Evaluation of protective and curative role of α-lipoic acid and selenium in gentamicin-induced nephrotoxicity in rabbits

Afeefa Tahira[1], Uzma Saleem[2], Saeed Mahmood[3], Furqan Khurshid Hashmi[1], Khalid Hussain[1], Nadeem Irfan Bukhari[1] and Bashir Ahmad[1*]

[1]University College of Pharmacy, University of the Punjab, Lahore-54000, Pakistan
[2]College of Pharmacy, G C University, Faisalabad, Pakistan, [3]Lahore General Hospital, Lahore, Pakistan

Abstract: Gentamicin induces nephrotoxicity, hence the present study explores protective and curative effects of α-lipoic acid and selenium alone and in combination in gentamicin-induced nephrotoxicity. Forty rabbits were randomly segregated into control, protective and curative groups. The groups A and B received water (10 ml/kg/day) and gentamicin (I/M, 80 mg/kg/day), respectively as normal and gentamicin controls. Four hours before gentamicin nephrotoxic dose, the protective subgroups C, D and E received α-lipoic acid, selenium and combination (50 mg/kg/day α-lipoic acid and 10 mg/kg/day selenium), respectively and then continued for 20 days. Nephrotoxicity was induced in curative subgroups F, G and H with gentamicin sulphate for 9 days and from 10th day onwards, followed the same treatments as for protective group for 26 days. Blood urea nitrogen (BUN), creatinine and antioxidant activity (AOA) were measured in all the groups. Combination of α-lipoic acid (50 mg/kg/day) and selenium (10 mg/kg/day) significantly reduced BUN (58.64%) and creatinine (17.48%) in protective subgroups treated for 20 days as compared to control without affecting AOA ($p<0.05$). Decrease of 82.19% BUN and 77.38% creatinine, and 46.66% increase in AOA was noted on day 26 in curative group treated with the combination of antioxidants. The combination of α-lipoic acid and selenium (50 mg/kg/day α-lipoic acid and 10 mg/kg/day selenium) was found to be effective in prevention and treatment of gentamicin-induced nephrotoxicity.

Keywords: α-lipoic acid; Selenium; Gentamicin-induced nephrotoxicity

INTRODUCTION

Certain drugs and their metabolites accelerate the production of reactive oxygen species (ROS) or interfere with the natural scavenging system resulting in oxidative stress (Brownlee *et al.,* 1998). Taber and Pasko (2008) have reported that about 18-27% of acute kidney diseases in the United States are due to usage of drugs. Gentamicin, a commonly used antibiotic, generates ROS which may cause ischemic acute renal failure, renal graft rejection, acute glomerulonephritis and toxic renal diseases. It is reported that oxidative stress manifests into a wide range of complications such as acute and chronic renal failure (Zager and Burkhart, 1997; Ozaki *et al.,* 1999; Handelman *et al.,* 2001), obstructive myopathy (Klahr, 1998), hyperlipidemia (Scheuer *et al.,* 2000; Sakatsume *et al.,* 2001) and glomerular damage (Kitamura and Ishikawa, 1999; Hahn *et al.,* 1999; Moreno-Manzano *et al.,* 2000).

The natural scavenging system of the living system, comprising enzymes such as superoxide dismutase and glutathione peroxidase, removes ROS and other harmful oxidants from the body and protects against oxidative damage (Stocker and Frei, 1991). Diet or use of antioxidants enhances the natural scavenging system by disrupting production of ROS and can prevent the oxidative stress, and thus renal failure may be avoided (Sing *et al.,* 2006). The protection of scavengers of ROS, iron chelaters and other antioxidants against gentamicin-induced nephrotoxicity has already been reported (Varzi *et al.,* 2007). Antioxidants alone or in combinations have been reported to counteract the nephrotoxic potential of aminoglycosides. Lipoic acid (Sandhya *et al.,* 1995) and selenium in combination with vitamin E (Ademuyiwa *et al.,* 1990) have been reported to attenuate the gentamicin nephrotoxicity.

The α-lipoic acid, a commonly used antioxidant, plays an essential role in mitochondrial dehyrogenase reactions (Packer *et al.,* 1995) and when taken up by eukaryotic cells, it is converted into dihydrolipoic acid (DHLA), which also has significant antioxidant activity by quenching various ROS.

