AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Nephroprotective Activity of Poly Herbal Methanolic Extract Against Gentamicin-Induced Nephrotoxicity and Renal Dysfunction In Experimental Rodents

V.V. Rajesham[1]*, Balusu Haarika[2], DVRN Bikshapathi[1], T. Rama Mohan Reddy[1], M.Srinivasa Chandramouli[3]

1.CMR College Pharmacy, Kandlakoya, Medchal road, Hyderabad-501401, Telangana state, India.

2.Sarojini Naidu Vanitha Pharmacy Maha Vidyalaya, Tarnaka, Secunderabad-500001, Telangana state, India.

3.Gandhi hospital, Musheerabad, Hyderabad, A.P., India.

ABSTRACT

To evaluate Nephrpotective potential of Polyherbal methanolic extraction (*Terminalia chebula, tinospora cordifolia, Phyllanthus emblica, Portulaca oleracea*) against gentamicin (GM) induced Nephrotoxicity and renal dysfunction. It was observed that the GM treatment induced significant elevation (P<0.001) in plasma and urine urea, creatinine, kidney weight, blood urea nitrogen, renal lipid peroxidation along with significant decrement (P<0.001) in urine output, renal enzymatic and non-enzymatic antioxidants. Polyherbal methanolic extraction 200 and 400 mg/kg treatment to GM treated rats recorded significant decrement (up to P<0.001) in plasma and urine urea and creatinine, renal lipid peroxidation along with significant increment (up to P<0.001) in renal enzymatic and non-enzymatic antioxidants. These finding powerfully supports that Polyherbal methanolic extraction acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of GM in the biochemical parameters and thus validates its ethnomedicinal use.

Keywords: Nephroprotective, *Terminalia chebula, tinospora cordifolia, Phyllanthus emblica, Portulaca oleracea.*

*Corresponding Author Email: vvrajesham@gmail.com

Received 20 September 2016, Accepted 06 October 2016

Please cite this article as: Rajesham VV *et al*., Nephroprotective Activity of Poly Herbal Methanolic Extract Against Gentamicin-Induced Nephrotoxicity and Renal Dysfunction In Experimental Rodents. American Journal of PharmTech Research 2016.

INTRODUCTION

Now-a-days significant basic and clinical research has been carried out on the medicinal plants and their formulations, with the state of[1] the art methods in a number of institutions or universities. Indian medicinal plants also provide a rich source for antioxidants that are[2] known to prevent or delay different diseased states. The medicinal plants also contain other beneficial compounds or ingredients[3] which can be used as food. Hence the global knowledge about ayurveda and Indian herbals will hopefully be enhanced by[4] information of the evidence-base of the plants.

A number of environmental contaminants, chemicals and drugs including antibiotics dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart and intestine[5]. Aminoglycoside antibiotics are frequently used in the treatment of severe infections of the abdomen and urinary tract[6]. Gentamicin (GM) is still considered to be an important aminoglycoside antibiotic against life threatening bacterial infections. However, Nephrotoxicity and ototoxicity remain major problems for its effective long term clinical use[7]. GM is known to cause a number of morphologic, metabolic and functional alterations in the kidney and the specificity of GM Nephrotoxicity is apparently related to its accumulation in the renal proximal convoluted tubules leading to tubular necrosis[8].

MATERIALS AND METHOD

Collection and Processing of Herbals

Almost all the plant materials (*Terminalia chebula, tinospora cordifolia, Phyllanthus emblica, Portulaca oleracea*))were collected from the forest region of Tamil Nadu and they were identified and authenticated by Dr. Vastavya S. Raju, Department of Botany, Kakatiya University, and Warangal. The parts proposed for this study were separated from the whole plant and kept for air drying under shadow (i.e. avoiding direct exposure to sunlight) and were subjected for size reduction.

Preparation of Poly Herbal Extract

A wide range of solvents with increasing polarity were chosen.

Step.1:

In a 5000ml round bottomed flask, weighed quantity of powdered drug mixture 1.5kg (containing all the selected plants) were macerated with the respective solvents and kept with occasional shaking for a period of 72-96 hrs. After the maceration process, the active ingredients present in the supernatant solvent were collected and concentrated under reduced pressure.

Step.2:

These extracts were labelled and its chemical constituents were identified, among the different solvent extracts, the extract possessing more number of active compounds were selected and prepared for bulk extraction similar as step 1.

Animal studies:

Animals

Albino rats (175-225gm) of either sex and of approximate same age used in the present studies were procured from Central Animal facility, Vijaya college of Pharmacy, Hyderabad, India. The animal was fed with standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours in darkness and light. The animals were acclimatized to the laboratory condition for a one week before starting the experiment. The experiment protocols were approved by Institutional Animal Ethics committee after securitization **(IAEC No: P22/VCP/IAEC/2013/3/VVR/AE2).** The animal received the drug treatment by oral gavage tube.

