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# Nephroprotective Effect of Nigella Sativa Linn (black seed) and Ramipril in Gentamicin Induced Nephrotoxic Rats: A Comparative Experimental Study

Mosfika Mahjabin[1], Zaida Rahman[2], Halima Begum[3], Sonia Akter[4], Sharmin Jahan Khan[5], Md Shamsur Rahman[6], Bhuiyan Mohammad Mahtab Uddin[7]

[1]Assistant Professor, Department of Pharmacology & Therapeutics, Enam Medical College, Savar, Dhaka, Bangladesh; [2]Professor, Department of Pharmacology & Therapeutics, Enam Medical College, Savar, Dhaka, Bangladesh; [3]Professor, Department of Pharmacology & Therapeutics, Enam Medical College, Savar, Dhaka, Bangladesh, [4]Associate Professor, Department of Surgery, Enam Medical College & Hospital, Savar, Dhaka, Bangladesh; [5]Assistant Professor, Department of Pharmacology & Therapeutics, Mugda Medical College, Dhaka, Bangladesh; [6]Consultant, Department of Physiotherapy, Unihealth Specialized Hospital Ltd, Dhaka, Bangladesh; [7]Associate Professor, Department of Microbiology, Enam Medical College, Savar, Dhaka, Bangladesh.

**Abstract**

**Background:** Acute kidney injury is one of the significant public health issues in patients treated with gentamicin in Bangladesh. Black seed has some nephroprotective effects that can alleviate gentamicin induced nephrotoxicity. **Objectives:** This study was carried out on a group of rats to evaluate the nephroprotective effect of black seed. **Methodology:** This experimental study was conducted to demonstrate the effect of ethanolic extract of black seed on blood urea and serum creatinine levels as well as histopathological changes in gentamicin induced nephrotoxic rats and the nephroprotective effects were compared to a nephroprotective drug, ramipril. **Results:** Blood urea and serum creatinine levels were significantly higher than the normal in gentamicin treated group. There were significant differences in blood urea and serum creatinine levels as well as histopathological changes in gentamicin to black seed and ramipril treated groups. Another test revealed no significant differences in blood urea and serum creatinine levels as well as histopathological changes between the black seed and ramipril treated groups. Nephroprotective effects were observed with ethanolic extract of black seed which is almost comparable to ramipril. **Conclusion:** black seed has nephroprotective effect that can alleviate gentamicin induced nephrotoxicity comparable to ramipril, though exact mechanism was not confirmed by this study. *[Journal of National Institute of Neurosciences Bangladesh, July 2025;11(2):170-175]*

**Keywords:** Gentamicin; Ramipril; Nigella sativa Linn; black seed; Nephrotoxicity; Blood Urea; Serum Creatinine; Histopathology

## Introduction

Acute kidney injury (AKI) is a common clinical problem associated with high mortality and can occur in normal renal function or patients with pre-existing renal diseases[1]. It refers to the sudden and usually reversible loss of kidney function which develops over a period of days or weeks[2] and characterized by accumulation of urea and other chemicals in the blood[3]. Metabolites of the drugs that are excreted through kidney cause cellular damage with histopathological changes leading to kidney dysfunction[4]. Gentamicin is an aminoglycoside antibiotic. It is effective against gram negative organisms and a popular choice for clinicians[5] and is one of the nephrotoxic drugs that can produce renal tubular damage which limits its frequent clinical prescription[6]. Amelioration of nephrotoxicity can increase its clinical use by the prescribers.

Gentamicin induces oxidative stress, apoptosis, necrosis, upregulation of transforming growth factor B and increase monocyte/macrophage infiltration and generates superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney which is responsible for acute kidney injury[6]. Different studies showed that antioxidants ameliorate gentamicin induced nephrotoxicity in rats. Antioxidants improve histopathological injuries such as tubular necrosis,

**Correspondence:** Dr. Mosfika Mahjabin, Assistant Professor, Department of Pharmacology & Therapeutics, Enam Medical College, Savar, Dhaka, Bangladesh; Cell No: +880 1711 385510; Email: mahjabin09@yahoo.com;
ORCID: https://orcid.org/0009-0000-1112-6619

tubular cell oedema and apoptosis in gentamicin treated rats[7]. Studies stated that multiple vegetables and fruit species may give nephroprotection against gentamicin induced nephrotoxicity[8]. The traditionally available remedies to prevent nephrotoxicity from natural sources in Southeast Asia are worth mentioning. The availability of certain local herbs with traditional medicine would be cheaper with fewer adverse effects if an effective remedy is provided to treat gentamicin induced nephrotoxicity. Herbal agent like black seed is known to possess properties to ameliorate gentamicin induced nephrotoxicity. It has potent antioxidant effect[9-12].

