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# Evaluation of the Nephroprotective Effect of Omega-3 Polyunsaturated Fatty Acids on Gentamicin-Induced Renal Toxicity in Albino Wistar Rats

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**Abstract:** The kidney performs several important functions in the body. Its failure leads to a dysfunction that deserves a solution. Thus, this study was initiated to investigate the protective effects of omega-3 fatty acids on nephrotoxicity. For this purpose, 48 Albino Wistar rats (male and female) were divided into 8 groups of 6 rats. The first group, serving as a control, received 1 mL/g body weight of distilled water daily by gavage and a 0.9% NaCl solution intraperitoneally one hour later. The second, received gentamicin at a dose of 80 mg/Kg/day by intraperitoneal injection for 7 days, the third, treated with the combination of omega 3 by gavage at a dose of 200 mg/Kg of body weight plus gentamicin by intraperitoneal injection at a dose of 80 mg/Kg for the same period. The fourth, treated with the combination of omega 3 by gavage at a dose of 600 mg/Kg body weight plus gentamycin by intraperitoneal injection at a dose of 80 mg/Kg for 7 days, the fifth, treated with the combination of vitamin E at a dose of 250 mg/kg/day body weight by gavage plus gentamicin by intraperitoneal injection at a dose of 80 mg/Kg for 7 days. The sixth and seventh received 200 mg/kg and 600 mg/kg body weight by gavage of omega-3 for 7 days, respectively. The last group received vitamin E at a dose of 250 mg/kg/day of body weight by gavage. Omega-3 at a dose of 600 mg/kg body weight exerts a protective effect against induced nephrotoxicity in rats (especially females), with a decrease in urea and creatinine levels. The consumption of food rich in omega 3 in the protection of the kidneys advised. Further studies could be envisaged to compare the protective effects of omega-3 with other polyunsaturated fatty acids (omega-6 and 9) on nephrotoxicity.

**Keywords:** Gentamicin, Nephrotoxicity, Kidney, Omega-3

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## 1. Introduction

The kidney is an essential organ in the body. It plays several essential roles, particularly in the elimination of waste products and exogenous chemical substances, thus participating in the homeostasis of the body. Renal failure or renal insufficiency has become today one of the public health problems affecting all classes of society from industrialized countries to developing countries (prevalence of renal disease very high in Africa) associated with a high morbi-mortality

[1, 2]. Indeed, renal pathologies are currently increasing due to the living conditions of men (age, lifestyle, chronic pathology, self-medication, environmental conditions, etc.) or the potential exposure to many nephrotoxic substances [3]. In order to face these major health problems, humans are looking for ways to effectively treat these serious diseases (cardiovascular disease, liver disease, kidney failure etc.) in order to prolong life, whenever possible, without creating additional risks or side effects [4]. Unfortunately, the high cost of synthetic drugs and food additives puts them out of reach of populations (especially those in developing

countries) [5]. Today, in order to fight against this major public health problem, several sensitizations (especially at the level of nutrition) are carried out among the populations in order to reduce this high morbi-mortality. Many synthetic antioxidants such as Butylated hydroxyanisole, Butylated hydroxytoluene and many others have been widely used as food additives and their use has given rise to several questions concerning their toxicity. In this sense, preventive medicine is increasingly interested in the development of antioxidants of natural origin. This is the case today with essential fatty acids such as omega-3 and omega-6 [6]. In recent years, polyunsaturated fatty acids (food additives), and more particularly the omega-3 and 6 series, have been the subject of numerous studies which have shown positive effects on heart, pancreas and kidney diseases, etc. Studies have also shown that omega-3 and 6 play an important role in the inflammatory response by allowing the synthesis of modulatory molecules (eicosanoids and cytokines). This interest was partly born from the observation that cardiovascular diseases and atherosclerosis only slightly affect populations whose diet is particularly rich in fish. It is in fact in fish oils that we find the highest concentration of omega-3. A German study has shown that the consumption of an average of 30g of fish per day reduced heart disease by 50%. [7, 8]. Today, omega-3 serve a large segment of the world's population as an essential means of medication. The importance of omegas as a very important platform deserves to be explored in the field of research of their nephroprotective actions [9, 8]. Therefore, in this study, it is necessary to study the polyunsaturated fatty acids of the n-3 series belonging to the lipidic compounds, which are widely used in countries to prevent several diseases.

