Original Article

The Effect of Nigella Sativa Linn (Kalajira) Extract on the Gentamicin Induced Nephrotoxicity in Experimental Rats

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Abstract:
The pathogenesis of gentamicin-induced nephrotoxicity has shown to generate free radicals. Several free radical scavengers are well recognized to ameliorate the nephrotoxicity. The seeds and oil of nigella sativa were reported to possess strong antioxidant properties and was effective against disease and chemically-induced hepatotoxicity and nephrotoxicity. The experiments were carried out in two parts, Experiment I and Experiment II, on a total of 35 rats of 8-12 weeks old and weighing between 200 and 230g. Nephrotoxicity and amelioration of nephrotoxicity was evaluated by measurement of concentrations of blood urea. Nephrotoxicity of the rats were induced by subcutaneous injection of gentamicin 100 mg/kg/day for 9 days and the rats were sacrificed on 10th day. The results indicated that gentamicin treatment caused marked renal tubular damage by significant increase (P<0.001) of blood urea concentrations when compared to those of control. When n-hexane extract of N. Sativa was administered as low and high dose with gentamicin and compared with the gentamicin treated groups, it was found that in these two groups there were significant decrease (P<0.001) of blood urea levels. When these two groups were compared with each other, it was observed that more amelioration occurred significantly in high dose treatment group than in the low dose treatment group. This study established that oral administration of n-hexane extract of N. sativa was able to produce considerable improvement from the nephrotoxic action of gentamicin in rats. The best amelioration was obtained in high dose treatment. Low dose treatment brought out the least amelioration of them all. Future works could better be directed towards obtaining the specific ingredient and the specific mechanism responsible for nephroprotection. We are hopeful that complete amelioration might not be impossible if given in proper doses or more effectively if we could extract the actual ingredients responsible for nephroprotection and can use them eventually.

Key words: blood urea, gentamicin, Nigella Sativa (Kalajira) seeds

Received: March 11, 2017; **Accepted:** May 20, 2017

Introduction:
Kidney diseases are one of the major health hazards both in terms of morbidity and mortality. Among the kidney diseases, acute renal failure (ARF) in children is a common and serious renal problem in our country [1]. Approximately 10,000-15,000 patients with ARF die each year in Bangladesh. Among the causes of ARF, the most important one is injury to the renal tubular cells (acute tubular necrosis) by toxin or ischemia [2].

The kidneys are the essential excretory organs of our body, and regulate the volume of body fluids and its composition (although the intestines, the lungs and the skin take part to some extent) [2]. The internal environment of the body is thus properly maintained by the appropriate function of the kidneys. The maintenance of hemodynamics also requires the excretion of various harmful product of metabolism. The kidneys bear the greatest responsibility of fluid and electrolyte reabsorption, secretion and excretion of excess as well as harmful solute and water [3]. Many products of metabolism of daily food intake, drugs and inhaled chemicals get their passage out of the body through the kidneys saving the mankind from many harmful diseases & toxicity [4].

The kidneys receive about 20% of the cardiac output. This high blood supply also makes the kidneys susceptible to toxic injuries by the drugs and toxins. Of the amount of blood going to each kidney, 90% or more of them is distributed to the cortical areas which contain the renal corpuscles and most parts of the renal tubules [5]. Thus, the exposure to circulating toxic substances is greater for the cortical tubules than that for most of other tissues.

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Among the drugs showing nephrotoxicity, the aminoglycoside gentamicin is well-recognized, reputed to produce renal tubular damage [6]. It is an effective drug because of its effects against gram-negative organisms and has been a favorable choice to the clinicians. Therefore, in spite of the nephrotoxicity it produces, its' low cost and reliable activity against all but the most resistant gram-negative aerobes has kept it as one of the most preferable drugs for gram-negative infections [7].

