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RESEARCH

Investigation of the Effect of Omega-3 Fatty Acids on Antioxidant System and Serum Aluminum, Zinc, and Iron Levels in Acute Aluminum Toxicity

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Abstract
Aluminum (Al), one of the three most prevalent metals in the Earth's crust, adversely impacts all metabolic systems of living organisms due to its extensive utilization by humans. It is known that omega-3 fatty acids (ω-3FA) protect the organism against diseases and have positive effects on the immune system. The aim of the study was to investigate the effect of ω-3FA on 8-OH-2-deoxyguanosine (8-OHdG), glutathione (GSH) levels and adenosine deaminase (ADA), paraoxonase (PON), and catalase (CAT) activities in rats with acute aluminum toxicity. The study also aimed to investigate the antioxidant system, as well as Al, zinc (Zn), and iron (Fe) levels. Forty Sprague–Dawley rats ($n=40$) were used in the study and the rats were divided into four equal groups ($n=10$). In group I, 0.5 mL of 0.9% saline solution (NaCI) was injected intraperitoneally. Group II was injected with 34 mg/kg aluminum chloride ($AlCI_3$) intraperitoneally. Group III received 400 mg/kg ω-3FA for 7 days and group IV received both $AlCI_3$ and 400 mg/kg ω-3FA for 7 days. At the end of the study, blood samples were obtained by cardiac puncture. The findings showed that Al exposure increased serum 8-OHdG and total oxidant status (TOS) levels, as well as ADA activity, which are markers associated with oxidative damage. Conversely, PON and CAT activities, GSH, and total antioxidant status (TAS) levels decreased compared to the control group. Furthermore, Zn and Fe levels decreased as Al levels increased. In conclusion, Al has the capacity to induce oxidative damage and lipid peroxidation, while ω-3 fatty acids may mitigate this damage through a regulatory mechanism. Moreover, ω-3-FA could be used as a therapeutic agent that reduces Al toxicity.

Keywords Aluminum · Omega-3 fatty acids · Heavy metals · Rat · Toxicity

Introduction

Aluminum (Al) is a silver-white, flexible metal that one of the most abundant elements in the environment. It is poorly absorbed and well eliminated in the body but if absorption does occur, it mainly distributes to the brain, liver, kidney, bone, testes, and blood, causing adverse effects on these organs [1, 2]. Al has a high affinity for participating in biochemical processes, particularly through strong binding with oxygen-based functional groups. This means that there are always numerous opportunities for Al to bind in any biological environment [3]. Chemical compounds containing Al, such as aluminum chloride, aluminum hydroxide, aluminum nitrate, aluminum phosphate, aluminum sulfate, aluminum potassium, aluminum ammonium sulfate, and aluminum silicate, are widely used in various products and processes [4, 5].

The main sources of Al exposure are drinking water, some foodstuffs (medicines, corn, cheddar cheese, salt, herbs, spices, tea), cosmetics and utensils. It enters the body through gastrointestinal and respiratory tracts and accumulates in all tissues, affecting lipid peroxidation and oxidative stress formation [6, 7]. It has been reported

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that exposure to Al disrupts cellular structure, causes cell death, induces the production of reactive oxygen species (ROS) and inflammation, and results in mitochondrial dysfunction, genetic damage and carcinogenicity [8]. Aluminum increases free radicals and changes the capacity of antioxidant enzymes. It has been stated that this situation disrupts the oxidant/antioxidant balance and lipid peroxidation, causing oxidative stress [9, 10]. As an index of lipid peroxidation, it has been reported that serum and tissue malondialdehyde (MDA) levels increase, antioxidant glutathione (GSH) content, glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) and catalase (CAT) decrease [11–15]. However, adenosine deaminase (ADA) activities and 8-hydroxy 2-deoxyguanosine (8-OHdG) levels have been shown to increase [16–19]. In addictionaly, it has been stated that exposure to aluminum compounds can also change the stability of the DNA structure and prevent DNA repair [20, 21]. Strong antioxidants, omega-3 fatty acids (ω-3FAs), are long-chain polyunsaturated fatty acids derived from plants and marine sources [22, 23]. Due to the fact that the human body does not produce these crucial fatty acids (EFA), they must be acquired through diet [24]. ω-3FAs exist in three different forms: alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). In recent years, numerous studies have focused on the role of these fatty acids in the human body [25]. In studies, it has been noted that ω-3FAs reduce oxidative stress, exhibit antioxidant properties, and inhibit ROS formation [26–31].