## 2. Material and Methods

### 2.1. Animal Material

The animal material consisted of albino rats of the wistar strain. They were provided by the laboratory of endocrinology of the University Félix Houphouët Boigny of Cocody, Abidjan.

### 2.2. Products Used

Gentamicin was used for the induction of nephrotoxicity. Vitamin E was used as a reference molecule in the treatment of the studied pathology.

### 2.3. Methods

#### 2.3.1. Experimental Conditions and Batch Constitution

Animals were raised in an animal facility at room temperature.

#### 2.3.2. Treatment of the Animals

Treatments were performed daily at the same time for 7 days for the nephrotoxicity test. The animals were deprived of food for 12 hours and water for only one hour before the treatments. They were fed one hour after the treatments and

weighed daily for the nephrotoxicity test during the experimental period [10, 11].

### 2.3.3. Experimentation

The experiment was performed with 48 adult rats. These animals were divided into 8 groups of 6 rats. The experimentation was done as follows:

Nephrotoxicity test:

Group 1 (control): This lot was treated by gavage with 1mL/g body weight of distilled water and intraperitoneally with 0.9% NaCl solution one hour later for 7 days.

Group 2 (negative control or gentamicin control): Rats in this batch were treated by gavage with 1 mL/g distilled water and then 1 h after gentamicin 80 mg/kg body weight intraperitoneally for 7 days.

Group 3: In this lot, rats were treated with 250 mg/kg/day body weight of vitamin E by gavage. And injected intraperitoneally with 80 mg/kg gentamicin 1h after for 7 days.

Group 4: Lot 6 was treated with 200 mg/kg/day body weight of omega-3 by gavage and then intraperitoneally with 80 mg/kg gentamicin 1 hour later for 7 days.

Group 5: Animals were treated by gavage with 600 mg/kg/day body weight of omega-3. 1h after 80 mg/kg gentamicin was injected to the animals intraperitoneally for seven days.

Group 6: animals were treated by gavage with 200 mg/kg/day of omega-3 for seven days.

Group 7: 600 mg/kg/day of omega-3 was used by gavage to treat the rats in this batch for seven days.

Group 8: This batch was treated with 1ml/g body weight of vitamin E at 250 mg/kg/day by gavage for seven days.

### 2.4. Sampling

All test animals were weighed and euthanized 24 hours after induction of gentamicin toxicity. 1mL of blood from each animal was collected in a tube without anticoagulant to assay biochemical parameters. Heart, kidney and liver were collected.

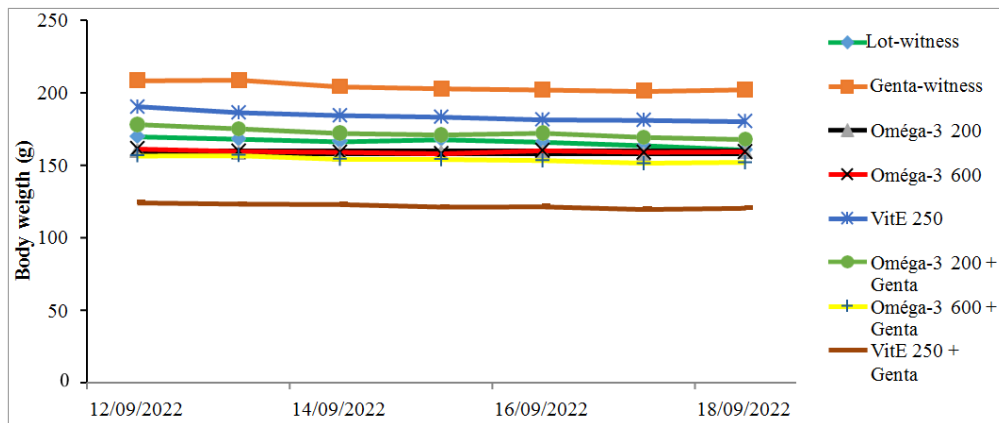
### 2.5. Serum Biomarker Assay

Blood samples collected in the anticoagulant-free tubes were centrifuged at 3000 rpm for 15 minutes. The collected sera were used to assay biochemical parameters such as urea, creatinine, and total protein using a Cobas C 311 HITACHI from ROCHE Diagnostic France.

