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Omega-3 fatty acids (EPA/DHA) act as a natural antioxidant to alleviate cadmium-induced oxidative stress and restore the oxidation/antioxidant balance in male Wistar mice

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ORIGINAL ARTICLE

Abstract

Omega-3 fatty acids are primarily derived from marine and plant sources and are commonly found in fatty fish and fish oils. The present study aimed to evaluate the *in vivo* antioxidant properties of omega-3 EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) and to investigate the effectiveness of exogenous omega-3 fatty acids in mitigating cadmium-induced oxidative stress. The experimental mice were allotted into four groups (n = 20), designated as untreated control, omega-3-treated, cadmium-exposed, and cadmium–omega-3 groups. The hematological and biochemical assays were performed to achieve the study's aim. Both hematological and biochemical profiles of cadmium-exposed mice (Group 3) manifested significant alterations, including increments and decrements, compared to that of untreated control mice. Concerning the biochemical profile (serum profile), group 2 animals (omega-3-treated group) demonstrated no significant changes, compared to the untreated control. Mice in group 4 (cadmium-exposed and omega-3-accessed) exhibited increased levels of total proteins, a significant increase in the levels of antioxidant markers, such as total thiols, glutathione, total antioxidant capacity, superoxide dismutase, glutathione peroxidase, and catalase, and a significant decrease in the levels of blood cadmium, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatinine, blood urea nitrogen, urea, bilirubin, and oxidation markers (hydrogen peroxide and malondialdehyde), compared to the animals exposed to cadmium (group 3). Tissue homogenates of the liver and kidney prepared from group 3 animals demonstrated parallel results to that revealed by serum biochemical analysis. It was concluded that omega-3 fatty acids (EPA/DHA) possess efficient antioxidant properties that effectively help to attenuate the oxidative stress induced by cadmium.

Keywords: antioxidant; biochemical profile; cadmium toxicity; omega-3 fatty acids

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Introduction

Omega-3 fatty acids (FAs) are primarily found in fatty fish, fish oils, and plant oils of marine and plant sources (Rahim *et al.*, 2023). These FAs are not produced in mammalian tissues (Saini *et al.,* 2021; Sarikaya *et al.,* 2023) and are mainly acquired through diet, especially from fish oil (Heshmati *et al.*, 2019; Ogłuszka *et al.,* 2024). They are long-chain polyunsaturated fatty acids (PUFAs) with double bonds in their carbon chain structure with carboxyl (COOH) and methyl ends (Heshmati *et al.,* 2019; Saini and Keum, 2018). The methyl end, also known as the omega end, is close to the first structural double bond, which is located at the third position from this end in the case of omega-3 (Smith *et al.,* 2011; Wang *et al.,* 2024). Omega-3 PUFAs consist of two primary acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Hajianfar *et al.,* 2013). Fatty fish, such as mackerel, salmon, and mullet, are the main dietary sources of these FAs (Detopoulou *et al.,* 2024).

Omega-3 EPA and DHA are involved in the synthesis of bioactive lipid compounds and have positive effects on growth, fetus development, vital organs, such as the heart and brain, as well as behavioral and mood status (Saini and Keum, 2018).

Moreover, omega-3 PUFAs help protect against a range of significant diseased conditions, such as atherosclerosis, cardiovascular diseases, diabetes, hypertension, autoimmune diseases, arthritis, cancer, and sickle cell disease (Saini and Keum, 2018; Smith *et al.,* 2011).

Clinical trials have proved the efficacy of omega-3 in reducing the risk of cardiovascular diseases, alleviating dyslipidemia, and inhibiting thrombosis (Saini and Keum, 2018). The positive effects of omega-3 PUFAs are involved in a range of bioactive processes, including thrombogenesis, inflammation, and metabolism of lipoproteins (Meital *et al.,* 2019). Omega-3 FAs are beneficial in ameliorating the complications of multiple diseased conditions, such as cardiovascular diseases, inflammatory processes, and dyslipidaemia (Tantipaiboonwong *et al.,* 2021).

The beneficial health effects of omega-3 FAs are mostly attributed to their anti-inflammatory, antioxidative, antitumor, cardioprotective, and neuroprotective properties (Ateya *et al.,* 2022; de Mattos *et al.,* 2017; Detopoulou *et al.,* 2024; Saini and Keum, 2018).

Antioxidants refer to the compounds that can counteract the oxidative damage caused by reactive oxygen species (ROS), and in this way combat oxidative stress (Saini and Keum, 2018).

The accumulated clinical and experimental evidence demonstrates the role of antioxidants in promotion of vital body functions, including that of the heart, immune system, and the brain as well as slowing the signs of aging. In many cases, regular foods are deficient in natural dietary antioxidants (antioxidant nutrients) that are best exemplified by vitamins C and E, selenium, zinc, polyphenols, and carotenoids. In such situations, there is an increasing demand for antioxidant supplements that support the body's bioactive processes and maintain healthy status. The potent supplementary antioxidant can restore a balance between oxidation and reduction reactions (redox status) (Mazereeuw *et al.,* 2017).

Oxidative stress is the status of imbalance between the rate of ROS production and efficacy of the endogenous antioxidant system to overcome the detrimental effects of ROS. Oxidative stress is one of the major contributors to the development of significant diseased conditions, such as cardiovascular diseases and cancer (Saboori *et al.,* 2016). Excess ROS damage cellular structural lipids, proteins, and deoxyribonucleic acid (DNA), and eventually lead to diseased conditions of significant medical concern, such as neurodegenerative disorders, cardiovascular diseases, cancer, diabetes, accelerated aging, and immune dysfunction (Anderson *et al.,* 2014). The role of a potent antioxidant is to limit the extent of ROS activity, contributing actively in maintaining redox status as a cytoprotective mechanism (Soleimani *et al.,* 2017).

The accumulated clinical evidence points to the properties of omega-3 PUFAs as an antioxidant to combat oxidative stress (Razavi *et al.,* 2017; Sharma *et al.,* 2015). Omega-3 PUFAs are found to be effective in mitigating oxidative stress in diabetic patients (Ateya *et al.,* 2022). Some researchers believe that the aforementioned beneficial effects of omega-3 PUFAs are mediated by the enhanced antioxidant defense system (Feng *et al.,* 2017). It has been reported that the diverse antioxidant properties of omega-3 FAs contribute significantly to protection against oxidative stress, as is the case in the brain, eye, liver, kidney, heart, skin, and testis (Meital *et al.,* 2019).

Cadmium (Cd) is a highly toxic heavy metal that naturally exists in the environment and is involved in a wide spectrum of industries. Exposure to Cd is either occupational or non-occupational (environmental). Occupational exposure is linked with the inhalation of industrial fumes, while non-occupational exposure is related to the ingestion of polluted feed and water. The hazardous effects of chronic Cd toxicity are progressive because the accumulation of this toxicant heavy metal is gradual (cumulative) in various body tissues. Cd toxicity is implicated in the causation of profound oxidative

stress associated with oxidative damaging effects in tissues and organs, particularly in the liver and kidney (Yong *et al.,* 2017).

