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Nephroprotective potential of *Trichosanthes dioica* ROXB. leaves extract against Gentamicin induced Nephropathy in albino Rats

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ABSTRACT

The purpose of this study is to evaluate nephroprotective potential of *Trichosanthes dioica* leaves extract against Gentamicin (GM) induced nephrotoxicity and renal dysfunction. Twenty-four Wistar rats were divided into four groups (n = 6). Control rats that received normal saline (i.p.) and 0.5% carboxymethyl cellulose (p.o.) per day for 8 days. Nephrotoxicity was induced in rats by intraperitoneally administration of GM (100 mg/kg/day for 8 days) and were treated with TLE (200 and 400 mg/kg/day (p.o.) for 8 days). Plasma and urine urea and creatinine, kidney weight, urine output, blood urea nitrogen, urinary sodium and potassium level, renal enzymatic and non-enzymatic antioxidants and lipid peroxidation was evaluated along with histopathological investigation in various experimental groups of rats. It was observed that the GM treatment induced significant elevation ($p < 0.001$) in plasma and urine urea, creatinine, kidney weight, blood urea nitrogen, plasma Na^+ and K^+ level, renal lipid peroxidation along with significant decrement ($p< 0.001$) in urine output, renal enzymatic and non-enzymatic antioxidants. TLE 200 and 400 mg/kg treatment to GM treated rats recorded significant decrement (up to $p < 0.001$) in plasma and urine urea and creatinine, urinary Na^+ and K^+ level, renal lipid peroxidation along with significant increment (up to $p < 0.001$) in renal enzymatic and non-enzymatic antioxidants. Histological observations of kidney tissues too correlated with the biochemical observations. These finding powerfully supports that *Trichosanthes dioica* leaves extract acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of GM both in the biochemical and histopathological parameters.

INTRODUCTION

Trichosanthes dioica R. *is an important medicinal herb.* In Charak Samhita, leaves and fruits used for treatment of alcoholism, jaundice, oedema and alopecia[1]. Over 20 species of *Trichosanthes* are recorded in Asia of which two namely *T. dioica* and *T. anguina* are cultivated as vegetable[2]. *Trichosanthes dioica (*Pointed gourd) is known by the name of *parwal*, *parmal, patol, potala* in different parts of India and Bangladesh and used as antipyretic, diuretic, cardiotonic and laxative [3]. The fruit and leaves is the edible part of the plant which is cooked in various ways either alone or in combination with other vegetables or meats[4]. Juice of leaves of *T. dioica* is used as tonic, febrifuge and in subacute cases of enlargement of liver and spleen[5]. The various chemical constituents present in *T. dioica* are vitamin A, vitamin C, tannins, saponins[6].

Aqueous extract of *T. dioica* fruits showed the anti-diabetes activity[7], cholesterol lowering activity in normal and streptozotocin diabetic rats[8], hepatoprotective activity against

ferrous sulphate-induced liver injury[9], skin disorder[10], the fixed oil of seeds of *Trichosanthes* species including *T. dioica* have antifungal property[11]. Rai *et al*. reported the *in-vitro* assessment of antimicrobial activity of *Trichosanthes dioica*[12]. Shivhare *et al.* evaluate the antioxidant activity of fruits of *Trichosanthes dioica*[13]. To the best of our knowledge there were no any scientific reports available in support of its nephroprotective potential. Therefore, present study was designed to demonstrate the effect of *Trichosanthes dioica leaves* extract (TLE) against Gentamicin induced renal damage in experimental animals.

MATERIALS AND METHODS

Chemicals

Gentamicin (Nicholas, Mumbai, India) from the source indicated in parentheses. All the chemicals used were of analytical grade and procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India.

Animals

Wistar rats (200-250 g) of either sex were procured from Royal college of Pharmacy, Health and Sciences Berhampur, Orissa. They were kept in departmental animal house in well cross ventilated room at 22 ± 2 °C with light and dark cycles of 12 h for 1 week before and during the experiments. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 1018C/06/CPCSEA).

