# Protective Effects of Omega-3 Supplement Against Doxorubicin-Induced Renal Injury in Male Rats: Histopathological Study

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**Abstract:**

***Background*:** Doxorubicin (DOX) is an effective chemotherapeutic agent but possesses a significant risk of inducing acute kidney injury (AKI). Unfortunately, it severely affects clinical outcomes and patient quality of life. Omega-3 is widely used as supplement due to its anti-oxidant, anti-inflammatory and anti-apoptotic properties.

***Objective*:** This study investigates the potential protective effects of Omega-3 fatty acids against DOX-induced renal injury in male rats. Using a controlled experimental design and evaluate the histopathological changes.

***Materials & Methods*:** Forty male rats were randomized into 5 equal groups each consisting of 8 animals as follows, Group I (control group), which received normal saline orally for 4 weeks followed by a single injection of NS intraperitoneally (IP), Group II (induction group), which received NS orally for 4 weeks followed by a single dose of DOX 15mg/ kg IP, Groups III, IV, V, which received oral doses of Omega-3 (100mg/ kg, 200mg/ kg, 400mg/ kg, respectively) for 4 weeks followed by a single dose of DOX 15mg/ kg IP. kidney from each rat were extracted and fixed in 10% neutral buffered formalin for histological examination using H&E stain.

***Results*:** Omega-3 supplementation significantly preserved renal histological integrity. Group IV and V showed approximately normal renal tubules with normal glomeruli & mild vascular congestion and the normal collecting tubule & thick segment of loop of Henle. While severe vascular degeneration of the lining cells of the renal tubules with necrosis and marked tubular dilation of collecting tubules in DOX group compared to control group.

***Conclusion:*** Omega-3 nephroprotective effects against DOX-induced toxicity reveals its multifaceted role in enhancing preserving histological integrity of renal tissues

**Kerwords:** Omega-3, nephroprotection, DOX, Histopathological changes**.**

# التأثير الوقائي للمكمل الغذائي أوميغا-3 ضد إصابة الكلى المستحثة بواسطة دوكسوروبيسين في الجرذان الذكور: دراسة نسيجية مرضية

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**خلاصة**

**الخلفية:** يعتبر دوكسوروبيسين (DOX) عامل كيميائي فعال ولكنه يشكل خطرًا كبيرًا في التسبب في إصابة الكلى الحادة (AKI)، مما يؤثر بشكل كبير على النتائج السريرية ونوعية حياة المرضى. يستخدم أوميغا-3 على نطاق واسع كمكمل غذائي بسبب خصائصه المضادة للأكسدة والمضادة للالتهابات والمضادة للاستماتة (الموت المبرمج للخلايا).

**الهدف:** تستهدف هذه الدراسة التحقيق في التأثيرات الوقائية المحتملة للأحماض الدهنية أوميغا-3 ضد إصابة الكلى الناتجة عن DOXفي الجرذان الذكور. يتم استخدام تصميم تجريبي محكم وتقييم التغيرات النسيجية المرضية.

**المواد والطريقة:** تم تقسيم الجرذان عشوائيًا إلى 5 مجموعات متساوية، كل مجموعة تتكون من 8 حيوانات كما يلي:

- المجموعة الأولى (مجموعة التحكم): تلقت محلول ملحي عادي عن طريق الفم لمدة 4 أسابيع تلتها حقنة واحدة من المحلول الملحي داخل الصفاق.(IP)
- المجموعة الثانية (مجموعة التحريض): تلقت محلول ملحي عادي عن طريق الفم لمدة 4 أسابيع تلتها جرعة واحدة من DOX 15ملغ/ كغ داخل الصفاق.
- المجموعات الثالثة والرابعة والخامسة: تلقت جرعات فموية من أوميغا-3 (100 ملغ/ كغ، 200 ملغ/ كغ، 400 ملغ/ كغ على التوالي) لمدة 4 أسابيع تلتها جرعة واحدة من DOX 15 ملغ/ كغ داخل الصفاق.

