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RESEARCH

Alleviation of Aluminum-Induced Oxidative Stress, Trace Element, and Mineral Levels in Rat Tissues Protective Role of Pomegranate Juice (*Punica Granatum* L.)

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Abstract

The present investigation examined the impact of pomegranate (*Punica granatum* L.) juice on trace elements, minerals, and oxidative stress in relation to the potential harm inflicted by aluminum chloride ($AlCl_3$) in rats. Rats were split into four groups at random for this purpose: control (C), pomegranate juice (PJ), aluminum chloride (A), and PJ + A. For 30 days, PJ was orally administered by gavage at a rate of 4 mL/kg every other day, whereas $AlCl_3$ was administered intraperitoneally at 8.3 mg/kg. Spectrophotometric analysis was used to measure the levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) enzyme activity in various tissues. In addition, high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) was used to determine the amounts of the elements Al, Cu, Fe, Mn, Zn, Ca, and Mg in the tissues. It was discovered that when PJ therapy was applied to all tissues, the antioxidant enzymes SOD and CAT activity increased, the GSH level rose, and the MDA level, a sign of lipid peroxidation, decreased. Al and Ca levels increased in the A group relative to the C group in all tissues, whereas they decreased in the A + PJ group relative to the A group. Group A exhibited a proportionate increase in Fe levels in the liver and renal tissues compared with group C. Furthermore, the A group's brain tissue had a higher Fe level than the C group's. The A + PJ group's brain tissue had a lower Fe level than the A group's. Our findings demonstrate that PJ therapy greatly decreased Al buildup and oxidative stress in tissues while controlling variations in trace element levels. In addition, it is concluded that PJ might have value as a strong chelating agent to prevent Al poisoning.

Keywords Aluminum · Pomegranate (*Punica granatum* L.) juice · Trace element · Antioxidant · Kidney · Liver · Brain

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Introduction

Heavy metals such as aluminum (Al) are widely present in our environment due to the rise in industrial activity, and the possible effects of these metals on human health are a subject of great interest [1]. The metallic element Al is widely distributed across the crust of the Earth [2]. Because Al is used so extensively in modern life from refining crude oil to purifying drinking water, food items, pharmaceuticals, medications, cosmetics, and electrical insulators, it is nearly difficult to completely avoid exposure to the metal [3, 4]. Estimates indicate that the average weekly consumption of Al is between 70 and 140 mg. Despite the extremely low (less than 1%) absorption rate from the gastrointestinal tract, Al steadily builds up in critical tissues such as the liver, brain, kidneys, heart, bones, and blood. Histopathologic (structural tissue changes) and serologic (blood-related) abnormalities may result from this buildup over time [5–8]. Al has been included in the Agency for Toxic Substances and Disease Registry (ATSDR) priority list of dangerous substances [9].

Additionally, at high concentrations, transition metals may produce excess nitrogen and oxygen free radicals. The body produces free radicals, which are more likely to react in the vicinity of their formation. The incapacity of the cell defense mechanisms to prevent oxidative damage to subcellular systems will occur if these reactive species are not neutralized. Similar to other transition metals, aluminum chloride ($AlCl_3$) can change the numbers of non-enzymatic antioxidants and their activity [10–12]. As a result, oxidative stress regarded as one of the main macromolecular damages can result from Al poisoning [13]. This increases the generation of free radicals, modifies sugar metabolism, and specifically impairs noradrenergic and cholinergic neurotransmission, which causes damage to neurons [6–14]. In addition, there is a chance that exposure to Al compounds will worsen hepatotoxicity, nephrotoxicity, and inflammation [15]. According to Lentini et al. [16], only a minor portion of the Al that builds up in the human body through the skin comes from tainted food and drink. Nephrotoxicity and renal failure result from the kidneys quickly eliminating most this Al. Al-Kahtani et al. [17] have reported that oxidative stress and apoptosis in rats are caused by Al buildup in liver tissue. In addition, Yu et al. [18] observed that Al buildup inhibits neurotransmitter synthesis and release, leading to an imbalance in metal homeostasis.

Certain critical element absorption and metabolism may be impacted by Al exposure. Research has demonstrated that long-term exposure to Al can upset the equilibrium of the elements [18, 19]. Specifically, introducing Al into the body can upset the body equilibrium of these elements or make it more difficult to absorb vital elements, including iron (Fe), zinc (Zn), magnesium (Mg), selenium (Se), and copper (Cu). Trace elements are necessary for the human body to function properly and are crucial for metabolic reactions vital to life, such as protein synthesis, cell division, and differentiation [20]. One of the key elements influencing significant pathophysiological mechanisms such as oxidative stress and inflammation is the disruption of trace element homeostasis [21]. Thus, preserving the body's health and ability to function depends on the balanced management of trace elements.

