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Renoprotective Effect of Corosolic Acid in Gentamicin-Induced Nephrotoxicity and Renal Dysfunction in Experimental Rats

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Abstract

Gentamicin is an aminoglycoside antibiotic that has a proven record of effectively treating various Gram negative bacterial infections. The selective accumulation of gentamicin in the renal proximal convoluted tubule results in the induction of nephrotoxicity. Gentamicin-induced nephrotoxicity is characterized by tubular necrosis and glomerular congestion, resulting in decreased glomerular filtration rate and renal dysfunction. In addition, induction of oxidative stress and inflammatory cascades play a key role in gentamicin nephrotoxicity. Numerous pharmacological agents have been identified to have a potential in preventing gentamicin-nephrotoxicity. However, we do not have a promising intervention clinically to blunt gentamicin-nephrotoxicity. Identification of a potent pharmacological intervention to satisfactorily halt gentamicin nephrotoxicity is imperative in clinical point-of-view. However, the effect of corosolic acid in gentamicin-nephrotoxicity is not yet known. Therefore, the present study was designed to investigate the possible effect of corosolic acid (CRA) in gentamicin-induced experimental nephrotoxicity in rats.

Keywords: Nephrotoxicity; Reactive oxygen species; Antioxidant enzymes; Corosolic acid

Abbreviations

NF-κB: Nuclear Factor-Kappa B; iNOS: Nitric Oxide Synthase Enzyme; MDA: Malondialdehyde, marker of lipid peroxidation; ROS: Reactive Oxygen Species

Introduction

Aminoglycosides are potent broad-spectrum antibiotics that kill the bacteria by binding to the 30s subunit of the bacterial ribosome and reducing the fidelity of protein synthesis [1]. Aminoglycosides antibiotic are commonly used because of their properties rapid concentration-dependent bactericidal effects, synergism with beta-lactam antibiotics, low rate of resistance, and low-cost therapy [2-19]. A major complication of the use of these drugs is nephrotoxicity accounting for 10% to 20% of all cases of acute renal failure [20]. Nephrotoxic substances damage different nephron cell types, although tubular epithelial cell necrosis, glomerular injuries and functional alterations also takes place [21]. Gentamicin (GM) is an aminoglycoside antibiotic with IUPAC name 2-(4,6-diamino-3-(3-amino-6-(1-(methylamino)propan-2-yl)-tetrahydro-*2H*-pyran-2-yloxy)-2-hydroxycyclohexyloxy)-5-methyl-4-(methylamino)-tetrahydro-*2H*-pyran-3,5-diol (Figure 1). Gentamicin has a proven record of effectively treating various Gram negative bacterial infections. However, its frequent clinical use is often limited with a criticism of its adverse action on the renal system and subsequent induction of nephrotoxicity by a direct dose-dependent mechanism [4-5,22]. The selective accumulation of gentamicin in the renal proximal convoluted tubule results in the induction of nephrotoxicity [6]. Gentamicin-induced nephrotoxicity occurs by selective accumulation of the drug in proximal renal convoluted tubules that leads to loss of its brush border integrity [23]. Occurrence of cellular desquamation, glomerular atrophy, tubular necrosis, tubular fibrosis, epithelial edema of proximal tubules, glomerular hypertrophy, perivascular edema, and inflammation and glomerular congestion are structural changes found in gentamicin induced-nephrotoxicity [24-35]. Gentamicin also acts as an iron chelator and the iron-gentamicin complex is a potent catalyst of radical generation [36]. ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage (Figure 2) [37-39]. Gentamicin induced free radicals-mediated lipid peroxidation (LPO) which changes the composition of lipid membranes (Figure 2) [28]. The alteration in kidney functions induced by

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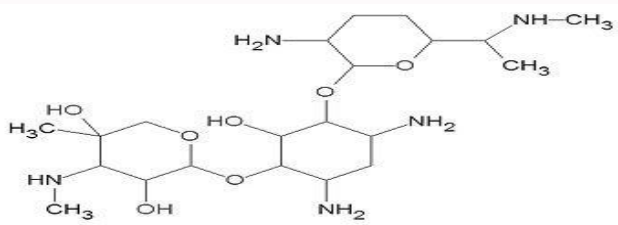

Figure 1: Chemical structure of gentamicin.

