ORIGINAL ARTICLE

Effect of Crude *Nigella sativa* Linn. (*kalajira*) on Gentamicin-induced Nephrotoxicity in Rats

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Abstract:

Reactive oxygen species are involved in gentamicin nephrotoxicity. This study investigated whether administration of crude Nigella sativa (kalajira) ameliorates gentamicin-induced nephrotoxicity. Crude N. sativa powder was given orally (10 gm/kg/day mixed in 5 ml deionised water) as pre-, post- and concomitant treatment for seven days in gentamicin-induced nephrotoxic rats (100 mg/kg/day for seven days). Nephrotoxicity was evaluated biochemically by measuring the concentrations of creatinine and urea in serum and reduced glutathione (GSH) in kidney cortex. The results indicated that gentamicin significantly increased the concentrations of creatinine and urea, and decreased GSH. Administration of crude N. sativa in gentamicin-induced nephrotoxic rats as pre-, post- and concomitant treatment demonstrated statistically significant amelioration of the biochemical indices of gentamicin nephrotoxicity. This amelioration of nephrotoxicity was more significant in post-treatment group than the concomitantly treated and the pretreatment groups. The result suggests that crude N. sativa may be useful in ameliorating signs of gentamicin nephrotoxicity in rats.

Introduction:

Kidney diseases are the fourth commonest cause of hospitalization in the medical college hospitals and second commonest cause of hospitalization in the paediatric units of Bangladesh[1]. Among the causes of acute renal failure (ARF), the most important is injury to the renal tubular cells by toxins or ischaemia. Aminoglycoside antibiotics, including gentamicin, are widely used in the treatment of gram-negative infections[2]. A major complication of the use of these drugs is nephrotoxicity accounting for 10-15% of all cases of ARF[3]. The specificity of gentamicin for renal toxicity is apparently related to its accumulation in the renal proximal convoluted tubules (50-100 times greater than serum) and lipid peroxidation, giving rise to free radicals, which are highly toxic to tissue[3].

Nigella sativa (*kalajira*) occupies a unique position among the herbal products of Southeast Asia as a natural remedy for a number of illnesses. Its antidiabetic[4], antibacterial[5], antihypertensive[6] and hypolipidaemic[7] properties have been reported. The seeds and oil of *N. sativa* was reported to possess strong antioxidant properties and was effective against diseases and chemically-induced hepatotoxicity and nephrotoxicity. In recent research, attempts

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were made to obtain certain agents that can ameliorate or potentiate the nephrotoxicity of gentamicin[8]. Among these agents, extract of medicinal plants like garlic[9], and *N. sativa* oil[10] have been reported to possess properties to ameliorate gentamicin-induced nephrotoxicity. One common feature of these herbal agents are that they all have antioxidant properties[11]. Gentamicin-induced nephrotoxicity has been shown to generate oxygen free radicals[3] and several free radical scavengers are well recognized to ameliorate the nephrotoxicity[12].

Therefore, in the present work, it was attempted to test and compare the possible pre-, post- and concomitant action of crude *N. sativa* on gentamicin nephrotoxicity in rats. A potential therapeutic approach to ameliorate gentamicin-induced renal damage would have very important clinical consequences in increasing the safety of drugs[8].

Materials and method:

Chemicals and reagents:

Gentamicin (80 mg/2 ml) was obtained from the Essential Drug Company Ltd. (Bangladesh). *N. sativa* was purchased from the local market. Chemicals and reagents for estimation of creatinine and serum urea were obtained from Human GmbH (Germany). Reduced glutathione was purchased from Loba Cheme (India), and 5,5-di-thiobis-2-nitrobenzoic acid (DTNB) was obtained from Sigma Chemicals (USA).

N. sativa (kalajira) powder:

The seeds of *N. sativa* (2000 g) were cleaned, washed and dried in air, incubated at 40°C for 24-48 hours, and finally, powdered by a blender to get a fine dried powder.

Animals:

Adult male rats aged between eight and 12 weeks, weighing 200-230 gm were obtained from the animal house of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Normal rat-feed, water *ad libitum* was provided under 12-hour light and 12-hour dark schedule at room temperature (24-28°C).

Experimental design:

Rats were randomly divided into seven equal groups (n=42) as follows:

Group 1 - untreated control: The rats were fed with normal rat diet and water *ad libitum* for seven days.

Group 2 - gentamicin treated: Rats received gentamicin subcutaneously at a dose of 100 mg/kg/day for seven days.

Group 3 - crude *N. sativa* treated: Rats were treated orally for seven days with *N. sativa* powder (10 gm/kg/day mixed in deionised water).

Rats of groups 1-3 were sacrificed on day 8.

Group 4 - gentamicin treated (control): Rats were treated as in group 2 and then kept untreated for another seven days.

Group 5 - crude *N. sativa* followed by gentamicin: Rats were treated as in group 3 and then they were treated as in group 2 for another seven days.