Using antioxidants from natural sources is crucial for minimizing the harmful effects of Al. The study of traditional medicine is considered crucial to the creation of novel compounds and contemporary medications [22]. Because natural sources such as plants, fruits, and vegetables contain healing qualities, including anticoagulants, anticancers, and antioxidants, many people choose natural remedies [23, 24]. The juice of pomegranate (PJ), which is widely consumed, has a high content of polyphenolic components, including vitamin C, anthocyanins, punicalagin, ellagic acid, and gallic acid [25]. Pomegranate has important bioactive properties such as anticancer, antiproliferative, antiapoptotic [26], HIV-I inhibition [27], antiinflammatory [28], cardioprotective effect [29], antihyperlipidemic [30], and antioxidant [31]. The application of plant extracts generated from polyphenols to mitigate the effects of Al exposure has been the subject of recent research. Hasan et al. investigated the preventive effect of blackberry juice (BBJ) on oxidative stress and neurological problems in rats exposed to sodium fluoride (NaF) and $AlCl_3$. They concluded that administering BBJ has a notable neuroprotective effect and might lessen oxidative stress. They did note, however, that serum levels of potassium (K) were dramatically elevated, whereas levels of sodium (Na), calcium (Ca), Cu, and Zn were significantly lowered [32]. Salem et al. demonstrated that plant polyphenolic compounds of ellagic acid and ferulic acid improved histological changes, liver dysfunctions, dyslipidemia, liver malondialdehyde (MDA), and protein carbonyl content levels; increased liver catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activity; increased glutathione (GSH) level; and increased Cu concentration while lowering liver Fe and Zn concentrations by reducing oxidative stress caused by γ radiation and $AlCl_3$ [33]. Aqueous pomegranate (*Punica granatum* L.) extract was found by Ali and Saeed to mitigate gentamicin-induced kidney oxidative damage [34]. Furthermore, El-Habibi [35] reported that the high phenolic content of PJ, along with the potential mechanism of action of scavenging and induction of various antioxidant enzymes, could be responsible for the improvement in renal physiology of rats administered PJ while they were on adenine treatment.

Few studies have examined kidney, liver, and brain tissues collectively, despite numerous studies on the bioactive qualities of pomegranates, plants, and fruits with comparable composition. Furthermore, there is not much research that concentrates on the homeostatic imbalance of trace elements in tissues. This study was designed to assess the potential protective effect of *Punica granatum* L. against $AlCl_3$-induced oxidative stress and its effects on the homeostasis of trace elements in tissues. This study was conducted considering the significance of protection against the harmful effects of $AlCl_3$ and to better understand the role and mechanism of natural products commonly consumed as inhibitors of oxidative stress.

Materials and Methods

Supply of Pomegranate Samples

Fresh pomegranate samples from Adiyaman in November 2019 were washed, strained, and divided into two. Pomegranate grains (white parts included) were broken down using a blender. It was kept in 1 mL Eppendorf tubes at − 20 °C until the study was conducted. The chemical content of pomegranate, a local product of Adıyaman province collected from the same region, was previously investigated by our researcher. The content of PJ used was determined as phenolic acid 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 [36].

Animal Models and Experimental Protocol

In the experimental study, 28 adult *Wistar albino* male rats weighing 200–250 g were used. They were randomly divided into 4 groups ($n = 7$) to minimize selection bias. Experimental animals were obtained from Adıyaman University Experimental Animals Production, Application, and Research Center (ADYU DEHAM). The ethics committee's decision on the experimental study was taken from the local ethics committee of Adiyaman University Animal Experiments (Ethics Committee No: 2019/038). The animals were treated according to national and international laws and policies on the care and use of experimental animals. The animals were kept in special cages in groups of 4 before and during the experiment under standard conditions (22–24 °C constant temperature and ventilated rooms; 12 h daylight and 12 h dark photoperiod). The rats were fed a standard laboratory diet (RT-FR-01, DSA Agrifood Products INC, Kırıkkale, Turkey) and water ad libitum.

- Control group (C): Saline administration (1 mL) was performed every other day for 30 days intraperitoneally (i.p).
- PJ group: PJ administration was performed by oral gavage at 4 mL/kg every other day for 30 days [37].
- $AlCl_3$ group (A): $AlCl_3.6H_2O$ was administered i.p. at 8.3 mg/kg every other day for 30 days [38].
- $AlCl_3$ + PJ group (A + PJ): $AlCl_3$ was administered at 8.3 mg/kg i.p., and PJ was administered at 4 mL/kg by oral gavage every other day for 30 days.

Collection of Tissue

At the end of the 30-day experiment, rats in all groups were decapitated under ketamine (75 mg/kg) + xylazine (10 mg/kg) anesthesia, and liver, kidney, and brain tissues were removed. Tissues were kept at − 80 °C until the study was conducted.