تم استخراج الكلى من كل جرذ وتثبيتها في محلول الفورمالين المعادل بنسبة 10% للفحص النسيجي باستخدام صبغة.H&E

**النتائج:** أظهرت النتائج أن مكملات أوميغا-3 حافظت بشكل كبير على سلامة النسيج الكلوي. أظهرت المجموعات الرابعة والخامسة أنيبيبات كلوية طبيعية تقريبًا مع كبيبات طبيعية واحتقان وعائي خفيف، وأنيبيبات تجميعية طبيعية والجزء السميك من حلقة هنلي. بينما أظهرت مجموعة DOX تدهورًا وعائيًا شديدًا في الخلايا المبطنة للأنيبيبات الكلوية مع نخر وتوسع أنبوبي ملحوظ للأنيبيبات التجميعية مقارنة بمجموعة التحكم.

**الاستنتاج:** توضح التأثيرات الوقائية لأوميغا-3 ضد سمية DOX دورها المتعدد الأوجه في تعزيز وحفظ سلامة النسيج الكلوي.

**الكلمات المفتاحية:** اوميغا-3 , دوكسوروبيسين , الوقاية الكلوية , التغييرات النسيجية المرضية

## Introduction

Acute kidney injury (AKI) is a significant global health issue that impacts a considerable number of individuals, with an estimated annual incidence of over 13 million cases. This condition is associated with substantial rates of morbidity and mortality [1]. The prevalence of AKI is elevated in underdeveloped nations. Many evidence based-researches have indicated that a range of 14-26% of reported cases of AKI can be attributed to drug-induced causes [2]. The primary factors contributing to the development of AKI pathogenesis are hypoxia, ischemia, nephrotoxicity and decrease in renal blood flow [3,4]. Inflammation represents a significant etiological factor contributing to the development of AKI in addition to damage of epithelial and endothelial cells [5]. Moreover, it can manifest in various compartments of the renal system, including the renal vasculature, glomerulus, tubule interstitium, and collecting ducts[6].

Doxorubicin (DOX) is a highly effective antineoplastic drug that is commonly administered either on its own or in conjunction with other medications, it is

exhibiting a broad range of therapeutic effects. In addition, DOX is utilized for the treatment of various types of solid tumors and hematological malignancies [7]. DOX, like other genotoxic chemicals, induces the activation of p53-DNA binding, which is facilitated by the activation of nuclear factor-kB (NF-kB)[8]. Nuclear Factor kappa B (NF-kB) is a heterodimeric transcription factor that controls the expression of genes involved in the stress response, including inflammation, oxidative damage, and apoptosis. It is suggested that DOX penetrates mitochondria, resulting in the generation of ROS. These ROS induce damage to mtDNA, leading to mitochondrial malfunction and ultimately contributing to the rapid progression of nephron destruction [9]. The precise mechanisms through which DOX causes nephrotoxicity are not completely comprehended. However, several studies propose that this toxicity is primarily facilitated by the creation of an iron-anthracycline complex. This complex then produces free radicals, which subsequently lead to oxidative damage on vital cellular elements [10].

Doxorubicin-induced kidney toxicity leads to significant histopathological changes including glomerular damage resulting in scarring and reduced kidney function [11]. Tubular injury is also found featuring tubular dilatation, vacuolation, loss of brush borders, and tubular necrosis, indicating severe cell damage. Another aspect is interstitial and vascular changes including inflammation, fibrosis, vascular congestion, and endothelial damage which are common and affect kidney structure and function [12].

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), known as omega 3 fatty acids, are present in various parts of the body, such as cell membranes. They have significant functions in cell signaling, as well as in anti-inflammatory and anti-oxidant processes and serve as substances that can be employed to produce both inflammatory and anti-inflammatory eicosanoids, such as prostaglandins and leukotrienes. As a result, they have been utilized to avoid various inflammatory conditions[13]. Moreover, they serve as precursors of highly active metabolites that provide a diverse range of therapeutic benefits for preventing and/ or treating various illnesses. Primarily, cardiovascular diseases[14] as well as a range of medical conditions, including cancer, therefore they are considered immunonutrients [15–16].

Omega-3 fatty acids have been studied for their potential benefits in renal diseases, showing promise in areas such as reducing proteinuria in chronic glomerular diseases, treating immunoglobulin A nephropathy, and preventing cyclosporine-induced nephrotoxicity. These benefits are attributed to Omega-3 capacity to increase renal vasodilatory capacity, reduce proinflammatory leukotrienes production, lower the transcapillary escape rate of albumin, and limit cyclosporine-related nephrotoxic effects[17,18]. Recently, one study stated that Omega-3 has potential protective agent against cardiotoxicity induced by Doxorubicin.

