

Nephroprotective potential of *Graptophyllum pictum* against renal injury induced by gentamicin

Keloth Kaitheri Srinivasan ¹, Jessy Elizabeth Mathew ^{2*}, Kerryn Joseph A.D'Silva ², Richard Lobo ³, Nimmy Kumar ³

¹ Department of Chemistry, Shri Madhwa Vadiraja Institute of Technology and Management, Udipi- 574115, India

² Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India

³ Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India

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ABSTRACT

Objective(s): To evaluate the effect of *Graptophyllum pictum* on lipid peroxidation and tissue antioxidant enzymes in liver and kidney of gentamicin induced nephrotoxic rats.

Materials and Methods: Animals were grouped into 6: Group 1 received gum acacia, Group 2 received *G. pictum* ethanol extract (300 mg/kg), Group 3 received gentamicin, Groups 4, 5, 6 received gentamicin along with *G. pictum* at 300, 150, 75 mg/kg, respectively. Nephroprotective activity was evaluated by measuring thiobarbituric acid-reactive substances (TBARS), biochemical markers Glutathione (GSH), Glutathione-S Transferase(GST), Superoxide dismutase (SOD), Catalase (CAT), serum urea and creatinine levels.

Results: Results obtained showed that gentamicin induced nephrotoxic rats exhibited lower activities of biochemical markers and raised levels of TBARS, serum creatinine and urea. Remarkably, after treatment with *G. pictum* extract, anomalous levels of biochemical markers, lipid peroxidation and serum creatinine were returned to normal.

Conclusion: The results propose that *G. pictum* has nephroprotective effects, and can be a promising natural source against gentamicin induced nephrotoxicity.

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Introduction

An aminoglycoside antibiotic gentamicin is primarily employed in the treatment of serious and life threatening Gram-negative infections caused by *Serratia*, *Proteus* and *Pseudomonas*. However, its use is restricted because it causes drastic damage to kidneys in 10 to 20% of cases (1). The damage is proposed to occur through pathological mechanisms such as apoptosis, necrosis, production of oxidative stress and by augmenting the levels of endothelin I and monocyte/macrophages infiltration. Gentamicin is thought to augment the production of reactive oxygen species (ROS) such as hydrogen peroxide, super oxide anions, hydroxyl radicals and reactive nitrogen species in the kidney (2). Since ROS are relatively unstable, they have a tendency to cause irreparable damage to cells and tissues. A dynamic equilibrium exists between the quantity of ROS and endogenous antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase etc. which detoxify and scavenge ROS and thereby protects the body against their damaging effect. This delicate balance is sometimes disturbed due to ROS build-up caused by gentamicin resulting in lipid peroxidation and

depletion of endogenous antioxidants (3). Hence, exogenous antioxidants are needed for protection against nephrotoxic agents. It is widely acknowledged that many medicinal plants are enriched with bioflavonoids, which are known to be potent antioxidants. As a result, mankind has turned to explore the alternative sources of indigenous system of medicine such as Ayurveda, Siddha, Unani and Naturopathy for investigation of nephroprotective agents possessing antioxidant property.

Graptophyllum pictum popularly known as 'Joseph's coat' because of the bicolor of its leaf is widely used in folk medicine for treatment of wound, swelling, ulcer and haemorrhoids (4). Several pharmacological activities such as analgesic, anti-inflammatory, uterotonic, abortifacient and hypoglycemic properties (5) have been reported in this plant. As there is no scientifically validated report to prove the nephroprotective activity of *G. pictum* on gentamicin-induced nephrotoxicity, the present study was carried out to establish the effectiveness of the selected plant in management of gentamicin-induced nephrotoxicity.

*Corresponding author: Jessy Elizabeth Mathew. Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal-576104, India. Tel: +91820 2922482 ext. 138; Fax: +91820 2571998; email: jessyelijabethmathew@gmail.com

Materials and Methods

Plant material

The leaves of *G. pictum* were collected from Manipal, Udupi district, Karnataka in September 2006 and authenticated by Dr Gopalakrishna Bhat, Department of Botany, Poorna Praja College, Udupi, Karnataka, India. A voucher specimen PP 710 was placed in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India.

Preparation of ethanol extract

The shade dried plant was coarsely powdered, extracted with 95% ethanol using soxhlet apparatus and concentrated by distillation *in vacuo*. The extract was prepared in 2% gum acacia solution for animal studies.

