

THE NEPHROPROTECTIVE ACTIVITY OF METHANOLIC EXTRACTS OF PHYLLANTHUS ACIDUS LEAVES AGAINST GENTAMYCIN-INDUCED NEPHROTOXICITY IN EXPERIMENTAL RODENTS.

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ABSTRACT

Objective: The existing drugs can cure most of the diseases. Still there is a never ending search for finding new drugs in the hope that it would yield drugs with lesser side effects and better therapeutic benefit than the existing drugs. This work was researched for evaluation of the Nephroprotective activity of methnolic leaf extracts of Phyllanthus acidus against gentamycin induced nephrotoxicity in albino rats.

Materials and Methods: Nephrotoxicity was induced in albino rats by intraperitoneal administration of gentamycin 100 mg/kg/day for 10 days and methanolic extracts of leaves of Phyllanthus acids at a dose of 50,100mg/kg & 200mg/kg body weight were concurrently given by oral. Serum creatinine, serum urea, SGOT, SGPT and ALT were determined on 11thday. Histopathological study of the kidney also done.

Results: The extracts produced significant nephroprotective activity in Gentamycin induced nephrotoxicity model as evident by a decrease in elevated Serum creatinine, serum urea, SGOT, SGPT and ALT which was further confirmed by histopathological study. Gentamycin induced glomerular congestion, blood vessel congestion, epithelial desquamation, accumulation of inflammatory cells and necrosis of the cells were found to be reduced in the groups receiving methanolic extracts of leaves of Phyllanthus acids along with gentamycin.

Conclusion: The results of this study have led to the conclusion that methnolic leaf extracts of Phyllanthus acidus possesses nephro protective activity against gentamycin induced nephrotoxicity in albino rats.

Keywords: Nephrotoxicity, Phyllanthus acidus, Nephroprotectivity, Gentamycin and Albino rats.

INTRODUCTION

Nephrotoxicity is a poisonous effect due to drugs and its overdosage on the kidneys. A number of antibiotics including the penicillin, cephalosporins, tetracyclines as well as aminoglycosides and sulfonamides, are potential nephrotoxicants. The drug induced nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion and lowering of glomerular filtration rate[1].Gentamycin is an antibiotic that exhibits a broad spectrum of activity and is particularly valuable in severe sepsis. Its use is restricted due to the development of ototoxicity and nephrotoxicity. At physiological pH, the drug is highly charged and water soluble, and therefore it is practically unable to diffuse through biologic membranes. Nephrotoxicity has been related to a selective accumulation of gentamycin in the renal cortex[2,3]. Approximately 8% to 26% of patients who receive aminoglycosides for more than 7 - 10 days develop mild renal impairment which is almost always reversible. It usually presents as gradually worsening non oliguric renal failure and sometimes acute tubular necrosis may occur. Phyllanthus acidus Skeels also called Malay goose berry, Tahitian goose berry, Country goose berry, Star goose berry, West India goose berry, simply goose berry tree, is one of the trees with edible small yellow berries fruits. Exhaustive literature survey showed that the plant is a good remedy for different types of ailments like emetic and purgative[4] hypertension and respiratory [5], hepatoprotective[6], psoriasis[7], anti-diabetics[8], anti nociceptive[9]. Poisoning, coughs asthma and bronchitis, poulticing and soles, cathartic [10], rehabilitation[11], addiction[12], liver tonic, laxative, urticaria eruptions & bronchial catarrh[13]. Sciatica, lumbago or rheumatism, sudorific & gonorrhoea, skin disorders[14], and antioxidant[15]. However, no work is reported relating to nephroprotective effect of Phyllanthus acids. Therefore, the present study was designed to investigate the nephroprotective effect of the methanolic extract of Phyllanthus acidus leaves on gentamicin induced nephrotoxicity in Wistar rats.

MATERIAL AND METHODS

Plant material

The fresh leaves of Phyllanthus acidus Linn. Was procured and authenticated by Head of Department, Botany, SV University,

Tirupathi, Andhrapradesh. A voucher specimen of the same has been deposited.

Preparation of the leaf extract

The authenticated leaves were shade dried and powdered coarsely. The powdered drug was extracted by extracting with pet-ether (60-800 C). Coarse powder of the leaf (500gm) was Soxhlet extracted with methanol. The extract obtained was concentrated under reduced pressure to yield a methanolic extract. The extract thus obtained was subjected to evaluation of nephroprotective activity. The test samples of methanolic extract were suspended in 1% gum acacia in distilled water prior to its use for animal studies.

