International Journal of
AGRICULTURE AND BIOSCIENCES
www.ijagbio.com P-ISSN: 2305-6622 editor@ ijagbio.com

RESEARCH ARTICLE

Gentamicin Induced Oxidative Stress on Renal Antioxidant Parameters and its Ameiloration by *Andrographis Paniculata* in Rats*

M Sivachandran[a] and P Hariharan[b]*

Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai-600 007, India

ARTICLE INFO

Received: October 27, 2012
Revised: October 29, 2012
Accepted: October 31, 2012

Key words:
Andrographis paniculata
Gentamicin,
Renal antioxidant parameters

***Corresponding Address:**
pharipharma123@gmail.com

ABSTRACT

The effect of *Andrographis paniculata* on renal antioxidant parameters were studied in a model of gentamicin induced toxicity in rats. An attempt was also made to correlate this with histopathological changes in kidney. Gentamicin was administered intraperitoneally at the dose of 80 mg/kg body weight, once daily for seven days. Significant reduction of the kidney antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) content with marked elevation in the level of lipid peroxidation (LPO) were observed in the present study. Alcoholic extract of *Andrographis paniculata* significantly restored the antioxidant status in kidney. Histopathological examination of kidney of rats treated with gentamicin revealed acute tubular necrosis, protein inclusion and cast in the proximal tubules in kidney. Groups treated with *Andrographis paniculata* showed restoration of gentamcin altered kidney architecture towards normal. Thus the results of the present study suggest that *Andrographis paniculata* plant extract can be used as a protective agent in gentamicin induced oxidative stress on renal tissues.

Cite This Article as: Sivachandran M and P Hariharan, 2012. Gentamicin induced oxidative stress on Renal Antioxidant Parameters and its Ameiloration by *Andrographis paniculata* in Rats. Inter J Agri Biosci, 1(1): 11-15. www.ijagbio.com

INTRODUCTION

Gentamicin is an aminoglycoside antibiotic that is still commonly used in the treatment of life-threatening infections. Its broad-spectrum activity against aerobic gram positive and gram negative organisms, chemical stability, and its rapid bactericidal action has often made it a first-line drug in a variety of clinical situations (Appel, 1990). Although the pathophysiology of gentamicin induced nephrotoxicity is multi-factorial, generation of oxygen-free radicals may be a major factor in its production (Ali, 1995; Garg *et al.*, 1996). However, there is no unanimity in the literature regarding the possible mechanism(s) of oxidative stress. The value of aminoglycosides, including gentamicin, in clinical practice would be greatly enhanced if some means could be found to protect the kidney from this undesirable side effect. Recently some medicinal plants with anti-oxidant properties have been shown to protect rats against gentamicin induced toxicity. A potential therapeutic approach to ameliorate, protect or reverse gentamicin induced renal toxicity would have very important clinical consequences. Keeping the above aspects in view, the present work was undertaken to study the effect of gentamicin on oxidative stress of renal tissues by analyzing its biomarkers and to probe into the possibility of protective effect of *Andrographis paniculata* (Green chirayta) on them and compare the same with that of standard drug silymarin. An attempt was also made to correlate these changes with histopathological study of kidney tissue.

MATERIALS AND METHODS

Inbred male albino rats of wistar strain weighing 120-150 g were obtained from Laboratory Animal Medicine, Tamilnadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai – 600 051, India. Animals were housed in cages, acclimatized to the laboratory conditions and fed with standard dry pellet feed and provided drinking water *ad libitum*. *Andrographis paniculata* (alcoholic extract) obtained from Natural Remedies, Bangalore, India, silymarin obtained from Microlabs, Goa, India and gentamicin sulphate procured

from Intas Pharmaceuticals, Matoda, Gujarat, India *as gratis* were used in the study. All other chemical reagents used were of analytical grade.