### Aim of the study

The presented study is designed to investigate the possible protective effect of Omega-3 against Doxorubicin-induced renal injury in male rats by Histopathological examination of kidney tissue changes using Hematoxylin & Eosin (H&E) stain.

## Materials &Methods

### Animals

Fifty-two adults male Wistar albino rats were obtained from the animal house department at the Iraqi Center for Cancer and Medical Genetics Research, aged 16 – 18 weeks weighing (240±10) g. They were properly

housed in suitable cages in a well-ventilated area within the animal house with optimum conditions and free access to food and water. They were left to be acclimatized for one week before the study started. Twelve rats were used in a pilot study to determine DOX dose for renal toxicity induction, while the other forty rats were randomized into five groups for the main study. Omega-3 doses used in this study were 100 mg/ kg, 200 mg/ kg and 400 mg/ kg. These doses were selected based on similar previous study[20]. Omega-3 oil was drained from the soft gel capsules and collected in a glass container to be ready to use for the study. Each capsule contains 1.5 ml of 882 mg of omega-3 (EPA+DHA) so 1 ml would be equivalent to 588 mg of omega-3.

**Animal groups for the main study**

The animals in the main study were randomly divided into five groups (N=8), as follows:

**Group I (Control group):** received normal saline through oral gavage for 28 days then received a single intraperitoneal dose of normal saline on the 29$^{th}$ day.

**Group II (Induction group):** received normal saline through oral gavage for 28 days then received a single dose of 15mg/kg DOX intraperitoneally on the 29$^{th}$ day.

**Group III (100mg/kg of Omega-3):** received 100mg/kg Omega-3 through oral gavage for 28 days then received a single dose of 15mg/kg DOX intraperitoneally on the 29$^{th}$ day.

**Group IV (200mg/kg of Omega-3):** received 200mg/kg Omega-3 through oral gavage for 28 days then received a single dose of 15mg/kg DOX intraperitoneally on the 29$^{th}$ day.

**Group V (400mg/kg of Omega-3):** received 400mg/kg Omega-3 through oral gavage for 28 days then received a single dose of 15mg/kg DOX intraperitoneally on the 29$^{th}$ day.

**Samples collection**

On day thirty-one of the study (after 48 hours from DOX injection, all rats were sacrificed and dissected and one kidney from each rat was harvested and stored in specimen containers with neutral buffered formalin solution 10% that was prepared from formalin and phosphate buffered saline solution[21].

**Histopathological study**

The following steps of the histopathological study were conducted as follow[20,21]:

A. **Fixation:** kidneys from rats were stored in neutral buffered formalin (NBF) which was prepared in 10% concentration. The kidneys were fixated in labeled cups containing 10% NBF in order to stop the degenerative enzymes.
B. **Dehydration**: In order to eliminate water from the renal tissue, the samples were subjected to incubation in jars containing alcohol solutions of several concentrations (50%, 70%, 95%, and 100%) for approximately two hours.
C. **Clearing:** The samples were immersed in two jars filled with xylene for approximately one hour in each to remove any residual alcohol and lipids present in the tissue.
D. **Infiltration:** samples were placed in embedding center and immersed in molten paraffin wax at 55°C resulting in the production of a kidney wax blocks. The blocks were appropriately labeled and stored at a temperature of -20°C in a freezer overnight to have them solidified and ready for sectioning.

E. **Sectioning:** wax blocks were cut by rotary microtome; sectioning thickness was set at 3µm then the trimmed section was held by forceps and placed on ordinary slides for H&E staining or on positively charged slides for IHC staining then the corresponding slides were placed in water at 45°C.
F. **Dewaxing:** the slides were placed in a slide basket and put into an oven at 65°C for a duration of 30 minutes to eliminate the wax content from the slides and facilitate the subsequent rehydration.
G. **Rehydration:** the slides were immersed in pre-warmed xylene jar at 55°C for five minutes. Then, the slides were transferred to xylene jar at room temperature for an additional five minutes. Following this, the slides were put in ethanol jars in a descending sequence starting from highest to lowest concentration (100%, 95%, 70%, and 50%) for two minutes in each jar. After that the slides were rinsed with water and were ready for staining.
H. **Hematoxylin and Eosin staining**: slides were stained with hematoxylin dye for 3 minutes and rinsed with water until the tissue color turned blue and then the slides were stained with eosin for 1 minute and rinsed with water and then dipped several times (5 dips) in alcohol jars in an ascending sequence according to concentration (50%, 70%, 95%, and 100%) and left to dry. After that the slides were covered and DPX mountant was used to fix them. The slides were left overnight to be ready for examination under light microscope by a specialized histopathologist.

