

## Original Article



# Effect of Fish Oil on The Serum Urea Level in Gentamicin Induced Nephrotoxicity in Long Evans Rats

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### Abstract

**Background:** Gentamicin is used worldwide as bactericidal agent against severe gram negative infections. Gentamicin induced nephrotoxicity can be minimized by administration dietary fish oil. **Objectives:** The present study was designed to observe the possible potential effect of Fish oil on gentamicin-induced nephrotoxicity in Long Evans rats. **Materials and Methods:** The experimental study was carried out in the department of anatomy at Dhaka Medical College (DMC) among 40 Long Evans rats of both sex with the weight ranges from 172-255 gm and the age ranges from 7 to 10 weeks. The rats were divided into four groups-Group A (normal control) received normal saline, group B, C and D received gentamicin for 6 days. Rats of group C received fish oil capsule for 9 days with gentamicin and group D received fish oil capsule with gentamicin for total 10 days. Serum urea level was measured at the end of experiment. **Results:** The serum urea (mean±SD) levels in group A, B, C and D was 4.79±0.32, 12.41±1.22, 6.95±0.91 and 6.18±1.00, respectively. The gentamicin treated rats (group B) had shown increased serum urea levels in comparison to fish oil (group C&D) treated rats. The difference between groups were highly significant ( $P<0.001$ ) for group A&B, A&C, B&C and B&D. The difference between groups A&D ( $P<0.01$ ) were moderately significant. Whereas the difference between C&D ( $P>0.05$ ) was not significant. **Conclusion:** Fish oil treatment showed some protective effects against gentamicin induced nephrotoxicity. The results also indicated that effectiveness of fish oil depends on a suitable duration of pretreatment.

**Key words:** Gentamicin, Fish oil, Nephrotoxicity.

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### Introduction

One of the major excretory organs of human body is the kidney. The kidneys contribute for the maintenance of homeostasis by a complex process that involves filtration, reabsorption, secretion and the ultimate final product is urine.<sup>1</sup> As the kidneys concentrate and excrete metabolic waste, chemicals and many drugs and it is often exposed to a toxic concentration of these substances and chemicals that can lead to nephrotoxicity.<sup>2</sup> A variety of drugs produces nephrotoxicity.<sup>3,4</sup> Drug induced nephrotoxicity can cause acute renal failure.<sup>5</sup> The global review of six regions, including Bangladesh has reported overall 14% prevalence of kidney diseases.<sup>6</sup> Out of 18 million 35,000 to 40,000 Chronic Kidney Disease (CKD) patients, develops kidney failure in each year in Bangladesh.<sup>7</sup> Drugs can cause nephrotoxicity by altering

the intra-glomerular hemodynamics and by decreasing GFR.<sup>8,9</sup> Certain drugs like ampicillin, ciprofloxacin, Nonsteroidal anti-inflammatory drug (NSAIDs), rifampicin, gentamicin etc. can cause nephrotoxicity.<sup>10</sup>

Gentamicin is an antibiotic that exhibits a broad spectrum of activity and is particularly valuable in severe sepsis.<sup>11</sup> Gentamicin is the first choice aminoglycosides because of its low cost and reliable activities against gram negatives aerobes.<sup>12</sup> This drug is excreted mainly through urine.<sup>13</sup> The range between effective use and toxic concentration level is narrow, thus more than 40% chances of nephrotoxic effects in aminoglycosides treated patients.<sup>14</sup> So monitoring of blood level is recommended for avoidance of toxicity with these drugs to assure effective therapy.<sup>15</sup>

