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Original Article

Evaluation Of Nephroprotective Potential of Aqueous Extract of *Hibiscus Rosa Sinensis* Against Gentamicin Induced Nephrotoxicity in Albino Rats

Barnwal A.C[1], Sharma M[2], Vishwakarma P[3], Gupta S[4], Saini M[5], Singh P[6]

[1,2,3,4,5,6]*Department of Pharmacology L.L.R.M Medical college Meerut*

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Corresponding Author:

D Barnwal A.C
Department of Pharmacology
L.L.R.M Medical college Meerut

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ABSTRACT

Background: Gentamicin, a widely used aminoglycoside antimicrobial, is known to cause nephrotoxicity primarily through oxidative stress and tubular damage. Medicinal plants with antioxidant properties have shown potential in mitigating drug-induced renal injury. *Hibiscus rosa-sinensis,* a traditionally used medicinal plant, possesses various pharmacological activities; however, its nephroprotective potential remains inadequately explored.

Objective: To evaluate the nephroprotective effect of aqueous extract of *Hibiscus rosa-sinensis* against gentamicin-induced nephrotoxicity in albino Wistar rats.

MATERIALS AND METHODS: Following approval from the Institutional Animal Ethics Committee of L.L.R.M. Medical College, Meerut (registered under CCSEA, India), the study was conducted in the Department of Pharmacology. During the study period of 21 days, 24 albino Wistar rats (150–200 g) were randomized into four groups of six animals each.

Group I received normal saline (1 ml/100 g, p.o.) for 21 days and served as control.
Group II received gentamicin (40 mg/kg, i.p.) for the last 5 days.
Group III received aqueous extract of *Hibiscus rosa-sinensis* (200 mg/kg, p.o.) for 21 days along with gentamicin for the last 5 days.
Group IV received aqueous extract of *Hibiscus rosa-sinensis* (400 mg/kg, p.o.) for 21 days along with gentamicin for the last 5 days.

After the experimental period, animals were sacrificed under ketamine (75 mg/kg) and xylazine (10 mg/kg) anaesthesia. Blood samples were collected from abdominal aorta for estimation of blood urea, BUN and serum creatinine. Kidneys were dissected out and processed for histopathological examination using Hematoxylin & Eosin (H&E) staining.

Data were expressed as Mean ± SEM and analyzed using ANOVA followed by post hoc test.

Results: Gentamicin administration resulted in a significant increase in blood urea, BUN, and serum creatinine levels ($p < 0.001$), indicating renal injury. Pretreatment with *Hibiscus rosa-sinensis* extract significantly attenuated these elevations in a dose-dependent manner. The higher dose (400 mg/kg) showed greater nephroprotective activity with values approaching those of the control group. Histopathological findings supported the biochemical results, demonstrating reduced tubular necrosis, inflammation, and congestion in treated groups.

Conclusion: Aqueous extract of *Hibiscus rosa-sinensis* exhibited significant nephroprotective activity against gentamicin-induced renal damage, likely due to its antioxidant and cytoprotective properties. The findings suggest its potential as a therapeutic agent in preventing drug-induced nephrotoxicity.

Keywords: *Nephroprotective Potential, Aqueous, Hibiscus Rosa Sinensis.*

INTRODUCTION

The human body functions as an integrated system in which multiple organs coordinate to maintain physiological homeostasis. Among these, the kidneys play a vital role in regulating fluid and electrolyte balance, maintaining blood pressure and eliminating metabolic waste products and toxins. Any impairment in renal function can disrupt systemic homeostasis and lead to serious complications. [1,2]

The kidneys depend on adequate blood flow for normal functioning and any reduction in renal perfusion may result in acute kidney injury (AKI) [3]. AKI is characterized by a rapid decline in renal function and may arise from intrinsic diseases or exposure to nephrotoxic agents such as drugs and chemicals. Although often reversible, repeated or prolonged injury can progress to chronic kidney disease (CKD).[4]

CKD is a progressive and irreversible condition associated with a gradual loss of kidney function over time. It significantly impairs the excretory and endocrine functions of the kidneys and may ultimately lead to end-stage kidney disease (ESKD), where renal replacement therapy becomes necessary.[5,6]

