

# Protective Effect of Pomegranate Juice on Lead Acetate-Induced Liver Toxicity in Male Rats

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Received: 2023-11-10

Accepted: 2023-12-26

Published Online: 2023-12-27

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## ABSTRACT

**Objective:** Lead has been reported to cause oxidative stress in liver tissues and cause histopathological changes. Studies have shown that pomegranate juice has antioxidant properties that prevent oxidative stress. In this study, the harmful effects of lead acetate on rat liver tissue and the efficacy of pomegranate juice against these effects were investigated.

**Methods:** 28 male Wistar albino rats were divided into four groups: control, lead acetate (50 mL/kg), pomegranate juice (1 mL/kg), and lead acetate + pomegranate juice (50 mL/kg+1 mL/kg). Lead acetate and pomegranate juice were administered orally.

**Results:** When compared with the control group, it was seen that the lead acetate had an increase in the malondialdehyde level and a decrease in reduced Glutathione, Glutathione S-transferase, and Carboxylesterases. Group lead acetate + pomegranate juice had a reduction in malondialdehyde level and an increase in Glutathione, Glutathione S-transferase, and Carboxylesterases compared with the group lead acetate. The lead level of group lead acetate + pomegranate juice decreased compared to the group lead acetate. Cellular degeneration and irregular hepatic cords were observed in group lead acetate's liver tissue, and the negative changes were lost in group lead acetate + pomegranate juice.

**Conclusion:** It was observed that pomegranate juice had a protective effect against liver toxicity caused by lead acetate.

**Keywords:** Antioxidant, Lead acetate, Liver, Pomegranate juice, Oxidative stress

## INTRODUCTION

Today, industrial activities cause an increase in environmental waste, such as heavy metals. Lead (Pb), one of these heavy metals, spreads to the biosphere in developing and industrialized societies due to its essential role in industry, causing high environmental exposure. Pb can enter the body through water, food, and breathing air, and can accumulate in the tissues causing toxic effects. The most common ways of

exposure are through consumption followed by inhalation. For this reason, Pb poisoning is amongst the most common heavy metal poisonings. Due to slow Pb excretion, even at low doses, living organisms can experience physiological, biochemical, and behavioral disorders as a result of Pb accumulation in their tissues. Pb has been reported in studies that cause neurological, immunological, renal, hepatic, and hematological disorders [1-3]. In the antioxidant system, Pb binds to sulfhydryl groups and

is replaced by essential cofactors, copper and zinc. Nearly 90% of Pb is found in blood and bone, while the rest is found in the liver and kidneys [4, 5]. In many studies, lipid peroxidation due to Pb poisoning; has been reported to cause inhibition of the antioxidant system by causing the release of reactive oxygen species and free radicals. Accordingly, Pb has been reported to cause oxidative stress in liver tissues and cause histopathological changes [6].

Pomegranate (*Punica granatum L.*) is a fruit of nutritious and therapeutic value, which has been widely used in the past. Its medicinal properties are related to the existence of polyphenols (Ellagitannins, flavonoids, phenolic acids, stilbenes, tannins, and anthocyanins) with free radical scavenging properties. Pomegranate has been reported in studies with anti-carcinogenic, cardioprotective, antihyperlipidemic, anti-inflammatory, and antioxidant effects [7, 8].

Tissues produce free oxygen radicals and antioxidants in a regulated manner. Excessive production of free radicals leads to the formation of harmful substances like malondialdehyde (MDA). Antioxidant defense systems develop against its harmful effects when overproducing free oxygen radicals. Antioxidant defense systems show their impact by eliminating the detrimental effects of radicals. Glutathione (GSH) and Glutathione S-transferase (GST) are important antioxidants [9]. Carboxylesterases (Ces) are a member of esterases and catalyses the hydrolysis of amides, esters, and thioesters. The enzymatic process of converting esters into carboxylic acid and hydroxylated products has been identified in various tissues, including the liver [10].

