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RESEARCH ARTICLE

Gentamicin Induced Hepatic Oxidative Stress and Its Amelioration using *Andrographis Paniculata* Extract in Rats

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ABSTRACT

Hepatoprotective effect of *Andrographis paniculata* was evaluated in a model of gentamicin-induced toxicity in rats. Gentamicin was administered intraperitoneally at the dose of 80 mg/kg body weight once daily for seven days. Significant reduction in the hepatic antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione in conjunction with elevated levels of lipid peroxidase (LPO)were observed after the administration of gentamicin. On the other hand, treatment with the aqueous extract of *Andrographis paniculata* significantly restored the levels of the antioxidant enzymes to near normal levels in the liver. Histopathological examination of the liver of gentamicin-treated rats revealed vonkupffer cell hyperplasia, obliteration of sinusoids with focal necrosis. On the other hand, histopathological examination of the liver from rats treated with *Andrographis paniculata* did not exhibit such lesions, demonstrating a hepatoprotective effect of the extract. Furthermore, the hepatoprotective activity of the extract of *Andrographis paniculata* was comparable to that of silymarin, an active moiety in *Silybum marianum*, known for its hepatic regenerative activity. Thus, our significant findings indicate that the extract of *Andrographis paniculata* can be used as a hepatoprotective agent for rescuing the gentamicin- induced toxicity.

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INTRODUCTION

Gentamicin is an aminoglycoside antibiotic that is commonly used to treat life-threatening bacterial infections. Its broad-spectrum activity against aerobic gram positive and gram negative bacteria, chemical stability and rapid onset of bactericidal action has often made it a drug of choice to treat a variety of clinical cases (Appel, 1990). However, one of the side effects of gentamicin usage is its potential to induce hepatotoxicity.

Although the pathophysiology of gentamicin-induced hepatotoxicity is multi-factorial, generation of oxygen free radicals is suggested to be a major factor responsible for the hepatotoxicity (Ali, 1995; Garg *et al*., 1996). Thus, the value of aminoglycosides including gentamicin could be greatly enhanced if we can protect the liver from their undesirable side effects. Hence, the goal of the present research work was to study if the effect of gentamicin-induced oxidative stress on hepatic tissue can be ameliorated by the use of *Andrographis paniculata* extract and also to compare the hepatoprotective effects of *Andrographis paniculata* extract with that of the commonly used hepatoprotective drug, silymarin. An attempt was also made to correlate the hepatoprotective effects of the extract with histopathological changes in the hepatic tissue.

MATERIALS AND METHODS

Inbred adult male albino rats of wistar strain weighing 120 – 150 g were obtained from the Laboratory Animal Medicine, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai–51. Rats were housed in cages, acclimatized to the laboratory conditions and fed with standard dry pellet feed and drinking water *ad libitum*. *Andrographis paniculata* (aqueous extract) obtained from Natural Remedies, Bangalore, silymarin obtained from Microlabs, Goa and gentamicin sulphate obtained from Intas Pharmaceuticals, Matoda, Gujarat *as gratis* were used in this study.

Thirty male rats were divided randomly into five groups of six in each group and were subjected to the following treatments.

Control	Negative control (Untreated group)
Gentamicin	Gentamicin sulphate 80 mg/kg body weight, intraperitoneal (b.w., i/p)
AP 125	Gentamicin sulphate 80 mg/kg b.w. i/p + *Andrographis paniculata* 125 mg/kg b.w. p/o
AP 250	Gentamicin sulphate 80 mg/kg b.w. i/p + *Andrographis paniculata* 250 mg/kg b.w. p/o.
Silymarin	Gentamicin sulphate 80 mg/kg b.w. i/p + silymarin 50 mg/kg b.w. p/o.

Several dosage rates have been reported for gentamicin administration by different researchers (Garg *et al*. 1989 and 1996). Since the study was intended to look for unequivocal proof of the protective effects of the plant in question, a higher dosage of gentamicin which is reported to cause moderate toxicity in rats (Ali, 2002), was used. Hence, we have administered gentamicin at the dose of 80 mg/kg/day for seven days i.p. instead of the normal dosage of 50 mg/kg body weight

After the treatment, the liver tissue was minced and thoroughly homogenized for use in the antioxidant assay; which included the estimation of thiobarbituric acid reactive substances (TBARS) (Yagi,1976), antioxidant enzymes such as 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). Liver samples were also subjected to histopathological examination.