Animals

Experiments were carried out in healthy adult Wistar albino rats (150 to 250 g) aged 60 to 90 days. The animals were sheltered two per cage, endowed with a temperature regulated and humidity controlled atmosphere. They had permitted access to standard food pellets and water. The study was performed after acquiring ethical committee approval from the Institutional Animal Ethics Committee of Kasturba Medical College, Manipal (IAEC/KMC/07/2007-2008).

Gentamicin induced renal injury

The rats were randomly assigned into six groups (n= 8 rats per group).

- Group 1 (Control group) received gum acacia orally for 23 days; Blood was withdrawn on 24th day
- Group 2 received *G. pictum* ethanol extract (300 mg/kg) per orally for 10 days; Blood was withdrawn on 11th day
- Group 3 (Disease control) received Gentamicin (40 mg/kg) subcutaneously for 13 days; Blood was withdrawn on 14th day
- Group 4, 5, 6 (Curative groups) received gentamicin (40 mg/kg) subcutaneously for 13 days, following they were treated orally with 300 mg/kg, 150 mg/kg and 75 mg/kg of *G. pictum* extract, respectively from 14th to 23rd day. Blood was withdrawn from animals in all 3 groups on 24th day.

Collection of samples and biochemical assays

At the end of the experimental period, blood samples were collected for measuring the biochemical parameters. The rats were anaesthetized and sacrificed by cervical dislocation. The kidney sections were stained with haematoxylin and eosin and observed under light microscope for histopathological studies. The kidney and liver were excised and homogenized with Tris-Hydrochloric buffer (pH 7.4). The

homogenates were used to determine thiobarbituric acid reactive substance, reduced glutathione, glutathione transferase, superoxide dismutase and catalase.

Determination of lipid peroxidation

The extent of lipid peroxidation was evaluated by measuring the amount of thiobarbituric acid-reactive substances (TBARS) (6).

GSH determination

Reduced glutathione (GSH) was measured by means of glutathione reductase 5, 5'-dithiobis- 2-nitrobenzoic acid (DTNB) recycling procedure (7).

Determination of Superoxide dismutase (SOD)

SOD activity was evaluated by its potential to impede reactions through the generation of O₂⁻ by a xanthine-xanthine oxidase system (8).

Catalase (CAT) determination

Catalase activity was resolved from the rate of decomposition of H₂O₂, assessed by decline in absorbance at 240 nm resulting from addition of tissue homogenate (9).

GST determination

GSH-S-transferase was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate according to an established method (10).

Serum urea and creatinine

Serum urea was determined using diacetylmonoxime (DAM) reagent (Modified Berthelot methodology) using a diagnostic kit (Roche Diagnostics, Hitech, Mangalore, India). Serum creatinine was determined by alkaline picric acid method using a diagnostic kit (Roche Diagnostics, Hitech, Mangalore, India).

Histopathological studies

Two animals from each group were sacrificed on the day of blood withdrawal, and kidneys were isolated. The sections were stained with haematoxylin and eosin and then observed under light microscope for histopathological changes.

Statistical analysis

Results were presented as values of mean ±SEM. Data was analyzed using one-way ANOVA followed by *post-hoc* Dunnett's test using SPSS computer software version 7.5 wherein *P*<0.05 was considered to be statistically significant.

Results

Effect on lipid peroxidation

The concentration of TBARS in the liver and kidney of normal, gentamicin induced nephrotoxic rats and *G. pictum* treated gentamicin induced rats

Table 1. Effect of *Graptophyllum pictum* ethanol extract on biochemical markers suggesting oxidative stress in gentamicin induced renal damage (kidney and liver)

Groups	GSH (U/G/min)		GST (U/G/min)		SOD (U/G)		CAT (μg of H_2O_2 /min/mg protein)	
	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
Control	13.42 \pm 0.76	12.97 \pm 2.1	20.43 \pm 1.01	18.8 \pm 0.96	395.05 \pm 12.43	391.8 \pm 11.3	376.18 \pm 1.89	356.9 \pm 14.4
Gp	13.48 \pm 0.92*	12.08 \pm 0.64*	21.87 \pm 1.81*	19.8 \pm 2.2*	384.46 \pm 10.28*	388.4 \pm 13.6*	373.2 \pm 1.98*	362.5 \pm 19.2*
Gentamicin	5.32 \pm 0.68#	4.3 \pm 1.2#	11.86 \pm 1.42#	6.8 \pm 0.64#	168 \pm 8.87#	167.8 \pm 9.86#	138.65 \pm 12.4#	146.8 \pm 13.56#
Gentamicin + Gp1	13.98 \pm 0.92*	13.05 \pm 1.3*	21.12 \pm 1.90*	18.5 \pm 2.2*	384 \pm 13.48*	386.08 \pm 10.92*	380.42 \pm 18.46*	370.4 \pm 18.2*
Gentamicin+ Gp2	12.8 \pm 1.12*	12.45 \pm 0.98*	19.88 \pm 1.22*	18.1 \pm 1.34*	371.23 \pm 12.2*#	382.45 \pm 14.1*	370 \pm 18.3*#	361.68 \pm 16.54*
Gentamicin+ Gp3	11.32 \pm 0.8*	11.86 \pm 0.54*	18.5 \pm 1.2*	14.87 \pm 1.08*	340 \pm 11.43*	365.48 \pm 10.7*	320.4 \pm 18.4*	358.9 \pm 21.92*

