Advance Research in Pharmaceuticals and Biologicals

(A Peer Reviewed International Journal for Pharmaceutical and Allied Research)

ISSN 2250-0774 USA CODEN: ARPBGZ

PROTECTIVE EFFECT OF *PHYLLANTHUS FRATERNUS* WEB ON CISPLATIN AND GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

***B. Kalyani[1], T. M. Joyti[2], S. R. Setty[3] and Y. H. Babu[4]**

[1]Department of Pharmacology HKE'S Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences M R Road Gulbarga-585105 Karnataka India.

[2]SCS College of Pharmacy Harapanahalli Karnataka India.

[3]Government College of Pharmacy Bangaluru Karnataka India.

[4]University College of Veterinary Sciences, Nadi Nagar Bidar-585402 Karnataka India.

Received on 11/08/2012 Revised on 1/09/2012 Accepted on 12/09/2012

ABSTRACT:

The investigation of Nephroprotective activity of plant *Phyllanthus fraternus* (PF) Web, euphorbiaceae aerial parts extract (70% methenolic) was tested against cisplatin (anti cancer agent) and gentamicin (amino glycoside antibiotics) induced nephrotoxicity in rats. The degree of protection was determined by estimating blood urea nitrogen (BUN), serum Creatinine (Scr) levels and initial and final body weight (BW) of the animals was determined. The treatment with 70% methanolic extracts of PF at doses 100 and 200 mg/kg body weight markedly reduced cisplatin and gentamicin induced elevation of serum nitrogen and creatinine levels and normalizes body weight of the animals. The comparative histopatholigcal study of kidneys exhibited almost normal architecture as compared to control group.

KEYWORDS: Phyllanthus fraternus; Nephroprotective; Blood Urea Nitrogen; Serum Creatinine.

***Corresponding Author:**
Kalyani B. Biradar.
HKES' Society College of Pharmacy
M.R. Road, Gulbarga-585 105,
Karnataka-India
Email: biradarkb76@gmail.com
09620631443, Fax: 08472-244662

INTRODUCTION

The kidneys are susceptible to toxicity from xenobiotics because they too have a high blood flow (22% of cardiac output) relative to their mass (0.5% of body mass). Cells of the tubular nephron also face double sided exposure, to agents in the blood on the basolateral side and in the filtered urine on the luminal side. Cells in the proximal tubule are generally the site of nephrotoxicity. These cells have greatest abundance of cytochrome P450 in the nephron and they have the ability to transport organic anions and cat ions from the blood in to the cells , chemically induced kidney damage in typically seen as acute tubular necrosis (ATN) the cells in the proximal tubule are affected , Re-absorption of water, electrolytes, glucose and amino acids is impaired[1].

There are several nephrotoxic drugs in the market for treating cancer and infectious diseases caused by gram negative bacteria namely cisplatin and gentamecin etc. Cisplatin is a potent anticancer drug used in the treatment various solid tumors. But the major dose limiting side effect associated with cisplatin is nephrotoxicity[2]. Similarly gentamicin a potent antibiotic effective against gram negative bacteria. However here also ototoxicity and nephrotoxicity associated with these types antibiotics are limity their use in clinical practice[3], Therefore it is thought that administrations of Nephroprotective agents like antioxidants along with such essential nephrotoxic drugs may prevent / reduce the severity of nephrotoxicity. Search for such nephroprotctive agents from synthetic sources has been less fruitful.

Therefore we thought of exploring a possibility of using Nephroprotective agents from natural sources along with nephrotoxic drugs so as to protect kidneys.

As a part of this concept survey of locally available medicinal plants was undertaken. It was observed that

the plant *Phyllanthus fraternus* is grown widely and abundantly. In addition, a native practioner has claimed that this plant is very useful Nephroprotective agent[4]. The other species of phyllanthus are known to posse's antioxidant and hepatoprotective properties[5]. Keeping all these facts in view the present study is aimed at giving a scientific basis for the native claims and traditional knowledge. In addition it is also thought to combine a natural Nephroprotective drugs with synthetic nephrotoxic drug so as to enhance the safety of treatment for cancer and infectious diseases caused by gram negative bacteria.

