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Role of Heme Oxygenase (HO)-1 Enzyme in the Protective and Therapeutic Effect of Omega 3 Fatty Acids on Cisplatin-induced Hepatic and Renal Toxicity in Rats

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Abstract

Background: **Cisplatin is frequently used as an anticancer medication. Nephrotoxicity and hepatotoxicity are triggered by its usage, causing patients to limit their long-term therapy.** ***Aim:*** **To investigate the underlying mechanism of omega-3 (fatty acids) effect on hepatorenal toxicity induced by cisplatin in rats and to detect whether it has a protective or therapeutic effect or both and the role of HO-1 enzyme in both effects.** ***Materials and Methods:*** **40 male rats were divided into four equal groups: Control group: received i.p. saline+ corn oil orally, Cisplatin Group: received i.p. CP (12 mg/kg)+ corn oil orally, Cisplatin+ ω-3 pretreatment group: received i.p. CP following 10 days of ω-3 pretreatment in dose {(270 mg/kg) EPA, (180 mg/kg)} and Cisplatin+ ω-3 post-treatment group: received i.p. CP followed by 10 days of ω-3 post-treatment in dose as the previous group. Liver and kidney function, serum (HO-1), serum& tissue (TNF-α, IL-10), tissue (NFkB, GSH, MDA), and NrF2 gene expressions were measured.** ***Results:*** **Cisplatin-induced marked hepatorenal failure; detected by elevation of serum: AST, ALT, creatinine, and urea. Also, serum& tissue (TNF-α, IL-10), and tissue (NFkB, GSH, MDA) were significantly changed with no change in NrF2 gene expressions as compared to the control. On other hand, pre or post-ω-3 intake significantly corrected the changed markers. Liver and renal histopathological and immunohistochemical changes confirmed the biochemical results in all groups.** ***Conclusion:*** **Cisplatin treatment impairs liver and kidney function, while ω-3 supplementation could avoid this toxicity, with the protective response appearing to be more beneficial than the therapeutic effect.**

Keywords: omega-3, cisplatin, heme oxygenase-1, hepatic toxicity and renal toxicity

Introduction

Cisplatin (CP) is a frequent and effective anti-neoplastic agent used to treat testicular, ovarian, cervical, and bladder cancers[(1)]. Due to substantial toxic side effects such as neurotoxicity, ototoxicity, and nephrotoxicity, the use of CP is restricted despite its usefulness[(2)]. Hepatotoxicity has also been recognized as a prominent dose-limiting side effect of chemotherapy based on CP[(3)]. Higher dos-

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ages of CP may be required for efficient tumor suppression during aggressive treatment protocols, which could contribute to hepatotoxicity, which is also seen under low-dose recurrent CP therapy. Furthermore, CP causes direct breaks in the DNA strands of the basal epithelium, resulting in the release of reactive oxygen species (ROS) and cell destruction. Indeed, oxidative stress is a symptom of mitochondrial damage and plays a significant role in this toxicity[4]. CP treatment is linked to renal toxicity in 28–36% of patients who receive a loading dose of CP 50–100 mg/m^2 body surface[5]. Nephrotoxicity is produced by the buildup of excessive levels of CP in the kidneys. Because of this significant consequence, its clinical use has been restricted[6]. CP-induced nephrotoxicity via apoptosis and necrosis, as well as vascular factors and tubule inflammation. The oxidative stress caused by CP leads to the development of renal tubule damage[7]. Furthermore, increased synthesis of cytokines, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) is a marker of inflammation that is an oxidative stress consequence. So, stress can induce both adaptive and apoptotic responses at the same time; the fate of the cell is determined by the balance between survival and death signals. The prevalent discovery of hepatotoxicity induced by CP in cancer patients necessitates the urgent development of a natural substance that improves clinical outcomes among those cancer patients[4]. Omega 3 (ω-3) is an essential fatty acid that must be obtained through diet because the human body does not manufacture it. Furthermore, because of their well-documented health advantages, they are now considered an essential component of the human diet. Fish oil is the primary source of the long-chain -3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[8]. Anti-inflammatory and antioxidant properties have been suggested for EPA and DHA[9]. Heme oxygenase (HO)-1 is a stress-inducible enzyme that protects against free radicals and has anti-inflammatory properties[10]. HO-1 stimulates heme oxidation to bilirubin and carbon monoxide, both of which are powerful antioxidants. The anti-inflammatory cytokine interleukin (IL-10) regulates the HO-1 pathway. HO-1 also inhibits the production of inflammatory cytokines such as TNF-α[11]. As an antioxidant, ω-3 fatty acids play a vital role in reducing oxidative damage and removing free radicals from our bodies. Several studies have reported the protective and therapeutic effects of ω-3 fatty acid supplementation on degenerative diseases like cancer, cardiovascular diseases, immune system disorders, cirrhosis, and nervous system disorders[12]. Therefore, the purpose of this study was to see if ω-3 fatty acid supplementation could protect against CP-induced hepatotoxicity and nephrotoxicity in a protective way when given prior to CP and or in a therapeutic way when given following CP administration, depending on its underlying biochemical and antioxidant qualities. It would increase the liver and kidney metabolism and antioxidant defense mechanisms.

Materials and Methods

Drugs and chemicals

Omega-3 (fatty acids)

It was derived in the form of a soft gelatin capsule (SEDICO Pharmaceutical Co., Egypt). Each capsule contains fish oil-1000 mg (13% eicosapentaenoic acid (EPA) and 9% docosahexaenoic acid (DHA)). Rats were given a dose of 270 mg/kg EPA and 180 mg/kg DHA once daily by oral gavage[9] (Each capsule contains 1ml of fish oil so 1 ml= 1000mg (130 mg EPA+ 90mg

DHA) and 0.1 ml= 100 mg (13mg EPA+ 9mg DHA). According to the weight of rats ranging from (190-200mg), each rat received 0.4 ml (400 mg) once daily.

Cisplatin

It was derived in the form of Cisplatin MYLAN ampules (OnePharmaMedics, Cairo, Egypt) (each ampule contains 50 mg/50 ml) Rats were given a dose of (12 mg/kg) as a single dose by intraperitoneal injection[4].

Animals

Forty male Albino rats of the local strain aged 24–30 weeks and weighing 190–200 g were kept under standard conditions (temperature (20±2 ℃) and (a 12-h light-dark cycle) with free access to food and water. The procedures and animal care were approved by the "Research Ethics Committee, Faculty of Medicine, Tanta University, Egypt" approval code NO 35274/2/22.

Experimental protocol

Randomly rats have divided into four groups ten animals in each as follows:

1. Control group: Rats received a single isotonic saline i.p. injection and corn oil orally daily for 10 consecutive days using oral gavage[13].

2. Cisplatin Group: Rats were injected with a single dose of Cisplatin (12 mg/kg i.p) on day 1 only[4] then the rats received corn oil orally daily for 10 consecutive days using oral gavage.

3. Cisplatin+ ω-3 pretreatment: Rats received ω-3 (270 mg/kg) EPA, (180 mg/kg) DHA using oral gavage, once daily for 10 days. On the 10th day, 2h after the last dose of ω-3, they received a single dose of cisplatin (12 mg/kg i.p)[14].

4. Cisplatin+ ω-3 post-treatment group: in addition to the injection of CP on day 1, 2 hours after cisplatin injection, rats received ω-3 (270 mg/kg) EPA and (180 mg/kg) DHA using oral gavage, once daily starting from day 1 for 10 days[9,13].

Mortality rate

No deaths were observed in the control group (0%), while the mortality rate in the cisplatin-treated group was increased up to (40%). On the other hand, in the cisplatin+ ω-3 pretreatment and cisplatin+ ω-3 post-treatment groups, it was only (10%).

Serum and tissue samples collection:

After one week, the rats were anesthetized with sodium barbiturate 60 mg/kg injected intraperitoneally[15]. The rats were sacrificed by cervical dislocation and the blood was collected and serum samples were stored and kept frozen at–20℃ for biochemical tests. For histological evaluations, small pieces of liver and kidney tissues were fixed in 10% buffered formalin. Another part of the liver and kidney tissues was homogenized in phosphate-buffered saline (PBS), centrifuged, then the supernatant was separated and stored at -20℃.

Biochemical liver and kidney functions

The colorimetric method was used for the estimation of concentrations of serum Alanine aminotransferase (ALT) (Egyptian Company for Biotechnology, Cairo, Egypt Cod No 264 002), serum aspartate aminotransferase (AST) (Egyptian Company for Biotechnology, Cairo, Egypt Cod No 260 002), serum creatinine (Egyptian Company for Biotechnology, Cairo, Egypt Cod No 235 003), serum urea (Egyptian Company for Biotechnology, Cairo, Egypt Cod No 318 002) according to described protocols.

Measurement of serum (HO-1), (TNF-α), and (IL-10)

Enzyme-linked immunoassay technique was used to measure concentrations of serum HO-1 using an ELISA Kit Catalog Number. CSB-E08267 (CUSABIO Houston, TX 77054, United States), TNF-α using ELISA kit (Shanghai Sunred Biological Technology Co. Ltd, China. Catalog no 201-11-0765), and IL-10 using ELISA Kit Catalog Number CSB-E04595r (CUSABIO Houston, TX 77054, United States) following the manufacturer's protocols.

Measurement of hepatic and renal NFkappa (NFκB), reduced glutathione (GSH), malondialdehyde (MDA), (TNF-α), and (IL-10)

Tissue homogenate

Ice-cold saline was used to wash the liver and kidney three times, then blotted on filter paper and homogenized in 50 mM potassium phosphate (pH 7.4). Centrifuged the homogenate in 7000×g for 10 min at 4°C and the supernatant was stored at -20°C and used for measurement of NFκB using ELISA kit (MyBioSource, San Diego, CA 92195-3308. USA. Catalog no MBS453975), GSH using Bio-diagnostic Kit No. GR2511 (Bio-diagnostic Co., Egypt), MDA using Bio-diagnostic Kit No MD 25 29 (Bio-diagnostic Co., Egypt), TNF-α using ELISA kit (Shanghai Sunred Biological Technology Co. Ltd, China. Catalog no 201-11-0765) and IL-10 using ELISA Kit Catalog Number CSB-E04595r (CUSABIO Houston, TX 77054, United States) following the manufacturer's protocols.

