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Investigating the potential protective effects of omega-3 against doxorubicin-induced renal injury in rats through modification of antioxidant markers

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Abstract

Introduction: Acute kidney injury (AKI) is a severe complication in patients undergoing chemotherapy that significantly impacts their clinical outcomes and quality of life. Doxorubicin (DOX), a potent chemotherapeutic agent, is known for its wide range of adverse effects, including nephrotoxicity. Omega-3 fatty acids, known for their anti-inflammatory and antioxidant properties, have shown potential to protect against such injuries.

Objectives: This study evaluated the nephroprotective effects of omega-3 fatty acids on rats with acute DOX-induced renal injury and examined the levels of associated antioxidant markers (*Nrf2*, *HO1*, *GPx*).

Materials and Methods: Forty rats were randomized into five equal groups: Group I (control group); Group II (DOX group), which received a single dose of 15 mg/kg DOX via IP (intraperitoneal injection); and groups III, IV, and V, which received oral doses of omega-3 (100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively) for 28 days followed by a single dose of 15 mg/kg DOX via IP. After 48 hours, blood samples were collected, and the gene expression levels of *Nrf2*, *HO1* and *GPx* were measured by quantitative reverse transcription polymerase chain reaction (qRT–PCR).

Results: *Nrf2* expression was highly significantly lower in the DOX group (3.415 ± 0.35; $P \leq 0.001$) than in the control group (28.059 ± 2.19), while the fold change was highly significantly greater in the omega-3 groups IV and V (8.692 ± 0.69, 13.645 ± 0.97; $P \leq 0.001$). *HO1* also exhibited a highly significant decrease in the fold change in the DOX group (1.623 ± 0.14; $P \leq 0.001$) compared with the control group (34.893 ± 2.07), while it exhibited a highly significant increase in the omega-3 groups IV and V (11.423 ± 0.57 and 12.301 ± 0.64, respectively; $P \leq 0.001$). *GPx* significantly decreased the fold change in the DOX group (0.195 ± 0.02; $P \leq 0.0017$) compared with that in the control group (1.792 ± 0.28), while it significantly increased in the omega-3 groups IV and V (0.729 ± 0.08, 1.081 ± 0.11; $P \leq 0.0017$).

Conclusion: These results demonstrate the potential role of omega-3 in enhancing antioxidant markers, highlighting the prophylactic potential of omega-3 as a complementary treatment strategy to mitigate the nephrotoxic effects of chemotherapeutic agents such as DOX, which could improve clinical outcomes for cancer patients undergoing chemotherapy.

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Introduction

Acute kidney injury (AKI) is characterized by a sudden loss of kidney function leading to the accumulation of waste products in the blood, elevated creatinine levels, and reduced urine output (1). The Kidney Disease Improving Global Outcomes (KDIGO) criteria for diagnosing AKI classify it into three stages; Stage 1; diagnosed with a serum creatinine level ≥1.3 times the baseline value or an increase of at least 0.3 mg/dL within 48 hours. A urine volume of less than 0.5 ml/kg for 6–12 hours is also indicative of this stage. Stage 2; defined by a serum creatinine level that is at least twice the baseline level or a urine volume of less than 0.5 ml/kg for at least 12 hours. Stage 3 was characterized by a serum creatinine level ≥3 times the baseline value or an increase to ≥4 mg/dL. A urine volume of less than 0.3 mL/kg for at least 24 hours is also indicative of this stage (2,3).

Acute kidney injury affects more than 13 million people annually and is associated

Key point

This study showed that omega-3 fatty acids have significant potential to protect against doxorubicin-induced nephrotoxicity in rats by enhancing the expression of antioxidant markers (*Nrf2, HO1, GPx*). This suggests that it has a role as a complementary pretreatment option to ameliorate the nephrotoxic effects of chemotherapy.

with significant morbidity and mortality, especially in developing countries (4). Drug-induced AKI accounts for 14%-26% of cases and can lead to high mortality rates (5). This condition can be classified as either prerenal AKI due to reduced blood flow to the kidneys, intrinsic renal AKI due to damage within the kidney itself, or postrenal AKI due to obstruction in the urinary tract (6,7). AKI affects various parts of the kidney, including the glomeruli and tubules (8). Drug-induced kidney injury is a common type of AKI that can be caused by many drugs, such as non-steroidal anti-inflammatory drugs, antibiotics, and chemotherapeutic agents. The pathophysiological mechanisms include decreased renal perfusion, tubular toxicity, and crystal nephropathy. Early intervention can prevent severe complications (9).