The results were analyzed by complete randomized design using SPSS software (version10) and comparison of the mean values of different groups was done using Duncan's post-hoc test (multiple comparison test).

RESULTS

Effect of *Andrographis paniculata* on gentamicin-induced oxidative stress was evaluated at two different dose levels *viz*., 125 and 250 mg/kg b.w. The doses were selected based upon the previous research work of several researchers (Trivedi and Rawal, 2000). Antioxidant activity of the liver was studied to assess the effect of *Andrographis paniculata* in ameliorating the oxidative stress. The mean ± S.E values of LPO, SOD, CAT, GPX and GSH in the liver tissue are shown in Table 1.

There was a significant (P<0.05) increase in liver LPO activity in the gentamicin treated group (248.18 ± 0.99) when compared with the control group (228.15 ± 4.02). *Andrographis paniculata* at low dose level (125 mg/kg b.w.) significantly reduced the LPO activity (221.03 ± 1.04) when compared with the high dose (250 mg/kg b.w.) group (233.63±3.08). Silymarin treated group too had shown significant (P<0.05) reduction of TBARS activity (215.65 ± 1.56) (Table-1)

Gentamicin administered group showed significant (P<0.05) reduction of SOD activity (1.91 ± 0.07) when compared to the control group (2.58±0.15). On the other hand, the SOD activity of AP 250 group (2.54 ± 0.17) was similar to that of the control group and was also comparable to that of silymarin group (2.66±0.31). (Table 1).

There was a significant (P<0.05) decrease in the hepatic CAT activity in the gentamicin treated group (79.07 ± 1.04) when compared to the control group (96.08 ± 1.66). Dose dependent significant (P<0.05) increase in CAT activity was observed in the plant extract treated groups. Silymarin also produced a similar effect (98.22 ± 1.52) (Table 1).

GPx activity of gentamicin treated and control groups were 9.26 ± 0.47 and 10.76 ± 0.17, respectively. Significant (P<0.05) decrease of GPx activity was found in the gentamicin group compared to the control. Although a significant increase in GPx activity was observed in *Andrographis paniculata* treated groups at both the dosage levels when compared to the gentamicin treated group, there were no statistically significant differences among the AP125 and AP250 groups. Silymarin treated group showed a significant increase in the GPx activity when compared to gentamicin treated group. However, *Andrographis paniculata* at a higher dose induced a significant (P<0.05) increase of GPx (10.64 ± 0.52) which is comparable to the control value (10.76 ± 0.17). Silymarin exhibited a maximal increase in the GPx activity (11.34 ± 0.70)

There was a significant (P<0.05) reduction in the liver GSH content in the gentamicin group (13.56 ± 0.55) when compared to the control group (15.53 ± 0.58). Even though *Andrographis paniculata* at the lower dose significantly increased the GSH content when compared to gentamicin group, they were unable to restore the liver GSH content back to that of the control level. Significant (P<0.05) restoration of GSH content towards the control levels were observed in the higher dose group of *Andrographis paniculata* (15.68 ± 0.53) which was also comparable to that of silymarin (16.02 ± 0.68) (Table-1).

The architecture of the liver section from the control rats (Plate 1) was compared to that from the other groups. Liver sections from the gentamicin group showed vonkupffer cell hyperplasia, obliteration of sinusoids (Plate 2) and focal necroses (Plate 3). In contrast, there were no appreciable lesions in the liver sections of rats from all the plant extracted treated (Plates 4 and 5) and silymarin treated groups (Plate 6).