Oxidative actions induced by certain metabolites or external toxins are among the main damaging insults that occur inside the living body. These undesired actions may be severe enough to eventually cause cell death and extensive tissue damage. Counteracting these actions, either by prevention or inhibition, plays a crucial role in relevant antioxidative mechanisms. The naturally operating and efficient endogenous antioxidant system detects oxidative metabolites, such as free radicals, and prevents their damaging effects (Case, 2017; Sepidarkish *et al.,* 2020). The beneficial effects of this essential bioactivity are the maintenance of a persistent balance between oxidation and antioxidant activities to protect cells and preserve the normal functioning of cells and tissues.

The issue of natural antioxidants that can be used as dietary supplements is of great concern. Their use has steadily grown as a prophylactic measure for some significant diseased conditions, such as cardiovascular diseases, neurodegenerative disorders, cancer, and arthritis, as well as in the alleviation of aging changes (Fazelian *et al.,* 2021; Pisoschi and Negulescu, 2011; Staudacher *et al.,* 2018).

The current study was conducted to test the potency of antioxidant properties of the employed omega-3 FAs. The antioxidative properties of omega-3 FAs were evaluated experimentally in a state of challenging oxidative stress induced by Cd toxicity in male Wistar mice.

Materials and Methods

Ethical considerations

The guidelines for the care and use of laboratory mice, as per institutional and national regulations, set by the Research Ethics Committee of Imam Mohammad Ibn Saud Islamic University (IMSIU), were diligently adhered to (LAB-rats-2024-0173). All participants were informed about their participation in the present research work.

Type of sampling and reasons for selection

In the present study, blood, harvested serum, and tissue homogenates were selected as the investigated samples. These samples were chosen to reflect alterations in the hematological and biochemical profiles of experimental mice.

Inclusion and exclusion criteria

Inclusion criteria

All mice in the different experimental groups were included in the conducted assays. Blood samples were collected from all experimental mice to carry out hematological assays to assess erythrocytic and leucocytic counts, hemoglobin (Hb) concentration, and packed cell volume (PCV). The harvested serum samples as well as the tissue homogenates obtained from all experimental mice were subjected to biochemical assay to estimate the levels of antioxidative and oxidative parameters.

Exclusion criteria

No exclusion criteria were applied in the current study. In other words, all experimental mice were included in the process of samples (blood and tissues) collection. Any exclusion criteria applied could alter the accuracy of performed analysis in different ways.

Experimental mice

In all, 80 adult male Wistar mice, aged 3 months and weighing 145–210 g, were used in this study. The mice were obtained from the inbred colonies of the animal house, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The mice were maintained under standard laboratory conditions (ambient temperature 24 ± 1°C; 12-h dark–light cycle; relative humidity (RH): 35–70%).

Omega-3 fatty acids

Omega-3 FAs were employed as EPA and DHA originated from fish oil (Sigma-Aldrich, Darmstadt, Germany). EPA (cis-5,8,11,14,17-eicosapentaenoic acid) (molecular weight [MW]: 302.45; CAS number: 10417-94-4). DHA (cis-4,7,10,13,16,19-docosahexaenoic acid) (MW: 328.49; product No. D2534).

Cadmium

Cadmium was used as cadmium chloride ($CdCl_2$) of analytical grade (product No. 655198; Merck, Darmstadt, Germany). Cd was dissolved in purified water to prepare the required aqueous solution.

Experimental design

Mice were acclimatized for 1 week, and then randomly and equally allotted into four groups of 20 animals

each, designated as groups 1–4. Mice in group 1 served as the untreated control, that is, they were not exposed to Cd and did not receive omega-3. Mice in group 2 were administered daily with omega-3 via oral route at a dose of 400 mg/kg body weight (bw) (EPA 240 mg, DHA 160 mg). Mice in group 3 received the aqueous solution of $CdCl_2$ by oral gavage at a final concentration of 3 mg/kg bw/day as 1 mL/kg bw. The control mice received an equal volume of saline via the same route. Mice in group 4 received Cd orally and administered with omega-3 at the above-mentioned dose. A 10-h gap was maintained between the daily administration of Cd and omega-3.

The experimental period was 8 weeks, during which dry feed (commercial pellets; Nesom Distributing, Envigo, USA) and drinking water were supplied *ad libitum.*

All experimental mice were observed for behavioral activity, feed consumption, water intake, and clinical signs.

Hematological and biochemical assays

On the termination day of the experiment, all animals were anesthetized (3% isoflurane) and blood samples were collected via cardiac puncture. Blood samples collected with anticoagulant ethylenediaminetetraacetic acid (EDTA) were used to estimate various hematological indices. Serum harvested from coagulated blood samples was removed immediately and stored at –20°C until a biochemical assay was performed. Mice were killed by decapitation, and liver and kidney tissues were removed and homogenized in 150-mM NaCl. Then the homogenates were centrifuged at 3,000 ×*g* at 4°C for 10 min. The collected supernatants were used to determine various biochemical parameters.

Blood cadmium level

To assess Cd levels in the blood, 1-mL blood sample was subjected to digestion using a mixture of $HClO_4^-$ and HNO_3; blood Cd level was determined using an atomic absorption spectrophotometer (CBC 906 AA).

Hematological assay

Blood samples collected with anticoagulant EDTA were used to estimate various hematological parameters, such as RBCs and total WBC counts, and erythrocytic indices, such as Hb concentration and PCV%. Erythrocytic and total leukocyte counts were measured using a convenient hemocytometer. The PCV% was determined using the micro-hematocrit method. Hb concentration was estimated using the Cyanmet-hemoglobin method.

Biochemical assay

Serum separated from coagulated blood samples was used to estimate the following: biochemical parameters, such as total proteins, albumin, globulin, creatinine, urea, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP); antioxidant markers, such as total thiols, catalase, glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total antioxidant capacity (TAC); and oxidation markers, such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2).

Total thiols were measured using a total thiol colorimetric assay kit (MET-5053; Cell Biolabs Inc., USA). GSH was estimated using a reduced glutathione colorimetric assay kit (E-BC-K030-S; ElabScience, USA). Catalase levels were determined using a catalase activity colorimetric assay kit (BioVision ab 83464; Abcam, UK). SOD activity was estimated using a SOD activity assay kit (Sigma-Aldrich). GSH-Px activity was assessed using a GSH-Px activity assay kit (Elabscience, Houston, TX, USA).

Total antioxidant capacity was assessed by employing TAC assay kit (MAK 187-1 KT; Sigma-Aldrich). The principle of this kit is to determine the concentration of combined proteins and small-molecule antioxidants or the concentration of only small-molecule antioxidants. Cu^{2+} ions are converted to Cu^{+} by small molecules and proteins. However, insertion of a protein mask as a component of the kit prevents the reduction of Cu^{2+} by proteins, enabling the analysis of only small-molecule antioxidants. Cu^{+} ions (reduced by the small molecules) were chelated with a colorimetric probe, and the resultant absorbance peak was proportional to antioxidant capacity.

