

# Protective Role of Omega-3 Fish Oil against the Toxicity of Ifosfamide in Male Rats

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## Abstract

Ifosfamide (IFO) is a cytotoxic alkylating drug used for the treatment of a variety of cancers but has reported to cause certain hematological, hepatotoxic and nephrotoxic side effects. For protection against these side effects, different antioxidants were used. This study is performed to evaluate the ability of omega-3 fatty acids (Omega-3 FAs) to attenuate ifosfamide (IFO) toxicity. Thirty male albino rats were randomly divided into six groups. Group 1: control, Group 2: omega-3 (4gm/kg diet), Group 3: IFO (50mg/kg b.wt.), Group 4: IFO(80mg/kg b.wt.), Group 5: IFO (50mg/kg b.wt.) plus omega-3, Group 6: IFO (80mg/kg b.wt.) plus omega-3. Ifosfamide was administrated intraperitoneally (i.p.), while omega-3 was given with the diet. The duration was five consecutive days for IFO, and six consecutive days for omega-3 oil. A significant increase in the body weight gain of the rats has been recorded after applying omega-3 to both doses of IFO when compared to each IFO group. In the IFO group, the levels of serum creatinine, phosphorous, glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and malondialdehyde (MDA) were increased, while serum glucose decreased significantly. In the Omega-3 and IFO plus omega-3 groups, these variations didn't show significant changes. Hematologically, Omega-3 has recovered the decrease in WBC, RBC and blood platelets caused by IFO after giving omega-3 to the IFO treated rats. IFO has induced many histological alterations in the liver such as degeneration of the cells, inflammatory cells infiltration and dilation in the sinusoidal lumen, while it caused severe inflammation, degeneration in the kidney tubule lining cells and hypertrophy of these tubules. The histological structure of liver and kidney was found to be protected from this effect of IFO when a combination of Omega-3 FAs and IFO was administrated. Interestingly, Omega-3 didn't interfere with the antimetabolic property of IFO, suggesting a very important role of this oil in the future of cancer chemotherapy.

**Keywords:** IFO, omega-3 fish oil, hepatotoxicity, Nephrotoxicity.

## 1. Introduction

Although Ifosfamide (IFO) is a highly effective chemotherapeutic agent for treating a variety of pediatric and adult solid tumors (Straka *et al.*, 2003), it has been shown to induce many side effects such as hepatotoxicity (Paschke *et al.*, 1988) and nephrotoxicity (Chen *et al.*, 2008; Hanly *et al.*, 2009). Nephrotoxicity may present in more severe cases as Fanconi syndrome (Skinner *et al.*, 1993; Loebstein and Koren, 1998). This renal disorder is characterized by urinary loss of amino acids, glucose, phosphate and bicarbonate (Loebstein *et al.*, 1999; Rossi *et al.*, 1999; Skinner, 2003). The clinical consequences may finally necessitate a kidney transplant (Skinner, 2003). The IFO-induced animal model of Fanconi syndrome was described by Nissim and Weinberg (1996).

IFO has been found to cause oxidative damage in renal and bladder tissues (Sener *et al.*, 2004; Knouzy *et al.*, 2010), for this reason, researchers for improving the therapeutic efficacy of IFO, used different antioxidants such as, taurine (Badary, 1998), L-Histidinol (Badary, 1999a), thymoquinone (Badary, 1999b), resveratrol (Şehirli *et al.*, 2007), N-Acetylcysteine (Chen *et al.*, 2008) and melatonin (Casado-Zapico *et al.*, 2010). Against neurotoxicity caused by IFO, methylene blue (Hamadani and Awan, 2006) and dexmedetomidine (Bernard *et al.*, 2010) have been used.

Omega-3 fatty acids (Omega-3 FAs) is considered as a strong antioxidant (Calviello and Serini, 2010) and its role as an anticancer agent has been extensively confirmed in

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most of the human malignancies (Shaikh *et al.*, 2010). Its role in enhancement cytotoxicity of anticancer drugs to tumor cells and protection of normal cells was previously reported (Pardini, 2006). Furthermore, the anti-inflammatory potential of long chain Omega-3 FAs in many chronic diseases has been suggested (Calder, 2009; Wall *et al.*, 2010). The role of Omega-3 FAs in inhibiting proliferation, inducing apoptosis and promoting differentiation in many cancers have been recently studied (Edwards and O'Flaherty, 2008; Sun *et al.*, 2009). In addition, recent findings indicate that omega3-FA acts synergistically with certain chemotherapeutic agents (Wendel and Heller, 2009). The present investigation was undertaken to study the protective role of omega-3 fish oil against the IFO toxicity in animal model which can be considered, as far we searched, as the first trial for this oil in this field.

## 2. Materials and Methods

### 2.1. Experimental animals

Thirty adult Wister albino male rats (8 weeks old weighing 200-250 gm) were used in this study. They fed a standard laboratory chow and allowed to drink water. They were divided randomly into six groups (each contained five rats). Group 1, control: injected daily with 0.9% NaCl (1 ml) (i.p.) for 5 days. Group 2: Omega-3: administered omega-3 in diet (4gm/kg diet) daily for 6 days. Group 3 and 4: two doses of IFO (50 and 80mg/kg b.wt., respectively) administered intraperitoneally (i.p.). Group 4: IFO (80mg/kg b.wt.). Group 5: Omega-3 + IFO (50mg/kg b.wt.). Group 6: Omega-3 + IFO (80mg/kg b.wt.).