Animals

The healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study. They were housed under controlled conditions of temperature (23±2°C), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee as per the CPCSEA guidelines, Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity studies

Acute toxicity studies for methanolic extracts of Phyllanthus acidus Linn. were conducted as per OECD guidelines 423 using albino Wistar rats. Each animal was administered the methanolic solution of the extract by oral route. The animals were observed for any changes continuously for the first 2h and up to 24 h for mortality[16].

Gentamycin induced nephrotoxicity in rats

The present animal study was conducted on gentamicin model; the rats were systematically randomized into six groups of 6 rats each such that the differences in the average weights between and within groups do not exceed ±20% of the average weights of all the rats. Twelve hours before the experiment began, the rats were fasted of feed but distilled water was made available ad libitum.

The study was carried out for ten days and treatment was given for eight days. Group I served as a control group and received 1% gum

acacia p.o. Group II served as gentamycin group. The gentamycin treated group received 100 mg/kg/day gentamycin by the intraperitoneal (i.p.) route. Group III received Cystone mg/kg B.w. Respectively. Group IV, V & VI received 50 and 100 & 200 mg/kg B.w. of Methanolic extract of *Phyllanthus acidus* Linn respectively.. Animals of all groups III to VI were administered 100 mg/kg b.w of gentamycin i.p. along with extracts p.o. for 10 days. After dosing on the day 10, blood samples were collected via retro-orbital puncture at the end of these 24h, the serum was rapidly separated and processed for determination of serum creatinine, serum urea, using of Span Diagnostic kits. Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination [17,18,19,20].

Histopathological studies of rat kidneys

Kidneys of sacrificed animals were identified and carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5m thick sections stained with hematoxylin-eosin and observed under a photomicroscope (magnification power-40X) [21].

STATISTICAL ANALYSIS

Results were expressed as the Mean \pm standard error means (S.E.M.). The comparison of data within groups was performed by the analysis of variance using ANOVA test. Significant difference

between control and experimental groups was assessed by Dunnett's test. A probability level of less than 1 % ($P < 0.001$) was considered significant. Statistical analysis was performed using Graph Pad prism.

RESULT AND DISCUSSION

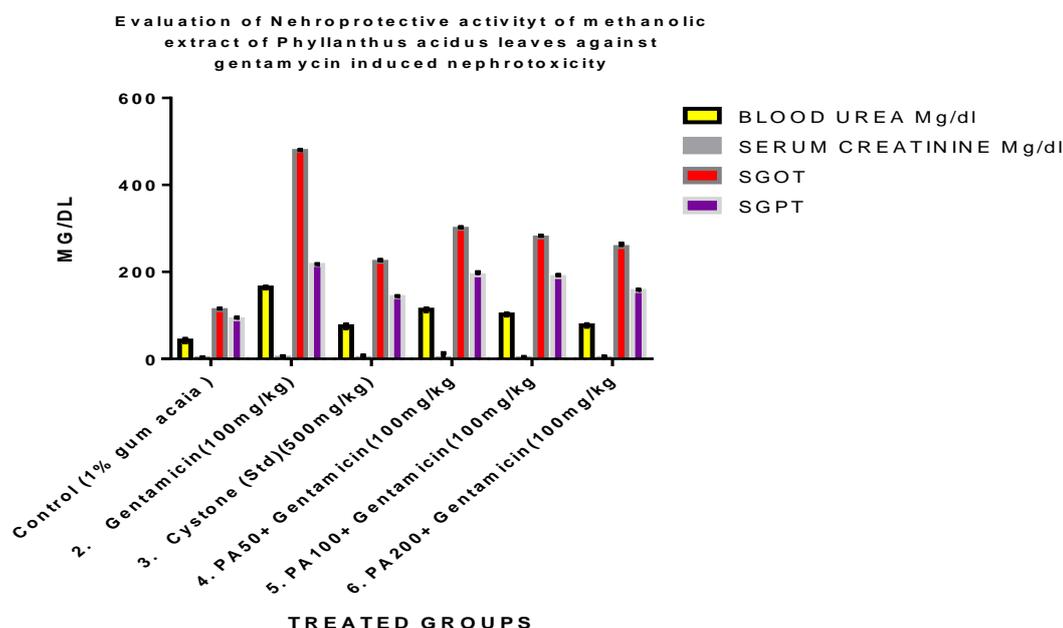
There was no change in the normal behavioral pattern of animals and no sign and symptoms of toxicity were observed during the first 2h and no mortality was observed till 24h. Extracts were safe up to a maximum dose of 2000 mg/ kg B.w. The biological evaluation was carried out at doses of 50,100 and 200 mg/kg b.w by oral route. Serum creatinine, serum urea and was found to be significant ($P < 0.001$) increased in rats treated with only gentamycin, whereas treatment with the Methanolic extracts of leaves of *Phyllanthus acidus* Linn. Reversed the effect of gentamycin indicating nephroprotective activity. [Table No. 1& Graph 1]. The impairment in kidney functions is accompanied by an increase in serum creatinine and urea level and kidney tissue MDA levels that indicates lipid peroxidation. It is one of the essential compounds for maintaining cell integrity participation in the cell metabolism. The significant and progressive weight loss in gentamycin treated rats may possibly be due to the injury of renal tubules and the subsequent loss of the tubular cells to reabsorb water, leading to dehydration and loss of body weight.. The extract showed dose depended protective effect. *Phyllanthus acidus* leaves might have exhibited nephroprotective activity by the virtue of its antioxidant activity.