Given the widespread sources of Al exposure, it is crucial to find effective therapeutic approaches to reduce toxicity. Therefore, this study aimed to investigate the protective effects of ω-3FAs against Al toxicity as well as associated biochemical changes, oxidative stress, and DNA damage.

Material and Methods

The required permission for the study was obtained from the Local Ethics Committee of Kafkas University Animal Experiments (Approval No: KAU-HADYEK/2016–079). Housing, maintenance, and experimental procedures were conducted at the Kafkas University Experimental Animals Production and Experimental Research Center. During the experiment, the rats were housed in cages and kept at a room temperature 25 °C with a relative humidity of 50–55%. They were exposed to a 12-h light and 12-h dark cycle and had access to food and water ad libitum. The animals were fed a regular pelleted diet (Bayramoğlu-Erzurum). The physical and chemical properties of feed are collectively presented in Table 1.

Table 1 Feed content

	Feed content
Humidity	12.80%
Crude protein	23.00%
Crude cellulose	3.70%
Crude ash	8.30%
Crude oil	1.70%
Sodium	0.50%
Manganese	96.00 mg/kg
Iron	31.00 mg/kg
Zinc	95.00 mg/kg
Cobalt	0.50 mg/kg
Selenium	0.30 mg/kg
Iodine	2.28 mg/kg
Vitamin A	12,000,000 IU/kg

Food pellets (Bayramoglu Feed and Flour Industry and Trade Inc., Erzurum, Turkey)

Experimental Design

Forty Albino Sprague–Dawley male rats six months old were used in the study. The rats were randomly divided into four groups and the live weight was weighed for the amount of dose to be administered to each rat. The up and down method was used because it allowed the use of fewer animals [32].

The groups were as follows:

Group I: Control ($n = 10$), during the experiment, 0.5 mL of saline solution (0.9%) was administered intraperitoneally daily.
Group II: Al ($n = 10$) The rats were given a single intraperitoneal dose of Al (34 mg/kg Al^{+3}) ($AlCl_3$ dissolved in 0.9% NaCl, pH 4.5) [19].
Group III: ω-3FA ($n = 10$) The rats were given ω-3FA (Marincap, Kocak Pharma, Turkey; the composition of the Marincap capsule was EPA 18% and DHA 12%) (400 mg/kg), for seven days [26].
Group IV: Al + ω-3FA ($n = 10$) The rats were given a single intraperitoneal dose of Al + ω-3FA (34 mg/kg Al^{+3} with 400 mg/kg ω-3FA). After 7 days, the animals were sacrificed under anesthesia, and intracardiac blood samples were taken. The samples were then centrifuged at 3000 rpm for 10 min to separate the serum, which was stored at − 45 °C until analysis.

8-OHdG Assay

The levels of 8-OHdG were determined using enzyme-linked immunosorbent assay commercial kits following to the manufacturer's procedures (SunLong and SunRed Bio, Shangai, China). All spectrophotometric analyses were performed on the microplate reader (Bio-Tek Eon, USA).

GSH Assay

GSH analysis in blood was performed according to the Beutler method [33]. Briefly, all proteins that do not carry the sulfhydryl (-SH) group in the samples were precipitated. The absorbance of the yellow complex resulting from the reaction of the proteins containing the -SH group with 5,5'-2-ditiobis nitro benzoic acid was then read at 412 nm in spectrophotometer.