Selenium, a component of several enzymes such as iodothyronine 5-deiodinase, thioredoxin reductase and glutathione peroxidase, plays an important role in living organisms. Studies have suggested the synergistic effect of multiple antioxidants like α-lipoic acid, selenium and *Silybum marianum* in hepatitis (Berkson *et al.,* 2006; Su *et al.,* 2008). However, Cakatay *et al*. (2008) have reported a decrease of selenium levels in serum, heart, brain, and muscle in aged rats receiving α-lipoic acid supplementation. Any significant change in selenium status may change the activity of sleno-enzymes which

**Corresponding author:* e-mail: dr_b_ahmad@hotmail.com

may result in abnormal oxidation-reduction systems (Bordoni *et al.*, 2007). Thus, combining selenium with α-lipoic acid may effectively prevent the drug-induced nephrotoxicity. The curative and protective role of α-lipoic acid and selenium in combination in gentamicin-induced nephrotoxicity has not been studied. Therefore, the present study has been undertaken to evaluate the protective and curative role of α-lipoic acid and selenium in combination in gentamicin-induced nephrotoxicity in rabbits.

Nephrotoxicity is characterized by elevated levels of electrolytes, BUN and creatinine. Thus, these are valuable markers for the assessment of renal functional and structural integrity (Baum *et al.,* 1997). In the present study, BUN, serum creatinine and antioxidant activity have been used to assess the protective and curative role of the antioxidants.

MATERIALS AND METHODS

Experimental Animals

Forty adult healthy albino rabbits, of either sex weighing between 0.9 to 1.7 ± 0.51 kg were housed in animal facility of the University College of Pharmacy, University of the Punjab Lahore, under controlled environmental conditions for acclimatization. Fresh green fodder was given to the animals twice daily whilst fresh water was made available *ad libitum*.

Materials

Gentamicin sulphate injection 80 mg/2ml (Tabros Pharma, Karachi, Pakistan), α-lipoic acid capsules 100 mg (General Nutrition Company, USA), selenium powder (Merck, Germany) and Randox kits (Randox Laboratories, UK) were procured from the local market.

Experimental design

The animals were randomly segregated into control, protective and curative groups. The control group was subdivided into two subgroups, A and B (n=5). Subgroup A served as a control for both protective and curative groups, and received 10 ml/kg tap water p.o. throughout the study, whereas, subgroup B served as a gentamicin control for both curative and protective groups and received gentamicin sulphate (I/M) 80 mg/kg/day throughout the study. The protective group was divided into subgroup C, D, and E (n=5) which received gentamicin 80 mg/kg I/M and after 4 h these subgroups were treated by oral administration of α-lipoic acid 50 mg/kg, selenium 10 mg/kg and combination of α-lipoic acid and selenium (50 mg/kg + 10 mg/kg), respectively throughout the study (for 20 days). The curative group was divided into subgroup F, G and H (n=5). Nephrotoxicity was induced in animals of subgroups F, G and H by administering gentamicin sulphate (I/M) 80 mg/kg/day for 9 days, which was confirmed on day 10 by measuring BUN, creatinine, AOA and kidney histology. Then α-lipoic acid (50 mg/kg), selenium (10 mg/kg) and combination of α-lipoic acid and selenium (50 mg/kg and 10 mg/kg) were administered orally once daily to animals of subgroups F, G and H respectively, throughout the study (for 26 days).

Collection of blood samples and sample processing

The blood samples were collected between 9 am and 11 am throughout the study. For evaluation of protective role blood samples were taken in non-heparinized collecting tubes from the marginal vein of the ear at day 0, 4, 8, 12, 16 and 20 whilst for curative role at day 0, 10, 14, 18, 22 and 26. The tubes were kept undisturbed for about 30 min to let the blood clot. Then the tubes were centrifuged at 3000 rpm for 25 min to separate the serum, which was transferred to clean serum tubes and frozen at –20°C until analyzed.

Determination of biochemical parameters

The blood samples were analyzed within 15 days after the collection for determining BUN and creatinine using diagnostic kits (Randox Laboratories Limited, UK). The total antioxidant activity (AOA) was determined on day 0, 8 and 20 for protective and on 0, 10 and 26 days for curative groups by the reported method (Koracevic *et al.,* 2001). Body weight was measured on day 0, 12 and 20 for assessing protective role and on day 0, 10 and 26 for determining curative role. The values taken on day 0 were considered as normal, while on day 10 as nephrotoxic.