Histopathologic score was achieved depending on 4 score to assess the damage happened in renal tissue depending on histopathological criteria including congestion, degeneration, cast, inflammation and fibrosis [22], as follow: 0 (no damage), 1 (mild), 2 (moderate), 3 (severe) and 4 (very severe). Kidney tissue samples from all groups were examined under light microscope with 100x and 400x magnification power using H&E stains by a specialized histopathologist Dr. Ban Abbas.

### Statistical analysis

All results were expressed as frequency and percentage. Chi-square test was used to compare between percentages. To compare the results among groups, P-value $\leq 0.05$ indicates significant difference, P-value $\leq 0.01$ indicates highly significant difference.

## Results

### Histopathological changes of kidney in rats received DOX and pretreated omega-3 groups using Hematoxylin and Eosin stain

Histopathological features are shown below for the groups:

A. **Control group (I):**
The histopathological examination of kidney section from the control group revealed approximately normal appearance and cytoarchitecture. Renal tubules and glomeruli were apparently normal, as shown in Figure 1 (A - C).
B. **Induction (DOX) group (II):**
Histopathological figures of the renal cortex and medulla showed severe vascular degeneration of the lining cells of the renal tubules with necrosis and marked tubular dilation of collecting tubules. In addition, the figures of the renal cortex showed marked dilation of Bowman spaces with hyper cellularity of glomerular mesangial cells as shown in Figure1 (D - F).
C. **OMG 100mg + DOX group (III):**
Histopathological figures of the renal cortex and medulla showed mild vascular degeneration of collecting

tubules without signs of necrosis and mild tubular dilation, the glomeruli had an approximately normal appearance, Figures 2 (A-C).

**D. OMG 200mg + DOX group (IV):** Regarding to the Figures 8 to 10, the renal cortex and medulla were apparently similar to those of control group. Slides showed approximately normal appearances of glomeruli and tubules with mild congestion, Figure 2 (D-F).

**E. OMG 400mg + DOX group V:** Histopathological figures of the renal cortex and medulla were apparently similar to those of control group and showed approximately normal appearances of their components, Figure 2 (G-I).