Detection of nuclear factor erythroid 2–related factor 2(NrF2) gene expressions in kidney and hepatic tissues by quantitative real-time PCR (qRT-PCR):

The Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA) was used to extract total RNA from hepatic and renal tissue homogenates in accordance with the manufacturer's guidelines. Revert Aid H Minus Reverse Transcriptase (Thermo Scientific, #EP0451, USA) was used to construct first-strand cDNA from 5 g of total RNA. Using Power SYBR Green PCR Master Mix (Life Technologies), PCR reactions were performed. The primer sequences were as follows: Rat NrF2 forward primer (5'-GGCTACGTTTCAGTCACTTG-3') and reverse primer (5'-AACTCAGGAAT GGATAATAG-3') (GenBank Accession No. NZ_WBOA 01000003) rat β-actin forward primer (5'-CGTTGACATCCGTAAAGACCTC-3') and reverse primer (5'-TAGGAGCCAGGGCAGTAATCT-3') (GenBank Accession No. NM_031144.3). The following was the cycling pattern: one ten-minute cycle at 95°C, followed by 40 amplification cycles at 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 1 minute. The relative gene expression was measured using the 2-ΔΔCt technique after the cycle threshold (Ct) values for target genes and the housekeeping gene were determined[16].

Histological examination of liver and kidney

Formalin-fixed liver and kidney tissues were embedded in paraffin, cut into 5 µm thick slices by microtone, and stained with Hematoxylin-Eosin (H&E) stain. Sections were then examined using an Olympus light microscope model BX43F.

Immunohistochemical examination of NFκB

(Cat# No: MBS9701121) was used for immunohistochemical assessment of NFκB in hepatic and renal tissue. The positive expression of NFκB was detected using an Olympus light microscope model BX53F, and it was assessed using an image J analysis program and represented as a percentage area[17]. Then, the sacrificed animals were packed in special packages according to safety precautions and infection control measures.

Statistical Analysis

Survival analysis was constructed by Kaplan-Meier analysis using the Log-Rank test as shown in Figure 1. Mean ± (SD) in tables and mean ± (SEM) in figures were expressed and comparisons in statistics were done using the one-way ANOVA test, followed by (Tukey's) post hoc test, with p values less than 0.05 indicating statistical significance. The analysis was conducted using the statistical package for social science software (SPSS 22.0).

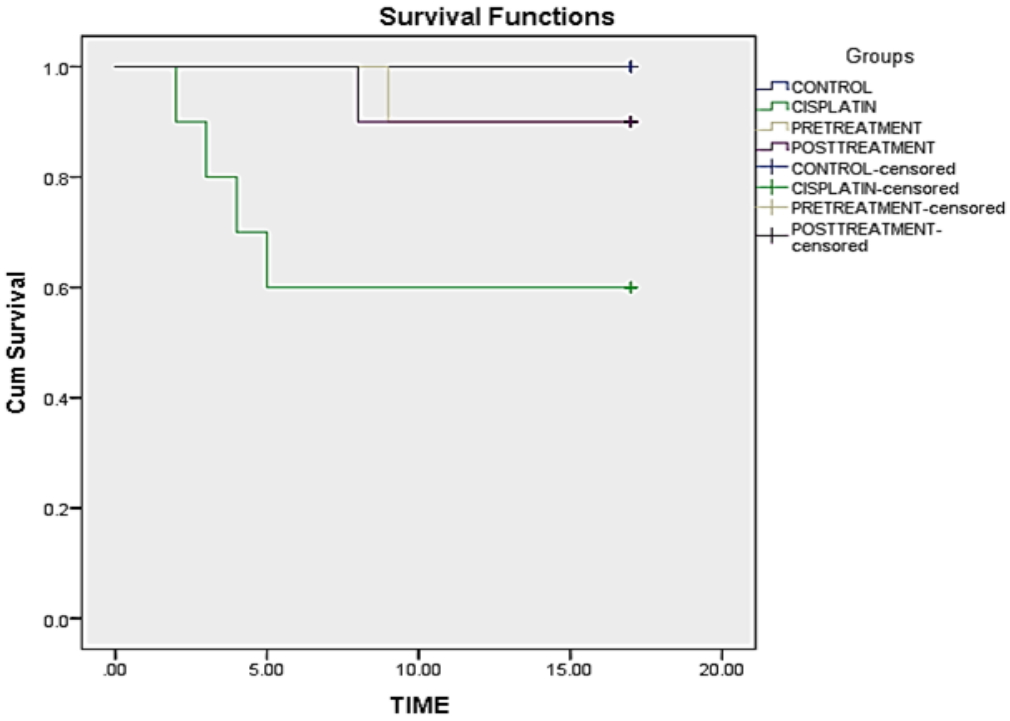

Figure 1: Survival analysis (Kaplan-Meier curve) of studied group P≤0.05

Results

Effects ofω-3 on liver functions

AST and ALT were measured to access liver function. There was a significant increase (p≤0.05) in ALT and AST in the CP group compared to other groups, while there was a significant decrease (p≤0.05) in ALT and AST in both CP+ ω-3 pretreatment and CP+ ω-3 post-treatment groups when compared with the CP group. ALT and AST showed no significant change (p≥0.05) in CP+ ω-3 pretreatment when compared with the control group and with the CP+ ω-3 post-treatment group. On the other hand, ALT and AST showed a significant increase (p≤0.05) in CP+ ω-3 post-treatment when compared with the control group and a non-significant change (p≥0.05) when compared with the CP+ ω-3 pretreatment group, as shown in Figure 2: A, B.

Effects of ω-3 on renal functions

Urea and creatinine were measured to access renal function. There was a significant increase (p≤0.05) in urea and creatinine in the CP group compared to other groups, while there was a significant decrease (p≤0.05) in urea and creatinine in both the CP+ ω-3 pretreatment and CP+ ω-3 post-treatment groups when compa-

ed with the CP group. Urea and creatinine showed no significant change (p≥0.05) in CP+ ω-3 pretreatment compared to the control group and with the CP+ ω-3 post-treatment group.

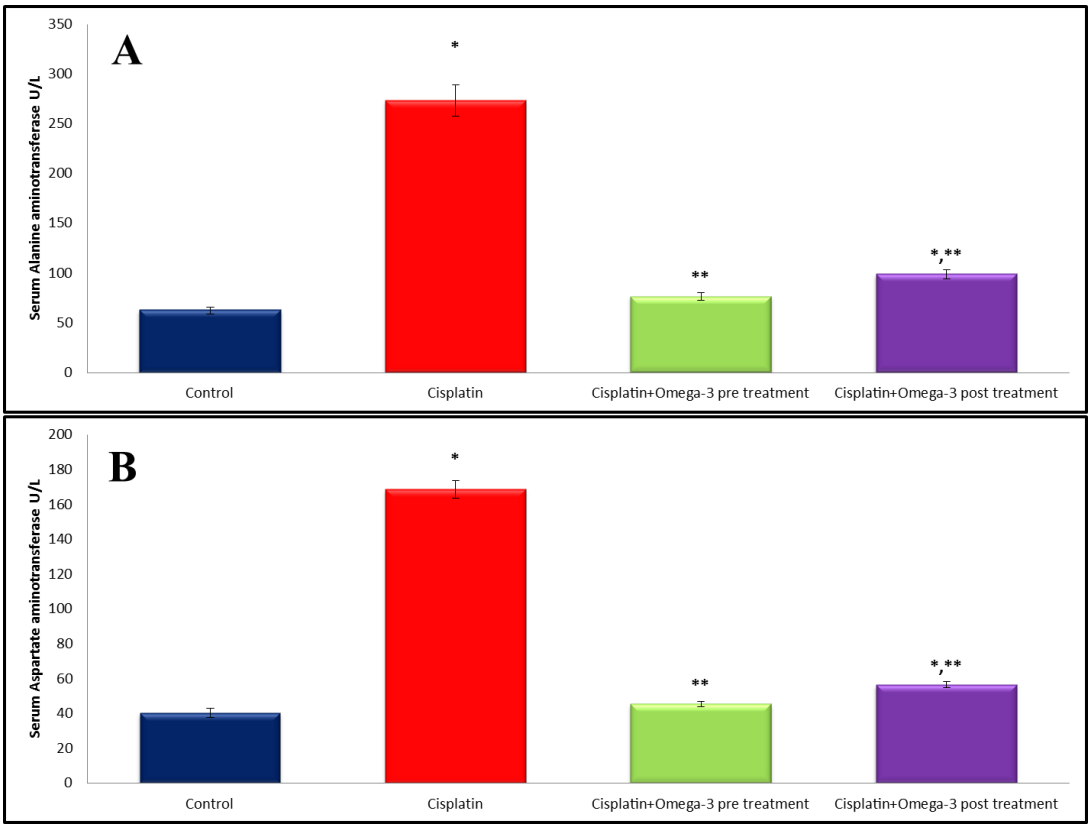

Figure 2: (A) Serum Alanine aminotransferase U/L in all studied groups **(B)** Serum Aspartate aminotransferase U/L in all studied groups (Mean value ±SEM)
* P≤0.05 versus control group, ** P≤0.05 versus Cisplatin group

In contrast, urea and creatinine showed a significant increase (p≤0.05) in CP+ ω-3 post-treatment when compared with the control group and a non-significant change (p≥0.05) when compared with the CP+ ω-3 pretreatment group (Figure 3A, 3B).