The H_2O_2 levels were determined using an H_2O_2 colorimetric assay kit (E-BC-K102-S; Elabscience, USA). MDA levels were estimated using a colorimetric assay kit for MDA (E-BC-K028-M; Elabscience).

Levels of ALT, AST, and ALP were measured using relevant diagnostic kits (catalog Nos.: ALT, ab105134; AST, ab105135; and ALP, ab83369; Abcam). Urea levels were determined using a colorimetric assay kit (BioVision, catalog No. K375-100; Biovision Incorporation, UK). BUN levels were estimated using a BUN colorimetric detection kit (Catalog No. EIABUN; ThermoFisher

Scientific, USA). Other biochemical parameters, such as total proteins, creatinine, bilirubin, albumin, and globulin, were assessed using relevant colorimetric diagnostic kits (catalog Nos.: total proteins, FT7250; creatinine, FT7040; bilirubin, FT 6920; albumin, FT 6760; and globulin FT 7253; Interchim Diagnostics Biochemistry Kits, France).

Liver and kidney homogenates were prepared to estimate the levels of total thiols, GSH, catalase, H_2O_2, MDA, and TAC in animal tissues. The same assay kits used to determine the serum levels of these parameters were used to assess their levels in tissue homogenates.

Histopathological examination

Kidney and liver tissues of proper size and thickness collected from the animals were immediately fixed by immersing in 10% neutral-buffered formalin and processed routinely for paraffin-embedding technique. The prepared tissue sections (4 µm) were stained with hematoxylin and eosin (H&E) staining.

Statistical analysis

The data presented in this study are expressed as means ± standard deviation (SD). To compare mean values between multiple groups, one-way ANOVA and the SPSS software (SPSS Inc. Chicago IL, USA) were used for statistical analysis. The normality and homogeneity of variances were checked, and the independence of observations was ensured. The normality of the data was verified using the Shapiro–Wilk test. Results with $p < 0.05$ were considered statistically significant.

Results

Starting from week 3 of the experiment, mice provided omega-3 and mice exposed to Cd and provided omega-3 showed normal behavior, activity, and food intake, compared to the untreated control mice. Mice exposed to Cd but not treated with omega-3 showed relatively decreased activity and reduced food intake, compared to the control group. No animal deaths were observed in any of the experimental groups.

In control mice, the blood Cd level was 0.0021 ± 0.0001 ppm, which increased significantly ($p < 0.05$) in mice exposed to Cd (0.581 ± 0.020 ppm). Blood Cd levels were comparatively lower in mice exposed to Cd and provided omega-3 (0.231 ± 0.018 ppm) than in Cd-exposed mice.

Regarding the assessed hematological parameters, mice that received omega-3 (group 2) showed no significant differences from the control group. The hematological profile of mice exposed to Cd and not provided omega-3 (group 3); showed varied decrements in its parameters, compared to the control group (Table 1 and Figure 1). Hb concentration and PCV% were significantly altered. Compared to the parameter decrements recorded in group 3, the hematological parameters estimated in mice exposed to Cd and provided omega-3 (group 4) were relatively improved and became closer to the control group levels.

Concerning the biochemical profile, mice that were provided omega-3 (group 2) showed no significant biochemical alterations, compared to the control group levels (Tables 2–5; and Figures 1–4). Comparable decrements in the estimated levels of total proteins, albumin, and globulin were observed in mice of group 3, which were

Table 1. Hematological parameters of mice that received omega-3, exposed to Cd, and exposed to Cd and provided omega-3, compared to the control mice.

Parameter	Untreated control	Omega-3	Cadmium	Cadmium and omega-3
RBCs count (10^6/mm^3)	5.51 ± 0.07	5.62 ± 0.09	4.04* ± 0.11	5.41** ± 0.02
Total leucocytic count (10^3/mm^3)	6.42 ± 0.36	6.49 ± 0.21	5.01* ± 0.25	6.27** ± 0.06
Hemoglobin (Hb) concentration (g/dL)	12.66 ± 0.31	12.70 ± 0.26	9.56* ± 0.34	12.11** ± 0.31
Packed cell volume (PCV %)	44.29 ± 0.27	45.02 ± 0.27	36.01* ± 0.53	43.59** ± 0.81

Notes: RBC count, total leucocytic count (TLC), hemoglobin concentration, and packed cell volume percentage of Cd-exposed mice were significantly lower, compared to the control mice. The measured hematological indices in omega-3-administered mice exhibited no significant differences, compared to the control mice. In Cd-exposed mice and those provided omega-3, the estimated hematological parameters showed significant increments, compared to Cd-exposed mice and were closer to the control levels.
Values are shown as means ± SD. Number of mice/group = 20.
*Significantly different mean values from that of untreated control mice ($p < 0.05$).
**Significantly different mean values from that of Cd-exposed mice.

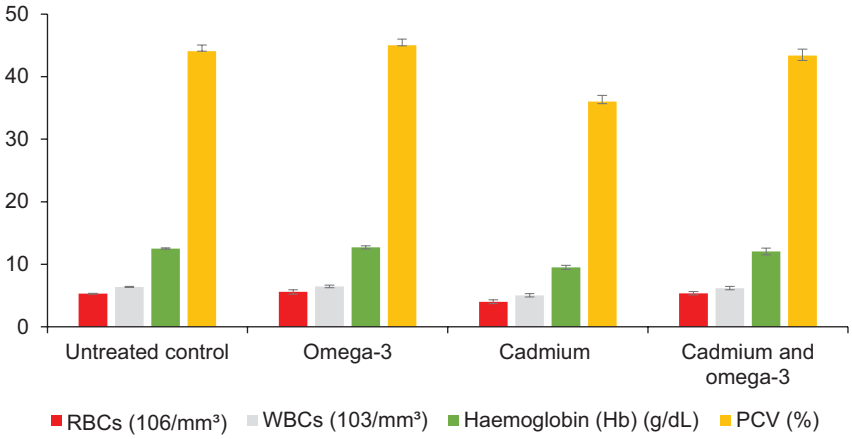

Figure 1. RBCs and WBCs indices in the control mice, omega-3-administered and Cd-exposed mice, and the mice that received both Cd and omega-3.

Table 2. Biochemical parameters of mice that received omega-3, exposed to Cd, and exposed to Cd and provided omega-3, compared to the untreated control mice.