The discovery of natural products and new substances has gained significant importance in treating diseases and toxic substances that threaten human health. Pomegranate and pomegranate juice (PJ), which contain phytochemicals that significantly increase

bioactivity, have also been the subject of recent studies [11]. In addition, it has been reported in experimental studies that PJ has a histological and biochemical hepatoprotective effect by reducing oxidative stress and inflammation [12-14]. Studies have shown that consuming pomegranate juice can help prevent non-alcoholic fatty liver disease and inhibit the development of unhealthy lipid profiles. Including pomegranate juice in the diet of individuals at risk of fatty liver and high cholesterol can be beneficial [15]. We conducted a study using light microscopy and biochemical analysis to investigate the impact of lead acetate (LA) on rat liver tissue and the potential protective effect of PJ on these changes. Therefore, in this study, the effects of PJ on MDA, GSH, GST, Ces, element levels, and histopathological parameters in rat liver tissues exposed to LA were investigated.

## MATERIALS AND METHODS

### Animals

The research was carried out in the Animal Experiment Center of Firat University. Ethics committee approval was obtained by Firat University Animal Experiments Local Ethics Committee with the 2018/04 protocol number and 09 decision number. 28 adult Wistar albino 200-250 g male rats were used in the research. The animals were divided into four groups, and experimental procedures were applied. Rats were housed at  $22 \pm 2^{\circ}\text{C}$  room temperature, 12 hours light, and 12 hours dark-light cycle, as feed and water ad-libitum.

### PJ Preparation

In our study, pomegranate grown in the Adiyaman region was used. After the pomegranates were washed and dried, their juice was obtained by pulling them out of the blender. PJ was kept at  $-20^{\circ}\text{C}$  until used.

### Chemical Content of PJ

The chemical composition of pomegranate grown in the Adiyaman region was investigated. The content of PJ was anthocyanin 137.1 mg/L, phenolic acid 490.75 mg/kg, ellagic acid 175 mg/100g, total flavonoids 63 mg/kg, and total antioxidants 1530 mg/kg [16].

### The Study Groups and Applications

Twenty-eight male Wistar albino rats were randomly divided into control groups, LA, PJ and LA+PJ (n=7 in each group). The cages were left untouched for a week prior to the start of the experiment to allow for adaptation.

### Main Points:

- A decrease in the liver function of the rats was observed as a result of the administration of LA, according to the findings of our research.
- Our research suggests that consuming PJ may alleviate the negative effects of LA-induced oxidative stress.

Control group: Rats were given distilled water for 30 days without any additional procedures conducted throughout the investigation.

LA group: 500 ppm Pb dissolved in acetic acid was mixed into drinking water per liter. Rats were administered 50 mL/kg of Pb through oral gavage per animal daily for 30 days, from a stock solution.

PJ group: PJ were given 1 mL/kg of PJ every two days for 30 days through oral gavage [16].

Oral gavage was applied to the rats at the same time each day. No complications resulted from these applications. The rats were sacrificed by intraperitoneal injections of xylazine hydrochloride and ketamine hydrochloride after the 30-day study period. The liver tissue samples were divided into two sections for the biochemical analysis and histopathological examination. One half of the samples were stored at a temperature of -80 °C, while the other half was fixed in 10% formaldehyde.

### Preparation of Tissue Homogenates

Tissue samples of the liver in cooled potassium phosphate buffer (0.1 M, pH 7.4; 0.15M KCl, 1.0 mM EDTA, 1.0 mM DTT) were homogenized with the device of the Homogenizer (Heidolph 2021). Five hundred microliters of homogenate were reserved for use in MDA analysis. The remaining homogenates were centrifuged for 20 minutes at +4 °C (Hettich 460 R), 16,000 x g, and supernatants (S16) were taken into Eppendorf tubes for measurements of biomarkers other than MDA.