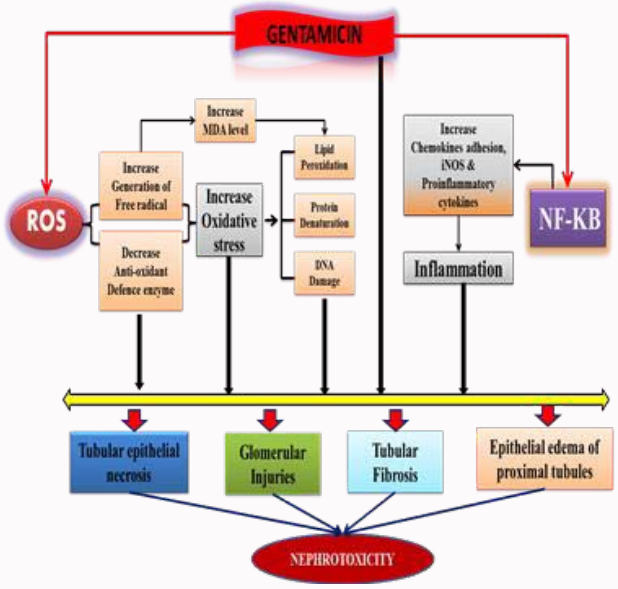

Figure 2: Various pathological mechanism pertaining gentamicin nephrotoxicity. Values were expressed as mean ± SD.*, p<0.001 versus Normal Control & Corosolic Acid 600 Perse; #, p<0.001 versus Gentamicin Control; $, p<0.001 versus Corosolic Acid 200; @, p<0.001 versus Corosolic Acid 400.

LPO is a proximal event in the injury cascade of gentamicin mediated nephrotoxicity. The elevated level of Malondialdehyde (MDA), a marker of lipid peroxidation, indicates increased free-radical generation in the gentamicin-induced nephrotoxicity (Figure 2) [40,41]. Nitric oxide (NO) may be required for maintaining normal renal function but excessive production of NO plays a major role in oxidant stress and tissue damage in the pathophysiology of acute renal failure [42,43]. GM results in an increased production of NO by inducing nitric oxide synthase, leading to the formation of toxic peroxy nitrite [44]. Studies suggest that ROS generated after gentamicin-administration brings deficiency in antioxidant defense enzymes (Figure 2) like superoxide dismutase and catalase [45-48]. Decreased manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT) antioxidant enzymes activity are involved in the sequence of events leading to GM nephrotoxicity [49-51]. Nuclear factor-kappa B (NF-κB) is a renal transcription factor, which plays a central role in inflammation by its ability to activate proinflammatory genes (Figure 2) [52]. NF-κB has been shown to be highly activated in several inflammatory diseases and trigger proinflammatory cytokines as Tumor necrosis factor-α (TNF-α), chemokines, adhesion molecules, and nitric oxide synthase enzyme (iNOS) [53]. Gentamicin-induced rats also demonstrated an increase in the levels of inducible iNOS and NF-κB (Figure 2) [54]. Increased serum creatinine and blood urea nitrogen, albuminuria and urinary loss of carnitine, decreased glomerular filtration rate, and renal dysfunction are marker which characterized gentamicin-induced nephrotoxicity [55-182]. Whether human-made or natural, the most important criteria for a medicine's use are safety, effectiveness and quality: identity, purity, potency and stability. The dissatisfaction with synthetic medicines is their inability to cure everything and pronounced side effects. Growing belief in herbal drugs due to their superiority over organic molecules, movement and their lower price prompt the researchers to explore their benefits. In the light of above we focused our review to highlight few herbal drugs with potent nephroprotective action against gentamicin.

Gentamicin induced nephrotoxicity & lagerstroemia speciosa (corosolic acid)

Lagerstroemia speciosa L. (Lythraceae) – (Banaba), commonly known as Crepe Myrtle, grows widely in tropical countries, including the Philippines, India, Malaysia, China, and Australia. *L. speciosa* is a popular folk medicine in Southeast Asia; in the Philippines, a tea from the leaves has been used for the treatment of diabetes mellitus [183,184]. In India it grows widely in Maharashtra and commonly called as Queen's flower and Pride of India. It is a deciduous tropical flowering tree, 5 m to 7 m high, sometimes growing to a height of 20 m. Leaves are large, spatulate, 2 to 4 inches in width, 5 to 8 inches in length. It sheds leaves in the first months of the year. Before shedding, the leaves are bright orange or red during which time it is thought to contain higher levels of corosolic acid, the active principle considered. Flowers are racemes, pink to lavender, flowering from March to June. After flowering, the tree bears large clumps of oval nut like fruits. The leaves contain large amounts of corosolic acid (CRA), which has previously been shown to possess anti-diabetic properties [185] and significant amounts of tannins [186]. Banaba leaves (*L. speciosa* L. Pers, Lythraceae) have been used in traditional medicine to treat diabetes mellitus in Southeast Asia for a many years.

Banaba extracts are also known to have anti-obesity [187], anti-oxidant [188] and anti-gout [189] effects. Corosolic acid, an active ingredient in these extracts, displays a potential anti-diabetic activity [190-196], as well as anti-oxidant, anti-inflammation, and anti-hypertension properties [197]. The hypoglycemic effect of *L. speciosa* has been demonstrated in animal and in vitro studies. When genetically diabetic mice (Type II) were fed a diet containing hot-water extract from *L. speciosa* for 5 weeks, their elevated blood glucose was significantly suppressed [190]. In another study, when obese diabetic rats were fed a diet containing the same extract for 12 weeks, their blood glucose levels were not suppressed, but their body weights were lowered significantly [187].

