PROTECTIVE MECHANISMS OF OMEGA-3 FATTY ACIDS AGAINST HEPATOTOXIC IMPACT OF CADMIUM EXPOSURE IN RATS

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ABSTRACT

The goal of this research was to explore the underlying hepatoprotective mechanisms of Omega-3 fatty acids against liver toxicity in response to cadmium chloride exposure (Cd) in rats. Cd (5 mg /kg b.w) was ingested to rats daily for 6 days. Omega-3 fatty acids (100 mg/kg b.w.) were ingested orally to Cd intoxicated rats simultaneously with or before Cd intoxication daily for 6 days. Data revealed that administration of Omega-3 fatty acids simultaneously with or before Cd intoxication , significantly diminished the increases in hepatic malondialdehyde (MDA) and ameliorated the depletion in antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transfrease (GST). The fatty acids also attenuated the increase in hepatic 8-hydroxy-2'-deoxyguanosine (8-OHdG, index of DNA damage), interferon gamma (IFNγ), nuclear factor kappa B (NF-κB) and heat shock protein-70KDs (HSP-70). The existing biochemical investigations were confirmed by histo-cytologic observation. Conclusion: the present investigation propose that the hepatoprotective impacts of Omega-3 fatty acids against Cd caused liver toxicity in rats may ascribe to their anti-oxidative stress, antioxidant and anti-inflammatory beneficial actions.

Introduction

Cadmium (Cd) is a major hazard carcinogenic dense metal because of its ability to accumulate in body vital organs including liver. It is actually a by-product produced during zinc production and can be termed as the most toxic element to which human being are exposed so far [1]. Cadmium can be found in corrosion-resistant plating on steel, and its compounds are used to stabilize plastic and in nickel / cadmium batteries and cadmium / telluride solar panels. Uncontaminated surface waters may contain an average of less than 1.0gCd/kg, while concentrations in contaminated waters may reach a value several magnitudes higher [2]. Human can be exposed to Cd through inhalation of cigarette smoke , environment, water and consuming of vegetables contaminated with Cd [3]. Exposure to Cd can cause many health problems such as kidney malfunction**,** hypertension, heart disease, hepato-toxicity, and tumor of many organs [4-6]. In fact, some authors demonstrated that high amount of these metals, particularly Cd, stores in the liver [7]. A number of aquatic species such as fishes, shellfishes can concentrate Cadmium in their bodies at mentionable levels which gets accumulated in human body too when consuming sea food. It leads to a number of diseases such as Hypertension, Osteomalacia, gastric dysfunction, Central nervous system dysfunction, and endocrine disorders in human [2]. Fat is stored in the liver in case of pancreas abnormalities [7,8] Many reports have explained the toxic mechanization of Cd. One of these mechanisms , is the ability of Cd to displace the essential divalent metals, including zinc and selenium requiring for enzyme activities, resulting in inactivation of these enzymes [9]. Also, it has been found that Cd can promotes oxidative stress (OS)

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throughOS) through accumulation of oxygen reactive elements and reduction of cellular antioxidants , causing membrane lipid and protein denaturation and DNA fragmentation [6, 10-11]. There has been a significant positive correlation found between tissue antioxidant enzyme activity and the inflammatory response when exposed to Cadmium [2] In addition, previous investigation has shown that Cd can take part in liver damage by stimulation of the inflammatory immune cells , leading to the formation of many molecules ,namely tumor necrosis factor-α (TNF-α), interleukin 1β (IL1β), and interleukin-8 (IL-8) which have damaging influences on liver [12] . The liver is one of the organs mostly affected by contaminants in the water [13].

 Supplementation with an agent with antioxidant and anti-inflammatory properties may be an efficient strategy in preventing or reducing the liver damage in response to Cd toxicity. Plants serve as source of food and they also contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds which have preventive and curative properties [14]. Cape gooseberry has a lot of phytochemical compounds in it which can prevent liver microsomes and hepatocytes from oxidative stress [15].