ADA Activity Assay

ADA activity in serum samples was measured using the method described by Giusti and Galanti [34]. Adenosine was used as a substrate and incubated with the sample at 37 °C for 30 min. The formation of ammonia resulted in the production of blue indophenol in the presence of sodium hypochlorite and phenol in an alkaline medium. In this experiment, sodium nitroprusside acted as a catalyst and the concentration of ammonia was directly proportional to the absorbance of indophenol. To perform the experiment, 1 mL of phosphate buffer and 50 µL of distilled water were added to the reagent blind tube, 1 mL of ammonium sulfate, and 50 µL of distilled water were added. In the sample tube, 1 mL of adenosine solution and 50 µL of the sample were added. Additionally, 1 mL of adenosine solution was added to the sample blind tube. The test tubes were closed and incubated at 37 °C for 1 h. Then, 3 mL of phenol/nitroprusside solution was added. After adding 0.05 mL of the sample to the sample blind tube, 3 mL of alkaline hypochlorite solution was added to all tubes. The tubes were closed again and incubated at 37 °C for 30 min. Following incubation, the absorbance of the tubes against the blind was measured at 625 nm. One unit of ADA activity was defined as the amount of enzyme that releases 1 µmol of ammonia from adenosine per minute.

PON Activity Assay

PON activity was determined using the method specified by Furlong et al. (1988). Paraoxon was used as the substrate in the measurement of PON activity. The absorbance of the resulting color, which occurs due to the hydrolysis of paraoxon at 37 °C, was determined at 412 nm [35].

CAT Activity Assay

CAT activity was determined using the method specified by Aebi (1984). This method was based on the principle that catalase neutralizes H_2O_2. The measurement of CAT activity was determined at 240 nm [36].

Determination of TAS and TOS Levels

TAS and TOS levels of serum were measured using the Bio-Tek Eon auto analyzer with the Rel-Assay test kit (Rel Assay®, Gaziantep, Turkey). TAS levels were measured using an automated colorimetric method, and the results were expressed as µmol Trolox Eq/L [37]. TOS levels were also measured using an automated colorimetric method. Calibration for the test was done using hydrogen peroxide (H_2O_2) and the results were expressed in micromolar hydrogen peroxide equivalents per liter (µmol H_2O_2 Eq/L) [38].

Determination of Al, Zn, and Fe Levels in Serum

Al, Zn, and Fe levels in the serum were analyzed using inductively coupled plasma–mass spectrometer (ICP-MS) (CPMS Nexion 300X) [39, 40]. Before use, tubes, pipette tips, glassware, and containers used in the study were soaked in 10% TraceMetal Grade HNO_3 for at least 24 h and rinsed multiple times with double-distilled water. To prevent potential Al contamination, only metal-free laboratory materials ($Al \leq 1$ ng/g) were used in all sample processing and analysis procedures.

Standards and Chemicals

Standard solutions and tuning solutions were purchased from Agilent Technologies (CA, USA). High-purity argon gas (99.999%) used for ICP-MS analysis was sourced from HABAŞ (Antalya, Turkey). Ultrapure water (18.2 M×cm) was produced using the ELGA DI Polishing System (High Wycombe, UK). HNO_3 (TraceMetal Grade) was purchased from Sigma.

Calibration Standards and Quality Controls

For the comparative analysis of trace element concentrations of Al, Zn, and Fe in serum, a serial dilution of the control samples and calibration was prepared by decreasing amounts (10, 50, 100, 200, 450, 700, and 1000 ng/mL) as calibration standards. The calibration standards were prepared daily in the surrogate matrix. We chose Al 27, Zn 66, and Fe 56 as the isotopes for our targeted analytes. The matrix-matched

protocol was used to eliminate mass matrix interference. The surrogate matrix used in this study was a 2% (v/v) HNO_3 solution prepared in ultrapure water. The kinetic energy discrimination mode was used for Al to eliminate polyatomic ion interference and prevent contamination with atmospheric particulates. Sample preparation and dilution of standards were performed under a clean hood.

