ORIGINAL ARTICLE

Evaluation of Nephroprotective Activity of Gallic Acid in Gentamicin-induced rat Model of Nephrotoxicity

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Abstract

Introduction: Renal damage due to gentamicin is associated with oxidative stress. Gallic acid is a phenolic compound that possesses antioxidant and anti-inflammatory properties. Hence, an attempt was made to explore the nephroprotective activity of gallic acid in gentamicin-induced nephrotoxicity. **Materials and Methods:** Wistar albino rats of either sex were used. Experimental nephrotoxicity was produced by intraperitoneal administration of gentamicin for 8 days. Rats were divided into five groups: Group I - normal control (normal Saline), Group II - gentamicin only treated group (100 mg/kg), Group III - gentamicin (100 mg/kg) + Vitamin C (200 mg/kg), Group IV (treatment group) - gentamicin (100 mg/kg) + gallic acid (200 mg/kg), and Group V - gentamicin (100 mg/kg) + gallic acid (400 mg/kg). The period of drug administration was of 8 days, in which animals were treated with Vitamin C and gallic acid. After the treatment for 8 days, the animals were sacrificed for the investigation of biochemical parameters and histopathological examination. **Results:** Gentamicin-induced nephrotoxicity was successfully reproduced. Concurrent administration of gallic acid along with gentamicin significantly prevented the rise in level of serum creatinine, serum urea, blood urea nitrogen, and total protein. Administration of gallic acid also leads to increase glutathione and superoxide dismutase level in the kidney. Therefore, gallic acid had significantly prevented nephrotoxicity as compared to the group receiving gentamicin drug alone. **Conclusion:** These results showed that gallic acid is effective as nephroprotective agent.

Key words: Gallic acid, gentamycin, nephrotoxicity

INTRODUCTION

Kidneys play a variety of important functions in the body. They help in maintaining the level of ions such as Na^+, K^+, Cl^-, Ca^{2+},and Mg^{2+} in blood plasma and excretion of nitrogenous waste products such as urea and creatinine from the body. Kidneys also play an important role in maintaining the acid-base balance in the body. Nephrotoxicity is defined as the renal damage and tubular injuries.[1] It produces harmful effects in our body and increases the level of ions, serum creatinine, serum urea, blood urea nitrogen (BUN), and serum total protein in the body.[2] Nephrotoxicity can be of various forms such as tubular necrosis, nephropathies, structural changes in kidney, and inflammation in renal cells.

Gentamicin is an aminoglycoside antibiotic that produces nephrotoxicity and 10–20% of people receiving aminoglycoside antibiotic gentamicin suffer from kidney disorders.[3] The mechanism of gentamicin-induced nephrotoxicity in rats is through generation of oxidative stress which is mainly due to the production of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide and inhibition of oxidative phosphorylation.[4,5]

ROS may result in the development of oxidative stress that may cause kidney damage.[6] Various inflammatory mediators responsible for inflammation may also result

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in tissue damage. There is also a report indicating that gentamicin increases oxidative stress in renal tissue which is a vital factor for the induction of nephrotoxicity.[7] Thus, agents having antioxidant and anti-inflammatory properties may be beneficial in preventing the gentamicin-induced nephrotoxicity.[8-10]

Gallic acid is generally found in vegetables, plants, and fruits such as grapes, raisins, spinach, and green tea.[11] Gallic acid is a phenolic compound and inhibits the inflammation and microbial infections. Gallic acid also inhibits the overproduction of ROS and therefore produces anti-oxidative effect in the body.[12-14] Since gallic acid has good antioxidant and anti-inflammatory properties, therefore the present study was designed to explore the nephroprotective activity of gallic acid.[15]

MATERIALS AND METHODS

Chemicals and kits

Gentamicin was purchased from CDH, Chemicals Pvt., Ltd., and gallic acid was purchased from BB, Chemicals Pvt., Ltd., India. Ascorbic acid was purchased from Erba Mannheim Pvt., Ltd. Serum creatinine, BUN, urea analysis, and total protein level kit were purchased from Erba Mannheim Private Limited, India.