### Determination of Liver MDA Level

MDA levels in liver tissue samples were determined using the technique established by Placer et al. [17]. A solution of 0.375% thiobarbituric acid and 15% trichloroacetic acid in 0.25 N hydrochloric acid was used. MDA reacts with thiobarbituric acid to form a pink-colored compound. The absorbance of the samples was read spectrophotometrically (ThermoTM Varioskan Flash, Finland) at 532 nm. In the study, MDA levels were shown as nmol/mg protein. This study was carried out on Thermo-3001 UV/VIS device with Microplate Attachment.

### Determination of Liver Reduced GSH Activity

GSH analysis in liver tissues was performed at 412 nm using the Sedlak and Lindsay method on a Thermo-3001 UV/VIS device with Microplate Attachment [18]. Tissues were precipitated with

50% TCA (Trichloroacetic acid) and centrifuged at 1000xg for 5 minutes. 0.5 ml was taken from the supernatant in Eppendorf tubes removed from the centrifuge, and 2 ml Tris EDTA buffer (0.2 M, pH: 8.9) and 0.1 ml 0.01 M 5,5'-dithio-bis-2 nitrobenzoic acid were added. This mixture was left at room temperature for 5 minutes and absorbance values were measured at 412 nm on the device. GSH levels were expressed as nmol/mg protein.

### Determination of Liver GST Activity

The examination of GST activity involved the preparation of a solution comprising 10 µL of supernatant, 100 µL of GSH, and 100 µL of phosphate buffer (0.1 M, pH 6.5). Following this, a solution of 20 mM 1-chloro-2,4 dinitrobenzene was prepared as a substrate, utilizing 96% ethanol, and subsequently transferred into the designated microplate wells. The microplates were placed within the reader system, and the corresponding alterations in absorbance were diligently recorded at 344 nm for a duration of 2 minutes, with a temperature of 25°C maintained throughout the process. Specific GST activity was determined from the nmol/min/mg protein type [19].

### Determination of Liver Ces

For the analysis of Ces activity, 26 mM of nitrophenol acetates were utilized as a substrate, which was prepared in 96% ethanol. The reaction solution, consisting of a mixture of 5 µL of sample and 250 µL of 50 M Mtrizma buffer (pH, 7.4), was incubated for 3 minutes at 25°C. To initiate the reaction, 5 µL of the substrate was added and monitored for 2 minutes at 25 °C, with readings taken at 405 nm. Ces activity was expressed as nmol/min/mg protein [20].

### Determination of Total Protein

The protein amount was measured as standard using bovine serum albumin (0–1.4 mg BSA/mL) [20].

### Determination of Liver Element Concentrations

Liver Pb, iron (Fe), manganese (Mn), zinc (Zn), calcium (Ca), and copper (Cu) levels were measured at Adiyaman University Central Research Laboratory. NexION 350 inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, MA, USA) was used [21]. The measurements were conducted in parts per billion (ppb) unit.

### Histopathological Evaluation

To perform histopathological analysis, liver samples taken were divided into 10% formaldehyde solution by dividing into

separate groups. Following a week of fixation, paraffin blocks were prepared in accordance with standard histological tissue protocol. Subsequently, 5-micron thick sections were extracted from the paraffin blocks. The stained sections were evaluated using images captured by the Carl Zeiss brand Axiocam ERc5 model digital camera attached to a microscope for histopathological analysis.

### Statistical Analysis

All statistical calculations were made using SPSS 22.0 program. The results have been calculated and presented as mean  $\pm$  SEM. One-way analysis of variance (ANOVA) was used for the statistical evaluation of the groups, and the Tukey-HSD test was used to identify the significant groups.

## RESULTS

### MDA, Reduced GSH, GST, and Ces Levels in the Liver

Liver tissue biochemical parameter levels are given in Table 1. Our study observed no statistically significant difference between the control group's MDA level and the PJ group ( $p > 0.05$ ). It was determined that there was an increase in the LA group's MDA level compared to the control group ( $p < 0.001$ ). In contrast, the LA+PJ group MDA level increased compared to the control group ( $p < 0.001$ ), and it was observed that the LA+PJ group MDA level reduced compared to the LA group ( $p < 0.01$ ). Furthermore, GSH level of LA group decreased compared to other groups ( $p < 0.001$ ;  $p < 0.01$ ).