Doxorubicin (DOX) is an anthracycline extracted from the pigment-producing Streptomyces peucetius. It is administered intravenously due to its instability in the stomach. DOX intercalates into DNA, inhibiting replication and transcription and leading to apoptosis (10). It also affects mitochondrial function and generates reactive oxygen species (ROS), such as hydrogen peroxide (11). This compound can cause nausea, vomiting, bone marrow suppression, and cardiotoxicity (12). It also causes nephrotoxicity by generating free radicals and damaging mitochondrial DNA in renal tissue (13).

Omega-3 fatty acids are primarily found in fish oil and have high bioavailability (14). They are absorbed in the intestines and incorporated into cell membranes. Omega-3 polyunsaturated fatty acids exhibit anti-inflammatory and antioxidant effects, impacting various cell types and reducing inflammatory responses (15). Omega-3 fatty acids are administered to prevent cardiovascular diseases, reduce blood pressure, and improve blood circulation (16). They also show potential for reducing proteinuria and preventing drug-induced nephrotoxicity (17). The increasing incidence of AKI in patients treated with DOX highlights the need for accessible protective agents such as omega-3 to prevent kidney damage.

The expression of antioxidant proteins, which prevent oxidative damage induced by inflammation and injury, is modulated by a transcription factor known as nuclear factor erythroid 2-related factor 2 *(Nrf2*) (18). In AKI, the activation of *Nrf2* induces cytoprotective and antioxidant genes including catalase (*CAT*), glutathione peroxidase (*GPx*), heme oxygenase 1 (*HO1*), and superoxide dismutase (*SOD*), which help to mitigate renal damage. Given its central role in modulating the oxidative stress response, research suggests that *Nrf2* activation can reduce the severity of AKI by enhancing kidney resistance to injury (19,20). Moreover, *HO1* is an enzyme with strong anti-inflammatory, antioxidant, and cytoprotective action. In AKI, elevated *HO1* expression in response to renal injury suggests its potential as a protective mechanism and a valuable marker for early diagnosis (21,22).

Objectives

This study investigated the protective effects of omega-3 on DOX-induced renal injury in male rats. The fold change in the gene expression of antioxidant markers (*Nrf2*, *HO1*, and *GPx*) was measured using a molecular technique known as quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR).

Materials and Methods

Drugs and chemicals

Doxorubicin (HCL) was obtained from Pfizer, US (Adriamycin). Omega-3 fatty acids were obtained from Sunshine Nutrition, USA. Other chemicals used in the study were obtained from well-known suppliers.

Animals

Forty adults male Wistar albino rats aged 16–18 weeks and weighing 240–10 g was used. The animals were properly handled according to the laboratory animal handling code of ethics and housed in suitable cages in a well-ventilated area within the animal house of the College of Pharmacy, Mustansiriyah University under optimum conditions (temperature 24 °C, humidity 50%) with free access to food and water. The rats were acclimatized for one week before the start of the study.

Experimental design

Group I, the control group, received normal saline (NS) orally by oral gavage for 28 days, followed by a single intraperitoneal (IP) injection of normal saline. Group II, the DOX group, also received NS orally via oral gavage for 28 days, followed by a single dose of 15 mg/kg IP DOX (23). Groups III, IV, and V received oral doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, of omega-3 via oral gavage (24) for 28 days, followed by a single dose of 15 mg/kg IP DOX. After a two-day period, blood samples were collected, and added to TRIzol for RNA extraction.

Gene expression analysis

One hundred microliters of blood sample was added to TRIzol reagent to enable RNA extraction and stored at -20 °C for molecular analysis through reverse transcription quantitative polymerase chain reaction (qRT-PCR) for *Nrf2*, *HO1*, and *GPx* gene expression using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene (reference gene). The gene primers (25) were synthesized by Bioer, Korea, as shown in Table 1.