### 2.2. Anesthesia, dissection and removal of organs

All animals were anesthetized with ketamine hydrochloride (100mg/Kg b.wt.) and sacrificed, blood was collected then liver and kidneys were surgically removed, cut into small pieces (approximately 0.5cm in thickness) and put in fixative. The body weight of the rats in all groups was recorded twice; at the beginning and at the end of the experiment for calculating the body weight gain.

### 2.3. Histological preparations

#### 2.3.1. Light Microscopy (Paraffin Method)

Samples of the organs were directly fixed in Bouin's fluid for 24 hours and then processed for paraffin method by dehydrating through ascending concentrations of ethanol (50%, 70%, 95% and 100%), cleared in xylene, and embedded in paraffin. The 4µm thick sections were stained by haematoxylin and eosin (H&E) (Murice-Lambert *et al.*, 1989).

#### 2.3.2. Light Microscopy (Resin Method)

Tissue samples (<1mm<sup>3</sup>) were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2 - 7.4 for 24 hours, postfixed in 1% osmium tetroxide for 1 hr., dehydrated through a graded series of ethanol (50%, 70%, 95%, and 100%), cleared in propylene oxide and embedded in Araldite mixture. Plastic sections (0.5-1µm) were stained by 1% toluidine blue in 1% Borax for light microscopy.

### 2.4. Bone marrow smear preparation

Another separate six groups of male rats (5 rats in each group) were used for this preparation. Rat femur was subcutaneously injected with colchicine (1.0mg/kg) 2 hours before sacrifice (El-Habit *et al.*, 2000). Bone marrow cells from control and experimental animals were processed for analysis of chromosomal aberrations by the method of Sharma and Sharma (1994). The bone marrow from the femurs was flushed into a centrifuge tube containing 0.9% saline and centrifuged at 500g for 5 min. The supernatant was removed and hypotonic KCl was added to the sediment. After incubation for 20 min at 37°C, the contents were centrifuged for 5 min and the sediment was fixed in methanol-acetic acid (3:1v/v). Three changes of fixative were given prior to slide preparation. The slides were air-dried, stained in 5% Giemsa solution and scored blindly. Bone marrow cells were examined for mitotic figures by scoring the number of cells in mitosis per 1000 bone marrow cells, then the percentage of mitotic cells were obtained.

### 2.5. Blood collection

Blood samples were taken from the rats through cardiac puncture some into chilled tubes with ethylene diaminetetraacetic acid (EDTA) for hematological parameters and another into chilled tubes without EDTA for serum collection (biochemical test); later centrifuged at 3000 rpm for 15 minutes at 4°C, then the sera were stored at -55°C.

### 2.6. Biochemical analysis

#### 2.6.1. Determination of serum malondialdehyde (MDA):

The level of serum MDA was determined spectrophotometrically by thiobarbituric acid (TBA) solution. In brief: 150µl serum sample was added to the followings: 1ml trichloroacetic acid (TCA) 17.5%, 1ml of 0.66% TBA, then mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added and left to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and the supernatant was taken out for scanning spectrophotometrically (Weinstein *et al.*, 2000).

#### 2.6.2. Determination of serum creatinine

Colorimetric reaction of creatinine with alkaline picurate was measured kinetically at 490 nm (490-510) nm. The kit was obtained from BIOLABO SA, Maizy, France.

#### 2.6.3. Determination of serum phosphorous

In an acid medium, phosphate ions form a phosphomolybdic complex with the ammonium molybdate. The absorbance measured at 340 nm is proportional to the concentration of phosphate ions in the specimen. The kit was obtained from BIOLABO SA, Maizy, France.

#### 2.6.4. Determination of serum glucose

The absorbance was measured at 505 nm. The kit was obtained from Plasmatic laboratory products LTD.

### 2.6.5. Determination of Serum glutamate-pyruvate transaminase (GPT):

The GPT, also called alanine aminotransferase (ALT), was determined in serum and it relies on the following principle: the  $\alpha$ -Oxoglutarate reacts with L-Alanine in the presence of ALT to form L-Glutamate plus Pyruvate. The kit was obtained from BIOLABO SA, Maizy, France. The absorbance was measured at 505 nm.

### 2.6.6. Determination of Serum glutamate-oxaloacetate transaminase (GOT)

The GOT is also known as aspartate aminotransferase (AST). The determination of serum GOT is based on the principle that the  $\alpha$ -Oxoglutarate reacts with L-Aspartate in the presence of AST to form L-Glutamate plus Oxaloacetate. The kit was obtained from BIOLABO SA, Maizy, France. The absorbance measured at 505 nm.