In a recent study, both hot water and methanol extracts of the leaves of this plant were shown to stimulate glucose uptake in 3T3-L1 cells in a manner similar to insulin, and to inhibit adipocyte differentiation induced by insulin and isobutyl-methyl-xanthin and dexamethasone, suggesting that plant extract may be useful for prevention and treatment of hyperglycemia and obesity in Type II diabetics [191]. In a bioassay-guided fractionation, employing glucose transport activity in Ehrlich ascites tumor cells, researchers in Japan isolated corosolic acid (2-hydroxy ursoloic acid, $C_{30}H_{48}O_4$) from the methanol extract of *L. speciosa* leaf, which showed a significant glucose transport-stimulating activity at a concentration of 1 μM [185].

Accordingly, extracts from the leaves of the plant were standardized to corosolic acid, and one such extract is Glucosol TM, which is standardized to contain 1% corosolic acid. In this paper,

Table 1: Drugs and chemicals.

Name of Drugs and Chemicals	Source
Reduced glutathione	SD fine, Mumbai, India
5,5'-dithiobis (2-nitrobenzoic acid) (DTNB)	Sigma-Aldrich, St, Louis, MO, USA.
Gentamicin	Parth Parentral Pvt. Ltd, Kalol, India
Corosolic Acid	Ex-Gratia
Thiobarbituric acid	*otto* Chemika-Biochemica, Mumbai, India
Estimation of serum creatinine by Jaffe's Method, Initial Rate	Transasia Boi-Medicals Pvt. Ltd, Baddi, Solan,(HP) India
Estimation of blood urea and Blood urea nitrogen by (GLDH Kinetic method)	Crest biosystems, Goa, India
Estimation of proteinuria by Pyrogallol red method	Crest biosystems, Goa, India

we report on the efficacy of this extract in balancing blood sugar in humans. Treatment with *L. speciosa* reduced blood glucose and insulin levels in diabetic KK-AY mice [198]. Thus, it is possible that *L. speciosa* mediates anti-cardiac hypertrophy through a mechanism that is not well defined. NF-κB is an inducible transcription factor that is activated by various carcinogens, inflammatory stimuli, and growth factors and controls the expression of genes linked with survival, proliferation, invasion, and metastasis of tumors [199]. Recent studies by several investigators have also implicated NF-κB's activation in the cardiac hypertrophic response, as modeled in cultured cardiac myocytes [200]. However, corosolic acid from Banaba leaves (*L. speciosa*) has been used as a traditional herbal medicine for kidney disorders.

Materials & Methods

Wistar albino rats of either sex weighing about 200 g to 300 g were used in the present study. The animals were acclimatized in the 'institutional animal house' and maintained on rat chow and tap water. Rats were allowed *ad libitum* access to food and water. They were exposed to normal day and night cycles. The experimental protocol employed in this study received approval from the 'Institutional Animal Ethics Committee' under the guidelines given by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)'.

Experimental protocol

There are six groups are employed in the present study each comprised 6 rats. The corosolic acid (CRA) used in the present study has an antioxidant and anti-inflammatory property.

- **Group 1** (Normal Control), rats are maintained on standard food and water and no treatment are given.
- **Group 2** (Gentamicin Control), rats are administered gentamicin (100 mg/kg/day, i.p.) for 14 days.
- **Group 3** (Corosolic acid – 600 per se), normal rats are administered Corosolic acid (600 mg/kg/day, per os) orally for 14 days.
- **Group 4** (Corosolic acid – 200+Gentamicin treated), rats administered gentamicin (100 mg/kg/day, i.p., 2 weeks) are treated with (200 mg/kg/day, per os) orally, and the treatment started 3 days before the administration of gentamicin and continued for 2 weeks from the day of administration of gentamicin.
- **Group 5** (Corosolic acid – 400+Gentamicin treated), rats administered gentamicin (100 mg/kg/day, i.p., 2 weeks) are treated with Corosolic acid (400 mg/kg/day, per os) orally, and the treatment started 3 days before the administration of gentamicin and continued for 2 weeks from the day of administration of gentamicin.
- **Group 6** (Corosolic acid – 600+Gentamicin treated), rats administered gentamicin (100 mg/kg/day, i.p., 2 weeks) are treated with Corosolic acid (600 mg/kg/day, per os) orally, and the treatment started 3 days before the administration of gentamicin and continued for 2 weeks from the day of administration of gentamicin.

Methods

Induction of experimental nephrotoxicity: Experimental nephrotoxicity is induced in rats by administration of gentamicin (GT) (100 mg/kg/day, i.p.) for 14 days.

Drugs and chemicals: DTNB purchased from Otto Kemi, Mumbai, India. The corosolic acid obtained as Ex-Gratia. Reduced glutathione purchased from SD fine, Mumbai, India. The enzymatic kits serum creatinine purchased from Trans asia Bio-Medicals Pvt. Ltd, Baddi, Dist. Solan, (HP) India,

Blood urea nitrogen obtained from Crest Biosystems, Goa, India and Proteinuria from Crest biosystems, Goa, India. Thiobarbituric acid purchased from Otto Chemika-Biochemika, Mumbai, India (Table 1). All other chemicals used in the present study are of analytical grade.

Assessment of nephrotoxicity

The development of nephrotoxicity, 2 weeks after the administration of gentamicin (GT) is assessed in rats by estimating serum creatinine, blood urea nitrogen and protein in urine using commercially available kits.

