

# NEPHROPROTECTIVE EFFICACY OF MORINGA OLEIFERA AQUEOUS UNRIPE FRUIT EXTRACT AGAINST GENTAMICIN-INDUCED NEPHROTOXICITY IN WISTAR RATS

Surbhi Jangir<sup>1</sup>, Neetu Chouhan<sup>2</sup>, Prashant Kumar Dhakad<sup>3</sup>

<sup>1</sup>Associate Professor, Jaipur College of Pharmacy, ISI - 15 RIICO Institutional Area, Tonk Rd, Sitapura, Jaipur, Rajasthan 302022, India.

<sup>2</sup>Research Scholar, Jaipur College of Pharmacy, ISI - 15 RIICO Institutional Area, Tonk Rd, Sitapura, Jaipur, Rajasthan 302022, India.

<sup>3</sup>Professor, Jaipur College of Pharmacy, ISI - 15 RIICO Institutional Area, Tonk Rd, Sitapura, Jaipur, Rajasthan 302022, India.

DOI: <https://www.doi.org/10.58257/IJPREMS31200>

## ABSTRACT

There has been a renaissance of interest in the study of medicinal plants in recent years due to their widespread application in the treatment of many human ailments. Traditional medicine employs a broad variety of herbs for their alleged ability to cure urinary issues. Many people with nephrolithiasis in India consume a decoction made from the seeds of the plant *Moringa oleifera*, which contains an aqueous extract of its unripe fruit. There is currently no evidence from scientific studies showing that this plant helps people with renal issues. At doses of 250 mg/kg and 500 mg/kg body weight i.p., the aqueous unripe fruit extract of *Moringa oleifera* was shown to be efficacious in both male and female rats as nephroprotection, without causing any harm. The diuretic effects of *Moringa oleifera* have been attributed to the plant's flavanoids, saponins, organic acids, and steroidal substances. Loss of fluid might account for the rats' dramatic weight loss; in the treated groups, urine volume rose. Rats given only gentamicin had significantly higher levels of urine creatinine, serum creatinine, blood urea, blood urea nitrogen, and kidney weights compared to control rats ( $p < 0.01$ ), while rats given an aqueous extract of *Moringa oleifera*'s unripe fruit were protected from gentamicin's nephrotoxicity.

**Keywords:** *Moringa oleifera*, Gentamicin, Herbal plants, Kidney, Nephroprotective

## 1. INTRODUCTION

Among the most enduring kidney issues is nephrotoxicity, which carries a lifetime risk of 8-15% in Europe, 2-5% in Asia, and 20% in the Middle East. Nephrotoxicity causes a rise in blood pressure and fluid retention due to a decrease in glomerular filtration rate and an increase in serum Creatinine and blood urea nitrogen. The kidney is one of the most common sites of drug toxicity. The kidneys are the key excretion bodies of the body, therefore they are inherently exposed to circulatory medications and chemicals. Overall, nephrotoxic medications lead to both higher morbidity and death as well as acute renal failure [1]. The epithelial cells of the renal proximal convoluted tubules (PCT) are an important target for nephrotoxicants due to their roles in glomerular concentrations, drug transport, and metabolism. The renal tubular epithelial cells are often harmed by nephrotoxic substances because of their reactions with membrane components and cellular macromolecules, both directly and indirectly. Direct renal damage was caused by polyene antibiotics (such as amphotericin), heavy metals (such as Pb and Hg), and organic cations (such as cationic amino acids and spermine), while cysteine conjugates, cisplatin, and acetaminophen formed metabolites such as oxalates and fluoride in hepatic metabolism [2]. The fast-growing, drought-resistant tree *Moringa oleifera* is a native to the Indian subcontinent and a member of the family Moringaceae. *Moringa* is also known as the drumstick tree because of its tall, thin, triangular seed-pods. The horseradish tree gets its name from the taste of its roots, which are similar to horseradish [3]. The maximum height for an *M. oleifera* tree is 12 metres (33-39 feet), and its trunk can get as wide as 45 centimetres (18 inches). The bark is a pale grey and is encased in a thick cork layer. The bark of young stems is either purple or greenish white and hairy. The tree's leaves grow in a fluffy foliage of tripinnate leaves, and its crown is open and made up of drooping, brittle branches. Five asymmetrical, lightly veined, yellowish-white petals enclose fragrant, hermaphroditic blooms. The blooms are roughly 2 cm in diameter and 1 cm in length. The flower clusters, which can be as wide as 25 centimetres (10 inches) or as long as 10 centimetres (4 inches), grow on thin, hairy stalks. Within the first six months after planting, you'll see flowers. Only in the late Spring and early Summer (April-June in the northern hemisphere, October-December in the southern hemisphere) can blossoming occur. With more stable weather patterns, plants can bloom twice or even continuously throughout the year [4]. The fruit is a brown, three-sided capsule that hangs from 20 to 45 centimetres long and contains dark brown, globose seeds