Preparation of the Samples

Digestion of samples (0.4–0.5 g) for elemental analysis of liver, kidney, and brain tissues was performed using temperature- and pressure-resistant polytetrafluorethylene (PTFE) containers in a microwave oven with a volume of 100 mL. Each sample was placed in PTFE containers, and solutions of 5 mL of nitric acid (65% HNO_3, (w/w)), 1 mL of perchloric acid (72% $HClO_4$ (w/w)), and 1 mL of hydrogen peroxide (30% H_2O_2 (w/w)) were added and left for 30 min to dissolve in the microwave digestion procedure. After the PTFE vessels were cooled to room temperature, the volume of the obtained clear mixture was completed to 10 mL with 0.1 mol/L HNO_3 solution. Microwave solubilization was applied again to the insoluble samples [39].

Tissue samples that became a clear solution were read in the Analytik Jena ContrAA 300 (GLE, Berlin, Germany) model high-resolution continuum source flame atomic

Table 1 HR CS-FAAS device variables

Variables	Al	Cu	Fe	Mn	Zn	Ca	Mg
Wavelength, nm	396.15	324.75	248.32	279.48	213.85	422.67	285.20
N_2O-C_2H_2 flow rate, L/h	215	0	0	0	0	215	0
C_2H_2-air flow rate, L/h	55	55	60	80	60	50	70
Flame head height, mm	7	6	5	8	8	6	5
Evaluation pixels, pm	3	3	3	3	3	3	3

absorption spectrometry (HR CS FAAS). HR-CS FAAS device variables are shown in Table 1, respectively.

To obtain the calibration graphs, metal stock solutions (Merck) with a concentration of 1000 mg/L were taken in certain volumes and completed to the appropriate volumes. The calibration variables obtained for the studied metals are shown in Table 2.

Homogenization of Samples

For the determination of MDA, GSH, SOD, and CAT, tissue homogenization was performed with a Potter–Elvehjem glass homogenizer using an ice-cooled 0.1 M K-phosphate buffer containing 0.15 M KCl, 1 mM EDTA, and 1 mM DTT in a 1:4 ratio of total tissue weight (w/v). After homogenization, the homogenates were transferred to Eppendorf tubes and centrifuged for 20 min at 16,000 g at 4 °C (Sigma 2-16 K, St. Louis, Missouri). After this process, the supernatant fraction was removed, and analyses were performed on these samples.

Determination of Malondialdehyde (MDA)

MDA levels were measured according to the method developed by Buege and Aust [40]. A total of 1 mL of the sample and 2 mL of trichloroacetic acid (TCA)-thiobarbituric acid (TBA)-hydrochloric acid (HCl) reagent [0.37% TBA, 15% TCA, and 0.24 N HCl] (in a ratio of 1:1:1) were transferred to the test tubes. The tubes were placed in boiling water for 15 min and then cooled. The tubes were then centrifuged at 5000 rpm for 10 min. The readings of the supernatants were obtained spectrophotometrically at 532 nm. The MDA level was calculated using a molar absorption coefficient of 1.56×10^5 1/mol cm.

Superoxide Dismutase (SOD) Activity

The SOD test was performed using an indirect method based on the SOD inhibitory effect of epinephrine autoxidation on the initial rate [41]. After 0.2 mL of the sample and 2.5 mL of carbonate buffer (0.05 M, pH 10.2) were transferred into the test tube, 0.3 mL of freshly prepared epinephrine was added. Absorbance was recorded every 30 s for 2 min using a spectrophotometer at 480 nm.

Table 2 Calibration curves by equation

Metal	Calibration equation (mg/L)	Correlation coefficient (R^2)
Al	$y = 0.0015638x - 0.0002724$	0.998439679
Cu	$y = 0.0965943x + 0.0006576$	0.998346131
Fe	$y = 0.0352571x + 0.0020019$	0.998499348
Mn	$y = 0.113278x + 0.0044163$	0.996893854
Ca	$y = 0.1231887x + 0.0116040$	0.990439556
Zn	$y = 0.1870560x + 0.0308181$	0.964975492

Glutathione (GSH) Analysis

Ellman [42] described a method to determine the GSH level. A total of 0.5 mL of the sample was transferred into the test tube and centrifuged after adding 2 mL of 10% TCA. Then, 1 mL of supernatant, 0.5 mL of Ellman reagent, and 3 mL of phosphate buffer were added. Absorbance values were read and recorded at 412 nm using a spectrophotometer.

Catalase (CAT) Activity

Claiborne [43] method was used to determine CAT activity. CAT divides H_2O_2 directly into H_20 and O_2. As a result of the reaction of 2 mL of H_2O_2 solution and 1 mL of the sample, absorbance values were read at 360 nm for 70 s. The degradation of H_2O_2 was calculated using the molar absorption coefficient $\mathcal{E} = 34.9$ 1/mol cm.

Statistical Assessment

Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's HSD test was applied using SPSS (20.0 software) to calculate the differences between groups; p values of < 0.05 and < 0.001 were considered statistically significant.