AKI affects more than 13.3 million people annually worldwide and is associated with high morbidity and mortality, particularly in low- and middle-income countries.[7] Furthermore, CKD affects nearly 697.5 million individuals globally, with a prevalence of 9.1%, and its incidence continues to rise, particularly in countries such as India and China.[8]

Drug-induced nephrotoxicity is one of the most common causes of renal injury. Gentamicin, an aminoglycoside antimicrobial widely used against gram-negative infections, is well known for its nephrotoxic potential. It accumulates in proximal tubular cells and induces renal damage through oxidative stress, inflammation, and apoptosis.[9]

Medicinal plants have gained increasing attention as potential nephroprotective agents due to their antioxidant and anti-inflammatory properties. Several plants, including *Aegle marmelos, Boerhavia diffusa,* and *Phyllanthus emblica,* have demonstrated protective effects against drug-induced renal injury.[10,11] The World Health Organization also recognizes herbal medicines as important components of primary healthcare. [4]

Hibiscus rosa-sinensis is a widely used medicinal plant in traditional system of medicine and has been reported to possess various pharmacological activities. However, its nephroprotective potential remains inadequately explored.[12]

Therefore, the present study was undertaken to evaluate the nephroprotective effect of aqueous extract of *Hibiscus rosa-sinensis* against gentamicin-induced nephrotoxicity in albino Wistar rats.

MATERIALS AND METHODS

STUDY DESIGN

A prospective, randomized experimental study was conducted in the Department of Pharmacology, L.L.R.M. Medical College, Meerut, India, after approval from the Institutional Animal Ethics Committee (IAEC). **(Registration No 819/GO/ReRcBiBt/S/04/CPCSEA).**

Experimental Animals

Adult albino Wistar rats (150–200 g) of either sex were used. Animals were maintained under standard laboratory conditions with free access to food and water.

Method of preparation of extract:

Aqueous extract of *Hibiscus rosa sinensis*: Dried flowers of *Hibiscus rosa-sinensis* were procured in powdered form and extracted with boiling distilled water (10% w/v) for 20 minutes. The extract was cooled, centrifuged, and filtered through a 0.2 µm membrane filter. The filtrate was evaporated to dryness at room temperature and stored in an airtight container. For experimental use, the dried extract was reconstituted in distilled water to obtain a concentration of 50 mg/ml.[13]

STUDY OUTLINE:

Evaluation of Nephroprotective activity:

The study was conducted on albino rats, of either sex, weighing 150-250 gm. During the study period of 21 days, the animals were randomly divided into six groups of six animals each. The groups were as described below:

Group 1 – This group was the control group and animals were administered normal saline (1 ml/100 g) orally once a day for test duration of 21days.

Group 2 – This group was given Gentamicin (40mg/kg) i.p once a day for last 5days.

Group 3 and 4- These groups were given aqueous extract of *Hibiscus rosa sinensis* orally in 2 graded doses respectively (200mg/kg and 400mg/kg) for 21 days and injection gentamicin (40mg/kg) i.p once a day for last 5 days.

After 24 hours of giving last dose of gentamicin, all the animals were sacrificed under ketamine (75mg/kg i.p.) and xylazine (10mg/kg i.p) anesthesia. Blood samples were collected from abdominal aorta (5ml) for kidney function test (Serum urea, Blood urea nitrogen, Serum creatinine) and kidneys were dissected out for histopathological examination.

STATISTICAL ANALYSIS

Mean ± SE was calculated for each group to observe the general trend. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc test. A value of $p < 0.05$ was considered statistically significant.

OBSERVATIONS AND RESULTS

The present study revealed significantly increased levels of renal biomarkers namely blood urea, blood urea nitrogen (BUN) and serum creatinine following gentamicin administration (40 mg/kg i.p), indicating renal dysfunction.