Table 1: Effect of methanolic leaf extract of *Phyllanthus acidus* on various serum parameters like serum creatinine, serum urea, SGOT & SGPT

Group	Serum Urea Mg/Dl	Serum Creatinine Mg/Dl	SGOT (IU)	SGPT (IU)
I	42 + 3.70	1.5 + 2.53	112 +.67	91 + 2.45
II	164 + 1.56	2.5 + 3.56	478 + 2.26	216 + 1.78
III	75 + 3.56**	1.56 + 5.56**	223 + 4.00**	142 + 2.34**
IV	113 + 1.19*	1.6 + 11.46*	29 + 3.56*	192 + 7.16*
V	102 + 2.12**	1.65 + 2.56**	279 + 4.12**	188 + 4.56**
VI	77 + 2.76**	1.76 + 3.24**	257 + 7.89**	156 + 2.67**

Values are expressed as Mean \pm SEM of 6 rats in each group.

**P < 0.001, *P < 0.01, compared with control group. The data was analyzed by two way ANOVA.



Graph I: Graphical representation of Effect of methanolic leaf extract of *Phyllanthus acidus* on various serum parameters like serum creatinine, serum urea, SGOT & SGPT

Histopathological studies

In the negative control group II, histopathological findings showed kidney structure distorted by severe necrosis of tubules. The stroma was edematous. The tissue was infiltrated by numerous chronic inflammatory cells. Engorged blood vessels and areas of hemorrhage were seen. Features suggested severe tubular necrosis. Renal histology in the Gentamycin treated group showing severe tubular necrosis.

In the group III, histopathological findings showed the stroma with a mild degree of edema. There was a mild degree of glomerular congestion. The tissue was sparsely infiltrated by inflammatory cells. Features suggested mild tubular damage.

In the group IV, histopathological examination showed that there was mild interstitial edema. moderate degree of congestion was

also seen in the glomeruli. Numerous engorged blood vessels were seen. Mild tubular changes were noted. The tissue was free from inflammatory cells. Renal histology in the A group IV showing moderate tubular necrosis with significant reversal of inflammatory changes.

In group V, histopathological findings showed mild interstitial edema, mild degree of peritubular and glomerular congestion and numerous engorged blood vessels. The tissue was free from inflammatory cells. Features suggested mild tubular changes.

In group VI histopathology showed mild interstitial edema, mild degree of glomerular congestion and few congested blood vessels. Mild tubular damage was observed. The tissue was sparsely infiltrated by chronic inflammatory cells.



Fig. 1: 1% Gum accaia suspension : In the plain control group I, microscopic examination of the kidney showed a good number of glomeruli. Numerous blood vessels were seen in both cortex and medulla. No histopathological abnormalities were observed. Renal histology in the plain control group showing normal structure of kidney.



Fig. 2: Gentamycin treated 100mg/kg:



Fig.3: Cystone (500mg/kg) + Gentamycin 100mg/kg):



Fig. 4: PA (50mg/kg) + Gentamycin 100mg/kg)



Fig. 5: PA (100mg/kg) + Gentamycin 100mg/kg)



Fig. 6: PA (200mg/kg) + Gentamycin 100mg/kg

Table 2 : Effect of methanolic leaf extract of *Phyllanthus acidus* on Histopathological features of Kidneys.