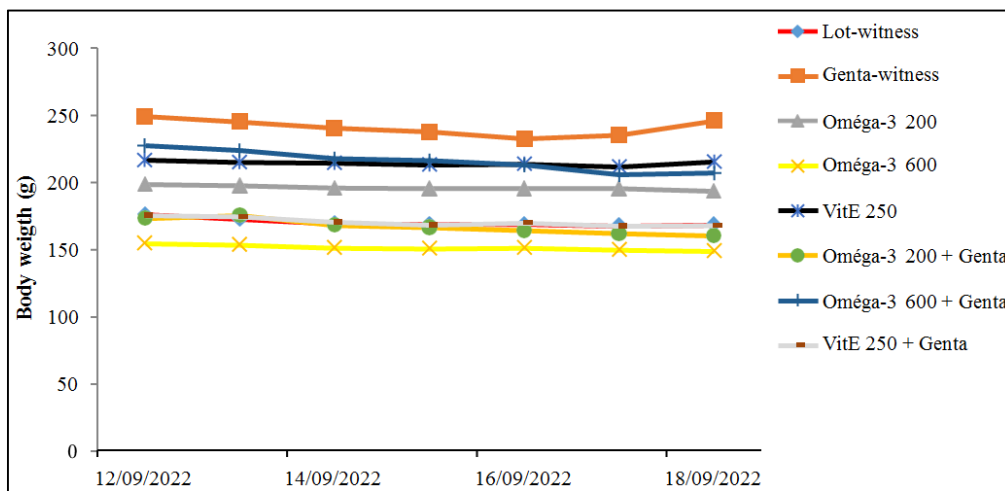
### 2.6. Statistical Analysis

The results were expressed as mean values with standard errors on the mean (Mean  $\pm$  SEM). The comparison of the different batches was performed using analysis of variance (ANOVA ONE WAY). Differences between means were determined using the Turkey multiple comparison test. These differences were considered significant when p-value was less than 0.05 ( $p < 0.05$ ). The results were presented in tables and figures. Graphical representation of the data was performed

using Graph Pad Prism 7.0 software (Microsoft USA).



a. Effet of oméga-3 and vitamin E on weighth gain in female rats.



b. Effet of oméga-3 and vitamin E on weighth gain in male rats.

**Figure 1.** Effet of oméga-3 and vitamin E on weighth gain.

valeurs are means ± ESM (standart error on the mean) with n= 6. \*P < 0,05; \*\*P < 0,01; \*\*\*P

< 0,001: significant difference from lot witness-Genta. # P < 0,05; ## P < 0,01; ### P < 0,001: significant difference from lot- witness

Witness: distilled water + NaCl; witness-Genta: distilled water + Gentamicin; Oméga-3 200: oméga-3 dosed at 200 mg/kg; Oméga-3 600: oméga-3 dosed at 600 mg/kg; Vit E 250: vitamin E dosed at 250 mg/kg; Oméga-3 200 + Genta: oméga-3 dosed at 200 mg/kg+ Gentamicin; Oméga-3 600 +Genta: oméga-3 dosed at 600 mg/kg + Gentamicin; Vit E 250 + Genta: vitamine E dosed at 250 mg/kg + Gentamicin

### 3. Result

#### 3.1. Effects of the Different Treatments on the Body Weight of the Animals

The variation of the body weight of the animals according to the treatments is presented in figure 1 (a: in females and b: in males). From the analysis of the results, it appears that all the animals lost weight from day 1 to day 6. From the 7th day of the experiment, a recovery of the animals' weight was observed.