Omega-3 fatty acids (Omega-3) as eiocosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are beneficial substances present in oil of fish, shellfish , seaweed and microphytes [24-25]. However, fishes affected by cadmium contamination can bring pathological alterations in metabolic process according to the literature [2] Omega-3 has a prophylactic impact against liver damage and steatosis [18]. Omega-3 have many other beneficial impacts such as , antioxidants , anti- stress , anti-inflammatory and hepato-prophylactic activities [19]. Omega-3 can interfere with the expression of inflammatory molecules such as chemokines, growth factors and matrix proteases [20].

No previous work has investigated the molecular protective mechanisms of omega -3 against Cd induced liver toxicity. So, the goal of this investigation was to illustrate the underlying molecular hepato-prophylactic mechanisms of Omega-3 fatty acids against liver damage in response to cadmium chloride exposure in rats. Animal models are also employed to study the Pharmacodynamics, mechanism of drug resistance and also used to study whether elileptogenesis alters the adverse effect potential of a given drug [21]. The preferred rodent species is the rat although other rodent species may be used for study. Normally female rats or mice are used [22].

Materials and Methods
Chemicals
Cadmium chloride was bought from Sigma –Aldrich company (USA) and dissolved in distilled water before administration. Omega -3 fatty acids was gotten from General Nutrition Center (GNC) in Saudi Arabia.
Animals
Forty male albino rats (150-200 g) were utilized for this Research. The animals were gotten from Laboratory Animal Production , King Fahd Research Centre , King Abdulaziz University. Rats were housed in a control room (22–24∘C and 12:12 h light-dark cycle) and provided with food and water ad libitum. Animals were conserved for one week before the experiment for acclimatization. Animal handling was performed in accordance to the roles of the King Abdul-Aziz University, Faculty of Science.
Experimental design
The rats were classified into 4 groups (n=10) as follows

Group 1: Control rats.

Group 2: Rats were ingested oral dose of Cd (5 mg /kg) for six sequent days dissolved in water [23].

Group 3: Rats were co administered with Cd (5 mg /kg) and Omega-3 (100 mg/kg b.w.) [24]for 6 sequent days.

Group 4: Rats were treated with Omega-3 (100 mg/kg b.w.) daily for 6 days followed by intoxication with Cd (5 mg /kg b.w) for 6 sequent days.

At the end of the experimental duration, rats were fasted for approximately 13 hours. Blood were taken in tubes for clotting and serum isolation . The rats were then scarified and the livers were taken , washed in saline and utilized for biochemical and histopathological investigations.
Serum analysis
 Serum ALT, AST, ALP, albumin were measured as a biomarkers of liver injury utilizing an automated analyzer
Biochemical investigations in the liver tissues :
Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferases (GST),) in the liver tissue homogenate were measured utilizing commercial kits (Nanjing, Jiancheng Co., China). 8- hydroxydeoxyguanosine (8-OHdG) and heat shock protein70 (Hsp70) were measured in liver homogenates using enzyme-linked immunosorbent assay (ELISA) kits (Uscn Life Science Inc., Wuhan, China) in accordance to the instructions supplied by manufacturer. Nuclear Factor-κB (NF-κB), and Interferon-γ (IFN-γ) were measured using an ELISA kit (a product of Thermo Scientific, Waltham, MA, USA) following the instructions of manufacturer.

Histopathological studies:

Small fractions of liver were fixed at once in formaldehyde (10%), dehydrated with graded concentrations of ethanol and then cleared in xylene. The samples were placed into paraffin wax, cut (3-5 µm) and stained with Haematoxylin and Eosin (H&E).

Statistical analysis

Comparisons between values were made by the one-way analysis of variance (ANOVA). Values were calculated as mean ± SD (n = 10). Data were statistically significant at $p \leq 0.05$.

Results

The influence of Omega-3 fatty acids on the level of OS and antioxidant indices are demonstrated in Figure 1.The data illustrated that subjection of rats to Cd toxicity (G2), pronouncedly boosted the level of hepatic MDA (index of membrane lipid oxidation) and diminished the hepatic antioxidant enzymes, namely SOD,CAT and GST with respect to control animals (G1). Ingestion of Omega-3 fatty acids simultaneously with (G3) or before Cd (G4) administration, successfully ameliorating the hepatic levels of OS index (MDA) and the enzymes of antioxidants in Cd intoxicated rats compared to intoxicated untreated ones.