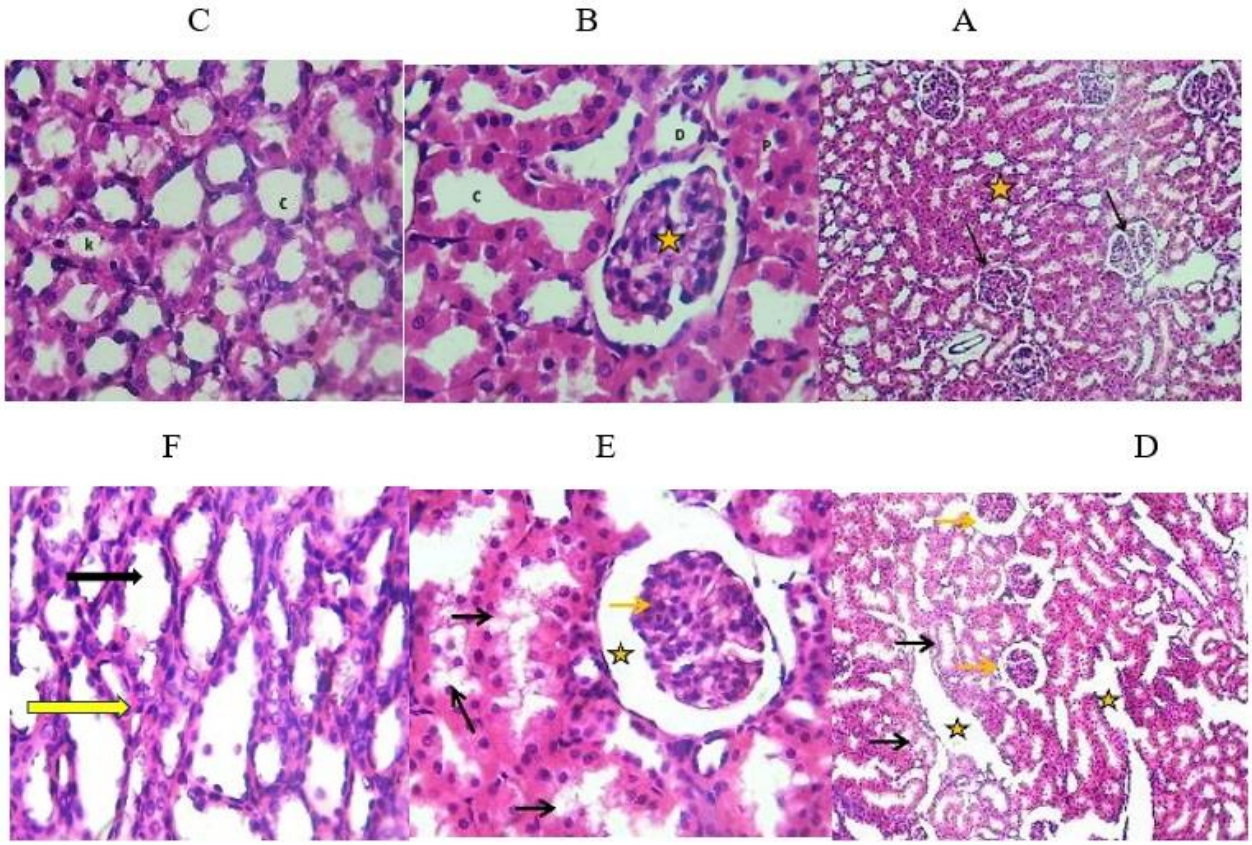


**Figure 1: A: Section of kidney (Group I) shows apparently normal renal tubules (asterisk) & glomeruli (black arrows), H&E stain 100x. B: Section of renal cortex (Group I) shows apparently normal collecting tubules (C), proximal (D), distal tubules (P) & glomerulus (asterisk), H&E stain 400x. C: Section of renal medulla (Group I) shows apparently normal collecting tubule (C) & thick segment of loop of Henle (K). H&E stain 400x.D: Section of kidney (Group II) shows severe vascular degeneration of renal tubules (black arrows) with marked tubular dilation of collecting tubules (asterisks), marked dilation of bowman spaces with hypercellularity of glomerular mesangial cells (yellow arrows), H&E stain 100x. E: Section of renal cortex (Group II) shows severe vascular degeneration of renal tubules (black arrows), marked dilation of bowman spaces (asterisk) & hypercellularity of glomerular mesangial cells (yellow arrows), H&E stain 400x.F:Section of renal medulla (Group II) shows mild vascular degeneration of renal tubules (yellow arrows), marked dilation of collecting tubules (black arrows), H&E stain 400x.**

