Nephro-protective effect of vitamin C and *Nigella sativa* oil on gentamicin associated nephrotoxicity in rabbits

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Abstract: Oxidative stress causes the generation of reactive oxygen species (ROS) that lead to nephrotoxicity. An aminoglycoside, gentamicin, has pronounced nephrotoxic effect in humans and animals and this study was planned to observe the nephro-protective effect of antioxidants, vitamin C and *Nigella sativa* oil. Serum creatinine, blood urea nitrogen, and antioxidant activity were measured as indicators of nephrotoxicity for all the groups of rabbits. Results showed that vitamin C and *Nigella sativa* oil both had nephro-protective effect as they lowered the values of nephrotoxicity indicators (serum creatinine, blood urea nitrogen, and antioxidant activity) as compared to gentamicin control group values. When these two antioxidants were given as combination, they proved to have synergistic nephro-protective effect.

Keyword: Gentamicin**,** Reactive oxygen species (ROS), *Nigella sativa*, vitamin C.

INTRODUCTION

The aminoglycosides such as gentamicin have been used for gram negative infections from many years (JI and Barza, 1987). Routine use of gentamicin at dose 80 mg/kg/body wt. for more than seven days is the major and common cause of renal toxicity associated with aminoglycosides in approximately 30% of the patients (Moore *et al*., 1984, Barclay and Begg., 1994, Pedraza-Chaverri *et al*., 2003). Nephrotoxicity with gentamicin is because of oxidative stress that leads to generation of free radicals within renal proximal convoluted tubules (Maldonado *et al*., 2003, Yanagida *et al*., 2004). Gentamicin is more nephrotoxic as compared to tobramycin and other drugs included in this group (Schentag *et al.*, 1981). *Nigella sativa* oil (Burits and Bucarand, 2012) and Vitamin-C have potentially effective antioxidant activity (Toama *et al*., 1974). The aim of study is to use free radical scavenging property of *Nigella sativa* oil and vitamin C in preventing the gentamicin induced nephrotoxicity. The study was conducted in animal model (rabbit). This study provided backbone for use of these antioxidants in human beings; animal dose can be converted into human dose by using conversion formula (Weblink 1, Reagan *et al*., 2007).

MATERIALS AND METHODS

Materials

Gentamicin sulphate intramuscular injections (80mg/2ml) (Tabros Pharma, Karachi, Pakistan), Vitamin-C as L (+)-Ascorbic acid (Merck, Germany) and Nigella sativa oil were purchased from local market of Lahore, Pakistan. All the chemicals used including Sodium phosphate buffer, sodium benzoate, sodium hydroxide, EDTA, $Fe(NH_4)_2SO_4$, H_2O_2, acetic acid, thiobarbituric acid, uric acid were of scientific grades and purchased from Merck, Germeny. Randox kits (Randox Laboratories, UK) were procured from the local market.

Biochemical and analytical parameters

Serum Creatinine level and Serum Urea level were measured by using analytical kits manufactured by Randox UK. Serum Antioxidant activity was evaluated in different body fluids. Control (A_0, sample blank) for each sample (A_1) was prepared. For each analysis a negative control (K_1 and K_0) was also prepared (in three sets) which contained the same reagents as A_1 or A_0, except that serum was replaced with phosphate buffer. For calibration 1mmol/litre uric acid (UA_1 and UA_0) was used as standard. Tubes were incubated for 60 minutes at 37^{o}C, and then different quantities of acetic acid and thiobarbituric acid were added. Tubes were then again incubated for 10 minutes at 100^{o}C in water bath and cooled by placing them in an ice bath. Absorbance was measured at 532 nm against deionised water and antioxidant activity was calculated using standard formula.

Experimental animals

Twenty five healthy male albino rabbits having weight between 1-1.5kg were purchased from local animal market. The rabbits were kept under controlled environmental conditions in animal house of University College of Pharmacy, University of the Punjab, Lahore,

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Pakistan for acclimatization for the period of one week prior to start of study. The rabbits were fed on green fodder and were provided with water ad libitum.