Toxicity studies

Albino rats (200-250gm) of either sex were selected and segregated in to 8 groups of 6 animals each. Single dose of methanolic extract of polyherbal formulation, starting from the minimal dose of 50mg/kg up to 2000mg/kg administered orally. The drug treated animals were observed carefully for its toxicity signs and mortality. From the maximum dose, $1/5^{th}$ and $1/10^{th}$ of the concentration was considered as therapeutic dose for further study.

Nephrpotective activity by Gentamicin Induced Nephrotoxicity in Rats [9, 10]:

The evaluation of the ethanolic and aqueous extracts for Nephrpotective activity was done according to the procedure given in the literature with minor modifications.

Total 36 animals were taken and 6 rats were allotted in each of the following groups;

Group I: Control group (Normal Vehicle only)

Group II: Gentamicin control group (60mg/kg)

Group III: Gentamicin + PHME (200mg/kg)

Group IV: Gentamicin + PHME (400mg/kg)

Activity profile of the test formulations in Gentamicin induced changes in different parameters:

Serum uric acid is the end product of purine catabolism. So, any defect in the glomerular filtration rate causes the rise in the level of uric acid in the blood .The raise after Gentamicin can be attributed to the GFR impairment. The decrease in the elevated uric acid by any substance may be due to the antagonism of Gentamicin induced disturbance in the glomerulus.

Creatinine clearance gives the glomerular filtration rate. Administration of Gentamicin leads to significant elevation of serum creatinine level indicating injury to the glomerular apparatus. The reversal of the elevation by any substance may be indicative of the reversal of the GFR impairment.

RESULTS AND DISCUSSION

Nephrpotective activity by Gentamicin induced Nephrotoxicity in albino rat's model:

In the present study the mean Blood urea, Serum creatinine, Total protein, Albumin and Globulin value of each group of rats at the 11th day of the experiment is compared with the values of Nephro-toxic control group. Mean levels of Blood urea, Serum creatinine, Total protein, Albumin and Globulin are presented in Table 2 and Table 3; the representing Charts in Figure 1, 2 and 3. In this study the rats included in Group IV (Nephrotoxic control) showed significant increase in Blood urea level on comparison with the values of Group I ($P < 0.001$). In the group II (Sirupeelai kudineer @ 270.0 mg/kg orally for 10 days) there was significant reduction in Blood urea levels as compared to that of Group IV ($P < 0.05$). In the group III (Sirupeelai kudineer @ 500.0mg/kg orally for 10 days) there was significant reduction in Blood urea levels as compared to that of Group IV ($P < 0.02$).

Gentamicin like other aminoglycoside antibiotics causes Nephrotoxicity by inhibiting protein synthesis in renal cells.

The serum creatinine and blood urea nitrogen (BUN) were found to be significantly increased in rats treated with only Gentamicin, whereas treatment with the Poly herbal Methanolic extract (PHME) the effect of Gentamicin indicating Nephrpotective activity. Among various doses, the aqueous extract of dose 400mg/kg has shown good Nephrpotective activity. Results were showed in Table 1, 2 and figure 1, 2, 3.

Table 1:% of Body weight change in Gentamicin induced Nephrotoxicity in albino rats.

Groups	% of body weight change
Normal control	3.44 ± 0.190
Toxic control	9.926± 0.448***
PHME (200mg/kg)	8.216 ± 0.410***
PHME (400mg/kg)	5.776 ± 0.463**

Values are expressed in mean±SEM where n = 6, Significant at $P < 0.05$*, 0.01** and 0.001***, compared to control group.