RNA was extracted from the blood-TRIzol samples by the FavorPrep™ Blood/Cultured Cell Total RNA Mini kit (Cat No. FABRK 001, Favorgen, Taiwan) according to the kit manual. The extracted RNA samples were conducted to synthesize complementary DNA (cDNA) by EasyScript® One-Step gDNA Removal and cDNA Synthesis Super Mix (Cat No. AE311-03, Transgene, China) according to the kit manual. Then, the synthesized cDNA samples were mixed with the gene primers plus the Luna® Universal qPCR

Table 1. Gene primers used in this study

Gene	Direction	Primer nucleotides sequence 5′ to 3′
Nrf2	Forward	CACATCCAGACAGACACCAGT
	Reverse	CTACAAATGGGAATGTCTCTGC
HO1	Forward	ACAGGGTGACAGAAGAGGCTAA
	Reverse	CTGTGAGGGACTCTGGTCTTTG
GPx	Forward	AGTTCGGACATCAGGAGAATGGCA
	Reverse	TCACCATTCACCTCGCACTTCTCA
GAPDH	Forward	TCTGCTCCTCCCTGTTCTAGAGACA
	Reverse	TTGTGAGGGAGATGCTCAGTGTTGG

Master Mix (Cat No. M3003L, New England BioLabs, USA). The final sample (20 µL) was made up of 10 µL of master mix, 0.5 µL of both forward and reverse gene primers, 5 µL of cDNA template that was made earlier from blood samples, and 4 µL of nuclease-free water. The final ready-for-reaction samples were run in an RT-PCR instrument (Bioer LineGene 9600 plus real time thermalcycler PCR, Japan) under the following conditions for amplification. The procedure involved holding the samples for one minute at 95 °C to activate the polymerase enzyme, followed by 45 cycles of PCR. Each cycle included 15 seconds at 95 °C for cDNA double-strand denaturation and 30 seconds at 60 °C for extension. A plate read was included at the end of the extension step, and melting curve analysis was performed based on the separation characteristics of the cDNA double strand during cycling. The $2^{-\Delta\Delta CT}$ method was employed to measure gene expression through threshold cycles (CTs) (26).

Statistical analysis

The Statistical Analysis System (SAS 2018) program was used to detect the effect of different factors on the study parameters. The least significant difference (LSD) test (analysis of variance-ANOVA) was conducted to compare the means of the groups in this study. In addition, Microsoft Excel was conducted to calculate all equations related to the fold change in gene expression. All results are expressed as the mean ± standard error (SE). A *P* value ≤0.01 indicated a significant difference among the groups, while a *P* value ≤0.001 indicated a highly significant difference.

Results

Nrf2 gene expression

The results showed a highly significant decrease in the fold change in *Nrf2* gene expression in the DOX group (3.415 ± 0.35; $P \leq 0.001$) compared with that in the control group (28.059 ± 2.19) and in groups IV and V (Omega-3), as there was a highly significant increase in *Nrf2* expression in these groups (8.692 ± 0.69, 13.645 ± 0.97; $P \leq 0.001$), respectively, compared with that in the DOX group, as shown in Table 2 and Figure 1.

HO1 gene expression

The results showed a highly significant decrease in the fold change in *HO1* gene expression in the DOX group (1.623 ± 0.14; $P \leq 0.001$) compared with that in the control (34.893 ± 2.07) and omega-3 groups IV and V, as there was a highly significant increase in *HO1* expression in these groups (11.423 ± 0.57, 12.301 ± 0.64; $P \leq 0.001$), respectively, compared to that in the DOX group, as shown in Table 2 and Figure 2.

GPx gene expression

The results showed a significant decrease in the fold change in *GPx* gene expression in the DOX group (0.195 ± 0.02; P ≤ 0.0017) compared with that in the control (1.792 ± 0.28) and omega-3 groups IV and V, as there was a significant increase in *GPx* expression in these groups (0.729 ± 0.08, 1.081 ± 0.11; $P \leq 0.0017$), respectively, compared to that in the DOX group, as shown in Table 2 and Figure 3.

Discussion

These findings showed that omega-3 pretreatment before DOX injection significantly increased the expression of antioxidant genes (*Nrf2*, *HO1*, and *GPx*) in a dose-dependent manner. *Nrf2* is a crucial regulator of cellular defense against oxidative damage, orchestrating the expression of numerous antioxidants and cytoprotective

Table 2. Changes in *Nrf2*, *HO1* and *GPx* gene expression levels are related to the effect of omega-3 on DOX-treated rats