Histopathological examination

The histological examination was carried out in an additional rabbit each for kidneys of control (day 0), nephrotoxic rabbits (day 9) and for protective and curative groups after concluding the studies (day 20 and 26). The kidneys were dissected out from the sacrificed animals, washed and fixed in 10% neutrally buffered formalin. The sections were prepared and stained with haemotoxylin and eosin for examination under the light microscope (Bancroft and Gamble, 2002).

STATISTICAL ANALYSIS

Results were presented as mean ± standard deviation (SD) for weights, BUN, creatinine and antioxidant activity of five animals. The data were analysed using one way analysis of variance (ANOVA) followed by post-Hoc Tukey's test for BUN and creatinine levels and Paired sample T-Test for antioxidant activity using statistical package SPSS version 12.0. Statistical significance was considered at $p \leq 0.05$.

RESULTS

Protective group

As shown in Table 1 administration of 50 mg/kg/day α-lipoic acid caused 34.55% decrease in BUN as compared

to the control. Selenium (10 mg/kg/day) showed a better protection than α-lipoic acid. The combination of α-lipoic acid and selenium (Group E) was highly protective which decreased 58.64% in BUN ($p<0.05$). The rabbits treated with gentamicin sulphate 80 mg/kg/day alone for 20 days serving as gentamicin-control gradually but significantly ($p<0.05$) increased BUN.

Creatinine in group B receiving nephrotoxic dose of gentamicin (80 mg/kg/day) was significantly higher (table 2), whereas α-lipoic acid and selenium alone played different protective roles on different days. There was 15.53% and 3.10% decrease in creatinine levels at the end of study in groups treated with α-lipoic acid and selenium, respectively. Combination of α-lipoic acid and selenium maintained the creatinine level almost near to the normal giving 17.48% decrease in creatinine that was significant statistically when compared to normal values at day 0 and gentamicin-control group (table 2).

The AOA remained unaffected in group A till the end of the study, while it was significantly decreased (50.43 %) in group B receiving gentamicin indicating nephrotoxicity (Table 3). Alpha-lipoic acid and selenium alone in groups C and D showed comparable protection at day 16, (above 18%), whereas combination of α- lipoic acid and selenium played protective role by restoring antioxidant activity ($p<0.05$). The IM administration of 80 mg/kg/day of gentamicin (Group B) resulted in progressive loss of the body weight of animals which continued till the end of the study. The treatment with antioxidants alone and in combination protected the animals against weight reduction (Table 4). Lipoic acid and selenium alone decreased 9% and 1.81% at day 20, respectively.

Table 1: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg + 50 mg/kg) on blood urea nitrogen (Mean ± SD; n=5)

Days	Group A (Placebo Control)	Group B (Gentamicin Control)	Group C (α-lipoic acid)	Group D (Selenium)	Group E (α-lipoic acid ±Selenium)
0	6.31±1.03	8.45±3.27	8.51±4.80	8.88±1.30	11.10±1.50
4	7.2±1.13	24.76±5.66* ↑ (193.01)%	7.40±2.20* ↑ (13.04)%	6.89±0.84* ↓ (22.40)%	9.11±1.48* ↓ (17.92)%
8	6.67±1.15	30.14±1.96* ↑ (256.69)%	5.57±0.41* ↓(34.55)%	5.17±0.72* ↓(41.78)%	8.15±1.17* ↓(26.57)%
12	5.10±0.77	34.15±0.76* ↑ (304.14)%	5.54±0.51* ↓(34.90)%	5.57±0.10* ↓(37.27)%	4.08±0.49* ↓(63.24)%
16	6.42±0.67	39.46±1.43* ↑ (366.98)%	5.57±0.25* ↓(34.55)%	5.54±0.51* ↓(37.61)%	4.59±0.45* ↓(58.64)%
20	6.67±0.02	46.49±3.85* ↑ (450.18)%	5.57±0.81* ↓(34.55)%	5.54±0.41* ↓(37.61)%	4.39±0.57* ↓(58.64)%

*Statistically significant at $P<0.05$ as compared to gentamicin-control group; ↑ % increase in BUN; ↓ % decrease in BUN as compared to values at day 0.

