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Research Article

Evaluation of Nephroprotective Activity of the Methanolic Extract of *Phyllanthus niruri* (Family - Euphorbiaceae)

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Abstract

The present study was undertaken to evaluate the Methanolic extract of *Phyllanthus niruri* leaves for its protective effects on gentamicin induced nephrotoxicity in rats. Rats were divided into 4 groups; normal saline, gentamicin 80 mg/kg, i.p. for 8 days, MEPN, 200mg/kg and 400 mg/kg p.o 3 days and concurrently with gentamicin for 8 days. Body weight, urine volume and kidney weights were measured. Serum protein, serum creatinine, serum urea, blood urea nitrogen levels were estimated. Kidneys were collected to perform tissue GSH, lipid peroxidation estimation and histopathological examination after the treatment. Gentamicin treatment caused nephrotoxicity as evidenced by marked changes in physical parameters, urinary and blood parameters. Co-administration of Methanolic extract of *Phyllanthus niruri* leaves with gentamicin have markedly improved all the parameters. There was a significant reduction in lipid peroxidation and rise in GSH levels with Methanolic extract of *Phyllanthus niruri* leaves treatment. Histopathological reports showed reduction in the damage of kidneys when treated with the extract. These results suggest that the Methanolic extract of *Phyllanthus niruri* leaves may be useful in reducing the gentamicin induced nephrotoxicity.

1. INTRODUCTION

Kidneys play an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis etc. The main functions of kidney can be categorised as formation of urine, water and electrolyte balance and production of hormones and enzymes. Kidneys have some delicate tasks, especially when they have to deal with unwanted substances, which they have to clear from the system¹. Nephrotoxicity is renal dysfunction that arises as a direct result of exposure to external agents such as drugs and environmental chemicals. It has been known from many years that toxic metals and heavy metals have toxic effects on kidney by accumulating and producing broad spectrum of morphological and functional effects of kidney.

A number of drugs and antibiotics including penicillin's, cephalosporin's, tetracycline, sulfonamides and amino glycosides are known to be potential nephrotoxins². Certain Indian Medicinal plants have been reported to exhibit protective effect of renal tissues against injuries³. Since there are only few researches made on this field of nephroprotection, this present study of nephroprotective activity of *Phyllanthus niruri* will satisfy the research for better and cost effective nephroprotection⁴. Thus renal diseases are one of the fatal diseases in the world today. They pose a serious challenge to international public health. Unfortunately, conventional or synthetic drugs used in the treatment of kidney diseases are inadequate and sometimes can have Hence, the research for ideal drugs still continues and has been extended to herbal drugs, About 600 commercial herbal formulations with claimed nephroprotective activity are being sold all over the world.

In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal

formulations^{5,6}. *Phyllanthus niruri* L., (Syn. *P. fraternus* Webster), *Euphorbiaceae*, is a common kharif (rainy season) weed found in both cultivated fields and wastelands. Although considered a problematic weed for farmers it is a valuable medicinal for herbalists and holds a reputed position in both Ayurvedic and Unani systems of medicine. This plant is popular in folk medicine, whole plant, fresh leaves and fruits are used in the treatment of various diseases, particularly hepatitis and other viral infection⁶. *Phyllanthus niruri* has many effective traditional uses for a wide variety of diseases.

Some of the medicinal uses have been supported in experimental models, suggesting that the plant extracts possess various pharmacological properties. Due to its impressive preclinical therapeutic potential, extracts of species of the genus *Phyllanthus* have been evaluated to treat hypertension, jaundice, diabetes, hypercalciuria, and urolithiasis⁷. Other studies revealed preclinical pharmacological activity and therapeutic potential of photochemicals isolated from *Phyllanthus niruri*. The species has demonstrated an antimutagenic and anticarcinogenic action⁸, antitumor⁹, antioxidant¹⁰, hepatoprotective¹¹, and antihyperuricemic properties¹², as well as antihyperlipemic activity^{13,15}. The methanol extracts of *Phyllanthus* species from India were reported to have strong antioxidant activity¹⁴. The root of *Phyllanthus acuminatus* inhibited the growth of murine P-388 lymphocytic leukemia and B-melanoma cell lines¹⁶. The alkaloidal extract of *P. niruri* is found to exhibit sensitive inhibitory response on cytopathic effects induced by both the strains of human immunodeficiency virus on human MT-4 cells in the tested concentrations¹⁷.