Group	Mean ± SE of *Nrf2* Fold change	Mean ± SE of *HO1* Fold change	Mean ± SE of *GPx* Fold change
Control (I)	28.059 ± 2.19 ^{a}	34.893 ± 2.07 ^{a}	1.792 ± 0.28 ^{a}
Dox (II)	3.415 ± 0.35 ^{b}	1.623 ± 0.14 ^{b}	0.195 ± 0.02 ^{b}
100 mg OMG-3 (III)	4.325 ± 0.32 ^{b}	1.739 ± 0.28 ^{b}	0.0568 ± 0.007 ^{b}
200 mg OMG-3 (IV)	8.692 ± 0.69 ^{c}	11.423 ± 0.57 ^{c}	0.729 ± 0.08 ^{c}
400 mg OMG-3 (V)	13.645 ± 0.97 ^{d}	12.301 ± 0.64 ^{c}	1.081 ± 0.11 ^{c}
LSD	3.906 **	4.529 **	0.529 *
P value	0.0001	0.0001	0.0017

DOX: Doxorubicin, LSD: Least significant difference, OMG-3: Omega-3, SE: Standard error.
Data were expressed as the mean ± SE, mean superscript with different lowercase letters (a, b, c, d) differ statistically, LSD used to compare mean among groups, *P* value ≤ 0.01 * (significant difference), *P* value ≤ 0.001 ** (highly significant difference).

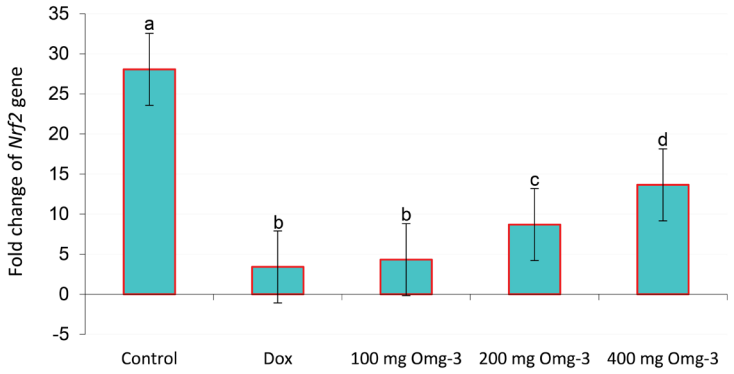

Figure 1. Fold change variation of *Nrf2* gene related with the effect of Omgea-3 on treated rats with DOX

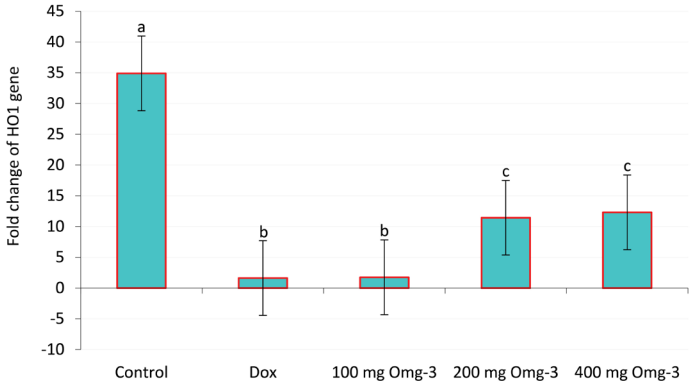

Figure 2. Fold change variation of *HO1* gene related with the effect of Omgea-3 on treated rats with DOX.

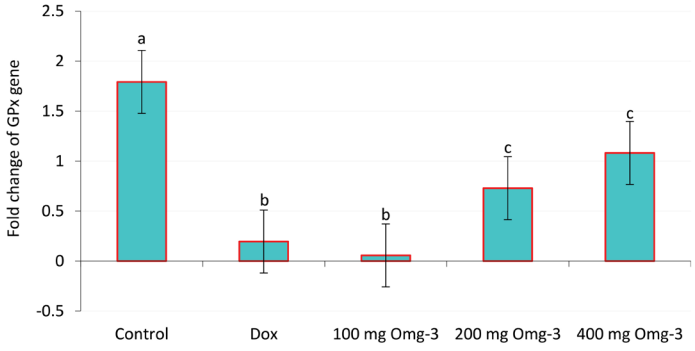

Figure 3. Fold change variation of *GPx* gene related with the effect of Omgea-3 on treated rats with DOX.