Experimental animals

Albino Wistar rats of both the sexes weighing 150–200 g were used, maintained at standard laboratory diet, and had free access to food and water throughout the study. They were housed in the department animal house in cages at ambient temperature 25 ± 2°C and were exposed to 12-h cycle of light and dark. The experimental protocol (CPCSEA/PCL/14/2016-17) was approved by the Institutional Animal Ethics Committee ,and care of the animals was carried out as per the guidelines of the committee for the purpose of the Control and Supervision of Experiments on Animals (CPCSEA) on animal experimentation.

Experimental design

Rats were randomly divided into five groups. Group 1 served as a control group received normal saline (0.9%w/v NaCl) i.p for 8 days. Group 2 served as gentamicin-treated negative control group and was administered with gentamicin (100 mg/kg; i.p) diluted in normal saline for 8 days.

Group 3 served as standard *per se* treated group and was administered ascorbic acid (200 mg/kg) p.o. for 8 days. Group 4 served as drug-treated group and had received gallic acid (200 mg/kg p.o., respectively) along with gentamicin (100 mg/kg; i.p) diluted in normal saline for 8 successive days. Group 5 also served as drug-treated group and had received gallic acid (400 mg/kg p.o., respectively) along with gentamicin (100 mg/kg; i.p) diluted in normal saline for 8 successive days.

At the end of the study, blood was collected for biochemical estimations (creatinine, BUN, and total protein). All animals were anesthetized and sacrificed to high dose of diethyl ether, and kidneys were taken out for oxidative stress assessment parameters (glutathione [GSH] and superoxide dismutase [SOD]) and histopathological examination.

Kidney tissue homogenate preparation for SOD and GSH estimations

The kidney was dissected and washed with ice-cold isotonic saline and weighed. The kidney was then minced, and a homogenate (10% w/v) was prepared in chilled 1.15% KCl. The homogenate was used for estimating SOD and GSH.[16]

Estimation of GSH

The supernatant of homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio, i.e. the mixture contained 400 µl of supernatant and 400 µl trichloroacetic acid. The tubes were centrifuged at 100 rpm for 10 min at 4°C. The supernatant (0.5 ml) obtained was mixed with disodium hydrogen phosphate (0.3 M; 2 ml). Then, freshly prepared 5, 5-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate (0.001 M; 0.25 ml) and absorbance was measured spectrophotometrically at 412 nm. A standard curve was plotted using 10–100 µM of reduced dorm of GSH, and concentration of GSH was expressed in micromoles of reduced GSH per mg of protein.

Estimation of SOD

SOD activity was measured in the Sephadex G 25 eluted PMS as described by Das *et al.* In this method, superoxide radical generation by photoreduction of riboflavin is combined with nitrite formation from hydroxylamine hydrochloride to detect superoxide radical.

Superoxide radicals are allowed to react with hydroxylamine hydrochloride to produce nitrite. The nitrite, in turn, reacts with sulfanilic acid to produce a diazonium compound that subsequently reacts with naphthylamine to produce a red azo compound. Briefly, 0.1 ml sample (PMS) was added to a cocktail mixture of seven reagents, namely (1) phosphate buffer (pH 7.4), (2) L-Methionine (20 mM), (3) hydroxylamine hydrochloride (10 mM), (4) EDTA (50 µM), (5) Triton X-100 (1%), (6) riboflavin 100 µM, and (7) Griess reagent (consisting of 0.1% napthethyldiamine and 1% sulfanilamide), and incubated at 370°C for 5 min. Riboflavin (80 µl) was added to each sample and further incubated in

a specially designed fluorescent illuminated light box for 10 min. Following the incubation, Griess reagent was added to each tube to develop color. Absorbance was measured at 543 nM in a spectrophotometer. The activity was expressed as U SOD/mg protein.[17]

Histopathological examination

The kidneys were sectioned longitudinally into two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax, and sections were taken using a microtome. These sections were stained with hemotoxylin and eosin and were observed under a computerized light microscope.[16]

Statistical analysis

All the data was entered into database program (Sigmastat 4.0). Data were expressed as mean ± standard error of the mean. Statistical analysis was done with one-way analysis of variance followed by Dunnett test. $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered as statistically significant.