Estimation of serum creatinine: The serum creatinine concentration is estimated by Jaffe's Method, initial rate using the commercially available kit (Trans asia Bio-Medicals Pvt. Ltd, Baddi, Dist. Solan, (HP) India). Briefly, 100 µL serum sample and 100 µL standard creatinine solutions (2 mg/dL) are taken separately in glass tubes, which are named as test (T) and standard (S), respectively. The working reagent (1,000 µL) containing alkaline picrate solution is added in both tubes, mixed and the reaction temperature is kept at 30°C. The absorbance of test and standard at 20 s (T1, S1) and again at 80 s (T2, S2) is noted against blank spectrophotometrically. The formation of a colored complex as a result of a reaction between creatinine present in serum sample and alkaline picrate present in working reagent is measured at 510 nm.

Estimation of blood urea nitrogen: The blood urea nitrogen is estimated by GLDH Kinetic method using the commercially available kit (Crest Biosystems, Goa India). Briefly, 10 µL standard solutions (40 mg/dL) and 10 µL serum sample are taken separately in standard (S) and test (T) glass tubes, respectively. The working enzyme reagent

(800 µL) (containing urease and a mixture of salicylate, hypochlorite and nitroprusside) is added in all glass tubes with thorough mixing. All glass tubes are incubated at 37°C for 5 min. Then, the working starter reagent (200 µL) (containing alkaline buffer) is added to all glass tubes, and they are again incubated at 37°C for 5 min. The absorbance of test and standard at 30 s (T1, S1) and again at 60 s (T2, S2) is noted against blank spectrophotometrically. The principle involved in this estimation follows. Urease hydrolyzes urea to ammonia and carbon dioxide. The ammonia formed further combines with α ketoglutrate and NADH to form glutamate and NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance in a fixed time which is proportional to the urea concentration in the sample. The intensity of the color produced is measured spectrophotometrically at 340 nm.

Estimation of protein in urine: The microproteinuria is assessed by pyrogallol red method using the commercially available kit (Crest Biosystems, Goa, India). The reagent (containing pyrogallol dye) (1,000 µL) is added to 10 µL distilled water, 10 µL standard protein and 10 µL urine sample to prepare blank, standard (S) and test (S), respectively. All test tubes are mixed and incubated at 37°C for 5 min. The absorbance of test and standard samples are noted against blank at 600 nm spectophotometrically within 30 min. When the pyrogallol red-molybedate complex binds to basic amino groups of protein molecules, there is a shift in reagent absorbance and forms a blue colored complex. The intensity of color formed is directly proportional to protein concentration present in the sample.

Assessment of renal oxidative stress

The development of oxidative stress in the rat kidney is assessed by estimating renal thiobarbituric acid reactive substances (TBARS) [201] and reduced form of glutathione (GSH) [202,203].

Preparation of renal homogenate: The kidney is excised and is hed with ice cold isotonic saline and weighed. The kidney weight to body weight ratio is calculated. The kidney is then minced, and a homogenate (10% w/v) is prepared in chilled 1.15% KCl. The homogenate is used for the estimation of renal TBARS and GSH.

Estimation of renal thiobarbituric acid reactive substances (TBARS): The renal thiobarbituric acid reactive substances, an index of lipid peroxidation, are estimated according to the method described earlier [201]. The reaction mixture is prepared by mixing 0.2 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid (TBA). The reaction mixture is made up to 4.0 mL with distilled water, and then incubated at 95°C for 60 min. After cooling in tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1 v/v) are added to reaction mixture and shaken vigorously using vortex shaker. The test tubes are centrifuged at 4,000 rpm for 10 min (REMI Cooling Centrifuge, India). The absorbance of developed pink color is measured spectrophotometrically at 532 nm. The standard curve using 1,1,3,3-tertramethoxyopropane (1 nM to 10 nM) is plotted to calculate the concentration of TBARS, and the results are expressed as nM/g wet weight of renal tissue.

Estimation of reduced glutathione: The renal GSH level is estimated using the methods described by Boyne and Ellman [203]. The renal homogenate of the rat is mixed with 10% w/v trichloroacetic acid in 1:1 ratio and centrifuged at 4°C for 10 min at 5,000 rpm. The supernatant (0.5 mL) is mixed with 2 mL of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 mL of distilled water. Then, 0.25 mL of 0.001 M freshly prepared DTNB (5, 5'-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate) is added to the reaction mixture, and then incubated for 10 min. The absorbance of the yellow colored complex is noted spectrophotometrically at 412 nm. A standard curve is plotted using the reduced form of glutathione (0.1 µM to 1 µM), and the results are expressed as µM/g wet weight of renal tissue.

