



# Ukrainian Journal of Nephrology and Dialysis

Scientific and Practical, Medical Journal

## Founders:

- State Institution «Institute of Nephrology NAMS of Ukraine»
- National Kidney Foundation of Ukraine

ISSN 2304-0238;  
eISSN 2616-7352

Journal homepage: <https://ukrjnd.com.ua>

## Research Article

Gunes Bolatli<sup>1</sup>, Mahinur Ulusoy<sup>2</sup>, Fatih Tas<sup>3</sup>, Naci Omer Alayunt<sup>4</sup>,  
Ismail Zarasiz<sup>5</sup>

doi: 10.31450/ukrjnd.4(72).2021.03

## Effect of omega-3 fatty acid on contrast-induced nephropathy

<sup>1</sup>Siirt University, Faculty of Medicine, Department of Anatomy, Siirt, Turkey

<sup>2</sup>Akşehir community health center, Konya, Turkey

<sup>3</sup>Siirt University, Faculty of Medicine, Department of Histology and Embryology, Siirt, Turkey

<sup>4</sup>Siirt University, Faculty of Medicine, Department of Biochemistry, Siirt, Turkey

<sup>5</sup>Girne American University, Faculty of Medicine, Department of Anatomy, Girne, Cyprus

## Citation:

Bolatli G, Ulusoy M, Tas F., Alayunt NO, Zarasiz I. Effect of omega-3 fatty acid on contrast-induced nephropathy. Ukr J Nephrol Dial. 2021;4(72):15-25. doi: 10.31450/ukrjnd.4(72).2021.03.

**Abstract.** Renal failure that develops acutely after the use of iodinated contrast material is called “contrast-induced nephropathy”. It is a complication with high morbidity and mortality risk. Current treatments are aimed at protecting kidney functions, new treatment methods are being researched. This study aims to demonstrate the therapeutic effects of omega-3 fatty acids on CIN, taking into account the possible clinical usage of iodinated contrast media and the benefits of omega-3 fatty acids.

**Methods.** A total of 30 rats were studied, divided into four groups. Only saline was administered by gavage to group 1, only IV urography to group 2, only 400 mg omega-3 to group 3, and urography and 400 mg omega-3 to group 4. At the end of the study, kidney tissue and serum oxidative and antioxidant markers, and creatinine levels were analyzed.

**Result.** While the degrees of glutathione peroxidase, catalase and total antioxidant capacity in kidney tissue and serum tests of rats treated with omega-3 fatty acid increased statistically; total oxidant capacity and malondialdehyde levels were found to be significantly lower. Furthermore, blood urea nitrogen and creatinine levels were found to be significantly lower in the omega-3 treated group.

**Conclusion.** Omega-3 fatty acids had therapeutic effects in the experimental CIN model. As a result, we believe omega-3 fatty acids can be used as an alternative to existing supportive medicines in this common disease with few therapy options.

**Key Words:** contrast-induced nephropathy, experimental animals, omega-3 fatty acid.

**Conflict of interest statement.** The authors declare no competing interest

## Article history:

Received September 9, 2021

Received in revised form

September 14, 2021

Accepted September 15, 2021

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Correspondence should be addressed to Gunes Bolatli: [gunesbolatli83@gmail.com](mailto:gunesbolatli83@gmail.com)



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УДК 616.61- 008.64-036.11-085

Гюнес Болатлі<sup>1</sup>, Махінур Улусой<sup>2</sup>, Фатіх Тас<sup>3</sup>, Начі Омер Алаюнт<sup>4</sup>, Ісмаїл Зарасіз<sup>5</sup>

## Вплив омега-3 жирної кислоти на контраст-індуковану нефропатію

<sup>1</sup>Університет Сірта, медичний факультет, кафедра анатомії, Сірт, Туреччина

<sup>2</sup>Оздоровчий центр громади Акшехір, Конья, Туреччина

<sup>3</sup>Університет Сірта, медичний факультет, кафедра гістології та ембріології, Сірт, Туреччина

<sup>4</sup>Університет Сірта, медичний факультет, кафедра біохімії, Сірт, Туреччина

<sup>5</sup>Американський університет Гірне, медичний факультет, кафедра анатомії, Гірне, Кіпр

**Резюме.** *Контраст-індукована нефропатія (КІН) має високий ризик захворюваності та смертності. Метою цього дослідження було продемонструвати терапевтичний вплив омега-3 жирних кислот на КІН, беручи до уваги переваги омега-3 жирних кислот.*

*Методи.* До дослідження залучено 30 щурів, розділених на чотири групи. Групі 1 за допомогою зонда вводили фізіологічний розчин, групі 2 доведено вводили контрастну речовину, групі 3 – лише 400 мг омега-3, а групі 4 – контрастну речовину та 400 мг омега-3. Наприкінці експериментального дослідження аналізували тканину нирок та сироватку крові щодо концентрації оксидативних й антиоксидантних маркерів та рівня креатиніну.