Sample Preparation for ICP-MS Analysis

Before analysis, 50 µL of the blood samples were placed in metal-free plastic containers that had been pre-cleaned with 2% (v/v) HNO_3. The samples were then digested with 8 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 in a microwave oven (MilestoneStard-D) in two steps. In the first step, the samples were heated to 180 °C for 10 min. Following this, the heating was continued for another 15 min at 180 °C [39]. After digestion, the samples were placed in the autosampler for analysis. To determine the levels of Al, Zn, and Fe in blood samples, an ICP-MS Nexion 300X system equipped with software was used [40]. The operational parameters of the ICP-MS were determined in plasma conditions: RF power of 1600 W, plasma gas flow of 15 L min^{-1}, carrier gas fow of 0.75 L min^{-1}, dilution gas fow of 1 L min^{-1}, and helium gas fow of 4.5 L min^{-1}. The sample uptake tubing had an inner diameter of 0.38 mm id (green/orange) and PVC drain tubing had an inner diameter 1.30 mm id (gray, gray). The autosampler tubes were metal-free conical tubes that had been prewashed with 2% (v/v) HNO_3 for sterilization. The ICP-MS was operated with an RF power of 1600 W and a helium fow of 4.5 L min^{-1} in the gas cell. The nebulizer fow rate, auxiliary gas fow rate, and plasma gas fow rate were 0.98, 1.2, and 15 L min^{-1}, respectively. The integration time was 0.1 s per point to obtain one point per mass. The autosampler was also equipped with a programmable rinse function. The assay was blindly run in triplicate for each sample, and the mean of three readings was used for further statistical analyses.

Statistical Analysis

Statistical analyses were performed in triplicate and average values with standard deviation (mean ± SD) are reported. The statistical significance was evaluated using the SPSS 20.0 software package (SPSS ver. 20.0 for Windows Professional Edition). A one-way analysis of variance (ANOVA) was performed, followed by Duncan's test to estimate significance at the 5% probability level. Tukey's post hoc test was also performed. The Mann–Whitney *U*-test was conducted to determine the source of significant differences among groups.

Results

The serum 8-OHdG level in the Al-administered group was significantly higher ($p < 0.001$) than in the control, ω-3FA, and Al + ω-3FA groups (Fig. 1a). The blood GSH level was found to be significantly lower ($p < 0.001$) in the Al-administered group compared to the control group and other experimental groups (Fig. 1b). Serum ADA activity in the Al-administered group was significantly higher ($p < 0.001$) than in the control and ω-3FA groups. However, no statistical difference was found in the Al + ω-3FA group ($p = 0.905$) (Fig. 1c). Serum PON activity in the Al-administered group was significantly lower ($p < 0.005$) than in the control, ω-3FA, and Al + ω-3FA groups (Fig. 1d). Serum CAT activity in the Al-administered group was significantly lower ($p < 0.001$) than in the control, ω-3FA, and Al + ω-3FA groups (Fig. 1e).

Serum TAS levels were lower in the Al group compared to the other groups ($p < 0.001$). It was observed that this level increased in the Al + ω-3FA group compared to the Al group ($p = 0.222$). Additionally, there was a significant increase in TAS levels compared to both the control and ω-3FA groups ($p < 0.005$, $p < 0.05$, respectively). There was no statistically significant difference in TAS levels in the ω-3FA group compared to the control ($p = 0.258$), but a significant difference was observed when compared to Al + ω-3FA ($p < 0.05$) (Fig. 2a).

Serum TOS levels were higher in the Al group compared to the other groups ($p < 0.05$). However, the Al + ω-3FA group showed a decrease in TOS levels compared to the Al group. Suprisingly, there was a significant increase in TOS levels compared to both the control and ω-3FA groups ($p < 0.05$). The ω-3FA group did not show a statistically significant difference in TOS levels compared to the control and Al + ω-3FA groups ($p = 0.841$, $p = 0.222$, respectively). However, TOS levels in the ω-3FA group were lower compared to the Al group, and this difference was statistically significant ($p < 0.05$). There was also a statistically significant difference between the Al and Al + ω-3FA groups ($p = 0.310$) (Fig. 2b).