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In Bangladesh the typical diet consists of rice, vegetables and fish. 64% of total food intake is rice, 30% is vegetables, 6% is animal food and 53% of animal foods are made up by fish, which contributes 23g/capita/d.<sup>16</sup> In our country fish is the main and cheapest animal protein source.<sup>17</sup> Fish are the excellent sources of vitamin A, minerals and fat.<sup>18</sup> Recent findings suggest that, intake of fish and fish oil have beneficial effects on human health.<sup>19,20</sup> A number of path-physiological disorders like coronary heart disease, atherosclerosis and hypertension are reduced due to fish oil intake. Because the fish oil contains characteristic long chain polyunsaturated fatty acid (PUFA) or omega-3 as the major nutritional components related to human health and they have effect on lowering plasma triglyceride level and increasing beneficial HDL-cholesterol level in man.<sup>20,21</sup> It is also found that fish oil can protect against cyclosporine nephrotoxicity in rats and in renal transplant recipient against proteinuria in passive Heymann nephritis.<sup>22,23,24</sup>

Gentamicin induced acute renal failure is one of the kidney diseases which is common worldwide. Each year many patients die due to acute renal failure in Bangladesh of which 60% causes are related to drugs. A large number of people are suffering from kidney disease and this number is increasing day by day. Gentamicin is the aminoglycoside of choice because of its low cost and its reliable activity against gram-negative aerobes. In case of poor patients amoxicillin, gentamicin and metronidazole therapy still the popular drug of choice after surgery and other serious gram-negative bacillary infections. In Bangladesh 53% of animal foods are made up by fish and it is the main and cheapest animal protein source. As the fish oil is composed of eicosapentaenoic acid, docosahexaenoic acid and many more may be one or more of these compounds has got nephro-protective action. Thus it is thought that it may help to gentamicin-induced nephrotoxicity. This study may ensure the clinicians about safe use of gentamicin among those patients who are resistant to other antibiotics.

## Materials and Methods

This experimental study was carried out in the Department of Anatomy in Dhaka Medical College, Dhaka, Bangladesh during the period from February 2005 to January 2006, on 40 healthy long Evans rats of both sex with the weight ranges from 172-255 grams and the age ranges from 7 to 10 weeks. They were allowed to live on normal room temperature under the conditions of normal natural light and dark schedule. They were fed on pellets of standard rat foods. Drugs used were gentamicin, fish oil and normal saline (0.9% NaCl). Distilled water was used as vehicle for both the drugs. The rats were divided into four groups and randomly selected. Grouping of the rats were done according to treatment pattern. Group A (Normal control): The rats of this group received normal diet and injections of normal saline intramuscularly (2ml/kg/day) for 6 days without any drugs treatment. All the rats were sacrificed on 7<sup>th</sup> day. Group B (Experimental

control): The rats of this group received normal diet and injection of gentamicin intramuscularly (80mg/kg/day) for 6 days. All the rats were sacrificed on the 7<sup>th</sup> day. Group C (Experimental): The rat of this group received normal diet and fish oil capsule orally (5.0 ml/kg/day) for total 9 days (pretreatment for 3 days + simultaneous treatment with gentamicin for 6 days) and gentamicin were injected intramuscularly (80 mg/kg/day) during the last 6 days of the 9-day period. All the rats were sacrificed on the 10th day that is 24 hours after gentamicin administration. Blood samples were collected by cardiac puncture from each rat in separate test tubes and serum was separated, centrifuged and investigated for biochemical parameters. Group D (Experimental): The rat of this group received normal diet and fish oil capsule orally (5.0 ml/kg/day) for total 10 days (pretreatment for 4 days + simultaneous treatment with gentamicin for 6 days) and gentamicin were injected intramuscularly during the last 6 days of the 10-day period. All the rats were sacrificed on the 11th day that is 24 hours after gentamicin administration. Blood samples were collected by cardiac puncture from each rat in separate test tubes and serum was separated, centrifuged and investigated for biochemical parameters. The animals were sacrificed on the fixed day under chloroform anesthesia by cervical dislocation. Kidneys were collected after the opening of abdomen. Blood was obtained by cardiac puncture using 5cc syringe from each rat in separate test tubes and allowed to clot for one hour at room temperature. The serum was separated and preserved. Estimation of serum urea was done by chemical method that is Diacetylmonoxime method. Data were analyzed by SPSS version 12.0 for Windows. Standard deviation (SD) and mean of the collected Data were calculated for paired student's 't' test and comparison between the groups were made by using ANOVA test.