RESULTS

Effect of Gallic acid on serum creatinine, BUN, and total protein

The levels of renal function parameters such as creatinine, BUN, and total protein were significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) increased in gentamicin-treated rats compared to normal rats. Oral administration of gallic acid (200 mg/kg and 400 mg/kg p.o.) to gentamicin-treated group significantly ($P < 0.001$) decreased the serum creatinine, BUN, and total protein level compared to gentamicin only treated group. However, ascorbic acid (200 mg/kg p.o.) is not produced a prominent effect on serum creatinine, BUN, and total protein compared to only gentamicin-treated group [Table 1].

Effect of gallic acid on GM-induced alterations in oxidative stress

Renal GSH and SOD levels were significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) decreased in gentamicin alone treated group when compared to normal saline group. The administration of gallic acid (200 mg/kg and 400 mg/kg p.o.) with gentamicin significantly ($P < 0.05$, $P < 0.01$ and $P < 0.001$) increased the renal GSH and SOD level compared to gentamicin only treated group. However, ascorbic acid did not produced significant effect on the renal GSH and SOD levels in gentamicin per se treated group [Table 2].

Histopathological estimations

- Group I: Normal control showed the normal renal cortex showing glomerulus and tubules and no damaged part of the cortex [Figure 1a].
- Group II: Which was treated with gentamicin 100 mg/kg showed that extravasations of erythrocytes in glomerulus and the renal tubules were observed with degenerative changes [Figure 1b].
- Group III: Which was given gentamicin with Vitamin C 200 mg/kg p.o showed that there is moderate recovery

Table 1: The values are expressed as mean±SEM

Group	Treatment	Creatinine (mg/dl)	BUN (mg/dl)	Total protein (g/dl)
I	Control	0.55±0.072	24.14±4.2	6.34±0.44
II	GM (100 mg/kg)	1.48±0.08	55±8.94	8.79±0.75
III	GM (100 mg/kg) + Ascorbic acid (200 mg/kg)	1.01±0.01	49±7.36	7.72±0.53
IV	GM (100 mg/kg) + GA (200 mg/kg)	0.91±0.12	40.2±3.32	7.06±0.49
V	GM (100 mg/kg) + GA (400 mg/kg)	0.74±0.06	27.16±1.42**,#	6.80±0.22*

*P<0.05: **P<0.01: ***P<0.001, when compared with negative control. #P<0.05; ##P<0.01, ###P<0.001, when compared with standard group. The data were analyzed by one-way ANOVA followed by Dunnett's test. SEM: Standard error of the mean, ANOVA: Analysis of variance

Table 2: The values are expressed as mean±SEM

Groups	Treatment	SOD (U/mg)	GSH (µmol/mg)
I	Control	532±32.23	6.95±0.08
II	GM (100 mg/kg)	281±32.07	3.56±0.50
III	GM (100 mg/kg) + Ascorbic acid (200 mg/kg)	352±18.98	5.16±0.34**
IV	GM (100 mg/kg) + GA (200 mg/kg)	425±33.01**	5.44±0.21***
V	GM (100 mg/kg) + GA (400 mg/kg)	460±49.06**	5.53±0.65***

*P<0.05: **P<0.01: ***P<0.001, when compared with negative control. #P<0.05; ##P<0.01, ###P<0.001, when compared with standard group. The data were analyzed by one-way ANOVA followed by Dunnett's test. SEM: Standard error of the mean, ANOVA: Analysis of variance

