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Research Article

ALOE VERA ATTENUATES GENTAMICIN-INDUCED NEPHROTOXICITY IN WISTAR ALBINO RATS: HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES

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ABSTRACT

Objectives: To evaluate the nephroprotective properties of ethanol extract of leaves of *Aloe vera* against gentamicin-induced nephrotoxicity in rats.

Methods: Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of gentamicin 40 mg/kg/days for 5 days. Effect of concurrent administration of ethanol extract of leaves of *A. vera* at a dose of 20 ml/kg/day given by oral route was determined using serum creatinine and blood urea nitrogen as biochemical indicators of renal damage; after 10, 20, and 30 days. The study included a control group which received oral saline only, gentamicin treated group, received gentamicin and oral saline and *A. vera* group, received oral *A. vera* prior to gentamicin administration. Each group had six rats.

Results: It was observed that in *A. vera* treated rats, prevented elevation of the biochemical indicators of nephrotoxicity and significantly reduced histopathological scores.

Conclusion: Ethanol extract of *A. vera* contains constituents with nephroprotective activities.

Keywords: Gentamicin-induced nephrotoxicity, *Aloe vera*, Wister rats, Histopathological change.

INTRODUCTION

Aloe vera (*Aloe barbadensis miller*) is in use as traditional medicine across geographical boundaries [1,2]. Interest in *A. vera* has greatly increased recently owing to their free radical scavenging properties [3]. Studies have demonstrated the presence of bioactive constituents, exhibiting antioxidant properties, including phenolic glycosides, polysaccharides, and triterpenes in *A. vera* [4,5]. However; systematic and scientific published literature on the nephroprotective effect of A. vera is very limited [6,7].

Nephrotoxicity is a potential adverse effect of aminoglycosides. Depending on the clinical scenario, in 7-58% of therapeutic courses, it leads to nephrotoxicity, which limits their frequent and prolonged clinical use [8-10]. Gentamicin-induced renal damage is widely used model for inducing nephrotoxicity in experimental animals. The mechanism of gentamicin-induced nephrotoxicity is not completely understood. Oxidative stress is implicated as central mechanism of the gentamicin-induced renal cell injury [11]. Accordingly, several compounds with antioxidant activity have been tested to prevent or ameliorate the gentamicin-induced nephrotoxicity in animals [12-16].

The present study was designed to determine duration dependent protective effect of ethanolic extract of leaves of *A. vera* on gentamicin-induced nephrotoxicity in Wistar albino rats.

METHODS

The experiments were conducted in pharmacology Department of LLRM Medical College, Meerut (Uttar Pradesh) India during the year 2011-12. The experimental protocol was approved by the Institutional Animal Ethical Committee (approval no. STP/2011/13) registration no. (819/04/CPCSEA).

Plant material

A. vera (*Aloe barbadensis miller*) plants were procured from the local market of Meerut, Uttar Pradesh, India. The plant was identified and authenticated by the scientists in Botany Department of Chaudhary Charan Singh University, Meerut, (UP) India.

Preparation of the plant extract

The *A. vera* plant extract was prepared as per the procedure described by Lawrence *et al*. (2009) [17] with slight modifications. In brief, the fully expanded leaves of *A. vera* were selected from the plants; washed with distilled water and were subjected to surface sterilization with 70% ethyl alcohol. The parenchymatous covering of the leaves were peeled and the gel drained out. The slurry was prepared with the help of pestle and mortar. For the preparation of ethanolic extract, fresh leaf gel was dried in the oven at 80°C for 48 hrs and then powdered. 20 g of this powder was soaked in 200 ml of ethanol for 24 and shaker was used to prevent sedimentation. It was then filtered through Whatman Filter No. 1, and the filtrate was concentrated by heating at 40°C, until complete evaporation of the solvent was achieved. 100 mg of this concentrated extract was dissolved in 1 ml of distilled water, and the resulting solution was administered to rats.