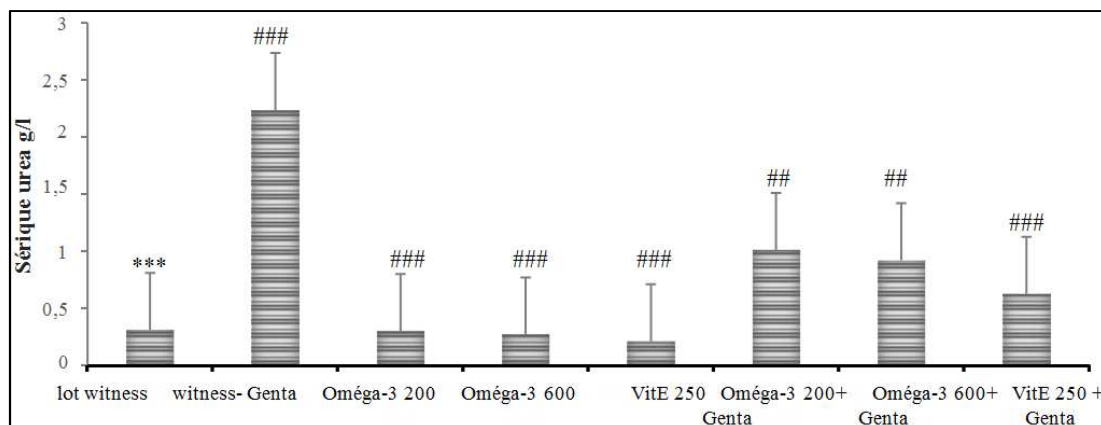
#### 3.2. Effects of Different Treatments on Serum Urea Levels

The serum urea levels of the animals during the treatments are shown in Figure 2 (a: females and b: males).

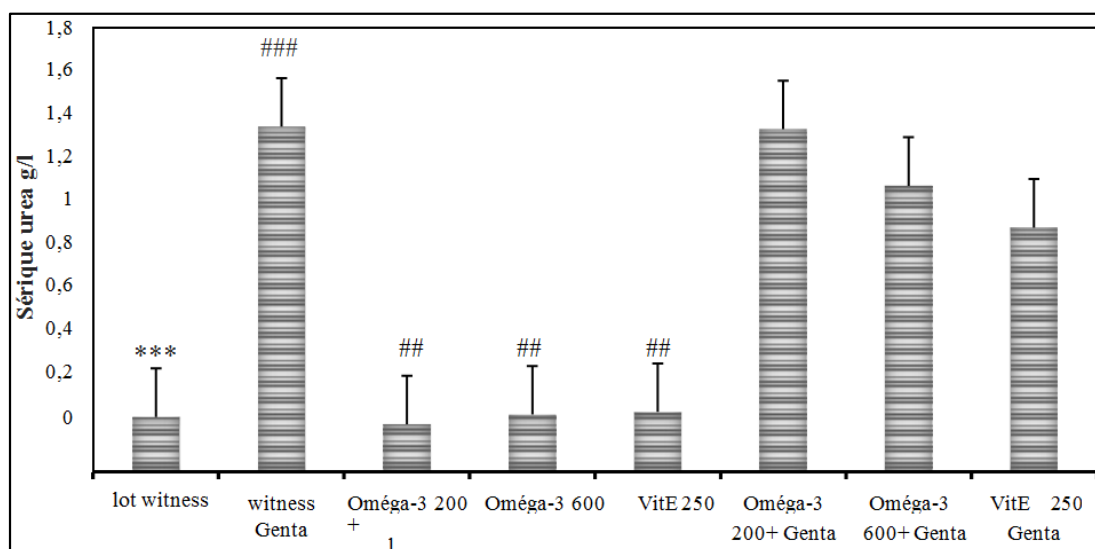
The results of the serum urea determination in female and male rats show that the urea level of gentamicin control rats (intoxicated with gentamicin and not treated; values 2.23 g/L in females and 1.4 g/L in males) is higher than that of unintoxicated (0.21 g/L in females and 0.24 g/L in males), intoxicated (0.62g/L in females and 0.99 g/L in males) and vitamin E treated controls. On the other hand, the serum urea level of animals treated with omega-3 (dosed at 200 and 600 mg/kg body weight) gave lower serum urea levels in the batches of intoxicated animals and those treated with the different doses of omega-3 than in the animals treated with gentamicin (males and females). The lot treated with omega-3 at 200 mg/kg body weight and not intoxicated had a serum urea level identical to that of the control without gentamicin. The lot treated with omega-3 at 600 mg/kg body weight and not intoxicated had a serum urea level

identical to that of the control without gentamicin, as did the lot treated with vitamin E (at 250 mg/kg body weight) and not intoxicated. Overall, Figure 2 shows that the 600

mg/kg body weight dose of omega-3 has a better effect than the 200 mg/kg body weight dose in both female and male rats.



a. Effet of oméga-3 and vitamin E on sérique concentration urea to female rats



b. Effet of oméga-3 and vitamin E on sérique concentration urea to male rats

Figure 2. Effect of omega-3 compounds; and vitamin E on serum urea concentration.