Figure 2 shows a significant elevation of the hepatic 8-OHdG , an indicator of DNA disfiguration , in Cd intoxicated rats with relation to control ones. Treatment of Cd intoxicated rats with Omega-3 fatty acids simultaneously with or before Cd administration , obviously modulated the hepatic increase in 8-OHdG compared to Cd intoxicated rats.

Results in Figure 3 illustrates the efficacy of Omega-3 fatty acids on the hepatic concentrations of inflammatory molecules, including IFN-γ and NF-κB, in Cd intoxicated rats. The data demonstrated that Cd toxicity stimulated the generation of these proteins in comparison to control animals. Intake Omega-3 fatty acids simultaneously with or before Cd administration, significantly depleted the hepatic IFN-γ and NF-κB in Cd intoxicated rats versus control ones.

The concentrations of HSP-70 in different Cd intoxicated rat groups are shown in Figure 4. Statistical analysis revealed that rats intoxicated with Cd showed high level of hepatic HSP-70 in comparable to control rats. Treatment with Omega-3 fatty acids simultaneously with or before Cd intoxication, markedly minimized the hepatic HSP-70 in comparable to control ones.

Data in Figure 5 reveals the serum concentrations of hepatic function indices (ALT,AST,ALP and albumin) in control and Cd intoxicated groups. The results showed that, Cd toxicity caused marked rising in ALT AST and ALP with a reduction in albumin versus control rats . Supplementation of Cd intoxicated rats with Omega-3 fatty acids simultaneously with or before Cd intoxication, markedly significantly minimized the serum activities of enzymes and ameliorated the serum albumin content versus Cd intoxicated animals .

Light microscope examination

The influence of Omega-3 fatty acids on the histomorphologic pictures of livers in Cd intoxicated rats is shown in Figure 6. Liver section of Cd intoxicated rat (Fig 6b) showed dilation in portal vein with infiltration of inflammatory cells at portal areas , cytoplasmic vacuolization, karyolysis, pyknosis, fatty degeneration and degeneration in hepatocytes . Liver sections of rats treated with Omega -3 simultaneously with (Fig 6c) or before (Fig 6d) Cd administration, showing normal liver architecture.
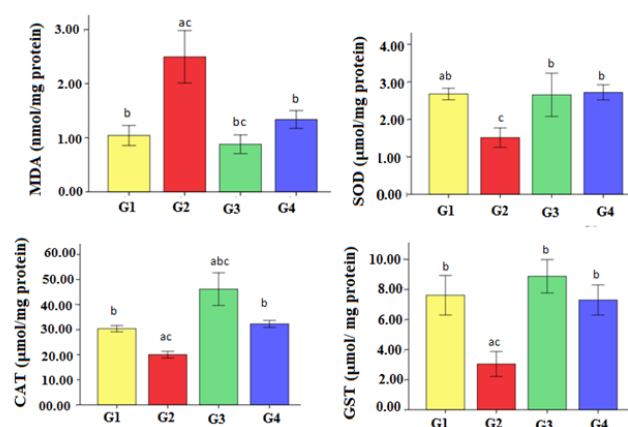

Fig 1: Effects of Omega-3 on hepatic lipid oxidation marker (MDA) and antioxidant markers (SOD, CAT and GST) in rats intoxicated with Cd. G1, Control; G2, Cd intoxicated rats; G3, Cd treated rats simultaneously with Omega-3; G4, Omega-3 treated rats before Cd intoxication. Values are expressed as mean ± S.D. (n=10). a , significantly different from G1; b, significantly different from G2; c, significantly different from G4.
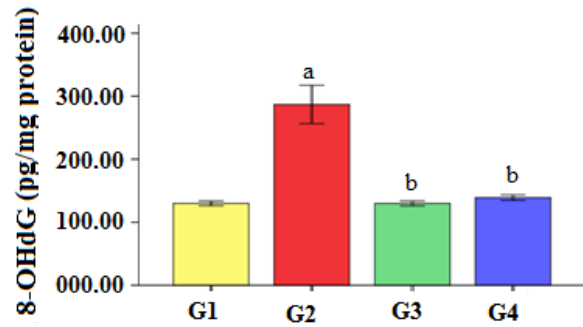
Fig 2: Effects of Omega-3 on hepatic DNA damage marker (8-OHdG) in rats intoxicated with Cd. G1, Control; G2, Cd intoxicated rats; G3, Cd treated rats simultaneously with Omega-3; G4, Omega-3 treated rats before Cd intoxication. Values are expressed as mean ± S.D. (n=10). a, significantly different from G 1 ; b, significantly different from G2.
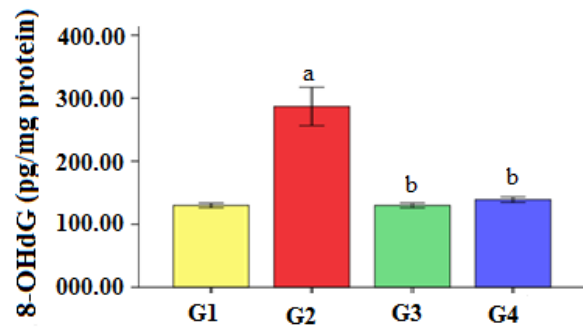