*Результати.* У той час як показники глутатіонпероксидази, каталази та загальної антиоксидантної здатності в нирковій тканині та сироватки щурів, які отримували омега-3 жирні кислоти, зросли статистично, загальна окислювальна здатність і рівні малонового діальдегіду значно знижувались. Крім того, було виявлено, що рівні азоту сечовини крові та креатиніну були значно нижчими в групі, яка отримувала омега-3.

*Висновок.* Омега-3 мала терапевтичний ефект у експериментальній моделі КІН. Ми вважаємо, що омега-3 жирні кислоти можна використовувати як альтернативу існуючим підтримуючим засобам лікування.

**Ключові слова:** *контраст-індукована нефропатія, експериментальні тварини, омега-3 жирна кислота.*

**Introduction.** In clinical radiology, “iodinated contrast agent” is used in diagnostic and therapeutic applications. Contrast-induced nephropathy (CIN) is a type of acute renal failure caused by the use of iodinated contrast agents. It is a significant complication that has a significantly high risk of morbidity and mortality. CIN is defined as a 25% or 0.5 mg/dl increase in serum basal creatinine level, detected 48 hours following radiocontrast agent exposure [1, 2].

CIN is the third most prevalent cause of hospital-acquired acute renal failure [1]. In addition, the incidence of CIN due to coronary invasive interventions varies between 2-4.47% in the general population, while this rate increases up to 40% in patients with chronic kidney diseases and using diuretics [3]. Therefore, although the use of “iodinated contrast material” has a low effect on kidney functions in the general population, its negative effect may be much higher in some patient groups [4]. In addition, it causes prolongation of the hospitalization period and an increase in morbidity and mortality rates [3].

The pathophysiology of the development of ne-

phropathy associated with contrast agent use is complex and one of the important mechanisms involved in the pathogenesis is renal tubular ischemia observed due to this contrast agent [5]. Due to renal tubular ischemia, a decrease is observed in medullary blood flow and ischemic damage and necrosis occur in tissues [6]. In addition, it is stated that the changes in adenosine metabolism, glomerular flow, endothelin and prostaglandin metabolism and oxidative stress are also effective [7-9]. It is thought that renal dysfunction due to oxidative damage in the kidney can be minimized with the use of antioxidants [10].

Because of their antioxidant and antibacterial properties, omega-3 fatty acids are becoming acknowledged as possible medicinal agents [11]. These acids are essential fatty acids that exist in the cell membrane's structure and are required for the cell to operate normally [12]. In addition, a diet enriched with omega-3 fatty acids has been shown to reduce the progression of certain types of cancer and cardiovascular, respiratory, inflammatory and nephrological diseases [13]. Signal transmission, cell membrane physiology, immunology, inflammation, and metabolic pathways are all affected by omega-3 fatty acids, which are particularly obtained from cold-water fish [14].

Omega-3 fatty acids' effects on a variety of clinical conditions, including chronic kidney disease, are still being researched [14]. This study aims to demonstrate the therapeutic effects of omega-3 fatty acids on CIN, taking into account the possible clinical usage of iodinated

**Gunes Bolatli**

**gunesbolatli83@gmail.com**

**Telephone number:5057611324**

ated contrast media and the benefits of omega-3 fatty acids.

**Materials and methods. Experimental animals.** The complete experimental study was carried out in line with the rules of the "Guide for the Care and Use of Laboratory Animals," with the permission number 2016/3 of the Sel uk University Experimental Medicine Research and Application Centre's Animal Experiments Ethics Committee.