Group 6 - gentamicin followed by crude *N. sativa*: Rats were treated as in group 2 and then they were treated as in group 3 for another seven days.

Group 7 - gentamicin and crude *N. sativa* concomitantly: Rats were treated as in groups 2 and 3 concomitantly and then kept untreated for another seven days.

Rats of groups 4-7 were sacrificed on day 15.

The animals were anaesthetized with light chloroform and rapidly decapitated, and 5 ml blood was collected in clean test tubes and centrifuged (4000 rpm for five minutes). The serum obtained was stored at 0-4°C for estimation of creatinine and urea concentrations. The kidneys were excised, blotted on a filter paper and weighed in pairs. The cortex was dissected out and a portion was processed for biochemical estimation of reduced GSH concentration.

Biochemical measurements:

Glutathione concentration was measured in homogenates of the renal cortex spectrophotometrically[13]. Serum creatinine concentration was measured by alkaline picrate method[14]. Serum urea concentration was measured by enzymatic method[15].

Protein analysis:

Protein content (renal cortex) was determined by Biuret method[16].

Statistical analysis:

The results obtained from the experiments were represented as mean±SEM of the number of samples. Data were analyzed by (a) unpaired Student's 't' test, and (b) significant difference between mean±SEM of the different groups were estimated using one-way analysis of variance (ANOVA), followed by unpaired Student's 't' test.

Results:

Table- I: Effects of gentamicin and crude *N.sativa* on renal parameters of rats

Group	Duration of treatment		n	Cortical GSH (mg/g protein)	Serum creatinine (mg/dl)	Serum urea (mg/dl)
	First week (day 1-7)	Second week (day8-14)				
1	Untreated control	-	6	2.21± 0.12	0.39± 0.05	26.2±3.10
2	Gentamicin	-	6	1.00± 0.01***	3.10 ± 0.40***	110.40±9.20***
3	Crude *N. sativa*	-	6	5.72± 0.10***	0.38± 0.04^{NS}	25.10±1.40^{NS}
	Sacrificed on day 8					
	First week (day1-7)	Second week (8-14)				
4	Gentamicin		6	1.07± 0.04^{NS}	2.90± 0.08^{NS}	108.00± 8.30^{NS}
5	Crude *N. sativa*	Gentamicin	6	1.68± 0.01***,#	0.73± 0.02***,#	41.00±0.40***,#
6	Gentamicin	Crude *N. sativa*	6	2.01± 0.05***	0.57± 0.01***	37.00±0.41***
7	Gentamicin and crude *N. sativa*			1.75± 0.01***	0.68± 0.01***	40.00±0.05***
	Sacrificed on day 15					

Doses: Gentamicin (100 mg/kg/day by subcutaneous injection); crude *N.sativa* (10 gm/kg/day orally mixed in deionised water); values are means ± SEM; n= number of rats in each group; GSH refers to reduced glutathione; *** indicates significant difference ($p<0.001$), groups: 1 vs 2, 1 vs 3, 2 vs 3, 4 vs 5, 4 vs 6, 4 vs 7, 5 vs 6; 6 vs 7 (unpaired Student's 't' test); # indicates significant difference ($P<0.05$), groups: 5 vs 7 (unpaired Student's 't' test); NS indicates no significant difference ($P>0.05$), groups: 1 vs 3, 2 vs 4 (unpaired Student's 't' test)

The group of rats injected subcutaneously with gentamicin (100 mg/kg/day) for seven days (group 2) and sacrificed on day 8 had serum creatinine and urea concentrations significantly ($p<0.001$) increased while the renal cortical reduced GSH concentrations of these group of rats were significantly decreased ($p<0.001$) compared to those of control rats (group 1). This suggested that these rats were made nephrotoxic (Table-I).

The crude *N. sativa* treated rats (group 3) differed from the control rats (group 1) by significantly elevated ($p<0.001$) concentration of renal cortical reduced GSH, while the other biochemical parameters were identical (no statistical difference could be obtained) almost to those of the control rats. The gentamicin-treated rats (group 2) differed from the crude *N. sativa*-treated rats (group 3) by significantly decreased ($p<0.001$) renal cortical GSH concentrations and significantly increased ($p<0.001$) serum creatinine and urea concentrations. There was no significant difference ($p>0.05$) between the gentamicin-treated groups sacrificed on day 8 and day 15 (groups 2 and 4) in all above parameters. Treatment of nephrotoxic rats with crude *N. sativa* pre-, post- and concomitantly mitigated the increases in serum creatinine and urea, and the decreases in GSH while compared to those of the gentamicin-treated group sacrificed on day 15 (group 4) (Table-I). But none of the parameters in any of these groups (pre-, post- and concomitant) were identical or closer to those of the control groups. However, post-treatment group (gentamicin followed by crude *N. sativa*) demonstrated significant reduction in serum creatinine and serum urea concentrations ($p<0.001$) and also a significant elevation ($p<0.001$) of renal cortical GSH concentrations compared to those observed in the pretreatment group (crude *N. sativa* followed by gentamicin) and the concomitantly treated (gentamicin and crude *N. sativa* concomitantly treated). When the pretreatment group and concomitantly-treated group were compared, significant improvements were seen in concomitantly-treated group. The post-treatment, therefore, remained as the most alleviated treatment group from toxic damage of gentamicin (Table-I).