Effects of ω-3 on serum HO-1, TNF-α, & IL-10
Serum concentrations of (HO-1), (TNF-α), and (IL-10) were measured to determine the mechanism of ω-3. In the CP group, HO-1 showed an insignificant change (p≥0.05) when compared with the control group, while it was significantly lower (p≤0.05) when compared with the CP+ ω-3 pretreatment and CP+ ω-3 post-treatment groups, as shown in Figure 4A. While there was a significant increase (p≤0.05) in CP+ ω-**3** pretreatment and CP+ ω-3 post-treatment groups when compared with CP and control groups, as shown in Figure 4A. In addition, in CP+ ω-**3** post-treatment group, HO-1 was significantly lower (p≤0.05) when compared with the CP+ ω-**3** pretreatment group, as shown in Figure 4A. TNF-α and IL-10 levels were significantly higher (p≤0.05) in the CP group when compared to the other groups, but significantly lower (p≤0.05) in the CP+ ω-**3** pretreatment and post-

treatment groups when compared to the CP group. Moreover, there was no significant change (p≥0.05) in these parameters in CP+ ω-3 pretreatment when compared with the control group and with the CP+ ω-3 post-treatment group.

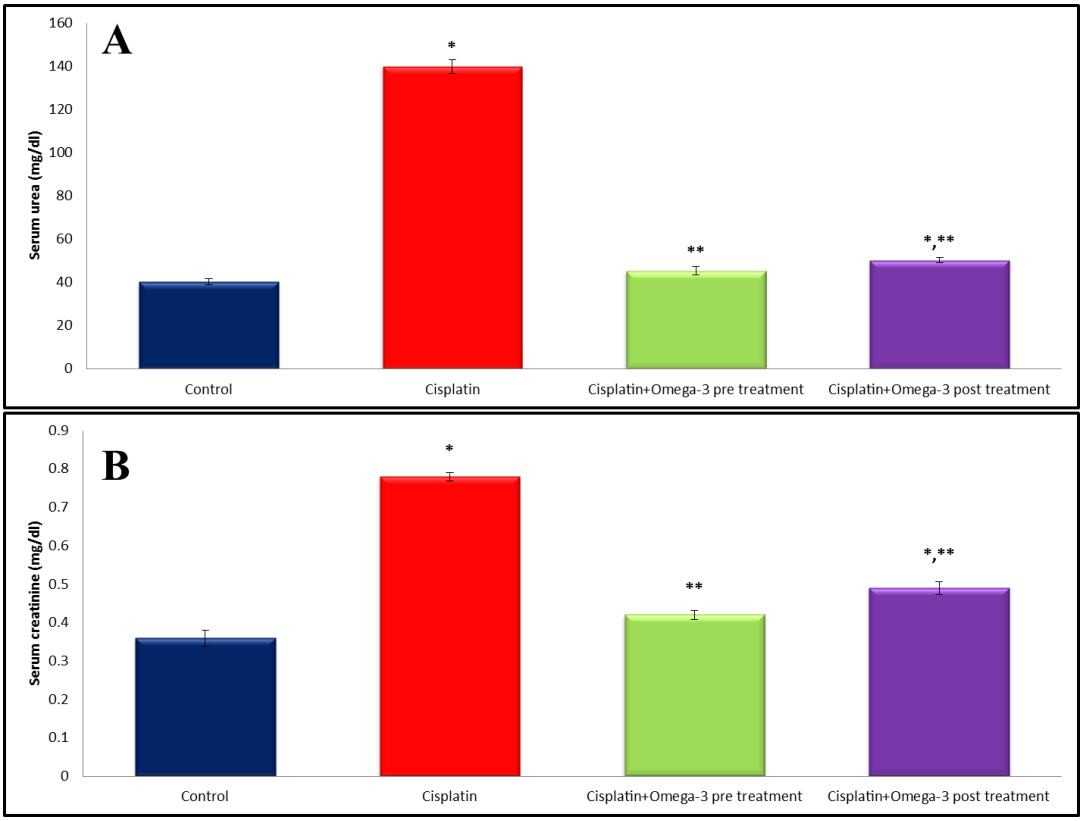

Figure 3: (A) Serum urea in all studied groups (B) Serum creatinine in all studied groups
(Mean value ±SEM). * P≤0.05 *vs.* control group, ** P≤0.05 *vs.* Cisplatin group

On the other hand, there was a significant increase (p≤0.05) in CP+ ω-**3** post-treatment when compared with the control group and a non-significant change (p≥0.05) when compared with the CP+ ω-**3** pretreatment group, as shown in Figure 4 (B and C).

Effects of ω-3 on hepatic and renal tissue NFκB, MDA, GSH, TNF-α, IL-10, and relative NrF2 expression

To detect lipid peroxidation and antioxidant capacity, NFκB, MDA, GSH, TNF-_α, and IL-10 were detected in hepatic and renal tissue. Hepatic and renal NFκB and MDA were significantly increased (p≤0.05) in the CP group when compared with other groups, while there was a significant decrease (p≤0.05) of these parameters in both CP+ ω-3 pretreatment and CP+ ω-3 post-treatment groups compared to the CP group. In addition, there was no significant change (p≥0.05) in these parameters in CP+ ω-3 pretreatment in comparison with the control group and with the CP+ ω-3 post-treatment group. On the other hand, there was a significant increase (p≤0.05) of these parameters in CP+ ω-3 post-treatment when compared with the control group and a non-significant

change (p≥0.05) when compared with the CP+ ω-3 pretreatment group (Table 1). Hepatic and renal GSH were significantly decreased (p≤0.05) in the CP group in comparison with other groups. It significantly increased (p≤0.05) in both the CP+ ω-3 pretreatment and CP+ ω-3 post-treatment groups compared to CP group.

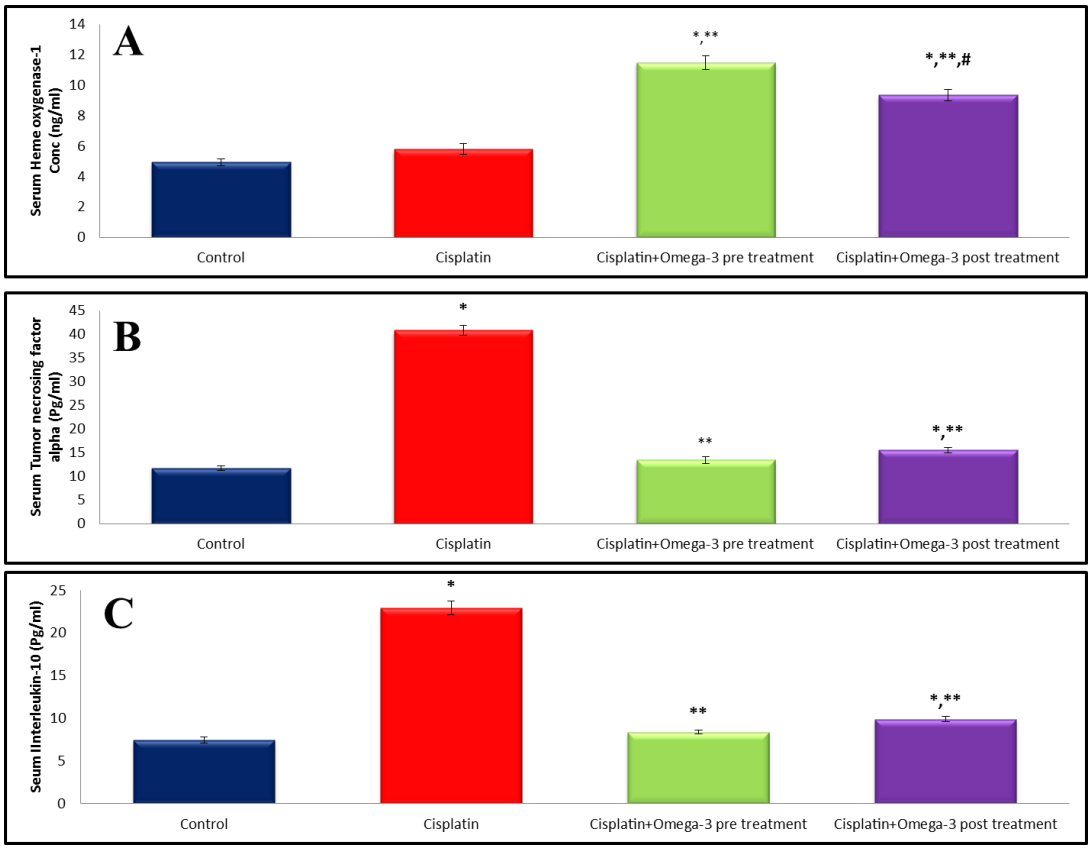

Figure 4: (A) Serum Heme Oxygenase-1 (B) Serum Tumor necrosing factor alpha (C) Serum IL-10
(Mean value ±SEM). * *P≤0.05* versus control group, ** *P≤0.05* versus Cisplatin group,
P≤0.05 versus Cisplatin+Omega-3 pretreatment group

Also, there was no significant change (p≥0.05) in CP+ ω-3 pretreatment in comparison with the control group and with the CP+ ω-3 post-treatment group. In contrast, it was significantly lower (p≤0.05) in CP+ ω-3 post-treatment in comparison with the control group and a non-significant change (p≥0.05) in comparison with the CP+ ω-3 pretreatment group, as shown in (Table: 1). In the CP group, hepatic and renal TNF-α and IL-10 levels were significantly higher (p≤0.05) than in the other groups, whereas in the CP+ ω -3 pretreatment and post-treatment groups, these levels were significantly lower (p≤0.05) than in the CP group. In addition, these parameters showed no significant change (p≥0.05) in CP+ ω-3 pretreatment when compared with the control group and with the CP+ ω-3 post-treatment group. On the other hand, there was a significant increase (p ≤0.05) of these parameters in CP+ ω-3 post-treatment when compared with the control group and a non-significant change (p≥0.05) compared to CP+ ω-3 pretreatment group (Table 1)