The study was realized on 30 Wistar Albino male rats (weighted 150-200 g). Rats were provided in a standard animal shelter under room humidity and temperature control (temperature:  $21 \pm 2^\circ \text{C}$  and humid-

ity: 50%), ad libitum with 12-hour light and dark cycle. During the experiment, the animals were put into polycarbonate transparent cages and were fed with fabricated pellet feed and tap water. Marincap branded fish oil containing 380 mg of eicosapentaenoic acid (EPA), 200 mg of docosahexaenoic acid (DHA) and a total of minimum 720 mg of omega 3-fatty acids [n-3-polyunsaturated fatty acids (PUFA) was used.

**Experimental design.** 4 groups were formed from the experimental animals as 6 rats in the control group and 8 rats in the other groups. Experiment groups, applied materials and application methods are summarized below (Fig. 1).

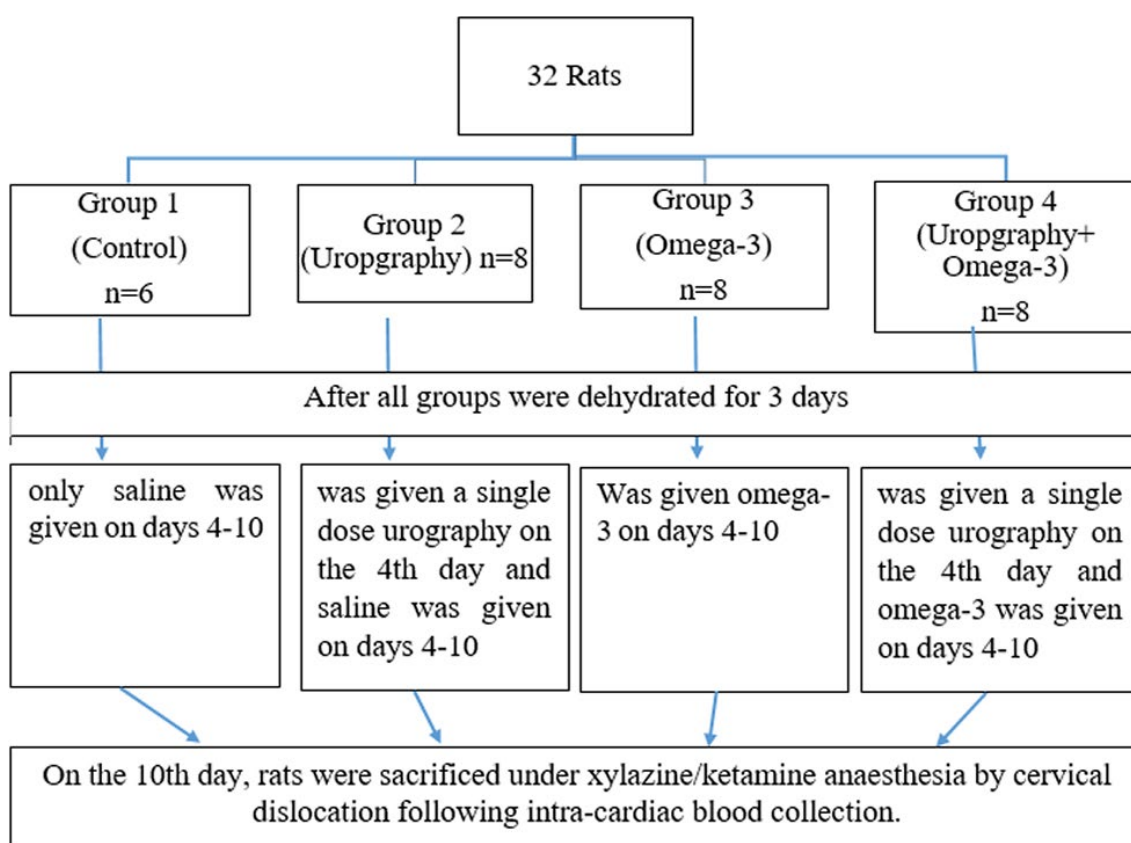


Fig. 1. Flow diagram of the experimental design.

Group 1 control group (n = 6)

Group 2 was given only IV urography (n = 8)

Group 3 was given only 400 mg/kg/day omega-3 (n = 8)

Group 4 was given urography and 400 mg/kg/day omega-3 (n = 8)

After all groups were dehydrated for 3 days [15];

Only saline was given by gavage method to Group 1, on days 4-10.

Group 2 was given a single dose of 6 mL/kg urography in 5 minutes with a slow infusion from the tail on the 4th day (under ether anesthesia) and experimental CIN was created [16] and saline was given by gavage method on days 4-10.