Compared to the control group, the highest serum Al level was found in the Al-administered group and the Al + ω-3FA-administered group ($p < 0.001$). There was no statistically significant difference between the Al levels in the ω-3FA-administered group and the control group ($p = 0.394$). Serum Zn concentration in the Al-applied group was significantly lower than the control group and other experimental groups ($p < 0.001$). Similarly, there was no statistically significant difference between the Al levels in the ω-3FA-administered group and the control group ($p = 0.177$). Serum Fe concentration in the Al-applied group was significantly lower than the control group and

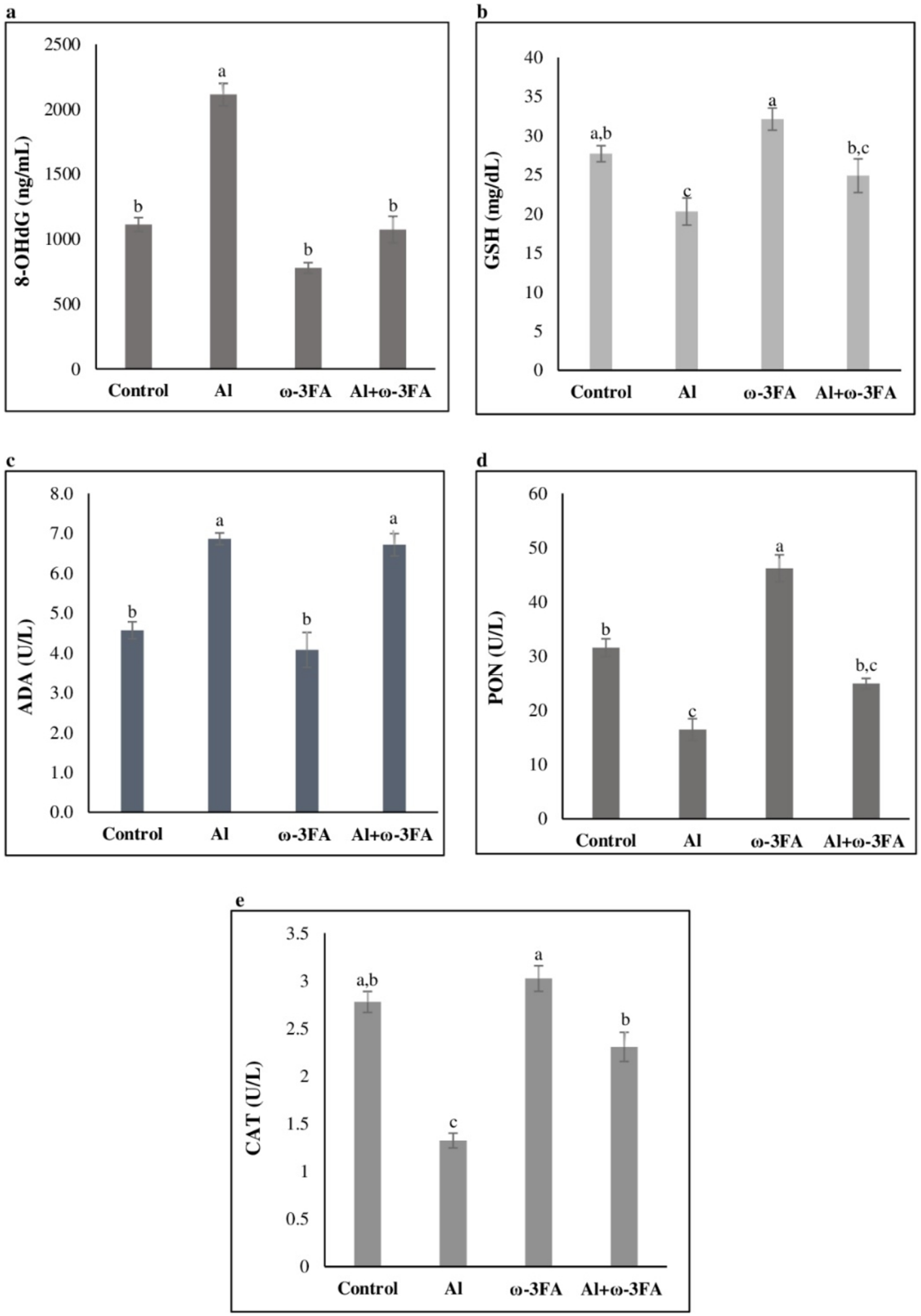


Fig. 1 8-OHdG and GSH levels, ADA, PON, and CAT activity, in serum. **a** and **c** Different superscripts in the same row show a statistical difference (**a** and **b**, $p < 0{,}001$). **b** Different superscripts in the same row show a statistical difference (**a**, **b**, and **c**, $p < 0.001$). **d** Different superscripts in the same row show a statistical difference (**a**, **b**, and **c**, $p < 0.005$)