Preparation of plant extract

Leaves of *T. dioica* were collected from local area of Berhampur (Orissa) India, during the month of October. The plant material was identified and the voucher specimen was deposited in the institutional herbarium for future references. The leaves of the Trichosanthes dioica were dried in an oven at 37°C and then powdered with a mechanical grinder, passing through sieve #40 and stored in an air tight container. The dried powdered material (1.0 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, in vacuo at 40°C to render the MeOH extract (300 g) and this extract is referred as TLE (*Trichosanthes dioica* leaves extract).

Gentamicin induced nephrotoxicity in rats

Twenty-four Wistar rats (150-170 g) were divided into 4 groups of 6 animals each. Group A: control rats (CON) that received normal saline (i.p.) and 0.5% carboxymethyl cellulose (CMC) (p.o.) for 8 days. Group B: Gentamicin-treated rats (GM) that received 100 mg/kg GM (i.p.) and 0.5% CMC (p.o.) for 8 days[14]. Group C: (GM + TLE 200) treated rats that received 100 mg/kg GM (i.p.) and 200 mg/kg TLE (p.o.) for 8 days. Group D: (GM + TLE 400) treated rats that received 100 mg/kg GM (i.p.) and 400 mg/kg TLE (p.o.) for 8 days. After collection of blood and urine animals were sacrificed by cervical dislocation under mild ether anaesthesia and kidneys were harvested, rinsed in saline and stored at -80 ⍰C till further biochemical analysis.

Plasma and urine markers of renal damage

Rats of each group were individually housed in metabolic cages for 24 h and urine was collected on the 8th day of the treatment. Blood samples were collected from these overnight fasted animals through retro-orbital plexus puncture in ethylene diamine tetra acetic acid coated vials and plasma was separated by cold centrifugation of vial at 3000 rpm for 10 min. Urea and creatinine were assayed in plasma and urine using commercially available kits (Reckon Diagnostics Ltd., Mumbai, India) as per instruction of the manufacturer and blood urea nitrogen (BUN) concentration was also measured as an indicator of renal function[15].

Preparation of renal homogenate

After the completion of the experiment, the kidneys were excised, weighed and homogenized in chilled Tris buffer (10mM, pH 7.4) at a concentration of 10% (w/v) (Potter-Elvenhjem glass homogenizer). Homogenates were then centrifuged at 12,000 rpm for 20 min (4 °C) in a high speed and supernatant and sediment were used for further biochemical estimations.

Measurement of renal lipid peroxidation

Measurement of malonaldehyde as an index for lipid peroxidation (LPO) was done using thiobarbituric acid assay as per Buege and Aust[16].

Measurement of renal antioxidants

Superoxide dismutase (SOD) was assayed in tissue supernatant by the method of Kakkar et al.[17] based on the inhibition of the formation of nicotinamide adenine dinucleotide-phenazine methosulfate-nitro blue tetrazolium formazan. Catalase, GSH and ascorbic acid assayed in tissue sediment[18,19,20].

Histopathological examination

Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50100%) alcohol and embedded in paraffin, cut into 4-5 µm thick sections and stained with hematoxylin-eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity.

Statistical analysis

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by NewmanKeuls test using Prism Pad software (version 3.0) for the determination of level of significance. The values of $p<0.05$ was considered statistically significant.

RESULTS

Effect of TLE on kidney weight

The effect various doses of TLE were studied on kidney wt. in GM intoxicated animals. Renal injury induced by GM caused significant increases the kidney wt. by 76.92% compared to control group. The percentage protection in kidney wt. of treated groups at 200 mg/kg as 17.39 ($P<0.05$) when compared to toxic group while maximum percentage protection in kidney wt. at the dose of 400 mg/kg *i.e.* 36.95 ($P<0.001$). Table 1 Shows that, GM treated rats' registered significant ($p< 0.001$) increment in kidney weight.