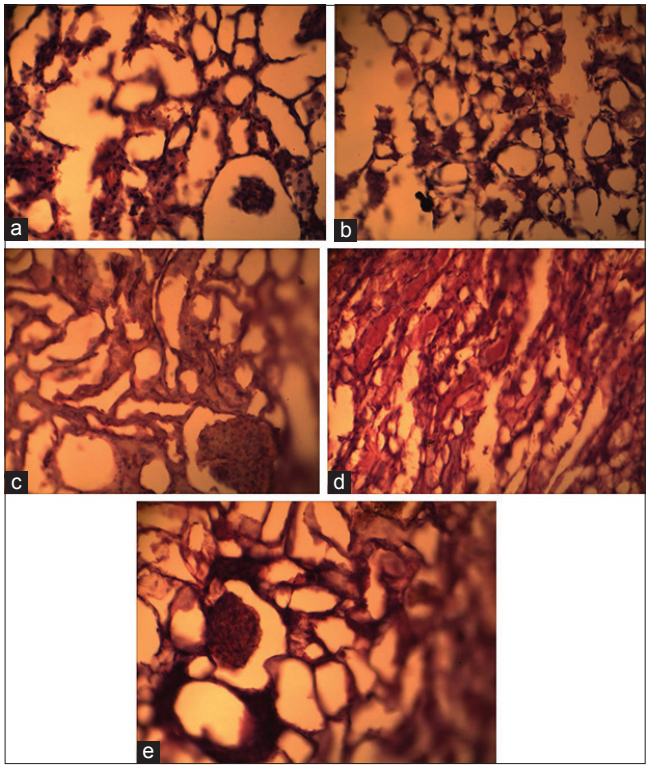

Figure 1: (a) Normal control, (b) gentamicin (100 mg/kg), (c) GM (100 mg/kg) + Vitamin C (200 mg/kg), (d) GM (100 mg/kg) + GA (200 mg/kg), (e) GM (100 mg/kg) + GA (400 mg/kg)

of tubular epithelial cells, glomerulus, and renal parenchyma remained intact [Figure 1c].

- Group IV: Which was given gentamicin with gallic acid 200 mg/kg low dose, the section showed evidence of the restoration of tissue damage in some aspects and the very little effect is seen in tubular repair with low doses [Figure 1d].
- Group V: Which was given gentamicin with gallic acid 400 mg/kg high dose showed intact renal parenchyma, glomerulus, and tubular regeneration of mild to moderate compared to standard [Figure 1e].

DISCUSSION

Nephrotoxicity occurs when the renal blood is exposed to a nephrotoxic drug or toxin that causes damage to the kidneys. This may lead to acute kidney failure. In this condition, the kidney function deteriorates and may lead to chronic kidney failure.[16,18] If unchecked, the kidney failure may lead to the death. ROS responsible for the generation of oxidative stress along with inflammation can contribute to kidney damage.[4,19,20]

Gentamicin is an aminoglycoside antibiotic mainly used to prevent severe Gram-negative infections, and one of its limitations is that it leads to nephrotoxicity.[21] Pathological mechanism for gentamicin-induced nephrotoxicity includes oxidative stress, apoptosis, necrosis, and increase of monocyte/macrophages infiltration.[8] Gentamicin-induced nephrotoxicity is associated with renal oxidative stress in the kidney which is due to overproduction of ROS,[12] reduction in the amount of renal antioxidant defense mechanism, and result in glomerular damage, renal inflammation, and tubular necrosis which are major complications produced in gentamicin nephrotoxicity.[16] Nephrotoxicity has been treated due to the damage and retention of aminoglycoside in the proximal convoluted tubules.[3,10] Gentamicin-induced nephrotoxicity is characterized by functionally increase in serum creatinine, increase in BUN, and decrease in glomerular filtration rate, proximal tubule epithelial desquamation, and tubular necrosis edema.

Gallic acid contains phenolic compounds and has the ability to attenuate the renal dysfunction, increase the antioxidant enzymes activity, and decrease lipid peroxidation[22-23] (Chilwant and Muglikar, 2012; Balagangadharan, 2012). It has also anti-inflammatory, anticancer, and antibacterial activity.[13-15,20] It has been found from the present study that gallic acid has a prominent effect against renal injury that may be due to increase in the level of GSH and SOD in renal cells and the nephroprotective effect was confirmed through biochemical parameter estimation in blood and histopathological examination of the kidney.

In view of the above findings, it is suggested that gallic acid could contribute to protection against gentamicin-induced nephrotoxicity possibly by inhibition of lipid peroxidation, enhancing renal GSH and antioxidant enzymes activity. Hence, gallic acid has potential therapeutic effect as a nephroprotective agent against gentamicin-induced nephrotoxicity.

CONCLUSION

The present study has shown that the gallic acid may be a potential therapeutic option in the effective management of nephrotoxicity by gentamicin because of its ability to reduce oxidative stress.

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