### 2.7. Statistical analysis

All data were expressed as means  $\pm$  standard error of mean ( $M \pm SE$ ) and statistical analysis was carried out using statistically available software (SPSS version 11.5). One-way analysis of variance (ANOVA) was performed to test for significance followed by Duncan's multiple range comparison tests for comparisons between the groups. P values  $\leq 0.05$  and  $0.01$  were considered significant.

## 3. Results

As shown in Figure 1, both IFO doses caused a significant decrease in the body weight gain of rats when compared to the control group. On contrast, both IFO plus omega-3 treated groups showed a significant increase in the body weight gain of rats in comparison to the IFO treated rats. Hair loss has been seen in all the treated groups except the control.

Table 1 shows some serum biochemical results (MDA, creatinine, phosphorous, glucose, GOT, and GPT) after administration of both doses of IFO in combination with omega-3. Compared to the control group, both IFO treated groups caused a significant increase in the level of serum MDA, while these high levels of serum MDA decreased significantly in both IFO plus omega-3 treated groups when compared to the IFO treated groups. Both IFO doses caused a significant increase in the level of serum creatinine when compared to the control group, while when omega-3 was added to the diet of IFO treated rats, these levels of creatinine were significantly decreased when compared to the IFO treated groups. With respect to phosphate level, both doses of IFO caused significant increase when compared to the control group, while these levels of serum phosphorous significantly decreased after giving omega-3 to the IFO treated groups. A significant decrease in serum glucose level of both IFO treated groups were reported when compared to the control group, while both IFO plus omega-3 treated groups showed a significant increase in the level of serum glucose when compared to the IFO treated groups. The level of serum GOT was significantly increased in both IFO treated groups when

compared to the control group, while both IFO plus omega-3 treated groups showed significant decrease in serum GOT level when compared to the IFO treated groups. Only the higher dose of IFO treated group showed statistical increase in serum GPT level when compared to the control group, while the lower dose of IFO treated group showed non-significant increase in serum GPT when compared to the control group. Similarly, both IFO plus omega-3 treated groups showed non-significant decrease of serum GPT in comparison to the IFO treated groups.

As shown in Table 2, both IFO treated groups caused significant decrease in blood WBC, RBC, HGB, and PLT count when compared to the control group. On the other hand, both IFO plus omega-3 treated groups showed a significant increase in the level of these blood parameters (except RBC) when compared to the IFO treated groups.

As shown in Figure 2, all groups that have received IFO alone or in combination with omega-3 showed approximately the absence of mitotic division, while omega-3 alone didn't cause significant decrease of mitotic division in the bone marrow when compared with the control group.

The histological figures have shown normal structure of rat liver in control group (Figure 3 A and B). On the other hand, histological figures of both doses of IFO treated rats showed degeneration of hepatocytes, in which the higher dose of IFO (i.e. 80mg/kg) has showed higher degenerative effect (Figure 3 C-F). In very rare occasions, few mitotic figures have been seen in IFO treated groups. Other histological alterations due to IFO administration included infiltration with inflammatory leukocytes, congested blood vessels (Figure 3 C).

Both paraffin and plastic sections showed well-designated glomeruli and tubuli (PCT and DCT) in the kidney of control group (Figure 4 A and B). Medullary region of the control kidney also showed normal appearance of tubules. The IFO treated groups showed various histological alterations such as dilatation of the cortical tubule lumens (Figure 4 C). Other histological changes included the appearance of inflammatory areas in the cortex and medulla (Figure 4 D and E). Dilatation in the lumen of kidney tubules has been appeared in higher level in the IFO 80mg/kg treated rats (Figure 4 F and G) as well as necrosis, congestion of blood vessels with blood cells (Figure 4 G) and anisonucleosis of the nuclei of kidney tubular cells (i.e. the nuclei sizes were variable) (Figure 4 H).

In comparison to the IFO treated groups, the histological figures of the IFO plus omega-3 treated rats showed that the structure of the glomeruli and cortical tubuli (PCT and DCT) has been approximately normalized (Figure 5 A-E). Although the kidney structure in the IFO 80mg/kg plus omega-3 showed approximately normal structure in which no inflammatory cells were detected (Figure 5 E), some alterations were still exist such as degeneration of some kidney tubular cells and shrinkage of glomerular tuft (Figure 5 F).