Table 2: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg +50 mg/kg) on blood creatinine (Mean ± SD; n=5)

Days	Group A (Placebo Control)	Group B (Gentamicin Control)	Group C (α-lipoic acid)	Group D (Selenium)	Group E (α-lipoic acid ±Selenium)
0	2.03±0.17	2.04±0.19	2.77±0.77	1.61±0.38	2.23±1.03
4	1.96±0.41	8.26±1.2* ↑(304.90)%	2.84±0.93* ↓(2.52)%	1.66±0.89* ↓(3.10)%	1.96±0.89* ↓(12.10)%
8	3.20±0.20	11.54±2.20* ↑(465.68)%	2.36±0.87* ↓(14.8)%	1.90±0.91* ↓(18.01)%	1.80±0.94* ↓(19.28)%
12	2.36±0.63	13.26±2.58* ↑(550.0)%	2.62±1.08* ↓(5.42)%	1.76±0.93* ↓(9.32)%	1.96±0.36* ↓(12.10)%
16	1.64±0.22	12.20±1.09* ↑(498.04)%	2.58±1.01* ↓(6.89)%	1.66±0.68* ↓(3.10)%	2.62±1.08* ↓(17.48)%
20	2.90±0.21	13.47±2.01* ↑(560.22)%	2.34±0.66* ↓(15.53)%	1.66±0.70* ↓(3.10)%	2.62±0.73* ↓(17.48)%

*Statistically significant at $P<0.05$ as compared to gentamicin-control group; ↑ % increase in BUN; ↓ % decrease in BUN as compared to values at day 0.

Combined α-lipoic acid and selenium protected weight in better way.

Curative group

As shown in Tables 5-8, 9 days treatment of animals with I/M gentamicin sulphate (80 mg/kg/day) caused 100% rise in BUN as compared to control indicating induction of nephrotoxicity. In gentamicin control (Group B) BUN was drastically increased to 193.01% on 10^{th} day and onward, gradually. In treated groups (F, G and H) BUN was lowered, and till day 14, the reduction of BUN in 3 groups was comparable. However, on day 18 and onward, selenium (10 mg/kg) (Group G) and selenium in combination with α-lipoic acid (Group H) effectively ($p<0.05$) reduced BUN (>80%) as compared to α-lipoic acid alone (about 59%) in Group G.

In group B, there was 62.92% increase in creatinine as compared to that of group A (Table 6), which was non-significant ($p<0.05$). Antioxidants in group F, G and H

Table 3: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg +50 mg/kg) on antioxidant activity (Mean ± SD; n=5)

Days	Group A (Placebo Control)	Group B (Gentamicin Control)	Group C (α-lipoic acid)	Group D (Selenium)	Group E (α-lipoic acid ±Selenium)
0	2.24±0.008	2.28± 0.11	2.37±0.02	2.3±0.15	2.18 ±0.08
8	2.2±0.008	1.48± 0.15 ↓ (35.08) %	1.64±0.03 (30.80) %	1.84± 0.02 (20)%	2.18±0.15 (0)%
20	2.25± 0.08	1.13± 0.02 ↓(50.43)%	1.92± 0.01 (18.98)%	1.87± 0.01 (18.69)%	2.2±0.04 (0.9)%

*Statistically significant at P< 0.05 as compared to gentamicin-control group; ↑ % increase in BUN; ↓ % decrease in BUN as compared to values at day 0.

Table 4: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg + 50mg/kg) on average weight (% change is in parentheses) (Mean ± SD; n=5)

Groups	Average weight		
	Day 0	Day 12	Day 20
Placebo (Group A)	1.06±0.11	1.08±0.083 (0.01) ↑	1.08±0.05 (0.01) ↑
Gentamicin control (Group B)	1.1±0.15	0.78±0.05 (29.09) ↓	0.74±0.089 (32.72) ↓
Lipoic acid (Group C)	1.1±0.07	1.1±0.15 ↔	1±0.07 (9.0) ↓
Selenium (Group D)	1.1±0.1	1.09±0.007(0.90) ↓	1.08±0.007 (1.81) ↓
Lipoic acid ± selenium (Group E)	1.2±0.07	1.19±0.01 (0.83) ↓	1.2±0.07↔

↑Increase; ↓decrease; ↔ no change

Table 5: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg + 50 mg/kg) on blood urea nitrogen (% change is in parentheses) (Mean ± SD; n=5)