Results

Metal levels in the liver, brain, and kidney tissues of rats exposed to $AlCl_3$ are shown in Tables 3, 4, and 5. When liver tissue trace element and mineral levels were examined (Table 3), there was no statistical difference among Cu levels in all groups. Manganese (Mn) levels in the A and A + PJ groups decreased compared with those in the C group ($p < 0.05$, $p < 0.001$). It was observed that the Ca level increased in the A group compared with the C group ($p < 0.001$), whereas the Ca level in the A + PJ group decreased compared with the A group ($p < 0.001$). Al level increased significantly in the A group compared with the C group ($p < 0.001$). It was observed that the Al level of the A + PJ group decreased compared with that of the A group ($p < 0.001$).

Trace element and mineral levels in kidney tissue are shown in Table 4. When the A group was compared with the C group, it was observed that the Cu level increased ($p < 0.01$).

Table 3 Liver tissue element concentrations (mean ± SD, µg/g) after PJ administration during $AlCl_3$ exposure

Elements/group	C	PJ	A	A + PJ
Al	22.15 ± 3.01	24.21 ± 4.15^{z}	88.57 ± 8.25^{c}	60.41 ± 7.15^{cz}
Cu	1.43 ± 0.03	1.41 ± 0.03	1.57 ± 0.05	1.50 ± 0.07
Fe	43.20 ± 2.17	41.50 ± 1.74	46.01 ± 2.43	40.53 ± 1.18
Mn	0.71 ± 0.02	0.76 ± 0.01	0.64 ± 0.01^{c}	0.69 ± 0.02^{a}
Ca	93.61 ± 2.78	84.25 ± 7.05^{z}	139.60 ± 4.12^{c}	99.46 ± 3.25^{z}
Mg	177.78 ± 4.87	184.94 ± 6.24	202.52 ± 9.93	191.18 ± 12.37
Zn	35.12 ± 1.37	35.14 ± 1.61	33.84 ± 1.97	35.42 ± 1.79

Comparison with the A group: x, $p < 0.05$; y, $p < 0.01$; z, $p < 0.001$

Comparison with the C group: a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$

Table 4 Kidney tissue element concentrations (mean ± SD, µg/g) after PJ administration during $AlCl_3$ exposure

Elements/group	C	PJ	A	A + PJ
Al	7.62 ± 0.27	8.67 ± 0.56	14.51 ± 0.42^{a}	6.44 ± 0.19^{z}
Cu	5.42 ± 0.48	6.00 ± 0.39	7.95 ± 0.17^{b}	6.56 ± 0.64^{x}
Fe	38.34 ± 3.09	39.38 ± 2.55	41.39 ± 1.26	43.05 ± 1.33
Mn	0.43 ± 0.03	0.45 ± 0.02	0.37 ± 0.02	0.48 ± 0.03
Ca	95.21 ± 5.71	94.05 ± 4.16^{x}	124.86 ± 7.46^{a}	63.12 ± 9.59^{az}
Mg	97.14 ± 2.16	102.48 ± 4.20	145.24 ± 10.16^{c}	121.96 ± 6.88
Zn	21.32 ± 1.31	21.70 ± 1.21	23.65 ± 1.13	23.85 ± 1.19

Comparison with the A group: x, $p < 0.05$; y, $p < 0.01$; z, $p < 0.001$

Comparison with the C group: a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$

Table 5 Brain tissue element concentrations (mean ± SD, µg/g) after PJ administration during $AlCl_3$ exposure

Elements/group	C	PJ	A	A + PJ
Al	28.00 ± 2.00	26.00 ± 3.00^{z}	50.01 ± 4.00^{c}	38.01 ± 3.00^{ax}
Cu	1.25 ± 0.06	1.19 ± 0.09	1.10 ± 0.10	1.15 ± 0.09
Fe	15.01 ± 0.50	17.00 ± 0.50^{x}	22.07 ± 1.21^{a}	16.02 ± 0.30^{x}
Mn	0.33 ± 0.01	0.30 ± 0.02^{x}	0.25 ± 0.01^{a}	0.26 ± 0.01^{a}
Ca	40.00 ± 4.00	45.00 ± 3.00^{z}	75.01 ± 6.00^{c}	59.01 ± 5.00^{ax}
Mg	140.00 ± 8.00	160.00 ± 7.00	155.00 ± 9.00	145.00 ± 6.00
Zn	9.00 ± 0.50	10.00 ± 0.20	10.02 ± 0.05	11.00 ± 0.80

Comparison with the A group: x, $p < 0.05$; y, $p < 0.01$; z, $p < 0.001$

Comparison with the C group: a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$

It was found that the Cu level decreased in the A + PJ group compared with the A group ($p < 0.05$). There was no statistical difference between the Fe, Mn, and Zn levels of all groups ($p > 0.05$). The Ca level was higher in the A group than in the C and A + PJ groups ($p < 0.05$, $p < 0.001$). The mg level of the A + PJ group increased significantly compared with that of the C group ($p < 0.001$). When the A group was compared with the C group, it was found that the Al level increased ($p < 0.05$). It was determined that the Al level of the A + PJ group decreased significantly compared with that of the A group ($p < 0.001$).