Experimental animals

The animals were obtained from the rat rearing unit of the central animal house of the institute. The healthy Wistar albino rats of either gender, weighing 150-200 g were selected for the study. After 1 week of quarantine and acclimatization, the animals were included in the study. The rats were housed in stainless steel metabolic cages under controlled conditions of temperature (25°C) and alternating periods of light and darkness of 12 hrs each. The rats had free access to standard rat pellet diet (Vetcare India Ltd.) and tap water *ad libitum.*

Acute toxicity study

The acute toxicity study for ethanolic extract of *A. vera* was carried out on Wistar rats as per the revised guidelines by Organization for Economic Co-operation and Development (OECD) and Committee for the purpose of control and supervision of experiments on animals [18,19]. Each animal was administered a single dose of the ethanolic extract of *A. vera*

by oral route The animals were observed for any changes continuously for 2 hrs and up to 24 hrs for any adverse effect.

Estimation of LD_{50}

$LD5_0$ of *A. vera* extract was determined on Wistar rats by standard procedure [20]. The acute oral toxicity study and estimation of LD_{50} was done according to the OECD guideline 423 [21].

Experimental design

The study was carried out in three phases. The basic experimental design of study remained same (Fig. 1) except that the *A. vera* was administered for the duration of 10, 20, and 30 days, respectively, in first, second, and third phase of the study. For the each test duration, eighteen rats were randomized into three groups of six rats each. Group I served as control group and the animals received normal saline (20 ml/kg/day) orally into two equally divided doses, spaced at 12 hrs, consecutively for test durations. Group II animals served as gentamicin group, and animals received normal saline (20 ml/kg/day) orally twice daily into two equally divided doses consecutively for treatment duration, as in Group I and in last 5 days gentamicin (Genticyn, Abott Healthcare Pvt. Ltd, India) was injected intraperitonealy at 8 AM, as a single dose of 80 mg/kg [22,23]. Group III animals received *A. vera* orally in a dose of 20 ml/kg/day into two equally divided doses, in similar way as oral saline in Group I, consecutively for test duration and in last 5 days gentamicin was injected intraperitonealy as a single dose of 80 mg/kg, as in Group II. The test compounds were administered by gavage method wherein animals were fasted for 3-4 hrs prior to and 1 hr after administration to ensure proper absorption. On completion of test duration (i.e., 10, 20, and 30 days in first, second, and third phase) animals of all the groups fasted for 24 hrs (during this period tap water remained freely available) before sacrificing under ketamine (75 mg/kg) and diazepam (10 mg/kg) anesthesia given intraperitoneally [24]. Blood samples were collected (3 ml) from anesthetized animals via retro-orbital plexus puncture. The serum was separated and processed for determination of biochemical parameters. After blood collection, the animals were sacrificed to dissect out both kidneys for histopathological examination.

Determination of biochemical parameters

The collected blood sample, after a standing time of ½ hr, was centrifuged in Remi R-8 centrifuge at 2500 rpm for 10 minutes. The serum so separated was used for evaluation of renal functions tests *viz*. blood urea nitrogen (BUN) and serum creatinine (SCr) [25]. Serum urea was estimated spectrophotometrically by Liquimax Urea Reagent Kit (marketed by Avecon Healthcare Pvt. Ltd). SCr was estimated spectrophotometrically by AutoZyme Creatinine Reagent Kit (marketed by Accurex Biomedical Pvt. Ltd).