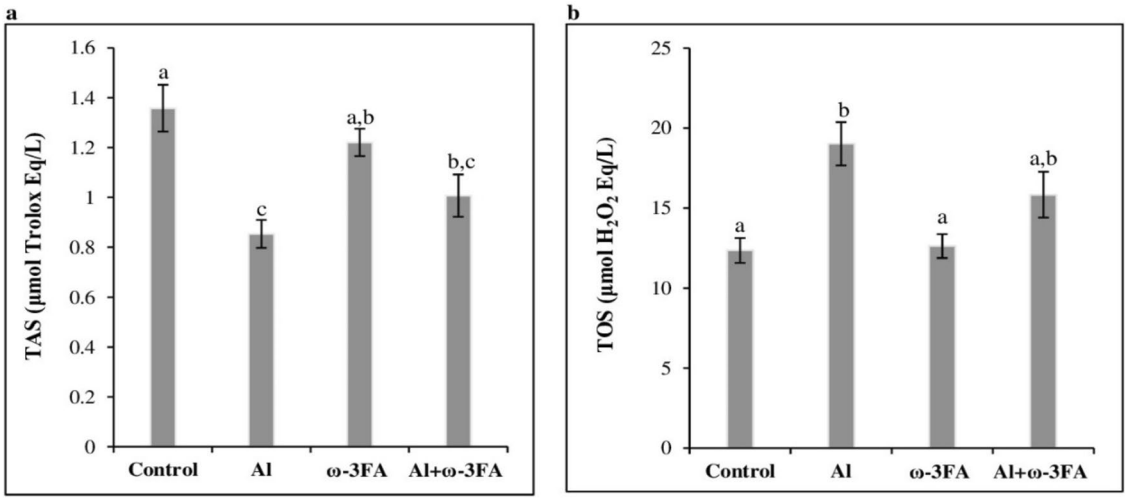


Fig. 2 Levels of serum TAS and TOS in the experimental groups. **a** Different superscripts in the same row show a statistical difference (**a**, **b**, and **c**, $p < 0.001$). **b** Different superscripts in the same row show a statistical difference (**a** and **b**, $p < 0.05$)

Table 2 Levels of serum aluminum, zinc, and iron in the experimental groups

Parameters	Control	Al	ω-3FA	Al + ω-3FA	*P*
Aluminum (ppm)	0.47^{c}	2.64^{a}	0.44^{c}	0.92^{b}	$p < 0.001$
Zinc (ppm)	0.96^{a}	0.47^{c}	1.08^{a}	0.70^{b}	$p < 0.001$
Iron (ppm)	0.98^{a}	0.44^{b}	$0.90^{a,b}$	$0.51^{a,b}$	$p < 0.05$

[a,b,c]The groups in the same line labeled different letters are statistically significant

other experimental groups ($p < 0.05$). While there was no statistically significant difference between the Al levels in the control group and the group given ω-3FA ($p = 0.792$), a statistically significant difference was found with the group given Al + ω-3FA, ($p < 0.05$) (Table 2).

Discussion and Conclusion

Metals such as aluminum are important contributors to environmental pollution. This toxic metal is not biodegradable as cells cannot break it down. Therefore, it poses a significant health hazard worldwide as it has the potential to contribute to the occurrence of a number of diseases [41]. Antioxidants have proven to be useful in conditions associated with oxidative stress. Studies on ω-3FAs have reported that they have antioxidant, anti-apoptotic, anti-inflammatory, and antihypertensive effects. They also reduce oxidative stress and prevent the formation of ROS [42, 43]. The aim of this study was to investigate the effect of ω-3FAs on 8-OHdG and GSH levels, ADA, PON, and CAT activities, as well as the antioxidant system, Al, Zn, and Fe levels in experimental Al toxicity.