Fig 2: Effects of Omega-3 on hepatic DNA damage marker (8-OHdG) in rats intoxicated with Cd. G1, Control; G2, Cd intoxicated rats; G3, Cd treated rats simultaneously with Omega-3; G4, Omega-3 treated rats before Cd intoxication. Values are expressed as mean ± S.D. (n=10). a, significantly different from G 1 ; b, significantly different from G2.
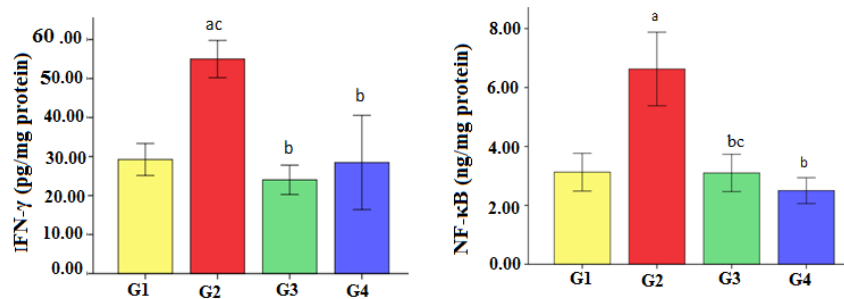

Fig 3: Effects of Omega-3 on hepatic inflammatory markers (IFN-γ and NF-κB) in rats intoxicated with Cd. G1, Control; G2 , Cd intoxicated rats ; G3, Cd treated rats simultaneously with Omega-3; G4, Omega-3 treated rats before Cd intoxication. Values are expressed as mean ± S.D. (n=10). a , significantly different from G 1 ; b, significantly different from G2; c, significantly different from G4.
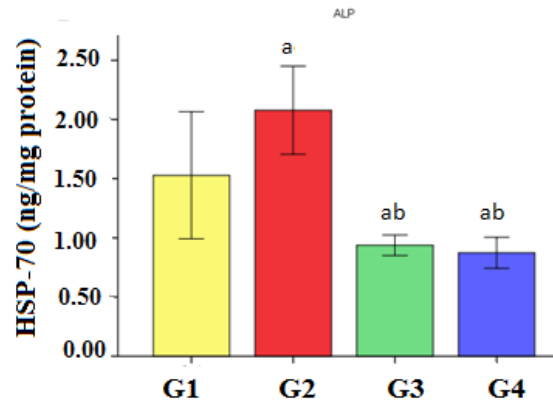
Fig 4: Effects of Omega-3 on hepatic HSP-70 in rats intoxicated with Cd. G1, Control; G2, Cd intoxicated rats; G3, Cd treated rats simultaneously with Omega-3; G4, Omega-3 treated rats before Cd intoxication. Values are expressed as mean ± S.D. (n=10). a , significantly different from G 1 ; b, significantly different from G2.
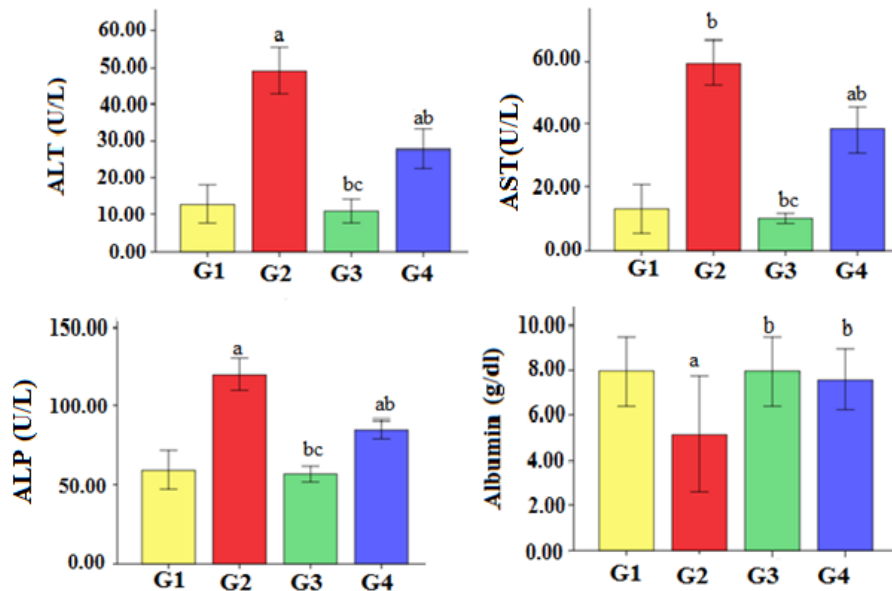