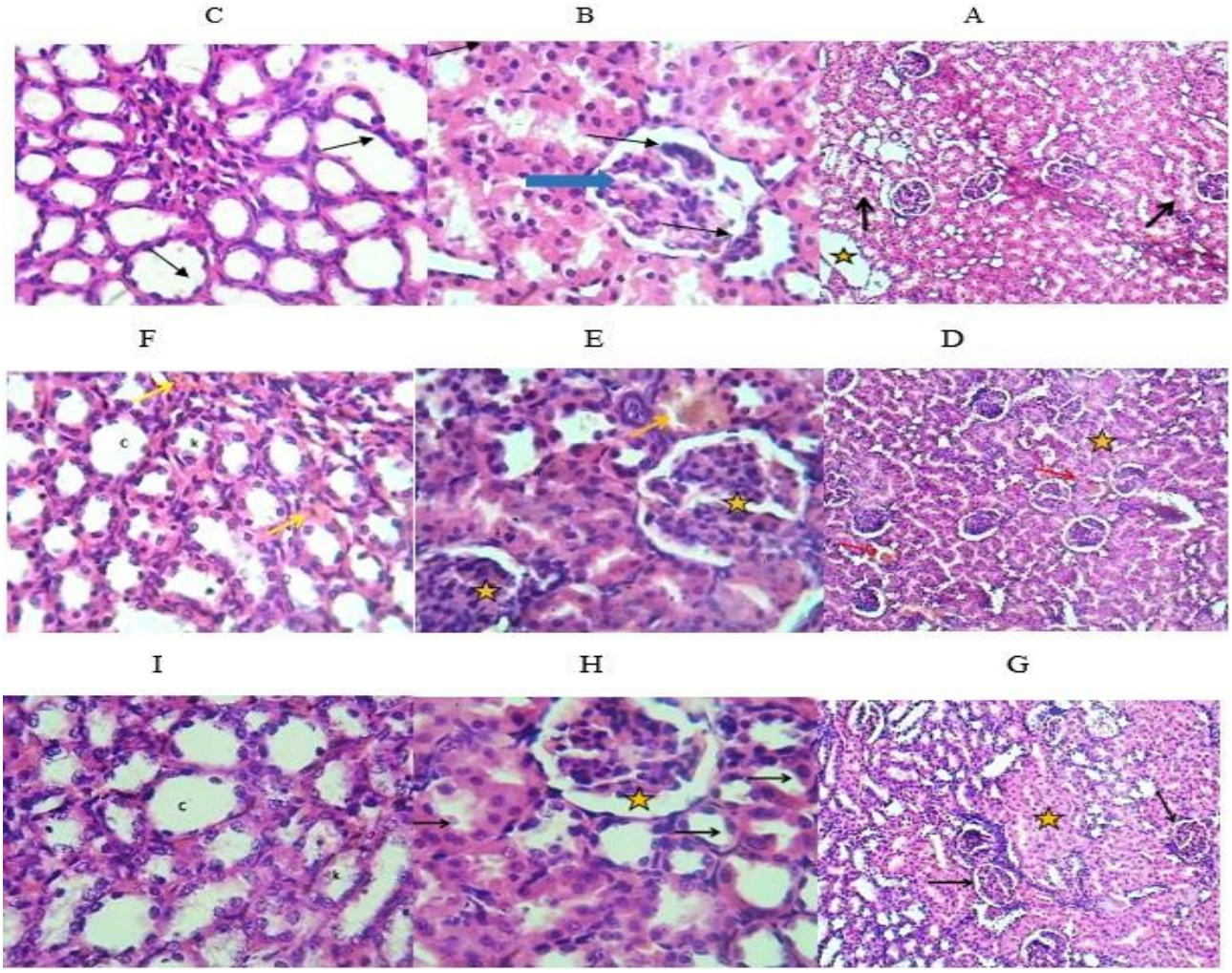


**Figure 2: A: Section of kidney (Group III) shows mild vascular degeneration of renal tubules (black arrows) with mild tubular dilation of collecting tubules (asterisk), & approximately normal glomeruli (blue arrows), H&E stain 100x. B: Section of renal cortex (Group III) shows mild vascular degeneration of lining cells of renal tubules without necrosis (black arrows) with approximately normal of glomerular cells (blue arrow), H&E stain 400x. C: Section of renal medulla (Group III) shows approximately normal lining cells of renal tubules, H&E stain 400x. D: Section of kidney (Group IV) shows approximately normal renal tubules (asterisk) mild intratubular vascular congestion (red arrows) & approximately glomeruli (black arrows), H&E stain 100x. E: Section of renal cortex (Group IV) shows approximately normal renal tubules with approximately normal glomeruli (asterisks) & mild vascular congestion (black arrow), H&E stain 400x. F: Section of renal medulla (Group IV) shows approximately normal collecting tubule (C) & thick segment of loop of Henle (K) with mild vascular congestion (yellow arrows), H&E stain 400x. G: Section of kidney (Group V) shows approximately normal renal tubules (asterisk) & glomeruli (black arrows), H&E stain 100x. H: Section of renal cortex (Group V) shows approximately normal renal tubules (black arrows) & glomerulus (asterisk), H&E stain 400x. I: Section of renal medulla (Group V) shows approximately normal collecting tubule (C) & thick segment of loop of Henle (K), H&E stain 400x.**

**Histopathological score evaluation:**
Histopathologic score was achieved depending on 4 score to assess the damage happened in renal tissue depending on histopathological criteria including congestion, degeneration, cast, inflammation and fibrosis (shown in **table 1)** [22], as follow: 0 (no damage), 1 (mild), 2 (moderate), 3 (severe) and 4 (very severe).

**Group I (Control Group):**

- Congestion: minimal or absent.
- Degeneration: minimal or absent.
- Cast: Higher frequency of low scores (score 0 frequency: 75%)
- Inflammation: Higher frequency of score 1: 75%
- Fibrosis: Almost absent, statistically non-significant.