It was observed that the GST enzyme activity level decreased in the LA group compared to the control group ( $p < 0.01$ ). In

comparison to the LA group, the GST enzyme activity level of the PJ group was decreased ( $p < 0.05$ ). In addition, there was a partial increase in the LA+PJ group GST enzyme activity level compared to the LA group. In comparison to the control group, the activity level of Ces enzyme was observed to decrease in the LA group ( $p < 0.001$ ). On the other hand, PJ and LA+PJ groups Ces enzyme activity levels were increased compared to the LA group ( $p < 0.01$ ).

### Findings on the Liver Element Analysis

The results of our study indicate that there was no statistically significant difference in the level of Pb between the control group and the groups that consumed PJ ( $p > 0.05$ ). However, it was observed that the group that received Pb showed a significant increase in Pb levels when compared to the control group ( $p < 0.001$ ). Also, LA+PJ group showed an increase in Pb levels in comparison to the control group ( $p < 0.001$ ). However, the Pb levels decreased in LA+PJ group when compared to the LA group ( $p < 0.01$ ) (Table 2).

There was no significant difference in Fe level between the groups ( $p > 0.05$ ). A significant decrease was found in the Mn level in the LA group compared to the control group ( $p < 0.05$ ). While the Zn level decreased in the LA group compared to the control ( $p < 0.001$ ), it increased in the PJ ( $p < 0.01$ ) and LA+PJ groups compared to the LA group ( $p < 0.05$ ). Upon conducting an analysis on the levels of Ca, it has been observed that there is a significant increase in the PJ group in comparison to the LA group ( $p < 0.01$ ). Also, there was no significant difference in Cu levels between the groups ( $p > 0.05$ ).

**Table 1.** Biochemical parameters in the rat hepatic tissues

Parameters	Control	LA group	PJ group	LA+PJ group
MDA (nmol/mg protein)	8.37 $\pm$ 0.47	20.5 $\pm$ 0.95 <sup>c</sup>	10.3 $\pm$ 0.47 <sup>z</sup>	15.1 $\pm$ 1.1 <sup>cy</sup>
GSH (nmol/mg protein)	97.8 $\pm$ 2.80	59.9 $\pm$ 1.44 <sup>c</sup>	73.8 $\pm$ 1.50 <sup>cy</sup>	77.6 $\pm$ 2.02 <sup>cz</sup>
GST (nmol/min/mg protein)	60.3 $\pm$ 1.89	45.7 $\pm$ 1.71 <sup>b</sup>	55.5 $\pm$ 3.27 <sup>x</sup>	52.7 $\pm$ 1.60
Ces (nmol/min/mg protein)	1.90 $\pm$ 0.06	1.30 $\pm$ 0.04 <sup>c</sup>	1.62 $\pm$ 0.05 <sup>y</sup>	1.61 $\pm$ 0.07 <sup>y</sup>

Values are expressed as means  $\pm$  SEM;

n=7 for each treatment group.

Comparison with group control.

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:  $p < 0.001$

**Table 2.** Element concentrations in the rat hepatic tissues

Parameters	Control	LA group	PJ group	LA+PJ group
Pb (ppb)	80.07±2.04	1337.21±35,56 <sup>c</sup>	86.22±4.39 <sup>z</sup>	287.37±20.22 <sup>cz</sup>
Fe	145095.99±5556.20	162619.31±4478.40	159101.43±6338,39	161356.43±5609.52
Mn	3265.83±166.51	2605.15±50.82 <sup>a</sup>	2971.49±100.98	2910.13±165.71
Zn	33504.42±1197.89	26729.02±30997 <sup>c</sup>	31798.02±897.79 <sup>y</sup>	31207.61±556.24 <sup>x</sup>
Ca	117905.01±2933.38	108197.37±3356.26	130237.61±5137.38 <sup>y</sup>	122698.83±3082.36
Cu	4160.26±243.01	3899.98±232.56	4247.64±52.59	4444.85±271.49

Values are expressed as means ± SEM; n=7 for each treatment group.