Group 3 was given 400 mg/kg/day omega-3 by gavage method on days 4-10.

Group 4 was given a single dose of 6 mL/kg urography in 5 minutes with a slow infusion from the tail on the 4th day (under ether anesthesia) and 400 mg/kg/day omega-3 by gavage method on days 4-10 (see Fig. 1).

On the 10th day, rats were sacrificed under xylazine/ketamine anesthesia by cervical dislocation following intra-cardiac blood collection. During the sacrifice process, 5 mL of blood was taken from all rats into gel plain biochemistry tubes, centrifuged at 5000 rpm for 5-10 minutes, then separated into serums and kept in Eppendorf tubes at  $-80^\circ \text{C}$  until the day of analysis.

Furthermore, after sacrifice and fixation in 4% formaldehyde, rat kidney tissues were carefully removed for biochemical analysis. The kidney tissues were isolated and kept at  $-80^\circ \text{C}$  in appropriate containers until the analysis day.

After all of the preliminary preparations for analysis were completed, serum and kidney tissues that had been maintained at  $-80^{\circ}\text{C}$  were dissolved at room temperature and serum samples were immediately analyzed, while tissue samples were prepared for analysis through sample preparation processes.

**Tissue Preparation.** Kidney samples weighed for kidney tissue analysis were trimmed with appropriate chopping tools, treated with 0.015 M phosphate buffer (pH 7.5). It was then homogenized with tissue solvent (TissueLyser II, Qiagen) at 1/20 (w/v) ratio. Finally, it was rested by vortexing at  $4^{\circ}\text{C}$  and centrifuged at  $20,000\times g$  for 15 minutes and the supernatant was separated.

**Measurement of serum blood urea nitrogen (BUN) and creatinine levels.** BUN and creatinine concentrations were measured on a Siemens Advia 2400 brand automated analyzer using commercial kits to evaluate renal function. Before the measurement, the calibration and control studies of the device were carried out.

**Tissue and serum Elisa Glutathione Peroxidase (GPx) and Glutathione (GSH) determination.** GPx enzyme activity of supernatant and serum samples obtained from tissue homogenates were determined using the ELISA method (BIOTEK ELx800) with commercial kits (Randox/Ransel, Crumlin, United Kingdom). GPx concentration is expressed in U/L. Commercial kits measuring the glutathione reductase enzyme were used for the determination of glutathione (GSH) concentration (Cayman, Michagen, USA). GSH concentration is expressed in  $\mu\text{g}/\text{mL}$ .

**Tissue and serum catalase (CAT) determination.** CAT activity of the supernatant and serum samples obtained from tissue homogenates was measured using an enzymatic assay kit (ZellBio GmbH CAT Colorimetric Assay Kit). All reagents and samples were added as described in the kit catalog and then incubated for 1 minute at  $37^{\circ}\text{C}$ . The absorbance of the final product, which turned into a chromogenic color with the addition of the final reagents, was read colorimetrically and the CAT activity was calculated according to the manufacturer's formula (Siemens Advia 2400 brand automatic analyzer). The concentration of CAT is expressed as k/mg protein.

**Tissue and serum total antioxidant capacity status (TAS).** TAS (Unit: mmol Torolox. Equiv/L) level analysis of the supernatant part and serum samples obtained from the separated tissues were analyzed at 660 nm with

the help of commercial kits (Rel Assay Diagnostics brand, TEST KIT Catalog no. RL0017 LOT.:RL024). It was measured colorimetrically with a Siemens Advia 2400 brand automatic analyzer [17, 18].

**Tissue and serum total oxidant capacity status (TOS).** TOS (Unit:  $\mu\text{mol H}_2\text{O}_2$  Equiv/L) level analysis of the supernatant part and serum samples obtained from the separated tissues were analyzed at 530 nm (Rel Assay Diagnostics brand, ASSAY KIT Catalog no. RL0024 LOT: RL026) with the help of commercial kits. It was measured colorimetrically with a Siemens Advia 2400 brand automatic analyzer [17, 18].