MATERIALS AND METHODS

The Plant material and Preparation of extract: The plant PF aerial parts were collected from the surrounding villages of Harapanahalli in the month of Sept-Oct. The plant was identified and authenticated by Department of Pharmacognosy, S. C. S. College of Pharmacy, Harapanahalli. The plant specimen has been deposited at the herbarium of the college. The aerial part of the plant was shade-dried and crushed at room temperature and pulverized. The powder obtained was subjected to soxhlet extraction with 70% methanol the extract were concentrated under reduced pressure and stored in a dedicator until further use[6].

Experimental animals: Adults Wister albino rats weighing between 140-190 gm and adult Swiss albino mice weighing between 20-25gms of either sex were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet (gold Mohr Lipton India Ltd) and water was given *ad libitum* under strict hygienic conditions and ethical clearance for animal use was obtained from institutional animal ethical committee prior to the activity the registration no for institutional animal house is 157/1999/CPCSEA.

Acute toxicity studies: The acute toxicity for test extract PF were determined on albino mice, the selected animals were divided in to eight group of six in each .the control group received 2ml /kg of vehicle orally, other groups received the extract as test drug in one of dose 100, 200, 400, 800, 1000, 2000 and 3000 mg / kg in a similar manner, after dosing the animals were observed continuously for first behavioral changes and mortality if any at the end of 24 hrs, 48 hrs and 72 hrs respectively[7].

Nephroprotective activities

Cisplatin induced nephrotoxicity: Six groups of six rats in each were selected; the group first was administered with equivalent volume of 2% gum acacia (op) for 15 days, the group is served as control. The remaining group was treated with cisplatin 5mg/kg BW intraperitonial (ip) for five days. The blood was withdrawn from the animal through retro-orbital vein on sixth day in second group, on the sixteenth day in group third to assess the renal functions. The group fourth and fifth animals were treated with extract of 100 and 200 mg/kg BW from sixth day to sixteenth day orally and blood was withdrawn on the sixteenth day to estimate. The group sixth was treated with 200mg/kg BW from the day of administration of cisplatin. The blood was withdrawn on the day sixth[8,9].

The results are shown in Table no.1.

Table No. 1: Effect of 70% methenolic extracts of PF in cisplatin induced renal damage in rats.

Group (n=6)	Treatment regimen	Change in (bw) (%)	Blood urea (mg/dl)	Serum creatine (mg/dl)
I	Vehicle treated (control)	5.00 ± 0.57	38.03 ± 1.31	0.54 ± 0.01
II	Cisplatin (5 mg/kg bw) for 5 days.	-13.83 ± 0.94***	71.37 ± 1.64***	2.25 ± 0.43***
III	Cisplatin for 5 days (5 mg/kg) and 70% alcoholic extract simultaneously administered for 5 days.	-4.66 ± 0.84***	51.67 ± 1.12***	1.91 ± 0.20**
IV	Cisplatin for 5 days (5 mg/kg) and 70%alcoholic extract (100 mg/kg, 6th to 16thday).	-2.66 ± 0.42***	48.56 ± 0.58***	1.50 ± 0.13*
V	Cisplatin for 5 days (5 mg/kg) and 70% alcoholic extract (200 mg/kg, 6th to 16thday).	-3.66 ± 0.88***	38.68 ± 2.05	0.85 ± 0.11

*P < 0.05 **P < 0.01, ***P<0.001 (vs. Control).

Gentamicin induced nephrotoxicity: Group one, which received only normal saline throughout the course of the experiment was used as control. The second group of animals received daily ip injection of generation (80mg/kg BW) for eight days. The animals of third group received 80 mg/kg of gentamicin i.p nearly for eight days in addition to this they also received 100 mg/kg BW PF orally. Group four animals were given 80 mg/kg BW of gentamicin i.p for eight days in addition to this they also received 200 mg/kg BW of PF orally which was started three days prior to the gentamicin

injections and continued with eight days gentamicin treatment[10, 11]. All the data's are given in Table no.2.

Table No. 2: Effect of 70% methanolic extracts of PF in Gentamicin induced renal damage in rats.