Comparison with group control. 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

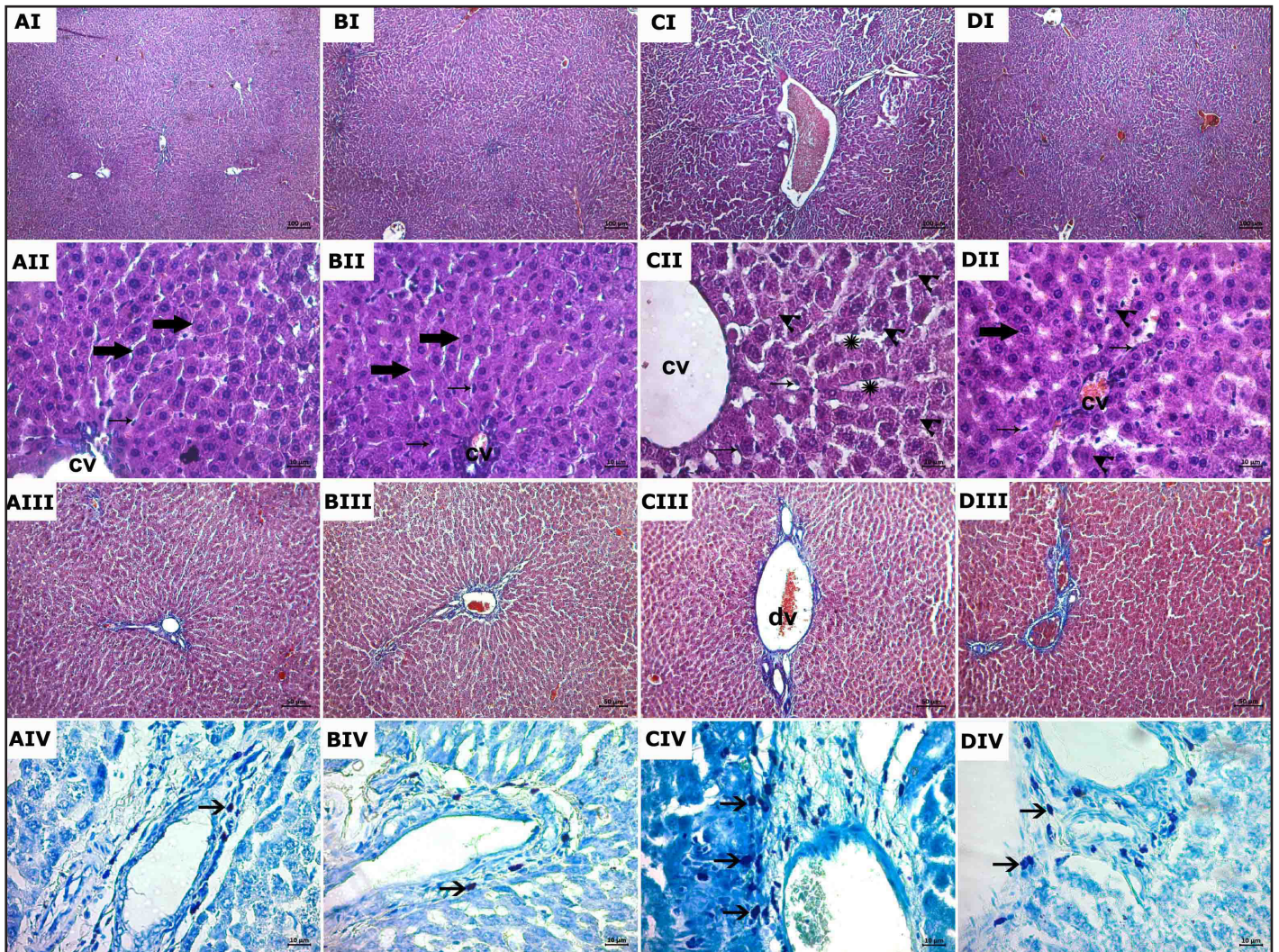
### Histologic Analysis of Liver Tissue

After analysing tissue sections belonging to both the control and PJ groups using hematoxylen eosin, it was observed that the hepatocyte cords extending from v. centralis to the periphery in the middle of the liver lobule, as well as the sinusoids located between these cords, appeared to be regular and consistent (Figures 1 AI and BI). The monitoring of polygonal-shaped liver cells revealed that the cytoplasm of hepatocytes exhibited an acidophilic staining feature that varied in density in accordance with cell activity levels. The nuclei of these cells were centrally located and characterized as large, round, and euchromatic. Additionally, some hepatocytes displayed a bi-nucleus and exhibited normal structural features (Figures 1 AII and BII). To assess the connective tissue density, we utilized Masson's triple staining method on liver tissues from both the control group and the group that received PJ. Our analysis revealed the presence of dense connective tissue around the v. centralis and periportal area (Figures 1 AIII and BIII). The observed density of mast cells in the connective tissue surrounding the vessel was within the average range (Figures 1 AIV and BIV).

Upon examination of rat liver tissues from the group treated with LA, a disruption in the arrangement of hepatocyte cords was observed around the vena centralis, resulting in an altered liver structure. At slight magnification, the lobule's structure and boundaries were indistinguishable. These findings may have significant implications in understanding the effects of LA on liver function (Figure 1 CI). It is of significance to note that in the enlarged views of the same group, the cytoplasmic boundaries of the hepatocytes composing the parenchyma are indistinguishable, the polygonal shapes are absent, and the