**Malondialdehyde (MDA) analysis in serum by HPLC.** Serum MDA analysis was studied in serum samples to be analyzed by HPLC under the appropriate mobile phase, column, flow rate and pressure. By taking 0.3 ml of serum sample and adding 0.3 ml 0.5 M  $\text{HClO}_4$ , the proteins were precipitated. Then, after this mixture was vortexed, pure water was added to the total volume of 1 mL. After centrifuging the mixture for 15 minutes at 2500 rpm, 20  $\mu\text{l}$  of the clear part of the samples was carefully removed and analyzed on HPLC (18). Analyses were performed in the mobile phase in a mixture of 30 mM  $\text{KH}_2\text{PO}_4$  - methanol (82.5-17.5%; pH:4) at 250 nm using an inertial 5  $\mu\text{C}$ -18 (15cmx4.6mm) column at flow rate mL/min.

**Malondialdehyde (MDA) analysis in tissue by HPLC.** Tissue MDA analysis was studied on supernatant samples obtained from kidney homogenates to be analyzed by HPLC under appropriate mobile phase, column, flow rate and pressure. 1.5 mL of 0.5 M  $\text{HClO}_4$  was added to approximately 0.3 grams of the crushed kidney tissue sample. After precipitation of the proteins, 1.5 mL of distilled water was added and the total volume was completed to 3 mL. The mixture was then centrifuged at 4500 rpm for 25 minutes, then carefully removed from the clear part and placed in vials. The injection volume was analyzed on 20  $\mu\text{L}$  HPLC [19].

**Statistical analysis.** All data from the experimental groups were analyzed using the SPSS (version 22.0) statistics program. To compare numerical variables in groups, the one-way ANOVA test was utilized. For pairwise comparisons, an independent t-test was used after a post hoc Bonferroni correction. During statistical analysis,  $p < 0.05$  was considered significant when all data were determined as mean standard deviation.

**Results.** Statistically significant differences were detected between the groups by calculating the mean values of biochemical findings (Table1).

Table 1

Serum biochemical analysis results of the experimental rats

	Group (n)	Mean ( $\pm$ SD)	F	p
BUN (mg/dL)	1 (6)	24.5 (0.08)	5746.139	0.000
	2 (8)	34.1 (0.10)		
	3 (8)	24.8 (0.27)		
	4 (8)	32.4 (0.19)		
	Total (30)	29.29 (4.41)		

Continuation of Table 1

	Group (n)	Mean ( $\pm$ SD)	F	p
Creatinin (mg/dL)	1 (6)	0.57 (0.02)	115.271	0.000
	2 (8)	0.69 (0.01)		
	3 (8)	0.54 (0.02)		
	4 (8)	0.60 (0.06)		
	Total (30)	0.60 (0.06)		
GPx (U/L)	1 (6)	18.8 (0.39)	358.801	0.000
	2 (8)	10.3 (0.75)		
	3 (8)	19.4 (0.56)		
	4 (8)	16.6 (0.63)		
	Total (30)	16.13 (3.76)		
GSH ( $\mu$ g/mL)	1 (6)	125.1 (3.39)	181.337	0.000
	2 (8)	92.1 (2.79)		
	3 (8)	123.0 (4.0)		
	4 (8)	111.3 (1.82)		
	Total (30)	112.13 (13.67)		
CAT (k/mg)	1 (6)	21.2 (0.5)	232.884	0.000
	2 (8)	14.4 (0.39)		
	3 (8)	19.3 (0.65)		
	4 (8)	16.5 (0.50)		
	Total (30)	17.67 (2.61)		
TAS ( $\mu$ mol Trolox Equiv./L)	1 (6)	1.8 (0.04)	223.850	0.000
	2 (8)	1.2 (0.04)		
	3 (8)	1.87 (0.06)		
	4 (8)	1.54 (0.08)		
	Total (30)	1.60 (0.29)		
TOS ( $\mu$ mol H <sub>2</sub> O <sub>2</sub> Equiv./L)	1 (6)	18.8 (0.8)	270.131	0.000
	2 (8)	30.5 (0.99)		
	3 (8)	18.1 (1.18)		
	4 (8)	24.5 (0.82)		
	Total (30)	23.31 (5.21)		
MDA (mg/lt)	1 (6)	0.14 (0.01)	217.452	0.000
	2 (8)	0.47 (0.03)		
	3 (8)	0.26 (0.03)		
	4 (8)	0.32 (0.02)		
	Total (30)	0.31 (0.12)		

**BUN level.** It was observed that there was a significant difference between groups in serum BUN level analysis. Group 2 BUN level was significantly higher than the other groups ( $p < 0.05$ ). In addition, Group 4 BUN level was significantly higher than Group 1 and Group 3, Group 3 BUN level was significantly higher than Group 1 ( $p < 0.05$ ).