The present study aimed at evaluates nephroprotective of *Phyllanthus niruri* against Gentamicin induced nephrotoxicity in rats with special reference to biochemical, antioxidants parameters and histopathological studies. Collection and authentication of *Phyllanthus niruri* having nephroprotective properties. Extraction of dried plant materials in a Soxhlet apparatus using Petroleum ether and methanol. Preliminary chemical tests of extracts to identify phytoconstituents. Effect plant extract against Gentamicin induced nephrotoxicity in rats by using following parameters

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2. MATERIALS AND METHODS

2.1 Plant material

Whole plant of *Phyllanthus niruri* was collected from Andhra Pradesh, South India in the month of February 2012. It was authenticated from botanist Dr. Dr. Vatsavaya S. Raju Senior professor, Former head and chairperson, BOS, Department of Botany Kakatiya University, Warangal, Andhra Pradesh.

2.2 Preparation of extract

The herb was shade dried and powdered. The coarse powder obtained was extracted with Petroleum ether and methanol in Soxhlet apparatus separately and filtered. The extract was concentrated under reduced temperature and pressure to get dark green colored dry residue. For preparing aqueous extract the coarsely powdered plant material was macerated for 7 days using Chloroform Water as solvent, with occasional shaking. After 7 days the solvent was decanted, filtered and concentrated under reduced temperature and pressure to get dark green colored dry residue.

2.3 Preliminary phytochemical screening

Following procedures were followed:

Test for alkaloids

Dragendorff's Test: To 1 ml of the extract, add 1 ml Dragendorff's reagent, and orange red precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Molisch Test: To 2 ml of the extract, add 1 ml of α -naphthol solution, and then concentrated sulphuric – acid through the sides of the test tube. Purple or Reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

Barfoed's Test: To 5ml of Barfoed's solution, add 1 ml of extract solution and heat to boil, formation of a red precipitate of copper oxide was formed, and that confirms the presence of carbohydrates in the test extract.

Test for Steroids and Sterols

Liebermann Burchard Test: Dissolve the extract in 2 ml of Chloroform in a dry test tube. Add Ten drops of acetic anhydride and two drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green, indicates the presence of steroids.

Test for Glycosides

Legal Test: Dissolve the extract in pyridine and add freshly prepared sodium nitroprusside solution to make it alkaline. The formation of pink to red color shows the presence of glycosides.

Test for Saponins

About 1 ml of alcoholic extract, dilute separately with 20ml of distilled water and shake in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicated the presence of saponins.

Test for Flavonoids

Shinoda Test: To 1 ml of the extract, add magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of pink or red colour shows the presence of flavonoids.

Test for Tannins and Phenolic Compounds

To 1 ml of the extract, add 5 percent neutral 5 percent ferric chloride, a dark blue colour product show the presence of tannins.

Test for Triterpenoids

Dissolve two to three granules of tin metal in 2 ml of thionyl chloride solution. Then add 1ml of the extract into the test tube. The formation of a pink colour indicates the presence of triterpenoids.

Test for Proteins and Amino Acids

Biuret Test: To 1 ml of the extract add 1ml of 40 percent sodium hydroxide solution and 2 drops of 1 percent copper sulphate solution. Presence of violet colour indicates the presence of proteins.

Test for fixed oils

Spot Test: Press a small quantity of extract between two filter papers. Oil stains on paper indicates the presence of fixed oils.

Saponification Test: To 1 ml of the extract add few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein, heat the mixture on a water bath for 1-3 hours. The formation of soap indicated the presence of fixed oils.

2.4 Experimental animals

Healthy, adult albino rats of Wistar strain 180-220g were obtained from the Animal House of Talla Padmavathi College of Pharmacy, Warangal. The animal house was well ventilated and the animals exposed to 12 hours day and night cycle with a temperature $25^{\circ}\pm 2^{\circ}\text{C}$. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and standard rat pellet obtained from Hindustan Lever Ltd. All experiments were carried out as per the guidelines of the Animal Ethical Committee.

2.5 Determination of acute toxicity (LD50)

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water ad libitum. A single dose of the test extract (2000 mg/kg, p.o.) was administered to the animals, as the test item was a source from herb. After administration of extract, food was withheld for 3–4 hrs.

2.6 Evaluation of nephroprotective activity in Gentamicin induced nephrotoxicity

The albino rats were divided in to 4 groups and each group contains 6 rats and treatment would be as follows:

Group I: Served as normal control and received equivalent volumes of 0.1 ml i.p of normal saline (0.9% w/v NaCl) for 9 days.

Group II: Served as toxicant control and received 80 mg/kg/day i.p of gentamicin for 9 days to induce nephrosis.

Group III: Served as treated group and received 80 mg/kg/day i.p of gentamicin for 9 days to induce nephrosis followed by MEPN 200mg/kg suspended in Tween 80 from 10th to 19th day of study.

Group IV: Served as treated group and received 80 mg/kg/day i.p of gentamicin for 9 days to induce nephrosis followed by MEPN 400mg/kg suspended in Tween 80 from 10th to 19th day of study. suspended in Tween 80 from 10th to 19th day of study.