variations in size and degenerative changes between the cells are eliminated. Certain regions of these cells exhibit a dark pyknotic core (Figure 1 CII). To evaluate the connective tissue density within the rat liver tissues of the LA group, Masson's triple staining method was employed. The results revealed the presence of non-dense connective tissue surrounding the v. centralis and periportal area, which was consistent with the control and PJ groups. Furthermore, the examination of this group revealed notable dilatation observations in the structures of the v. centralis, portal vein, and sinusoid (Figure 1 CIII). The study observed a significant increase in mast cell density within the vascular connective tissue, as compared to both the control and PJ groups (Figure 1 CIV).

When the sections of rat liver tissues belonging to the LA and PJ treated groups are examined with H.E, hepatocytes form parenchyma in slight magnification, similar to control and PJ groups. It has been noted that there exists a stable structure surrounding the v. centralis (Figure 1 DI). It was noted that the hepatocytes, the primary cells of the liver parenchyma, possess a consistent structure and maintain their acidophilic properties even in instances of significant enlargement within the same group. It was observed that the presence of hepatocytes degenerated in places among hepatocytes still continues, but their density decreases (Figure 1 DII). With the triple painting method of Masson, it was observed that the connective tissue density in the v. centralis and periportal areas was similar to in all other groups (Figure 1 DIII). The density of mast cells was slightly lower in the LA+PJ group in comparison to the LA group (Figure 1 DIV).



**Figure 1.** Histological examination of the effects of LA and PJ on the rat liver tissues

**AI-AIV** C group, **BI-BIV** PJ group, **CI-CIV** LA group, **DI-DIV** LA and PJ group.

**AI, BI, CI and DI:** General image of the groups at x4 magnification;

**AII, BII, CII and DII:** Images of groups at x40 magnification-Hematoxylin and Eosin Staining;

**AIII, BIII, CIII and DIII:** Images of the groups at x10 magnification-Masson Trichrome Staining;

**AIV, BIV, CIV and DIV:** Images of the groups at x40 magnification-Toluidin Blue Staining; **cv**, v. centralis; **dv**, dilated vein; **thick arrow**, healthy hepatocyte; **thin arrow**, kuppfer cell; **arrowhead**, degenerated hepatocyte cells; **star**, dilated sinusoid; **middle thick arrow**, mast cell.