**Group II (DOX-group)**

- Congestion: Higher scores were more frequent (score 3: 50%, score 4: 25%)
- Degeneration: Higher scores were more frequent (score 3: 62.5%, score 4: 25%)
- Cast: Higher scores observed but not statistically significant (score 3: 25%, score 2: 37.5%)
- Inflammation: Moderate scores more frequent (score 2: 50%, score 3: 37.5%)
- Fibrosis: Almost absent, statistically non-significant.

**Group III (Omega-3 100 mg)**

- Congestion: Higher scores were more frequent (score 3: 50%)
- Degeneration: Higher scores observed (score 3: 25%)
- Cast: Higher frequency of score 1: 50%
- Inflammation: Moderate scores more frequent (score 2: 37.5%, score 3: 37.5%)
- Fibrosis: Almost absent, statistically non-significant.

**Group IV (Omega-3 200 mg)**

- Congestion: minimal or absent.
- Degeneration: minimal or absent.
- Cast: Higher frequency of score 1: 87.5%
- Inflammation: Higher frequency of score 1: 75%
- Fibrosis: Almost absent, statistically non-significant.

**Group V (Omega-3 400 mg)**

- Congestion: minimal or absent (similar to Group I).
- Degeneration: minimal or absent (similar to Group I).
- Cast: Higher frequency of low scores (score 0: 25%, score 1: 62.5%)
- Inflammation: Higher frequency of score 1: 75%
- Fibrosis: Almost absent, statistically non-significant.

**Table 1: Frequencies & percentages of histopathology scores for all the study groups**

**Discussion**

| | Score | Group I | Group II | Group III | Group IV | Group V | Chi-square | P-value |
|---|---|---|---|---|---|---|---|---|
| Congestion | 0 | 3 | 0 | 0 | 0 | 2 | 1.28 | 0.0078 ** |
| | | 37.5% | 0.0% | 0.0% | 0.0% | 25.0% | | |
| | 1 | 5 | 0 | 0 | 2 | 6 | 5.94 | 0.0094 ** |
| | | 62.5% | 0.0% | 0.0% | 25.0% | 75.0% | | |
| | 2 | 0 | 2 | 4 | 3 | 0 | 3.89 | 0.041 * |
| | | 0.0% | 25.0% | 50.0% | 37.5% | 0.0% | | |
| | 3 | 0 | 4 | 4 | 3 | 0 | 3.82 | 0.047 * |
| | | 0.0% | 50.0% | 50.0% | 37.5% | 0.0% | | |
| | 4 | 0 | 2 | 0 | 0 | 0 | 0.784 | 0.169 NS |
| | | 0.0% | 25.0% | 0.0% | 0.0% | 0.0% | | |
| Degeneration | 0 | 7 | 0 | 0 | 0 | 5 | 6.26 | 0.0037 ** |
| | | 87.5% | 0.0% | 0.0% | 0.0% | 62.5% | | |
| | 1 | 1 | 0 | 0 | 6 | 2 | 6.07 | 0.0091 ** |
| | | 12.5% | 0.0% | 0.0% | 75.0% | 25.0% | | |
| | 2 | 0 | 1 | 6 | 1 | 1 | 6.38 | 0.0082 ** |
| | | 0.0% | 12.5% | 75.0% | 12.5% | 12.5% | | |
| | 3 | 0 | 5 | 2 | 1 | 0 | 5.26 | 0.0096 ** |
| | | 0.0% | 62.5% | 25.0% | 12.5% | 0.0% | | |
| | 4 | 0 | 2 | 0 | 0 | 0 | 1.09 | 0.359 NS |
| | | 0.0% | 25.0% | 0.0% | 0.0% | 0.0% | | |
| Cast | 0 | 6 | 0 | 1 | 0 | 2 | 6.41 | 0.0076 ** |
| | | 75.0% | 0.0% | 12.5% | 0.0% | 25.0% | | |
| | 1 | 0 | 3 | 4 | 7 | 5 | 7.02 | 0.004 ** |
| | | 0.0% | 37.5% | 50.0% | 87.5% | 62.5% | | |
| | 2 | 2 | 3 | 3 | 1 | 1 | 0.837 | 0.405 NS |
| | | 25.0% | 37.5% | 37.5% | 12.5% | 12.5% | | |
| | 3 | 0 | 2 | 0 | 0 | 0 | 1.09 | 0.359 NS |
| | | 0.0% | 25.0% | 0.0% | 0.0% | 0.0% | | |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 NS |
| | | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | | |
| Inflammation | 0 | 2 | 0 | 0 | 1 | 2 | 0.807 | 0.217 NS |
| | | 25.0% | 0.0% | 0.0% | 12.5% | 25.0% | | |
| | 1 | 6 | 1 | 2 | 6 | 6 | 6.29 | 0.0072 ** |
| | | 75.0% | 12.5% | 25.0% | 75.0% | 75.0% | | |
| | 2 | 0 | 4 | 3 | 1 | 0 | 3.87 | 0.035 * |
| | | 0.0% | 50.0% | 37.5% | 12.5% | 0.0% | | |
| | 3 | 0 | 3 | 3 | 0 | 0 | 1.28 | 0.072 NS |
| | | 0.0% | 37.5% | 37.5% | 0.0% | 0.0% | | |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 NS |
| | | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | | |
| Fibrosis | 0 | 8 | 7 | 8 | 8 | 8 | 0.794 | 0.711 NS |
| | | 100.0% | 87.5% | 100.0% | 100.0% | 100.0% | | |
| | 1 | 0 | 1 | 0 | 0 | 0 | 0.705 | 0.796 NS |
| | | 0.0% | 12.5% | 0.0% | 0.0% | 0.0% | | |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 NS |
| | | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | | |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 NS |
| | | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | | |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 NS |
| | | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | | |