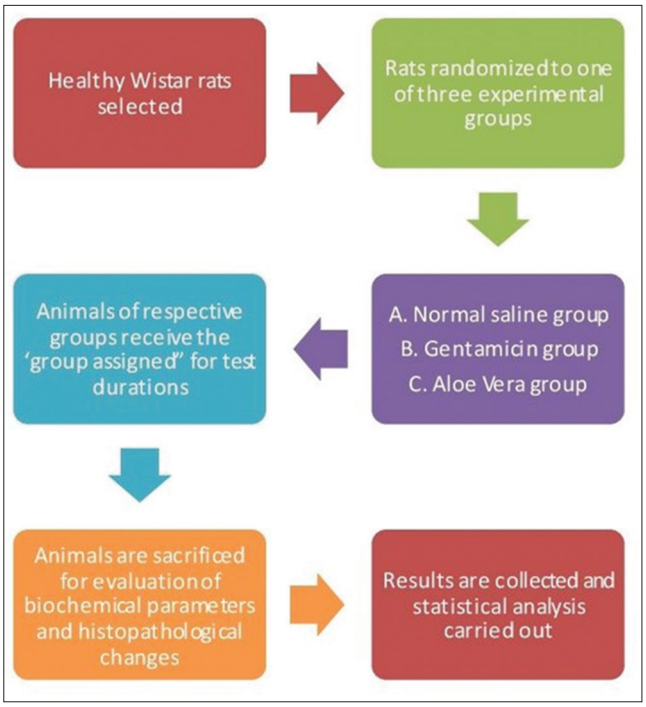

Fig. 1: Flowchart of the steps of the experimental study

Histopathological examination

The kidney was excised from the animals and washed with the normal saline. Whole of kidney was placed in 10% neutral formalin for 12-24 hrs. It was then dehydrated and cleared with ethanol and xylene, respectively; followed by embedding in paraffin wax from which blocks were prepared. Sections of 5 µm thickness were prepared from the blocks using a microtome [26].

These were processed in alcohol-xylene series and were stained with Harris hematoxylin and eosin stain and subjected to histopathological examination [27]. Histopathological examination was according to the scoring system [28].

Score 0: Normal score

Score 1: Areas of focal granulovacuolar debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (<1% of the total tubular population).

Score 2: Tubular epithelial necrosis and desquamation easily seen but involving <½ of cortical tubules.

Score 3: More than half of proximal tubules showing desquamation necrosis but involved tubules easily found.

Score 4: Complete or almost complete tubular necrosis.

Statistical analysis

Results of quantitative parameters were expressed as a mean±standard deviation. The Statistical analysis was carried out using one-way Analysis of Variation followed by Student's *t*-test. $p<0.05$ were considered to be statistically significant.

RESULTS

Acute toxicity study and LD_{50}

There was no observed change in behavioral pattern or signs and symptoms of toxicity with ethanolic extracts of *A. vera* in animals up to 24 hrs. Doses of 20 ml/kg to 100 ml/kg in increments of 20 ml/kg were given to determine the LD_{50} of the *A. vera* in Wistar rats [20]. There was no mortality at all doses levels. The pharmacological evaluation was carried out at 20 ml/kg body weight into two equally divided doses by the oral route.

EFFECT ON BIOCHEMICAL PARAMETERS

The mean BUN in animals of saline treated control group ranged between 23.8±1.62 and 25.1±1.34 mg/dl for the different treatment durations ($p=0.310$). Administration of gentamicin in animals resulted in significant ($p<0.001$) increase in BUN level in gentamicin group compared to control group (Table 1). *A. vera* kept the BUN to

Table 1: BUN levels in control group, gentamicin treated and AVT groups

Duration of treatment (days)	BUN (mg/dl) (mean±SD)		
	C	GT	AVT
10	25.1±1.34	66.16±3.31#	40.5±7.14*
20	24.4±1.20	64.83±1.72#	29.66±2.50*
30	23.8±1.62	66.6±3.14#	26±2.41*

(#$p<0.001$ compared to control group; *$p<0.001$ compared to gentamicin group) n=6, AVT: *Aloe vera* treated, GT: Gentamicin treated, BUN: Blood urea nitrogen, SD: Standard deviation

significantly lower level (p<0.001) in group III animals when compared to gentamicin treated group II animals. Administration of *A. vera* exhibited the duration dependent limitation of the BUN elevation (Fig. 2). The 30 days treatment significantly (p=0.005) prevented a rise in BUN level (26±2.41 mg/dl), and kept it almost near to normal level, compared to that after 10 days treatment (40.5±7.14 mg/dl).