### Histopathological Scoring

In this study, a semi-quantitative evaluation was made using the histopathological scoring system adapted from Bekheet [21]. The evaluation was graded as 0 (absent), 1 (slight), 2 (moderate) and 3 (severe). Inflammation, hemorrhage, fibrosis, cell damage, and sinusoidal dilatation findings were examined. Each parameter was evaluated independently and blindly by an expert histologist.

In the evaluation, a statistically significant difference was found between the LA group with the control group and the PJ in terms of cell damage and sinusoidal dilatation ( $p < 0.005$ ). No statistically significant difference was detected between the groups in terms of fibrosis, inflammation and hemorrhage ( $p > 0.05$ ). Statistical data regarding the scoring obtained because of the evaluation are shown in Table 3.

**Table 3.** Histopathological Scoring

Parameters	Control	LA	PJ	LA+PJ
Cell damage	0.57 ± 0.535 <sup>a</sup>	2.14 ± 1.069	0.43 ± 0.535 <sup>b</sup>	1.00 ± 0.816
Sinusoidal Dilatation	0.71 ± 0.488 <sup>c</sup>	2.29 ± 0.756	0.86 ± 0.378 <sup>d</sup>	1.14 ± 0.690
Fibrosis	0.29 ± 0.488	0.43 ± 0.535	0.14 ± 0.378	0.71 ± 0.488
Inflammation	0.43 ± 0.535	0.57 ± 0.535	0.29 ± 0.488	0.71 ± 0.488
Hemorrhage	0.29 ± 0.488	0.57 ± 0.535	0.29 ± 0.488	0.57 ± 0.535

Statistical significancy compared to the LA group: a:  $p < 0.05$ ; b:  $p < 0.05$ , c:  $p = 0.005$ , d:  $p = 0.01$ .

Scoring data obtained because of semi-quantitative evaluation (Mean ± SD)

## DISCUSSION

Lead causes oxidative stress, and consequently, free radical levels increase which induces lipid peroxidation [22]. As a result, the MDA, a lipid peroxidation product, increases [1]. Liver tissue shows elevated levels of MDA and oxidative stress as a result of lead exposure, as indicated by previous studies [1, 23]. The results of these studies are similar to those of our research. Our investigation determined that the MDA level was reduced in the LA+PJ group compared to the LA group.

Flavonoids and phenolic compounds in PJ have been reported to have high antioxidant activity [24]. GSH and GST are essential antioxidants [25]. In our study, a statistically significant decrease was observed in the GST and GSH levels of rats treated with LA compared to the control group. Numerous studies have demonstrated that exposure to lead in laboratory animals results in a reduction of GSH levels in liver tissues [1, 2, 26]. In line with our findings, there have been studies indicating a decrease in GST levels as a result of exposure to lead [1, 27]. The GST enzyme has many functions in the cell. Compounds such as hydroxyalkanel, acroneils, and hydro peroxides formed in the cell are destroyed by the antioxidant effect of the GST enzyme. The presence of the GSH molecule is essential for optimal GST enzyme activity [27]. Our research has revealed a notable decrease in both GSH molecules and GST enzymes in liver tissues of rats exposed to lead. This decline in GST enzyme levels may be attributed to the increased activation and excessive utilization of GSH molecules required for this activity.

Changing enzyme activities in tissues is clinically critical [10, 28]. In a study conducted by Ozkaya et al. [1] on rats, the application of Pb resulted in a decrease in the activity level of the Ces enzyme in the rats' livers. These findings parallel the

results of our research. In our study, while Ces enzyme activity levels in the LA group decreased compared to the control group; it increased in the PJ and LA+PJ groups compared to the LA group. Based on our analysis, it appears that the detoxifying properties of the Pb molecules may be responsible for a decrease in enzyme activity. This suggests that Ces may be impacted by these effects.