Parameter	Untreated control	Omega-3	Cadmium	Cadmium and omega-3
Total proteins	7.55 ± 0.15	7.57 ± 0.21	5.28* ± 0.16	7.08** ± 0.13
Albumin	3.31 ± 0.02	3371 ± 0.16	2.33* ± 0.14	3.05** ± 0.15
Globulin	3.82 ± 0.08	3.89 ± 0.17	2.31* ± 0.16	3.51** ± 0.28
Creatinine	0.54 ± 0.13	0.57 ± 0.13	0.91* ± 0.07	0.61** ± 0.27
Urea	40.61 ± 0.41	40.65 ± 0.38	74.16* ± 0.61	48.13** ± 0.64
BUN	15.31 ± 1.02	15.81 ± 1.05	29.31* ± 1.32	17.71 ± 1.46
Bilirubin	6.37 ± 0.41	6.49 ± 0.37	11.57* ± 0.24	7.14** ± 0.41

Notes: Serum levels of total proteins (g/dL), albumin (g/dL), and globulin (g/dL) were significantly lower in Cd-exposed mice, compared to the untreated control mice. Levels of creatinine (mg/dL), urea (mg/dL), BUN (mg/dL), and bilirubin (mg/dL) in the Cd-exposed mice were significantly increased, compared to the untreated control mice. Mice administered with omega-3 demonstrated no significant differences in their biochemical parameters, compared to the untreated control mice. The estimated biochemical parameters in Cd-exposed and omega-3-treated mice exhibited improvements toward the control levels and were significantly different from those estimated in the Cd-exposed mice.
Values are shown as means ± SD.
Number of mice/group = 20.
*Significantly different means from that of untreated control mice ($p < 0.05$).
**Significantly different mean values from that of Cd-exposed mice.

Table 3. Serum levels of alanine transferase (ALT) (IU/L), aspartate transferase (AST) (IU/L), and alkaline phosphatase (ALP) (IU/L) in the different groups.

Parameter	Untreated control	Omega-3	Cadmium	Cadmium and omega-3
ALT	26.61 ± 1.12	26.51 ± 1.08	69.16* ± 1.13	30.56** ± 1.07
AST	41.30 ± 1.09	41.35 ± 1.07	139.12* ± 3.41	53.13** ± 1.19
ALP	25.19 ± 1.21	25.17 ± 1.03	75.78* ± 1.38	28.19** ± 1.59

Notes: The Cd-exposed mice had significantly increased levels of all measured biochemical parameters. No significant differences were recorded in omega-3 group, compared to the control group. In the group of mice exposed to Cd and [provided omega-3, the biochemical parameters displayed improvements toward the control levels and were significantly decreased, compared to the Cd-exposed mice.
Values are shown as means ± SD.
Number of mice/group = 20.
*Significantly different means from that of untreated control mice ($p < 0.05$).
**Significantly different means from that of Cd-exposed mice.

Table 4. Serum levels of total thiols (mmol/L), glutathione (GSH) (µg/mL), catalase (IU/L), superoxide dismutase (SOD) (U/mL), glutathione peroxidase (GSH-Px) (U/mL), malondialdehyde (MDA) (nmol/mL), hydrogen peroxide (H_2O_2) (mmol/L), and total antioxidant capacity (TAC) (nmol/mL) in different groups.

Parameter	Untreated control	Omega-3	Cadmium	Cadmium and omega-3
Total thiols	2.41 ± 0.27	2.31 ± 0.21	0.27* ± 0.03	2.03** ± 0.81
Glutathione	40.91 ± 1.13	41.59 ± 1.08	13.93* ± 0.63	36.94** ± 1.29
Catalase	51.82 ± 1.52	52.64 ± 1.49	28.88* ± 1.05	46.81** ± 1.21
SOD	6.31 ± 1.71	6.27 ± 1.61	3.44 ± 1.29	5. 71 ± 1.34
GSH-Px	136 ± 2.40	128 ± 2.11	76 ± 1.67	120 ± 1.64
MDA	317.17 ± 3.04	314.61 ± 3.12	447.31* ± 3.41	331.23** ± 3.51
H_2O_2	40.70 ± 1.51	40.61 ± 1.43	90.11* ± 1.14	49. 16** ± 1.03
TAC	35.40 ± 1.02	35.76 ± 1.09	16.75* ± 1.04	29.67** ± 1.08

Notes: Compared to the untreated control mice, the Cd-exposed mice exhibited significantly decreased levels of total thiols, GSH, catalase, and TAC. The levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were significantly increased in Cd-exposed mice, compared to the untreated control mice. Omega-3-treated mice showed no significant differences in the measured biochemical parameters, compared to the untreated control mice. In the mice exposed to Cd and treated with omega-3, the biochemical parameters showed improvements toward the control levels and were significantly different, compared to those of the Cd-exposed mice.
Values are shown as means ± SD.
Number of mice/group = 20.
*Significantly different means from that of untreated control mice ($p < 0.05$).
**Significantly different means from that of Cd-exposed mice.

exposed to Cd but had no access to omega-3. Mice in this group showed a significant increase in creatinine, urea, and BUN.

The serum and tissue levels (tissue homogenates) of total thiols, GSH, SOD, GSH-Px, and catalase were significantly decreased in the mice exposed to Cd but not provided omega-3 (group 3). TAC in serum and tissues was significantly reduced in the mice exposed to Cd, and relatively improved in the mice exposed to Cd and provided omega-3. Levels of MDA and H_2O_2 in serum and tissues of the mice exposed to Cd without access to omega-3 were significantly increased, compared to the control group levels.

The recorded parameters of the altered biochemical profile (serum and tissues) were observed closer to the control levels in the mice exposed to Cd and provided omega-3 (group 4).

The histopathological changes observed in kidney and liver tissues of the Cd-exposed mice, compared to the corresponding tissues of the control mice are shown in Figures 5–8.

Discussion

Omega-3 long-chain PUFAs are not produced in the body, so it is essential to obtain them from dietary sources to meet body's requirements. In simpler terms, consuming omega-3 FAs from outside sources is vital for maintaining good health and supporting body's metabolic processes.

In the present study, a dietary source of omega-3 FAs was used along with the administration of Cd, a highly toxic heavy metal known to induce a state of oxidative stress (Temleton and Liu, 2010). The objective of the present study was to test the efficacy of these FAs to improve both hematological and biochemical alterations raised as sequelae of Cd-induced oxidative stress.

Oxidative overload, commonly termed oxidative stress, is the state of imbalance between oxidation reactions and antioxidative activities (redox status) with the loss of efficacy of the endogenous antioxidant system to overcome the hazardous effects of ROS (Klaudia and Marian, 2011). Any factor, either pathological or environmental, that disrupt this balance is considered initiator of oxidative stress (Klaus and Heribert, 2004). The main feature of oxidative stress is the generation of excess ROS, which include free radicals, such as hydroxyl and superoxide radicals, and non-radicals represented mainly by H_2O_2 (Mruk *et al.,* 2002). A controlled level of free radicals contributes to physiological metabolic processes, such as cell respiration. Excess of free radicals are eradicated by the endogenous antioxidant system to prevent, block, or inhibit the damaging effects of free radicals (Alfonso-Prieto *et al.,* 2009). The antioxidant system is composed of antioxidative enzymes, such as SOD,

Table 5. Levels of total thiols (mmol/L), glutathione (GSH) (µg/mL), catalase (IU/L), superoxide dismutase (SOD) (U/mL), glutathione peroxidase (GSH-Px) (U/mL), malondialdehyde (MDA) (nmol/mL), hydrogen peroxide (H_2O_2) (mmol/L), and total antioxidant capacity (TAC) (nmol/mL) in the tissue homogenates of different groups.