Effect on Blood Urea Levels

Blood urea levels increased significantly from 55.67 ± 2.3 mg/dl in the normal saline-treated group to 124.67 ± 2.9 mg/dl following gentamicin administration ($p < 0.001$). Pretreatment with aqueous extract of *Hibiscus rosa-sinensis* limited this increase to 69.33 ± 1.5 mg/dl and 57.67 ± 1.5 mg/dl at doses of 200 and 400 mg/kg/day respectively ($p < 0.001$ vs gentamicin-treated group).

Effect on BUN Level

BUN levels increased from 43.33 ± 2.3 mg/dl in the normal saline-treated group to 58.83 ± 1.8 mg/dl following gentamicin administration ($p < 0.001$). Pretreatment with aqueous extract of *Hibiscus rosa-sinensis* limited the increase to 50.77 ± 2.1 mg/dl and 39.00 ± 1.7 mg/dl at 200 and 400 mg/kg/day, respectively.

Effect on Serum Creatinine Level

Serum creatinine levels increased significantly from 0.63 ± 0.08 mg/dl in the normal saline-treated group to 3.70 ± 0.20 mg/dl after gentamicin administration ($p < 0.001$), while pretreatment with aqueous extract of *Hibiscus rosa-sinensis* limited the increase to 1.29 ± 0.06 mg/dl and 0.84 ± 0.08 mg/dl at 200 and 400 mg/kg/day respectively.

Histopathological examination further supported the biochemical findings, showing reduced tubular necrosis, inflammation, and vascular congestion in treated groups compared to the gentamicin control group. The higher dose (400 mg/kg) demonstrated greater nephroprotective efficacy, suggesting a dose-dependent response

Table 1: Effect of aqueous extract of *Hibiscus rosa-sinensis* in different doses on gentamicin-induced changes in blood urea, blood urea nitrogen (BUN), and serum creatinine levels in albino Wistar rats (n = 6)

TABLE 1: EFFECT ON RENAL PARAMETERS

Group	Treatment	*Blood urea (mg/dl)* (mean ±SE)	*Serum creatinine (mg/dl)* (mean ± SE)	*BUN (mg/dl)* (mean ± SE)
1	Normal saline (1 ml/kg p.o.)	55.67±2.3	0.63±0.08	43.33±2.3
2	Gentamicin (40 mg/kg i.p.)	124.67±2.9$^{\alpha}$	3.70±0.20$^{\alpha}$	58.83±1.8$^{\alpha}$
3	*Hibiscus rosa-sinensis* (200 mg/kg p.o.)	69.33±1.5$^{\beta}$	1.29 ± 0.06$^{\gamma}$	50.77±2.1$^{\beta}$
4	*Hibiscus rosa- sinensis* (400 mg/kg p.o.)	57.67±1.5$^{\beta}$	1.41 ±0.03$^{\beta}$	39.00±1.7$^{\beta}$

α $p < 0.001$ as compared to normal saline group
β $p < 0.001$ as compared to gentamicin treated group