**Serum Creatinine level.** A statistically significant difference was observed between the groups in serum creatinine level analysis. Group 2 creatinine level was statistically significantly higher than other groups ( $p < 0.05$ ). Group 4 creatinine level was significantly higher than Group 1 and Group 3.

**Serum and Tissue GPx levels.** It was observed that there were statistically significant differences between groups in serum GPx level analysis. GPx level of Group 1 and Group 3 was statistically significantly higher than Group 2 and Group 4, and Group 4 GPx level was significantly higher than Group 2 ( $p < 0.05$ ) (see Table 1).

A statistically significant difference was observed between groups in tissue GPx level analysis. Group 1 GPx level was significantly higher than the other groups ( $p < 0.05$ ). In addition, Group 3 GPx level was significantly higher than Group 2 and Group 4, Group 4 GPx level was significantly higher than Group 2 ( $p < 0.05$ ) (Table 2).

Table 2

## Tissue biochemical analysis results of the experimental rats

	Group (n)	Mean ( $\pm$ SD)	F	p
GPx (U/L)	1 (6)	34.1 (0.44)	276.014	0.000
	2 (8)	28.0 (0.37)		
	3 (8)	33.6 (0.60)		
	4 (8)	30.2 (0.45)		
	Total (30)	31.3 (2.57)		
GSH ( $\mu$ g/mL)	1 (6)	34.2 (1.67)	92.618	0.000
	2 (8)	24.4 (0.91)		
	3 (8)	31.6 (0.53)		
	4 (8)	28.9 (1.39)		
	Total (30)	29.5 (3.76)		
CAT (k/mg)	1 (6)	28.3 (0.17)	1953.755	0.000
	2 (8)	17.4 (0.33)		
	3 (8)	28.5 (0.40)		
	4 (8)	24.4 (0.32)		
	Total (30)	24.4 (4.66)		
TAS ( $\mu$ mol Trolox Equiv./L)	1 (6)	0.05 (0.00)	871.535	0.000
	2 (8)	0.01 (0.00)		
	3 (8)	0.04 (0.00)		
	4 (8)	0.03 (0.00)		
	Total (30)	0.03 (0.02)		
TOS ( $\mu$ mol H <sub>2</sub> O <sub>2</sub> Equiv./L)	1 (6)	0.11 (0.00)	330.720	0.000
	2 (8)	0.14 (0.00)		
	3 (8)	0.12 (0.00)		
	4 (8)	0.13 (0.00)		
	Total (30)	0.13 (0.01)		
MDA (mg/lt)	1 (6)	0.75 (0.01)	1301.564	0.000
	2 (8)	0.95 (0.01)		
	3 (8)	0.72 (0.01)		
	4 (8)	0.86 (0.01)		
	Total (30)	0.82 (0.09)		

**Serum and Tissue GSH levels.** It was observed that there was a statistically significant difference between groups in serum GSH level analysis. GSH level of Group 1 and Group 3 was statistically significantly higher than Group 2 and Group 4 ( $p < 0.05$ ). Group 4 GSH level was statistically significantly higher than Group 2 ( $p < 0.05$ ) (see Table 1).

In the tissue GSH level analysis, it was observed that there was a statistically significant difference between the groups. Group 1 GSH level was significantly higher than the other groups ( $p < 0.05$ ). In addition, Group 3 GSH level was significantly higher than Group 2 and Group 4, Group 4 GSH level was significantly higher than Group 2 ( $p < 0.05$ ) (see Table 2).

**Serum and tissue CAT levels.** It was observed that there was a statistically significant difference between the groups in serum CAT level analysis. Group 1 CAT level was statistically significantly higher than other groups ( $p < 0.05$ ). In addition, Group 3 CAT level was significantly higher than Group 2 and Group 4, Group 4 CAT level was significantly higher than Group 2 ( $p < 0.05$ ) (see Table 1).

It was observed that there was a statistically significant difference between the groups in tissue CAT level analysis. Group 1 and Group 3 CAT level was statistically significantly higher than Group 2 and Group 4 ( $p < 0.05$ ). Group 4 CAT level was significantly higher than Group 2 ( $p < 0.05$ ) (see Table 2).