Assessment of renal hypertrophy and renal fibrosis

Estimation of kidney weight/body weight (%): Both left and right kidneys are isolated, renal fascia is removed and kidneys are weighed individually. Kidney weight/body weight (%) is calculated according to following formula [204,205]:

$$\text{Calculation} = \frac{\text{Left kidney weight (gm)} + \text{Right kidney weight (gm)}\ \text{X}\ 100}{\text{Body weight (gm)}}$$

Histopathological study

Gentamicin-induced renal structural changes in glomeruli and tubules were assessed histologically where the kidney was excised and immersed in 10% formalin solution. The kidney was then dehydrated in graded concentration of alcohol, immersed in xylene and embedded in paraffin. From the paraffin blocks, sections of 5 µm in thickness were made and stained with hematoxylin and eosin to assess the pathological changes that have occurred in glomeruli and tubules using light microscopy at 40X (Motic Digital Microscope BA310, USA).

Statistical analysis

All values are expressed as mean ± SD. The data obtained from various groups are statistically analyzed using one way ANOVA, followed by Tukey's multiple comparison tests. A 'p' value of less than 0.05 is considered statistically significant and the 'p' values are of two tailed.

Results

Administration of corosolic acid (200, 400 & 600 mg/kg/day, per os, 14 days) to normal rats did not produce statistically significant per se effect on various parameters assessed in the normal rats in present study. Corosolic acid (200, 400 & 600 mg/kg/day, per os, 14 days) treatment was started before 3 days of gentamicin administration and its treatment was continued for 14 days. All the parameters were assessed at the end of 14 days of experimental protocol.

Effect of corosolic acid on the kidney weight to body weight ratio

An increase in the kidney weight to body weight ratio was noted in gentamicin control rats as compared to normal rats. Treatment with corosolic acid (200, 400 & 600 mg/kg/day, per os, 14 days) significantly reduced gentamicin-induced increase in the kidney weight to body weight ratio (Figure 3, Table 2).

Effect of corosolic acid on serum creatinine and blood urea nitrogen

The serum creatinine level was noted to be markedly increased in gentamicin administered rats as compared to normal rats. Likewise, blood urea nitrogen level was significantly increased in gentamicin administered rats as compared to normal rats. However, treatment with corosolic acid significantly reduced gentamicin induced elevation of serum creatinine and blood urea nitrogen levels (Figure 4 and 5, Table 3 and 4).

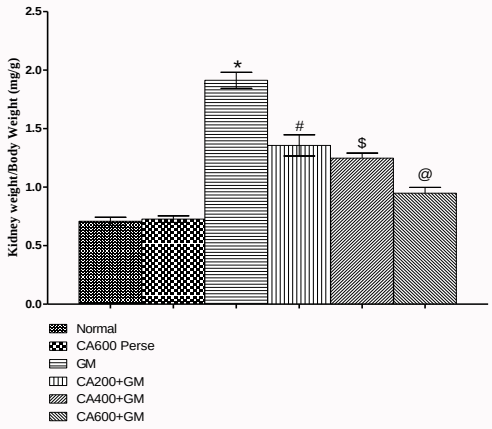

Figure 3: Effect of corosolic acid on kidney weight/body weight (mg/g). Values were expressed as mean ± SD. *, p<0.001 versus Normal Control & Corosolic Acid 600 Perse; #, p<0.001 versus Gentamicin Control; $, p<0.001 versus Corosolic Acid 200; @, p<0.001 versus Corosolic Acid 400.

Table 2: Effect of corosolic acid on kidney weight/body weight (mg/g).

Groups	Weight/body weight (mg/g)
Normal	0.7083 ± 0.03488
CA600 Perse	0.7267 ± 0.02805
GM	1.913 ± 0.06861*
CA200+GM	1.357 ± 0.09048#
CA400+GM	1.248 ± 0.04262$
CA600+GM	0.9483 ± 0.04997@

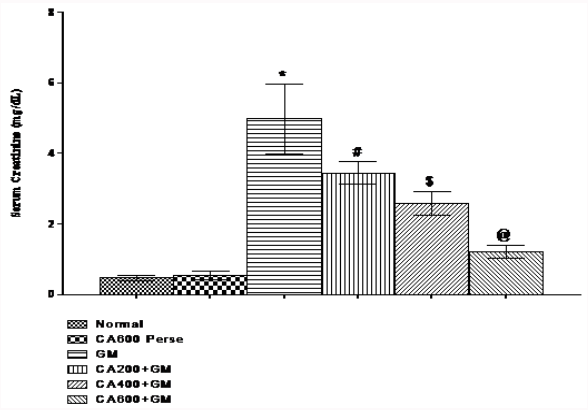

Figure 4: Effect of corosolic acid on serum creatinine concentration (mg/dL). Values were expressed as mean ± SD. *, p<0.001 versus Normal Control & Corosolic Acid 600 Perse; #, p<0.001 versus Gentamicin Control; $, p<0.001 versus Corosolic Acid 200; @, p<0.001 versus Corosolic Acid 400.

Table 3: Effect of corosolic acid on serum creatinine concentration (mg/dL).

Groups	Serum creatinine concentration (mg/dL)
Normal	0.4650 ± 0.06775
CA600 Perse	0.5483 ± 0.1194
GM	4.977 ± 0.9841*
CA200+GM	3.442 ± 0.3205#
CA400+GM	2.573 ± 0.34084$
CA600+GM	1.198 ± 0.1830@

Effect of corosolic acid on microproteinuria

An increase in the occurrence of microproteinuria was noted in gentamicin treated rats as compared to normal rats. Treatment with corosolic acid (200, 400 & 600 mg/kg/day, per os, 14 days) significantly reduced gentamicin-induced increase in the occurrence

Table 4: Effect of corosolic acid on blood urea nitrogen concentration (mg/dL).