Trace element and mineral levels in brain tissue are shown in Table 5. No statistical difference existed between Cu, Mg, and Zn levels of all groups. The Ca level in the A group was found to be higher than in the C and A + PJ groups ($p < 0.05$, $p < 0.001$). It was observed that the Al level increased significantly in the A group compared with the C group ($p < 0.001$). It was determined that the Al level of the A + PJ group decreased significantly compared with that of the A group ($p < 0.05$). It was observed that the Mn level of the A + PJ and A groups decreased compared with that of the C group ($p < 0.05$). It was found that the Fe level of the C group showed a significant increase in the A group ($p < 0.05$). It was found that the Fe level of the PJ and A + PJ groups decreased significantly compared with that of the A group ($p < 0.05$).

The manifested $AlCl_3$-induced oxidative stress in liver, kidney, and brain tissues was caused by significant

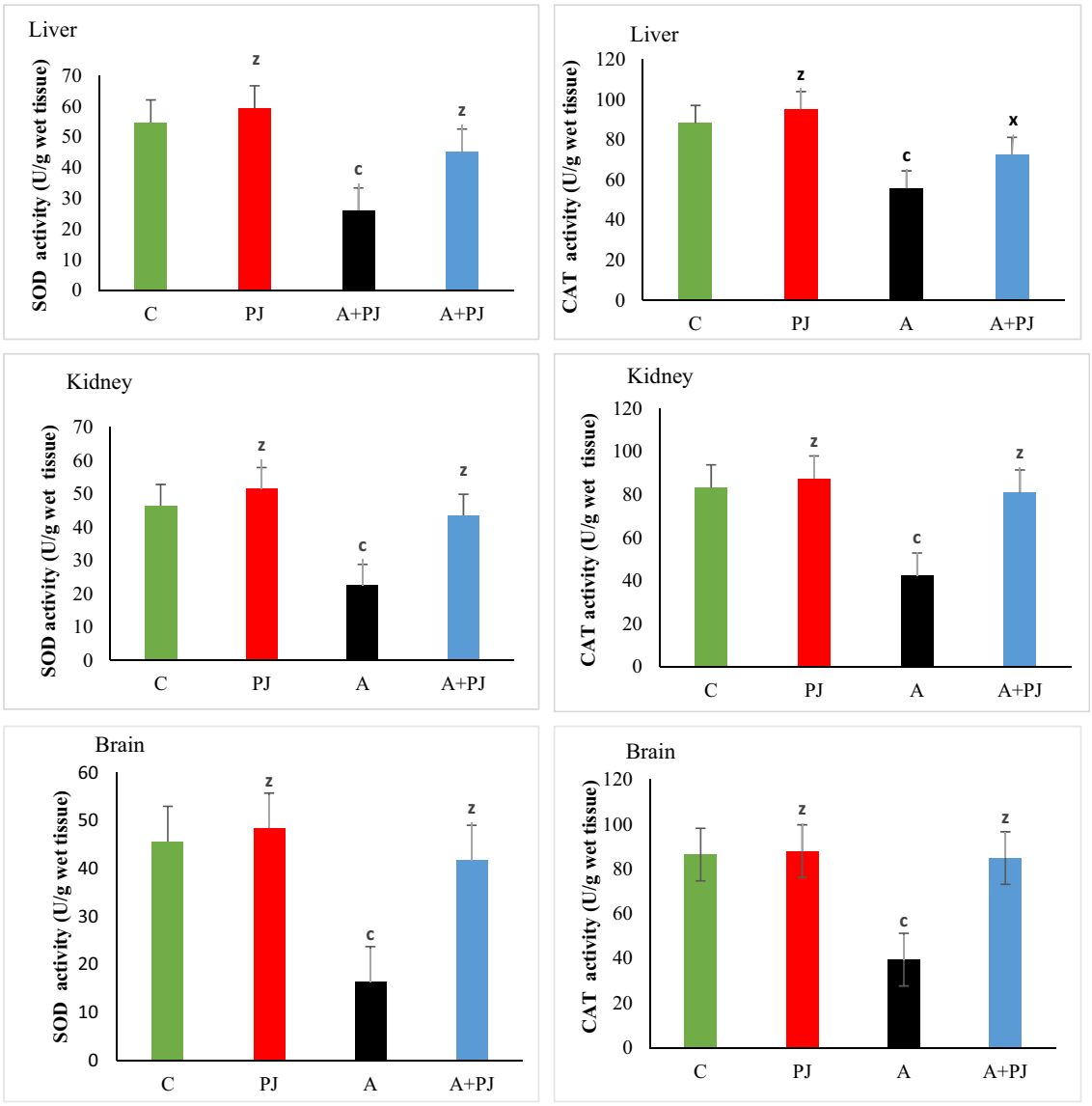
($p < 0.001$) increases in the levels of MDA concurrently with a significant ($p < 0.001$) decrease in GSH contents and inhibition of antioxidant enzyme activities compared with the C group.

Treatment with PJ significantly improved these changes in GSH levels (brain ($p < 0.01$), liver and kidney ($p < 0.001$), and antioxidant enzyme activities ($p < 0.001$) and decreased MDA levels, as shown in Figs. 1 and 2 ($p < 0.001$).