Days	Group A (Placebo Control)	Group B (Gentamicin Control)	Group F (α-lipoic acid)	Group G (Selenium)	Group H (α-lipoic acid ±Selenium)
0	6.31±1.03	8.45±3.27	8.88±2.89	7.64±3.12	11.11±1.51
10	7.24±1.13	24.76±5.66* ↑ (193.01)%	36.58±7.26* ↑ (311.93)%	40.0±7.44* ↑ (423.56)%	43.91±3.08* ↑ (295.22)%
14	6.67±1.51	30.14±1.96* ↑ (21.72)%	30.15±15.98* ↓ (17.57)%	33.50±4.82* ↓(16.25)%	35.31±7.27* ↓(19.59)%
18	5.10±0.77	34.15±0.76* ↑(37.9)%	15.50±1.72* ↓(48.59)%	15.13±1.51* ↓(62.18)%	13.67±0.38* ↓(68.87)%
22	6.42±0.67	39.46±1.43* ↑(59.36)%	12.32±2.06* ↓(59.13)%	11.51±0.86* ↓(72.13)%	10.43±1.22* ↓(76.25)%
26	6.67±0.02	46.49±3.85* ↑(87.76)%	12.32±2.06* ↓(59.13)%	7.50±1.28* ↓(81.25)%	7.82±0.99* ↓(82.19)%

↑ and ↓ shows %age increase and decrease in BUN as compared to the 10^{th} day values
*Significantly different when compared to gentamicin-control group

significantly decreased creatinine as 55.04%, 55.37% and 77.38%, respectively. In group H, receiving combination of antioxidants, significant decrease in creatinine which was comparable to the value of creatinine observed in the control (Group A) was observed.

The gentamicin treatment significantly reduced antioxidant activity at day 9 in all the curative groups except in group A. None of the antioxidants' treatments alone was able to treat nephrotoxicity because only 18.66% and 14.28% increase in AOA was observed by α-lipoic acid and selenium, respectively on day 26. The combination of the above antioxidants caused 46.66% increase in AOA. However, there was a significant increase in body weights from 30.0% in groups C and D and up to 42.85% in group E.

Table 6: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg + 50 mg/kg) on serum creatinine levels (μ mol/L) (% change is in parentheses) (Mean ± SD; n=5)

Days	Group A (Placebo Control)	Group B (Gentamicin Control)	Group F (α-lipoic acid)	Group G (Selenium)	Group H (α-lipoic acid +Selenium)
0	2.03±0.17	2.04±0.19	2.78±0.78	1.61±0.38	2.23±1.03
10	1.96±0.41	8.82±1.44* ↑(333.99)%	6.54±2.40* ↑ (135.25)%	7.26±1.47* ↑ (350.93)%	9.11±1.54* ↑ (308.52)%
14	3.20±0.20	11.54±2.20* ↑(30.84)%	5.66±0.90* ↓(13.46)%	6.00±1.77* ↓ (17.35)%	4.61±2.44* ↓ (49.39)%
18	2.36±0.63	13.26±2.58* ↑ (50.34)%	4.96±1.02* ↓(24.16)%	6.54±0.41* ↓(9.92)%	5.22±1.67* ↓ (42.70)%
22	1.64±0.22	13.48±2.03* ↑(52.83)%	4.36±1.51* ↓(33.33)%	5.04±1.03* ↓ (30.58)%	3.42±0.94* ↓ (62.46)%
26	2.90±0.21	14.37±2.08* ↑(62.92)%	2.94±1.47* ↓(55.04)%	3.24±1.77* ↓ (55.37)%	2.06±0.96* ↓ (77.38)%

↑ and ↓ shows %age increase and decrease in creatinine as compared to the 10th day values; *Significantly different when compared to gentamicin- control group

Table 7: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg + 50 mg/kg) on antioxidant activity (% change is in parentheses) (Mean ± SD; n=5)

Days	Group A (Placebo Control)	Group B (Gentamicin Control)	Group F (α-lipoic acid)	Group G (Selenium)	Group H (α-lipoic acid +Selenium)
0	2.25±0.44	2.22±0.41	2.38±0.20	2.3±0.32	2.2±0.28
10	2.25±0.43	1.62+0.34* ↓ (27.02)	1.5±0.3* ↓(36.97)	1.44±0.18* ↓(37.39)	1.5±0.27* ↑ (31.81)
26	2.26±0.42	1.12± 0.17* ↓(49.54)	1.78±0.21* ↑(18.66)	1.64± 0.27* ↑(14.28)	2.2±0.29* ↑ (46.66)