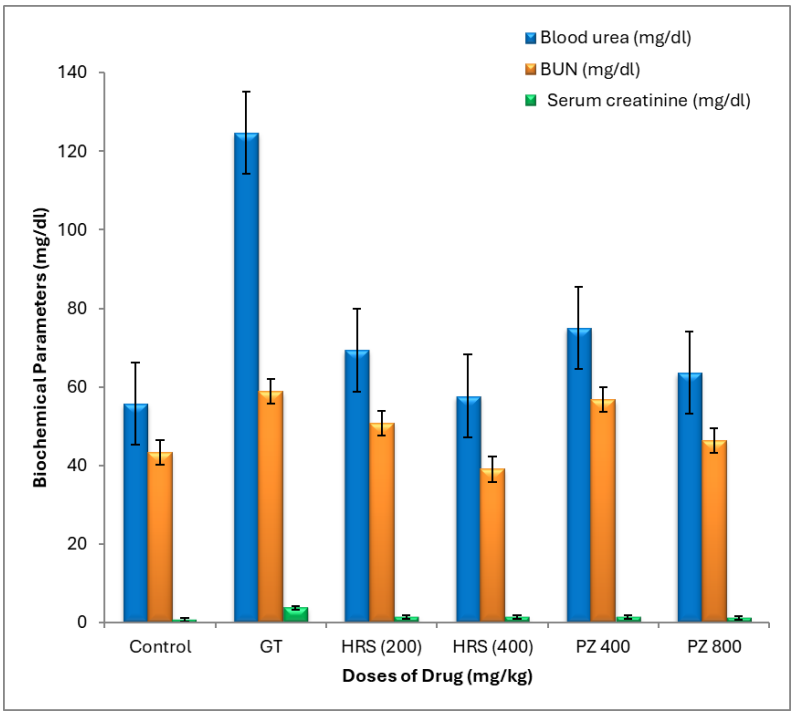

FIGURE 1: BAR GRAPH

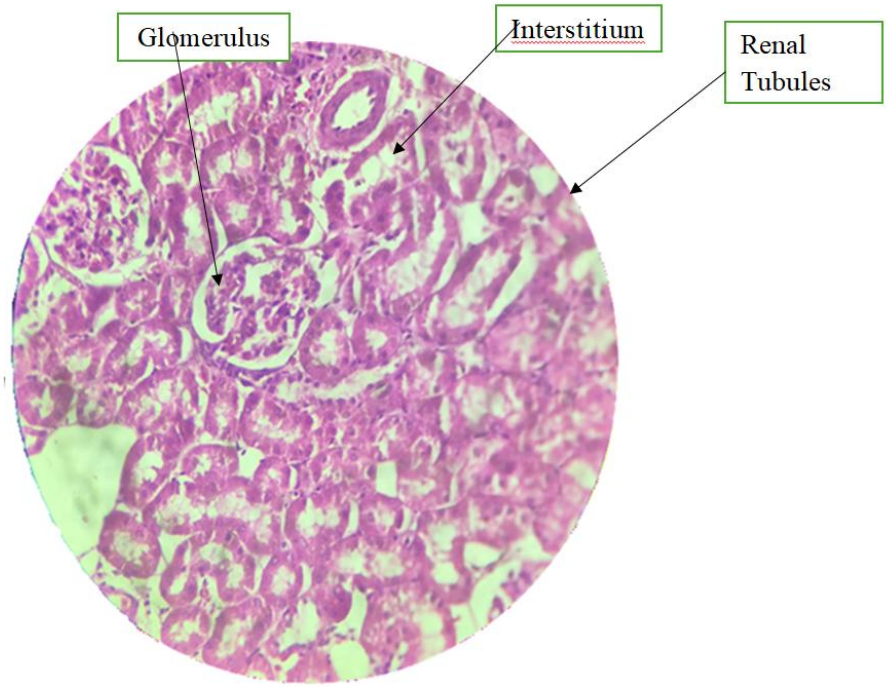

Figure 2: Microscopic features of kidney stained with Hematoxylin & Eosin (40X) in normal saline treated groups

Normal histological architecture of glomerulus, renal tubules and interstitium are marked with arrow heads.