Effect of TLE on urine output

The effect various doses of TLE were studied on urine outflow (Table 1) in GM intoxicated animals. GM caused significant decreases the urine outflow by 43.83% compared to control group. The percentage augmentation in urine outflow of treated groups at 200 mg/kg as 36.58 ($P<0.01$) when compared to toxic group while maximum percentage augmentation in urine outflow at the dose of 400 mg/kg *i.e.* 60.97 ($P<0.001$).

Table 1. : Effect of TLE on kidney weight, urine output, plasma urea, plasma creatinine, urine urea and urine creatinine level against GM induced nephropathy in rats.

Groups	Kidney weight (mg/100 body weight)	Urine output (ml/h)	Plasma		Urine	
			Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
CON	0.52 ± 0.04	0.73 ± 0.06	21.42 ± 1.2	0.82 ± 0.13	58.63 ±2.02	12.03 ± 1.3
GM	0.92 ± 0.06*	0.41 ± 0.03*	58.6 ± 2.3*	2.1 ±0.11*	123.4±3.2*	38.23 ±3.1*
GM + TLE 200	0.76 ± 0.05a	0.56 ± 0.03b	51.6 ± 2.8a	1.62 ±0.15a	112.5±3.1a	30.2. ± 2.8a
GM + TLE 400	0.58 ± 0.05c	0.66 ± 0.05c	31.72 ± 1.9c	1.22 ±0.12c	71.63 ± 2.5c	19.9 ± 1.1c

Values are mean ± S.E.M. of 6 rats in each group
P values: *<0.001 compared with respective control group CON
P values: a<0.05, b<0.01, c<0.001 compared with group (GM)

Table 1. : Effect of TLE on SOD (Units/mg protein), CAT (µmol of H2O2 consumed/mg protein), GSH (µg/mg protein), LPO (nmol of malonaldehyde formed/mg protein), ascorbic acid AA (mg/g of tissue) and blood urea nitrogen BUN (mg/dl) level against GM induced nephropathy in rats.

Groups	SOD	CAT	GSH	LPO	AA	BUN	Urinary Na^+ level (mmole/L)	Urinary K^+ level (mmole/L)
CON	15.6 ± 0.9	39.46 = 3.1	98.2 ± 4.2	14.21 ± 0.9	1.73 ± 0.03	13.32 ± 0.4	134.6±4.5	5.23=0.2
GM	6.9 ± 0.3*	14.35 = 1.2*	42.71 ± 2.3*	41.17 ± 1.4*	1.32 ± 0.05*	22.02 ± 0.7*	173.6±6.4*	8.12= 0.4*
GM + SXE 200	8.8 ± 0.5a	21.56 = 2.0a	56.75 ± 3.1a	36.2 ± 1.1b	1.47 ± 0.04a	19.21 ± 0.8b	164.5±3.9ns	7.1 ±0.3a
GM + SXE 400	11.89 ± 0.7c	29.72 = 2.4c	81.31 ± 4.2c	22.78 ± 1.2c	1.61 ± 0.06c	16.63 ± 0.7c	142.8±4.2c	5.98=0.4c

Values are mean ± S.E.M. of 6 rats in each group
P values: *<0.001 compared with respective control group CON
P values: a<0.05, b<0.01, c<0.001 compared with group (GM)