Table 1: Effect of *Andrographis paniculata* extracts on the liver antioxidant parameters in experimentally-induced gentamicin toxicity

Groups	LPO	SOD	CAT	GPx	GSH
Control	228.15^{b}±4.02	2.58^{b}±0.15	96.08^{de}±1.66	10.76^{bc}±0.17	15.53^{c}±0.58
Gentamicin	248.18^{c}±0.99	1.91^{a}±0.07	79.07^{a}±1.04	9.26^{a}±0.47	13.56^{a}±0.55
AP125	221.03^{a}±1.04	2.18^{ab}±0.10	90.80^{c}±0.74	10.28^{abc}±0.34	14.89^{ab}±.60
AP250	233.63^{b}±3.08	2.54^{b}±0.17	93.12^{cd}±1.05	10.64^{bc}±0.52	15.68^{c}±0.53
Silymarin	215.65^{a}±1.56	2.66^{b}±0.31	98.22^{e}±1.52	11.34^{c}±0.70	16.02^{c}±0.68

Means bearing different superscripts in the same column differ significantly (p<0.05); All values are expressed as Mean ± S.E, n=6; LPO unit of activity (an indicator of TBARS activity) is expressed as nM of MDA/g tissue; SOD is expressed as unit of the 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 of liver tissue; GPx is expressed as U/g protein.

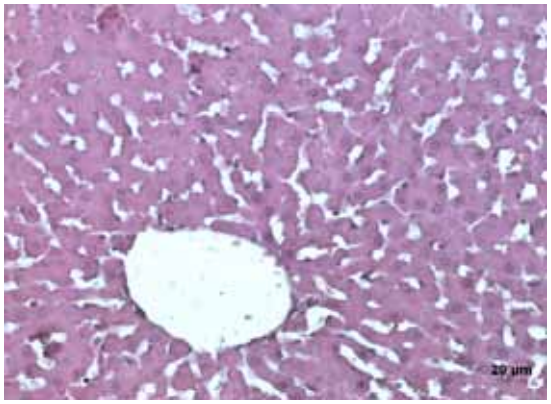

Plate 1: Liver from normal rats showing the presence of sinusoids (indicated by white spaces in the plate)

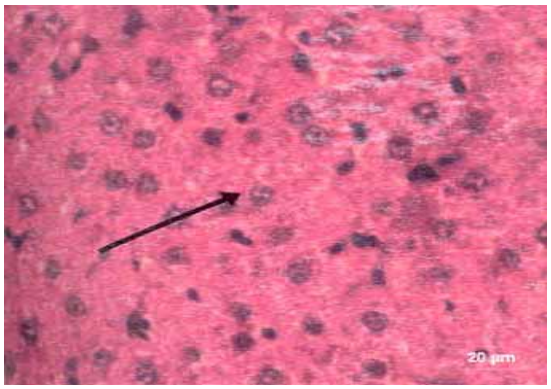

Plate 2: Liver from Gentamicin-treated rats showing Vonkupffer cell hyperplasia and obliteration of sinusoids

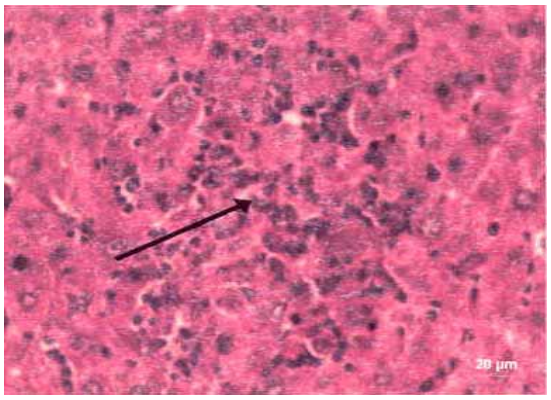

Plate 3: Liver from gentamicin-treated rats showing focal necrosis

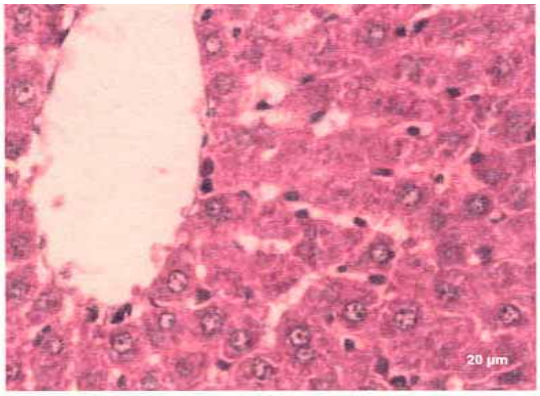

Plate 4: *Andrographis paniculata* 125-treated- Liver showing no appreciable lesions and with near-normal architecture