Ramipril is an angiotensin converting enzyme inhibitor that inhibits the renin angiotensin aldosterone system. Ramipril has specific nephroprotective effect, probably because of the role of angiotensin II in driving the underlying glomerular hyperfiltration[13]. Losartan is an angiotensin II receptor blocker and has nephroprotective effect on gentamicin induced nephrotoxic rats[14]. In this background a scientific evaluation of nephroprotective effect of black seed should be explored and develop a natural source that is readily available and low cost.

In this study an attempt has been made to evaluate the nephroprotective effect of ethanolic extract of black seed in gentamicin induced nephrotoxic rats and nephroprotective effects were compared with ramipril. The study was carried out in the department of Pharmacology & Therapeutics at Dhaka Medical College, Dhaka.

## Methodology

**Study Settings:** This experimental study was carried out in the department of Pharmacology and Therapeutics, Dhaka Medical College. 28 healthy Wistar Albino rats of both sexes weighing from 180 to 250 grams were collected from ICDDR'B. Gentamicin was purchased from a pharmacy which was manufactured by Opsonin Pharmaceuticals Ltd. Black seed, which DACB accession number was 45092, were collected from a super shop and identified and authenticated by National Herbarium, Mirpur, Dhaka. Ethanol extracts were prepared in Drug Research Laboratory of Centre for Advanced Research of Sciences (CARS) of Dhaka University. Ramipril was purchased from a pharmacy which was manufactured by Opsonin Pharmaceuticals Ltd. The experimental study was designed to demonstrate the effect of ethanolic extract of black seed on blood urea and serum creatinine levels as well as histopathological changes on gentamicin induced nephrotoxic rats and to compare the nephroprotective effects of ethanolic extract of black seed and ramipril.

Study Procedure: The rats were divided into four groups (A, B, C & D) comprising 7 rats in each group. Rats were treated for nine days. Group A was served as control group. Rats were given a standard diet for 9 days and were sacrificed on day 10. Group B was gentamicin treated group. Nephrotoxicity was induced by intraperitoneal injection of gentamicin at a dose of 100mg/kg body weight for 9 days[11,15]. The rats were allowed for the usual diet for the same duration and then sacrificed on day 10. Group C was treated with ethanolic extract of black seed (1.0 ml/kg/day)[16,17] by gastric intubation and gentamicin (100mg/kg/day) intraperitoneally for 9 days with usual rat diet and then sacrificed on day 10. Group D was treated with ramipril (1mg/kg/day)[18] by gastric intubation and gentamicin (100mg/kg/day) intraperitoneally for 9 days with usual rat diet and then sacrificed on day 10. On day 10, blood samples were collected through cardiac puncture and sent to the department of biochemistry of Dhaka Medical College for biochemical analysis. The rats were then sacrificed and kidneys were dissected and stained with haematoxylin & eosin for histopathological examination.

**Statistical Analysis:** All results were appropriately recorded in computer. Statistical analysis was done by SPSS version 17 software. The variables were expressed as mean SD. 'Unpaired students t test, ANOVA test and Post Hoc test were done for comparison of means.

**Ethical Consideration:** Ethical approval for this study was obtained from the Ethical Review Committee, Dhaka Medical College. As this was an experimental study, all methods were performed in accordance with the relevant guidelines and regulations.

## Results

Obtained data on blood urea and serum creatinine of the four different groups (A, B, C & D) were separately recorded and analyzed. Data were expressed as mean ± SE and presented accordingly in tables of a variable of the specific group. The results of the interventional groups were compared with that of the control. At first, the data on groups A and B were compared to observe the effect of gentamicin on their blood urea and serum creatinine level as well as histopathological changes. Secondly, the data on blood urea and serum creatinine level as well as histopathological changes of groups B, C, and D were compared to observe the effect of gentamicin, black seed and ramipril respectively. Finally, the blood urea and serum creatinine level as

well as histopathological changes of group C and D were compared to observe the effects of black seed and ramipril in gentamicin treated rats.