The study examined the possible connections between levels of aluminum, oxidative stress, and DNA damage in workers who are regularly exposed to aluminum in their occupation. The study revealed that the levels of serum Al and urinary 8-OHdG were markedly elevated ($p < 0.001$) in the group exposed to Al, while the plasma TAS levels were dramatically reduced ($p < 0.001$) compared to the group not exposed to Al. According to these data, it was shown that higher levels of Al resulted in oxidative DNA damage in workers exposed to Al [18]. Al is known to increase oxidative stress in the brains of rats, which speeds up the accumulation of amyloid beta and the formation of plaques. In a study on Al-induced neurotoxicity in Wistar rats, the rats were exposed to $AlCl_3$ through oral administration of 100 mg/kg for 21 days. This exposure caused a significant increase in levels of nitric oxide (NO) and 8-OHdG levels ($p < 0.01$). It was also found that the levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly reduced ($p < 0.01$). However, when eugenol, which is identified in clove oil and has phytochemical properties, was administered to rats, there was a significant decrease in NO and 8-OHdG levels ($p < 0.01$), and it was observed that SOD and GPx levels increased significantly ($p < 0.05$) [44].

When evaluating the antioxidant status of SOD, CAT, and GPx enzymes in the brain tissues of rats, it was found that after camellia oil which is known to contain omega-3 fatty acids application reduced the effects of oral $AlCl_3$ exposure. The researchers observed a significant decrease in $AlCl_3$ levels and an increase in malondialdehyde (MDA) level in rats compared to the control group ($p < 0.05$). Additionally, the application of camellia oil was found to significantly

reduce inflammatory cytokines and induce antioxidant activity in rats with $AlCl_3$-induced cognitive impairment. Furthermore, both aqueous and alcoholic extracts of *Echinacea purpurea* were found to inhibit the production of NO and MDA in oxidative stressors, compared to the Al group ($p < 0.001$). These extracts significantly altered the levels of antioxidants such as catalase, SOD, and GSH ($p < 0.001$). In addition, prolonged exposure to aluminum has been shown to cause neurotoxicity. Among the effects observed, it was noted that it caused an increase in lipid peroxidation, significant increases in DNA fragmentation, increased oxidative stress in the hippocampus, increased mitochondrial activity, impaired outer membrane permeability, significant decreases in antioxidant enzyme activity, and significant changes in neurotransmitter concentrations [45–49].

Atakisi et al. noted that there were limited studies investigating the impact of heavy metals on ADA activity. They found that ADA activity was higher in the group treated with Al compared to the control group, indicating a positive correlation with increased oxidative stress [19]. In our study, we observed a significant increase in 8-OHdG levels following Al application. Additionally, there was a significant increase in ADA activity, a metabolic enzyme, and a significant decrease in GSH levels. However, treatment with ω-3FA resulted in a decrease in cellular 8-OHdG levels and ADA activity, while increasing GSH level. This suggests that ω-3FAs treatment offers protection against oxidative damage to mitochondrial DNA caused by ROS. Similar studies have observed that levels of serum MDA significantly increased, while values of serum GSH significantly decreased in the group treated with Al. This decrease in GSH levels may affect the synthesis of GSH by reducing the activity of glutathione-synthase. Additionally, it has been reported that treatment with ω-3FA can reduce the severity of Al-induced neurotoxicity and improve glutathione levels. These findings align with the results of our study [50, 30, 51].

Oxidative stress occurs when there is an imbalance between the production of ROS and the antioxidative processes, favoring radical production. In the present study, a noteworthy reduction in the activities of GSH, PON, and CAT antioxidant enzymes indicates the inability of the antioxidant defense system to counteract the increased influx of ROS induced by exposure to $AlCl_3$. These findings support the finding of Cheraghi and Roshanaei [52], who also reported a significant decrease in the activities of GSH, PON, and CAT after treatment with $AlCl_3$. Al has toxic effects due to the high risk of daily exposure. Studies have reported that Al causes oxidative damage by reducing antioxidant parameters and increasing oxidant parameters [19, 53]. Similarly, our study revealed that Al may induce oxidative stress by decreasing the TAS level and increasing the TOS level. The application of ω-3FA provides insights in to the mechanisms through which Al exposure may cause toxicity.

