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Evaluation of nephroprotective and antioxidant activity of ethanolic extracts of *Momordica dioica* leaves

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ABSTRACT

Since kidneys are the vital excretory organs carrying diverse functions, effective and safer drug treatments are required to treat renal disorders. Oxidative renal stress due to generation of reactive oxygen species is the reason for gentamycin induced nephrotoxicity. Our work was researched to evaluate the nephroprotective and antioxidant activity of ethanolic leaf extracts of momordica dioica. Group I control group received distilled water. Gentamycin(80 mg/kg, ip) was injected to group II rats once daily for 8 days (negative control). Cystone drug induced rats were used as positive control (group III). Group IV and V rats were orally pretreated for 6 weeks with ethanolic leaf extracts of plant along with gentamycin induction during the last 8 days. After the experimental period serum renal biomarkers levels and renal antioxidant enzymes levels were estimated. Gentamycin treated rats showed after 8 days reduced activity of renal antioxidant enzymes and increased levels of serum biomarkers. Orally pre-treated rats for 6 weeks with plant extracts, after experimental period produced significant nephroprotective and antioxidant activity in gentamycin induced nephrotoxic rats by reversing all these parameters levels. Nephroprotective and antioxidant activity of ethanolic leaf extracts of Momordica dioica have been proved.

Keywords: Antioxidant, *Momordica dioica* leaves, Nephroprotective, Gentamycin toxicity and Albino rats.

INTRODUCTION

Drugs are a common source of acute nephrotoxicity. Drug toxicity [1] remains an important cause of acute kidney injury that in many circumstances can be prevented or at least minimized by vigilance and early intervention. Nephrotoxic drugs are penicillin, cephalosporins,tetracyclines and aminoglycosides..The drug induced nephrotoxicity[2] is manifested functionally by oliguria, enzymuria, proteinuria, glycosuria and proximal tubular dysfunction and lowering of glomerular filtration rate.

Gentamycin is a broad spectrum antibiotic [3] frequently used due to its high efficacy against gram negative bacteria. The principal side effect of this class of antibiotics is nephrotoxicity. Its use is restricted due to the development of ototoxicity and nephrotoxicity. *Momordica dioica* plant is also called as pazhupaagal .The plant belongs [4] to cucurbitaceae family. This plant is a good remedy for hepatic disorders, cancer, malaria and ulcer.

Since no work was reported relating to nephroprotective effect of *Momordica dioica* plant leaves, the present study was designed to investigate the nephroprotective effect of the ethanolic extract of *Momordica dioica* leaves on gentamicin induced nephrotoxicity in wistar rats.

MATERIALS AND METHODS

Plant material
The fresh leaves of *Momordica dioica* was procured and authenticated by Botanist Dr.P.Jayaraman, Chennai, india.

Preparation of the leaf extract
The authenticated leaves were shade dried and powdered coarsely. The powdered drug (500gm) was extracted in a soxhlet with pet-ether, chloroform and then using ethanol. The extract obtained was concentrated and evaporated until solvents were removed. The extract thus obtained was subjected to evaluation of nephroprotective activity.

Animals
The healthy Wister albino rats of either sex weighing between 150-200 gram were taken for the study. They were housed under controlled conditions of temperature (23±2^{o}c), humidity (55±5%) and 12hour light and 12hour 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 (Committee for the purpose of control and supervision of experiments on animals) guidelines, Ministry of Social Justice and Empowerment, Government of India. (IAEC approval no: IAEC/I/05/CLBMCP/2013 dated 15.2.13)

Acute toxicity study
Acute toxicity study [5] for ethanolic extracts of *Momordica dioica* leaves was conducted as per OECD guidelines 423 using albino wistar rats. Each animal was administered with the ethanolic solution of the plant extract by oral route (2000 mg/kg was used as starting dose). The animals were observed for any changes continuously for the first 2h and up to 24 h for mortality.