Drug treatment was continued for seven days. At the end of the experiment, blood samples were collected from all the rats, by cardiac puncture in a sterile tube and then they were sacrificed. Serum was separated by centrifuging at 800 g for five minutes for biochemical estimations. Kidney tissue weighing one gram was used for antioxidant assay which included lipid peroxidation by estimation of thiobarbituric acid reactive substances (TBARS) (Yagi,1976), estimation of enzymatic antioxidants like superoxide dismutase (SOD) **(**Marklund and Marklund, 1974), catalase (CAT) **(**Caliborne, 1985), glutathione peroxidase (GPx) (Rotruck *et al*. 1973) and non-enzymatic antioxidants like reduced glutathione (GSH) (Moron *et al.*, 1979). They were minced and homogenized in 0.05 M ice cold phosphate buffer (pH 7.4) by using a tissue homogenizer to make 10% homogenate. A 0.2 ml portion of the homogenate was used for lipid peroxidation assay. The remaining part of homogenate was mixed with 10% trichloroacetic acid in the ratio of 1:1, centrifuged at 5000 g for 10 min, at 4° C and the supernatant was used for estimation of reduced glutathione and glutathione peroxidase . The remaining part of the homogenate was centrifuged at 15,000 g for 60 min at 4 °C and the supernatant obtained was used for estimation of superoxide dismutase and catalase. A piece of kidney tissue fixed in 10 % formalin, routinely processed, paraffin embedded and sectioned to 5 µ thickness for histopathological examination

The results were analyzed by complete randomized design using SPSS software (version 10) and comparison of the means was done by using Duncan's Post-Hoc test (multiple comparison test). This experimental trial was approved by the Institutional Animal Ethics committee of Madras Veterinary College, Chennai-600 007, India.

RESULTS

The mean values of LPO (expressed as nM of MDA/g tissue), SOD (expressed as unit of enzyme required to inhibit 50 % pyrogallol autooxidation/min/mg protein), Catalase activity (expressed as units per mg protein i.e. One unit is nM of H_2O_2 decomposed/Min/mg protein), GPX (expressed as U/g protein) and GSH (expressed as µg GSH/g tissue) are furnished in Table 1.

LPO activity in gentamicin treated group (90.35±1.01) was significantly (P<0.05) higher when compared with control (64.19±1.14). However *Andrographis paniculata* at both the doses restored the LPO activity to control levels (79.92±1.20 and 70.90±0.96 respectively). Silymarin also produced a similar effect (77.53±1.45) (Table1).

Those animals which received gentamicin alone exhibited significant decrease (p<0.05) in SOD (1.08 ± 0.04), catalase (46.02 ± 0.73), GPx (4.81± 0.37) and GSH (8.77 ± -.39) activity in renal tissue when compared to their corresponding control values (Table -1) When compared to gentamicin treated group *Andrographis paniculata* at both doses produced a significant increase (P<0.05) in the following parameters studied

- Kidney SOD activity (1.35 ± 0.04 and 1.47 ± 0.04 respectively)
- A dose dependent increase in renal catalase activity (53.33 ± 0.74 and 63.53 ± 0.90 respectively) and GPx activity (4.81 ± 0.37 and 6.16 ± 0.26 respectively) with the higher dose producing a better effect and
- An increase in the renal level of GSH (8.77 ± 0.39 and 10.77 ± 0.44 respectively)

Silymarin also produced an increase the renal SOD, CAT, GPx and GSH activity (1.57 ±0.07, 59.85 ± 0.43, 7.81 ± 0.29 and 10.53 ± 0.67 respectively) when compared to Gentamicin treated group.

The renal architecture appeared to be normal in the control group of rats. In gentamicin administered rats, there was acute tubular degeneration with protein inclusions (Plate 1). Scarce eosinophilic homogenous casts were seen in the lumen of the tubules (Plate 2). In *Andrographis paniculata* 125 mg group mild tubular damage (Plate 3) was observed whereas in *Andrographis paniculata* 250 mg group the tubular epithelial degeneration as well as necrosis was minimal (Plate 4) and the structure was practically normal. Silymarin treated rats revealed mild tubular degeneration with protein inclusion and eosinophilic homogenous casts in the lumen (plate 5). Regenerating tubules were also noticed (Plate 6).