Several renal insufficiencies have been reported in patients with previously normal blood urea nitrogen (BUN) and urinalysis while on usual or therapeutic doses (2 mg per kg body weight) of gentamicin. Increasing the duration of gentamicin would increase the percentage of patients with nephrotoxic effects. Less than 15% of the patients receiving Aminoglycosides for 5 days or less developed nephrotoxicity and the percentage was more than 40% with treatment for 14 days or more. Some patients treated with gentamicin may progress to Acute Renal Failure (ARF), and among them, a significant number had received gentamicin before the onset of the syndrome. 10 to 15 % of all cases of ARF were users of Aminoglycosides [8].

The traditionally available remedies for gentamicin-induced nephrotoxicity from natural sources from the Southeast Asia may be worth mentioning at this point. Because of local and easy availability of certain herbs, treatment of gentamicin-induced nephrotoxicity with traditional medicine would probably be cheaper and less harmful if effective remedy could be provided [9].

Nigella sativa Linn. seeds locally called kalajira in Bangladesh is one such herbal product. It is a well-known spice of Southeast Asia, especially in Bangladesh. Its disease alleviating properties have strengthened its use as one of the food ingredients in our country. Its antidiabetic effect, lipid-lowering potentialities, and antibacterial potentialities had been observed [6,7].

Pharmacologically active constituents in the oil of Nigella sativa seeds were thymoquinine (TQ), dithymoquinine (DTQ) and thymol (TOH). It has been shown that both the fixed oil of N. sativa and thymoquinine, the main compound of the essential oil, inhibit non-enzymatic lipid peroxidation in liposomes and possess strong antioxidant properties. The essential oil of N. sativa seeds considered to have antioxidant activity shown to modify gentamicin induced nephrotoxicity. Prophylaxis of rats orally with N. sativa extract resulted in a significant decrease in renal microsomal lipid peroxidation, Gama- glulamyl- trans- peptidase, hydrogen peroxide and xanthine oxidase. There was significant recovery of renal glutathione content and antioxidant enzymes. There was also reversal in the enhancement of blood urea nitrogen, serum creatinine. N. sativa oil produced a dose dependent amelioration of harmful biochemical of gentamicin nephrotoxicity. N. sativa increased the amount of reduced glutathione and enhancement of total antioxidant status concentrations in renal cortex. This reduced glutathione no longer cause lipid peroxidation and acts as a free radical scavenger [7,8].

With these background information, in this study, attempt has been made to evaluate the nephroprotective role of N. sativa on experimentally induced nephrotoxicity in rats. Gentamicin has been chosen to induce the nephrotoxicity.

Materials and Methods:

The study was carried out in the Department of Pharmacology, Dhaka Medical College, Dhaka on 32 albino rats with Nigella sativa (kalajira) extract. Kalajira were collected from market and its ethanolic extract was prepared in CARS. Gentamycin were collected from Square pharmaceutical.

Total 35 albino rats were collected from icddr'b Dhaka. They were of either sex, weighing about 150-200 gm. Rats were randomly divided into 5 groups of 7 in each group and the study was carried out in 2 stages, experiment 1 and experiment 2.

In experiment 1 Group A served as control group that received distilled water 1 ml orally and normal rat diet daily for 9 days and Group B received gentamicin subcutaneously (100 mg/kg body weight/day) for 9 days.

In experiment 2 rats were divided into 3 groups, Group C, Group D and Group E and each group contained 7 rats. Group C received gentamicin subcutaneously (100 mg/kg body weight/day) with normal diet for 9 days and served as experimental control group. Group D received gentamicin (100 mg/kg body weight/day) subcutaneously for 9 days along with N. sativa extract (10 mg/kg body weight/day) mixed in 5 ml deionized water, orally with normal diet for 9 days and Group E received gentamicin subcutaneously (100 mg/kg body weight/day) for 9 days along with N. sativa extract orally (20 mg/kg body weight/day) mixed in 5ml deionized water for 9 days.

On 10th day of both experiment 1 and 2, blood samples were collected from rats through cardiac puncture, and sent for haematological analysis by automated haematology analyzer.

Data were expressed as Mean ± SE. Statistical analysis were done by Student's unpaired t-test using the SPSS software version 12.