Group (n=6)	Treatment regimen	Change in (bw) (%)	Blood urea nitrogen (mg/dl)	Serum creatinine (mg/dl)
I	Vehicle treated (control).	5.50 ± 0.67	29.21 ± 0.46	0.90 ± 0.03
II	Gentamicin 80mg/kg/7days.	-13.33 ± 1.28***	53.52 ± 2.08***	1.63 ± 0.10***
III	Gentamicin 80 mg/kg/8 days with 70% alcoholic extract 200 mg/kg simultaneously	-9.16 ± 2.02***	36.62 ± 2.09*	1.12 ± 0.07
IV	Gentamicin 80 mg/kg/8 days and 70% alcoholic extract 100 mg/kg 9thday to 16thday.	-9.83 ± 1.24***	47.99 ± 1.10***	1.62 ± 0.09***
V	Gentamicin 80 mg/kg/8 days and 70% alcoholic extract 200 mg/kg after 9thday to 16thday.	-3.50 ± 0.92***	31.63 ± 1.79	1.02 ± 0.06

*P < 0.05 **P < 0.01, ***P<0.001 (vs. Control).

Parameter assess for the renal functions:

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 and the effects are shown through graphs i.e. fig.1,2,3,4,5,6.

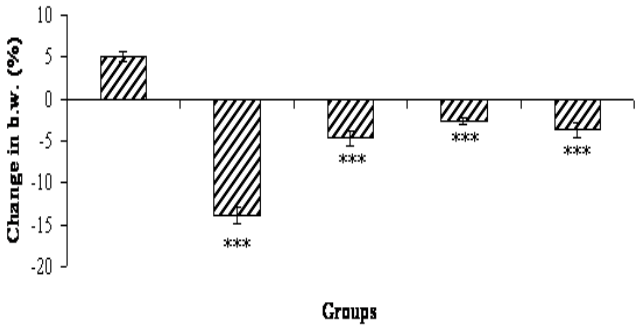


Fig. 1: Effect of 70% alcoholic extract of PF on cisplatin induced reduction in body weight.

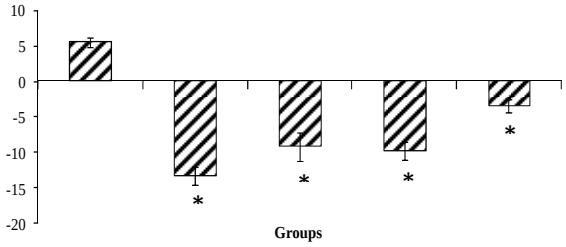


Fig. 4: Effect of 70% alcoholic extract of PF on gentamicin induced reduction in body weight

Blood urea nitrogen: The BUN concentration in blood serum was estimated by enzymatic method using urea's enzyme kit. (Berthelot's method)[12].

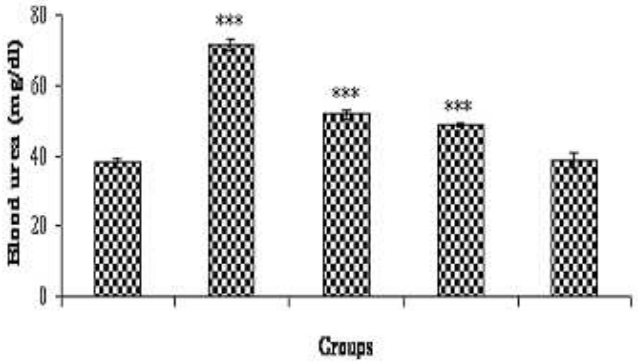


Fig. 2: Effect of 70% alcoholic extract of PF on cisplatin induced elevated blood urea nitrogen level.

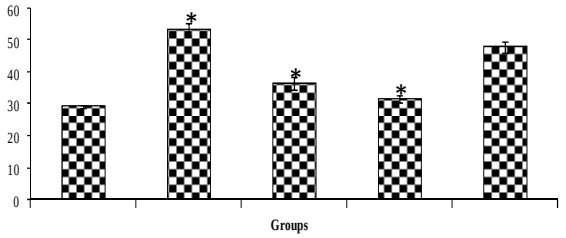


Fig. 5: Effect of 70% alcoholic extract of PF on gentamicin induced elevated blood urea nitrogen level

Serum creatine: The blood Scr level was estimated by alkaline picrate method using creatine kit. (Jiffies method)[13].