The mean SCr level in the control group did not change significantly (p=0.803) for the different treatment durations. There was significant (p=<0.001) increase in SCr level in group II gentamicin treated animals (Table 2). In Group III animals, *A. vera* kept the SCr level to significantly (p<0.001) lower level when compared to group II gentamicin treated animals. Similar to BUN duration dependent limitation of the SCr level was observed for progressively increasing test durations (Fig. 3). The 30 days treatment resulted in SCr level (0.53±0.07) significantly lower (p<0.001) than after 10 days treatment (0.97±0.06).

Histopathological changes

Control group animals showed glomerular and tubular histology. The gentamicin treatment resulted in diffuse cell necrosis in proximal tubules of rat kidneys. The observed histopathological changes included renal congestion with mononuclear cell infiltration, tubular necrosis, hyaline, and granular casts (Fig. 4). The concurrent *A. vera* administration in Group III animals resulted in the preservation of the tubular histology by significantly (p<0.05) reducing the scores of histopathological damage compared to gentamicin treated group (Table 3).

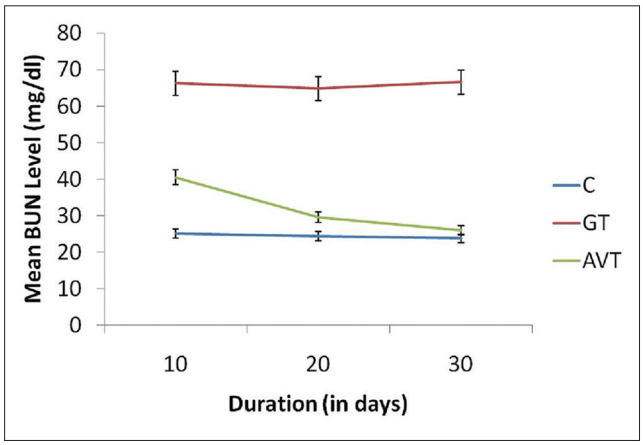

Fig. 2: Blood urea nitrogen levels in control group, gentamicin treated and *Aloe vera* treated groups. (*p<0.001 compared to gentamicin group)

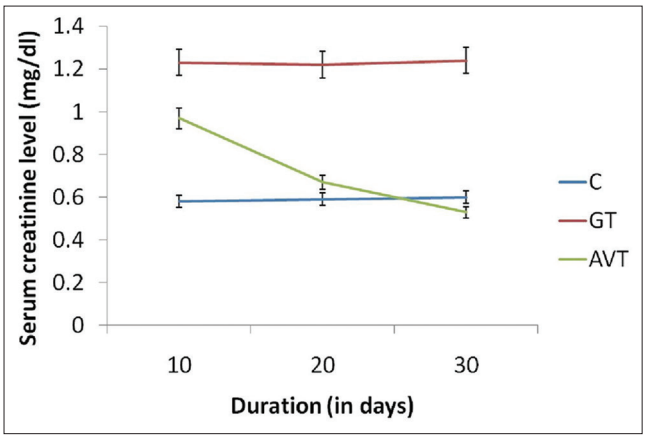

Fig. 3: Serum creatinine levels in control group, gentamicin treated and *Aloe vera* treated groups (n=6). (*p<0.001 compared to gentamicin group)

DISCUSSION

Drugs are a major contributor to acute kidney insult. Renal toxicity attributive to aminoglycosides is of major clinical concern because of their widespread use [29-31]. Thus, amelioration of aminoglycoside nephrotoxicity by any means would be of great clinical significance.