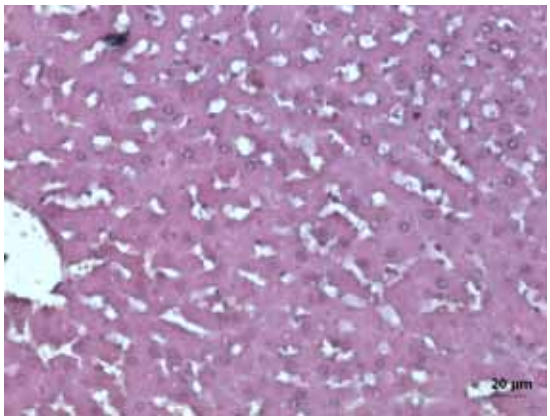

Plate 5: *Andrographis paniculata* 250 -treated Liver showing no appreciable lesion with near normal architecture

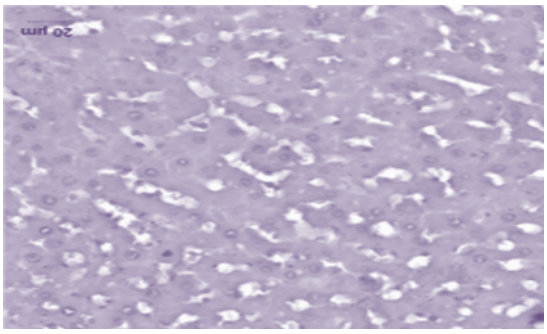

Plate 6: Silymarin-treated Liver showing no appreciable lesions and with near-normal architecture

DISCUSSION

The free radicals generated by the administration of gentamicin initiate not only the peroxidation of polyunsaturated fatty acids in the cell membrane, but also covalently bind to microsomal lipids and proteins. (Manna *et al*., 2006) This phenomenon results in the generation of reactive oxygen species (ROS) such as the superoxide anion, H_2O_2 and OH. Various enzymatic and non-enzymatic pathways are utilized by the cell to cope up with the ROS and other free radicals. However, once a condition of oxidative stress is established in the cell, the cellular defense mechanisms against ROS become inadequate. It has been reported that SOD, CAT and GST constitute a mutually supportive team of defense against ROS. The decreased activity of SOD in the liver of gentamicin treated animals may be due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. Increased utilization of GSH by the free radicals generated due to gentamicin toxicity might lead to a decreased GSH content (Manna *et al*., 2006). Biological membranes are rich in unsaturated fatty acids and are bathed in oxygen-rich metal containing fluids, and hence are highly susceptible to peroxidative attack (Bafna and Balaraman, 2005).

Gentamicin treatment significantly increased the TBARS levels in the liver. Increase in TBARS levels can be predominantly attributed to the damage of the Kupffer cells. The increased levels of TBARS of liver indicate enhanced lipid peroxidation due to tissue injury and failure of the antioxidant defense mechanisms, which prevent the formation of excess free radicals (Bafna and Balaraman, 2005). LPO levels in liver, which were increased upon gentamicin treatment, were restored back to normal in the groups treated with *Andrographis paniculata* (Table 1). Silymarin treatment also produced a similar response. Evidence for such effects of *Andrographis paniculata* comes from the studies of Triwedi and Rawal (2000) who reported that administration of *Andrographis paniculata* reversed the benzene hexachloride-induced elevated levels of LPO. They also suggested that hepatoprotective activity of *Andrographis paniculata* may be due to its antioxidant properties and our results concur with their observations.

SOD is an important cellular antioxidant enzyme (a metallo protein) which catalyses the conversion of superoxide radical to hydrogen peroxide and oxygen. In the present study, SOD activity of the liver was significantly decreased in the gentamicin-treated group when compared to the control (Table 1). These observations correlate with the findings of Ramasammy *et al*. (1987) who observed similar effects in their study in rats. In our study the higher dose of *Andrographis paniculata* produced a further increase in the SOD levels in the liver than the lower dose. However, silymarin treated group produced the maximum increase in the SOD level. Ramarperumalsamy *et al*. (2008) reported that *Andrographis paniculata* crude extract and its purified fractions could restore the snake venom-induced decrease in the serum SOD activity in mice and also suggested that the antivenom action of *Andrographis paniculata* may be due to its antioxidant activity.