Values are means  $\pm$  SEM (standard error on the mean) with  $n=6$ .

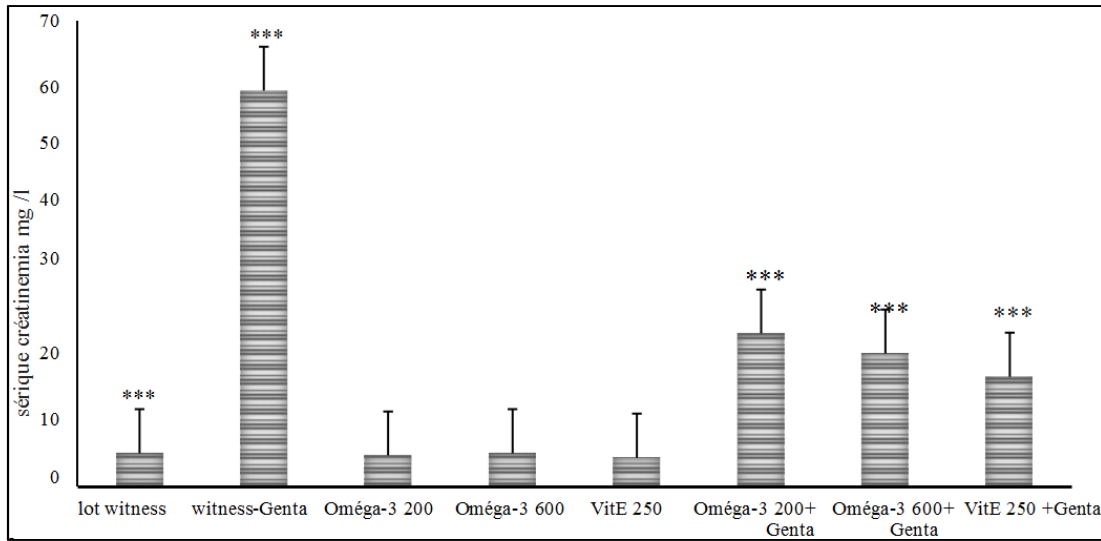
\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; # Significant difference from T Genta batch. #  $P < 0.05$ ; ##  $P < 0.01$ ; ###  $P < 0.001$ : \*Significant difference from batch T Control: distilled water + NaCl; Genta control: distilled water + Gentamicin; Omega-3 200 alone: omega-3 at 200 mg/kg alone; Omega-3 600 alone: omega-3 at 600 mg/kg alone; Vit E 250 alone: vitamin E at 250 mg/kg alone; Omega-3 200 + Genta: omega-3 at 200 mg/kg+ Gentamicin; Omega-3 600 +Genta: omega-3 at 600 mg/kg + Gentamicin; Vit E 250 Genta: vitamin E at 250 mg/kg + Gentamicin