Parameter	Untreated control		Omega-3		Cadmium		Cadmium and omega-3	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Total thiols	1.91 ± 0.26	1.07 ± 0.26	1.71 ± 0.26	1.08 ± 0.27	0.22* ± 0.05	0.21* ± 0.04	1.81** ± 0.35	1.66** ± 0.45
Glutathione	16.57 ± 1.13	14.71 ± 1.13	16.51 ± 1.07	14.71 ± 1.03	5.02* ± 0.61	4.91* ± 0.69	14.79** ± 1.39	14.83** ± 1.44
Catalase	20.28 ± 1.52	19.14 ± 1.21	19.87 ± 1.32	18.88 ± 1.11	9.17* ± 1.03	9.13* ± 1.07	17.75** ± 1.11	17.43** ± 1.56
SOD	6.21± 1.71	6.19 ± 1.74	6.13 ± 1.66	6.12 ± 1.61	3.49 ± 1.56	3.18 ± 1.58	5.21 ± 1.81	5.06 ± 1.64
GSH-Px	131 ± 2.41	128 ± 2.33	130 ± 2.21	113 ± 2.37	80 ± 1.48	78 ± 1.89	117 ± 1.78	114 ± 1.38
MDA	125.18 ± 3.18	121.11 ± 3.75	124.0 ± 13.13	121.11 ± 3.15	413.61* ± 3.39	491.31* ± 3.64	148.28** ± 3.11	147.08** ± 3.61
H_2O_2	15.92 ± 1.49	13.84 ± 1.13	15.49 ± 1.17	13.41 ± 1.09	87.91* ± 1.17	97.11* ± 1.62	19.44** ± 1.01	18.22** ± 1.08
TAC	14.72 ± 1.12	13.60 ± 1.09	14.18 ± 1.02	13.89 ± 1.03	6.38* ± 1.09	6.63* ± 1.08	12.77** ± 1.09	12.17** ± 1.01

Notes: The levels of total thiols, GSH, catalase, and TAC were significantly decreased in the tissue homogenates of Cd-exposed mice, compared to untreated control mice. MDA and H_2O_2 levels were significantly increased in the tissue homogenates of Cd-exposed mice, compared to untreated control mice. Omega-3-treated mice showed no significant differences, compared to untreated control mice. The estimated biochemical parameters in tissue homogenates of mice exposed to Cd and provided omega-3 demonstrated improvements toward the control levels and were significantly different, compared to those measured in the Cd-exposed mice.
Values are shown as means ± SD.
Number of mice/group = 20.
*Significantly different means from that of untreated control mice ($p < 0.05$).
**Significantly different means from that of Cd-exposed mice.

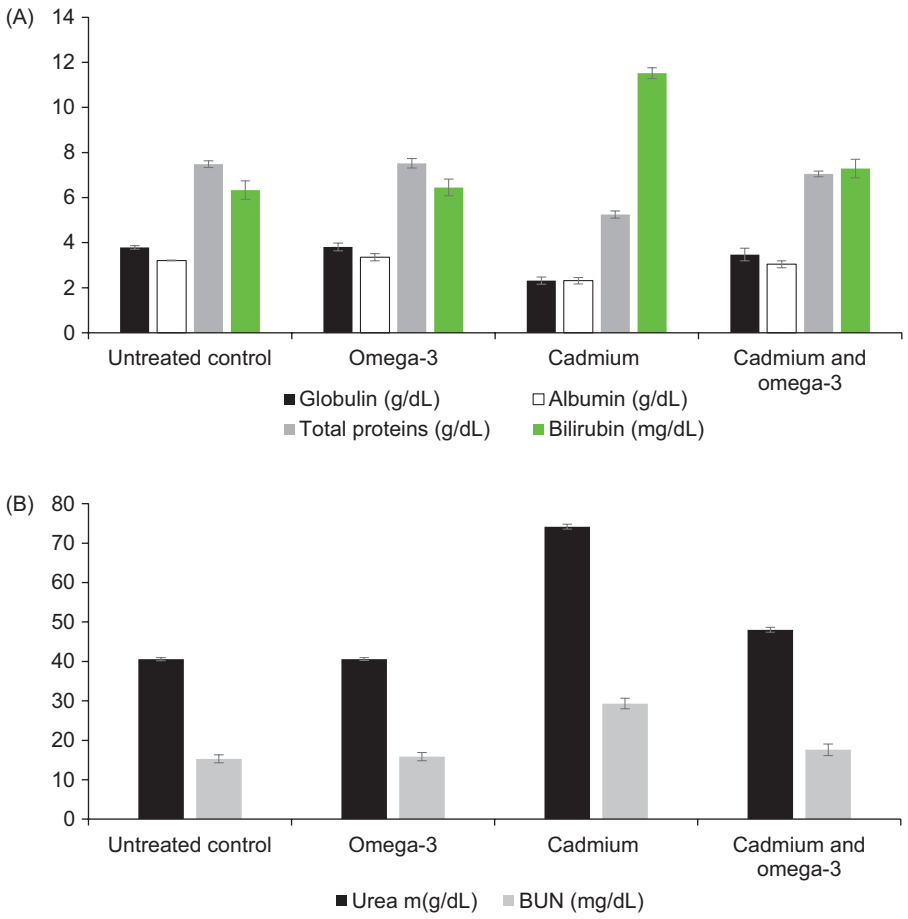

Figure 2. (A) Levels of globulin, albumin, total proteins, and bilirubin in the control mice, omega-3-administered and Cd-exposed mice, and the mice that received both Cd and omega-3. (B) Levels of urea and blood urea nitrogen (BUN) in the control mice, omega-3-administered and Cd-exposed mice, and the mice that received both Cd and omega-3.

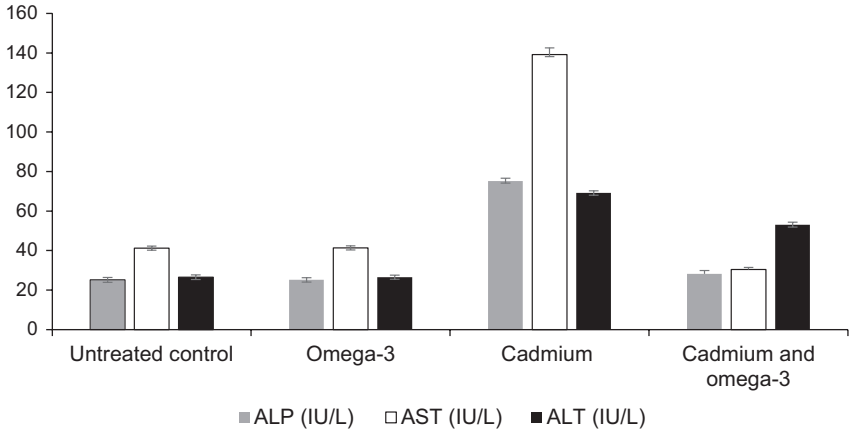

Figure 3. Levels of serum enzymes: ALP, AST, and ALT in the control mice, omega-3-administered and Cd-exposed mice, and the mice that received both Cd and omega-3.