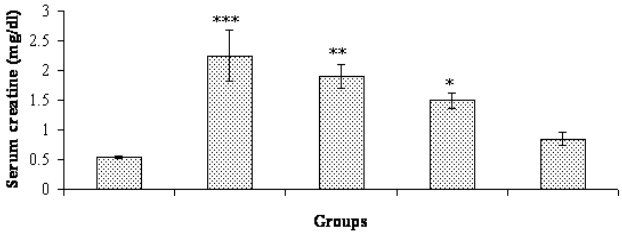


Fig. 3: Effect of 70% alcoholic extract of PF on cisplatin induced elevated serum creatinine levels.

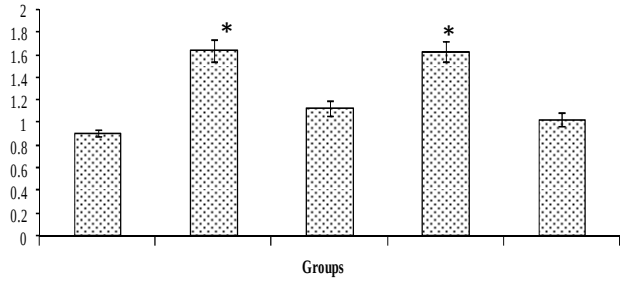


Fig. 6: Effect of 70% alcoholic extract of PF on gentamicin induced elevated serum creatinine levels.

Histopathological examination: Two animals from each group were sacrificed on the day of blood withdrawal and kidneys were isolated. It was washed with saline and preserved in 10% formaldehyde solution. The kidneys were process and embedded in paraffin wax. The sections were stained with Hematoxylin and Eosin and observed under light microscope[14].

Histopathological photography of cisplatin treated groups of rat's kidneys (**Fig. 7)**

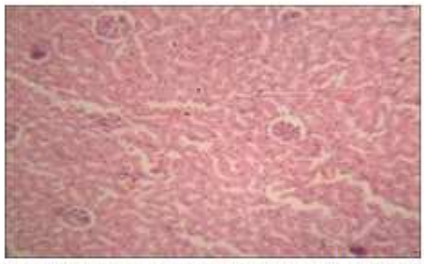
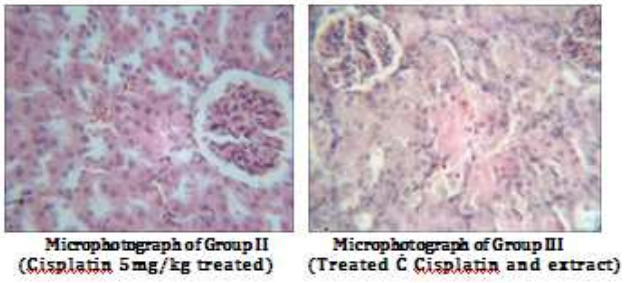
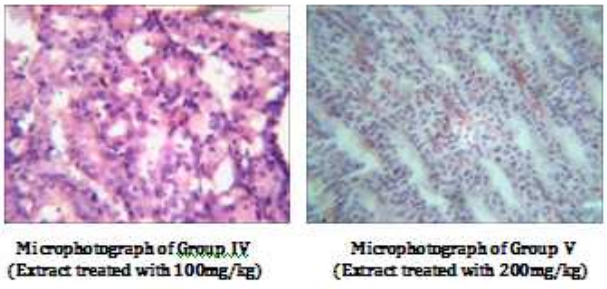


Fig.7. Microphotograph of rat kidney group I treated (Control)



Microphotograph of Group II (Cisplatin 5mg/kg treated)

Microphotograph of Group III (Treated C Cisplatin and extract)



Microphotograph of Group IV (Extract treated with 100mg/kg)

Microphotograph of Group V (Extract treated with 200mg/kg)

Histopathological photography of gentamicin treated groups of rat's kidneys **(Fig.8)**