Fig 5: Effects of Omega-3 on serum hepatic function markers in rats intoxicated with Cd. G1, Control; G2 , Cd intoxicated rats ; G3, Cd treated rats simultaneously with Omega-3; G4, Omega-3 treated rats before Cd intoxication. Values are expressed as mean ± S.D. (n=10). a, significantly different from G 1 ; b, significantly different from G2; c , significantly different from G4.
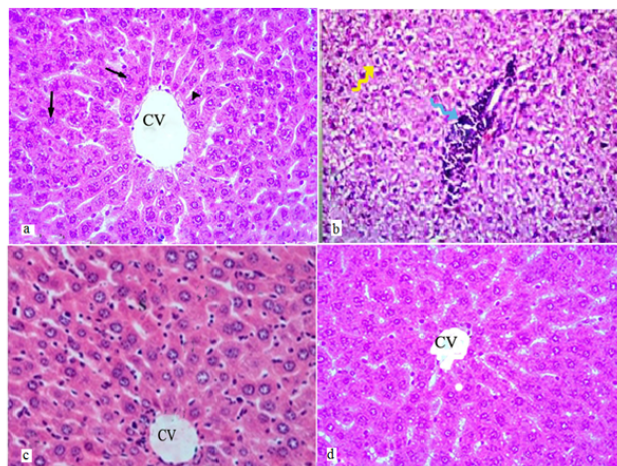
Fig 6 : Light micrograph showing the impact of Omega-3 on hepatic histo-morphological pictures in rats exposed to Cd toxicity. (a) Liver section of control rats, showing normal architecture of the liver, central vein (CV) in the center of the hepatic lobule, hepatocytes (arrows) are arranged in the form of cords and separated by sinusoids (arrowhead). (b) Liver section of rat treated with Cd, showing dilation in portal vein with infiltration of inflammatory cells at portal areas (blue arrow), cytoplasmic vacuolization, karyolysis, pyknosis, fatty degeneration and degeneration in hepatocytes (yellow arrow). (c& d) Liver sections of rats treated with Omega -3 simultaneously with (c) or before (d) Cd administration, showing normal liver architecture.