Table 2: SCr levels in in control group, gentamicin treated and AVT groups (n=6)

Duration of treatment (days)	Serum creatinine (mg/dl) (mean±SD)		
	C	GT	AVT
10	0.58±0.04	1.23±0.10#	0.97±0.06*
20	0.59±0.07	1.22±0.07#	0.67±0.05*
30	0.60±0.04	1.24±0.09#	0.53±0.07*

(#p<0.001 compared to control group; *p<0.001 compared to gentamicin group) n=6, AVT: *Aloe vera* treated, GT: Gentamicin treated, SD: Standard deviation, SCr: Serum creatinine

Table 3: Semi quantitative comparison and scores of the histopathological damage of the renal tissue of rats of different treatment groups normal saline treated (C), GT, AVT (n=6)

Histopathological features	C	GT	AVT
Glomerular congestion	-	+++	+
Peritubular congestion	-	+++	+
Epithelial desquamation	-	+	-
Blood vessels congestion	-	+++	+
Interstitial edema	-	+	+
Inflammatory cells	-	+++	-
Tubular casts	-	+	+
Necrosis	-	+	+
Histo-pathological score	0	2.9±0.4**	1.4±0.15**

**Gentamicin treated group versus *Aloe vera* pre-treated group (p<0.05), AVT: *Aloe vera* treated, GT: Gentamicin treated, SD: Standard deviation

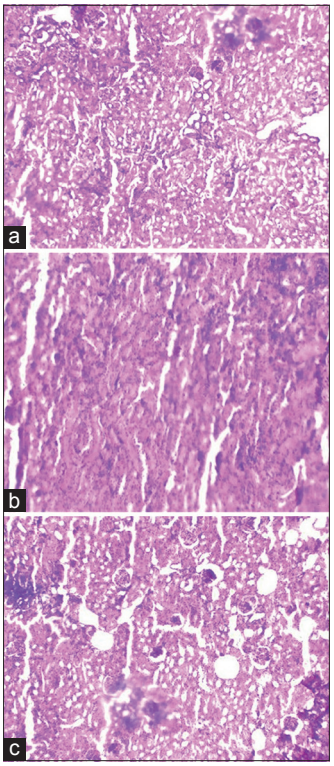

Fig. 4: Light microscopy of the renal tissue from the rats (H&E, ×100): (a) Normal saline group, (b) Gentamicin treated and (c) *Aloe vera* treated

Gentamicin-induced nephrotoxicity is an established experimental model of acute renal failure caused by oxidative stress generated by free radicals [32,33].

The results of the present study revealed that the ethanolic extract of *A. vera* possesses significant nephroprotective effect against gentamicin-induced nephrotoxicity. Co-administration of *A. vera* extract resulted in significantly prevented elevation of serum urea and creatinine level. Gentamicin has been shown to induce renal injury as evident from the elevated serum urea and creatinine levels and also from the histopathological features of acute renal failure [34-36]. The histopathological examination in gentamicin treated group revealed extensive tubular necrosis which is typical of gentamicin-induced nephrotoxicity [11,36]. The concurrent *A. vera* administration in Group III animals resulted in the preservation of the tubular histology compared to gentamicin treated group.

Gentamicin-induced nephrotoxicity is known to result from free radical induced oxidative damage [9,37,38]. Agents with free radical scavenging property can either inhibit or attenuate the renal damage induced by drugs [39]. Several chemicals and phyto derived compounds (because of their antioxidant properties) have been experimentally used to reduce the gentamicin nephrotoxicity [12-14]. Plant derived phenolic compounds have been demonstrated to confer excellent protection against reactive oxygen species induced renal damage by exhibiting good scavenging activity [14]. Several clinical and experimental studies have shown the antioxidant potential of *A. vera* [15,40] through both direct free radical scavenging as well as by regulating xenobiotic mechanisms [41]. The nephroprotective properties of ethanolic extract of *A. vera* seems to be due to its antioxidant properties.

Three treatment duration phases were used to study the relationship between duration of prophylaxis with *A. vera* and degree of amelioration of the gentamicin nephrotoxicity. Evaluation of the results suggested a dose-effect relationship. Prophylactic administration of the *A. vera* for a longer duration (30 days) was associated with the least disruption of renal function parameters and renal histology. However to determine the optimum duration of pre-exposure prophylaxis, further studies are required.

CONCLUSION

The results of this study suggest that the ethanol extract of *A. vera* contains constituents with nephroprotective activities. Based on the observations in the present study *A. vera* extract supplementation may provide a cushion against drug-induced nephrotoxicity without harmful side effects. However, further investigations are required to identify and isolate the specific nephroprotective and antioxidant constituent in *A. vera*, as well as to delineate the mechanism of alleviation of gentamicin nephrotoxicity.