DISCUSSION

Free radicals generated by gentamicin administration initiate the peroxidation of membrane polyunsaturated fatty acids and covalently bind to microsomal lipids and proteins. This phenomenon results in the generation of reactive oxygen species (like the superoxide anion, H_2O_2 and OH^-). Various enzymatic and non enzymatic systems have been developed by the cell to cope up with the ROS and other free radicals. However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient. It has been reported that SOD, CAT and GST constitute a mutually supportive team of

Table 1: Effect of *Andrographis paniculata* extract on renal antioxidant parameters in experimentally induced gentamicin toxicity in rats

Groups	LPO	SOD	CATALASE	GPx	GSH
Control	64.19 ± 1.14^{a}	1.75 ± 0.04^{d}	70.68 ± 1.29^{f}	7.87 ± 0.36^{c}	13.00 ± 0.55^{c}
Gentamicin	90.35 ± 1.01^{e}	1.08 ± 0.04^{a}	46.02 ± 0.73^{a}	4.81 ± 0.37^{a}	8.77 ± 0.39^{a}
AP125	79.92 ± 1.20^{cd}	1.35 ± 0.04^{b}	53.33 ± 0.74^{b}	6.16 ± 0.26^{b}	10.77 ± 0.44^{b}
AP250	70.90 ± 0.96^{b}	1.47 ± 0.04^{bc}	63.53 ± 0.90^{e}	7.51 ± 0.27^{c}	10.93 ± 0.60^{b}
Silymarin	77.53 ± 1.45^{c}	1.57 ± 0.09^{cd}	59.85 ± 0.43^{d}	$7.81^{c}\pm0.29^{c}$	10.53 ± 0.67^{b}

Means bearing different superscripts in the same column differ significantly (p<0.05); All values are expressed as Mean ± S.E, n=6; SOD is expressed as unit of enzyme required to inhibit 50% pyrogallol autooxidation/min/mg protein; Catalase activity is expressed as units per mg protein. One unit is nM of H_2O_2 decomposed/Min/mg protein; GSH is expressed as µg GSH/g tissue; GPx is expressed as U/g protein; LPO unit of activity is expressed as nM of MDA/g tissue

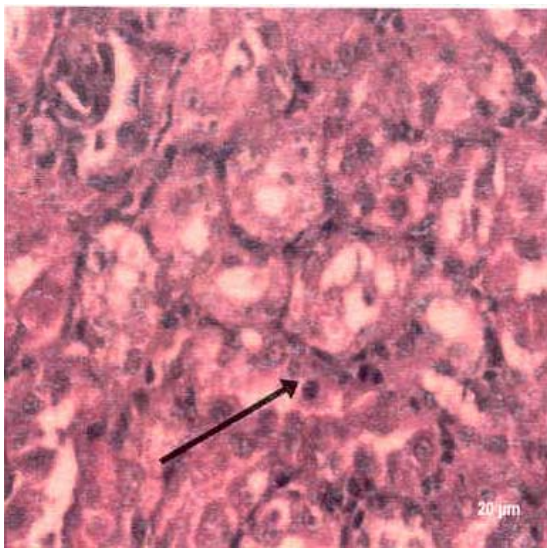

Plate 1: Kidney (gentamicin treated) : Acute tubular damage and protein inclusions