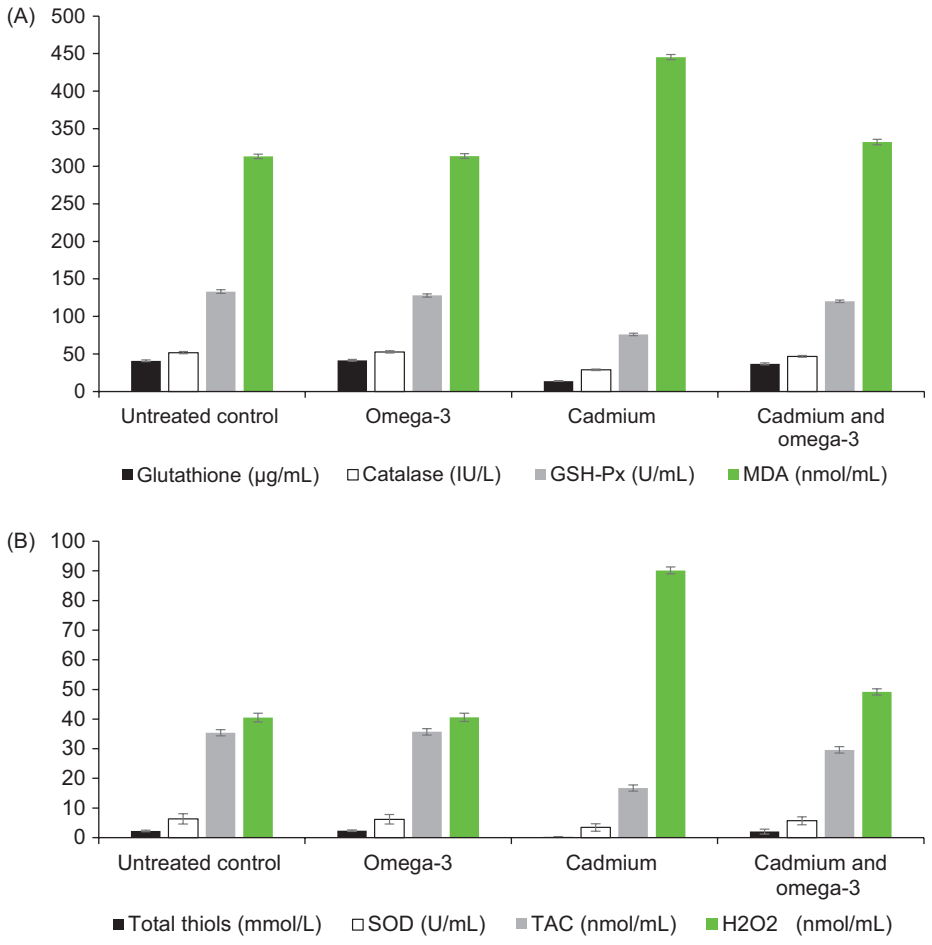

Figure 4. (A) Levels of glutathione, catalase, glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in the control mice, omega-3-administered and Cd-exposed mice, and the mice that received both Cd and omega-3. (B) Levels of total thiols, super oxide dismutase (SOD), total antioxidant capacity (TAC), and hydrogen peroxide (H_2O_2) in the control mice, omega-3-administered and Cd-exposed mice, and the mice that received both Cd and omega-3.

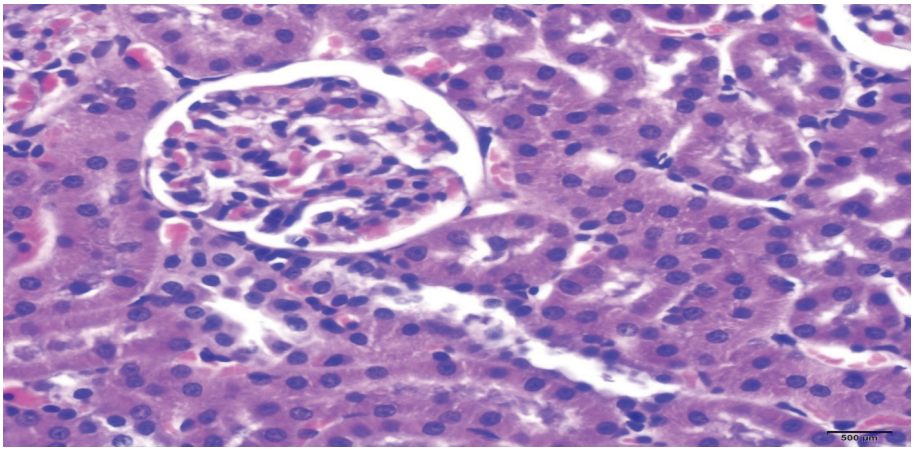

Figure 5. Kidney tissue of a control mouse showing the normal histological structure involving the glomerulus, tubules, and interstitial tissue (H&E staining).

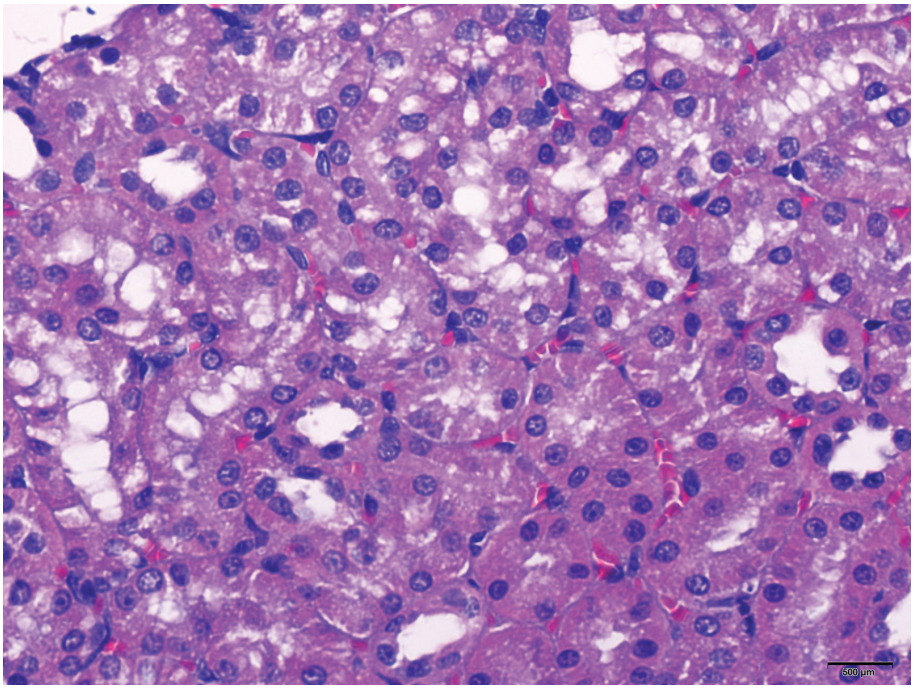

Figure 6. Kidney tissue of a Cd-exposed mouse showing swelling and hydropic degeneration of the epithelial tubular cells associated with narrowing of the tubular lumina (H&E stain).