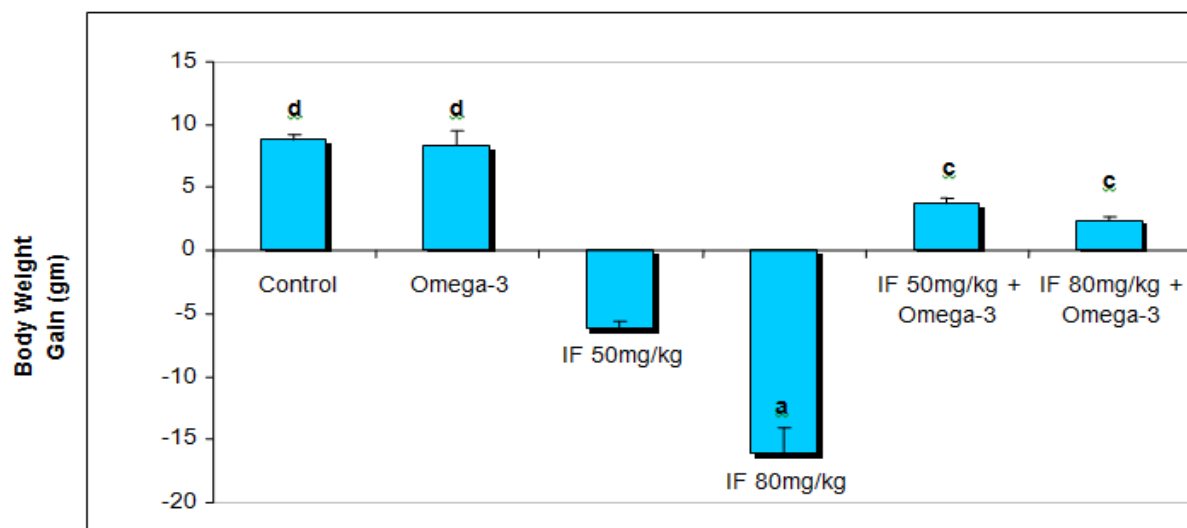


Figure 1. Effect of IFO and/or Omega-3 on body weight gain

**Table 1.** Effect of IFO and/or Omega-3 on Some Biochemical Parameters.

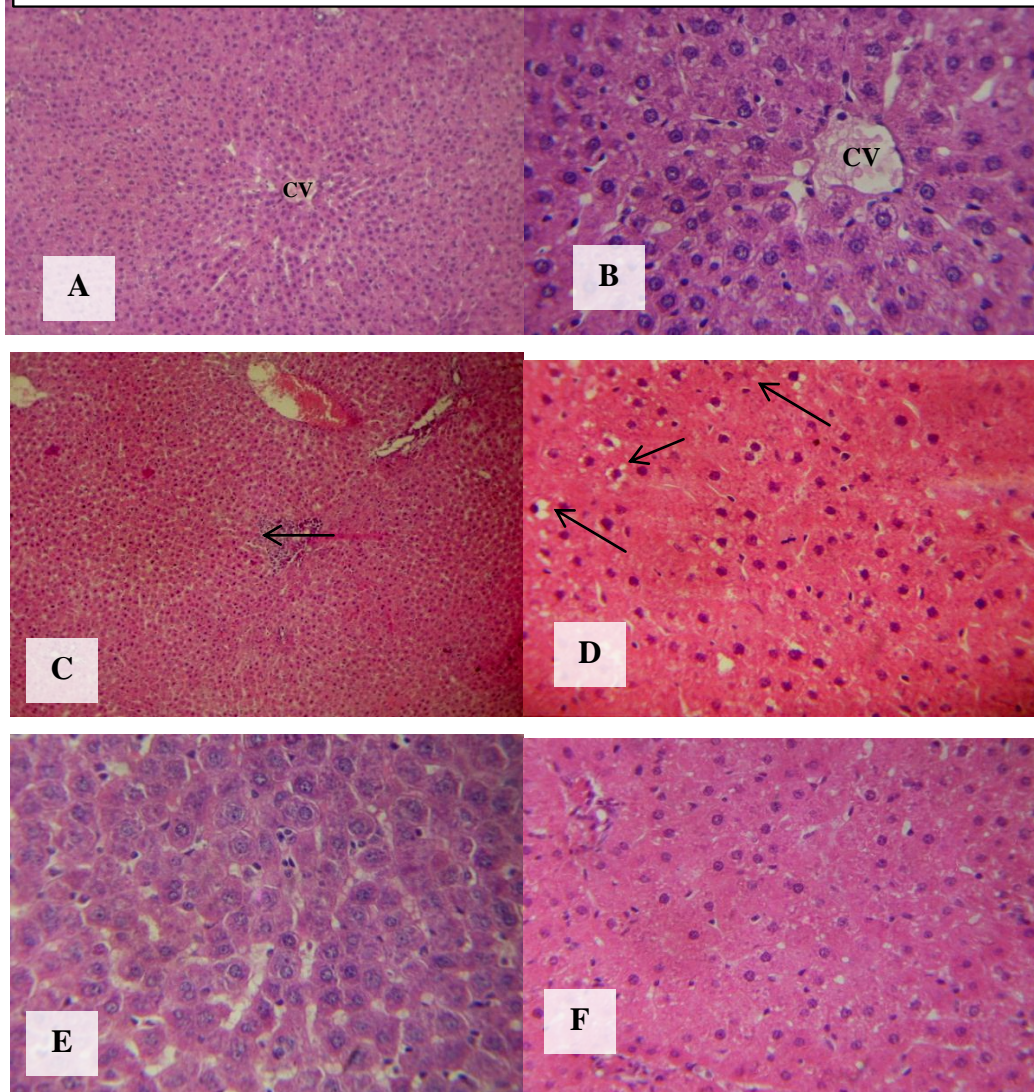
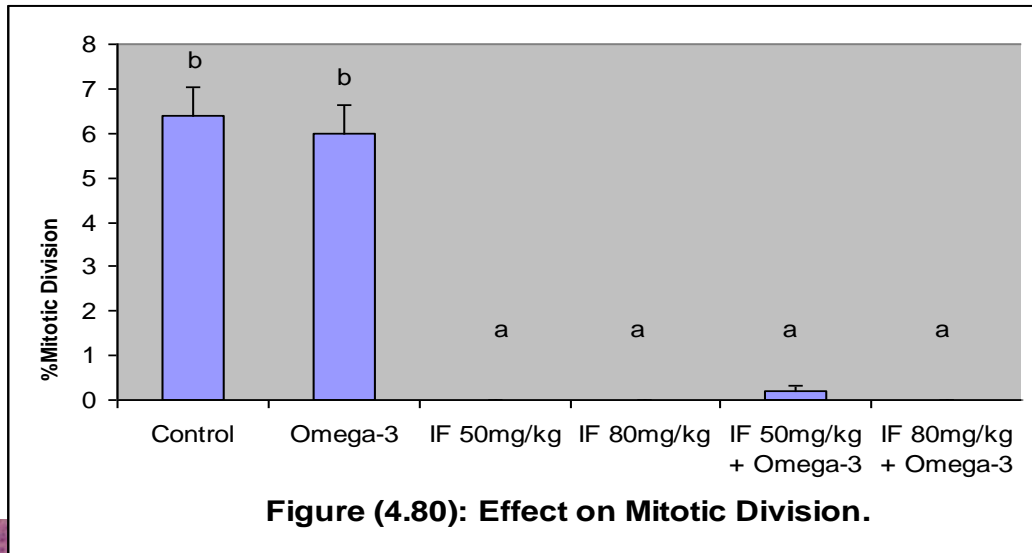
Groups	MDA µmol/L*	Creatinine Mg/dl**	Phosphor Mg/dl**	Glucose Mg/dl**	GOT IU/L*	GPTU/L**
Control	1.63±0.14 <sup>a</sup>	0.52±0.07 <sup>a</sup>	5.98±0.21 <sup>a</sup>	158.16±1.9 <sup>c</sup>	64.21±4.47 <sup>a</sup>	7.71±2.37 <sup>a</sup>
Omega-3	1.74±0.11 <sup>a</sup>	0.58±0.04 <sup>ab</sup>	7.8±0.16 <sup>b</sup>	152.83±0.89 <sup>c</sup>	77.01 ±7.93 <sup>ab</sup>	7.52±1.14 <sup>a</sup>
IF(50mg/kg)	2.23±0.22 <sup>bc</sup>	1.26±0.04 <sup>d</sup>	12.1±0.41 <sup>d</sup>	114.16±1.84 <sup>b</sup>	134.03±6.08 <sup>c</sup>	11.41±0.66 <sup>a</sup>
IF(80mg/kg)	2.54±0.1 <sup>c</sup>	1.52±0.06 <sup>c</sup>	12.88±0.16 <sup>d</sup>	98.36±1.48 <sup>a</sup>	185.08±14.78 <sup>d</sup>	20.39±1.02 <sup>b</sup>
IF(50mg/kg)+Omega-3	1.84±0.13 <sup>ab</sup>	0.76±0.02 <sup>bc</sup>	9.88±0.52 <sup>c</sup>	141.96±0.59 <sup>d</sup>	67.36±1.39 <sup>a</sup>	7.22±1.05 <sup>a</sup>
IF(80mg/kg)+Omega-3	1.93±0.05 <sup>ab</sup>	0.84±0.07 <sup>c</sup>	10.32±0.08 <sup>c</sup>	130.7±0.51 <sup>c</sup>	112.63±11.86 <sup>bc</sup>	17.26±1.14 <sup>b</sup>