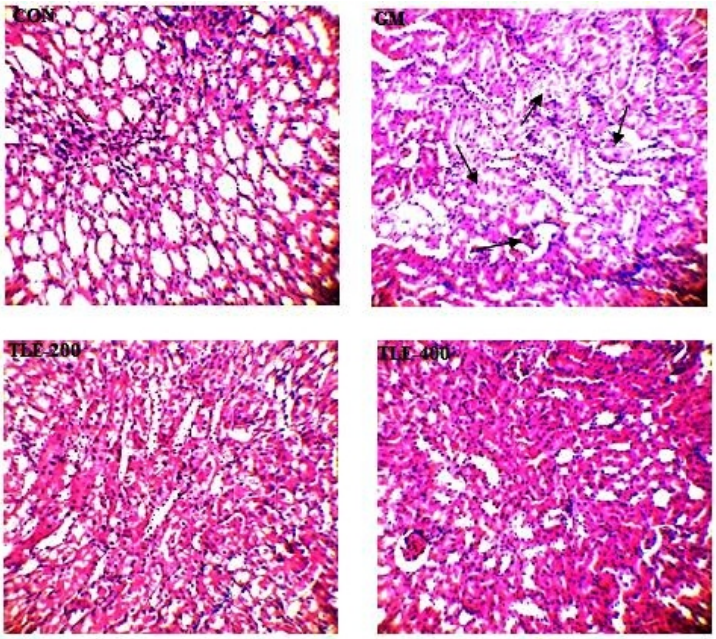

Fig 1. : Histological study of kidney tissue in control and experimental groups of rats. (A) No morphological damage was observed in rats in the control group. (B) Histopathological view of renal sections in GM treated group showed the epithelial cells of proximal tubules presented vacuoles and hydropic changes, atrophy and inflammatory cell infiltration, degeneration and desquamation (indicated by arrows) as compared to control group. (C) Animal treated with TLE-200 mg/kg showed considerably fewer lesions in the tubules and interstitium compared to the group GM treated rats. (D) Animal treated with TLE-400 mg/kg showed regeneration in tubular epithelial cells.

Effect of TLE on urea and Creatinine in plasma and urine

The effect various doses of TLE were studied on urea and Creatinine in GM intoxicated animals. Renal injury induced by GM caused significant changed in renal marker in plasma as urea by 173.57%, Creatinine by 156.09% but in case of urea and Creatinine in urine 110.47% and 217.78% respectively compared to control group. The percentage protection in renal marker in plasma of treated groups at 200 mg/kg as urea 11.94 (P<0.05) , Creatinine 22.87 (P<0.05), while these markers in urine 8.83 (P<0.05) and 21.0 (P<0.05) respectively when compared to toxic group while maximum percentage protection in renal markers in plasma at the dose of 400 mg/kg as urea by 45.87% (P<0.001), Creatinine by 41.9% (P<0.001) but in case of urea and Creatinine in urine 41.95% (P<0.001) and 43.97% (P<0.001) respectively (Table 1).

Effect of TLE on urinary Na^+ and K^+ concentration

The effect various doses of TLE were studied on Na^+ and K^+ level in GM intoxicated animals. Renal injury induced by GM caused significant changed in electrolyte concentration in urine as Na^+ by 28.97% and K^+ by 55.25% compared to control group. The percentage protection in urine concentration of treated groups at 200 mg/kg as Na^+ 5.24 (ns) and K^+ 12.56 (P<0.05) when compared to toxic group while maximum percentage protection in urine concentration at the dose of 400 mg/kg as Na^+ by 17.74% (P<0.001) and K^+ by 26.35% (P<0.001) were observed. (Table 2)

Estimation of renal LPO

The results in table 2 showed clear significant percentage change in the levels of LPO in GM intoxicated rats as 193.52 (P< 0.001) compared to control group. Treatment with TLE at the doses of 200 and 400 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 12.07 (P< 0.01) and 44.66 (P< 0.001) respectively.

Estimation of renal antioxidants

The percentage changed of SOD, CAT, GSH and ascorbic acid (Table 2) in GM intoxicated group were as 55.76 (P< 0.001), 63.63 (P< 0.001), 56.5 (P< 0.001) and 23.69 (P< 0.001) respectively. The percentage protection in SOD as 27.53 (P< 0.05), 72.31 (P< 0.001), CAT 50.24 (P< 0.05), 107.1 (P< 0.001) and GSH 32.87 (P< 0.05), 90.37 (P< 0.001) while in ascorbic acid 11.36 (P< 0.05), 21.96 (P< 0.001) at the doses levels 200 and 400 mg/kg, respectively. In different doses level of TLE, 400 mg/kg has shown maximum protection which was almost comparable to those of the normal control.