### 3.3. Effects of the Different Treatments on Serum Creatinine Levels

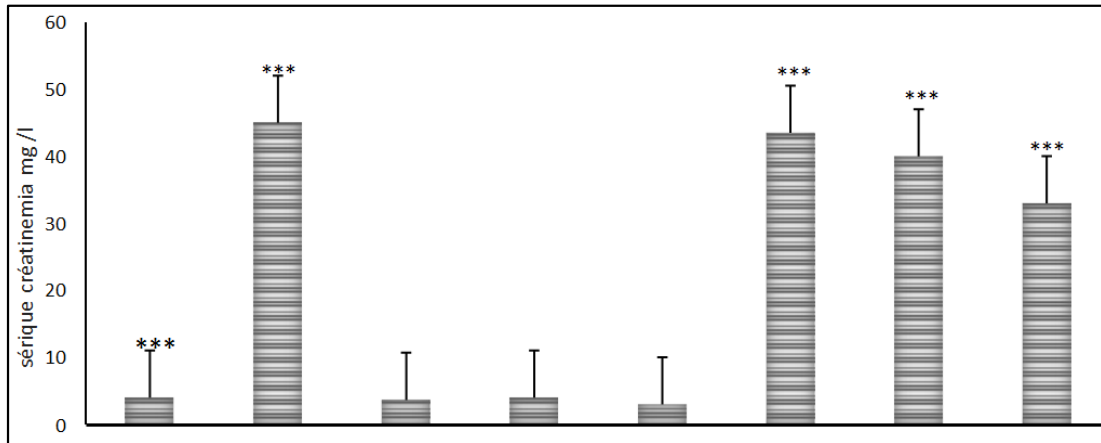
The serum creatinine levels of the animals during the treatments are shown in Figure 3 (a: females and b: males). Results of serum creatinine determinations in female and male rats show that the creatinine level of the untreated gentamicin-intoxicated control (59.5 mg/L in females and 45 mg/L in males) was higher than that of the unintoxicated (4.33 mg/L in females and 3 mg/L in males) and vitamin E-treated (16.5 mg/L in females and 24.66 mg/L in males) controls. On the other hand, administration of omega-3 at

200 and 600 mg/kg body weight to intoxicated animals resulted in low serum creatinine levels. These levels were lower than those of the gentamicin-intoxicated control animals (males and females). The lot treated with omega-3 at 200 mg/kg body weight and not intoxicated had a serum creatinine level identical to that of the control without gentamicin. The lot treated with omega-3 at 600 mg/kg body weight and not intoxicated had a serum creatinine level identical to that of the control treated with distilled water and that of the lot treated with vitamin E (at 250 mg/kg body weight) and not intoxicated. Overall, the 600 mg/kg body weight dose of omega-3 had a better effect than the 200

mg/kg body weight dose in both female and male rats.



a. Effet of oméga-3 and vitamin E on sérique concentration of créatinemia to female rats

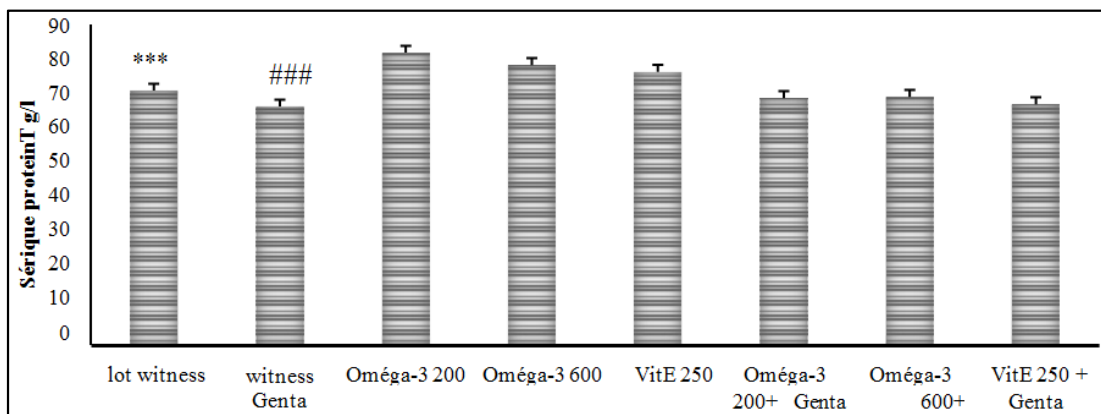


b. Effet of oméga-3 and vitamin E on sérique concentration of créatinemia to male rats