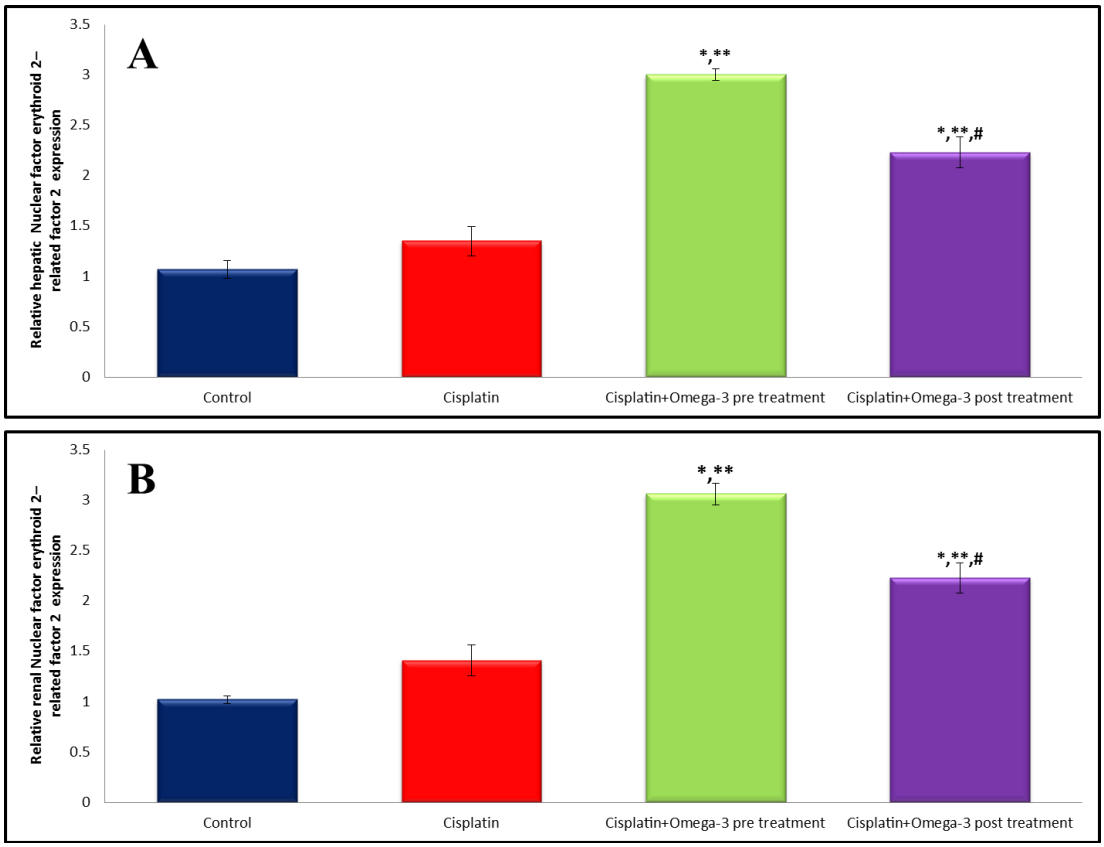

Figure 5: (A) Relative hepatic NrF2 and (B) Relative renal NrF2 expression in the studied groups (Mean value ±SEM). * P≤0.05 versus control group, ** P≤0.05 versus Cisplatin group, # P≤0.05 versus Cisplatin+Omega-3 pretreatment group

Relative hepatic and renal NrF2 expression in the CP group showed an insignificant change (p≥0.05) when compared with the control group; while it was significantly lower (p≤0.05) when compared with CP+ ω-3 pretreatment and with the CP+ ω-3 post-treatment group. While its expression in CP+ ω-3 pretreatment and CP+ ω-3 post-treatment group was significantly increased (p≤0.05) when compared with control and CP groups, Furthermore, relative hepatic and renal NrF2 expression was significantly lower (p≤0.05) in the CP+ ω -3 post-treatment group when compared to the CP+ ω-3 pre-treatment group, as shown in Figure 5: A, B

Effects of ω-3 on liver histopathology

The control group showed normal hepatic architecture formed of hepatic cords radiating from the central vein and separated by the hepatic sinusoids (S) with pericentral zone and midzone hepatocytes. The portal tract consists of a branch of the portal vein, a hepatic artery, and a bile ductule and is surrounded by a periportal zone of hepatocytes (Figure 6). CP group showed loss of normal hepatic architecture with congested dilated central vein, compressed hepatic sinusoids, multiple pyknotic nuclei, and diffuse vacuolar degeneration of hepatocytes. In addition, there were multiple large vacuoles, and ballooned hepatocytes can be seen (Figure 7). The CP+ω-3 pretreatment resulted in the restoration of normal hepatic architecture (Figure 8). While there was residual vacuolation and apoptotic bodies in CP+ω-3 post-treatment group (Figure 9).

Table 1: Hepatic and renal NFκB, MDA, GSH, TNF-α and IL-10 in the studied groups				
Group Parameter	Control group	Cisplatin group	Cisplatin+Omega-3 pre-treatment group	Cisplatin+Omega-3 post-treatment group
Liver NFκB (ng/gm tissue)	21.28 ± 2.58	62.55 ± 2.84*	22.30 ± 2.37**	25.63 ± 1.37*,**
Renal NFκB (ng/gm tissue)	31.43 ± 2.67	73.01± 3.00*	33.23± 2.49**	37.06± 1.84*,**
Liver MDA (µmol/mg tissue protein)	0.34± 0.02	0.54± 0.03*	0.35± 0.02**	0. 39± 0.02***
Renal MDA (µmol/mg tissue protein)	0.23± 0.02	0.62± 0.03*	0.24± 0.02**	0.29± 0.02*,**
Liver GSH (nmol/mg tissue protein)	33.60± 2.50	12.66± .50*	34.58± 2.45**	37.56± 1.97*,**
Renal GSH (nmol/mg tissue protein)	8.06± 0.41	4.03± 0.51*	8.85± 0.53**	9.51± 0.69*,**
Hepatic TNF-α (pg/mg protein)	40.66± 2.16	81.00± 4.28*	43.66± 2.58**	45.66± 2.58*,**
Renal TNF-α (pg/mg protein)	89.33± 1.75	128.33± 5.60*	92.50± 2.16**	94.83± 2.13*,**
Hepatic IL-10 (pg/mg protein)	35.83± 2.48	66.33± 4.03*	38.16± 2.13**	41.16± 3.06*,**
Renal IL-10 (pg/mg protein)	45.50± 3.08	120.16± 2.31*	47.50± 3.08**	50.50± 2.73*,**

NFκB: nuclear factor kappa-B, **MDA**: malondialdehyde, **GSH**: reduced glutathione, **TNF-α**: Tumor necrosing factor alpha, **IL-10**: Interleukin-10", * *P≤0.05 vs.* control group, ** *P≤0.05 vs.* Cisplatin group

Effects of ω-3 on Renal histopathology

The control group showed the normal appearance of renal corpuscles with a glomerular tuft and urinary space. Proximal convoluted tubules are lined by cuboidal cells with an acidophilic cytoplasm. Distal convoluted tubules are lined by cuboidal.
cells in renal glomeruli and tubules (Figure 10). In the CP group, there was tubular degeneration lined with low cuboidal cells with pyknotic nuclei and vacuolated cytoplasm. The dilated lumens of tubules contain sloughing necrotic cells. In addition, there was shrinkage of the renal corpuscle with wide Bowman's space (Figure 11). The CP+ω-3 pretreatment group showed that most of the renal glomeruli and tubules were like those of the control group. The brush border was preserved in most of the proximal and distal tubules (Fig. 12). The CP+ ω-3 post-treatment group showed normal glomeruli with moderate degeneration of renal tubules (Figure 13).

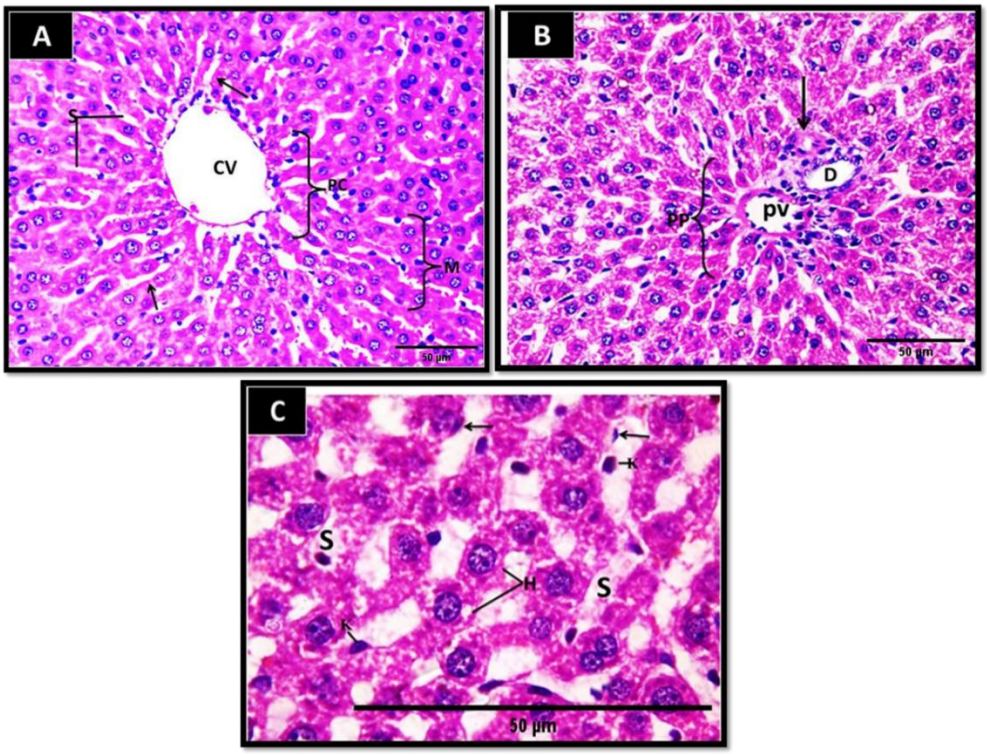

Figure 6: A photomicrograph of a liver section of an adult albino rat from the control group
A) Hepatic cords (arrows) radiating from the central vein (CV) and separated by the hepatic sinusoids (S) with pericentral zone (PC) and midzone (M) hepatocytes. B) Portal tract consists of a branch of the portal vein (p), hepatic artery (arrow), and bile ductule (D) and is surrounded by the periportal (PP) zone of hepatocytes. C) Polyhydral hepatocytes (H) appear with rounded vesicular nuclei and granular eosinophilic cytoplasm separated by sinusoids (S) which are lined with endothelial cells (arrows) and Kupffer cells (K). (H&E x400, x400, x1000)