Groups	Urea nitrogen concentration (mg/dL)
Normal	14.68 ± 0.8341
CA600 Perse	14.74 ± 1.159
GM	53.51 ± 7.941*
CA200+GM	36.33 ± 3.266#
CA400+GM	28.83 ± 1.941$
CA600+GM	21.71 ± 1.064@

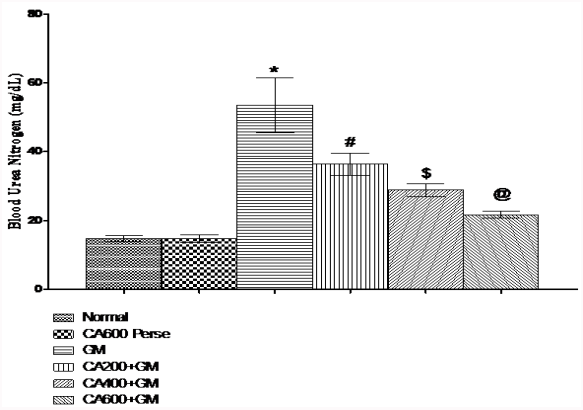

Figure 5: Effect of corosolic acid on blood urea nitrogen concentration (mg/dL). Values were expressed as mean ± SD.*, p<0.001 versus Normal Control & Corosolic Acid 600 Perse; #, p<0.001 versus Gentamicin Control; $, p<0.001 versus Corosolic Acid 200; @, p<0.001 versus Corosolic Acid 400.

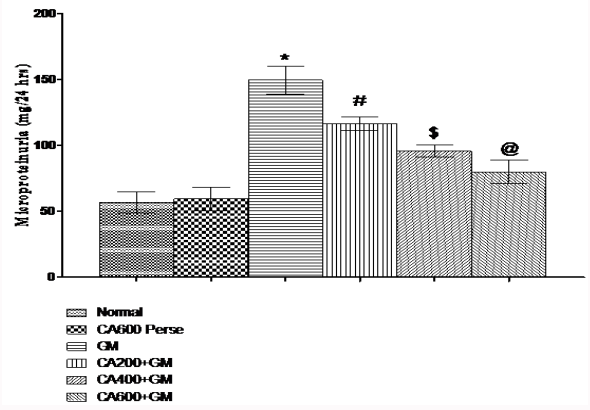

Figure 6: Effect of corosolic acid on microproteinuria concentration (mg/ 24 h). Values were expressed as mean ± SD.*, p<0.001 versus Normal Control & Corosolic Acid 600 Perse; #, p<0.001 versus Gentamicin Control; $, p<0.001 versus Corosolic Acid 200; @, p<0.001 versus Corosolic Acid 400.

Table 5: Effect of corosolic acid on microproteinuria concentration (mg/ 24 h).

Groups	Microproteinuria concentration (mg/ 24 h)
Normal	56.82 ± 7.945
CA600 Perse	59.34 ± 8.857
GM	149.4 ± 10.67*
CA200+GM	116.5 ± 5.167#
CA400+GM	95.50 ± 4.506$
CA600+GM	79.86 ± 8.950@

of microproteinuria (Figure 6, Table 5).

Effect of corosolic acid on renal TBARS

A marked increase in renal TBARS was noted in gentamicin treated rats as compared to normal rats. Treatment with corosolic acid (200, 400 & 600 mg/kg/day, per os, 14 days) prevented the gentamicin induced increase in renal TBARS (Figure 7, Table 6).

Effect of corosolic acid on renal GSH

Nephrotoxic rats, after 14 days of gentamicin administration showed a marked decrease in renal concentration of GSH as compared to normal rats. Treatment with corosolic acid (200, 400 & 600 mg/kg/day, per os, 14 days) significantly attenuated gentamicin induced decrease in renal GSH (Figure 8, Table 7).

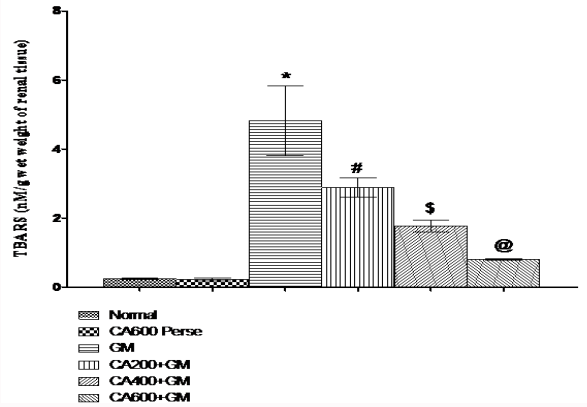

Figure 7: Effect of corosolic acid on TBARS level (nM/g wet weight of renal tissue). Values were expressed as mean ± SD.*, p<0.001 versus Normal Control & Corosolic Acid 600 Perse; #, p<0.001 versus Gentamicin Control; $, p<0.001 versus Corosolic Acid 200; @, p<0.001 versus Corosolic Acid 400.