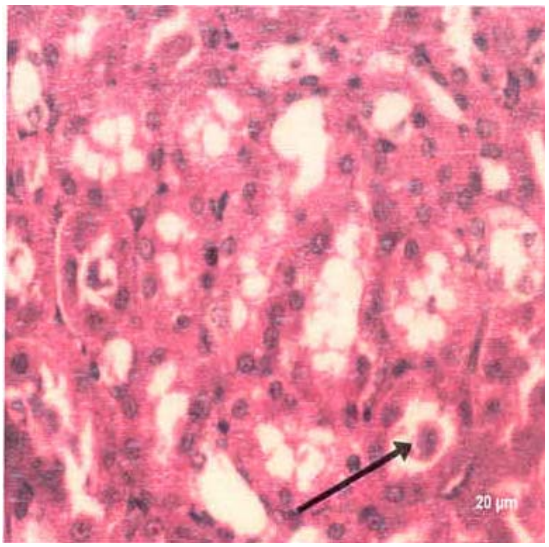

Plate 2: Kidney (gentamicin treated): Cast in lumen of tubules

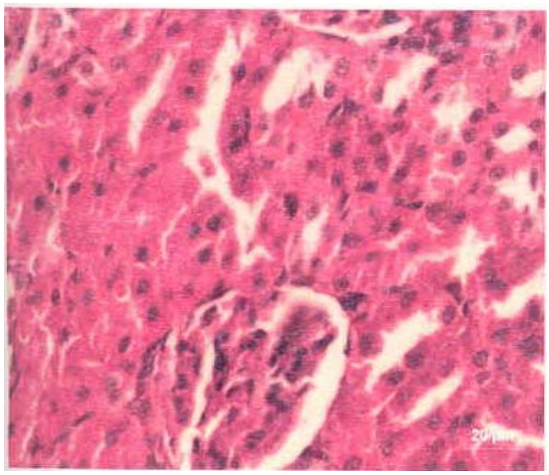

Plate 3: Kidney (AP 125 mg treated): Mild tubular damage

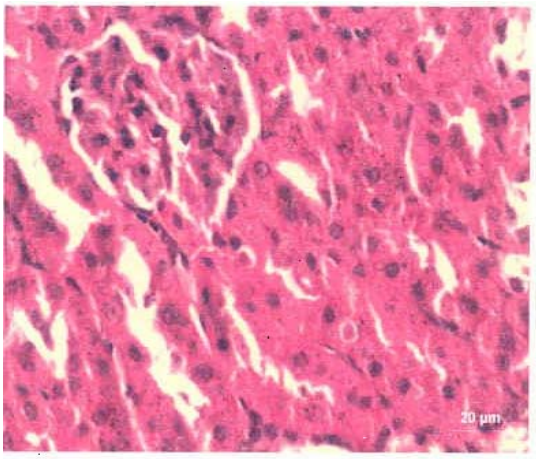

Plate 4: Kidney (AP 250 mg treated): Mild damage

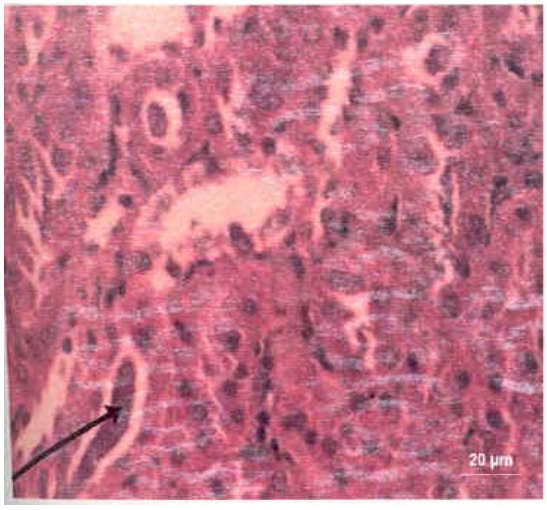

Plate 5: Kidney (Silymarin treated) : Cast in lumen

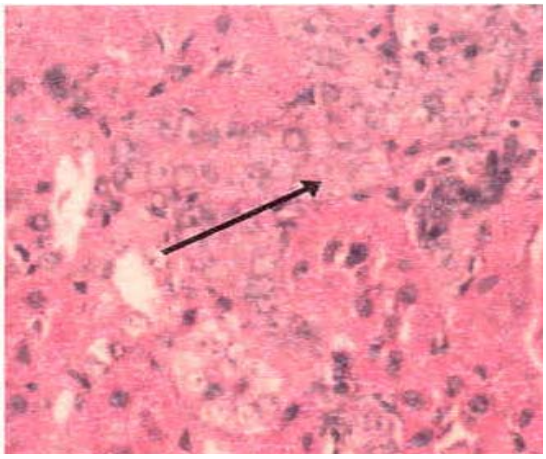

Plate 6: Kidney (Silymarin treated) : Regenerating tubules

defense against ROS. The decreased activity of SOD in kidney in gentamicin treated mice reported by Manna *et al*. (2006) may be due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. They also suggested that increased utilization of GSH by free

radicals generated by gentamicin might be the cause of decreased GSH content.