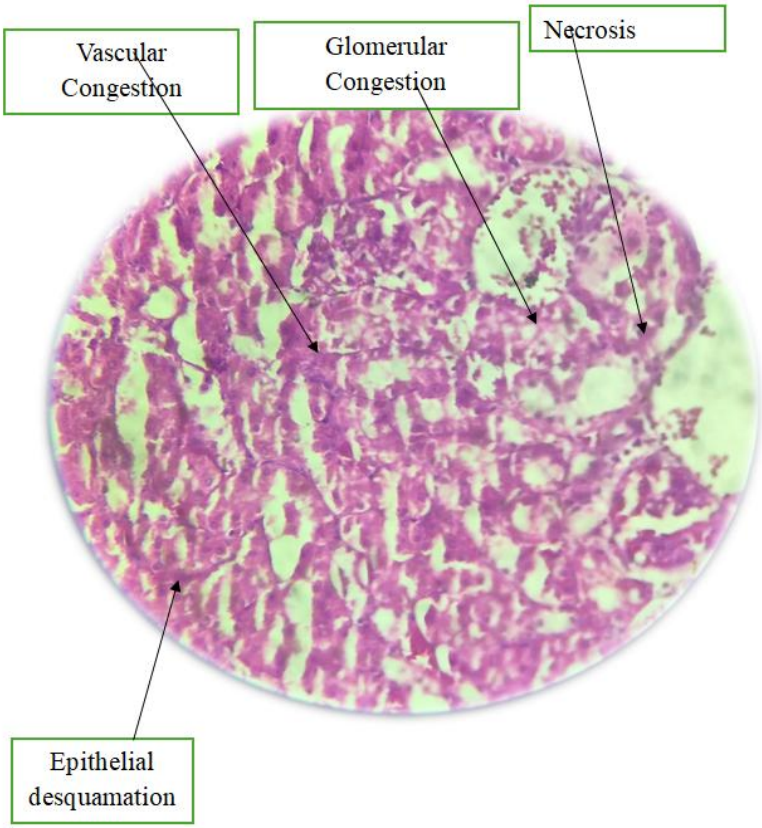

Figure 3: Microscopic features of kidney stained with Hematoxylin & Eosin (40X) in Gentamicin (40mg/kg i.p for last 5 days) treated group.

Vascular congestion, glomerular congestion, necrosis and epithelial desquamation are marked with arrow head.

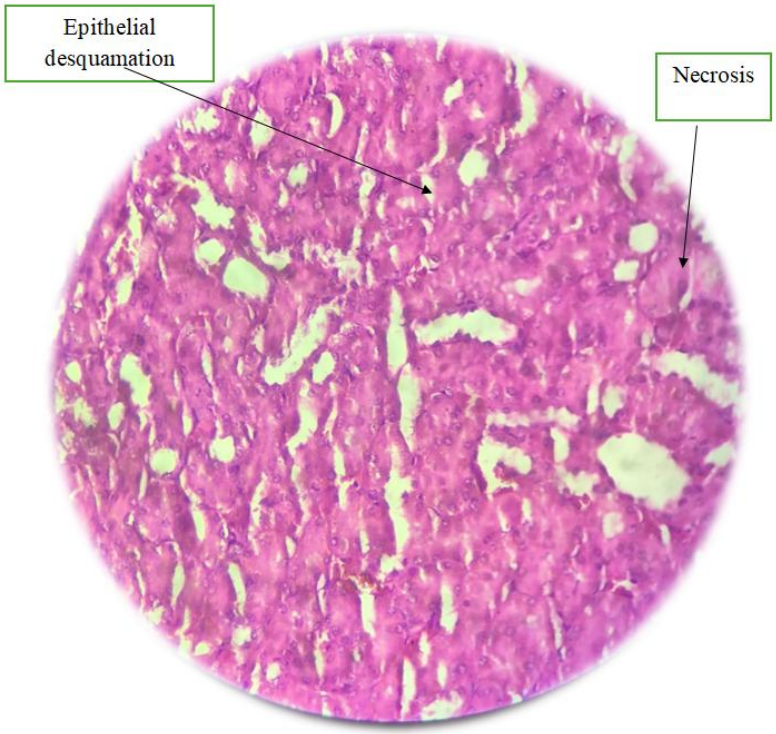

Figure 4: Microscopic features of kidney stained with Hematoxylin & Eosin (40X) (40X) in *Hibiscus rosa sinensis* (200mg/kg/day p.o for 21days with Gentamicin 40mg/kg/day i.p. for last 5 days) treated group