Results:
Obtained data on blood urea concentration was recorded & compiled. Data were expressed as mean ± SEM and tabulated & presented accordingly in tables & figure. Line diagram represented dose dependent change of a variable. Results of intervention groups were compared with that of control group in tables.

In Experiment 1 Group A (Control) and Group B (Experiment), n = Total number of subjects. Result are Significant at P <0.05; Not significant (P >0.05).

Table-I: Comparison of blood urea between Group A & Group B of Experiment 1

Group	Number of rats	Mean blood urea (mg/dl)	P value
A	7	19.29±1.23	<0.001
B	7	37.29±2.67	

In Experiment 2 Group C (Experimental Control), Group D (Low dose kalajira) and Group E (High dose kalajira); n = Total number of subjects. Result are Significant at P <0.05; Not significant (P >0.05).

Table-II: Comparison of blood urea between Group C & Group D, Group C & Group E and Group D & Group E of Experiment 2

Group	Number of rats	Mean blood urea (mg/dl)	P value
C	7	38.00±2.39	<0.01
D	7	33.00±2.13	
C	7	38.00±2.39	<0.001
E	7	20.86±1.12	
D	7	33.00±2.13	0.002
E	7	20.86±1.12	

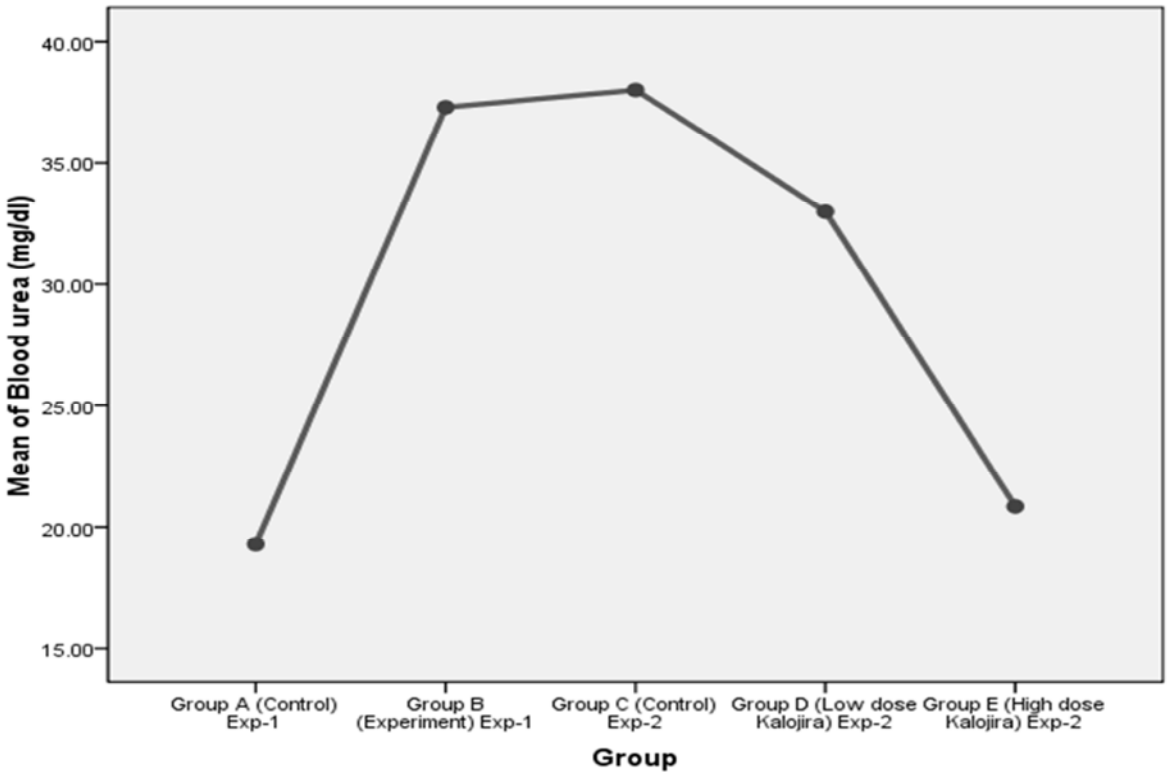

Figure-1: Line diagram showing the mean blood urea (mg/dl) in different study group.