Effects of ω-3 fatty acids on tissue expression of NFκB

Based on immunohistochemistry, the CP group significantly increased NFκB tissue expression in both hepatic and renal tissues compared to the control group ($p \leq 0.05$). Compared to the CP, pre, and post-treatment with ω-3 fatty acids resulted in a significant decrease in hepatic and renal NFκB expression ($p \leq 0.05$). Furthermore, both hepatic and renal NFκB expression were nearly normalized by the effect of pretreatment with ω-3 fatty acids (Figure 14, 15).

Discussion

CP is one of the abundant chemotherapeutics for cancer treatment, the use of CP in cancer patients is strictly regulated due to a number of unfavorable side effects. When a patient's kidney function is compromised, doctors usually instruct them to use lower doses of CP because excessive levels have been linked to nephrotoxicity. It's important to keep in mind that nephrotoxicity is a dose-dependent side effect[18]. A higher CP dose can also trigger liver toxicity. Because of a deep awareness of the severity of CP-induced liver toxicity, many anti-toxicity medicines, including vitamin E and selenium, have been advocated for patient protection. However, toxicity, which causes liver damage, remains a major obstacle to cisplatin's usefulness as an anti-cancer treatment[19]. The degree of nega-

tive impact differs from one patient to another, so researchers and practitioners should take precautions while using the medicine.

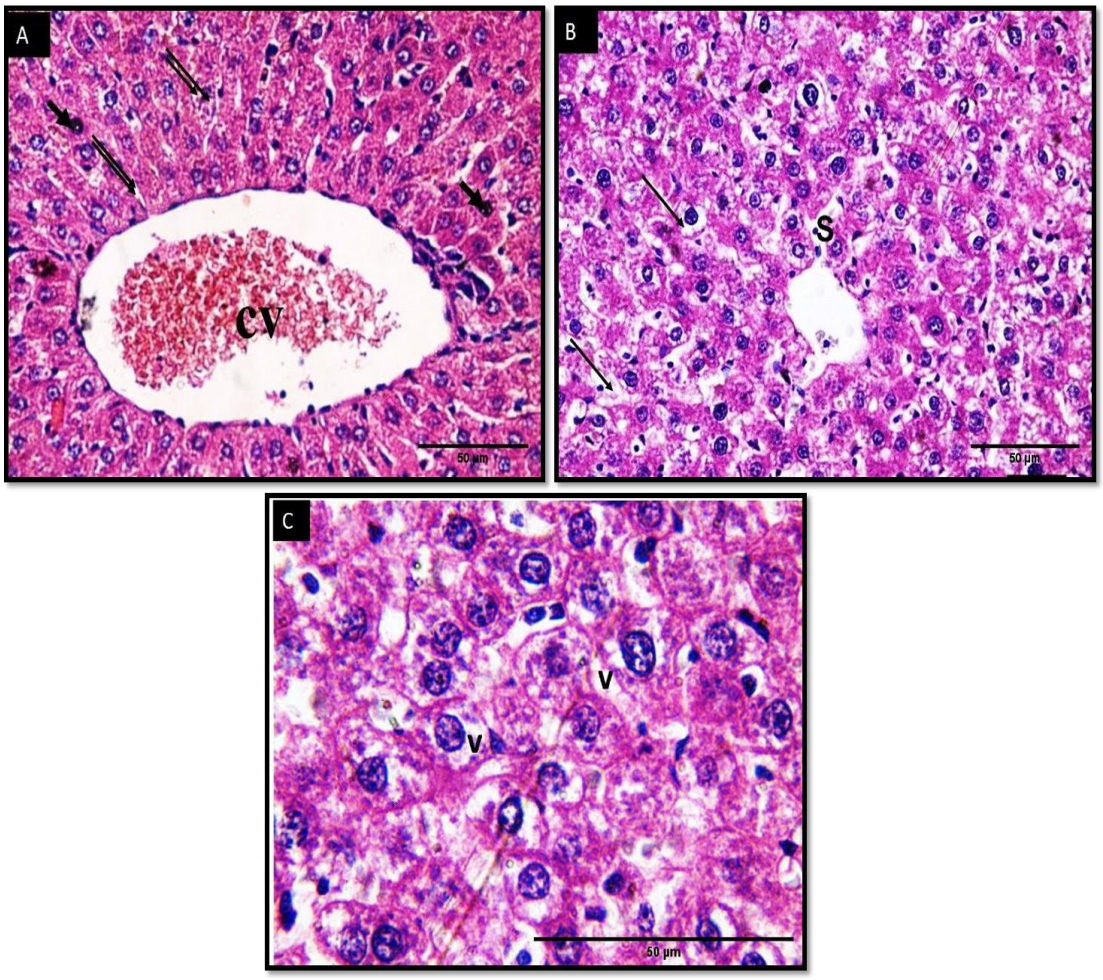

Figure 7: A photomicrograph of a liver section of an adult albino rat from the cisplatin group
A) Loss of normal hepatic architecture with the congested dilated central vein (CV). Notice the compressed hepatic sinusoids (double black arrows) and multiple pyknotic nuclei (short thick arrow). B) Loss of normal hepatic architecture with dilated sinusoids (S) and diffuse vacular degeneration of hepatocytes (arrow). There are numerous large vacuoles (V) and ballooned hepatocytes visible.
(H&E staining x400, x400, x1000)

Researchers suggested that combining CP with other medications is useful not only in overcoming drug resistance but also in lowering toxicity[20]. In the current study, we explored the possible protective and therapeutic effects of ω-3 against hepato renal toxicity induced by CP through anti-oxidative and anti-inflammatory effects.

Previously, a single dose (12 mg/kg) of CP was used by i.p injection to induce hepatorenal toxicity. The toxic effect of CP appears after 7 days[21]. In this study, CP-induced hepato and nephrotoxicity were evidenced by biochemical measurements and histopathological changes that have been reported by other investigators[22].

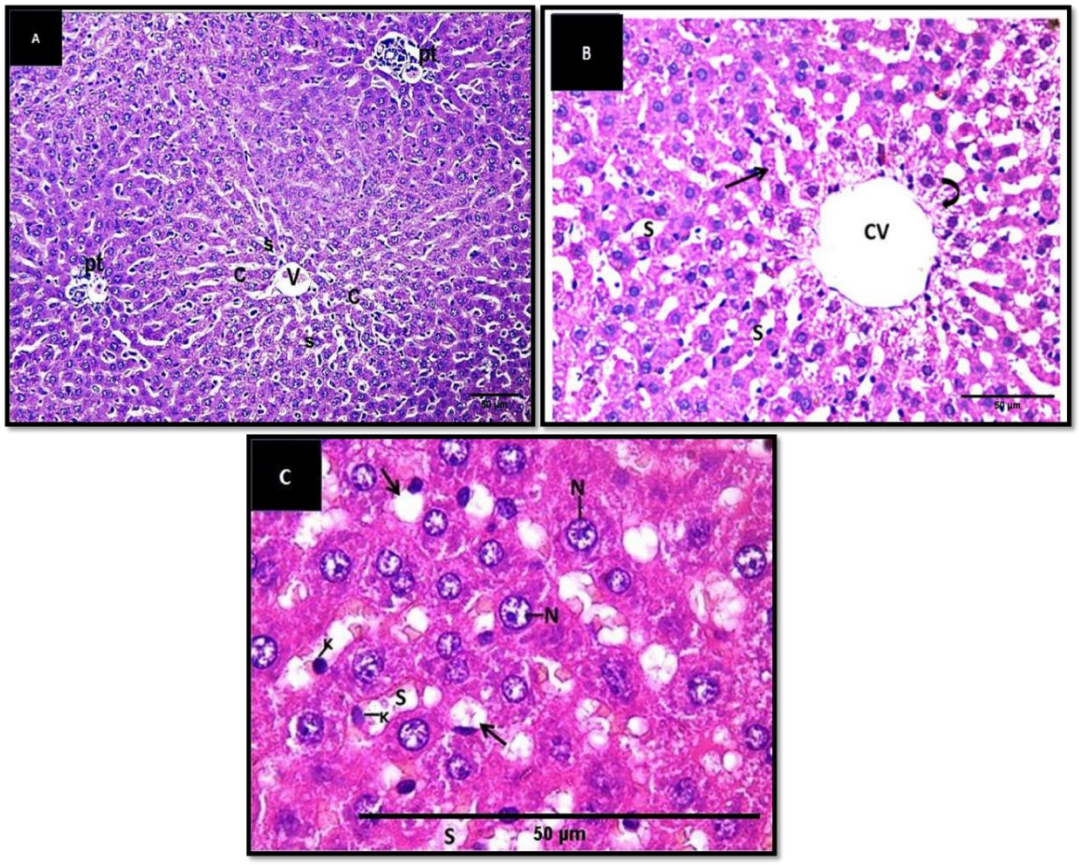

Figure 8: A photomicrograph of a liver section of an adult albino rat with Cisplatin+ω3 pretreatment