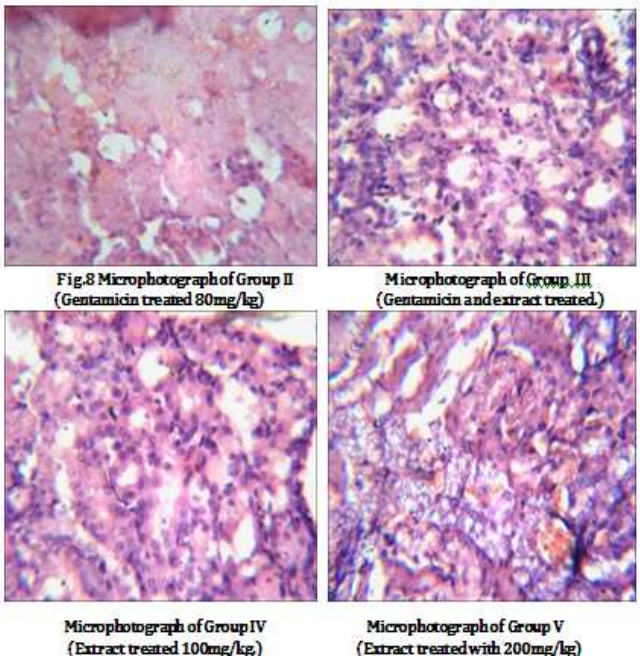


Fig.8 Microphotograph of Group II (Gentamicin treated 80mg/kg)

Microphotograph of Group III (Gentamicin and extract treated.)

Microphotograph of Group IV (Extract treated 100mg/kg.)

Microphotograph of Group V (Extract treated with 200mg/kg)

Statistical analysis: The data was analyzed using ANOVA followed by student's 'T' test.

RESULTS AND DISCUSSION

In the toxic doses study extracts of PF Since no mortality was observed at 2000 mg/kg. It was thought that 2000mg/kg was the cut off dose. Therefore 1/10th and 1/20th dose (i.e. 200mg/kg and 100mg/kg) were selected for the activity.

In cisplatin groups it was found that body weight was elevated to 5% $\pm$ 0.57 gm, BUN was 35.03$\pm$1.31 mg/dl serum Creatinine 0.54$\pm$0.01 mg/dl in control animals. Histopathological observations revealed that the morphology of kidney was normal and even the tubular stretch are normal, there were no necrotic sites. However upon the cisplatin 5 mg/kg for 5 days is reduced the body weight of 13.83$\pm$ 0.94 gm and elevated the BUN levels to 71.37 $\pm$ 1.64 mg/dl and serum Creatinine levels to be 2.25 $\pm$ 0.43 mg/dl histopathological observations reveals that there is a glomerular congestion, infiltration, inflammatory cells, tubular necrosis, peritubular necrosis and presence of casts. The group third and fifth has significantly reduced the severity of cisplatin induced nephrotoxicity in dose dependent manner. In treatment with 100 mg/kg dose a mild paritubular congested are persisted. However the higher dose 200 mg/kg animal's kidneys have shown almost complete recovery from nephrotoxicity. In gentamicin treated groups it was found that body weight was increased to 5.5% $\pm$ 0.67 gm, BUN level was 29.21$\pm$0.46 mg/dl and serum Creatinine 0.90 $\pm$ 0.03 mg/dl in control group animals. Histopathological observations revealed that the morphology of kidney

was normal. However upon the gentamicin 80 mg/kg for 8 days is reduced the body weight of 13.33 ± 1.28 gm and elevated the BUN levels to 53.52 ± 2.08 mg/dl, serum creatinine levels were 1.63±0.1 mg/dl and histopathological observations showed that there is a glomerular congestion, infiltration, inflammatory cells, tubular necrosis, peritubular necrosis and presence of casts. Upon treatment with 200 mg/kg along with gentamicin has reduced the severity of nephrotoxicity. Similarly treatments with 100 mg/kg and 200mg/kg after gentamicin toxicity have significantly reduced the severity of nephrotoxicity in dose dependent manner.