Essential elements such as Zn and Fe are microelements found in the blood and tissues. They are crucial for enzymatic activities, metabolic processes, and vital functions. Zn plays a significant role in protein, RNA, and DNA synthesis, while Fe is considered the most important element in transfering oxygen to tissues. However, the presence of toxic metals like Al in the body can interfere with the metabolic use of these essential elements [54, 55]. Our experimental study also revealed that Al toxicity led to a significant increase in Al levels in rat serum while simultaneoulsly causing a significant decrease in Zn and Fe levels. This aligns with previous findings, which demonstrated, it has been shown that plasma Al concentration increases significantly after different application methods and doses of Al, while Zn levels decrease [19, 56–59]. It has been suggested that high plasma Al induces metallothionein, which binds essential elements like Zn and other heavy metals [60]. Our results are consistent with those of other studies that have confirmed the impact of serum Al and Zn concentration on metabolism, serum concentrations, and the distribution of essential metals in body tissues. It is known that Al limits the binding of Fe^{2+} by binding to transferrin, which is protein that carries Fe^{3+}. Peroxidation of membrane lipids occurs when there is an increase in free intracellular Fe^{2+} leading to membrane damage [61]. There is believed that to be a clear connection between Al toxicity and dysregulation of redox metals, such as Fe. Formation of Al-transferrin complex disrupts iron homeostasis. Fe homeostasis effectively regulates the intake and distribution of Fe due to the body's inability to excrete it. Additionally, the production of Fe-binding proteins is regulated by the Fe-sensitive element/Fe regulatory protein (IRE/IRP) network, which prevents the formation of free Fe^{2+}. Al^{3+} can bind to IRP and affect the expression of IREs and iron-binding proteins, resulting in increased iron concentration [62–65]. Free Fe ions released from biological complexes by Al can catalyze the decomposition of hydroperoxides into hydroxyl radicals through the Fenton reaction, initiating peroxidation of membrane lipids and causing membrane damage [66]. Our experiment showed that Al toxicity significantly decreased Fe levels in rat serum, indicating a direct relationship between Al and Fe concentrations. On the other hand, the present study showed that treatment of rats with Al + ω-3FA decreased serum Al level and enhanced serum Zn and Fe levels compared to Al intoxicated group. This effect of ω-3FA application can be considered as an indication that it protects cellular membrane integrity from Al-induced oxidative damage.

It is known that the mode of action of ω-3FA is to block and clear the formation of reactive oxygen and increase endogenous antioxidant abilities [67]. It has been revealed in parallel with previous results that the power of ω-3FAs

to resist oxidative damage against Al toxicity is due to their antioxidant properties. Serum 8-OHdG level also supports these results [29, 68]. It has been shown that ω-3FA treatment increases PON and CAT activities as well as GSH content, while reducing the level of TOS. The decrease in TOS level is attributed to the antioxidant properties of ω-3FA, which inhibit lipid peroxidation, and the increase in TAS level supports this. This oxidant/antioxidant theory may explain the protective role of ω-3FAs against Al toxicity.

The study findings demonstrated that the presence of Al could induce notable alterations in some biochemical indicators of oxidative stress and impede the functioning of the antioxidant system. It was determined that ω-3FAs can mitigate this damage and provide a protective function. Therefore, it is crucial to focus on minimizing the sources of aluminum exposure. Consuming a diet that is high in ω-3FAs can help reduce the harmful effects of aluminum intoxication.

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Authors' Contributions E.A, O.A, K.Y.D, M.O and C.O. conceived the original idea and built the project. E.A and O.A. supervised the research, while experiments were performed by E.A., O.A., K.Y.D., and M.O. All authors have read and approved the fnal manuscript, which was written by K.Y.D., C.O and M.O.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval The study protocol was approved Kafkas University Animal Experimentation Ethics Board (KAU-HADYEK 2016–079).

Competing Interests The authors declare no competing interests.

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