Discussion

Toxicological works are increasing rapidly on environmental heavy metals due to their severe toxicity to human being [25]

OS is one of the mechanization of Cd toxicity [25] The current work revealed a significant elevation in MDA (an index of cellular membrane lipid oxidation) accompanied with pronounced depletion in the antioxidant enzymes, namely SOD, CAT and GST in livers of rats subjected to Cd toxicity in comparable to control rats. This result is a potential indicator of hepatic OS induced in rats under the effect of Cd exposure. Our result is coped with a previous work has illustrated that oral exposure to CdCl2 caused increase in hepatic lipid peroxidation [26]. Another study by Elbekai and El-Kadi [27] has revealed that exposure to a high dose of Cd for 24h , markedly increased the oxygen reactive species (ORS) and reduced the antioxidants in Hepa lclc7 cells. It has suggested that stimulation of lipid peroxidation due to Cd toxicity is a consequence of antioxidant suppression [10]. In acute exposure, Cd can directly stimulate ORS generation. However, chronic exposure, may be an important contributor to many health problems, particularly organ carcinogenesis [28]. Intake of Omega-3 fatty acids simultaneously with or before Cd administration, effectively restoring the hepatic levels of MDA as well as the antioxidant enzymes in Cd intoxicated rats compared to intoxicated untreated ones. Similarly, a former investigation stated that Omega-3 fatty acids has a potential efficacy in minimizing oxidation of lipids in experimental animal model [29]

Formation of ORS with depletion in antioxidants may cause pathological damage at the molecular levels. The nucleotide pool is one of the targets of ORS attacking, and guanine is specifically susceptive to oxidation due to its low redox potential [30] 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the major form of the DNA adduct. Evaluation of the concentration of this DNA adduct is utilized as an indicator of oxidative DNA disfiguration [30] Parallel with a former report , The current research illustrated that exposure of animals to Cd toxicity promoted hepatic DNA damage as proved by increased 8-OHdG concentration with respect to control rats [31]. The correlation between Cd-generated ORS and DNA damage has been contributed to the production of 8-OHdG , a fundamental signal for ORS formation and tumorigenesis [32] A correlation between Cd and 8-OHdG generation has been found in workers of glass production [33] . Also , the advancement of tumor in organs and the production of 8-OHdGs has been documented in human being and experimental animals [34-35]. Oxidative DNA damage caused by Cd exposure may attribute to the capability of Cd to displace the essential divalent metals, zinc, requiring for enzymes that maintaining DNA integrity, resulting in inactivation of these enzymes [9, 31] . Hepatic DNA damage by Cd may promote cell cycle stop and finally cell death. Progressive cell death causes organ dysfunction, and finally death [36]. Our result may suggest that production of 8-OHdGs due to Cd-toxicity is a pivotal risk factor in the development of liver malignancy .

Supplementation of rats with Omega-3 fatty acids simultaneously with or before Cd toxicity, significantly attenuated the hepatic increase in 8-OHdG compared to Cd intoxicated rats. In line with our result, some authors reported that Omega-3 fatty acids could modulate the rise in serum 8-OHdG in cigarette smokers [37]. The present study suggests that the ability of Omega -3 fatty acids to counteract the oxidative alteration of DNA may due to their antioxidant potential impacts.

In experimental animals, the genesis of cytokines by the dense metals is a sign of inflammatory reactions [38] Result of the current work demonstrated that exposure of rats to Cd induced inflammation in their livers as observed by over - expression of hepatic IFN-γ in Cd treated rats compared with control animals. It has reported that exposure to Cd can cause systemic inflammation due to the downstream impact of cadmium- promoted OS [38]. Experimental models have illustrated that production of IFN-γ has a fundamental role in the liver illness [39]. It has been demonstrated that expression of IFN- γ stimulates the release of chemokines by hepatocytes. The produced chemokines recruit CD8+ cytotoxic T cells and natural killer which have a key role in liver cytolysis [39] Also some authors declared that IFN- γ can induce the production of NO that mediates hepatic damage via many mechanisms , including suppression of mitochondrial respiratory chain, inactivation of proteinase suppressors , and formation of ORS [40]

NF-κB is an important inflammatory inducible transcription factor. Activation of this factor regulates the expression of many inflammatory molecules [41] . The present work depicted that over- expression of hepatic NF-κB in Cd treated rats versus control animals. It has reported that exposure to low-dosage of Cd can activate NF-κB in HeLa cells and experimental animals [42] Also some authors showed that mice exposed to Cd toxicity induced over generation of NF-κB and inflammatory cytokines [26,43]. NF-κB has a key function in the cellular signaling mechanisms for inflammation in different pathological states [44] NF-κB activates many inflammatory genes, resulting in tissue damage [44]. At normal physiological conditions, NF-κB is inactive by binding to cytoplasmic specific inhibitor (IkB) . However, formation of inflammatory molecules can activate the NF-κB signalling pathways [45] , resulting in the transcription of many genes such as TNF-α and IL-6 [45] . Beside, NF-κB has been found to have a fundamental role in generation of NO via the generation of inducible NO synthase [46] . NF-kB activation is also coupled with the transformation of hepatic stellate cells (HSCs) into myofibroblasts, resulting in production of extracellular matrix (ECM) proteins , a pivotal event in the fibrogenesis [46]