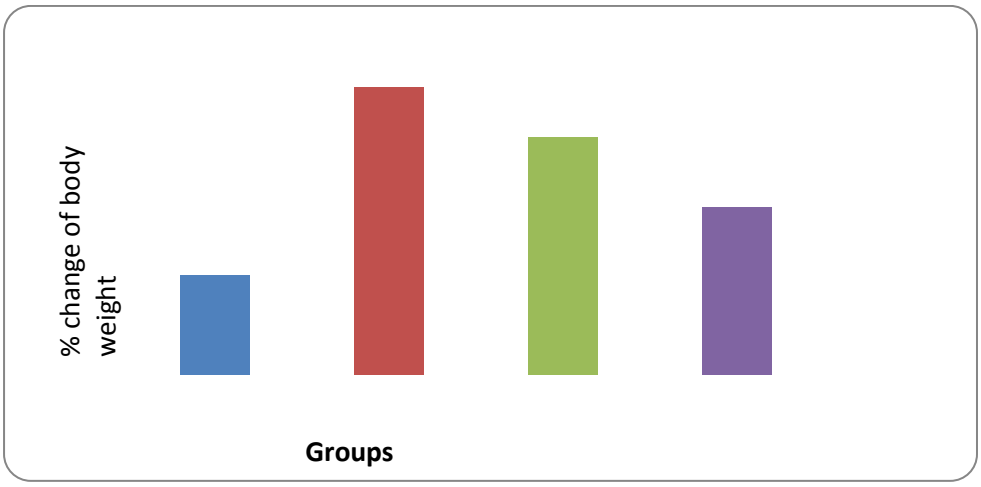

Figure 1: % of Body weight changes in different groups by Gentamicin induced Nephrotoxicity model

Table 2: Blood urea and serum creatinine levels of different groups by Gentamicin induced Nephrotoxicity

Groups	Blood urea	Serum creatinine
Normal control	31.03± 5.018	1.023± 0.053
Toxic control	70.591 ± 4.064***	2.05± 0.081***
PHME (200mg/kg)	57.385 ± 11.724**	1.918± 0.07***
PHME (400mg/kg)	45.916 ± 11.65*	1.53 ± 0.11***

Values are expressed in mean ± SEM where n = 6, Significant at P < 0.05*, 0.01** and 0.001***, .compared to control group.

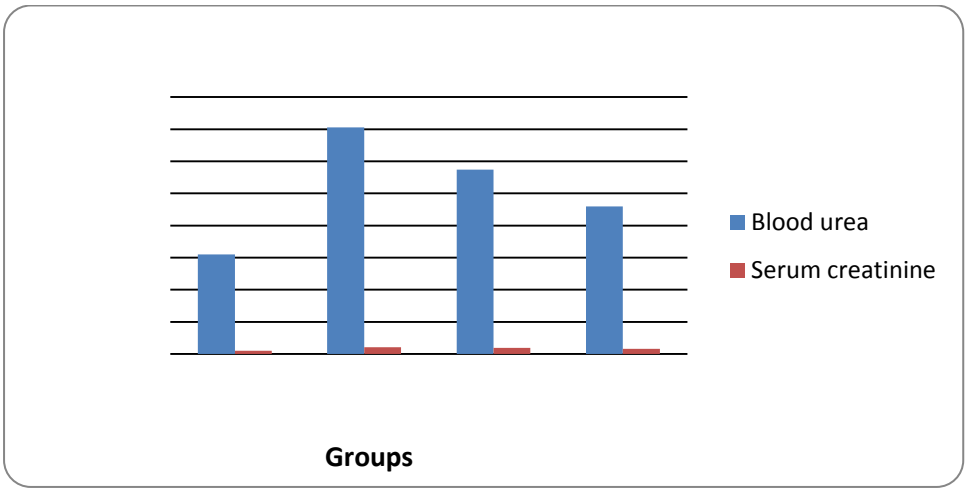

Figure 2: Blood urea level of different groups in Gentamicin induced Nephrotoxicity model

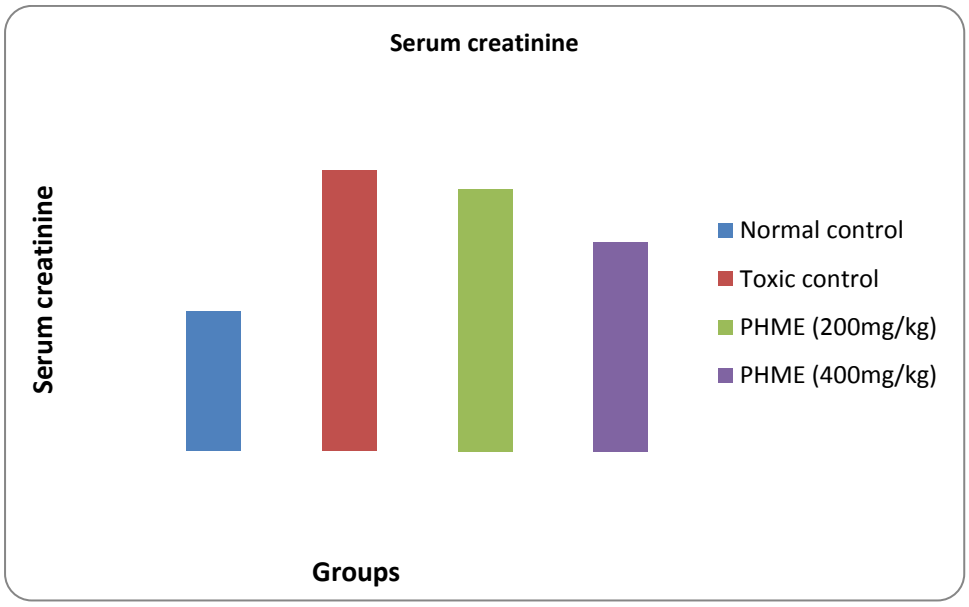

Figure 3: Serum creatinine level of different groups in Gentamicin induced Nephrotoxicity model