**Table 1: Effects of Gentamicin on blood urea and serum creatinine level in group A & B at day 10 (mean±SD)**

| Groups | Blood Urea Level (mg/dl) | P value | Serum Creatinine level (mg/dl) | P value |
|---|---|---|---|---|
| Group A | 21.43 ± 2.15 | <0.001 | 0.49 ± 0.05 | <0.001 |
| Group B | 84.43 ± 6.87 | | 3.37 ± 0.55 | |

*ANOVA test was done

The mean blood urea and serum creatinine levels were higher in group B than in group A. This difference was statistically significant (p<0.001). An unpaired t test was done to find out the difference between individual groups (Table 1).

**Table 2: Comparison of Blood Urea and Serum Creatinine Levels Between Different Groups (group B with C and D) at day 10 (mean±SD)**

| Groups | Blood Urea Level (mg/dl) | P value | Serum Creatinine level (mg/dl) | P value |
|---|---|---|---|---|
| Group B | 84.43 ± 6.87 | <0.001 | 3.37 ± 0.56 | <0.001 |
| Group C | 64.71 ± 13.46 | | 1.74 ± 0.13 | |
| Group D | 53.85 ± 11.55 | | 1.67 ± 0.26 | |

*ANOVA test was done

The mean blood urea and serum creatinine level was higher in group B and lower in group C and D. These difference was statistically significant (p<0.001). ANOVA test was done to determine the difference between individual groups (Table 2).

There was a highly significant difference in blood urea and serum creatinine level of group B compared with group C and group D (Table 3).

Another Post Hoc test (Games-Howell) was done to compare blood urea and serum creatinine levels between groups D and C; no significant difference was observed (Table 4).

In group A there is typical appearance of glomeruli, tubules and interstitium. No inflammatory cell infiltration or vascular changes were observed (Figure I).

**Table 4: Comparison of Blood Urea and Serum Creatinine Level Between Group D with group C (mean± SE)**

| Groups | Blood Urea Level (mg/dl) | P value | Serum Creatinine level (mg/dl) | P value |
|---|---|---|---|---|
| Group D vs. C | 10.86 ± 6.71 | 0.603 | 0.07 ± 0.11 | 0.987 |

*Post Hoc test (Games-Howell) was done

Histopathological observations

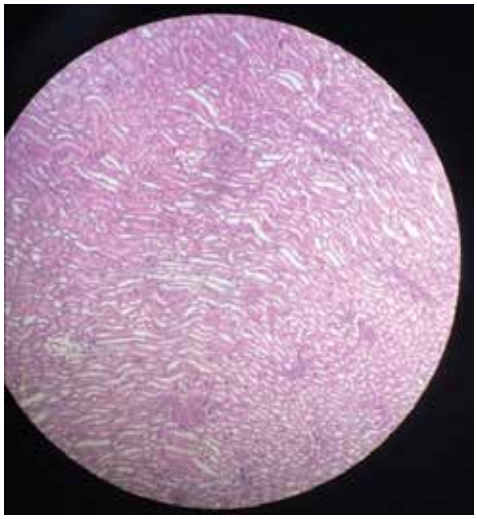

**Figure I: Microscopic Photograph of Group A**

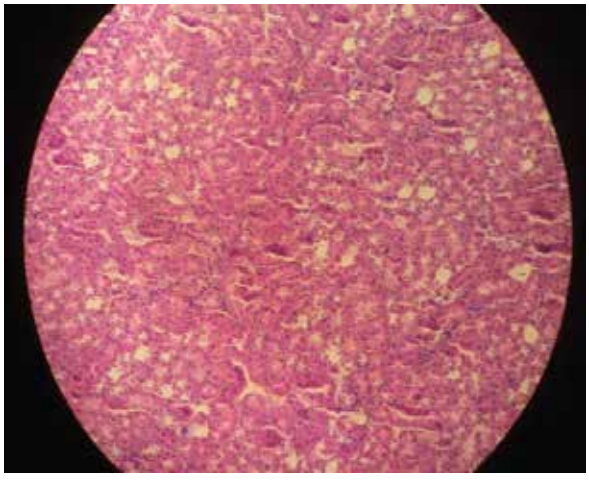

**Figure II: Microscopic Photograph of Group B**

**Table 3: Comparison of Blood Urea and Serum Creatinine Level Between Group B and Groups C and D at day 10**

| Groups | mean± SE in blood urea level (mg/dl) | P value | mean ± SE in blood creatinine level (mg/dl) | P value |
|---|---|---|---|---|
| Group B vs. C | 19.71 ± 5.71 | <0.058 | 1.63 ± 0.22 | <0.001 |
| Group B vs. D | 30.57 ± 5.08 | <0.001 | 1.70 ± 0.23 | <0.001 |

*Post Hoc test (Games-Howell) was done

In group B there are moderate to severe distortion of renal architecture as well as glomerular congestion, early tubular necrosis and the interstitium was infiltrated with many lymphocytes (Figure II).