Effect of TLE on serum blood urea nitrogen

The effect various doses of TLE were studied on serum blood urea nitrogen in GM intoxicated animals. Renal injury induced by GM caused significant changed BUN in plasma by 65.31% compared to control group. The percentage protection in blood urea nitrogen of treated groups at 200 mg/kg as 13.5 (P<0.01) when compared to toxic group while maximum percentage protection in blood urea nitrogen at the dose of 400 mg/kg as by 24.47 (P<0.001) respectively.

Histopathological observations

The histological changes in kidneys and pathological manifestations are presented in Figure.1. The nephrotoxicity were confirmed by evaluating the pathological symptoms such epithelial cells of proximal tubules presented vacuoles and hydropic changes, atrophy and inflammatory cell infiltrate, degeneration and desquamation. Treatment with the TLE extract 200 and 400 mg/kg body weight ameliorated the toxic manifestations in the kidney. The histopathological observations supported this conclusion.

DISCUSSION

Gentamicin-induced nephrotoxicity is a well-documented event involving some functional and cellular mechanisms such as Glomerular lesions that interfere with Glomerular hemodynamic, and altered tubular transport, with the injury ranging from solely functional lesions to the occurrence of necrosis. Most chemotherapy drugs targets pathways that are essential to dividing cells[21]. Several studies have now documented the importance of reactive oxygen metabolites (ROM) in Gentamicin induced renal damage[22]. Nephrotoxicity of the drugs is usually associated with their accumulation in renal cortex, dependent upon their affinity to kidneys and on kinetics of drug trapping process[23]. Several studies have reported that oxygen-free radicals are considered to be important mediators of GM-induced acute renal failure. GM induced nephrotoxicity is characterized by elevated levels of urea and creatinine in plasma as well as urine, severe proximal tubular necrosis, renal failure[24] and weight of kidney were found to be significantly increased in rats treated with only GM[25]. Gentamicin (100 mg/kg, i.p) also increased urinary Na^+ and K^+ level that indicating the nephrotoxicity[26]. Similar pattern of changes were also observed in our study following GM treatment. TLE supplementation to GM treated rats recorded decrement in levels of urea and creatinine in plasma as well as urine these observations indicates at an improved renal function in form of effective clearance of urea and creatinine. Increment of urinary Na^+ and K^+ level in TLE supplemented animal also a good indication of improved renal function. GM-treated rats showed that there was a significant decrease in urine volume as compared to control. But after the treatment with TLE the urine volume was significantly increase as compared to GM group. In fact, 400 mg/kg TLE increased the urine volume to the level of the control. Decrement in activity levels of renal SOD, CAT and GSH following GM treatment are in accordance with previous report on GM induced suppression of endogenous enzymatic antioxidant machinery[27]. TLE treatment efficiently prevented GM induced decrease in activity levels of SOD, CAT and GSH. A relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models[28]. The elevated level of MDA, a marker of lipid peroxidation, indicates increased free-radical generation in the GM-induced nephrotoxicity[29]. GM-induced increment in MDA content of plasma was significantly prevented by TLE treatment in the present study. GM-induced increment in MDA content of plasma was significantly prevented by TLE treatment in the present study. Therefore, the significantly lower levels of MDA in the tissues of treated groups as compared with the GM group indicate attenuation of lipid peroxidation. This was probably due to less damage by oxygen-free radicals with TLE. The involvement of oxygen-free radicals in tissue injuries is well established[30]. Also, GM + TLE (200 and 400) groups recorded significantly higher levels of AA as compared to GM group, thus indicating that TLE treatment prevents GM induced depletion in levels of renal non-enzymatic antioxidants. GM administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in serum BUN[31]. TLE supplementation to GM treated rats recorded decrement in levels of blood urea nitrogen in plasma.