The values represented by mean ± SE, N=5, Duncan's test used to compare between groups. Different letters in the same column refer to significant changes, while similar letters refer to non-significant changes. \*\* (p<0.01) and \* (p<0.05).

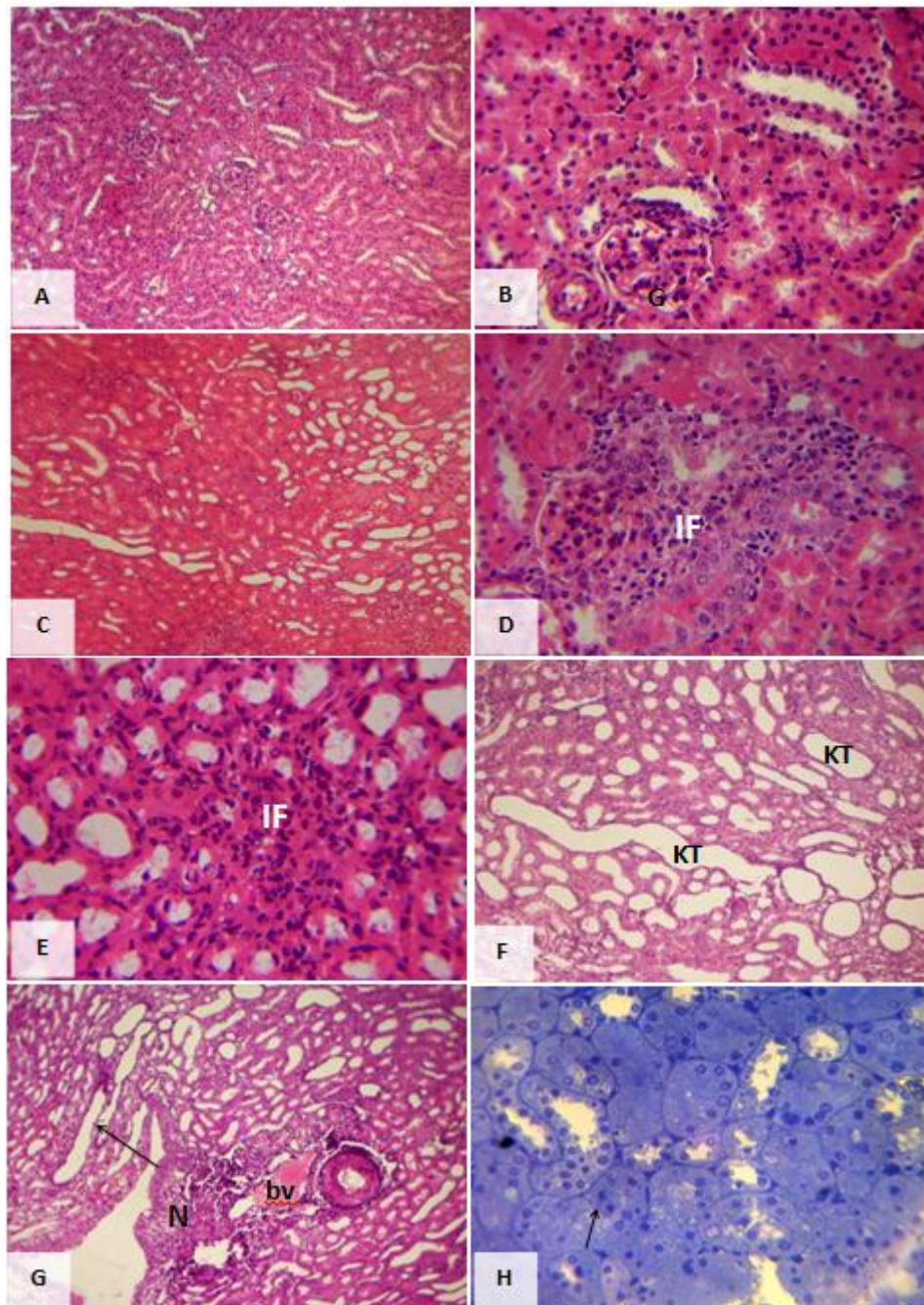
**Table 2.** Effect of IFO and/or Omega-3 on Some Hematological Parameters.

Groups	WBC**	RBC*	HGB*	PLT**
Control	6.52 ± 0.49 <sup>c</sup>	7.52 ± 0.17 <sup>b</sup>	14.2 ± 0.28 <sup>c</sup>	508.66 ± 21.36 <sup>b</sup>
Omega-3	5.86 ± 0.6 <sup>c</sup>	7.38 ± 0.22 <sup>b</sup>	13.76 ± 0.31 <sup>c</sup>	489.33 ± 31.13 <sup>b</sup>
IF(50mg/kg)	0.63 ± 0.08 <sup>a</sup>	5.87 ± 0.17 <sup>a</sup>	10.2 ± 0.35 <sup>a</sup>	361 ± 27.75 <sup>a</sup>
IF(80mg/kg)	0.52 ± 0.02 <sup>a</sup>	5.65 ± 0.13 <sup>a</sup>	9.95 ± 0.12 <sup>a</sup>	345 ± 21.33 <sup>a</sup>
IF(50mg/kg) + Omega-3	4.43 ± 1.53 <sup>b</sup>	6.45 ± 0.29 <sup>a</sup>	11.76 ± 0.76 <sup>b</sup>	573 ± 19.73 <sup>b</sup>
IF(80mg/kg) + Omega-3	3.8 ± 0.9 <sup>b</sup>	6.13 ± 0.17 <sup>a</sup>	10.9 ± 0.46 <sup>b</sup>	432 ± 11.07 <sup>b</sup>

