

## Research Article

# Ameliorative Effect of Rutin on Gentamicin-Induced Nephrotoxicity in Murine Model

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## Abstract

Gentamicin is one of the widely used antibiotics. However, it suffers from drawback of nephrotoxicity, which is predisposed due to oxidative stress. Rutin is one of the major classes of flavonoids and phyto-antioxidant. It has demonstrated cardioprotective, hepatoprotective and neuroprotective effects. However, no studies pertaining to its nephroprotective activity have still performed. Present work was aimed to determine nephroprotective activity of Rutin. Dose of 100 mg/ kg and 200 mg/ kg was taken in present study. Gentamicin (80 mg/ kg) was used to induce nephrotoxicity. At the end of the study body weight, kidney weight, serum creatinine, serum urea, blood urea nitrogen and urine creatinine were determined along with histopathological studies. Administration of Rutin restored the levels of biomarkers. Histopathological studies also demonstrated protective effect on kidney. Thus it could be concluded that rutin demonstrates nephroprotective potential.

**Keywords:** Rutin; Nephroprotective; Histopathology; Markers

## Introduction

Since last 50 years, aminoglycosides are being effectively used as an integral part of antimicrobial chemotherapy [1] and these drugs are still useful to treat various life threatening infections [2]. Low resistance rate, dose dependent effects, along with low cost are some of its merits [3]. However, Gentamicin causes dose dependent nephrotoxicity [4,5].

Gentamicin induced nephrotoxicity is a multifaceted process that is observed by augmentation of plasma urea and creatinine levels along with necrosis of renal tubules that ultimately leads to renal failure [6,7]. One of the reasons behind this manifestation includes increased oxidative stress [9]. Thus anticipation and diminution of oxidative stress seems to be a novel strategy in preventing such situation.

Flavonoids are polyphenol obtained from plants [9]. They are found abundantly in grains, fruits, vegetables, barks, roots, stem, flower, tea and wine [10]. These compounds have benzo-pyran nucleus. Flavonoids are produced by plants as a result of response to preclusion of microbial infection [11]. However, due to high antioxidant capacity, they are believed to have health promoting properties [12,13].

Rutin (3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside; Vitamin P) is an important bioflavonoid has demonstrated a number of beneficial pharmacological effects [14]. Rutin is known for various therapeutic effects. It has effectively prevented cognitive impairments by ameliorating oxidative stress and neuro-inflammation in rat model of sporadic dementia of Alzheimer type [15] and established protective effect against cognitive deficits and brain damage in rats with chronic cerebral hypoperfusion [16]. Rutin demonstrated protective effect against reflux oesophagitis by the inhibition of gastric acid secretion, oxidative stress and inflammatory cytokine production [17].

It has reported to be neuroprotective [18], cardioprotective [19], nephroprotective, and protective effect against reproductive toxicity [20] along with anti-inflammatory [21] and antihyperglycemic [22] effects in experimental animals.

In the present context, *in vivo* protective activity of Rutin against Gentamicin induced nephrotoxicity was evaluated in albino Wistar rats.

## Experimental

### Drugs and chemicals

Rutin and Gentamicin were purchased from Central Drug House, Mumbai, India. All the other chemicals used were of analytical grade.

### Animals

Healthy adult male wistar albino rats (5-6 month; 250-300 g) were used. The animals were housed in polypropylene cages and maintained under standard conditions. They were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai India) and water *ad libitum*. All the animal experimental protocols were approved by Institutional Animal Ethics Committee.

### Selection of dose

In accordance to the dose as determined by Motamedshariaty et al., [23], rutin in the dose of 100 and 200 mg/kg orally was used in the present study.

### Experimental protocols [24]

Animals were divided into four groups containing five animals each and grouped as

- |           |   |
|-----------|---|
| Group I   | : Control                                 |
| Group II  | : Gentamicin (80mg/kg)                    |
| Group III | : Gentamicin (80mg/kg) + Rutin (100mg/kg) |

Group IV : Gentamicin (80mg/kg) + Rutin (200mg/kg)

Except group I animals, other three groups received 100 mg/kg/day gentamicin by the intraperitoneal (i.p.) route.

After daily dosing, on the day 10, individual rats were placed in separate metabolic cages for 24 h for urine collection to determine urine creatinine content.

### Body weight

Body weights of these rats were monitored on 10<sup>th</sup> day

Kidney function test

Blood urea nitrogen

Blood urea nitrogen was estimated as per manufacturer's instructions (Span Diagnostic Ltd).