In addition, PJ application alone improved the GSH content and antioxidant enzyme activities of liver, kidney, and brain tissues compared with group C, while decreasing MDA levels ($p < 0.001$).

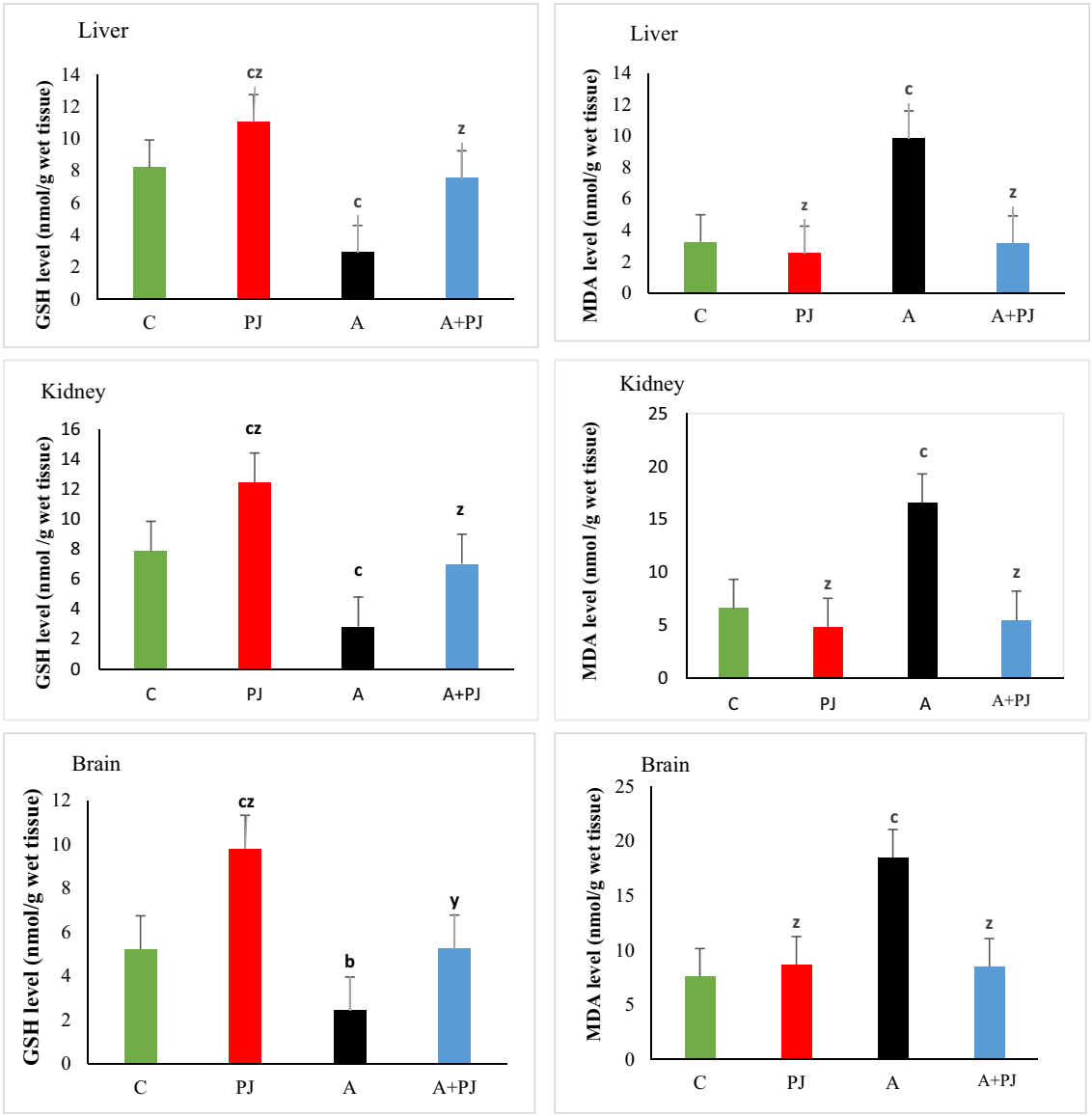
Discussion

Al is easily absorbed by living organisms through their skin, digestion, and breathing. Al builds up in the kidneys, brain, heart, and other organs as it enters the body. Al exposure can directly result in mineral level disruptions that lead to electrolyte imbalance, particularly in target organs such as the liver and brain [44–47]. It has been noted that each mineral tested may contribute differently to the pathological consequences of Al-induced toxicity because of the variety of mineral distribution and organ-specific functions [48]. Nevertheless, data assessing the correlation between Al exposure and the