## Results

The (mean±SD) serum urea levels in group A, B, C and D was 4.79±0.32, 12.41±1.22, 6.95±0.91 and 6.18±1.00, respectively. The gentamicin treated rats (group B) showed increased serum urea levels in comparison to fish oil (group C&D) treated rats. The differences between groups were highly significant ( $P<0.001$ ) for groups A&B, A&C, B&C and B&D. The difference between groups A&D ( $P<0.01$ ) was moderately significant, whereas the difference between C&D ( $P>0.05$ ) was not significant. The results indicating that the normal saline control group (group A) of rat's serum urea level was within the normal limit (4.79 mmol/l). But the gentamicin treated rats (group B) showing the blood urea level abnormally high (12.41mmol/l). On the other hand the fish oil treated rats showed the values were relatively lower in comparison to gentamicin treated rats. But the values of group C was significantly higher ( $P<0.001$ ) in comparison to normal saline control rats and only group D showed that the value was moderately significant ( $P<0.01$ ) in relation to normal saline control rats (group A). (Table-1)

**Table I:** Serum urea levels in different group of rats

Group (n=10)	Serum urea in mmol/l (Mean±SD)
A	4.79±0.32 (4.3-05.40)
B	12.41 ± 1.22 (10.80-14.60)
C	6.95±0.91 (5.40-7.80)
D	6.18±1.00 (4.50-7.60)
P value	
A vs B <0.001***	B vs C <0.001*** C vs D >0.05 ns
A vs C <0.001***	B vs D <0.001***
A vs D <0.01**	

Values in parenthesis indicates range

Statistical analysis done by ANOVA (multiple comparisons)

ns = not significant \*/\*\*/\*\*\* = significant

Group A : Normal Control (normal saline only)

Group B : Experimental control (gentamicin only)

Group C : Experimental (fish oil + gentamicin)

Group D : Experimental (fish oil + gentamicin)

## Discussion

Gentamicin is still used as a first and second choice of drug in clinical practice due to low cost despite of having nephrotoxic effects. Moreover, this aminoglycosides has been widely used to study the nephrotoxicity, both in experimental animals and human beings.<sup>25</sup> Present study revealed the severity of nephrotoxicity by assessing the serum urea level among different group of rats. It was found that the mean serum urea level was high in gentamicin treated rats than comparison to normal control group. This biochemical proof of nephrotoxicity produced by gentamicin was seen in several research works.<sup>26,27</sup>

Present study also showed that, serum urea concentrations in case of fish oil with gentamicin group were not significantly different from those of normal saline control or only fish oil groups, but significantly lower than those of only gentamicin treated group. But fish oil at a dose of 5.0 ml/kg/day orally for 10 days partially protected against nephrotoxicity that was induced by gentamicin during the last 6 days of treatment with fish oil. Similar finding was observed in several studies.<sup>28,29</sup>

Present study showed that fish oil has partial protection against the nephrotoxicity caused by gentamicin. During the last 6 days of treatment of nephrotoxicity with fish oil showed that the urea concentration was returning to nearly normal. The results also indicate that fish oil was less effective in less duration of pretreatment, which means the pretreatment duration must be increased to a suitable period for better protection against gentamicin induced nephrotoxicity.

## Conclusion

Fish oil treatment showed some protective effects against gentamicin induced nephrotoxicity. The results also indicated that effectiveness of fish oil depends on a suitable duration of pretreatment. Further study with large sample may evaluate more beneficial effects of omega-3 of fish oil which is recommended for the health issue among the gentamicin induced kidney failure patients.

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