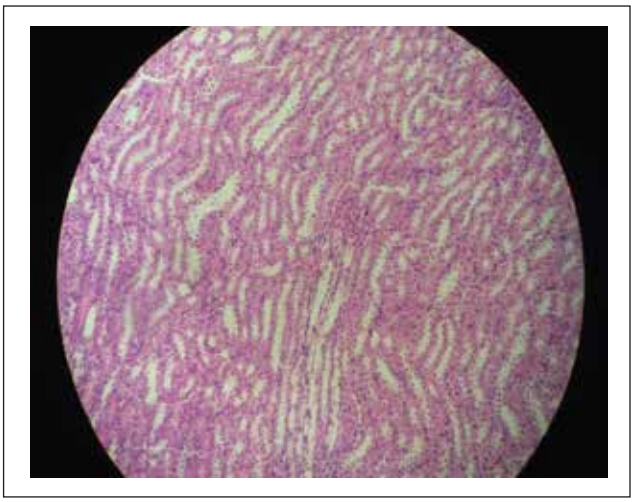

**Figure III: Microscopic Photograph of Group C**

Group C showed normal renal architecture with few to very few areas with glomerular congestion and the interstitium infiltrated by few to occasional lymphocytes (Figure III).

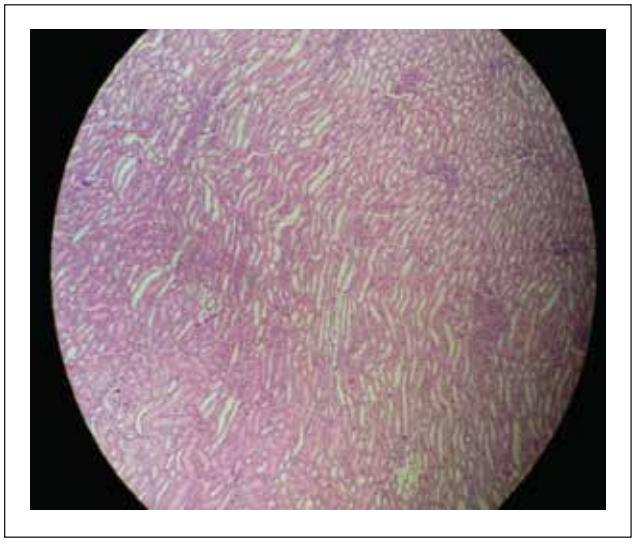

**Figure IV: Microscopic Photograph of Group D**

Group D showed preserved renal architecture with very few areas with glomerular congestion and the interstitium infiltrated with occasional number of lymphocytes (Figure IV).

## Discussion

The study was carried out to evaluate the nephroprotective effect of ethanolic extract of black seed with a comparison to ramipril on experimentally induced nephrotoxic rats. Nephrotoxicity was generated by the intraperitoneal injection of gentamicin in this study. 28 healthy adult Wistar Albino rats of both sex weighing 180 to 250 grams were selected for this study. The rats were divided into 4 groups (A, B, C, D), each comprising 7 rats. They were treated for 9 days. Group A served as the control group where the rats were given a standard rat diet for 9 days. Group B was gentamicin treated group and intraperitoneal administration of gentamicin (100mg/kg/day), and a standard rat diet were given for 9 days to them. Group C rats were treated with ethanol extract of black seed (1.0 ml/kg/day) by gastric intubation for 9 days, along with gentamicin (100mg/kg/day) intraperitoneally and standard rat diet. Lastly, group D rats were treated with ramipril (1mg/kg/day) by gastric intubation for the same period, along with gentamicin (100mg/kg/day) intraperitoneally and standard rat diet.

The duration of the study was 10 days. On day 10, blood urea and serum creatinine level estimations were done. Then, the rats were sacrificed and the kidneys were preserved and prepared for histopathological study. In this study, we found that the mean blood urea and serum creatinine level was 21.43 ± 2.15 and 0.49 ± 0.05 in group A which is almost similar with an earlier study[16]. Accumulation of gentamicin leads to pathological events in gentamicin induced nephrotoxicity and subsequent renal dysfunction[19]. Gentamicin induced nephrotoxicity is functionally evident by the elevated serum levels of urea, creatinine and structurally characterized by tubular necrosis, glomerular atrophy, mononuclear cell infiltration, intratubular hemorrhage, and hyaline casts[20,21]. In this study, we found that the mean blood urea and serum creatinine level was 84.43 ± 6.87 and 3.37 ± 0.55 respectively with a p value of <0.001, and histopathologicaly there were severe distortion of renal architecture, glomerular congestion, early tubular necrosis and interstitial infiltration with more lymphocytes in gentamicin treated rats in group B which is statistically significant and also reported by another study[22].