CONCLUSION

In the study it was observed that there was a significant elevation in BUN levels with reduction in body weight of animals in cisplatin induced toxicity. This is in conformity with the earlier report. This is due to the reactive oxygen species, increased nitric oxide synthatase activity and hydroxyl radical generation. These free radicals may deplete or over powers lipid peroxidation preventing enzymes like superoxide dismutase, glutathione transferase, catalase etc. The plant PF reported a potent antioxidant property. Similarly it has also protected that kidneys from cisplatin induced nephrotoxicity, therefore it seems free radical scavenging property may be responsible for nephroprotective activity against cisplatin induced nephrotoxicity.

Similarly gentamicin also induces nephrotoxicity by generation of free radical species like superoxide, hydroxyl and hydrogen peroxide. In our study methanolic extract of PF has protected animals against gentamicin induced nephrotoxicity and also shown anti-oxidant properties. Therefore in this model also nephroprotective effect may be due to the anti-oxidant property of the plant.

Treatment with PF extract has protected the kidney from cisplatin and gentamicin challenge. This was demonstrated by reducing the elevated levels of BUN, Scr and improves the body weight of animals. In addition histopathological observations have shown that there is an improvement in this architecture of the kidney due to the treatment with test extracts in both models. Our study has apparently justified the claim of the native practitioner that the aerial parts of *Phyllanthus fraternus* web are useful in treating kidney disorders. There is a need to undertake further studies to isolate phytoconstituents responsible for nephroprotection so as to identify the lead molecule responsible for the Nephroprotective activity.

ACKNOWLEDGEMENT

The Authors are extended very thanks to Dr. P Sheshikala Head of the Department and Dr. Dandinkkopa Professor in Pathology, S.S. Institute of Medical sciences and research Davangere for providing facilities for histopathological studies of rat kidneys.

REFERENCES

1. M. J. Reasor, M. E. Davis. Modern Pharmacology with clinical applications, 5th Ed. 1994, pp. 73.
2. P. L. Munson. Principles of Pharmacology, Basic concepts and clinical applications. Chapman and Hall ITP an international Thomson publishing company New York, 685.
3. H. P. Rang, M. M. Dale, J. M. Ritter, P. K. Moore. 5th Ed. Pharmacology. Churchill living stone; 2003, pp. 728.
4. J. C. Kurian. Plants that heal, Pune. Oriental Wat Duman Publishing House, 1999, 207.
5. A. A. Khan, A. V. Khan. Dept of Botany Aligarh University Aligarh, Medico-ethanobotanical uses of Phyllanthus Fractomus (Family-Euphorbiaceae) from western UP India. J Nat Rem, 4: 73-76 (2004).
6. V. Rajpal. Standardization of Botanicals. Vol I. Eastern Publishers G-59. Greater Kailash-2, New Delhi India, 184-92.
7. P. Veeraraghavan, Expert Consultant, CPCSEA, OECD guideline No. 420. OECD Environment, Health and safety publications environment directorate 2001, 423, 425.
8. A. Shriwaiker, S. Malini, S. Chandrika Kumari. Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamian induced nephrotoxicity in rats. Ind J Exp Biol 41: 58-62 (2003).
9. M. Rao, M. N. A. Rao. Protective effect of Selcomethionine against Cisplatin induced renal toxicity in mice and rats, J Pharm Pharmacol 50: 687-91 (1998).
10. Vijay Kumar K, M. U. R. Naidu, A. Anwar, K. S. Ratnakar. Probucol protects against gentamicin induced nephrotoxicity in rats, Ind J Pharmacol 32: 108-13 (2000).
11. D. Beauchamp, G. Laurent, P. Maldague, S. Abid, B. K. Kishore, P. M. Tulkens. Protection against gentamicin induced renal alterations (Phospholipidosis, increased DNA synthesis) by co administration of poly-L-aspurtic acid, J Pharmacol Exptl Ther. 255: 858-66 (1990).
12. A. L. Chaney, E. P. Marbach. Modified Reagents for Determination of Urea and Ammonia, Clin Chem 8: 130 (1962).
13. T. R. Mitchell. Clin Chem 19: 908 (1973).
14. D. C. Dobyan, J. Levi, L. C. Jacobs, J. Kosek, M. W. Weiner. Mechanisam of Cisplatin nephrotoxicity: II morphological observations, J Pharmacol Expt Ther 213: 551(1980).