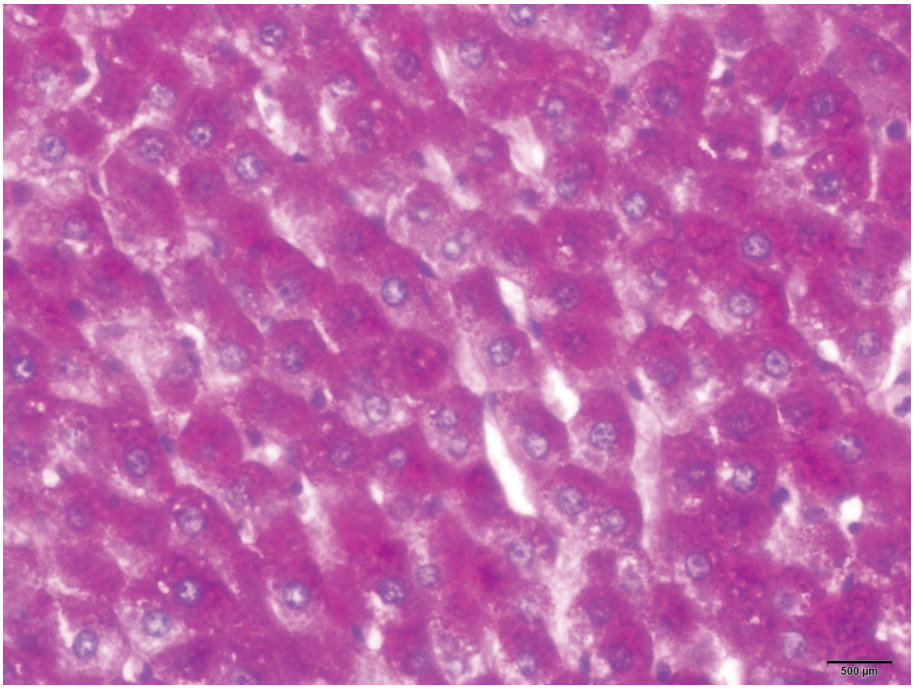

Figure 7. Liver tissue of a control mouse showing normal histological architecture and morphology of the hepatic cords and hepatocytes.

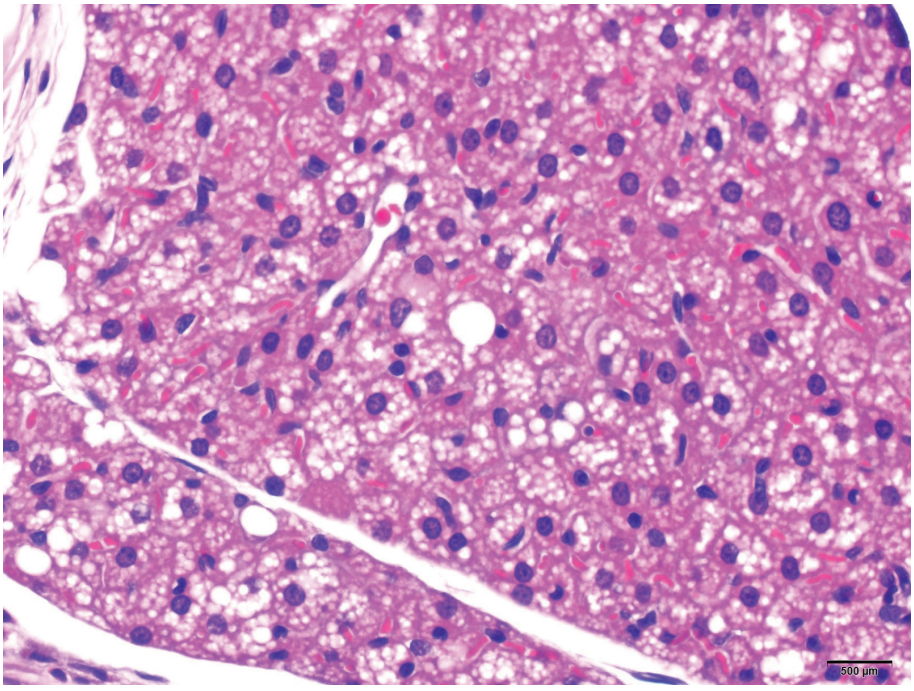

Figure 8. Liver tissue of a Cd-exposed mouse showing evident vacuolization of hepatocytes associated with disrupted hepatic cords arrangement (H&E staining).

catalase and GSH-Px, as well as active non-enzymatic molecules (Pham-Huy *et al.,* 2008). Each of these antioxidant enzymes targets one or more free radicals and acts in different ways to prevent the damaging effects of free radicals and to help maintain equilibrium between oxidative reactions and antioxidative activity (Dorta *et al.,* 2003). However, in the absence of an efficient antioxidant system, the generation of excessive free radicals can lead to deleterious effects. Owing to their structure as unstable molecules, excess of free radicals can oxidize other cellular biomolecules, with the development of oxidative stress that accounts for damaging cell proteins and DNA, and eventually may result in cell death (Feng *et al.,* 2016). Lipid peroxidation, which mainly affects cell membranes, is one of the paramount features of oxidative damage (Nichole *et al.*, 2008).

In the current research, Cd, a distinguished heavy metal toxicant, served as an inducer of oxidative stress. Especially in the liver and kidney, Cd indirectly initiates the generation of ROS that cause massive tissue damage (Sepidarkish *et al.,* 2020). Cd-induced toxicity results in lipid peroxidation of cell membranes (Huang *et al.,* 2009) associated with a significant decrease in the mitochondrial production of adenosine triphosphate (ATP) and GSH levels (Benharrat *et al.,* 2022). Additionally, Cd toxicity inhibits the activity of antioxidative enzymes, and the oxidative stress is exacerbated. At the final stage of Cd-induced cytotoxicity, apoptosis takes place because of caspase activation (Ghadiri *et al.*, 2023).