that are about 1 centimetre in diameter. The seeds are carried by the wind and water on three papery wings of a pale colour [5].

## 2. METHODOLOGY

**Collection and Authentication of Plant-** Unripe fruits of Plant was collected from Nehru Garden, Jaipur. The plant was authenticated as *Moringa oleifera* by Botanical Survey of India Jodhpur, Rajasthan.

**Extraction of the processed plant material-** *Moringa oleifera* fruits powders were extracted by aqueous extraction method in soxhlet apparatus at 35-40°C temperature, 500g of the powder were mixed with distilled water. The mixture were filtered in Buchner funnel and dried over water bath till dryness and percentage yield were determined. The percentage yield obtained was 36.4%.

**Preliminary Phytochemical screening-** Preliminary tests were carried out for the presence or absence of phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Glycosides, Reducing sugars, Saponins, sterols, Anthocyanins, Terpenes and Tannins. A description of methods adopted for performing the tests are summarized below.

### IN-VIVO PHARMACOLOGICAL INVESTIGATIONS

**Induction of Nephrotoxicity by Gentamicin-** The Nephrotoxicity in this model were induced by gentamicin 40 mg/kg body weight of animal by intra peritoneal route of administration. The study period is of 14 days [6].

**Table-1:** Experimental animals grouping and treatment protocol for a period of 15th day.

Five groups of six rats each were used for the study.

Group	Treatment
1.	Normal basal diet + 1 mL/kg of distilled water
2.	40 mg/kg IP gentamicin + 1 mL/kg of distilled water
3.	40 mg/kg IP gentamicin + silymarin 200 mg/kg
4.	40 mg/kg IP gentamicin + 250- mg/kg of MOE
5.	40 mg/kg IP gentamicin + 500- mg/kg of MOE

The 4<sup>th</sup> and 5<sup>th</sup> groups are studied for preventive regimen whereas 3<sup>rd</sup> group is studied for standard regimen. Blood were withdrawn from the rat by retro orbital puncture method and animals were sacrificed for isolation of organs on 14<sup>th</sup> day.

**Statistical Analysis-** The obtained results were expressed as Mean ± SEM. Comparison between control and treatment groups were performed by one way analysis of variance (ANOVA) followed by Dunnet's test. The statistical significance criterion were p < 0.05 (95% level). P < 0.05 is considered as significant.

## 3. RESULTS AND DISCUSSION

**Preliminary Phytochemical Screening-** Alkaloids, carbohydrates, flavonoids, glycosides, saponins, sterols, anthocyanins, terpenes, and tannins were all tested for in preliminary phytochemical analysis.