(A & B) regaining regular hepatic architecture. Each lobule contains a central vein (V) and a portal tract (pt) at the corner. Many hepatic cords (C) appear radiating from the central vein towards the portal tract and are separated by the hepatic sinusoids (s). C) Normal-appearing hepatocytes with rounded vesicular nuclei (N) and granular eosinophilic cytoplasm separated by sinusoids (S) lined with Kupffer cells (K). (H& E staining x200, x400, x1000)

Severe hepatic damage in the cisplatin group was indicated by the elevation of the serum levels of (ALT, and AST). Renal damage was proved by the elevation of serum urea and creatinine. These changes are in line with prior research findings[6,23,24]. Cisplatin's hepatorenal cytotoxicity is generated by mitochondrial dysfunction and the generation of superoxide anion and hydroxyl radical, which are ROS, along with lipid peroxidation[14]. Free radical production by CP and impairment of tissue oxidant-antioxidant balance were confirmed by the reduction of renal and hepatic GSH and elevation of renal and hepatic MDA. This is in accordance with other studies[22]. Normal biological functions are disturbed by exposure to oxidative stress. ROS causes membrane lipid peroxidation and polyunsaturated fatty acid breakage, resulting in cellular damage. The significant increase in hepatic and renal MDA concentrations is an indicator of the peroxidation of lipids as previously reported[25]. The generation of ROS is a major source of oxidative stress, which leads to a decrease in natural antioxidant capacity. Other changes include the production of non-enzymatic compounds and antioxidant enzymes, as well as a decrease in glutathione levels, all of which contribute to large changes in CP toxicity[26].

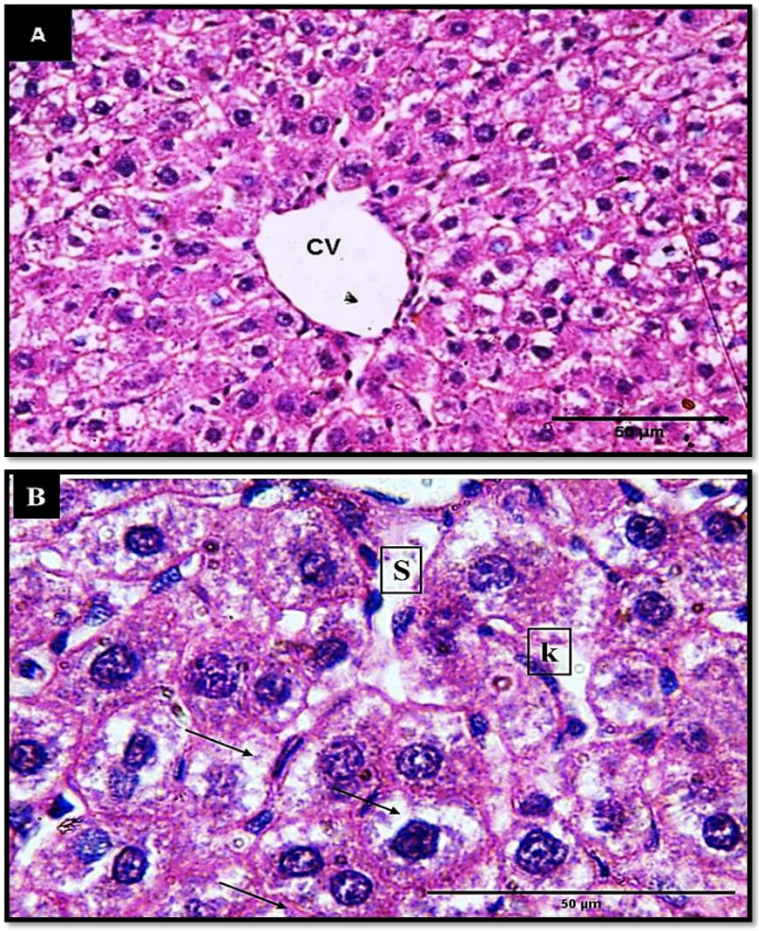

Figure 9: A photomicrograph of a liver section of an adult albino rat with Cisplatin+ω3 posttreatment

A) mild dilatation of the central vein (CV) with irregular hepatic cords. B) There is multiple vacuolation within hepatocytes (black arrows) and normal sinusoids (S) lined with Kupffer cells (K) (H & E staining x400, x1000)

In addition, CP accumulates in target organs by covalently attaching to their proteins. This may have an influence on their antioxidant enzymes, which are the cells' initial line of defense against oxidative stress[14]. This is in accordance with Sarin et al.[27], who noted that the kidney is the principal route of CP excretion and that the kidney has a higher tendency to accumulate CP than any other organ in the body, including the liver. CP accumulates in the proximal tubular epithelial cells at a rate of about 5 times greater than that of the serum levels. Cisplatin's excessive retention in kidney tissues causes CP-induced nephrotoxicity. Other research claims that CP interacts with DNA by forming covalent adducts between specific DNA bases and the platinum molecule, resulting in cell cytotoxicity[28]. On the other hand, several studies suggest that omega-3 fatty acids are effective in the treatment of cancer, cardiovascular disease, autoimmune disorders, cirrhosis, and nervous system diseases. Furthermore, ω-3 essential fatty acids are said to have antioxidant characteristics, lowering oxidative stress and preventing ROS from being produced[12]. In the present study, pre-and post-treatment with ω-3 signifi-

cantly improves hepatic and renal function.

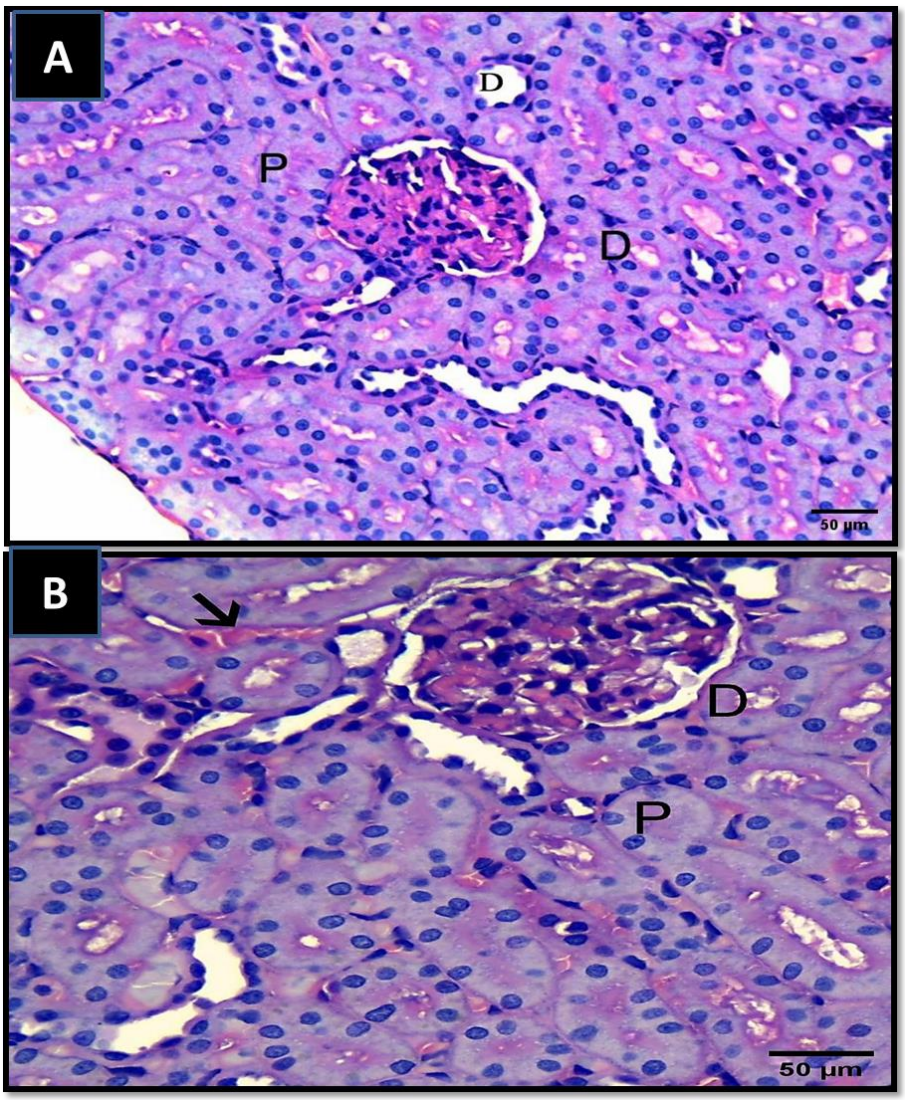

Figure 10: A photomicrograph of a kidney section of an adult albino rat from the control group
A & B) renal corpuscles with a glomerular tuft (G) and urinary space (arrow). Proximal convoluted tubules (P) are lined by cuboidal cells with an acidophilic cytoplasm. Distal convoluted tubules (D) are lined by cuboidal cells (H & E staining x200, x400)

This hepatorenal protective effect has also been reported[9]. However, in ω-3 post-treatment group, hepatic and renal function improved when compared to the CP group, but still significant when compared to the control group. The efficacy of ω -3 fatty acids to lower MDA levels, limit lipid peroxidation, and increase GSH in the liver and kidney demonstrated their antioxidant potential in both ω-3 pre and post-treatment groups. The protective effect of ω-3 is in agreement with other studies that have also shown the antioxidant and anti-inflammatory protective effect ofω-3 fatty acids against hepato-renal toxicity[29]. ω-3 intake in ω-3 post-treatment group significantly decreased hepato-renal MDA and increased hepatic and re-

nal tissue GSH in comparison to the CP group, but still significant when compared to the control group. Several studies at tribute the antioxidant and anti-inflammatory effects of ω-3 to its link to the HO-1 pathway[9]. This is evidenced in the present study as serum HO-1 enzyme was significantly high in both ω-3 pre and post-treatment groups compared to the cisplatin group or the control. First of all, HO-1 can be used as a biomarker for liver toxicity or damage with 100% sensitivity[30].