**Serum and tissue TAS levels.** It was observed that the difference between the groups in serum TAS level analysis was statistically significant. TAS level of Group 1 and Group 3 was statistically significantly higher than Group 2 and Group 4 ( $p < 0.05$ ). Group 4 TAS level was statistically significantly higher than Group 2 ( $p < 0.05$ ) (see Table 1).

It was observed that there was a statistically significant difference between the groups in tissue TAS level analysis. Group 1 TAS level was statistically significantly higher than other groups ( $p < 0.05$ ). Group 3 TAS level was statistically significantly higher than Group 4 and Group 2, Group 4 TAS level was statistically significantly higher than Group 2 ( $p < 0.05$ ) (see Table 2).

**Serum and tissue TOS levels.** It was observed that there was a statistically significant difference between the groups in serum TOS level analysis. Group 2 TOS level was significantly higher than the other groups ( $p < 0.05$ ). In addition, Group 4 TOS level is significantly higher than Group 1 and Group 3 ( $p < 0.05$ ) (see Table 1).

It was observed that there was a statistically significant difference between the groups in tissue TOS level analysis. Group 2 TOS level was statistically significantly higher than other groups ( $p < 0.05$ ). Group 4 TOS level is significantly higher than Group 1 and Group 3, Group 3 TOS level is significantly higher than Group 1 ( $p < 0.05$ ) (see Table 2).

**Serum and tissue MDA levels.** It was observed that the difference between groups in Serum MDA level analysis was statistically significant. Group 2 MDA level was significantly higher than other groups ( $p < 0.05$ ). Group 4 MDA level was significantly higher than Group 1 and Group 3, Group 3 MDA level was significantly higher than Group 1 ( $p < 0.05$ ) (see Table 1).

It was observed that the difference between groups in MDA level analysis was statistically significant. Group 2 MDA level was significantly higher than other groups ( $p < 0.05$ ). Group 4 MDA level was significantly higher than Group 1 and Group 3, Group 1 MDA level was significantly higher than Group 3 ( $p < 0.05$ ) (see Table 2).

**Discussion.** CIN is a renal dysfunction and the pathophysiology of this common nephropathy is not yet fully understood [16]. Different alternative treatment methods are being investigated for the treatment of CIN such as ozone, thymoquinone [20-24]. Omega-3 fatty acids also have healing effects on a variety of kidney diseases [25, 26]. It is known that omega-3 fatty acids, which are important in terms of maintaining cellular functions, provide a protective effect by replacing PUFA (polyunsaturated fatty acids) that decrease in tissue due to oxidative damage [27, 28]. However, according to the available literature results, there is no study showing the healing effects of omega-3 fatty acids on CIN. Therefore, we think that the study will contribute to the literature.

CIN risk factors include chronic diabetes, congestive heart failure, renal failure advanced age ( $>70$ ), gen-

der (female), dehydration and excessive use of contrast material [1]. In addition, captopril, an angiotensin-converting enzyme inhibitor, has been stated to be a risk factor for CIN [29]. Although various risk factors have been identified for this disease, the cumulative effect of their combination is not known enough [4]. In addition, since CIN treatment is quite limited, it causes complications that lead to long-term hospitalizations. The treatments performed are mostly supportive of kidney functions, and in some cases, temporary or permanent haemodialysis may be required [4]. For these reasons, it is important to pay attention to risk factors and to study alternative treatments in preventing the development of CIN.

Various applications are used for treatment and prevention in CIN, where oxidative stress and renal vasoconstriction play an important role [16, 30, 31]. Among these applications, vasodilator agents, diuretics, increasing the extracellular volume, adjusting the type and amount of contrast agent before the procedure can be given as examples [21-24]. Hydration increases renal blood flow and reduces renal ischemia and toxicity [6, 32]. Although there is no specific treatment for this disease, the main method used in its prevention is hydration [33]. In addition, antioxidants such as statins and N-acetylcysteine used in treatment can be considered in preventive treatment, but cannot replace hydration applications [33]. Although other applications such as ascorbic acid, calcium channel blockers, furosemide, prostaglandin analogs, endothelin agonists, adenosine antagonists, l-arginine, hypertonic mannitol, dopamine and theophylline have been tried in the treatment of the disease [34, 35], but their effectiveness on kidney toxicity caused by CIN controversial [33]. It has been shown in this study that omega 3 fatty acids are supportive in the treatment of this disease. At this point, omega-3 fatty acids known to have protective effects on kidney tissue [36], may be considered to have curative effects on CIN.