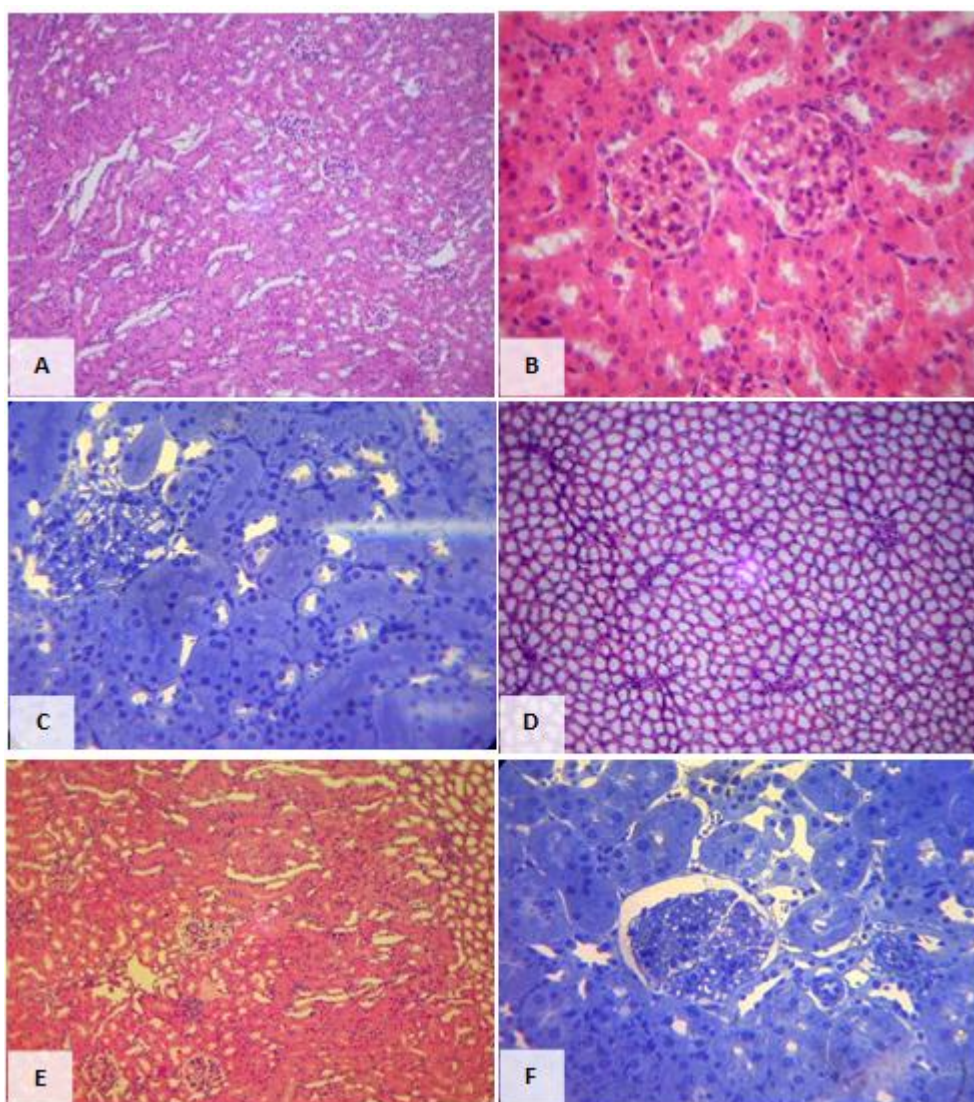
The values represented by mean ± SE, N=5, Duncan's test used to compare between groups. Different letters in the same column refer to significant changes, while similar letters refer to non-significant changes. \*\* (p<0.01) and \* (p<0.05).



**Figure 3.** Paraffin sections through the liver of rats: A) control, 100X, B) control,400X, (CV):central vein, C):group3,IFO(50mg/kg.b.wt) showing inflammatory leucocyte infiltration (arrow),100X, D) Group 4, IFO(80mg/kg.b.wt) showing degenerated hepatocytes with vacuolated cytoplasm and condensed nuclei(arrows),400X,E)Group 5, IFO(50mg/kg.b.wt) plus omega-3 showing well protected liver structure,400X, F) Group6: (80mg/kg.b.wt) plus omega-3 showing approximately protected liver structure, although some unhealthy hepatocytes still exist,400X.



**Figure 4.** Sections through the kidney of rats: A) & B) Control group showing the normal structure of kidney in the cortical region, 100X & 400X respectively ,C) Group3: IFO(50mg/kg.b.wt) showing dilatation of kidney tubules,100X,D) & E) Group3: IFO(50mg/kg.b.wt) large area of inflammatory cells(IF) in the cortex and medullary regions respectively,400X, F) Group 4: IFO(80mg/kg.b.wt) showing highly dilated kidney tubules(KT),100X, G) same later group with dilatation of kidney tubules(arrow),necrosis of the kidney cells(N) and congestion of blood vessels(bv),100X, H) Plastic section of the kidney tubular epithelial cells in the later group showing high number of dead cells(arrow),notice the nuclei of kidney tubular cells showing anisonucleosis,400X.



**Figure 5:** Sections through the kidney of rats treated with IFO plus omega-3 oil: A) & B) IFO (50mg/kg.b.wt) plus omega-3 showing approximately normal structure of the cortical region especially the glomeruli, 100X and 400X respectively, C) plastic section of the kidney in the later group showing approximately healthy kidney tubular epithelial cells, 400X, D) Normal cortical structure in the kidney of the later group, 100X, E) Group 6, (80mg/kg.b.wt) plus omega-3 showing approximately normal structure with no inflammation or dilation of kidney tubule lumen, 100X, F) same later group showing slight alteration in the structure of the kidney compared to control, notice the degeneration of some kidney tubule cells and slightly shrunken glomerular tuft, 400X.

#### 4. Discussion

The significant dose dependent decrease in body weight due to ifosfamide (IF) treatment, which is found in the present work and also recorded by other investigators (Springate and Van Liew, 1995) may be related to the effect of exposure to this drug on the appetite of the rats and also wastage in muscle mass as a result of the induced physiological changes (Chen *et al.*, 2008). As it will be declared later, degeneration of the liver and kidney which caused by IFO treatment may another reason for this decrease in the body weight gain.