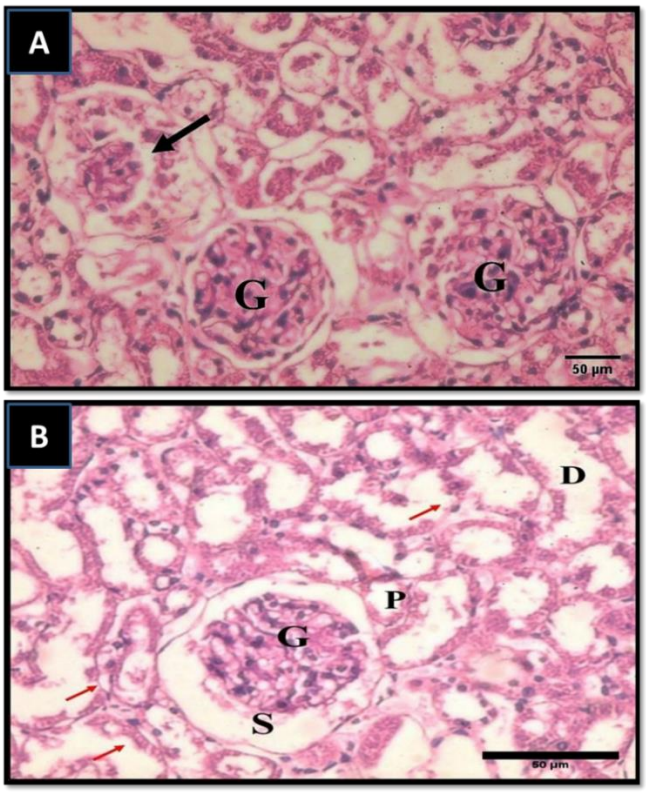

Figure 11: A photomicrograph of a kidney section of an adult albino rat of the cisplatin group
A) obliteration of the capsular space (arrow) with hypercellularity. B) A magnified part showing degenerated renal tubules lined with low cuboidal cells with pyknotic nuclei and a vacuolated cytoplasm (red arrows). The dilated lumens of tubules contain sloughing necrotic cells. Note shrinkage of the renal corpuscle with wide Bowman's space (S) (H & E staining x200, x400)

In addition, the expression of HO-1 in acute kidney damage models and plasma HO-1 levels in individuals with acute renal damage were found to be higher, suggesting that HO-1 could be used as a biomarker in this condition[31]. This is attributable to the reality that HO-1 is triggered by a variety of oxidative stress stimuli and serves a protective role by regulating antioxidant and anti-inflammatory actions. In this study, there was an elevation in HO-1 concentration in the serum of the CP group as compared to the control group, but still in a non-significant range as reported by others[32].

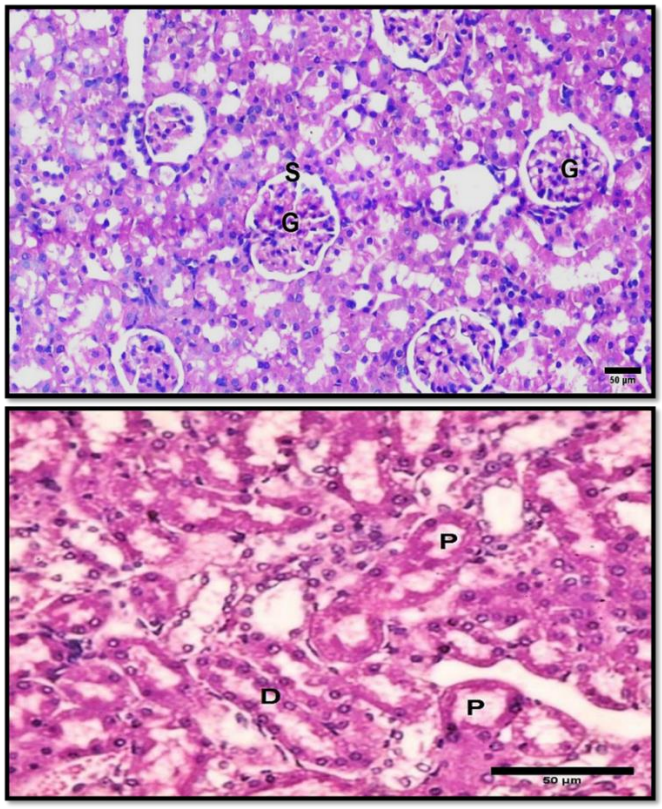

Figure 12: A photomicrograph of a kidney section of an adult albino rat from the Cisplatin+ ω3 pretreatment group

A & B) most of the renal glomeruli and tubules are more or less like those of the control group. The preserved brush border in most of the proximal and distal tubules (P) & (D), respectively (H & E staining x200, x400)

HO-1 breaks down heme to produce biliverdin, carbon dioxide, and iron. Biliverdin reductase converts biliverdin to bilirubin, and both of these bile pigments are effective peroxyl radical scavengers[33]. As a result, the HO-1 enzyme is hypothesized to be the rate-limiting enzyme in heme deconstruction, preventing heme toxicity. The transcription factor BACH1 suppresses the HO-1 gene, which is activated by free heme and heme-independent oxidative stress[34]. Nrf2 regulates HO-1 expression. Under normal circumstances, Nrf2 is found in the cytoplasm where it forms the Keap1/Nrf2 inactive complex with the Kelch-like ECH-associated repressor protein 1 (Keap1). After being released from the Keap1/Nrf2 complex, Nrf2 translocates into the nucleus and, by binding to the antioxidant response element (ARE), it induces cytoprotective gene transcription as HO-1[35].

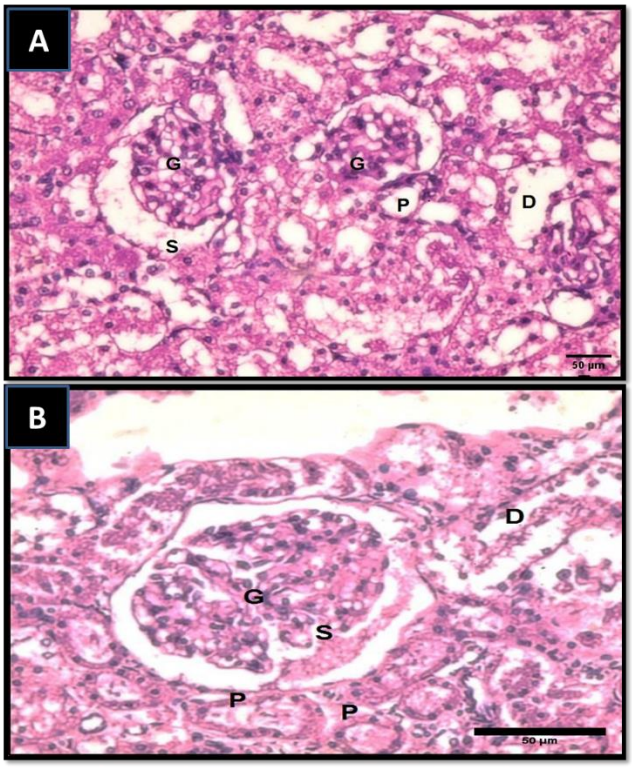

Figure 13: A photomicrograph of a kidney section of an adult albino rat from the Cisplatin+ ω3 posttreatment group

A & B) normal glomeruli (G) surrounded by Bowman's (S) space and moderate degeneration of proximal and distal renal tubules. (H & E staining x200, x400)

Under normal conditions, BACH1 forms a heterodimer with tiny Maf proteins, which then binds to the Maf recognition element (MARE) in the promoter region of HO-1 to limit transcription[36]. BACH1-Maf is released from MARE during oxidative stress and in the presence of excess free heme, allowing transcriptional activation of HO-1 by Nrf2-Maf heterodimers, leading to HO-1 synthesis and heme breakdown. As a consequence, the BACH1/HO-1 pathway acts as a feedback mechanism to maintain heme homeostasis in the face of oxidative stress[37]. In the current study, the effect of ω-3 intake significantly increased serum HO-1 and hepatic and renal tissue Nrf2 expression in both ω-3 pretreatment and post treatment groups when compared with the control group and CP group. Also, ω-3 pretreatment group showed a significant increase in relation to the post-treatment group. Sakai et al.[38] suggested that EPA and DHA may protect vascular endothelial cells from oxidative stress-induced DNA damage by up-regulating the Nrf2-mediated antioxidant response. Also, Tatsumi et al.[39] report that ω-3 fatty acids exert anti-oxidant effects by translocating Nrf2 from the cytoplasm to the nucleus, where it latches onto ARE and triggers the production of anti-oxidant enzymes like HO-1. Furthermore, Nrf2 has been demonstrated to change the transcription of several genes that control the antioxidant and anti-inflammatory pathways in the cell. It has also been hypothesized that Nrf2 and NFκB interact[40]. In the current study, the role of inflammatory mediators was evidenced by the elevation of NFκB in hepatic and renal tissue.