Histopathological results demonstrating structural changes in renal tissue of aminoglycoside antibiotics such as GM were reported by some researchers [32]. Histopathological view of renal sections in GM treated group showed the epithelial cells of proximal tubules presented vacuoles and hydropic changes, atrophy and inflammatory cell infiltrate, degeneration and desquamation. Vacuoles and hydropic changes, atrophy and inflammatory cell infiltrate, degeneration and desquamation and epithelial changes were considerably mild in the groups treated with GM + TLE-200 while in case of animal treated with TLE 400 mg/kg showed regeneration in tubular epithelial cells. We think that, morphological changes in kidneys were because of GM injection, but these changes tended to be considerably mild in GM plus TLE treatment.

CONCLUSION

In the present study, *Trichosanthes dioica* exhibited potent nephroprotective activity which might be useful for the therapy or management of disorders involving ROS-mediated pathology. Our data indicate that GM-induced nephrotoxicity might be related to oxidative damage. Co-administration of TLE lessened the negative effects of GM-induced nephrotoxicity possibly by inhibiting free radical mediated process. Further investigation of these promising protective effects of TLE against GM-induced renal injury may have a considerable impact on developing clinically feasible strategies to treat patients with renal failure.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

REFERENCES

1. Ahmed S, MM Hasan, Ahmed SW, Mahmood ZA, Azhar I, Habtemariam S. Anti-emetic effects of bioactive natural products. Phytopharmacology. 2013; 4(2): 390-433.
2. Kaikini AA, Dhande SR, Kadam VJ. Overview of Indian medicinal tree *Bambusa bambos* (druce). Int Res J Pharm. 2013; 4(8): 51-56.
3. Kumavat UC, Shimpi SN, Jagdale SP. Hypoglycemic activity of *Cassia javanica* Linn. in normal and streptozotocin-induced diabetic rats. J Adv Pharm Technol Res. 2012; 3(1): 4751.
4. Toshniwal V, Misal R, Metkar V, Toshniwal A, Patil G, Wandhare M. *Trichosanthes dioica* Roxb. A Review. Asian J Pharm Inven. 2013; 1(1): 1-10.
5. Sujatha RK, Rajan, Gokila M, Jency P, Brindha P. Pharmacognostical and preliminary phytochemical properties of *Aegle Marmelos* Correa fruit pulp. IJPSR.2011; 2(5): 1232-1236.
6. Namrta Choudhary N,Siddiqui MB, Azmat S, Khatoon S. *Tinospora cordifolia* Ethnobotany, Phytopharmacology and Phytochemistry aspects. IJPSR. 2013; 4(3): 891-899.
7. Rai DK, Rai PK, Jaiswal D, Sharma B, Watal G. Effect of water extract of *Trichosanthes dioica* fruits in streptozotocin induced diabetic rats. Indian J Clin Biochem. 2008; 23: 387-390.
8. Sharmila BG, Kumar G, Rajasekhara PM. Cholesterol-lowering activity of the aqueous fruit extract of *Trichosanthes dioica* in normal and streptozotocin diabetic rats. J Clin Dia Res.2007; 1: 561-569.
9. Ghaisas MM, Tanwar MB, Ninave PB, Navghare VV, Deshpande T. Hepatoprotective activity of aqueous & ethanolic extract of *T. dioica* in ferrous sulphate induced liver injury. Pharmacologyonline. 2008; 3: 127-135.
10. Galani VJ, Goswami SS, Shah MB. Antiulcer Activity of Patol Churna against Experimental Gastro-duodenal Ulcers in Rats. J Homeopat Ayurv Med. 2012; 1: 1-4.
11. Bhadoriyal SS, Mandoriya N. Immunomodulatory effect of *Tricosanthes Dioica* Roxb. Asian Pac J Trop Biomed.2012; 673-678.
12. Rai PK, Mehta S, Gupta RK and Watal G. A Novel Antimicrobial Agents *Trichosanthes dioica*. International J Pharm Bio Sci. 2010; 1: 1-9.
13. Shivhare Y, Singh P, Rajak H, Patil UK, Pawar RS. Antioxidant potential of *Trichosanthes dioica* Roxb (fruits). Pharmacognosy J. 2010; 2: 107-111.
14. Yaman I, Balikci E. Protective effects of *Nigella sativa* against Gentamicin induced nephrotoxicity in rats. Exp Toxicol Pathol. 2010; 62: 183190.
15. Hussain T, Gupta RK, Sweety K, Eswaran B, Vijayakumar M, Rao CV. Nephroprotective activity of *Solanum xanthocarpum* fruit extract against Gentamicininduced nephrotoxicity and renal dysfunction in experimental rodents. Asian Pac J Trop Med. 2012; 5(9): 686-691.
16. Braun N, Lima RLD, Flora FD, Lang ME, Bauermann LDF, Loro VL, Baldisserotto B. Lipid peroxidation and superoxide dismutase activity in silver catfish *(Rhamdia quelen)* juveniles exposed to different dissolved oxygen levels. Ciência Animal Brasileira. 2008; 9: 811-814.
17. Kakkar P, Das B, Viswanathan PN. Amodified spectrophotometric assay of SOD. Indian J Biochem Biophys.1984; 21: 130-132.
18. Sinha KA. 1972. Colorimetric assay of catalase. Anal Biochem., 47: 389-394.
19. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61: 882888.
20. Roe JH, Küether CA. The determination of ascorbic acid in whole blood and urine through 2-4 dinitrophenyl hydrazine derivative of dehydro ascorbic acid. J Biol Chem. 1943; 147: 399407.
21. Hanigan MH, Devarajan P. Cisplatin induced nephrotoxicity: molecular mechanisms. Cancer Ther. 2003; 1: 4761.
22. Chatterjee P, Mukherjee A, Nandy S. Protective effects of the aqueous leaf extracts of *Aloe barbadensis* on gentamicin and cisplatin-induced nephrotoxic rats. Asian Pac J Trop Biomed. 2012; 1754-1763.