SOD: Superoxide dismutase, CAT: Catalase

Fig. 1 Analysis of variance and Tukey HSD results (mean ± SD) of antioxidant enzyme activities (SOD, CAT) in liver, kidney, and brain tissues following PJ administration during $AlCl_3$ exposure ($n = 7$). Values are presented as mean ± SD, representing significant difference compared with group A: x, $p < 0.05$; y, $p < 0.01$; and z, $p < 0.001$ and group C: a, $p < 0.05$; b, $p < 0.01$; and c, $p < 0.001$, respectively



MDA: Malondialdehyde, GSH: Glutathione

Fig. 2 Analysis of variance and Tukey HSD results (mean ± SD) of oxidative stress markers (MDA and GSH) in liver, kidney, and brain tissues following PJ administration during $AlCl_3$ exposure ($n = 7$). Values are presented as mean ± SD, representing significant difference compared with group A: x, $p < 0.05$; y, $p < 0.01$; and z, $p < 0.001$ and group C: a, $p < 0.05$; b, $p < 0.01$; and c, $p < 0.001$, respectively

simultaneous concentrations of these elements in three distinct tissues are currently lacking. To this end, the effects of PJ on the homeostasis of trace elements in tissues were investigated to assess any potential defenses against oxidative stress caused by $AlCl_3$.

The findings demonstrate that the $AlCl_3$ treatment increased lipid peroxidation levels and reduced antioxidant enzyme activity in tissues compared with the C group. This suggests that oxidative damage is increasing. The $AlCl_3$ therapy likewise affected the concentrations of other metal ions in tissues simultaneously.

As necessary cofactors for antioxidant enzymes such as SOD (Cu, Zn, Mn) and CAT (Fe, Mn), trace metals, including Cu, Zn, Fe, Mn, and Mg, can lessen or repair oxidative damage [49].

It is well known that Al causes oxidative stress by exhibiting pro-oxidant characteristics that reduce the activity of antioxidant enzymes such as SOD, CAT, and GSH-Px. The ionic radius of the Al^{3+} ion is particularly similar to Fe^{3+}. Therefore, the appearance of Al^{3+} at Fe^{3+} sites is possible. Al is bound by the protein transferrin, known as the Fe^{3+} transporter, thereby reducing Fe^{2+} binding. This can lead to

increased levels of free Fe^{2+} within the cell, which in turn leads to Fe^{2+} peroxidation. This affects membrane lipids and can therefore cause membrane damage [50, 51]. Numerous investigations have demonstrated a clear correlation between oxidative stress and $AlCl_3$ [52–54]. The liver, kidney, and brain tissues of rats treated with $AlCl_3$ in this study showed elevated MDA levels, indicating that the treatment may cause the production of free radicals that initiate lipid peroxidation. Our findings also showed that the injection of $AlCl_3$ reduced GSH levels, SOD, and CAT activities. These results support the theory that peroxidation damage may be the source of elevated oxidative stress and impaired antioxidant defense enzyme activity.

Research in the literature demonstrates that $AlCl_3$ lowers GSH levels, increases MDA levels, and lowers SOD and CAT activity. These results corroborate our study findings [55–57]. According to our research, PJ therapy raised antioxidant markers such as GSH, SOD, and CAT while lowering MDA levels across the board in all tissues. The chelating ability of pomegranate polyphenols, which can sequester Al, reduce its bioavailability, and mitigate its harmful effects, is a possible mechanism for this action [55–58]. In addition, it has been determined that PJ functions as a powerful antioxidant in the detoxification of unsaturated fatty acids and free radicals because of its polyphenol and anthocyanin content [36, 59]. PJ maintains internal antioxidant balance and is associated with an increase in oxygenated respiration levels, particularly in response to increased energy demand from Al. This increase indicates its ability to reduce oxidative stress by scavenging accumulated high levels of free radicals, which in turn exert a protective effect on tissues [56–58].

In high Al exposure, nutritional strategies containing trace elements are applied [20]. In this context, detailed information on tissue mineral levels is required to effectively manage Al toxicity. In general, the uptake, distribution, and accumulation of metals in tissues depend on several factors, such as the properties, forms, route of uptake, dose, duration of exposure, ligand binding ability, and cell type [60]. According to our results, $AlCl_3$ accumulation occurred in the tissues as kidney < brain < liver, respectively.

PJ treatment significantly decreased Al accumulation in all three tissues. Reduction of Al levels may be the first protective mechanism of PJ against chronic tissue damage caused by Al exposure. Furthermore, the dietary intake of excess Al may affect the bioavailability of other trace elements such as Fe, Mg, Zn, and Ca, disrupting the overall metal homeostasis [8].

When we examined the elemental levels in liver tissue, we found a significant increase in Ca and a decrease in Mn levels in the Al exposure group compared with the C group. On the other hand, relatively less change was observed in Zn, Mg, Fe, and Cu levels.

The literature review reports that a rapid increase in Ca^{2+} concentration in the cell cytoplasm is usually observed under oxidative stress conditions [61–63]. Sun et al. [64] found that CCl_4 treatment increased Ca content in the liver and mitochondria. The transient increase in cellular Ca concentration is associated with cell death [65]. It is also likely that Ca metabolism is damaged by respiratory chain damage in mitochondria [66].