Evaluation of Neproprotective Activity in Gentamycin Induced Nephrotoxicity in Rats
Gentamicin induced model [6, 7] was used in this study. The rats were systematically divided into five groups of 6 rats each. Twelve hours before the experiment began, the rats were fasted of feed but distilled water was made available ad libitum.

Group I served as a control group and received distilled water p.o for 8 days. Group II served as gentamycin group. The gentamycin group received 80 mg/kg/day gentamycin by the intraperitoneal (i.p) route daily once for 8 days. Group III received Cystone 500mg/kg/day p.o as reference standard once daily for 6 weeks and alongwith gentamycin during last 8 days. Group IV rats received 200mg/kg b.w of ethanolic extracts of *Momordica dioica* leaves ($EEMD_{200}$) once daily orally for 6 weeks and alongwith gentamycin during last 8 days. Group-V received 400 mg/kg b.w of ethanolic extracts of *Momordica dioica* leaves ($EEMD_{400}$) once daily orally for 6 weeks and alongwith gentamycin during last 8 days respectively.

The weight of the animals in grams was noted on the first and last day of treatments. All animals were kept in individual metabolic cages and urine volume (24 hour urine) was measured on the first and last day of treatments. On the final day of the study, blood samples were collected via retro-orbital puncture and at the end of this 24 hour the serum was rapidly separated and processed for determination [8] of serum creatinine, serum urea, using diagnostic kits in a semi auto analyser. Blood urea nitrogen (BUN) concentration (mg/dl) was also measured as an indicator of renal function.

Kidney tissue specimens were collected and stored at -70 degree centigrade till used for estimation of renal antioxidant enzymes [9]. Collected 1gms of kidney tissue was washed with 1.15% potassium chloride solution and buffered 50 Mm potassium phosphate solution (pH 7.4) to yield 10% homogenate. Homogenization was done by using homogenizer and centrifuged at 8000 rpm for 20 minutes at 4 degree centigrade and the supernatant was collected for bio-chemical assays.

Statistical Analysis
Results were expressed as the Mean ± 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 ($P < 0.05$) was considered significant (*). Statistical analysis was performed using Graph Pad prism.

RESULTS

There was no change in the normal behavioural pattern of animals and no sign and symptoms of toxicity were observed during the first 2 hour and no mortality was observed till 24 hour. Extracts were safe up to a maximum

dose of 2000 mg/ kg b.w. The biological testing was carried out at doses of 200 and 400 mg/kg b.w of plant extracts by oral route.
When compared with control group, body weight of animals and daily urine volume were reduced in gentamycin in-toxicated animal group. When compared with gentamycin in-toxicated animal group, body weight of animals and daily urine volume were elevated to mormal in animals treated with standard drug cystone (Group-III) and in animals treated with plant extracts (Group-IV and V), described in Table I.

Values of Table II showed that Serum creatinine and serum urea levels were significantly increased in rats treated with only gentamycin, whereas treatment with the cystone and ethanolic extracts of leaves of *Momordica dioica* for 6 weeks reversed the effect of gentamycin indicating nephroprotective activity. Gentamycin administration to rats produced a pattern of nephrotoxicity infested by marked increase in serum BUN. Cystone or plant extracts supplementation to gentamycin treated rats recorded decrease in levels of serum blood urea nitrogen.The plant extract showed dose dependent nephroprotective effect. Values of Table II also showed that Serum albumin and serum total proteins levels were significantly reduced in rats treated with only gentamycin, whereas treatment with the cystone and ethanolic extracts of leaves of *Momordica dioica* for 6 weeks reversed the effect.

Values of Table III showed that antioxidants enzyme levels were significantly decreased in rats treated with only gentamycin, whereas treatment with the cystone or ethanolic extracts of leaves of *Momordica dioica* for 6 weeks, alongwith gentamycin in last 8 days reversed this effect indicating antioxidant and nephroprotective activity of cystone and ethanolic extracts of leaves of *Momordica dioica* .