Table 6: Effect of corosolic acid on TBARS level (nM/g wet weight of renal tissue).

Groups	TBARS level (nM/g wet weight of renal tissue)
Normal	0.2358 ± 0.02285
CA600 Perse	0.2172 ± 0.04134
GM	4.823 ± 1.008*
CA200+GM	2.883 ± 0.2787#
CA400+GM	1.772 ± 0.1778$
CA600+GM	0.8017 ± 0.02927@

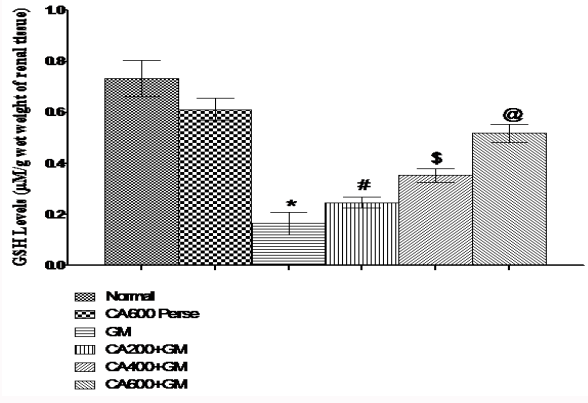

Figure 8: Effect of corosolic acid on GSH level (µM/g wet weight of renal tissue).

Table 7: Effect of corosolic acid on GSH level (µM/g wet weight of renal tissue).

Groups	GSH level (µM/g wet weight of renal tissue)
Normal	0.7337 ± 0.07081
CA600 Perse	0.6118 ± 0.04358
GM	0.1650 ± 0.04466*
CA200+GM	0.2467 ± 0.02160#
CA400+GM	0.3533 ± 0.02733$
CA600+GM	0.5185 ± 0.03631@

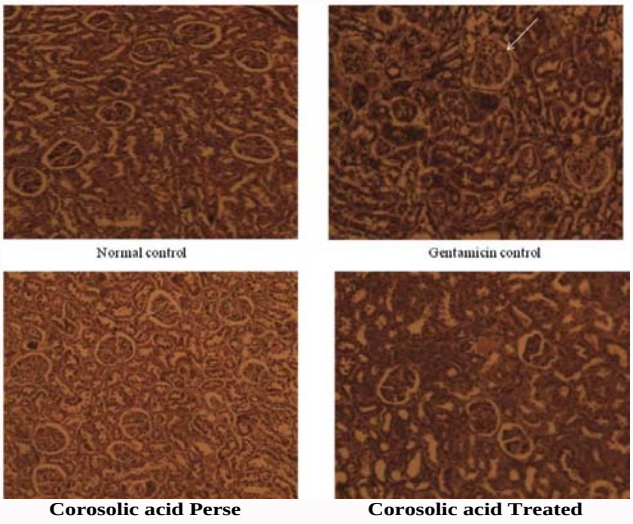

Figure 9: Effect of corosolic acid on pathological changes in the glomerulus of the kidney. The kidney of the gentamicin-administered rat developed pathological changes in the glomerulus such as degeneration in glomerular wall and mild hypertrophy. Treatment with corosolic acid markedly reduced these pathological changes of the glomerulus.

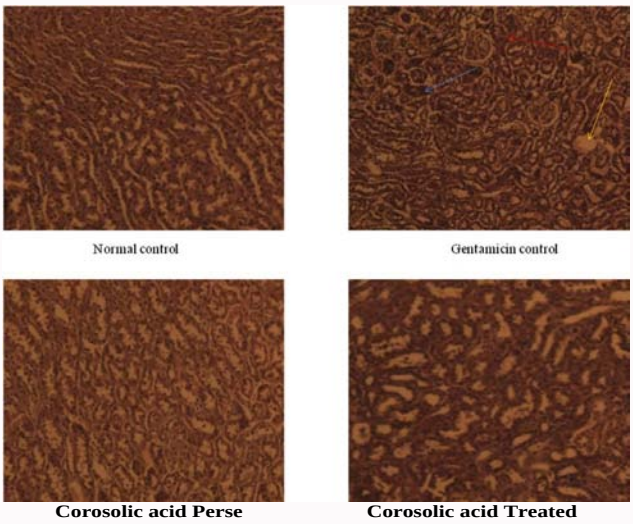

Figure 10: Effect of corosolic acid on pathological changes in tubules of the kidney. The kidney of the gentamicin-administered rat developed pathological changes in the tubules such as mononuclear cell infiltration, degeneration in epithelial layer, intertubular hemorrhage hyaline casts and protein desorption as compared to normal rats. Treatment with corosolic acid markedly reduced the pathological changes of tubules.