The presently demonstrated renal and hepatic histopathological changes in Cd-exposed mice indicate the profound effects of Cd toxicity. As a reflection of the Cd-induced hepatotoxicity, the levels of ALT, AST, and ALP serum enzymes were significantly increased in intoxicated mice. This finding can be interpreted by the resultant lipid peroxidation-induced lysosomal damage that allows the leakage of lysosomal enzymes and subsequent elevation of blood levels (Ayat *et al.,* 2024). On the other hand, the Cd-induced nephrotoxicity in mice leads to a significant increase in blood urea and creatinine levels. The histopathological changes were remarkably alleviated in the mice provided both omega-3 and Cd.

The presently employed markers of antioxidative status, such as total thiols, GSH, SOD, GSH-Px, catalase, and TAC, were significantly decreased as an expression of the effect of oxidative overload on these antioxidants in the mice administered with Cd. Oxidation of these antioxidant molecules by the generated ROS is suggested to be the cause of their alteration and loss of function.

Some endogenous antioxidants, such as GSH and catalase, act to directly scavenge free radicals (Ghadiri *et al.,* 2023) and their oxidation undoubtedly results

in the accumulation of free radicals and accentuation of oxidative stress. In this context, catalase decomposes H_2O_2 that accounts for lipid peroxidation, and the inhibited catalase activity allows H_2O_2 to exhibit its potent oxidizing activity in mice. H_2O_2 action constitutes the base of the Fenton reaction that releases extremely hazardous hydroxyl radical OH (Ayat *et al.*, 2024).

Significant elevation in MDA levels in Cd-intoxicated mice is ascribed to lipid peroxidation of cell membranes. MDA is a lipid peroxidation marker, a serious consequence of oxidative damage (Benharrat *et al.,* 2022).

The present results demonstrate that the endogenous antioxidant system was remarkably affected along with hematological and biochemical alterations because of Cd toxicity. Antioxidant molecules were depleted and the antioxidative activity was inhibited associated with disruption of physiological oxidant–antioxidant equilibrium. In such cases of a depressed antioxidant endogenous system, exogenous antioxidants are crucial to reset a balance between oxidant and antioxidant molecules. The supplementary antioxidants are supposed to overcome oxidative damage through rapid eradication and/or neutralization of excessive free radicals and strengthening of the endogenous antioxidative system (Temleton and Liu, 2010).

Of note, the resultant metabolites, because of the reaction of antioxidant molecules with free radicals, have the potential to scavenge more radicals with the accentuation of antioxidative capacity. Subsequently, exogenous antioxidants function synergistically with endogenous antioxidants to neutralize and eliminate free radicals (Ayat *et al.,* 2024). The properties of omega-3 FAs comply with these criteria of an efficient exogenous antioxidant, as they actively help enhance the antioxidant system that counteracts oxidative stress.

Some previous studies focused on the potential of omega-3 PUFAs in combating oxidative stress and the associated oxidative damage, and maintaining redox status (Mruk *et al.*, 2002). Omega-3 FAs enhanced the activity and expression of key antioxidant enzymes, scavenged free radicals, and decreased ROS generation in the mitochondria (Feng *et al.,* 2017; Rahim *et al.,* 2023; Tantipaiboonwong *et al.,* 2021). The antioxidant property of omega-3 FAs is partially through suppression of lipid peroxidation (Saini and Keum, 2018).

Omega-3 FAs are enhancers of the antioxidant system and significantly upregulate the expression of antioxidant enzymes such as GSH-Px (antioxidant markers), decrease the expression of pro-oxidative enzymes, and reduce the level of MDA (lipid peroxidation marker) (Ogłuszka *et al.,* 2024; Saini and Keum, 2018). In diabetic and cardiac disease patients, omega-3 FAs ameliorate oxidative stress by decreasing the production of free radicals, enhancing the activity of antioxidant enzymes, increasing antioxidant capacity (Ateya *et al.,* 2022; Mruk *et al.,* 2002), decreasing MDA level, and maintaining redox status (Mruk *et al.,* 2002; Ogłuszka *et al.,* 2024). The antioxidant activity of omega-3 FAs has been proved *in vitro* through the evaluation of ferric-reducing antioxidant power assay (FRAP), radical scavenging activity, and evaluation of ROS generation (Rahim *et al.,* 2023; Saboori *et al.,* 2016; Wang *et al.,* 2024).

The present results are in line with the aforementioned published studies as evidenced by the currently recorded improved levels of antioxidant markers (total thiols, GSH, catalase, and TAC) in mice exposed to Cd and provided omega-3 FAs. These findings confirm the positive effect of omega-3 FAs on the expression of antioxidant molecules and consequently reinforcing significant effect on endogenous antioxidant activity.

Omega-3 PUFAs provide their antioxidant protection through diverse mechanistic pathways. These pathways include donating electrons to neutralize and scavenge free radicals, interrupting free radical-induced lipid peroxidation by their double bonds, and inhibiting ROS generation. Moreover, omega-3 PUFAs are incorporated into lipoproteins of cell membranes rendering the double bonds of these structures less accessible for the attack of free radicals (Ogłuszka *et al.*, 2024). Upregulation of antioxidant enzymes and inhibition of pro-oxidant activity are among the antioxidative mechanisms of omega-3 FAs (Ateya *et al.,* 2024). Furthermore, Omega-3 FAs have the ability to replace arachidonic acid (an omega-6 polyunsaturated fatty acid) in cell membranes; thereafter the ROS-induced cleavage products of omega-3 FAs have much less damaging effects than that of arachidonic acid (Mruk *et al.,* 2002).

A significant decrease in Cd blood levels in Cd-intoxicated mice and provided omega-3 FAs may be interpreted by the supposed chelation of Cd. Chelation of pro-oxidant metals and protection against metal-induced lipid peroxidation is one of the antioxidative activities of omega-3 FAs. Cd is a trigger of oxidative overload and decreasing its level in the blood greatly limits the initiation of oxidative stress and thus helps to recover the endogenous antioxidant system.

The current hematological and biochemical assays in mice exposed to Cd and provided omega-3 FAs exhibited improved parameters that were relatively reversed and became closer to the control group levels. These findings may reinforce the proposed synergistic antioxidant role of omega-4 FAs in alleviating Cd-induced oxidative stress.

Conclusions

The present study provides evidence of the antioxidant properties of omega-3 FAs against the pronounced oxidative stress and the resultant oxidative damage induced by Cd toxicity. However, more detailed research work is recommended to elucidate the exact molecular mechanisms involved in the antioxidant bioactivity of omega-3 FAs in case of heavy metal toxicity.

Availability of Data

Data are available on request.

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Author Contributions

Mohammed Al-Zharani, Hassan Rudayni, Fahd A. Nasr, Abdullah S. Alawam, and Amin A. Al-Doaiss performed the laboratory work. Mohammed S. Al-Eissa and Saad Alkahtani supervised the experiment and were responsible for resources. Shaikha Albatli was responsible for laboratory work, software, and statistical data analysis. Mohammed Mubarak designed the study, supervised the methodology, and wrote the manuscript.

Conflict of Interest

The authors declared no conflict of interest.

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