Table 8: Effect of placebo and α-lipoic acid (50 mg/kg) and selenium (10 mg/kg) alone and combined selenium + α-lipoic acid (10 mg/kg + 50mg/kg) on the average weight of animals (% change is in parentheses) (Mean ± SD; n=5)

Groups	Day 0	Day 10	Day 26
Group A	1.06±0.11	1.08±0.08	1.08±0.01
Placebo control		↑ (0.01)	↔ (0.01)
Group B	1.1±0.15	0.78±0.05	0.74±0.08
Gentamicin-control		↓ (29.09)	(32.72) ↓
Group F	1.16±0.16	0.77±0.05	1.06±0.02
Lipoic acid		↓ (33.62)	↑ (8.6)
Group G	1.1±0.18	0.77±0.01	0.89±0.17
Selenium		↓ (30.00)	↓ (19.09)
Group H	1.28±0.19	0.77±0.02	1.1±0.16
α-lipoic acid+Selenium		↓ (39.84)	↓ (14.06)

↑ and ↓ percent increase, decrease and ↔ no change, respectively

Histological examination

The results of histological examination of various groups are given in fig. 1. As compared to the normal rabbit's kidney (fig. 1a), the nephrotoxic rabbit's kidney in gentamicin group induced with oxidative stress shows tissue injury, characterized by tubular necrosis and damaged cells in lumen and glomerulus (fig 2b). The damaged renal tissues in protective gentamicin control and curative groups were improved by the combination of antioxidants since there was no evidence of lesions in the kidneys in fig. 1c and 1d respectively.

DISCUSSION

Gentamicin, a broad spectrum antibacterial causes nephrotoxicity (Barry, 2001) which is correlated to oxidative stress (Devipriya and Shymaladevi, 1999). Nephrotoxicity increases BUN, creatinine and acute tubular necrosis and decreases AOA (Verpooten *et al.,* 1998) and the same was noticed on administering nephrotoxic dose of gentamicin in this study. In the present study the nephrotoxicity was induced in 9 days, contrary to 6 days and 14 days reported previously (Nordstrom *et al*., 1990; Ben Ismail *et al.,* 1994), might be due to differences in laboratory conditions and animal species. The dose of α-lipoic acid as 50 mg/kg/day was selected for rabbits since 25 mg/kg had been suggested as effective antioxidant in rats (Sandhya *et al.,* 1995). A dose of selenium 10 mg/kg (4×10^{-3}g)/body weight was selected as reported (Ademuyiwa *et al.,* 1990).

The assay used to determine AOA measures capacity of serum to inhibit the production of thiobarbituric acid reactive species (TBARS) from sodium benzoate under the influence of oxygen free radicals derived from Fenton's reaction. The antioxidants present in the test sample suppressed the production of TBARS. A significant decrease in the antioxidant activity was noted in nephrotoxic rabbits. A progressively significant weight loss in gentamicin-induced nephrotoxic rabbits was observed in protective and curative groups. Some rabbits also showed slower movements indicating lethargy and less consumption of food, especially in group B which resulted in weight loss. In the treatment groups, antioxidants protected animals against weight loss as reported (Banday *et al.,* 2008).