Values are presented as mean \pm SEM (n=8); * P <0.05 compared to Gentamicin group, # P <0.05 compared to Control group; Gp- *G. pictum*, Gp1- *G. pictum* extract (300mg/kg), Gp2- *G. pictum* extract (150mg/kg), Gp3- *G. pictum* extract (75 mg/kg), GSH-Glutathione, GST- Glutathione transferase, SOD- Superoxide dismutase, CAT- Catalase

(at various doses) are shown in Figure 1. It was distinctly noted that gentamicin caused a significant (P <0.05) elevation in lipid peroxide (TBARS) levels in both renal and liver tissues when compared to the control group. Remarkably, treatment with *G. pictum* was able to significantly (P <0.05) lower the raised TBARS levels in both kidney and liver.

Effect on biochemical markers (GSH, GST, SOD and CAT)

A significant (P <0.05) decline was found in GSH and GST levels in kidney and liver of nephrotoxic rats treated with gentamicin alone. In sharp contradistinction, GSH and GST content were significantly (P <0.05) raised in nephrotoxic rats receiving *G. pictum* treatment at 300 mg/kg, 150 mg/kg and 75 mg/kg.

Likewise, SOD and catalase levels were significantly (P <0.05) diminished in gentamicin only treated rats. Conversely, SOD and CAT levels were appreciably (P <0.05) elevated in *G. pictum* treated groups receiving the plant extract at all three doses of 300 mg/kg, 150 mg/kg and 75 mg/kg (Table 1).

Effect on serum urea and creatinine

Levels of Serum creatinine and urea were significantly (P <0.05) elevated in the gentamicin

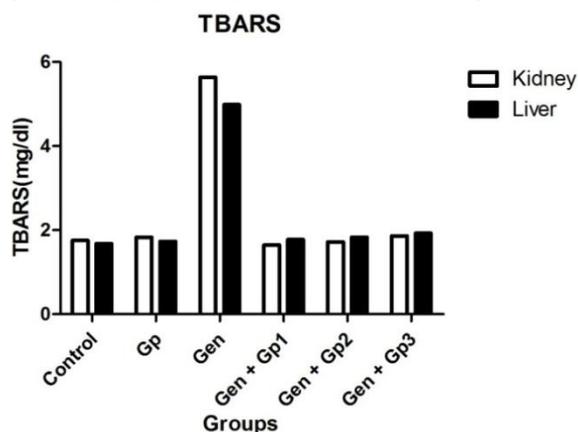


Figure 1. Protective effect of *Graptophyllum pictum* ethanol extracts on lipid peroxidation (TBARS) in gentamicin induced rats TBARS - Thiobarbituric Acid Reactive Substance, Gp - *G. pictum*, Gen - Gentamicin, Gp1 - *G. pictum* extract (300 mg/kg), Gp2 - *G. pictum* extract (150 mg/kg), Gp3 - *G. pictum* extract (75 mg/kg)

treated animals when compared to control group. This rise in serum creatinine and urea levels was brought down significantly (P <0.05) after treatment with *G. pictum* ethanol extract (Table 2).

Effect on histopathological injuries

Several features of acute tubular necrosis such as the appearance of peritubular and glomerular congestion, interstitial edema, tubular casts, epithelial degeneration, blood vessel congestion and infiltration by inflammatory cells were noted in the histopathological sections of gentamicin treated rats (Figure 2B, C). Notably, the *G. pictum* extract reversed acute tubular necrosis and other features of damage (Figure 2D).

Discussion

The purpose of the current study was to assess the antioxidant effect of oral administration of *G. pictum* ethanol extract on lipid peroxidation and tissue antioxidant enzymes in liver and kidney of gentamicin induced nephrotoxic rats. Results obtained indicated that gentamicin induced rats demonstrated lower activities of biochemical markers such as superoxide dismutase, catalase, glutathione and glutathione transferase. An appreciable increase in the levels of lipid peroxide (TBARS), serum creatinine and urea was noted in this particular group. Remarkably, following treatment with *G. pictum* extract, we found normal levels of biochemical markers, TBARS, serum creatinine and urea in all treated groups.