Supplementation of Omega-3 fatty acids to Cd intoxicated animals , pronouncedly diminished the increase in the hepatic IFN- γ and NF-κB in comparison to Cd intoxicated rats. This result imply the potential anti-inflammatory and immunomodulatory functions of Omega-3 fatty acids. The anti-inflammatory beneficial impact of Omega-3 has been confirmed [20]. Suppression of hepatic IFN- γ and NF-κB activation by Omega-3 presented in the current study is viewed as a potential therapy for inflammatory liver injury.

The present work showed a clear rise in the hepatic HSP-70 of Cd treated rats with relation to control ones. Elevation in this protein in hepatic of Cd intoxicated rats is regarded as another sign for liver stress (oxidative stress and inflammatory stress). This result is confirmed by Jing et al. [48] who have stated that overexpression of HSP-70 in livers of Tanichthys albonubes fish exposed to Cd toxicity. Heat shock proteins (HSPs) are a group of intracellular molecules with different molecular weights [49] . They are commonly stimulated under the influence of a wide range of stressors, including metal toxicity [50-51]. These proteins act as cyto-protective , marinating cell survival by preventing the misfolding or degradation of proteins in different stress conditions [52] . It was reported that HSP70 particularly promoted due to stress and participated in survival of cells under the efficacy of different stressful states [53]. The current investigation may suggest that the increase of hepatic HSP70 in Cd treated rats is considered as a detoxification defense mechanism against Cd toxicity. Intake of Omega-3 fatty acids simultaneously with or before Cd administration, markedly reduced the hepatic increase in HSP70 level versus Cd untreated rats. This investigation provides the first prove that Omega -3 fatty acids can modulate the hepatic expression of HSP70 in rats under the toxic effect of Cd. The modulating impact of Omega-3 fatty acids on hepatic HSP70 may ascribe to their ability to ameliorate the hepatic OS and inflammation in response to Cd toxicity.

The serum hepatic enzymes (ALT, AST and ALP) , as well as albumin are indices used for diagnosis of hepatic cellular injury . Marked increases in these enzymes and a reduction in albumin were recorded in the current investigation in Cd exposed rats in comparable with control animals. These data are parallel with a former work has stated the alteration in these indices in rats exposed to Cd toxicity [54] . The rising in serum liver enzymes is a clue of hepatic cellular leakage in response to Cd hepatotoxicity. The decrease in serum albumin may ascribe to metabolic disorder in hepatic protein in response to Cd toxicity. Haptic tissue damage induced in rats exposed to Cd toxicity is confirmed by histopathological observation. Liver section of rats treated with Cd showed many histopathological changes, including dilation in portal vein with infiltration of inflammatory cells at portal areas. Cytoplasmic vacuolization, karyolysis, pyknosis , fatty degeneration and degeneration in hepatocytes were also observed. Similar hepatic histological changes have been reported by some authors in experimental animals in response to Cd toxicity [55]. Ingestion of Omega-3 fatty acids simultaneously with or before Cd toxicity, significantly ameliorated the alteration in the serum function parameters (ALT, AST, ALP and albumin) and could protect the liver from the histological alterations induced in rats under the effect of Cd toxicity as observed by normal liver architecture.

Conclusion

The current investigation has proved that Omega-3 fatty acids have potential hepatoprotective impact against Cd toxicity. They have different protective mechanisms by which they could mitigate the hepatotoxic impact of Cd , including

antioxidant, antioxidative stress , anti-inflammation and immunomodulation . Our investigation may candidate the use of Omega-3 fatty acids as a potential hepato-protective supplement against liver damage induced by heavy metal toxicity .

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