The element Mn plays an important role, especially by forming cofactors for antioxidant enzymes such as SOD and GPx [67]. In the present study, a significant decrease in Mn levels was observed in the liver of Al-exposed rats. This is thought to be due to increased antioxidant enzyme activity. In addition, it is also known that heavy metals such as Al, Cd, As, and Pb can replace Mn in antioxidant enzymes and reduce the activity of these enzymes [68]. Treatment with PJ restored Mn and Ca levels in the liver of Al-exposed rats. These results emphasize the potential protective effects of PJ on the restoration of antioxidant mineral levels. A previous study showed that a mineral mixture of Ca/P, Zn, and Fe replaces heavy metals in the body [69]. This report agrees with our current results because PJ also contains these minerals. These minerals may combine with antioxidant enzymes in the body to reduce or eliminate the harmful effects of heavy metals [70, 71].

When we examined the elemental levels in kidney tissue, we observed that Ca, Cu, and Mg levels were significantly increased, and Mn, Zn, and Fe levels were partially changed in the high-Al exposure group compared with the C group.

Al may replace Mg in the active sites of regulatory enzymes [72]. In addition, Al exposure did not affect hepatic Cu levels, which raises the possibility that Al may interfere with renal trace element levels in particular. Al and Cu may have a functional relationship according to their distribution in the kidney [73], but the exact mechanism is unclear.

The observed increase in renal Cu content in Al-treated rats appears to be consistent with previous data. Experimental animal studies have shown that Cu levels increase with oxidative stress [74]. Devipriya et al. [75] reported that Cu levels increased in an alcohol-fed rat group. The presence of excessive amounts of Cu in rats triggers increased oxidative damage to membrane lipids and DNA of liver and kidney tissues and may ultimately cause degenerative disorders [38]. Moreover, our results support the increased MDA levels observed in the oxidative stress state.

When PJ was administered, the Ca and Cu levels were significantly lower than those in the A group, although the Mg levels were relatively lower. The intake of PJ may mitigate any harm resulting from the disruption of element homeostasis in the kidneys of rats exposed to $AlCl_3$ by bringing the concentrations of Mg, Ca, and Cu back to normal. In a previous study, it was reported that Cu and Ca levels were altered by $AlCl_3$ treatment [76]. The return of Cu to basal

levels is important for the maintenance of proper cell signaling, cellular integrity, and antioxidative defense [77]. When resveratrol was administered to elderly rats loaded with Al and given therapy, Muselin et al. [78] found that the Mg content of the treatment group's liver and renal tissues was significantly lower than that of the Al-treated group. These outcomes also support our findings.

Because of their high Fe content, high oxygen consumption rate (20%), abundance of polyunsaturated fatty acids in cell membranes, and low anti-oxidative enzyme activity, brain tissues are especially vulnerable to oxidative damage [32].

Essential trace elements Fe and Ca were found to be higher in the brain tissues of rats in the A group of this study, but Mn levels were found to be lower. This could be because Al mimics Fe and modifies the expression of proteins that bind Fe [6]. Moreover, oxidative stress, Ca release from intracellular reserves, and compromised mitochondrial function have all been linked to some studies that have demonstrated critical steps in the mechanisms causing Al-induced neuronal cell death [79, 80]. Rats exposed to $AlCl_3$ had their brain elemental concentrations returned to normal after receiving PJ. This suggests that pomegranates can preserve and control element homeostasis in brain tissue.

The findings of this study suggest that PJ supplementation may interact with metal ions, decreasing their absorption, changing their distribution and storage, and increasing their excretion in rats exposed to high doses of Al. This could aid in reducing oxidative events and adjusting the elemental balance.

Conclusion

The levels of Ca and Mn in the liver; Cu, Ca, and Mg in the kidney; and Fe, Mn, and Ca in the brain were the most significant findings of PJ. Its capacity to scavenge free radicals was also established. PJ has a positive impact, as evidenced by the normalization of the imbalances in trace element concentrations observed in all organs of the PJ-treated group. This illustrates how the bioactive constituents of pomegranate can control the cellular absorption, distribution, and excretion of elements. The data obtained from this study provide a theoretical basis for future research by revealing the regulatory role of pomegranate in elemental homeostasis and its antioxidant potential, in line with similar studies in the literature. In addition, further research is needed to elucidate the mechanisms of action and synergistic effects of the phytochemicals contained in pomegranate. In the future, clinical trials should be conducted to understand the potential of pomegranate in the prevention of a wide range of pathological conditions and to support treatment strategies.

Author Contribution HC, HK, and ZAE conceived the experiments. HK, CER, KO, and AO carried out the experiments. HK, CER, KO, and AO contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Data Availability Data for this study is available inside the manuscript.

Declarations

Ethics Approval and Consent to Participate This study has been approved by the Ethics Committee of the of University Adiyaman Local Ethics Committee for Experimental Animals (2019/038).

Competing Interests The authors declare no competing interests.

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