Gentamicin is widely used in clinical practice because of their bactericidal efficiency against gram-negative bacterial infections; synergistic activity with

Table 2. Effect of *Graptophyllum pictum* ethanol extract on serum urea and creatinine level

Group	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Control	36.06 \pm 3.53	0.32 \pm 0.016
Gp	35.09 \pm 1.65*	0.53 \pm 0.049*
Gentamicin	60.96 \pm 1.2#	1.71 \pm 0.054#
Gentamicin + Gp1	37.42 \pm 0.43*	1.03 \pm 0.04*#
Gentamicin + Gp2	41.40 \pm 1.51*	1.47 \pm 0.079#
Gentamicin + Gp3	42.32 \pm 2.68*	1.24 \pm 0.1*#

Values are presented as mean \pm SEM (n=8); * P < 0.05 compared to Gentamicin group, # P < 0.05 compared to Control group; Gp- *G. pictum*, Gp1- *G. pictum* extract (300 mg/kg), Gp2- *G. pictum* extract (150 mg/kg), Gp3- *G. pictum* extract (75 mg/kg)

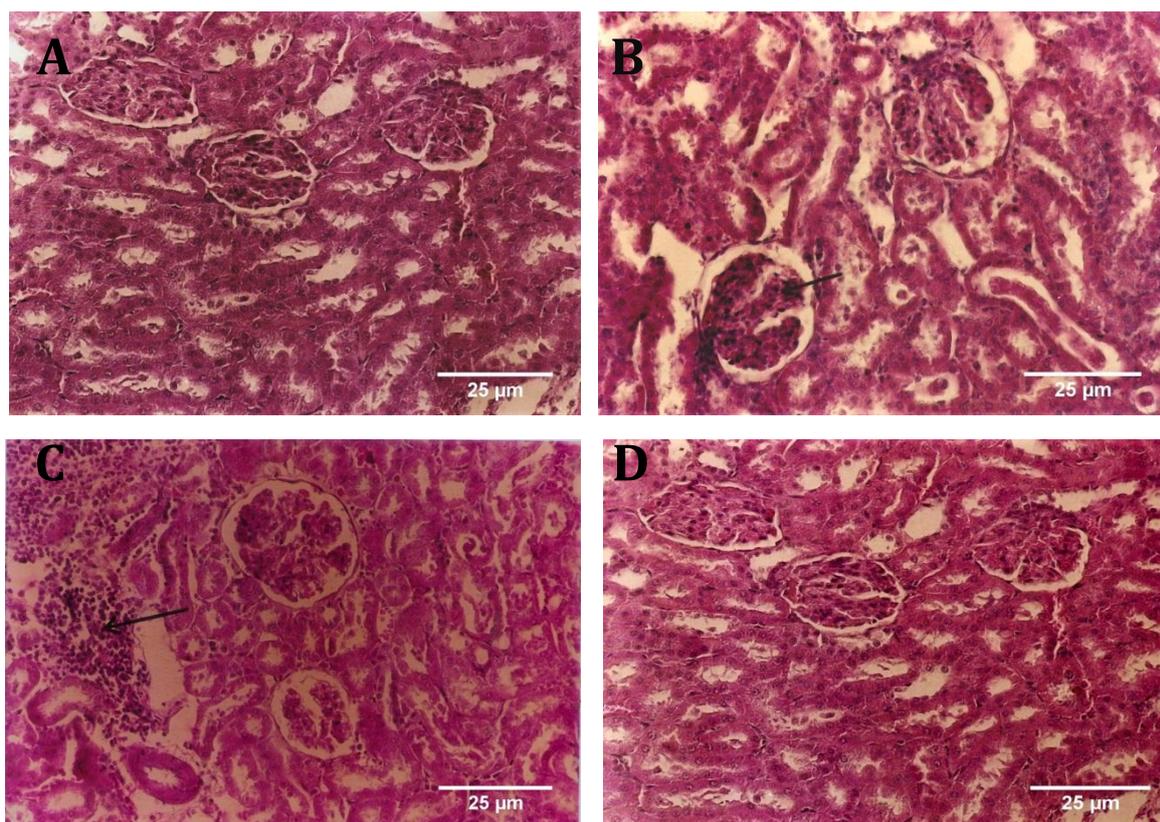


Figure 2. Light microscope photomicrograph depicting the nephroprotective effect of *Graptophyllum pictum* ethanol extract on gentamicin induced renal injury

