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Effect of *Tecoma Stans* Leaves Extract on Experimentally Induced Renal Injury In Various Animal Models

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ABSTRACT

In the current investigation, we have assessed the nephroprotective activity of the 70% ethanolic extract of *Tecoma stans* leaves (EETSL) against cisplatin, gentamicin and paracetamol (nephrotoxicants) induced nephrotoxicity in rats. Nephrotoxicity is confirmed by elevated kidney weight, blood urea, serum creatinine, lipid peroxidation (LPO), decreased tissue glutathione (GSH) and body weight levels at the administered doses. The ethanolic extract produced reduction in kidney weight, blood urea, serum creatinine, LPO levels and reversed the depleted GSH levels and body weight. This is further confirmed by histopathological studies. The results of the present study reveals that the ethanolic extract of *Tecoma stans* leaves significantly inhibited the cisplatin, gentamicin and paracetamol induced renal damage in rats and this may be attributed to its antioxidant properties.

Key words: *Tecoma stans*, Lipid peroxidation, GSH, Cisplatin, Gentamicin, Paracetamol, Renal injury.

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INTRODUCTION

Role of free radicals in the causation of diseases has been well established. Several substances have been known to produce excessive free radicals and thereby produce tissue damage[1]. Kidneys are among the highly vascularised organs receiving around 20-25% of cardiac output. Because of this reason they are constantly exposed to xenobiotics including environmental pollutants, many a times leading to impairment and damage to the kidney[2]. Reactive oxygen species produce deleterious effect on membrane lipids of the cellular components thereby producing lipid peroxidation leading to cell death[3]. Therefore, protection of kidney from pro-oxidants is essential by administering extracts of natural products and dietary antioxidants against nephrotoxicity[4].

Tecoma stans Linn (fam.: Bignoniaceae) is an erect shrub or small tree commonly known as Gante hoo in kannada and in Tamil Manja rali, Sonna patii, Swarna patii, which is planted in gardens in the plains throughout India in the hills upto an altitude 1,500mts[5]. It is recommended in diabetes mellitus, bacterial infections[6- 8], arterial hypotension, GIT disorders and various cancers. The plant is an effective remedy for snake and rat bites. It is also used as vermifuge and tonic[9, 10]. The literature revealed the presence of triterpenes, hydrocarbons, resins and volatile oils. The leaf contains flavonoids, tannins, traces of saponins, alkaloids, tecomine, tecostidine, beta carotene and zeaxantine[11, 12].

However no scientific information was available regarding nephroprotective activity, hence the present study is focused to evaluate the therapeutic effect of *Tecoma stans* leaves against cisplatin, gentamicin and paracetamol induced renal injury.

MATERIALS AND METHODS

Collection of plant material and extraction

Fresh leaves of *Tecoma stans* were collected locally and authenticated by Prof. K. Prabhu, Dept. of Pharmacognosy, S.C.S College of Pharmacy, Harapanahalli, India. A herbarium **specimen No. SCSCOP.Ph.Col Herb.No.012/2006-2007** was preserved in our college museum. The bark was shade dried separately at room temperature and pulverized. The powder was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. pet. Ether (60-80°), chloroform (59.5-61.5°), 70% ethanol (64.5-65.5°) and water. The 70% ethanol extract which was used for biological investigations and *in vivo* antioxidant studies, after subjecting it to preliminary qualitative phytochemical studies. The extracts were concentrated to a small volume using rotary flash evaporator.

Chemicals:

Cisplatin is obtained from Biochem labs, Mumbai, gentamicin from Sorus labs, Pithampur, paracetamol from Micro labs limited, Bangalore, the biochemical kits from Erba Manheim, Germany and all other chemicals were of analytical grade.

Animals:

Wistar albino rats (weighing 150-250g) and *albino mice* (weighing 20-25g) of either sex were used in this study. They were procured from Sri Venkateshwara Enterprises, Bangalore. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27° ± 2° with 12 hour dark/light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum*. The husk in the cages was renewed thrice a week to ensure hygeinity and maximum comfort for animals. The experimental protocols approved by Institutional Animal Ethics Committee (Ref.No.SCSCOP/665/2008-09 dated 24.11.2008) and the guidelines were followed accordingly.

Acute toxicity study:

Acute toxicity study was conducted on albino mice (20-25g) for EETSL as per OECD guideline No. 420 of CPCSEA[13.] The extract was found to be devoid of mortality at 2000mg/kg. Hence, 2500 mg/kg was considered as LD_{50} cutoff value. The doses at 1/ 10^{th} (250 mg/kg, p.o.) and 1/ 5^{th} (500mg/kg, p.o.) were selected for the evaluation of nephroprotective activity.

Experimental Design:

Healthy albino wistar rats were randomly assigned to 4 different groups having six animals in each group in all the models.