### Serum creatinine

Creatinine was estimated using the method described previously by Broad and Sirota, using Jaffe's reaction. To 1.0mL of serum and kidney homogenates, 8.0 ml of double-distilled water, 0.5mL of 2/3N sulfuric acid and 0.5ml of 10% sodium tungstate were added. This mixture was centrifuged at 4000 rpm for 15 min at 4°C, 5.0ml of the clear supernatant was taken to which 1.5ml of saturated picric acid and 1.5ml of 0.75N sodium hydroxide were added. The absorbance was read at 460 nm after 15 min in a Shimadzu UV-1700spectrophotometer. Standard and blank were also processed similarly and the creatinine levels were expressed in milligram per deciliter [25,26].

Blood urea nitrogen [25] and serum creatinine [26] were estimated by method reported elsewhere.

### Urea estimation

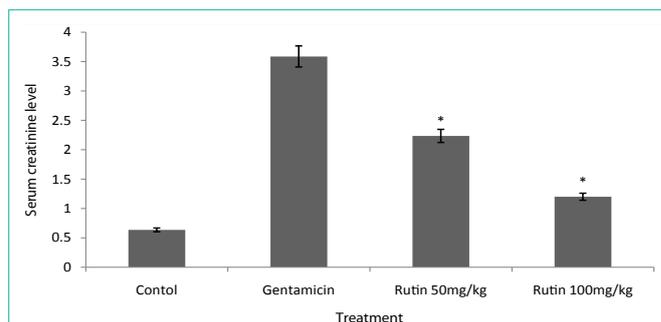
Urea was estimated by method reported by Natelson et al. [25]. Urea was estimated by the reported method. To 0.1ml of serum and kidney homogenates, 3.3ml of double-distilled water, 0.3ml of 10% sodium tungstate and 0.3mL of 0.67N sulfuric acids were added. The samples were centrifuged (4000 rpm, 15 min, 4°C) and to 1ml of the supernatant, 1ml of double-distilled water, 0.4ml of diacetyl monoxime reagent and 0.6ml of sulfuric acid- phosphoric acid mixture were added. The samples were incubated in a boiling water bath for 30 min, cooled to room temperature and the absorbance was read at 480 nm in a Shimadzu UV-1700spectrophotometer (Tokyo, Japan) [27].

### Histopathological inspection of kidney

Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination. The kidney were excised quickly and fixed in 10 % formalin and stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of nephrotoxicity if any.

### Statistical Analysis

The results were expressed as mean  $\pm$  SEM. Statistical analysis was carried out by using One way ANOVA followed by Dunnett's' test and  $p < 0.01$ ,  $p < 0.001$  was considered significant.



**Figure 1:** Effect of ethanol extract of Rutin on serum creatinine in gentamicin treated rats. Results are given as mean  $\pm$  SEM of five animals in each group. Drug treated group compared with rest the extract treated groups. Significance at \* $p < 0.05$ .

## Results

### Body weight

Administration of Gentamicin (80 mg/kg) caused decrement in weight of animals. However, administration of Rutin (200 mg/kg) prevented a significant reduction ( $P < 0.01$ ) in weight loss as evident from Figure 1. Weight gain in group II animals were observed, which was prevented up to an extent significantly ( $P < 0.01$ ) by administration of Rutin (200 mg/kg) (Figure 1).

### Kidney function test

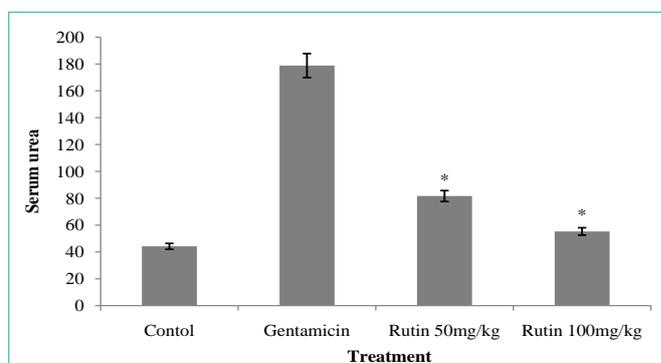
Serum creatinine levels were augmented in gentamicin treated animals; however, Rutin at dose 200 mg/kg significantly ( $P < 0.01$ ) decreased creatinine levels in animals (Figure 2). Blood urea nitrogen was increased in rats treated with only gentamicin; however, treatment with the Rutin at dose 200 mg/kg significantly ( $P < 0.01$ ) reversed the effect of gentamicin signifying nephroprotective activity. Urine creatinine levels were also significantly ( $P < 0.01$ ) reduced in Rutin-treated group (200 mg/kg) (Figure 3).