23. Karahan I, Atessahin A, Yilmaz S, Ceribasi AO, Sakin F. Protective effect of lycopene on Gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicology. 2005; 215: 198204.

24. Erdem A, Gundogan NU, Usubutun A, Kilinc K, Erdem SR, Kara A, Bozkurt A, et al. The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. Nephrol Dial Transplant. 2009; 15: 11751182.

25. Gaurav VH, Chandragauda RP, Mahesh RP. Protective effect of *Kalanchoe pinnata* pers. (Crassulaceae) on Gentamicin induced nephrotoxicity in rats. Indian J Pharmacol. 2007; 39: 201-205.

26. Patel NM, Swamy BMV, Swamy KMV. Protective Activity of *Psidium Guajava* Linn. Leaves Extract Against Gentamicin Induced Nephrotoxicity In Rats. J Pharm Sci. 2012; 2(4): 66-72.

27. Thounaojam MC, Jadeja RN, Devkar RV, Ramachandran AV. *Sida rhomboidea*. Roxb leaf extract ameliorates Gentamicin induced nephrotoxicity and renal dysfunction in rats. J Ethnopharmacol. 2010; 132: 365367.

28. Devipriya S, Shyamaladevim CS. Protective effect of quercetin in Cisplatin induced cell injury in the rat kidney. Indian J Pharmacol. 1999; 31: 422-426.

29. Kuhad A, Tirkey N, Pilkhwal S, Chopra K. Effect of Spirulina, blue green algae, on Gentamicin-induced oxidative stres and renal dysfunction in rats. Fundam Clin Pharmacol. 2006; 20: 121128.

30. Fox RB. Prevention of granulocyte-mediated oxidant lung injury in rats by a hydroxyl radical scavenger di- methyl thiourea. J Clin Invest. 1984; 74: 1456146.

31. Banday AA, Farooq N, Priyamvada S, Yusufi ANK, Khan F. Time dependent effects of Gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sci. 2008; 82: 450459.

32. Sayed-Ahmed MM, Nagi N. Thymoquinone supplementation prevents the development of Gentamicin-induced acute renal toxicity in rats. Clin Exp Pharmacol Physiol. 2007; 34: 399-405.