Table 1: Effect of ethanolic leaf extract of *Momordica dioica* on body weight and urine volume

Groups	Change in body weight(gm)	Urine volume(ml)
Normal control	11.22 ± 1.22	6.88 ± 0.46
Toxic control	-24.56 ± 0.68*	2.46 ± 0.24*
Gentamycin + Standard	8.54 ± 0.88*	4.66 ± 1.44*
Gentamycin + EEMD$_{200}$	2.66 ± 1.24*	2.64 ± 0.88*
Gentamycin + EEMD$_{400}$	7.68 ± 0.44*	4.22 ± 0.26*

Table 2: Effect of ethanolic leaf extract of *Momordica dioica* on serum urea, serum creatinine, Serum albumin, Serum total protein and BUN (Blood Urea Nitrogen)

Group	Serumurea mg/dl	Serum creatinine (mg/dl)	Serum albumin(g/dl)	Serum total protein(g/dl)	BUN(mg/dl)
Normal control	24.26±0.42	0.56 ± 0.04	3.74 ± 1.22	5.88 ± 0.22	17.34 ± 0.88
Toxic control	78.98±1.34*	4.44±0.46*	2.24 ± 0.48*	3.44 ± 0.44*	58.44±1.40*
Gentamycin+Standard	33.46±1.44*	1.54±0.06*	3.44 ± 1.64*	5.56 ± 0.56*	22.22±1.20*
Gentamycin+EEMD$_{200}$	49.64±0.88*	2.64±0.68*	2.48 ± 0.24*	5.22 ± 1.46*	24.34 ± 44*
Gentamycin+EEMD$_{400}$	38.86±1.54*	1.88±0.98*	2.88 ± 0.68*	5.34 ± 0.34*	18.68±0.64*

(P < 0.05) was considered significant (*)
All the values are expressed as Mean ± SEM. One way Anova followed by Dunnets 't test

TABLE 3: Estimation of antioxidant enzymes in kidney homogenate

Group	Superoxide dismutase (SOD) Units/mg protein	Catalase (mmol/min/mg protein)	Glutathione peroxidise (mmol/min/ mgprotein)	Reduced glutathione (mmol/min/mgprotein)
Normal control	9.6 ± 0.46	0.64 ± 0.66	8.88 ± 1.20	4.66 ± 0.66
Toxic control	6.4 ± 0.98*	0.44± 1.64*	5.34 ± 1.33*	2.88± 0.46*
Gentamycin+Standard	8.7 ± 1.26*	0.9 ± 0.88*	7.66 ± 0.64*	3.34± 0.27*
Gentamycin+EEMD$_{200}$	6.4 ± 1.22*	1.55± 0.64*	8.64 ± 0.44*	3.22± 0.44*
Gentamycin+EEMD$_{400}$	9.5 ± 0.64*	0.83± 0.93*	7.77 ± 1.06*	3.14± 0.66*

DISCUSSION

Abnormalities of renal functions in nephrotoxic rats induced by gentamycin are decrease in urine concentrating capacity, increased urinary protein excretion (proteinuria), increased urinary excretion of lysosomal enzymes (enzymuria), alterations of proximal tubular cell transport processes, mild glucosuria, depression of glomerular filtration rate and tubular cell necrosis. Rise in serum creatinine is used as an indicator of nephrotoxicity. Elevated Reactive Oxygen Species generation by the gentamycin toxicity induces oxidative stress. *Momordica dioica* plant leaf ethanolic extracts treatment in rats efficiently reversed all these manifestations after the experimental period. Antioxidant activity of *Momordica dioica* leaves is the reason for the nephroprotective activity of this plant.

CONCLUSION

The results of our study revealed that the ethanolic extracts of *Momordica dioica* leaves possesses antioxidant and nephroprotective potential on the dose dependant manner and substantiate the therapeutic utility in renal injury. Further research is needed to elucidate the exact mechanism of nephroprotective action of the *Momordica dioica* plant leaves.

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