Immunoglobulin A nephropathy, the most common primary glomerulonephritis in the world, mostly affects young adults and leads to progressive kidney disease. It is known that omega-3 fatty acids decrease renal inflammation and glomerulosclerosis, which are the hallmarks of this disease [13, 25]. In addition, it is known that omega-3 fatty acids have a protective effect on kidney damage caused by formaldehyde toxicity [26]. In this study, we observed that omega 3 fatty acids have healing effects in the experimentally created CIN.

Many methods have been tried to treat CIN [16, 37]. Ozone therapy on CIN was examined and it was reported that serum BUN and creatinine levels and tubular necrosis decreased [16]. Again, in a study investigating the effects of thymoquinone on CIN, it was observed that BUN and creatinine levels decreased [37]. We also found that omega-3 fatty acids decreased serum BUN and creatinine levels on the created experimental CIN model. The decrease in serum BUN and creatinine levels indicate that omega-3 protects kidney functions.

Although the pathogenesis of CIN is complex, the role of oxidative damage in this process is important [38]. Oxidative stress occurs as a result of an imbalance between the formation of free radicals and antioxidant defense mechanisms [39, 40]. Therefore, examining the products of free radical reactions and defense systems is preferred by many researchers [40]. The increase in free radical formation also leads to an increase in MDA levels. Organisms are protected by enzymatic and non-enzymatic antioxidant systems against oxidative damage resulting from this, and these antioxidant systems include GPx, GSH and CAT [36, 41].

GPx is an enzyme that reduces hydroperoxides. It also plays a major role in defense against free oxygen radicals, peroxides and carcinogens [42, 43]. GSH is an important tripeptide antioxidant used by antioxidant enzymes to neutralize free radicals [44]. CAT is among the important enzymes that prevent free radical accumulation and lipid peroxidation [45]. It has been stated that olive leaf extract, which is a natural antioxidant, increases the levels of GPx, GSH and CAT in kidney damage induced in rats [46]. Similarly, in our study, an increase in GPx, GSH and CAT enzyme activity levels was observed in rats treated with omega-3 fatty acids. It is suggested that this effect is due to the fact that omega-3 fatty acids strengthen the renal antioxidant defense mechanisms.

Studies show that TAS, TOS and MDA parameters are important markers for diseases associated with oxidative stress [44, 47]. While the increase in TAS value indicates antioxidant activity, the increase in TOS values is an indicator of oxidant activity [48]. MDA is one of the molecules that arise as a result of lipid peroxidation and is one of the parameters used to mark the oxidative damage that occurs in the tissue [47]. The antioxidant defense system is involved in clearing reactive oxygen species, which play a major role in the initiation of lipid peroxidation [49]. It is known that CIN causes oxidative stress and increases MDA levels in serum and

tissue [50, 51]. We observed a decrease in both tissue and serum MDA levels in rats treated with omega-3 fatty acids. In addition, while the TOS level was higher in the urography group compared to the control group, a decrease in TAS level was observed. Conversely, in the group treated with omega-3, the TAS level increased and the TOS level decreased. As a result, it was determined that urography exposure caused oxidative stress in tissues and this situation was improved with omega-3 treatment.

**Limitations.** There are certain limitations to be mentioned in this study. First, this study is an experimental animal model and the number of rats was kept low for ethical reasons. Second, the efficacy of omega-3 fatty acids on CIN has been studied by biochemical methods and is not supported by histopathological findings. Histopathological studies on this subject are needed. We applied the omega-3 dose as 400 mg/kg. New studies with different doses are needed in order to determine optimal dose ranges and toxic dose levels. This is the third limitation.

**Conclusions.** In this study, which we conducted at the biochemical level on serum and kidney tissues of rats, we observed that omega-3 fatty acids have therapeutic effects on CIN. Therefore, we consider that omega-3 fatty acids can be used as an alternative to other supportive therapies in CIN. However, further studies are needed to use these acids in optimal dose ranges in the treatment of CIN.

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Financial Disclosure:** All authors declare no financial support.

#### Author Contributions

**Gunes Bolatli:** Data collection, analyses, write up;

**Mahinur Ulusoy:** Design, data collection;

**Fatih Tas:** Literature review, write up;

**Naci Omer Alayunt:** Analyses, write up;

**Ismail Zarasiz:** Concept, data collection.

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