genes. When DOX is used alone, impaired *Nrf2* signaling can lead to unchecked oxidative stress and renal injury (27). This study revealed that omega-3 pretreatment significantly restored *Nrf2* expression in DOX-treated rats, indicating enhanced activation of the cellular antioxidant defense system. The highest dose of omega-3 fatty acids (200 and 400 mg) had the most notable effect, which resembles previous research studies that demonstrated a significant upregulation of *Nrf2* in groups pretreated with omega-3 fatty acids (28,29). In addition, *Nrf2* releases its cytoplasmic inhibitor protein in response to oxidative stress; this protein enters the nucleus and stimulates a number of genes associated with antioxidant defense, including *CAT*, *GPx*, *HO1* and, *SOD* (30,31). Research has shown that DOX treatment minimizes both the mRNA and protein expression of *Nrf2*, consequently, the expression of downstream antioxidant genes and proteins, resulting in nephrotoxicity (32).

HO1 is a protective enzyme against oxidative injury, promoting the degradation of the pro-oxidant heme into biliverdin, iron, and carbon monoxide. The upregulation of *HO1* in response to DOX treatment reflects the kidney's attempt to combat oxidative stress (22). Omega-3 pretreatment was found to significantly enhance *HO1* expression, demonstrating its efficacy in reducing oxidative stress and inflammation, which are vital for renal

protection. The highest dose of omega-3 (200 and 400 mg) had the most profound effect, consistent with findings from previous studies (28,29).

GPx plays a critical role in detoxifying H2O2 into water, thereby protecting cells from oxidative damage (21). This study revealed that DOX-treated rats had decreased *GPx* expression, which is an indicator of oxidative stress. However, omega-3 pretreatment dose-dependently normalized *GPx* expression. The highest dose (200 and 400 mg) effectively restored *GPx* levels close to those of the control group, highlighting the potent antioxidant action of omega-3 fatty acids. These findings are supported by previous research showing that omega-3 fatty acids increase *GPx* levels, reinforcing the antioxidant defense system and preserving renal function (33).

Conclusion

This study revealed that omega-3 fatty acids exhibit a significant nephroprotective effect against DOX-induced nephrotoxicity in a dose-dependent manner. The key antioxidant defense markers *Nrf2*, *HO1*, and *GPx* were upregulated in the omega-3 pretreatment groups, while they were downregulated in the DOX-only group, contributing to renal injury. The ability of omega-3 to modulate the expression of these genes, as well as its potential as a protective agent during chemotherapeutic treatment regimens, was demonstrated.

Limitations of the study

Although these findings are promising, translating preclinical results to clinical applications entails inherent challenges. The dose–response relationship, long-term safety, and efficacy of omega-3 supplementation in humans require thorough investigation through clinical trials, particularly concerning varying chemotherapeutic regimens and renal health statuses. Further research is necessary to optimize omega-3 dosing strategies, elucidate its mechanisms of action in human physiology, and confirm its therapeutic value in clinical settings.

Recommendations

Future studies should focus on clinical trials to validate these findings in humans, exploring the therapeutic potential and optimal dosing of omega-3 fatty acids. Additionally, investigating the protective effects of omega-3 in combination with other nephrotoxic chemotherapeutic agents could broaden its utilization in oncology.

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Authors' contribution

Conceptualization: Suhad Faisal Hatem Al-Mugdadi.
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Formal analysis: Ahmed Sabah Malik Al-Dabbagh, Suhad Faisal Hatem Al-Mugdadi.
Investigation: Ahmed Sabah Malik Al-Dabbagh.
Methodology: Ahmed Sabah Malik Al-Dabbagh.
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Resources: Ahmed Sabah Malik Al-Dabbagh.
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Supervision: Bahir Abdul Razzaq Mshemish, Suhad Faisal Hatem Al-Mugdadi.
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Visualization: Ahmed Sabah Malik Al-Dabbagh.
Writing–original draft: Ahmed Sabah Malik Al-Dabbagh.
Writing–review & editing: Suhad Faisal Hatem Al-Mugdadi

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research and protocol for this study adhered to the guidelines for animal studies and received approval from the Ethics Committee of the College of Pharmacy at Mustansiriyah University (Reference No. 217, 2023). Accordingly, we conducted the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). Additionally, This study was extracted from the MSc thesis of Ahmed Sabah Malik Al-Dabbagh at the department of Pharmacology and Toxicology in College of Pharmacy, Mustansiriyah University (Thesis title: investigating the possible protective effect of omega-3 against DOX-induced renal injury in male rats). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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