In current study, we found that mean blood urea and serum creatinine level in group C were 64.71 ± 13.46 SD and 1.74 ± 0.13 SD respectively with a p value of <0.001 which is statistically significant and

histopathologicaly there were preserved renal architecture, few areas of glomerular congestion and infiltration of interstitium by few lymphocytes. Black seed has nephroprotective effect in gentamicin induced nephrotoxic rats which was also observed by other studies[11,12,16]. We also observed that mean blood urea and serum creatinine level in group D were 53.85 ± 11.55 SD and 1.67 ± 0.26 SD with a p value of <0.001 which is statistically significant. Losartan has nephroprotective effect of gentamicin induced nephrotoxic rats which was observed in a study[14]. In this study, we tried to find out the nephroprotective effect of ramipril and found that it has nephroprotective effects in gentamicin induced nephrotoxic rats.

In our study, we found that the difference between mean blood urea and serum creatinine level in group C and group D were 10.86 ± 6.71 SE and 0.07 ± 0.11 SE with a p value of 0.603 and 0.987 respectively which is statistically insignificant and in histopathologicaly there were preserved renal architecture, few areas of glomerular congestion and infiltration of interstitium by few lymphocytes with black seed treated rats and in ramipril treated rats showed preserved renal architecture with very few area of glomerular congestion and infiltration of interstitium by very few lymphocytes. The nephroprotective effect of black seed was demonstrated in gentamicin induced nephrotoxic rats and comparable with the nephroprotective effect of ramipril. Increased blood urea and serum creatinine levels were significantly protected and histological architecture observed almost normal in experimentally nephrotoxic group when treated with ethanolic extract of black seed and ramipril.

Antioxidants can inhibit or ameliorate gentamicin induced nephrotoxicity in rats. They improve histological injuries such as tubular necrosis, tubular cell oedema and apoptosis in gentamicin treated rats[7]. This study observed that the ethanolic extract of black seed has a nephroprotective effect on gentamicin induced nephrotoxic rats. Results of this study suggest that black seed may be an easily available, low cost and beneficial nephroprotective agent in our southeast Asia. However, more studies would be necessary to validate the claim, explore the mechanism of action as well as application in population.

There are few limitation of the study. Sample size was small and gender variation could not be evaluated. Modern drugs and herbal products were used to influence the biological system. Natural system has certain limitations like individual variation and interference in response to the system which might have interfered with results. So, results obtained in this study may differ somewhat from the exact effect.

## Conclusion

Despite all limitations, interpretation of results obtained in this study was made cautiously and carefully. Concurrent administration of black seed may ameliorate the pathological changes of gentamicin induce nephrotoxicity as like as ramipril. Further studies are needed to identify and characterize before being establish it in clinical setting.

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**Conflict of interest:** All authors declare that they have no competing interest.

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**Contribution to authors:** Mahjabin M, Rahman Z, Begum H, conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Rahman Z, Begum H, Akter S involved in the manuscript review and editing; Mahjabin M, Rahman Z conceived and manuscript writing. All authors read and approved the final manuscript.

**Data Availability**
The datasets generated and analyzed during this study are not publicly available due to patient confidentiality and institutional data protection policies. However, anonymized data may be made available from the corresponding author upon reasonable request, subject to approval by the institutional ethics committee.

**Ethics Approval and Consent to Participate**
Ethical approval for the study was obtained from the Institutional Review Board.

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**ORCID:**
Mosfika Mahjabin: https://orcid.org/0009-0000-1112-6619

Zaida Rahman: https://orcid.org/0009-0000-6658-5425
Halima Begum: https://orcid.org/0009-0004-6768-3931
Sonia Akter: https://orcid.org/0000-0003-3897-9819
Sharmin Jahan Khan: https://orcid.org/0009-0001-4324-0982
Md Shamsur Rahman: https://orcid.org/0000-0001-8826-6492
Bhuiyan Mohammad Mahtab Uddin:
https://orcid.org/0000-0002-5109-9851

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