**Table 2:** Results of phytochemical tests of aqueous unripe fruit extract of *Moringa oleifera* (AUFEMO)

Phytochemical constituents	Presence in aqueous unripe fruit extract of <i>Moringa oleifera</i>
Alkaloids	+
Anthocyanins	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Reducing sugars	+
Saponin	+
Steroids	+
Terpinoids	+
Tannins	+
Proteins	+

+ = presence, - = absence

### Gentamicin Induced Model

Serum concentrations of urea, creatinine, uric acid, and total protein, as well as urinary concentrations of urea, uric acid, and creatinine, were all significantly higher in the gentamicin-treated group (2) of animals compared to the control group (1), indicating severe nephrotoxicity. Serum urea, creatinine, uric acid, total protein, and urea, uric acid, and creatinine concentrations were all significantly lower in the AUFEMO aqueous extract-treated groups (groups 3 and 4) compared to the gentamicin-treated group (2) (p0.001). SOD and glutathione peroxidase activity were significantly lower in gentamicin-treated mice (group 2) compared to untreated animals (group 1). Compared to rats given gentamicin, those given AUFEMO (groups 3 and 4) substantially maintained higher levels of SOD and GPx activity due to the aqueous extract of MU. Despite this, lipid peroxidase activity was significantly elevated in gentamicin-treated rats. Lipid peroxidase levels were maintained in groups 3 and 4 after treatment with MU aqueous extract. As a result, its antioxidant properties greatly suppress lipid peroxidation in vitro. Groups 3 and 4 treated with an aqueous extract of MU were significantly protected against gentamicin-induced toxicity.

When compared to the normal control group, the control groups' urine PH is significantly higher and their urine volume is significantly lower. However, the urine volume increases and the urine pH decreases significantly (p0.05 for ethanol extract) in the extract-treated groups.

**Table 1:** Effect of 40 mg/kg/day subcutaneous gentamicin and AUFEMO on serum creatinine, urea, uric acid and Total protein treated in rats for 14 days Gentamicin induced nephrotoxicity induced Model

Group	Treatment	Creatinine	Urea	Uric acid	Total protein
Control	Tap water	0.62±0.253	22.23±1.02	1.82±0.22	9.30±0.24
Gentamicin Induced	100 mg/kg	0.98±0.229***	45.38±0.46***	3.29±0.06***	4.22±0.44***
Preventive test-1	250mg/kg	0.82±0.435***	35.24±0.24***	2.67±0.22***	7.22±0.45***
Preventive test-2	500mg/kg	0.68±0.023***	26.35±0.67***	1.96±0.05	8.27±0.40**
Standard (Silymarin)	200 mg/kg	0.64±0.04***	23.4±1.23***	1.88±0.42***	8.89±0.20***

Above table showed the Effects of Treatments on Urinary protein in different animals groups. Induced group-2 showed significantly increase (p<0.01) in urinary protein level when compared to normal group-1. Treated group-3 and group-4 reversed the gentamicin induced increased urinary protein level significantly as compared to induced group-2 (p< 0.01).

**Table 2:** Effect of 40 mg/kg/day subcutaneous gentamicin and AUFEMO on Body weight, Urine volume, UrinePh and Kidney weight treated in rats for 24 days--Gentamicin Model

Group	Drug Treatment	Body weight	Urine volume	Urine pH	Kidney weight
Normal	Distilled water 1ml/kg	250.45±0.26	28.42±0.25	5.82±0.35	1.02±0.22
Induced Gentamicin	40mg/kg	180.26±1.03***	16.19±0.12*	8.50±0.34***	1.85±0.25**
Preventive test-1	250mg/kg	210.23±1.04***	22.42±0.24*	7.24±0.35*	1.52±0.29**
Preventive test-2	500mg/kg	228.23±0.96**	24.22±0.27	6.73±0.32**	1.42±0.24***
Standard	40mg/kg	240.23±1.01***	26.21±0.40*	6.30±0.27***	1.35±0.05***

Considerably decrease in activity of SOD and glutathione peroxidase in gentamicin treated animals (2nd) when compared to normal animals (group 1). Treating (group 3 & 4) with AUFEMO significantly prevented decrease in the level of SOD, GST activity compared to gentamicin treated rats (2nd).