Chi-square test was used to compare between percentages, NS= Non-significant, P≤0.05* (significant difference), P≤0.01** (highly significant difference)

Doxorubicin (DOX), a widely used chemotherapeutic agent, presents a significant risk of inducing AKI [23]. The nephrotoxic effects of DOX necessitate the exploration of protective strategies to mitigate renal damage without compromising its anti-cancer efficacy.[24] It is suggested that DOX penetrates mitochondria, resulting in the generation of reactive oxygen species (ROS). These ROS led to damage of mitochondrial DNA and ultimately contributing to the rapid progression of nephron destruction [25]. DOX was also reported to increase in mutations in mtDNA. This could be attributed to the suppression of topoisomerases II and/ or an elevation in oxidative stress[26] In this context, Omega-3 fatty acids have gained attention for their potential nephroprotective properties. Their known anti-inflammatory, antioxidative, and anti-apoptotic capabilities offer a promising approach to safeguard renal function during chemotherapeutic treatment. This discussion goes through the effects of Omega-3 on histopathological changes in DOX-treated rats, highlighting its role as a protective agent against DOX-induced nephrotoxicity.

To study histological modifications in acute DOX renal toxicity with omega-3 pretreatment, kidney tissue sections were stained with H&E to show renal congestion, degeneration, cast, inflammation and fibrosis. These changes were ranged quantitatively from no changes to very severe by pathologist depending on the number of renal cells with score as shown in Figures (1 &2).

Regarding to the Table (1), congestion & Degeneration with high scores was higher in Group II (DOX-group) and Group III (Omega-3 100 mg) than the other groups with the significant difference while the renal cast was very higher in Group II than the other groups (for score 2) without significant difference with score 2, 3, 4. Inflammation frequency for score 3 was higher frequency in Groups II and III than the other groups but the difference was not statistically significant .Low scores frequency was higher in Groups I , Group IV and Group V for score 1than Groups II and III and the difference was highly significant statistically for score 0 & 1 (P-value $\leq 0.01$).

Histopathological analysis provides a visual and structural insight into the protective effects of Omega-3 against DOX-induced renal damage. The examination revealed significant mitigation of tubular necrosis, glomerular degeneration, and interstitial inflammation in Omega-3 supplemented groups compared to the DOX-only group. These findings not only corroborate the molecular protective effects of Omega-3 but also vividly demonstrate its capacity to preserve renal histological integrity, crucial for maintaining functional renal capacity. This was similar to findings from previous studies[27,28], as renal tissue examined from the Omega-3 pre-treated groups showed almost similar texture to the control group contrary to the renal tissue from DOX group which showed significant changes in the tissue due to inflammation and necrosis.

## Conclusion

The investigation into Omega-3 nephroprotective effects against DOX-induced toxicity reveals its multifaceted role in enhancing preserving histological integrity of renal tissues. These findings highlight the prophylactic potential of Omega-3 fatty acids as a complementary treatment strategy to mitigate the nephrotoxic effects of chemotherapeutic agents.

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