Effect of corosolic acid on renal histopathology

Renal structural pathological abnormalities in the glomerulus and tubules were observed in gentamicin administered rats. As

compared to normal rats, degeneration in glomerular wall and mild hypertrophy in glomerulus, while in the tubules, mononuclear cell infiltration, degeneration in epithelial layer, inter-tubular hemorrhage and hyaline casts were found in gentamicin administered rats. Studies revealed the presence of dilated capillaries indicating protein desorption material in gentamicin treated group. The administration of corosolic acid markedly reduced these renal pathological changes. Corosolic acid treated group showed normal glomeruli, and blood vessels and no cast were identified (Figure 9 and 10).

Discussion

Gentamicin, an effective aminoglycoside antibiotic against severe Gram negative bacterial infections, is known to be potentially a nephrotoxic agent. In this study, we found that the corosolic acid could serve as a preventive agent against gentamicin induced nephrotoxicity.

Renal dysfunction is often manifested with elevation in serum levels of creatinine and urea nitrogen [206,207]. Creatinine is a non-protein waste product of creatine phosphate metabolism of muscles, and it is filtered by the glomerulus of the kidney. Conversely, the level of creatinine might increase in the circulation provided weakness in renal filtration process as a result of declined renal function. The elevation of serum creatinine concentration is therefore an indication of reduced glomerular filtration rate and renal dysfunction [208]. The elevated level of blood urea nitrogen is also an index of renal dysfunction because urea formed by the liver is cleared from blood by the kidney. In addition, incidence of microproteinuria is considered an index of early stage nephropathy [209]. Taken together, elevation of serum creatinine and urea nitrogen, and occurrence of microproteinuria are an index of renal dysfunction. We observed in the present study that rat's administered gentamicin exhibited a marked elevation in serum creatinine and urea nitrogen. In addition, a marked incidence of microproteinuria was observed in gentamicin administered rats. These results suggest the induction of nephrotoxicity with renal functional abnormalities in gentamicin administered rats.

These renal functional abnormalities were noted to be accompanied with high renal oxidative stress as assessed in terms of a marked decrease in renal GSH. Moreover, a marked increase in renal lipid peroxidation as assessed in terms of significant elevation in renal TBARS was noted in gentamicin-administered rats. These results confirm that gentamicin-induced nephrotoxicity is correlated with an induction of high renal oxidative stress.

In the present study, corosolic acid treatment significantly prevented the elevated levels of serum creatinine and urea nitrogen in gentamicin administered rats. Moreover, Corosolic Acid treatment partially, but significantly reduced the occurrence of microproteinuria in gentamicin administered rats. These results have pointed out a potent nephroprotective potential of corosolic acid against gentamicin-induced nephrotoxicity. In the present study, corosolic acid treatment was noted to improve the diminished level of renal GSH and also noted to decrease the elevated renal TBARS level in gentamicin-administered rats, showing its potent anti-oxidant action. A marked direct renal anti-oxidant action of corosolic acid might have therefore chiefly contributed to its nephroprotective action against gentamicin-induced nephrotoxicity.

These results strongly support the renoprotective potential of corosolic acid that was noted in the present study in gentamicin induced nephrotoxic rats. Moreover, in the present study, treatment with corosolic acid significantly reduced gentamicin induced increase in the kidney weight to body weight ratio. Taken together, the fact that nephrotoxicity induced by gentamicin administration largely involves high renal oxidative stress, the potent anti-oxidant action of catechin hydrate might explain the possible mechanism involved in corosolic acid mediated alleviation of gentamicin induced renal structural and functional abnormalities.

Histopathological studies revealed renal structural pathological abnormalities in the glomerulus and tubules in gentamicin administered rats. As compared to normal rats, degeneration in glomerular wall and mild hypertrophy in glomerulus were noted in gentamicin administered rats. In addition, in the tubules, mononuclear cell infiltration, degeneration in epithelial layer, inter-tubular hemorrhage, hyaline casts and protein desorption were found in gentamicin administered rats. However, treatment with corosolic acid markedly reduced these pathological changes of the kidney. Moreover, in the present study, treatment with corosolic acid significantly reduced gentamicin-induced increase in the kidney weight to body weight ratio. Taken together, the fact that nephrotoxicity induced by gentamicin administration largely involves high renal oxidative stress, the potent anti-oxidant action of corosolic acid might explain the possible mechanism involved in corosolic acid mediated alleviation of gentamicin induced renal structural and functional abnormalities.

On the basis of above discussion, it may be concluded that corosolic acid has a potential to prevent gentamicin induced renal structural and functional abnormalities. The renoprotective effect of corosolic acid against gentamicin induced experimental nephrotoxicity might be mediated through its potent anti-oxidant action and direct nephroprotective actions.

Conclusion

It is concluded that gentamicin induced oxidative stress could play a key role in the induction of nephrotoxicity. Gentamicin induced nephrotoxicity is mainly associated with an increase in serum creatinine, blood urea nitrogen, microproteinuria, and renal oxidative stress (TBARS) and decreases in renal glutathione. Corosolic acid decreases serum creatinine, blood urea nitrogen, microproteinuria, and renal TBARS and increase renal glutathione. Thus, corosolic acid has potential to prevent gentamicin induced nephrotoxicity possibly by protecting the kidney from lipid peroxidation and oxidative stress.

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