Group V: Served as positive control and received 80mg/kg/day i.p of gentamicin for 9 days to induce nephrosis followed by Standard drug Cystone at 2ml/kg, p.o from 10th to 19th day of study.

After 19th day of last dose animals were sacrificed after blood collection under ether anesthesia. Kidneys will be removed and used for estimation of lipid peroxidation and histopathological studies.

2.7 Physical parameters

Body Weight: The weight of the animals before starting and at the end of the treatment was measured and percentage change in body weight was calculated of the rats.

Kidney Weight: The weight of the kidneys of the animals at the end of the treatment was measured of the rats.

Urine Volume: The urine volume of the animals was measured of the rats.

2.8 Estimation of biochemical parameters

The following parameters were estimated by using standard procedures of Excel, Beacon and Transasia diagnostics estimating kits: Urinary parameters: sodium, potassium, creatinine, glucose and Blood parameters: urea, keratinize, total protein.

3. RESULTS

Table 1: Phytoconstituents in methanolic extract of leaves of *Phyllanthus niruri*

S. No	Tests	Methanolic Extract
1	Alkaloids	+
2	Carbohydrates	+
3	Steroids and Sterols	+
4	Glycosides	+
5	Saponins	+
6	Flavanoids	+
7	Tannins and Phenolic	+
8	Triterpenoids	+
9	Proteins and Amino Acids	+
10	Fixed Oils	+

'+' represent presence and '-' represent absence of phytoconstituents

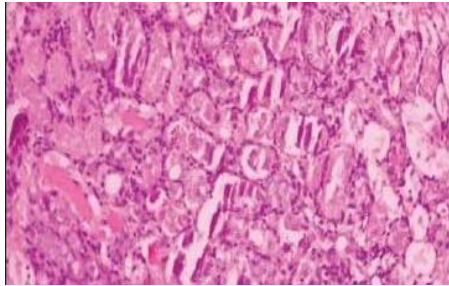


Figure 1: Kidney tissue of control animal with normal glomeruli

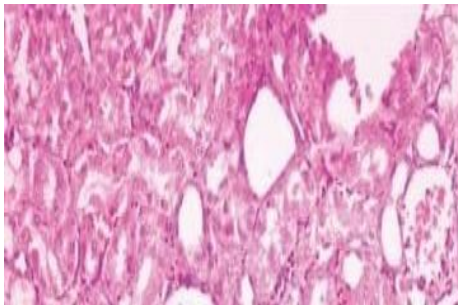


Figure 2: Kidney tissue of animal treated with gentamicin with glomerular congestion

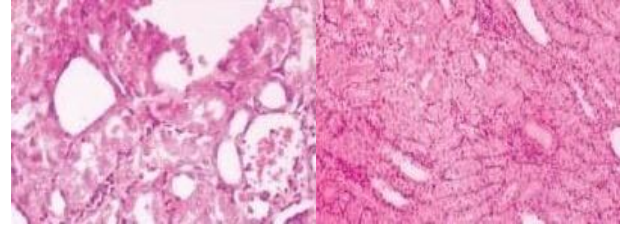


Figure 3: Kidney tissue treated with MEPN showing moderate tubular degeneration with normal glomeruli and Bowman's capsule

Effect of MEPN on change in body weight, Urine Volume and Kidney Weight

Body weight and urine volume were found to be decreased and kidney weight was found to be increased in the rats treated with only gentamicin (group-II); whereas treatment with MEPN in both doses (200 mg/kg and 400 mg/kg p.o.) was found to protect the rats from such effects. As shown in Table-2, the body weight and urine volume were significantly increased ($p < 0.001$) of the rats treated with MEPN (200 mg/kg and 400 mg/kg p.o.). Kidney weight was reduced of the rats treated with MEPN (200 mg/kg and 400 mg/kg p.o.).

Table 2: Effect of MEPN on change in body weight, urine volume and kidney weight in Gentamicin induced nephrotoxic rats