Discussion:

In the present study, nephrotoxicity induced by gentamicin was evidenced by depletion of renal cortical GSH and increases in serum creatinine and serum urea concentrations. These observations were in agreement with those of previous workers[10,12], who have reported similar biochemical changes suggestive of nephrotoxicity. Gentamicin alone caused accelerated lipid peroxidation evident by depression of total GSH and shift from reduced to oxidized GSH[17]. The crude *N. sativa* posseses strong antioxidant properties[11]. The toxic free radicals[3], which develop in the course of gentamicin administration, causing oxidative damage to renal cortex would be antagonized by *N. sativa*, which is why they were used in this study. Reports about similar ameliorating action of antioxidants upon gentamicin nephrotoxicity are available[10,12]. The mechanism of the ameliorative action of crude *N. sativa* is not certain, but may involve an antioxidant action against free radicals that are known to be generated during gentamicin nephrotoxicity[3]. Previous studies have shown that *N. sativa* and compounds isolated from it were able to ameliorate several models of nephrotoxicity and common features among these conditions are the generation of free radicals[18].

The present results suggested that the post-treatment (gentamicin followed by crude *N. sativa*) demonstrated the better protection compared to those of pretreatment (crude *N. sativa* followed by gentamicin) and concomitantly treated (gentamicin and crude *N. sativa* concomitantly treated). It may be due to that *N. sativa* was able to exert its antioxidant action better in presence of oxidative damage. Prior supply of antioxidant may not protect the tissues to the expected degree in the absence of free radicals. This may appear to be one

mechanism through which *N. sativa* ameliorates gentamicin nephrotoxicity. Further studies on this aspect are warranted.

References:

1. Rashid HU. Kidney diseases as a major health problem in Bangladesh. Bangladesh Renal J 1995; 14: 59-60.
2. Ali BH. Gentamicin nephrotoxicity in humans and animals: Some recent research. Gen Pharmacol 1995; 26: 1477-87.
3. Walker PD, Shah SV. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. J Clin Invest 1988; 81: 334-41.
4. Uddin N, Dewan ZF, Zaman M, *et al.* Effects of *Nigella sativa* Linn. (kalajira) on serum glucose concentration in streptozotocin-induced diabetic rats. Bangladesh J Physiol Pharmacol 2002; 18: 6-9.
5. Ara N. Antimicrobial activity of the volatile oil of *Nigella sativa* Linn. seeds [M.Phil thesis]. Dhaka: Bangabandhu Sheikh Mujib Medical University, 1999.
6. Rashid MA, Rahman S, Chowdhury SAR, *et al.* Primary screening of some herbal products of Bangladesh for antihypertensive activity. Bangladesh J Neurol 1987; 3: 56-58.
7. Saha RR, Dewan ZF, Uddin N. Effect of *Nigella sativa* Linn. (kalajira) on serum lipid profile of hyperlipidemic rats. Bangladesh J Physiol Pharmacol 2004; 20: 36-38.
8. Mingeot-Leclercq M, Tulkens PM. Aminoglycosides: Nephrotoxicity. Antimicrob Agents Chemotherp 1999; 43: 1003-12.
9. Pedraza-Chaverri J, Maldonado PD, Medina-Champos N, *et al.* Garlic ameliorates gentamicin nephrotoxicity: Relation to antioxidant-enzymes. Free Rad Biol Med 2000; 29: 602-11.
10. Ali BH. The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. Am J Clin Med 2004; 32: 49-55.
11. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytotherp Res 2000; 14: 323-28.
12. Ali BH, Mousa HM. Effect of dimethyl sulfoxide on gentamicin-induced nephrotoxicity in rats. Human Exp Toxical 2001; 20: 199-203.
13. Sedlak J, Lindsay RH. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Elman's reagent. Anal Biochem 1968; 25: 192-205.
14. Spencer K. Analytical reviews in clinical biochemistry: The estimation of creatinine. Ann Clin Biochem 1986; 23: 1-25.
15. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol 1960; 13: 156.
16. Weichselbaum TE. Estimation of serum total protein by Biuret method. Am J Clin Pathol 1946; 16: 40-48.
17. Ramsammy LS, Josepovitz C, Ling KY, *et al.* Failure of inhibition of lipid peroxidation by vitamin E to protect against gentamicin nephrotoxicity in the rat. Biochem Pharmacol 1987; 36: 2125-32.
18. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytotherp Res 2003; 17: 299-305.