In the present study, the CAT levels of liver significantly decreased in the gentamicin group when compared to control. Previously, Karahan *et al*. (2005) observed significant reduction of the CAT activity in gentamicin treated group when compared to the control. Significant improvement in the activity of liver CAT was observed in the *Andrographis paniculata* treated groups when compared to the gentamicin group. Silymarin treatment also produced a similar effect. Support for our findings comes from the studies of Nibha verma and Manjula vinayak, (2007) who also reported that the administration of aqueous extract of *Andrographis paniculata* caused a significant increase in the CAT activity in lymphoma bearing mouse livers.

GPx is a selenium-containing metalloenzyme that catalyses the oxidation of GSH by hydrogen peroxide to form water and oxidized glutathione. In the present study, GPx level of liver was significantly decreased in the gentamicin-treated group when compared to the control. In this context, it is important to note that Farombi and Ekor (2006) also reported a similar finding, wherein pretreatment with curcumin at 200 mg/kg for two weeks significantly restored the GPx as compared to control group in rats. In our study, *Andrographis paniculata* at both the doses significantly improved the liver GPx level, whereas silymarin produced a better response.

In the present study, GSH level of liver was significantly decreased in the gentamicin treated group when compared to the control. On the other hand, *Andrographis paniculata* at both doses significantly increased the liver GSH content in a dose dependant manner. Silymarin also produced an increase in the GSH level.

Our results regarding the antioxidant activity of *Andrographis paniculata* suggest that the extract of this plant possess significant membrane protective effect as can be inferred from the decrease in LPO activity in different groups, an effect that restores the liver LPO levels back to normal (Table 1). However, when it comes to the effect on SOD activity; significant activity was observed with *Andrographis paniculata* in a dose dependent fashion with a higher dose producing a better response, which was comparable to that of silymarin.

Based on the results for GPx activity and GSH content, we can infer that the extract of *Andrographis paniculata* was able to reverse the gentamicin induced oxidative stress, with the higher doses having a greater free radical scavenging activity, an effect comparable to that of silymarin.

Collectively, the above results suggest that *Andrographis paniculata* at the dose of 250 mg/kg body weight is preferable since it has a better activity on SOD levels apart from other beneficial features.

Decreased anti-oxidant activity upon gentamicin administration, may be attributed either to overproduction of free radicals or to poor neutralization of generated free radicals (Bafna and Balaraman, 2005). Hence the liver showed enhanced susceptibility to lipid peroxidation. The effects of *Andrographis paniculata* at a higher dose (250 mg/kg) indicates a marked protection against gentamicin induced plasma membrane damage and oxidative stress as shown by the restoration of biochemical parameters as well as the preservation of endogenous anti-oxidants (Table 1).

Our findings are also substantiated by the histopathological studies. The degenerative and necrotic changes observed in the gentamicin treated rats (plate 2), have been found to be reversed in both the doses of *Andrographis paniculata* treated rats as well as in the silymarin-treated rats (plates 3, 4, 5 and 6). The protective effects of *Andrographis paniculata* may be attributed to an increase in the antioxidant activity and regeneration of cellular membrane (Bafna and Balaraman, 2005). Thus, *Andrographis paniculata* was able to induce regeneration and reparative processes of the cellular membrane and upregulate antioxidant enzyme status, thus restoring the functional balance between pro-oxidant and anti-oxidant pathways.

Conclusion

In summary, findings from our study demonstrate that administration of gentamicin in rats at 80 mg/kg body weight i.p. for seven days produced toxic manifestations in the liver with alterations in the antioxidant status, an effect that was supported by histopathological lesions. Treatment with the extract of *Andrographis paniculata* was able to rescue the gentamicin induced toxicity, probably via augmenting the antioxidative defense mechanisms. Thus, significant findings from our study

indicate that this protective activity of the plant extract helps in maintaining the integrity of plasma membrane and also enhances the regenerative and reparative capacity of the liver. These properties could be attributed to the presence of active principles like andrographolide and arabinogalactan proteins, in *Andrographis paniculata*. Further research is required to fully characterize the responsible active principle(s) present in the plant and to clearly elucidate the possible mode(s) of action.

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