- A) Section of kidney showing normal glomeruli and tubules
 B) Renal section of gentamicin treated rats on 14th day exhibiting glomerular congestion, tubular casts, epithelial desquamation and peritubular congestion. The arrow indicates glomerular congestion
 C) Section of kidney of gentamicin treated rats on 24th day revealing infiltration of inflammatory cells. Epithelial degeneration can be observed (as indicated by the arrow)
 D) Kidney section from gentamicin + *Graptophyllum pictum* (curative group) treated rats showing normal glomeruli with mild glomerular congestion with no tubular casts or epithelial desquamation

β -lactam antibiotics, reduced cost and limited bacterial resistance. However, recent reports have shown that around 30% patients treated with gentamicin for more than a week exhibited certain symptoms of renal impairment (11). Oxidative stresses are believed to be the primary cause for gentamicin induced nephrotoxicity. It has been suggested that ROS, formed due to oxidative stress have a crucial role in mechanistic pathway of renal tubular necrosis. ROS are capable of activating nuclear factor kappa β that can in turn trigger the initiation of inflammatory process. In brief, the fundamental role of gentamicin-induced nephrotoxicity is oxidative stress and inflammation (12).

Free oxygen radicals can also bring about lipid peroxidation in cells. It was noted in the current study that gentamicin triggered a significant elevation of TBARS level in the kidney and liver in the gentamicin group, which decreased upon administration of *G. pictum* ethanol extract (at different doses) in the therapeutic groups as shown in Figure 1.

According to our results, biochemical markers (GSH, GST, catalase and SOD) declined significantly in the gentamicin-only treated group when compared to the control group. This was consistent with results reported in other studies (13). Glutathione (GSH) provides protection against oxygen free radical damage by providing reducing equivalents for several enzymes; it is also a scavenger of hydroxyl radicals and singlet oxygen. In the present study, the levels of GSH in rat kidney tissues were significantly reduced in the gentamicin administered group when compared to the control group. A possible rationalization of reduction in GSH level after treatment with gentamicin is the increased consumption of GSH in non-enzymatic removal of ROS generated as a result of gentamicin induced nephrotoxicity. In curative groups 4, 5, 6 which received *G. pictum* ethanol extract at 300 mg/kg, 150 mg/kg and 75 mg/kg along with gentamicin, the GSH and GST levels were significantly raised. It has been reported that prior treatment with antioxidants can appreciably increase the GSH level (14). Accordingly, our results were consistent with this finding as

administration of *G. pictum* along with gentamicin drastically increased the GSH levels in the curative groups. We observed that activities of SOD and CAT enzymes were vastly reduced in gentamicin treated rats when compared to the control group. Treatment with *G. pictum* extract in curative groups 4, 5, 6 brought back the SOD and CAT levels to normal.

Prominent increase in serum creatinine and urea concentration is suggested as a sign of significant functional impairment of kidney in gentamicin induced nephrotoxicity (13). In the current study, we measured both serum creatinine and urea after administration of gentamicin. We noted a significant increase in serum creatinine and urea; this was established in other studies as well (15). Administration of *G. pictum* along with gentamicin in the curative groups helped in restoring the serum creatinine and urea concentration to near normal levels. This probably points towards the antioxidant property of *G. pictum*.

Sections from control group shows normal histological structure of the glomeruli and renal tubules in the cortex as evidently depicted in Figure 2A. In contrast, the gentamicin treated rats produced glomerular congestion with tubular casts and epithelial degeneration; this was associated with peritubular congestion and edema with infiltration of inflammatory cells. The microscopic changes were observed even after 10 days following 14 days of gentamicin administration (Figure 2B and C). Intriguingly, ethanol extract of *G. pictum* at 300, 150, 75 mg/kg body weight produced mild glomerular congestion with no tubular casts or epithelial desquamation. There was no inflammation or peritubular congestion microscopically. There were no inflammatory cells in the interstitium (Figure 2D).

Conclusion

G. pictum extract protects gentamicin-induced nephrotoxicity probably by inhibiting lipid peroxidation and improving glutathione content and enzymatic activity of antioxidants in liver and kidney. The findings thus suggest that *G. pictum* could have a crucial role in the management of acute renal damage and could be developed as a therapeutic option against kidney failure caused by nephrotoxins like gentamicin. However, further studies with larger sample size are necessary to understand the mechanism of action in chronic experimental nephrotoxicity.

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