### Urea

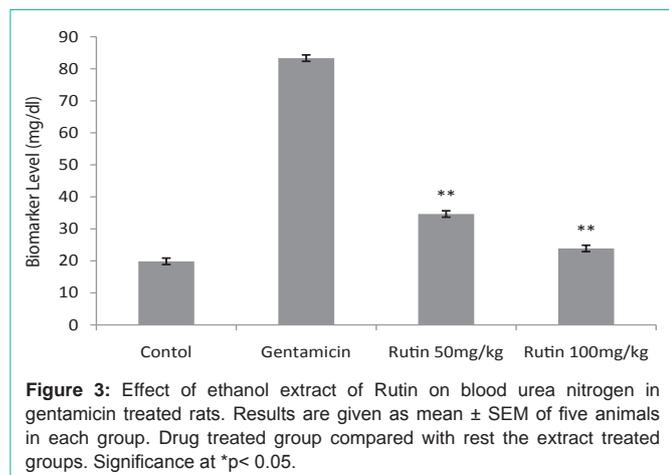
Treatment (Group III & IV) with Rutin (200 mg/kg) of demonstrated a significant ( $P < 0.001$ ) decrease ( $P < 0.001$ ) urea levels as compared to gentamicin treated (Group II) (Figure 3).

### Histopathological inspection of kidney

The normal control group of rats illustrated normal histology



**Figure 2:** Effect of ethanol extract of Rutin on serum urea in gentamicin treated rats. Results are given as mean  $\pm$  SEM of five animals in each group. Drug treated group compared with rest the extract treated groups. Significance at \* $p < 0.05$ .



**Figure 3:** Effect of ethanol extract of Rutin on blood urea nitrogen in gentamicin treated rats. Results are given as mean  $\pm$  SEM of five animals in each group. Drug treated group compared with rest the extract treated groups. Significance at \* $p < 0.05$ .

of rat kidney while Gentamicin treated group (Group II) showed peritubular, cortical glomerular, blood vessel congestion, and interstitial inflammation. Groups treated with Rutin (Group III & IV) were observed to diminish such alterations in kidney histology caused by Gentamicin (Figure 4).

## Discussion

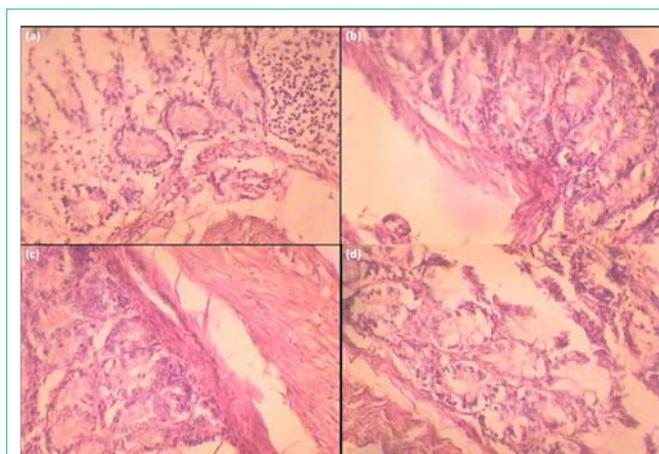
Gentamicin is one of the important antibiotics widely used to treat gram negative bacterial infections. Nephrotoxicity is a major side effect associated with it which is observed in two steps. Transportation and accumulation of Gentamicin at high concentration by 'renal proximal tubular cells' is observed in the first step of nephrotoxicity. During the second step, cellular damage due to interaction in between these polycationic drugs is observed [27]. The resulting oxidative stress seems to be the major reasons for such pathogenesis.

The present study aimed to evaluate the protective effect of Rutin against Gentamicin induced nephropathy in rats. Administration of Gentamicin to rats caused 'acute kidney dysfunction' that is evident by rise in levels of serum urea and creatinine.

Body weight of animals and weight of kidneys were determined to observe nephrotoxic effect of Gentamicin. It is generally observed that 'toxic kidneys' put on weight when damage augments [28,29] along with reduction in total body weight [30]. Such effects were also observed in the present study. Rutin aided in prevention of significant reduction in weight loss and weight gain by kidneys.

Damage to kidneys is also observed by alterations in levels of urea, uric acid and creatinine. Administration of rutin in gentamicin treated rats caused significant restoration of these markers. Histopathological assessment of kidney of normal control rat confirmed healthy anatomical features. Gentamicin treated rat's kidney illustrated presence of inflammatory depositions and cell necrosis. Kidney of rats treated with rutin showed minimal necrosis and least inflammatory accumulations with normal kidney anatomy, which revealed nephroprotective effect.

Rutin is reputed as an effective antioxidant in biological system. In the present work, administration of rutin to rats treated with gentamicin demonstrated nephroprotective activity in murine model which reconfirms its nephroprotective effect [31]. However, chronic studies are necessary to study long term effects.



**Figure 4:** Histopathological evidence of protective effect of Rutin extract on wistar rats (a) Control (b) Toxicant (c) Extract treated 50 mg/kg, po (d) Extract treated 100 mg/kg, po.

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