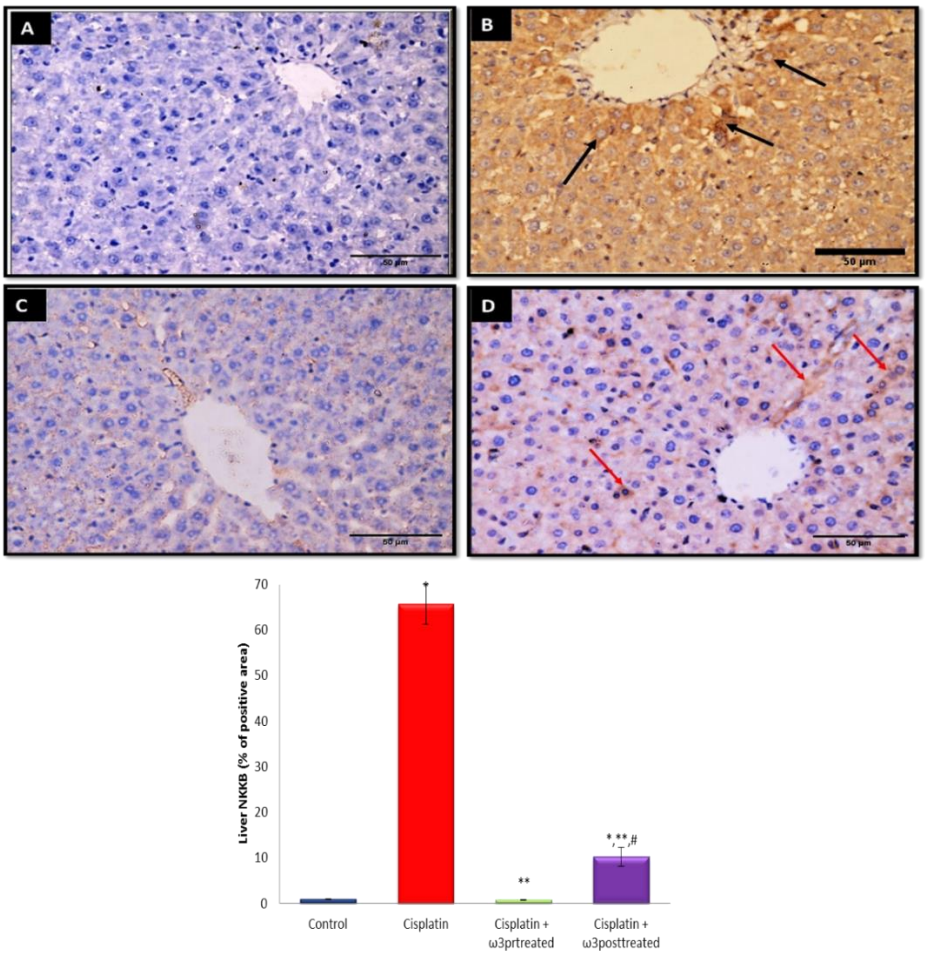

Figure 14: Effect of ω3 on immunohistochemical analysis and immune reactivity of liver NFκB. Immunohistochemical analysis of NFκB expression in the liver

(A) control group shows a negative NF-κB reaction within the cytoplasm of hepatocytes. B) the cisplatin group exhibited a strong positive NF-B reaction in the form of brown staining within the cytoplasm of hepatocytes (black arrows).C) negative NF-κB reaction in cisplatin+ ω3 pretreatment D) cisplatin+ ω3 post-treatment showing few NF-κB positive cells (red arrows) compared to cisplatin group (magnification x400) Data are presented as mean ± SEM. * P≤0.05 versus control group, ** P≤0.05 versus Cisplatin group, # P≤0.05 versus Cisplatin+Omega-3 pretreatment group

In addition, there was an elevation of serum and tissue TNF-α and IL-10 in CP group. This is in agreement with others[9]. Sun et al.[41] reported that TNF-α is a type of inflammatory cytokine that is induced by NFκB. In addition, inflammatory cytokines cause greater nuclear translocation and activation of NFκB, resulting in a positive-feedback process that amplifies the inflammatory condition[42]. The anti-inflammatory effect of ω-3 was proved by the decrease NFκB in hepatic and renal tissue, in addition to the reduction of serum and tissue TNFα and IL10 in ω-3 pre and post-treatment groups. The protective effect is in accordance with others[43].

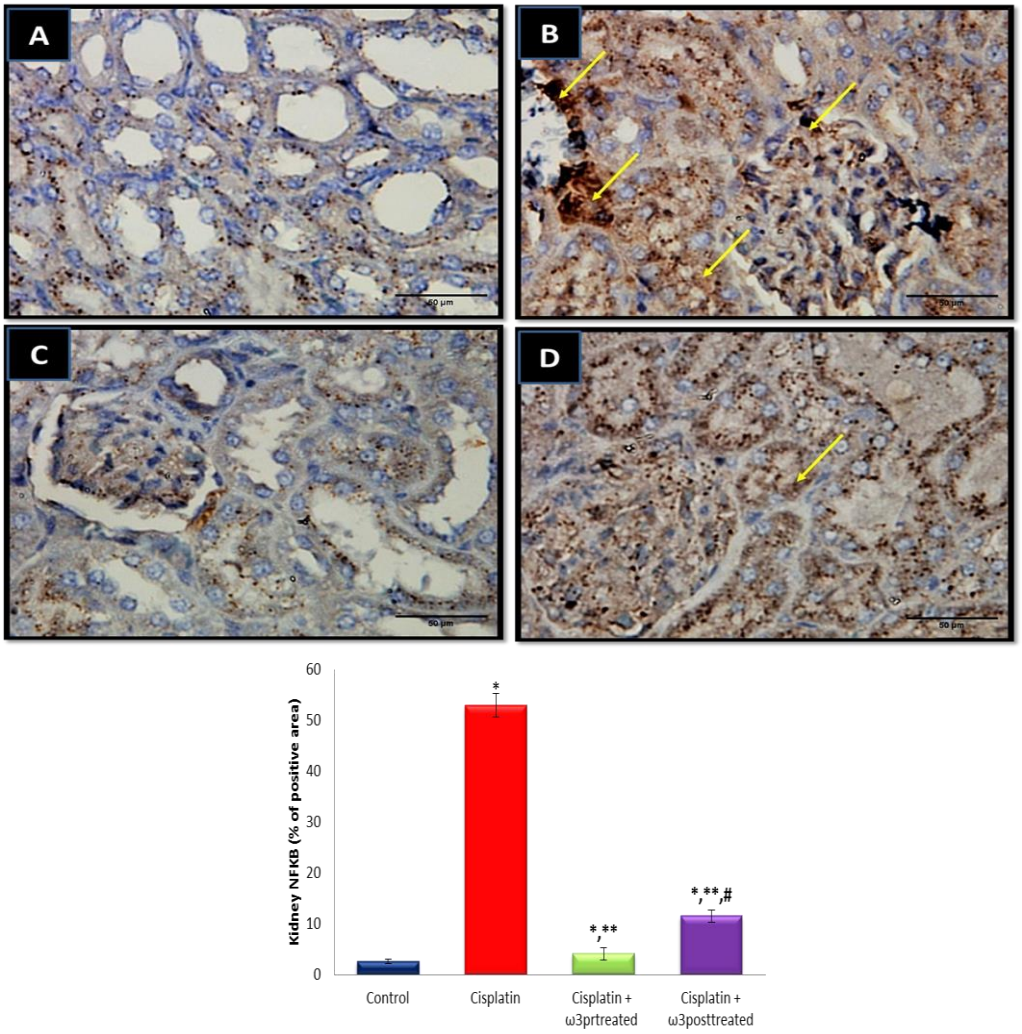

Figure 15: Effect of ω3 on immunohistochemical analysis and immune reactivity of kidney NFκB. (A) control group shows a negative NF-κB reaction. B) cisplatin group showing strong positive NF-κB reaction in the form of brown stain within the cytoplasm (yellow arrows). C) negative NF-κB reaction in cisplatin+ ω3 pretreatment D) cisplatin+ ω3 posttreatment showing NF-κB weak positive reaction (yellow arrows) compared to cisplatin group (Magnification x400) Data are presented as mean ± SEM. ***** P≤0.05 *vs.*control group, ****** P≤0.05 *vs.* Cisplatin group, # P≤0.05 *vs.*Cisplatin+Omega-3 pretreatment group

In addition, NFκB, TNF, and IL-10 were significantly decreased in ω-3 post-treatment group in comparison with the CP group, but still significantly elevated in comparison with the control group. Reduction of NFκB is the cause of the reduction of TNF-α and IL-10 in both ω-3 pretreatment and post-treatment groups, and this could be related to the ω-3 capacity to suppress NFκB nuclear translocation and so reduce inflammatory gene expression by down-regulating hepatic and renal NFκB tissue expression[44]. Biochemical results of the present study were confirmed by histopathological and immunohistochemical changes as CP induces hepatic damage and disturbed normal liver morphology in addition to renal damage by degeneration of glomeruli and renal tubules, these findings are in agreement with other studies[45,46]. While the antioxidant and anti-inflammatory effect of ω-3 was proved by

restoration of normal hepatic and renal architecture, this is in accordance with previous studies[14]. As regards immunohistological changes, CP increases NFκB expression in hepatic and renal tissue and this is in accordance with previous studies[45,47]. The anti-inflammatory effect of ω-3 was confirmed by immunohistochemical changes by suppression of tissue NFκB expression and this is in agreement with[9]. When the biochemical results of the current study were analyzed together with the histopathological and immunohistochemical changes, it was clear that ω -3 prevented hepato-renal toxicity via antioxidant effects via the Nrf2-BACH1-HO-1 pathway adjustment and anti-inflammatory effects via down-regulation of the NFκB-TNF-α pathway.

Conclusion

ω-3 has both protective and therapeutic effects against CP-induced hepato-renal toxicity, but its protective effect is more evident than its therapeutic effect, and these effects appear to be linked to the activation of the Ho-1 enzyme through the activation of Nrf2. This highlights the use of ω3 as a protective supplement together with cisplatin. Further studies are needed to identify the underlying mechanism of ω3 as a therapeutic agent.

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