Lead is one of the toxic elements that can accumulate in the liver. Therefore, as we expected in our study, the Pb level in the liver tissue increased in the LA group compared to the control group. It has been reported in previous studies that PJ reduces lead accumulation in tissues [12, 16]. Similarly, in our research, the Pb level decreased in the LA+PJ group compared to the LA group. Aksu et al. [12] reported in their study that LA increases the amount of Fe in the liver and does not change the Zn level. However, in our study, no significant difference was found between the groups in the Fe level. Also, the amount of Zn in the liver was decreased in the LA group. Fe and Pb tend to accumulate in similar environments within the body, leading to a competition between the two elements. In our study, it's possible that the absorption of Pb may have restricted the accumulation of Fe. Furthermore, it should be noted that the liver plays a significant role in Zn metabolism [29]. Therefore, it is possible that Pb-induced liver damage may affect the amount of Zn in the liver tissue.

PJ contains a significant amount of Fe, Ca, and Zn minerals [12, 30]. In this study, although Zn increased in the LA+PJ group compared to the LA group, no significant difference was found in the Fe level. Although the Ca level increased in the PJ group compared to LA, this increase was not significant in the LA+PJ group. It is known that the amount of Zn in the tissues decreases

in liver damage [31]. It is possible that the antioxidant properties of Zn, a component found in PJ, may have counteracted the decrease of Zn in the liver caused by Pb. Unlike our study, Aksu et al. [12] found that PJ decreased Fe and Zn levels in the liver even lower than the control group. Different concentration of PJ in our study compared to theirs may explain the difference between our results.

In this study, histopathological changes such as cellular degeneration and irregular hepatic cords were observed in the light microscopic examination of the liver tissue of rats exposed to Pb. Previous studies have reported that Pb causes cellular degeneration and irregular hepatic cords in liver tissue [1, 26, 32]. The histopathological findings we found in the liver regarding lead exposure are coherent with the results of the above studies. In addition, in our research, it was seen that the toxic effects of LA decreased with the application of PJ.

### Limitations

Our study focused on the impact of PJ solely on male rats, and it's worth noting that the effects could vary in female rats due to hormonal differences. Further research on larger sample sizes across different age ranges would provide more comprehensive results. Expanding the scope of biochemical and histological parameters could also lead to more effective outcomes. It's worth mentioning that we did not include an analysis of the total weight of liver tissues in our study, which could be a pertinent factor in evaluating other parameters.

### CONCLUSIONS

Our research findings indicate that the administration of LA resulted in a decline in the liver function of the rats. It was found that these toxic effects of LA can be prevented by the PJ. Toxic substances cause oxidative stress and cause cell and tissue damage. Antioxidants are an effective treatment method for preventing tissue damage caused by oxidative stress. Discovering natural products and new substances has gained significant importance in treating the adverse effects of diseases and toxic substances that threaten human health. Our research has indicated that consumption of PJ may offer potential benefits in mitigating the negative effects of LA-induced oxidative stress.

**Acknowledgments:** We express our gratitude to the staff of Firat University Experimental Animal Center.

**Conflict of Interest:** The authors declare no conflict of interest, financial or otherwise.

**Informed Consent:** We declare that we participated in the study and the development of the manuscript titled "Protective effect of pomegranate juice on lead acetate-induced liver toxicity in male rats". We have read the final version and consent to publish the article.

**Funding:** This study has no financial resources and no sponsors.

**Ethical Approval:** The research was carried out in the Animal Experiment Center of Firat University. Ethics committee approval was obtained by Firat University Animal Experiments Local Ethics Committee with the 2018/04 protocol number and 09 decision number.

**Author Contributions:** Study concept and design: HP, EA; Acquisition of subjects and data, analysis and interpretation of data: HP, EA, ÖB, AA; Preparation of manuscript: HP, BZ, MA.

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**How to Cite;**

Pekmez H, Annaç E, Bulmuş Ö, Zencirci B, Aydın M, Aydın A (2024) Protective Effect of Pomegranate Juice on Lead Acetate-Induced Liver Toxicity in Male Rats. 30(2):160-169. *Eur J Ther.* <https://doi.org/10.58600/eurjther1927>