In our study, extract of *Andrographis paniculata* showed significant dose dependent reduction of LPO in kidney. Silymarin also produced significant reduction in LPO level in kidney. Triwedi and Rawal (2000) reported that administration of *Andrographis paniculata* extract reversed benzene hexa chloride induced elevated levels of LPO.

SOD is an important cellular antioxidant enzyme (a metal protein) which catalyses the conversion of superoxide radical to hydrogen peroxide and oxygen. In the present study,

SOD activity of kidney was significantly decreased in gentamicin treated group when compared to control. These observations were in correlation with the findings of Ramasammy *et al*. (1987).

Andrographis paniculata at both the doses significantly restored the gentamicin induced decreased renal SOD activity towards the control levels. Silymarin also significantly increased the SOD activity when compared to gentamicin treated group. Samy et *al*. (2008) reported that *Andrographis paniculata* crude extract and its purified fractions could restore the snake venom induced decrease of serum SOD activity in mice and further suggested that antivenom action of *Andrographis paniculata* may be due to its antioxidant activity.

In the present study, CAT level of kidney significantly decreased in gentamicin group when compared to control. In a similar study, Karahan *et al*. (2005) observed significantly reduced activity of catalase in gentamicin treated group as compared to control. Significant and dose dependent improvement in the activity of kidney catalase was observed in *Andrographis paniculata* treated groups when compared to gentamicin group. Silymarin also significantly increased the CAT activity when compared to gentamicin treated group.

Glutathione peroxidase is a selenium containing metalloenzyme that catalyses the oxidation of reduced glutathione by peroxide to form water and oxidized glutathione. In the present study, GPx level of kidney significantly decreased in gentamicin treated group when compared to control. *Andrographis paniculata* improved the renal GPx activity towards control levels in a dose dependant manner. Silymarin significantly increased the GPx activity more towards control group. Farombi and Ekor (2006) also reported a similar finding wherein pretreatment of curcumin at 200 mg/kg for two weeks significantly restored the GPx as compared to control group.

In the present study, GSH level of kidney was significantly decreased in gentamicin treated group when compared to control. These observations were in concurrence with the findings of Ali *et al*. (1992) who found significantly decreased level of GSH content in kidney homogenate treated with gentamicin at the rate of 80 mg/kg/day for six days in male Wistar rats.

Similarly significant improvement in the level of kidney GSH was observed in *Andrographis paniculata* treated groups and silymarin treated group when compared to gentamicin group. Triwedi *et al*. (2000) have also reported that administration of *Andrographis paniculata* extract reversed benzene hexa chloride induced diminished levels of GSH which was concordant with our results.

The results on antioxidant activity of this plant suggest that the extract of the plant possess significant membrane protective effect as can be inferred from the LPO activity in different groups. *Andrographis paniculata* at the higher dose of 250 mg/kg possesses maximal activity even better than the standard drug silymarin on LPO. Significant increase in SOD activity was observed with the extracts of Andrographis *paniculata* at both the doses when compared to gentamicin group. A much better results were obtained with silymarin.

Renal catalase activity which has been inhibited by gentamicin was restored by *Andrographis paniculata* that too at the higher dose. Silymarin also produced restoration of renal catalase activity when compared to gentamicin treated group.

On analyzing the results obtained for GPx activity we can infer that the plant extract at both the doses were able to reverse the gentamicin induced oxidative stress. However, the higher doses showed greater free radical scavenging activity which was quite comparable to that of silymarin.

Andrographis paniculata extract at both the doses and silymarin increased the GSH activity when compared to gentamicin treated group

On the whole, the above results suggest that though the extract of the plant at both the doses were able to significantly reverse oxidative stress induced by gentamicin on renal tissues. *Andrographis paniculata* 250 mg/kg body weight is preferable since it has better restorative effect on many antioxidant parameters studied. *Andrographis paniculata* at the higher dose (250 mg/kg) showed marked protection against gentamicin induced plasma membrane damage and oxidative stress as observed by restoration of biochemical parameters and preservation of endogenous anti-oxidants.