Experimental design

Twenty five rabbits were used in this study. The blood samples were collected by puncturing the marginal vein of rabbit's ear, for the determination of Serum Creatinine and Blood Urea Nitrogen levels (BUN) and Serum Antioxidant activity at day zero. The rabbits were reated with vitamin-C (Vit-C), *Nigella sativa* oil (NSO), and Vitamin-C plus *Nigella sativa oil* (Vit-C+NSO) along with the IM injections of gentamicin sulphate (GS) 80mg/kg. In the beginning (before start of therapies) the blood samples of all the animals were collected to draw the baseline readings. Nephrotoxicity was induced by administering GS 80 mg/kg for ten days. The animals were divided into five groups which are as follows: Group A**:** Control group**,** all rabbits in this group received normal saline. Group B**:** Gentamicin control group**,** this group received intramuscular injections of GS 80mg/kg for twenty-six days with out any other treatment. Group C**:** This group received 250mg vitamin-C per day orally along with the intramuscular injection of gentamicin sulphate 80mg/kg body weight for twenty-six days. Group D: This group received 2ml of NSO per oral (p.o.) per day along with intramuscular injection of GS 80mg/kg body weight for twenty-six days. Group E: This group was given the combination of 250 mg of Vit-C and 2ml of NSO p.o./day along with intramuscular injection of GS 80mg/kg body weight for twenty-six days.

STATISTICAL ANALYSIS

The results are expressed as Mean $\pm$ Standard Deviation with n=5. The results were interpreted by using one-way analysis of variance (ANOVA) in SPSS Version 13, followed by multiple comparisons. The P values $\leq$ 0.05 are considered as significant.

RESULTS

Table 1 showed that serum Creatinine levels (mg/dl) were significantly increased (5.78$\pm$1.170) at day four in group B(Gent-control) as compared with the values of group A (control group) (1.99$\pm$0.600). On the other hand, all other groups showed significant decrease in levels of Serum Creatinine when compared with group B (Gent-control). On day eight and twelve all treated groups followed the same pattern of decreased Serum Creatinine level, with Group D receiving NSO, having the least Creatinine value (1.61$\pm$.620) on day twelve, when compared to levels of Gent-control (4.20$\pm$1.380). At the end of study, on day twenty, the groups receiving Vit-C, NSO and combination of both Vit-C+NSO showed significantly decreased Creatinine levels i.e. (1.83$\pm$0.670), (1.77$\pm$0.460), (1.36$\pm$0.480) respectively when compared to the gentamicin-treated group (4.16$\pm$.490). Lowest Creatinine levels were found in group E.

Table 2 depicted that BUN levels (mmol/l) were significant raised (17.54$\pm$2.600) at day four in Group B (Gent-control) as compared to Group A (control group) i.e. 3.92$\pm$1.920. Day eight also followed the same pattern as day ten i.e. the gentamicin-treated group, illustrated increased values of BUN levels (18.05$\pm$4.430). At this day of treatment, Group D, showed the lowest BUN level (4.81$\pm$0.980). At day twelve there was no rise in levels of BUN in any of the groups except the one treated with Gentamicin only (Group B), showing the highest BUN value. All other groups compared to Gentamicin treated group (Group B), showed significantly low levels of BUN. The BUN values of groups receiving Vit-C, NSO and combination of both Vit-C+NSO on day twelve were 5.39$\pm$1.060, 4.46$\pm$0.670, 4.6$\pm$0.660 respectively. All groups on day sixteen followed the same pattern of decreased in BUN levels, Group D possessing the lowest BUN value 3.90$\pm$0.590, when compared to group B(Gent-control group) BUN level i.e. 20.50$\pm$8.130. On day 20th, all the groups showed significantly low levels of BUN as compared to gent-control group (Group B) which illustrated highest levels, (3.90$\pm$0.590).

Table 3 showed serum antioxidant activity (mmol/l). At day twenty group B had significant decrease in serum antioxidant activity as compared to zero day value. All other groups showed no significant change in serum antioxidant activity in comparison to zero day values.

DISCUSSION

In the present study animals that received only Gentamicin showed rise in serum Creatinine and BUN with decrease in Antioxidant activity, indicating nephrotoxicity, as compared to other groups in which gentamicin was given along with vitamin C and *Nigella sativa* oil. One of the major mechanisms involve in the induction of renal toxicity was probably the accumulation of drug in tubules of nephrons and subsequent reactive oxygen species (ROS) generation that leads to oxidative stress and decrease in renal function, a decrease in the level of total serum antioxidant activity in animals treated with gentamicin as compared with the levels of control animals were observed during the study. Vitamin-C and *Nigella sativa oil* helped in lowering the high levels of Serum Creatinine and BUN and increased serum antioxidant activity (Frei *et al*., 1989, Harapanhalli *et al*., 1996). Group-D received Gentamicin sulphate + NSO had potential Renal-protective effects as compared to gentamicin-control group B**.** Group-C received Gentamicin sulphate +Vit-C also protected against raised Serum Creatinine and BUN. Group-E received combination showed to have the most pronounced and potential protective effects by maintaining levels of

Serum Creatinine and BUN along with increasing serum antioxidant activity with P<0.05 (tables 1-3). It is proposed that the cooperation of antioxidants (used in this study) in human serum provides greater protection means show synergism against attacks by free radicals than any antioxidant alone (Koracevic *et al*., 2001, Wayner *et al*., 1987).