The present study is being designated to protect the kidney damage induced by amino glycoside (AG) like Gentamicin in rat model. AG on intracellular bio-activation produces reactive oxygen species and lipid peroxidation in kidneys. The results of various studies suggested that treatment of rats with hydroxyl radical scavengers protect against Gentamicin induced renal damage. The Biochemical analysis of the drug Polyherbal methanolic extraction reveals the presence of minerals namely Selenium, Manganese, Lead, Copper, Potassium and Calcium. Wherein Polyherbal methanolic extraction may prevent lipid peroxidation and protects the cells against the free radicals including super oxide; maintain structural integrity of biological membranes. Manganese is a component of the antioxidant enzyme manganese superoxide dismutase (MnSOD) which can neutralize free radicals and may reduce or even help to prevent some of the damages of organs they cause and inhibits lipid peroxidation. Zinc also comprises the structural role of copper/zinc-superoxide dismutase (Cu/ZnSOD), it may also have antioxidant activity via its association with the copper-binding protein metallothionein. Copper, also contributes to the function of very many antioxidants, assisting the "mopping up" of the free radicals that cause cell damage. In general, calcium reduces Amino Glycoside (AG) Nephrotoxicity. Potassium also plays a vital role in the preservation of the permeability of cells. Long standing potassium deficiency may cause injury to myocardium and severe renal damage leading to chronic renal diseases. The presence of such minerals in the drug may be supportive or inducing of the antioxidant and free radical scavenging activity. In Pharmacological aspect, Rat is a suitable animal model for studies

of AG Nephrotoxicity in humans since pharmacokinetics and toxicology of AG are remarkably similar in rats and human. Hence the Nephrpotective activity of the said drug is being evaluated in rat models using Wistar albino rats. The study demonstrates renal damage in toxic groups, evidenced by elevated Blood Urea, Serum Creatinine levels most of the synthetic antioxidants like flavanoids, synthetic vitamins and minerals are not working as antioxidants while the natural products containing crude drugs with fibrous products act as anti oxidant and free radical scavengers. In Siddha system of medicine many of the drugs are used in crude forms as natural products, they may act better than synthetic drugs.

CONCLUSION

Nephrotoxicity is the major adverse effect of different drugs. So it is a drug induced disease. This toxicity has been induced because of the release of the oxidants in kidney. Thus damaging or destructing the nephrons which are the basic functional units of kidney. The present study throws light on the effect of the plant Bauhinia variegata in reducing the nephrotoxic effect that has been induced by Gentamicin which is a broad spectrum antibiotic used to treat many ailments. This study gives the idea that when we use the plant along with the Gentamicin like antibiotics will reduce the incidence of Nephrotoxicity. Further detailed scientific investigation of the plant will be helpful in treatment of drug induced toxicity.

REFERENCES

1. The wealth of India, A dictionary of Indian raw materials and industrial products. Vol 2, CSIR New Delhi, 1959, 56-57.
2. Maheshwari JK. Flora of Delhi-council of scientific and industrial research, 3rd edition, 137.
3. Ravindra S. Medicinal plants of India an encyclopedia, 2nd edition, 2003, 32-33.
4. Kirtikar R, Basu BD. Indian medicinal plants 1999; 1: 892- 901.
5. Kohn S, Fradis M, Robinson E, Iancu TC. Hepatotoxicity of combined treatment with cisplatin and gentamicin in the guinea pig. Ultrastruct Pathol 2005; 29: 129-137.
6. Nagai J, Takano M. Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokin 2004; 19: 159-170.
7. Khan SA, Priyamvada S, Farooq N, Khan S, Wasim KM, Yusufi ANK. Protective effect of green tea extract on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Pharmacol Res 2009; 59: 254-262.

8. Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Grandos-Silvestre M, Hernandez-Pando R, et al. Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidants. Free Radic Biol Med 2000; 29: 602-611.
9. Shivalinge KP and Vrushabendra BM. Study of nephroprotective activities of stem bark extracts of Ficus racemosa in gentamicin induced acute renal failure in rats International Journal of Pharmaceutical and Chemical Sciences 2012;1(1):314-318.
10. Saifuddin K, Hakeemuddin K, Aaftab A and Shahid A.Nephroprotective effect of the ethanolic extract of Lantana camara Linn flower on acute dose of Cisplatin induced renal injured rats; Rajiv Gandhi university of Health Sciences Journal of Pharmaceutical Sciences; 2012;2(2):68-77.