Cisplatin induced nephrotoxicity in rats[14]

Group 1(normal control) and 2 (positive control) received saline 1ml/kg p.o for 7days. Group 3 and 4 received 70% EETSL 250 and 500mg/kg b.w p.o for 7days respectively. On 2^{nd} day, 30 min after administration of scheduled doses to corresponding groups, the animals of groups 2, 3, and 4 cisplatin (6 mg/kg, i.v) was administered. The animals were sacrificed on 7^{th} day under mild ether anesthesia. Kidney tissues and blood samples were collected and assessed for tissue GSH as per the method of Ellman *et al*, lipid peroxidation according to the method of Ohkawa *et al*, blood urea and serum creatinine respectively.

Gentamicin induced nephrotoxicity in rats[15]

1^{st} group animals were administered saline (1 ml/kg, p.o) for 8 days. The 2^{nd} group of animals received gentamicin (80 mg/kg, i.p) for 8 days. The group 3^{rd} and 4^{th} received gentamicin (80 mg/kg i.p) for eight days and 70% EETSL (250 and 500 mg/kg p.o) was started three days prior

to the gentamicin injections and continued for eight days with gentamicin. At the end of experiment the animals were sacrificed under mild ether anesthesia, the kidney tissues and blood samples were collected. The samples of kidney tissue were analyzed for tissue GSH and lipid peroxidation and blood samples for estimation of biochemical parameters.

Paracetamol induced nephrotoxicity in rats[16]

Group1 (normal control) administered saline (1ml/kg p.o) daily for 7days. Group 2 (positive control) animals were similarly administered saline as group1. Group 3 and 4 animals were administered 70% EETSL 250mg/kg and 500 mg/kg p.o for 7days, respectively. On the 5th day, 30min after respective administration to groups 2, 3 and 4, paracetamol 2gm/kg was given p.o. After 48 hr of paracetamol administration rats were subjected to mild ether anesthesia and blood sample collected for evaluating the serum biochemical parameters. The kidney tissues were analyzed for tissue GSH and lipid peroxidation.

Histopathology[17]:

Pieces of kidney from each group in all the 3 experimental models were fixed immediately in 10% neutral formalin for a period of atleast 24 h, dehydrated in graded (50–100%) alcohol, embedded in paraffin, cut into 4–5 μm thick sections and stained with hematoxylin- eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

Statistical Analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Tukey's Kramer comparison test by using Graph Pad Instat Software. P value less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Effects of 70% EETSL of *Tecoma stans* leaves on cisplatin, paracetamol and gentamicin induced nephrotoxicity

Cisplatin, gentamicin and paracetamol treated rats showed a significant increase in serum marker enzymes like blood urea, serum creatinine and increased kidney weight and also there is marked depletion of tissue GSH levels, body weight and increased lipid peroxidation levels when compared with control. 70% ethanol extract pretreated rats showed significantly ($P<0.001$) decreased levels of serum marker enzymes, kidney weight, restoration of tissue GSH and inhibition of lipid peroxidation levels when compared with the nephrotoxicants treated rats. However, there was prevention of reduction in body weight significantly in a dose dependent manner. The results are summarized in table I to III.

Table I. Effect of 70% EETSL in Cisplatin induced renal damage in rats

(n=6)	Treatment regimen	Kidney weight (g/100g)	Change in b.w. (%)	Blood urea (mg/dl)	Serum creatinine (mg/dl)	GSH levels		LPO levels	
						Absorbance Mean ± SEM	% Increase	Absorbance Mean ± SEM	% Inhibition
1	Vehicle treatment (Negative control)	0.90± 0.06	7.3±0.667	34.16±2.92	0.53±0.02	0.88 ±0.015	--	0.121±0.025	--
2	Cisplatin 6mg/kg i.v. on 2nd day (Positive control)	1.08±0.04	-16.3±1.75	71.58±1.71	1.95±0.105	0.390±0.023	--	0.484±0.018	--
3	Cisplatin 6 mg/kg i.v. on 2nd day +70% ethanolic extract 250 mg/kg p.o. for 6 days	1.28±0.001***	-9.25±0.289**	32.16±0.83***	0.66±0.049***	0.483±0.01***	23.89	0.200±0.01***	58.67%
4	Cisplatin 6 mg/kg i.v. on 2nd day +70% ethanolic extract 500 mg/kg p.o. for 6 days	1.34±0.004***	-6.78±1.18***	31.83±1.49***	0.63±0.061***	0.495±0.01***	26.92	0.177±0.01***	63.42%

Values are the Mean ± S.E.M. of six rats / treatment
Significance ***P<0.05, ***P<0.001 (vs. Control)., b.w. – Body weight