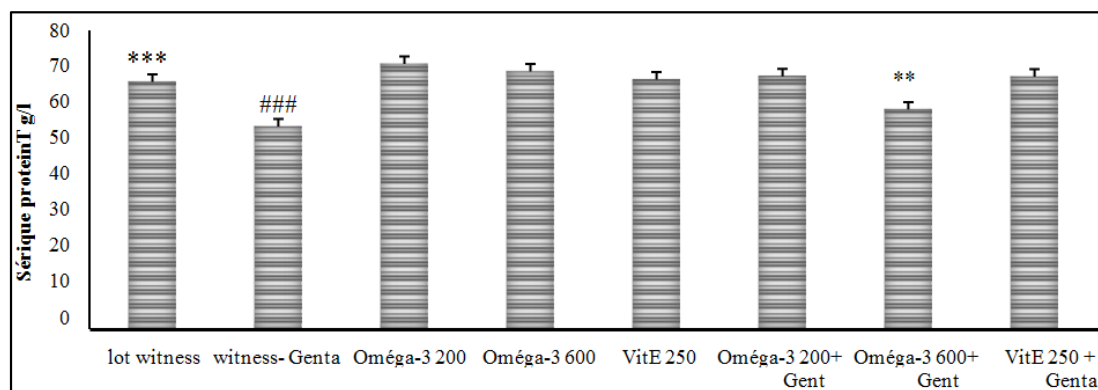
**Figure 3.** Effect of omega-3 compounds; and vitamin E on serum creatinine concentration.

Values are means ± SEM (standard error on the mean) with n= 6. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; #Significant difference from T Genta batch. # P < 0.05; ## P < 0.01; ### P < 0.001; \*Significant difference compared to lot T

Control: distilled water + NaCl; Genta control: distilled water+ Gentamicin; Omega-3 200 alone: omega-3 at 200 mg/kg alone; Omega-3 600 alone: omega-3 at 600 mg/kg alone; Vit E 250 alone: vitamin E at 250 mg/kg alone; Omega-3 200 + Genta: omega-3 at 200 mg/kg+ Gentamicin; Omega-3 600 +Genta: omega-3 at 600 mg/kg + Gentamicin; Vit E 250 Genta: vitamin E at 250 mg/kg + Gen



a. Effet of oméga-3 and vitamin E on sérique concentration of total protein to female rats



b. Effet de oméga-3 and vitamin E on sérique concentration of total protein to male rats

**Figure 4.** Effect of omega-3 compounds and vitamin E on serum total protein concentration in male and female rats.

Control: distilled water + NaCl; Genta control: distilled water + Gentamicin; Omega-3 200 alone: omega-3 at 200 mg/kg alone; Omega-3 600 alone: omega-3 at 600 mg/kg alone; Vit E 250 alone: vitamin E at 250 mg/kg alone; Omega-3 200 + Genta: omega-3 at 200 mg/kg+ Gentamicin; Omega-3 600 +Genta: omega-3 at 600 mg/kg + Gentamicin; Vit E 250 Genta: vitamin E at 250 mg/kg + Gen

### 3.4. Effects of Different Treatments on Serum Total Protein Levels

Figure 4 (A: in females and B: in males) shows the results of serum total protein assay of treated animals. From the analysis of the results, it appears that the total protein level in female and male rats in the gentamicin-treated control lot is low (67 g/L in females and 54.9 g/L in males) compared to the distilled water-treated control lot (71.4 g/L females and 66.26 g/L males) and the vitamin E-treated intoxicated lot (78.9 g/L females and 68.63 g/L males). Also, the results reveal that the omega-3 and vitamin E treated batches, all intoxicated and not intoxicated, have a total protein level statically identical ( $p > 0.05$ ) to that of the control treated with distilled water.

## 4. Discussion

Studies of omega-3s on the kidney suggest beneficial effects from consumption of marine products rich in polyunsaturated fatty acids [12]. Gentamicin is a bactericidal antibiotic of the aminoglycoside family. It has been shown that up to 30% of patients treated with gentamicin for more than 7 days show signs of renal failure. The nephrotoxicity of gentamicin has been studied in several experimental models such as rabbits, mice and rats. Different researchers have used different doses, ranging from 8 to 80 mg/kg/day to produce renal damage [13]. In an attempt to protect or reverse the renal damage caused by gentamicin, several strategies and various agents have been used along with the antibiotic, with varying degrees of success. But at present, there is no specific treatment with reliable protection against gentamicin-induced nephrotoxicity. In addition, several *in vivo* and *in vitro* experimental studies have been published on the involvement of renal mitochondria in gentamicin nephrotoxicity and the role of molecules with antioxidant powers from medicinal plants and flavonoids in either