Necrosis and epithelial desquamation are marked with arrow head

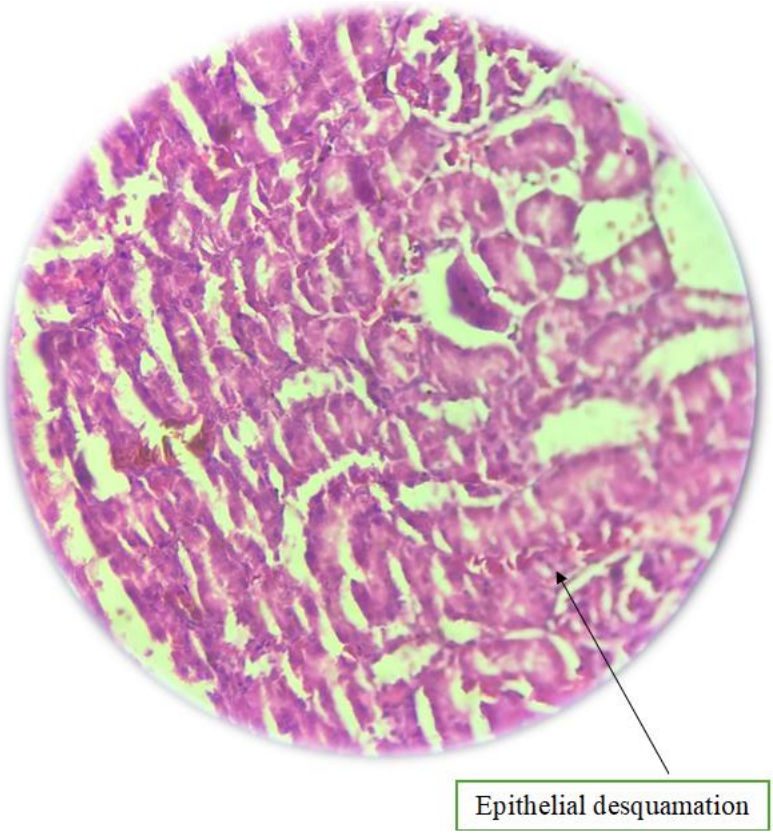

Figure 5: Microscopic features of kidney stained with Hematoxylin & Eosin (40X) in *Hibiscus rosa sinensis* (400mg/kg/day p.o for 21days with Gentamicin 40mg/kg/day i.p. for last 5 days) treated group

Epithelial desquamation is marked with arrow head

DISCUSSION

The present study demonstrated that gentamicin administration resulted in significant renal injury, as evidenced by elevated levels of blood urea, blood urea nitrogen (BUN), and serum creatinine. These findings are consistent with previous reports indicating that aminoglycosides induce nephrotoxicity through accumulation in proximal tubular cells. [10]

Gentamicin-induced nephrotoxicity is primarily mediated through oxidative stress, leading to the generation of reactive oxygen species (ROS), lipid peroxidation, mitochondrial dysfunction, and apoptosis of renal tubular epithelial cells.[9] These mechanisms result in impaired renal function and structural damage.

In the present study, pretreatment with aqueous extract of *Hibiscus rosa-sinensis* significantly attenuated the biochemical and histopathological alterations induced by gentamicin. The attenuation of elevated blood urea, BUN, and serum creatinine levels and preservation of renal architecture following treatment suggests a protective effect on renal function. The observed nephroprotective effect may be attributed to the presence of bioactive phytoconstituents such as flavonoids, anthocyanins, and phenolic compounds, which possess strong antioxidant properties. These compounds are known to neutralize free radicals and reduce oxidative stress, thereby protecting renal tissues from damage. [12]

Apart from its nephroprotective potential, *Hibiscus rosa-sinensis* has also demonstrated significant cardioprotective [14] and hepatoprotective [15]activities. Studies have shown that its aqueous extract protects against doxorubicin-induced cardiotoxicity and chemically induced hepatotoxicity by reducing tissue damage, improving biochemical parameters, and enhancing antioxidant defense mechanisms. These protective effects are primarily attributed to its potent antioxidant and free radical scavenging properties.

CONCLUSION

The present study demonstrates that aqueous extract of *Hibiscus rosa-sinensis* possesses significant nephroprotective activity against gentamicin-induced renal toxicity in albino Wistar rats.

Pretreatment with the extract effectively reduced elevated levels of blood urea, blood urea nitrogen, and serum creatinine and improved histopathological changes in renal tissues. The nephroprotective effect is likely mediated through antioxidant and cytoprotective mechanisms.

The findings suggest that *Hibiscus rosa-sinensis* may serve as a potential therapeutic agent for preventing drug-induced nephrotoxicity. However, further studies are required to isolate active constituents and elucidate the exact mechanisms of action.

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Conflict of interest: None declared

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