REFERENCES

1. Parthipan M, Thomas B, Rajendran A. Habitat diversity, morphological and systematic analysis of multipotential species of *Aloe barbadensis* Mill. (Liliaceae) from Southeren Westran Ghats of Tamil Nadu, India. J Res Biol 2011;1:237-41.
2. Available from: http://www.who.int/medicines/publications/traditional/MedPlantsMonograph1.pdf.
3. Davis RH, Donato JJ, Hartman GM, Haas RC. Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. J Am Podiatr Med Assoc 1994;84(2):77-81.
4. Hu Y, Xu J, Hu Q. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis miller*) extracts. J Agric Food Chem 2003;51(26):7788-91.
5. Hamman JH. Composition and applications of *Aloe vera* leaf gel. Molecules 2008;13(8):1599-616.
6. Bolkent S, Akev N, Ozsoy N, Sengezer-Inceli M, Can A, Alper O, *et al.* Effect of *Aloe vera* (L.) Burm. fil. leaf gel and pulp extracts on kidney in type-II diabetic rat models. Indian J Exp Biol 2004;42(1):48-52.
7. Chatterjee P, Mukherjee A, Nandy S. Protective effect of the aqeous extract of *Aloe barbedensis* on gentamicin and cisplatin induced nephrotoxic rats. Asian Pac J Trop Biomed 2012;2:S1754-63.
8. Laurent G, Kishore BK, Tulkens PM. Aminoglycoside-induced renal phospholipidosis and nephrotoxicity. Biochem Pharmacol 1990;40(11):2383-92.
9. Bertino JS Jr, Booker LA, Franck PA, Jenkins PL, Franck KR, Nafziger AN. Incidence of and significant risk factors for aminoglycoside-associated nephrotoxicity in patients dosed by using individualized pharmacokinetic monitoring. J Infect Dis 1993;167(1):173-9.
10. Oliveira JF, Silva CA, Barbieri CD, Oliveira GM, Zanetta DM, Burdmann EA. Prevalence and risk factors for aminoglycoside nephrotoxicity in intensive care units. Antimicrob Agents Chemother 2009;53(7):2887-91.
11. Walker PD, Barri Y, Shah SV. Oxidant mechanisms in gentamicin nephrotoxicity. Ren Fail 1999;21(3-4):433-42.
12. Ali BH. Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: Some recent research. Food Chem Toxicol 2003;41(11):1447-52.
13. Lakshmi BV, Sudhakar M. Protective effect of *Zingiber officinale* on gentamicin-induced nephrotoxicity in rats. Int J Pharmacol 2010;6:58-62.
14. Sandhya P, Mohandass S, Varalakshmi P. Role of DL alpha-lipoic acid in gentamicin induced nephrotoxicity. Mol Cell Biochem 1995;145(1):11-7.
15. Pedraza-Chaverrí J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Granados-Silvestre MA, Hernández-Pando R, *et al.* Garlic ameliorates gentamicin nephrotoxicity: Relation to antioxidant enzymes. Free Radic Biol Med 2000;29(7):602-11.
16. Randjelovic P, Veljkovic S, Stojiljkovic N, Jankovic-Velickovic L, Sokolovic D, Stoiljkovic M, *et al.* Salicylic acid attenuates gentamicin-induced nephrotoxicity in rats. ScientificWorldJournal 2012;2012:390613.
17. Lawrence R, Tripathi P, Jeyakumar E. Isolation, purification and evaluation of antibacterial agents from *Aloe vera*. Braz J Microbiol 2009;40(4):906-15.
18. Available from: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals_chem_guide_pkg-en.
19. CPCSEA Guidelines for Laboratory Animal Facility. Available from: http://www.envfor.nic.in/divisions/awd/cpcsea_laboratory.pdf.
20. Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics. Vol. 1. New York, N.Y: Academic Press; 1964. p. 135.
21. OECD Guidelines for Testing Chemicals. Acute Oral Toxicity-Acute Toxic Class Method. Available from: http://www.iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf.
22. Bennett WM, Plamp CE, Gilbert DN, Parker RA, Porter GA. The influence of dosage regimen on experimental gentamicin nephrotoxicity: Dissociation of peak serum levels from renal failure. J Infect Dis 1979;140(4):576-80.
23. Priyamvada S, Priyadarshini M, Arivarasu NA, Farooq N, Khan S, Khan SA, *et al.* Studies on the protective effect of dietary fish oil on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Prostaglandins Leukot Essent Fatty Acids 2008;78(6):369-81.
24. Wixson SK, White WJ, Hughes HC Jr, Lang CM, Marshall WK. A comparison of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam anesthesia in adult male rats. Lab Anim Sci 1987;37(6):726-30.
25. Pardue HL. Kinetic aspects of analytical chemistry. Anal Chim Acta 1989;216:69-107.
26. Talib VH, editor. A Handbook of Medical Laboratory Technology. 2nd ed. New Delhi: CBS Publishers and Distributors; 2007. p. 155-67.
27. Clayden EC, editor. Practical Section Cutting and Staining - Part 1. London: Churchill Livingstone; 1971.
28. Houghton DC, Plamp CE 3rd, DeFehr JM, Bennett WM, Porter G, Gilbert D. Gentamicin and tobramycin nephrotoxicity. A morphologic and functional comparison in the rat. Am J Pathol 1978;93(1):137-52.
29. Bellomo R. The epidemiology of acute renal failure: 1975 versus 2005. Curr Opin Crit Care 2006;12(6):557-60.
30. Guo X, Nzerue C. How to prevent, recognize, and treat drug-induced nephrotoxicity. Cleve Clin J Med 2002;69(4):289-90, 293-4, 296-7 passim.
31. Begg EJ, Barclay ML. Aminoglycosides-50 years on. Br J Clin Pharmacol 1995;39(6):597-603.
32. Ozbek E, Ilbey YO, Simsek A, Cekmen M, Mete F, Somay A. Rosiglitazone, peroxisome proliferator receptor-gamma agonist, ameliorates gentamicin-induced nephrotoxicity in rats. Int Urol Nephrol 2010;42(3):579-87.