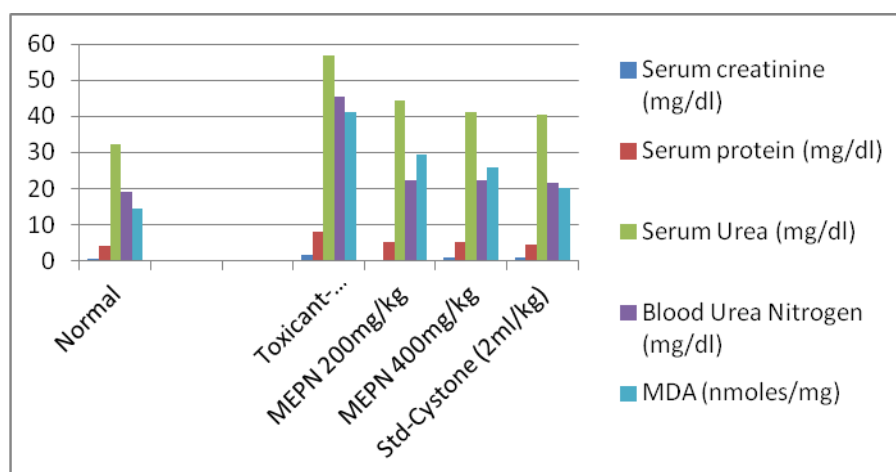
Group	Treatment	Change in body weight (g)	Urine volume (ml)	Kidney weight (g)
I	Vehicle	8.852 ± 0.3772	6.333 ± 0.3221	0.6450 ± 0.0197
II	Gentamicin 80 mg/kg i.p	-7.477 ± 0.8157	4.167 ± 0.4507	0.9850 ± 0.0170
III	Gentamicin 80 mg/kg i.p +200 mg/kg p.o MEPN	-4.345 ± 0.2797***	5.100 ± 0.2503	0.7300 ± 0.0177***
IV	Gentamicin 80 mg/kg i.p +400 mg/kg p.o MEPN	-3.272 ± 0.3239***	6.367 ± 0.3981**	0.6033 ± 0.0145***

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's 't' test, Note: n=6 in each group (*P value <0.05, **P value <0.01, ***P value <0.001). MEPN : Methanolic extract of leaves of *Phyllanthus niruri*.

Table 3: Nephroprotective effect of methanolic extract of leaves of the *Phyllanthus niruri* on serum parameters

Group	Treatment	Serum creatinin(mg/dl)	Serum protein (mg/dl)	Serum Urine (mg/dl)	Blood urea nitrogen (mg/dl)	MDA (nmoles/mg)
I	Vehicle	0.69 ± 0.48	4.26 ± 0.94	32.4 ± 0.43	19.24±0.95	14.3±0.05
II	Gentamicin 80 mg/kg i.p	1.72 ± 1.21**	7.94 ± 1.10**	56.8 ± 11.6**	45.40±1.24**	41.23±0.10**
III	Gentamicin 80 mg/kg i.p +200 mg/kg p.o MEPN	0.83 ±0.46*	5.28 ± 2.63**	44.5 ± 0.94**	22.19±1.54**	29.53±0.11**
IV	Gentamicin 80 mg/kg i.p + 400 mg/kg p.o MEPN	0.79 ± 0.44**	5.09 ± 8.1**	41.28 ± 2.22*	22.43±2.12**	25.68±2.8**

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's 't' test, Note: n=6 in each group (*P value <0.05, **P value <0.01, ***P value <0.001). MEPN : Methanolic extract of leaves of *Phyllanthus niruri*.



Graph 1: Nephroprotective effect of methanolic extract of leaves of the *Phyllanthus niruri* on serum parameters

4. DISCUSSION

Gentamicin induced nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydrolases such as sphingomylinase and phospholipases in addition to causing oxidative stress¹⁸. Drug induced nephrotoxicity are often associated with marked elevation in blood urea, serum creatinine and acute tubular necrosis. So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animal and ma¹⁹. In the present study drug induced nephrotoxicity were established by single daily intraperitoneal injection of the gentamicin, for 10 days. Gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR²⁰. Gentamicin is known to decrease the activities of catalase, glutathione peroxidase and the level of reduced glutathione²¹. Therefore it is no doubt to assume that the nephron protection showed by *phyllanthus niruri* extract in gentamicin induced nephrotoxicity is mediated through its potent antioxidant effect. A relation between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. In these studies both the agents prevented gentamicin induced lipid peroxidation. Gentamicin treated group showing diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion. While treatment group shows Focal glomerular congestion, Peritubular congestion, Focal hydrophic degeneration of tubular epithelial cells and treatment group shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. From histopathological results we can conclude that *Phyllanthus niruri* have protective effect on gentamicin induced nephrotoxicity. The findings suggest the potential use of methanol extract of *Phyllanthus niruri* a therapeutically useful nephroprotective agent. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

5. CONCLUSION

The result of present study shows that gentamicin induces nephrotoxicity. This ultimately affects kidneys and its important functions. In contrast MEPN has reduced the damages occurred to the kidneys caused by gentamicin. The beneficial effects of MEPN were able to ameliorate gentamicin-induced nephrotoxicity. Based on our present observations, we propose that *phyllanthus niruri* leaves may provide a therapeutic option against drug-induced nephrotoxicity without harmful side effects. Further studies will be necessary to establish the probable mechanism of action of the nephroprotective activity of the leaf extract *phyllanthus niruri* Linn.

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