The findings also gain support from the histopathological studies. The degenerative and necrotic changes observed in the kidney of gentamicin treated rats, have been found to be reversed in *Andrographis paniculata* treated rats (Plates 1 to 4) and silymarin also produced similar effects (Plates 5 & 6). This may be attributed to *Andrographis paniculata* induced increase in antioxidant activities, regeneration and reparative processes of cellular membrane, and up regulated anti-oxidant enzyme status, thus restoring the functional balance between pro-oxidant and anti-oxidant pathways.

Conclusion

Administration of gentamicin at 80 mg/kg body weight i.p. for seven days produced toxic manifestations in the kidney with alteration in the level of antioxidant status with corresponding histopathological lesions. The extract of *Andrographis paniculata* administered at two doses *viz*. 125 and 250 mg/kg body weight were able to offer protection against gentamicin induced renal toxicity probably by increasing the antioxidative defense activities which help in protecting the integrity of plasma membrane and also increase the regenerative and reparative capacity of kidney. This could be attributed to presence of active principles like andrographolide and arabinogalactan in the plant under study. Further studies

are needed to fully characterize the responsible active principle(s) present in this plant and elucidate its possible mode of action

REFERENCES

Ali BH, 1995. Gentamicin nephrotoxicity in humans and animals: some recent research. General Pharmacology 26 (7)**:** 1477–1487.

Ali BH, AAA Gayoum and A A Bashir, 1992. Gentamicin nephrotoxicity in rat: some biochemical correlates. Pharmacology and Toxicology, 70**:** 419-423

Appel GB, 1990. Aminoglycoside nephrotoxicity. American J of Medicine**,** 88**:** 165-205.

Bafna VA and R Balaraman, 2005. Anti-ulcer and anti-oxidant activity of pepticase, a herbomineral formulation. Phytomedicine, 12**:** 264-270.

Caliborne AL, 1985. Assay of catalase, in: Greenwald, R.A (editor), Handbook of Oxygen Radical Research. CRC Press, Baco- Raton.

Farombi EO and M Ekor, 2006. Curcumin attenuates gentamicin- induced renal oxidative damage in rats. Food and chemical toxicology, 44**:** 1443-1448.

Garg SK, SK Rastogi, C Varshneya, SP Verma, RP Uppal and DN Sharma, 1996.

Biochemical and histopathological alterations and tissue residue of gentamicin in rabbits following repeated parenteral administration. Indian J of Toxicology, 3(1): 45-50.

Karahan I, A Atessahin, S Yilmaz, AO Ceribasi and F Sakin, 2005. Protective effect of lycopene on gentamicin- induced oxidative stress and nephrotoxicity in rats. Toxicology, 215:198-204.

Manna P, M Sinha and PC Sil, 2006. Aqueous extract of *Terminalia arjuna* prevents carbon etrachloride induced hepatic and renal disorders. *BMC* complementary and alternative medicine, 6**:** 33.

Marklund SL and Marklund, 1974. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. European J of Biochemistry, 47**:** 469-474.

Moron MS, JW Depierre and B Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Acta Biochemistry and Biophysics, 582**:** 67-78.

Nibha Verma and Manjula Vinayak .2008. Antioxidant action of *Andographis paniculata* on Lymphoma, Molecular Biology Reports, 35: 535-540.

Ramasammy LS, C Josepovitz, KY Ling, BP Lane and GJ Kaloyarides, 1987. Failure of inhibition of lipid peroxidation by vitamin E to protect against gentamicin nephrotoxicity in the rat. Biochemical Pharmacology, 36**:** 2125–2132.

Rotruck JD, AL Pope, HE Ganther, AB Swanson, DG Hafeman and Hekstra, 1973.

Selenium: biochemical role as a component of glutathione peroxidase and assay. Science, 179**:** 588-590.

Samy RP. MM Thwin, P Gopalakrishnakone and S Ignacimuthu, 2008. Ethnobotanical survey of folk plants for the treatment of snakebites in southern part of Tamilnadu, India. Journal of Ethnopharmacology, 115**:** 302-312.

Trivedi N and UM Rawal, 2000. Hepatoprotective and toxicological evaluation of *Andrographis paniculata* on severe liver damage**.** Indian J of pharmacology, 32**:** 288- 293.

Yagi K, 1976. Simple fluorimetric assay for lipid peroxides in blood plasma. Biochemical Medicine, 87: 212-216.