mitigating or preventing this nephrotoxicity [14, 15]. The literature on Polyunsaturated Fatty Acids (PUFAs) of the omega-3 series has revealed that these molecules possess antioxidant properties. In view of these findings, the objective of this study was to investigate the nephroprotective activity of omega-3 on rats affected by nephrotoxicity by gentamicin administration by measuring biochemical parameters such as urea, creatinine and total protein in blood. Body weight analysis of the animals indicated a progressive loss of body weight in all animals with a slow recovery of body weight from day 6 in male rats. Indeed, this loss is due to the conditions of the treatments (12h of fasting before the treatment). This shows that Vitamin E and Gentamicin had no effect on the body weight of the animals during the treatment. Gentamicin induces a nephrotoxicity characterized by the elevation of biochemical markers, such as urea and creatinine, which are the main diagnostic criteria of renal failure. Indeed, the results obtained are in agreement with those obtained by Zaoui & Bouleghlimat and Gilbert *et al.* [14, 16] whose daily administration of gentamicin at a dose of 80 mg/kg for a period of 7 days caused a highly significant increase ( $P < 0.05$ ) in the concentration of creatinine and plasma urea in rats treated with Gentamicin compared to the control lot. Furthermore, plasma creatinine concentration is a more powerful indicator than urea in the evaluation of nephropathy [16]. However, the significant elevation of urea and creatinine in the gentamicin-treated group of rats represents an indicator of severe tubular necrosis [17]. Also, the serum total protein concentration in the same batch undergoes a significant decrease compared to the other batches. In this study, it was found that oral administration of omega-3 at a daily dose of 200 mg/kg/body weight and 600 mg/kg/body weight (omega-3 batch 200+Genta and omega-3 batch 600+Genta) significantly attenuated gentamicin-induced nephrotoxicity, especially in female rats. Better still, it significantly ( $P < 0.01$ ) decreased serum urea and creatinine levels (in females and from in males)

compared to gentamicin-treated controls. In In 2012, Khan *et al.* [18] during their work studied the protective effects of n-3 unsaturated fatty acids against oxidative nitrite-induced nephric damage and toxicity in rats. Their results showed that omega-3 rich fish oil reduced oxidative damage and sodium nitrite-induced nephrotoxicity by lowering plasma creatinine and urea levels. In the study conducted by Fassett *et al.* [19], omega-3 fatty acids were shown to be beneficial in the treatment of kidney disease. The results of this work are in agreement with those obtained in the present study. The results obtained with the animals treated with vitamin E +Gentamicin showed that vitamin E protected the rats (male and female) against Gentamicin-induced nephrotoxicity. In fact, vitamin E significantly reduced plasma creatinine and urea levels compared to the control treated with Gentamicin alone. These results confirm several studies which concluded that vitamin E was a powerful antioxidant preventing the peroxidation of unsaturated fatty acids and the deposition of lipid residues in tissues. Indeed, a deficiency in vitamin E would lead to numerous signs such as amyotrophy, muscle degeneration, absence of spermatogenesis, infertility, stillbirth, brown pigmentation of intestinal smooth muscles and a decrease in immune defenses [20]. The results also showed that female rats were better protected than males and the omega-3 dose of 600mg/kg/body weight/day had a better effect than that of 200 mg/kg/body weight/day. In fact, this high protection of female rats is due to several environmental and internal factors, one of which is the production of hormones that promote the activity of omega-3s.

## 5. Conclusion

Nephrotoxicity in this study was demonstrated in rats treated with nephropathy-inducing gentamicin in order to evaluate the nephroprotective effect of omega-3. The present study showed that omega-3s dosed at 600 mg/kg body weight exerted a protective effect against nephropathy induced in rats (especially females), with a significant effect on urea and creatinine. Omega-3s are part of the foods we can consume daily. Even though current studies do not always agree on the statistical significance of the protective effect of these nutrients on the various pathologies tested, it would be good to raise public awareness of the need to consume more fish, algae, rapeseed and shellfish, as has been done for some time for fruit and vegetables.

## Conflicts of Interest

The author declares no conflict of interest regarding the publication of this article.

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