Histopathological Features	GroupI	GroupII	GroupIII	GroupIV	GroupV	GroupVI
Glomerular congestion	-	+++	-	++	+	+
Tubular casts	-	+++	-	-	-	-
Epithelial desquamation	-	+++	-	++	-	-
Blood vessel congestion	-	+++	-	+	+	-
Interstitial oedema	-	+++	-	+	-	+
Inflammatory cells	-	+++	-	+	+	-

(-): normal, (+): little effect, (++) : appreciable effect, (+++) : severe effect

DISCUSSION

Gentamycin is a known nephrotoxic agent reported to induce a significant degree of nephro-toxicity at different dose levels. Its nephro-toxic potential was established at a dose level of 80 mg/kg in albino rats [22,23]. In the present study, this was evidenced by significant ($P < 0.001$) elevations in the serum urea and creatinine concentrations when compared with the plain control group I. The nephrotoxic effect was further corroborated by the histological findings in which many of the glomeruli showed diffuse eosinophilic sclerosis, engorged blood vessels and areas of hemorrhage, indicating severe tubular necrosis [Figure 2] and [Figure 4], while in the plain control group I, normal histopathological features were seen [Figure 1]. This functional and structural derangement caused by the toxic agent is in agreement with other reports showing its nephrotoxic effects. The markers of kidney function and structure in this study were grossly seen to be within the normal limits in the groups of animals treated with PA thus demonstrating nephroprotective effect. In the group III biochemical markers of kidney function were found to be significantly lower than in the control group I, while histological examination showed features suggestive of mild tubular damage [Figure 3]. Similarly, the animals in the groupIV demonstrated a significant decrease in serum urea and creatinine levels as compared with the Gentamycin group ($P < 0.001$). Microscopic examination demonstrated mild degree of tubular necrosis [Figure 5&6]. Thus, PA treated groups showed produced a significant degree of nephroprotection based on the biochemical markers of kidney function and the histopathological features in inter-group comparison. An almost similar pattern was observed in groups treated with a higher dose as both the indices of kidney function and its matrix were found within the normal limits and dose-dependent response was observed as compared with that of low-dose therapy ($P < 0.001$). The test drug, by not allowing the biochemical markers of kidney function as well as structural integrity to change beyond the normal limits even on administration of a high dose of gentamycin, clearly indicated that it possessed a striking nephroprotective effect. The findings further suggested that PA at a higher dose was more effective, In another study, PA has been reported to produce an anti-inflammatory effect. The role of anti-inflammatory effect apparently looks counter-productive to an injured organ, as anti-inflammatory agents usually delay the healing process [21]. Thus, an anti-inflammatory property in this condition may help improve the kidney function by neutralizing the pro-inflammatory mediators.

On the basis of our findings, it can be concluded that the PA possessed significant nephroprotective effect against gentamycin, a known nephrotoxic agent.

CONCLUSION

The present study aimed to evaluate the protective effect of methanolic Extract *Phyllanthus acidus* leaves against Gentamycin - induced nephropathy in rats. Gentamycin -administered rats

(control group) had encountered acute kidney dysfunction as evidenced by elevation in serum urea and creatinine and with multiple histological damages. Treatment with the *Phyllanthus acidus* leaves at the dose level of 50, 100, and 200mg/kg b.w, for 10 days significantly lowered the serum level of creatinine, urea when compared with the control group. The histological damages in the *Phyllanthus acidus* treated group were minimal in contrast to the toxic rats. The statistical significance of the nephroprotective activity of *phyllanthus acidus* treated group and the Cystone treated group were compared against control were found almost equal as both groups gained significance ($P < 0.001$) against the control group in most of the parameters including serum urea and creatinine. Out of three doses of *phyllanthus acidus* higher doses showed striking nephro-protective activity than lower doses but both are showed significant nephroprotective activity. These biochemical results were supported by hisptopathological data. The results of our study suggest that the *phyllanthus acidus* Possesses nephroprotective potential on the dose dependant manner and substantiate the therapeutic utility in renal injury. Extensive further research is needed to elucidate the exact mechanism of nephroprotective action of the *Phyllanthus acidus*. According to the pathological result it can be inferred that *Phyllanthus acidus* had a protective effect against degenerative injury.

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