33. Emin O. Induction of oxidative stress in kidney. Int J Nephrol 2012;2012:Article ID 465897, 9 pages, 2012. doi:10.1155/2012/465897.
34. Dahlgren JG, Anderson ET, Hewitt WL. Gentamicin blood levels: A guide to nephrotoxicity. Antimicrob Agents Chemother 1975;8(1):58-62.
35. Dehghani F, Namavar MR, Noorafshan A, Karbalay-Doust S, Esmaeilpour T. Evaluation of the kidney extract on gentamicin induced-nephrotoxicity in rat. Kidney Res J 2011;1:24-32.
36. Houghton DC, Plamp CE 3rd, DeFehr JM, Bennett WM, Porter G, Gilbert D. Gentamicin and tobramycin nephrotoxicity. A morphologic and functional comparison in the rat. Am J Pathol 1978;93(1):137-52.
37. Sha SH, Schacht J. Stimulation of free radical formation by aminoglycoside antibiotics. Hear Res 1999;128(1-2):112-8.
38. Yang CL, Du XH, Han YX. Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. Ren Fail 1995;17(1):21-6.
39. Abdelmeguid NE, Chmaisse HN, Abuo Zeinab NS. Protective effect of silymarin on cisplatin-induced nephrotoxicity in rats. Pak J Nutr 2010;9:624-36.
40. Saada HN, Ussama ZS, Mahdy AM. Effectiveness of *Aloe vera* on the antioxidant status of different tissues in irradiated rats. Pharmazie 2003;58(12):929-31.
41. Singh RP, Dhanalakshmi S, Rao AR. Chemomodulatory action of *Aloe vera* on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in mice. Phytomedicine 2000;7(3):209-19.