatum (pomegranate) on sperm production in male rats treated with lead acetate. *Toxicol Mech Methods,* 21(6): 495-502.

LI N, ZHAO Y, SHEN Y, CHENG Y, QIAO M, SONG L, HUANG X (2021) Protective effects of folic acid on oxidative damage of rat spleen induced by lead acetate. *Ecotoxicol Environ Saf*, 211: 111917.

LOWRY E, BEARD D, HEWIT L, BARNER J (1946) Tumor of the testicle: analysis of one hundred cases: a preliminary report. *J Urol*, 55(4): 373-384.

MAYUONI-KIRSHENBAUM L, BAR-YA'AKOV I, HATIB K, HOLLAND D, PORAT R (2013) Genetic diversity and sensory preference in pomegranate fruits. *Fruits,* 68(6): 517-524.

MORON MS, DEPIERRE JW, MANNERVIK B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta,* 582(1): 67-78.

OWOLABI J, WILLIAMS F, FABIYI O (2014) Evaluation of moringa's effects against lead-induced disruption of the hippocampus in animal models. *World J Life Sci Med Res,* 3(2): 39.

OYEYEMI WA, AKINOLA AO, DARAMOLA O-O, AIKPITANYI I, DUROTOLUWA OT, ALELE P-GO, OGIERIAKHI IO, OKORO TD (2022) Vitamin E and quercetin attenuated the reproductive toxicity mediated by lead acetate in male Wistar. *Bull Natl Res Cent*, 46(1): 22.

OZKAYA A, SAHIN Z, DAG U, OZKARACA M (2016) Effects of naringenin on oxidative stress and histopathological changes in the liver of lead acetate administered rats. *J Biochem Mol Toxicol,* 30(5): 243-248.

OZKAYA A, SAHIN Z, KUZU M, SAGLAM YS, OZKARACA M, UCKUN M, YOLOGLU E, COMAKLI V, DEMIRDAG R, YOLOGLU S (2018) Role of geraniol against lead acetate-mediated hepatic damage and their interaction with liver carboxylesterase activity in rats. *Arch Physiol Biochem,* 124(1): 80-87.

ÖZKAYA A, ŞAHIN Z, ŞAHIN Y, BULMUŞ Ö, UÇKUN M, YOLOĞLU E (2021) Evaluating effects of black carrot extract on testicular carboxylesterase activity and oxidative stress parameters in rats exposed to bisphenol A. *Adıyaman University J Sci,* 11(1): 73-86.

PEPE G, RAPA SF, SALVIATI E, BERTAMINO A, AURIEMMA G, CASCIOFERRO S, AUTORE G, QUARONI A, CAMPIGLIA P, MARZOCCO S (2020) Bioactive polyphenols from pomegranate juice reduce 5-Fluorouracil-induced intestinal mucositis in intestinal epithelial cells. *Antioxidants*, 9(8): 699.

QURESHI IZ, ABBAS Q (2013) Modulation of testicular and whole blood trace element concentrations in conjunction with testosterone release following kisspeptin administration in male rabbits (Oryctolagus cuniculus). *Biol Trace Elem Res,* 154: 210-216.

YOUSEF AO, FAHAD A, ABDEL MONEIM AE, METWALLY DM, EL-KHADRAGY MF, KASSAB RB (2019) The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities. *Int J Environ Res Public Health,* 16(16): 2895.

SAMARGHANDIAN S, SHIRAZI FM, SAEEDI F, ROSHANRAVAN B, POURBAGHER-SHAHRI AM, KHORASANI EY, FARKHONDEH T, AASETH JO, ABDOLLAHI M, MEHRPOUR O (2021) A systematic review of clinical and laboratory findings of lead poisoning: lessons from case reports. *Toxicol Appl Pharmacol,* 429: 115681.

SUDJARWO SA, SUDJARWO GW (2017) Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. *Res Pharm Sci,* 12(5): 381.

TÜRK G, SÖNMEZ M, AYDIN M, YÜCE A, GÜR S, YÜKSEL M, AKSU EH, AKSOY H (2008) Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clin Nutr,* 27(2): 289-296.

WANG Y, FANG J, HUANG S, CHEN L, FAN G, WANG C (2013) The chronic effects of low lead level on the expressions of Nrf2 and Mrp1 of the testes in the rats. *Environ Toxicol Pharmacol,* 35(1): 109-116.

XING H, WANG J, LI J, FAN Z, WANG M, XU S (2010) Effects of atrazine and chlorpyrifos on acetylcholinesterase and carboxylesterase in brain and muscle of common carp. *Environl Toxicol Pharmacol,* 30(1): 26-30.