Discussion:
The present study was aimed to ameliorate the renal toxicity of a commonly used aminoglycoside antibiotic gentamicin with the administration of N. sativa (Kalajira), which is a widely used herbal spice of Southeast Asia and the Middle East. N. sativa has been renowned for its various beneficial effects in the human[7,8].

Aminoglycoside antibiotic Gentamicin was administered subcutaneously on the nape of the neck of Adult male rats (weighing 200-230g) at a dose of 100 mg/kg body weight/day for 9 days (sacrificed on day 10). Nephrotoxicity and the ameliorating effects were estimated by biochemical findings.

Blood urea is more sensitive indicator renal dysfunction and the elevation suggests renal proximal tubule damage [8,9].

Experiment I was conducted prior to the commencement of the main study (Experiment II).

In Experiment I, Group A served as control group and they will be provided with normal rat diet and water ad libitum for 9 days and were sacrificed on 10th day. Group B is gentamicin treated group. Rats of this group were given gentamicin subcutaneously (100 mg/kg body weight/day) for 9 days. The rats were also allowed usual rat diet and water ad libitum for the same days (first week) and were sacrificed on day 10.

In Experiment II, n-hexane extract of N. sativa (kalajira) was administered orally through Ryle's tube at a dose of 10 mg/kg body weight/day to the group D and 20 mg/kg body weight/day to the group E of adult male rats for 9 days. All the rats were adult, male and of same species with the expectation that the interpretation of the findings could be easier and moreover, intergroup comparisons of the findings would be more relevant. In the present study, male rats were selected because male rats are believed to be more prone to gentamicin-induced nephrotoxicity than female rats [9,10].

The control group (group A) of rats were given normal rat diet and water ad libitum and sacrificed on day 10. The effects on blood urea concentrations were observed. The values obtained from the above parameters were similar to values in normal adult rats reported in earlier studies [9,10].

Group B (gentamicin treated, sacrificed on day-10) was compared with group A (control), it was found that there was significant increase ($P<0.001$) of blood urea concentrations (Table-I). These findings also indicate that gentamicin administration has induced damage to the renal tubules which has been the reason of increased urea level obtained in our samples. Several study reported similar biochemical changes and have described them as suggestive of nephrotoxicity [11,12].

In group C (gentamicin-treated, sacrificed on day 10) taken as control group of experiment II could be obtained in biochemical variables. The effects of low and high dose administration of N. Sativa (Kalajira) on gentamicin-induced nephrotoxic rats were obtained by estimation of blood urea in the group D (gentamicin and low dose N. sativa concomitantly) and the group E (gentamicin and high dose N. sativa concomitantly). In our observation, group E had showed significant ($P<0.001$) decrease in blood urea compared to those observed in the group D. These findings were similar to those of previous studies [13,14].

Cumulating these evidences together, the findings of the study suggest that Nigella sativa (kalajira) was able to produce considerable alleviation from the nephrotoxic action of gentamicin in adult male rats [15].

Conclusion:

The results of this study indicate that oral administration of n-hexane extract of N. sativa was able to produce considerable alleviation from the nephrotoxic action of gentamicin in rats. The best amelioration was obtained by high dose treatment. Low dose treatment brought out the least amelioration of them all. Future works could be better directed towards obtaining the specific ingredient and the specific mechanism (if any, other than the antioxidant action). We are hopeful that complete amelioration might not be impossible.

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Citation of this article:
Quadir R, Eva EO, Khanam M, Zabir SM. The Effect of Nigella Sativa Linn (Kalajira) Extract on the Gentamicin Induced Nephrotoxicity in Experimental Rats. Eastern Med Coll J. 2017; 2 (2): 17-21.