In curative group, selenium alone lowered effectively

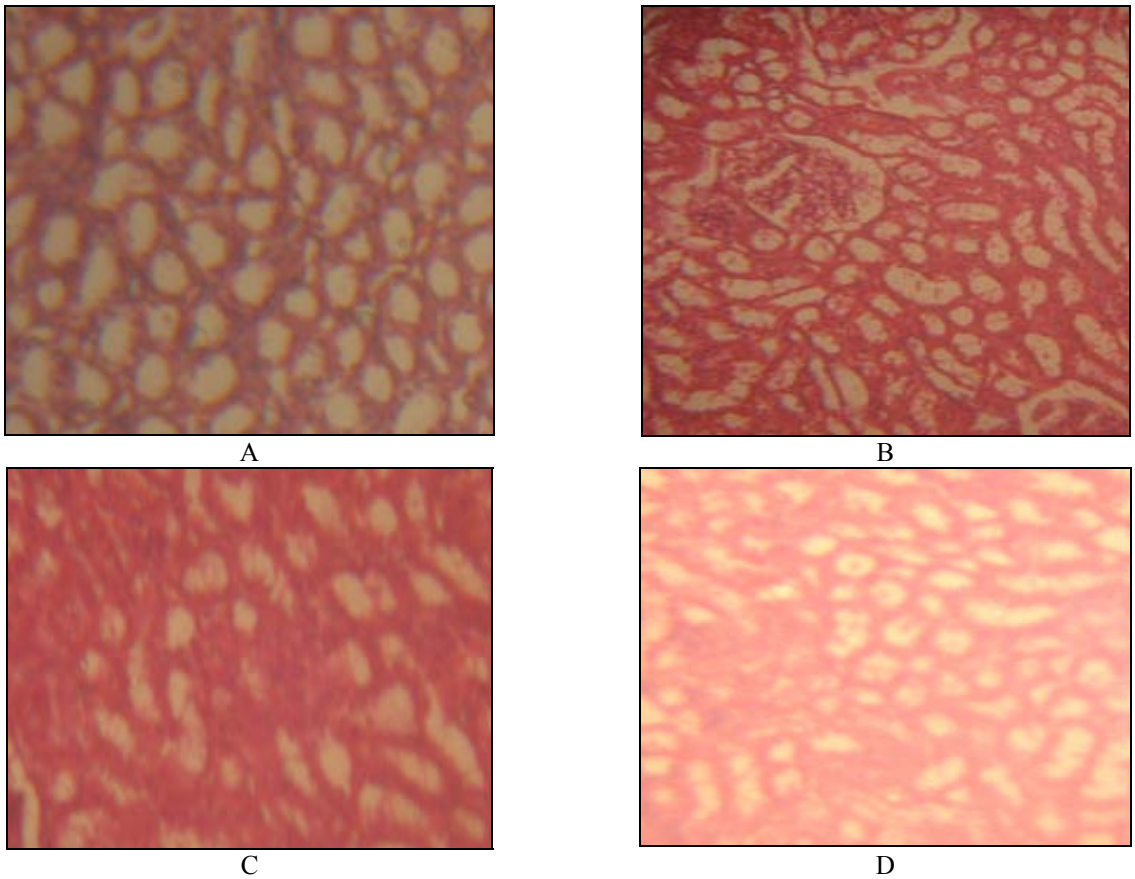

Fig. 1: Histology presentation of normal rabbits (A), nephrotoxic rabbits at day 9, showing oxidative stress-led tubular necrosis, damaged cells in lumen and glomerulus injury/necrosis (B), improvement in lesions with combined α-lipoic acid and selenium at the end of study in protective and curative study (C and D, respectively).

BUN and creatinine and raised AOA as compared to α-lipoic acid but, in combination more effectively cured nephrotoxicity which was contrary to a previous study (Cakatay *et al.,* 2008). However, the findings were in line with another study (Berkson, 1999) reporting synergistic effect of multiple antioxidants in hepatitis C treatment. Lipoic acid, a strong antioxidant protects against drug-induced damage to kidneys, liver and inner ear (Kagen *et al.,* 1992). In the present study, selenium supplemented the curative potential of α-lipoic acid. However, in accordance with previous study (Weijl *et al.,* 2004) superior effect of selenium was observed when combined with α-lipioc acid. The synergistic effect of α- lipoic acid and selenium is possibly due to restoration of diminished activities of catalases, super oxide dismutases and glutathione reductases, resulting in suppression of the elevated lipid peroxidation in kidney (Rybak *et al.,* 1999; Somani *et al.,* 2000). Selenium also functions as a co-factor for the enzymes such as glutathione and thioredoxin that remove ROS and enhances antioxidant functionality. Since antioxidants protect cell and tissue injury against ROS and free radicals damaging effect (Marcello *et al.,* 1993), α-lipoic acid and selenium synergistically prevented the damage of renal brush borders in proximal tubules in this study. Camorgo *et al.,* (2001) showed that sodium selenate protects from early proximal tubular injury in kidneys.

CONCLUSION

The α-lipoic acid and selenium have protective and curative effects against gentamicin-induced nephro-toxicity. Moreover, in combination, both the antioxidants showed synergism in protecting and curing gentamicin-induced nephrotoxicity.

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