As revealed by the current investigation, both doses of IFO (50 and 80 mg/kg.b.wt.) have caused Fanconi syndrome which included the higher levels of serum creatinine and phosphorous and the lower level of glucose in the IFO treated groups compared with the control

(Skinner *et al.*, 1993; Loebstein and Koren, 1998; Rossi *et al.*, 1999; Skinner, 2003; Şehirli *et al.*, 2007; Chen *et al.*, 2008). The degeneration of kidney tubule cells due to IFO action may be the reason for this renal dysfunction (Chen *et al.*, 2008).

The levels of serum MDA in the IFO treated rats were significantly higher than those of the control group. This elevation in MDA (thereby elevation in lipid peroxide level) indicates the presence of oxidative tissue damage as a result of impaired antioxidant defense mechanism (Şehirli *et al.*, 2007) and this oxidative stress may cause the death of cells in general (Ramaekers *et al.*, 1997).

Elevation of the two liver enzymes GOT and GPT in the serum of IFO treated rats refers to the hepatic cellular injury (Green and Flamm, 2002; Clark *et al.*, 2003) because damaged liver cells develop leaky membranes, allowing for escape of intracellular enzymes (including GOT, GPT and other enzymes) into the bloodstream and

this will raise the levels of these enzymes in the serum (Amacher, 1998).

The degeneration of hepatocytes which has been detected in the IFO treated rats may be related to the toxic metabolites of IFO, especially chloroacetaldehyde (CAA), which may induce cell death through the depletion of hepatocellular GSH, ATP and enhanced lipid peroxidation rate (Sood and O'Brien, 1994), although the pathophysiology of this toxicity is not fully understood (Knouzy *et al.*, 2010). It has been found that CAA collapsed the mitochondrial membrane, induced the release of cytochrome C from mitochondria to the cytosol and significantly reduced cellular ATP level that triggers cell death. The mechanism of such cell death follows the apoptotic cell death (Takahashi *et al.*, 2007), although necrotic mechanism of cell death has also been mentioned by other workers (Daniel *et al.*, 1992).

Similarly, the death of PCT cells in the IFO treated rats are mainly due to the toxic effects of IFO metabolites, acroline and CAA, and not by IFO itself (Schwerdt *et al.*, 2005) through a possible mechanism which is the depletion of reduced GSH and ATP (Nissim *et al.*, 2006; Brüggemann *et al.*, 2006; Dubourg *et al.*, 2001), because the depletion of tissue GSH is one of the primary factors that permit lipid peroxidation to occur in cell membranes, devastating the functional integrity of the cellular structure, and if the damage is severe, cell death is inevitable (Şehirli *et al.*, 2007). Elevation of MDA level reported by the present work confirms the above mechanism.

In accordance with the present results, some further histological changes in IFO treated rats which include inflammation in the renal tissue and degeneration of proximal tubules with desquamated epithelium was also observed by other investigators (Şehirli *et al.*, 2007; Chen *et al.*, 2008). This inflammation which has been seen in the liver and kidney is due to the necrotic mechanism caused by IFO (Schwerdt *et al.*, 2007; Chen *et al.*, 2008).

On the other hand, Omega-3 FAs through their antioxidant properties reduced the toxicity of IFO metabolites especially CAA. This was also detected in previous works that the toxicity of IFO lowered by using certain antioxidants (Nissim and Weinberg, 1996; Badary, 1998, 1999a, 1999b; Şehirli *et al.*, 2007; Chen *et al.*, 2008; Casado-Zapico *et al.*, 2010). The antioxidant and/ or anti-inflammatory effects of omega-3 FA through scavenging of free radicals and inhibiting lipid peroxidation have been reported previously (Ernest and Magdalena, 2008). This oxidant/antioxidant theory may explain the protection role of Omega-3 FAs against the hepatotoxicity and nephrotoxicity of IFO. The anti-inflammatory property of omega-3 fatty acids (Omega-3 FAs) is due to the action of eicosapentaenoic acid, which is one of the components of omega-3. The anti-inflammatory action is achieved by reducing the pro-inflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF $\alpha$ ) (Wardle, 2000) and it is effects on transcription factors that regulate inflammatory gene expression (e.g. Nuclear Factor Kappa B) (Calder, 2009).

The most peculiar result in the present work was the normal existence of mitotic division in the bone marrow of omega-3 group and the absence of mitotic division in the IFO plus omega-3 group. The antimitotic effect of Omega-

3 oil has been mentioned previously (Hardman 2002). If we consider this and the anticancer activity of this oil (Simopoulos, 2003) and its role in increasing the differentiation of the myeloid progenitor cells in the bone marrow without having an adverse effect on peripheral white blood cells counts (Varney *et al.*, 2009) beside the findings of the current work which included the protective role of this oil against the side effects of IFO (i.e. hepatotoxicity, nephrotoxicity, changes in hematological parameters and the oxidative stress), a unique protective agent against IFO toxicity can be suggested in comparison to the previously studied antioxidants.

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