## 4. CONCLUSION

This work used a rat model of Gentamicin-induced nephrotoxicity to show that aqueous unripe fruit extract of Moringa oleifera (AUFEMO) had dose-dependent nephroprotective effect. Kidney histology and biochemical tests on the serum and urine showed that kidney impairment might be avoided with pretreatment using an aqueous extract of Moringa oleifera's unripe fruit (AUFEMO). It has diuretic and antioxidant properties that shield the kidneys from damage caused by free radicals. The research supports its potential as a treatment for urinary tract disorders. This proves the herb has use in traditional medicine for treating nephrotoxicity. Histopathological tests added further support to the findings, showing that AUFEMO (aqueous unripe fruit extract of Moringa oleifera) protected kidney

cells against oxidative stress and damage. In conclusion, gentamicin-induced nephrotoxicity may be mitigated by aqueous extract of unripe *Moringa oleifera* fruit (AUFEMO).

Safer alternatives to synthetic medications include those derived from natural ingredients. Kidney disease is widespread worldwide, regardless of economic status. The Indian traditional medical system makes extensive use of medicinal plants for the treatment of kidney disorders. Medicinal plants like *Aerva lanata*, *Boerhavia diffusa*, *Cichorium intybus*, *Curcuma longa*, *Glyceryrhiza glabra*, *Moringa oleifera*, *Salvia officinalis*, *Solanum nigrum*, *Tinospora cordifolia*, *Tribulus terrestris*, *Withania somnifera*, and *Zingiber officinale* and their active components have been shown to have nephroprotective effects against different nephrotoxic agents. More research is needed to make sure these plants are safe and useful for people. This means that these plants might be prescribed for nephrological issues either as a standalone treatment or in addition to standard pharmaceuticals. The alternative is to conduct synergistic research to find ways to combine botanicals with conventional drugs. When it comes to nephroprotection, medicinal plants' antioxidant activity is among their most important roles. The nephroprotective effects of these medicinal herbs are mostly attributable to their active components. Therefore, the active elements of medicinal plants used in the Indian traditional medical system have the potential to enhance the prognosis for a sizable population of patients dealing with nephrological difficulties.

## 5. REFERENCES

- [1] Yadav N, Sharma S, Sharma S, et al. Critical analysis of protective role of plants against gentamicin induced nephrotoxicity. *Indian J Environ Sci.* 2017;21:1–34.
- [2] Guo H, Deng N, Dou L, et al. 3-D human renal tubular organoids generated from urine-derived stem cells for nephrotoxicity screening. *ACS Biomate Sci Eng.* 2020; 6(12):6701–6709.
- [3] Karatas Y, Secilmis MA, Karayaylali I, Doran F, Buyukafsar K, Singirik E, et al. Effect of tempol (4-hydroxy tempo) on gentamicin-induced nephrotoxicity in rats. *Fundam Clin Pharmacol.* 2004;18:79–83.
- [4] Morales AI, Buitrago JM, Santiago JM, Fernández-Tagarro M, López-Novoa JM, Pérez-Barriocanal F. Protective effect of trans-resveratrol on gentamicin-induced nephrotoxicity. *Antioxid Redox Signal.* 2002;4:893–8.
- [5] [https://en.wikipedia.org/wiki/Moringa\\_oleifera](https://en.wikipedia.org/wiki/Moringa_oleifera)
- [6] M.A laxmi devi, M.Yaso Deepika , B.Nagaraju , K.Prasad. Evaluation of Nephroprotective Activity of Ethanolic Extract of *Annona reticulata* in Gentamicin and Cisplatin Induced Nephrotoxicity in Rats. *J. Pharm. Sci. & Res.* Vol. 8(9), 2016, 995-1007