CONCLUSION

Gentamicin inj. 80mg/kg body wt. caused renal toxicity due to generation of free radicals within renal proximal convoluted tubules (if administered for more than seven days). *Nigella sativa* oil and Vitamin-C (alone and in combination) possessed free radical scavenging property. The use of *Nigella sativa* oil and Vitamin-C along with gentamicin injection prevents the occurrence of nephrotoxicity. So we concluded that *Nigella sativa* oil and Vitamin-C had synergistic nephron-protective effect in animal model (rabbit). We can use this combination (*Nigella sativa* oil and Vitamin-C) in human beings to get nephroprotective effect against gentamicin nephrotoxicity by converting animal into human doses using dose conversion formula given by Reagan *et al*., 2007.

Table 1: Serum Creatinine (mg/dl) level in rabbits

Days	Group-A (Control)	Group-B (Gentamicin – Control)	Group-C (vit-C+ Gentamicin Sulphate)	Group-D (NSO+ Gentamicin Sulphate)	Group-E (Vit-C+NSO+ Gentamicin Sulphate)
0	1.81±0.670	1.99±.650	2.13±.830	2.42±.750	2.08±.770
4	1.99±.600	5.78±1.170 ^{o}	2.58±1.280 $^{\bullet}$	2.31±.970 $^{\bullet}$	1.75±.410 $^{\bullet}$
8	1.91±.65	4.75±1.410	2.35±1.310 $^{\bullet}$	2.19±.860 $^{\bullet}$	1.87±.790 $^{\bullet}$
12	1.63±.690	4.20±1.380	1.95±0.630 $^{\bullet}$	1.61±.620 $^{\bullet}$	1.66±.560 $^{\bullet}$
16	1.86±.920	4.35±1.130	2.00±.840 $^{\bullet}$	1.92±.740 $^{\bullet}$	1.76±.640 $^{\bullet}$
20	1.69±.440	4.16±.490	1.83±0.670 $^{\bullet}$	1.77±.460 $^{\bullet}$	1.36±.480 $^{\bullet}$

Values are presented as Mean ± SD, n=5; $^{\bullet}$ p<0.05 when compared with values of Control (Group A)
^{o} p<0.05 when compared with values of Gentamicin Control (Group B)

Table 2: Blood Urea Nitrogen (mmol/l) levels in rabbits

Days	Group-A (Control)	Group-B (Gentamicin – Control)	Group-C (vit-C+ Gentamicin Sulphate)	Group-D (NSO+ Gentamicin Sulphate)	Group-E (Vit-C+NSO+ Gentamicin Sulphate)
0	5.24±2.500	4.07±2.190	5.50±2.280	3.54±1.350	5.08±2.580
4	3.92±1.920	17.54±2.600^{o}	6.00±1.580$^{\bullet}$	5.42±1.090$^{\bullet}$	5.34±1.110$^{\bullet}$
8	5.67±1.960	18.05±4.430	5.69±1.220$^{\bullet}$	4.81±0.980$^{\bullet}$	5.07±0.730$^{\bullet}$
12	5.41±2.360	18.58±7.870	5.39±1.060$^{\bullet}$	4.46±0.670$^{\bullet}$	4.6±0.660$^{\bullet}$
16	5.62±2.550	20.50±8.130	4.95±0.780$^{\bullet}$	3.90±0.590$^{\bullet}$	3.93±0.660$^{\bullet}$
20	5.74±2.650	17.90±1.500	3.84±0.820$^{\bullet}$	3.08±1.010$^{\bullet}$	2.88±0.710$^{\bullet}$

Values are presented as Mean ± SD, n =5; (p<0.05 when compared with values of Control (Group A)
(p<0.05 when compared with values of Gentamicin Control (Group B)

Table 3: Serum antioxidant activity (mmol/l) in rabbits

Days	Group-A (Control)	Group-B (Gentamicin – Control)	Group-C (vit-C+ Gentamicin Sulphate)	Group-D (NSO+ Gentamicin Sulphate)	Group-E (Vit-C+NSO+ Gentamicin Sulphate)
0	2.02±1.020	1.80±0.910	1.65±1.130	1.52±0.820	1.98±1.190
4	1.66±0.850	0.42±0.780	1.58 ±0.790	1.82±0.690	2.09±0.640
20	1.78±0.480	0.14±0.710$^{\bullet}$	1.64±0.780	1.96±0.330	2.13±0.520

Values are presented as Mean ± SD, n =5; $^{\bullet}$ p<0.05 when compared with values of Day_0

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