Table II. Effect of 70% EETSL in Gentamicin induced renal damage in rats

(n=6)	Treatment regimen	Kidney weight (g/100g)	Change in b.w. (%)	Blood urea (mg/dl)	Serum creatinine (mg/dl)	GSH levels		LPO levels	
						Absorbance Mean±SEM	% Increase	Absorbance Mean± SEM	% Inhibition
1	Vehicle treatment (Negative control)	1.61±0.12	13.74±1.26	45.09 ± 3.02	0.74±0.05	0.70± 0.01	--	0.167±0.003	--
2	Gentamicin 80 mg/kg i.p. for 8days (Positive control)	1.03±0.06	-7.59±1.01	81.47±6.22	1.60±0.07	0.25±0.01	--	0.462±0.029	--
3	Gentamicin 80 mg/kg i.p. for last 8 days + 70% ethanolic extract 250 mg/kg p.o. for 11 days	1.27±0.06*	-4.51±1.2*	21.10±1.0***	1.05±0.13**	0.41±0.03***	64.00%	0.280±0.08*	39.39%
4	Gentamicin 80 mg/kg i.p. for last 8 days + 70% ethanolic extract 500 mg/kg p.o. for 11 days	1.37±0.04**	-1.95±1.31**	14.78±1.47***	1.01±0.15**	0.49±0.01***	96.00%	0.129±0.00***	72.07%

Values are the Mean ± S.E.M. of six rats / treatment
Significance ***P<0.05, ***P<0.001 (vs. Control)., b.w. – Body weight

Table III. Effect of EETSL in paracetamol induced renal damage in rats

Gr. (n=6)	Treatment regimen	Blood urea (mg/dl)	Serum creatinine (mg/dl)	GSH levels		LPO levels	
				Absorbance Mean ± SEM	% Increase	Absorbance Mean ± SEM	% Inhibition
1	Negative Control (1ml vehicle)	36.22±3.30	0.58±0.02	0.815±0.005	--	0.269±0.017	--
2	Positive Control Paracetamol (2 g/kg p.o.)	86.56±3.52	1.88±0.03	0.395±0.003	--	0.317±0.010	--
3	Paracetamol + 70% ethanolic extract (2 g/kg p.o. +250 mg/kg p.o.)	43.56±3.27***	1.05±0.143**	0.480±0.00***	21.51%	0.193±0.03***	39.31%
4	Paracetamol + 70% ethanolic extract (2 g/kg p.o. +500 mg/kg p.o.)	43.10±3.38***	0.93±0.182***	0.530±0.00***	34.17%	0.148±0.03***	53.31%

Values are the Mean ± S.E.M. of six rats / treatment
Significance ***P<0.05, ***P<0.001 (vs. Control)., b.w. – Body weight

Histopathological Studies in cisplatin, paracetamol and gentamicin induced nephrotoxicity

Fig. 1, 5 and 9 shows the light micrograph of control kidney showing normal architecture with normal glomeruli. The nephrotoxicants induced rat kidney showed the glomerular congestion and severe interstitial congestions (Fig.2, 6 and 10). Pretreatment with 70% EETSL 250 and 500mg/kg demonstrated marked improvement with maintained glomeruli and no interstitial congestion (Fig. 3, 4, 7, 8, 11 and 12) as compared to nephrotoxicants administered groups.

Histopathological Studies in cisplatin, paracetamol and gentamicin induced nephrotoxicity

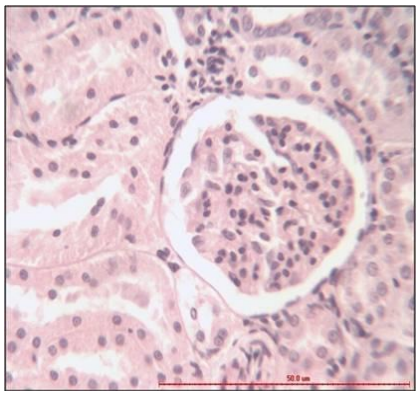

Figure.1(vehicle)

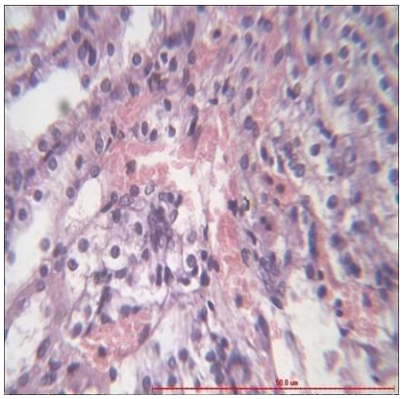

Figure.2 (Cisplatin)

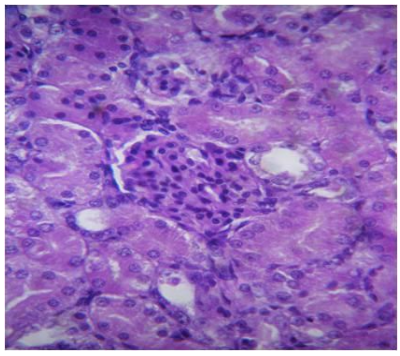

Figure.3(Cisplatin +250mg/kg70% EETSL)

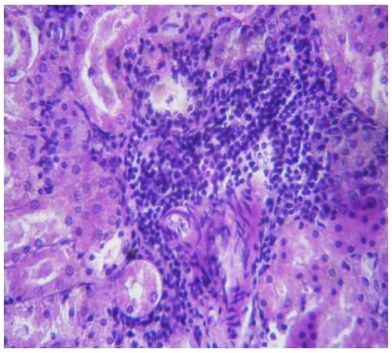

Figure.4(Cisplatin + 500mg/kg70% EETSL)

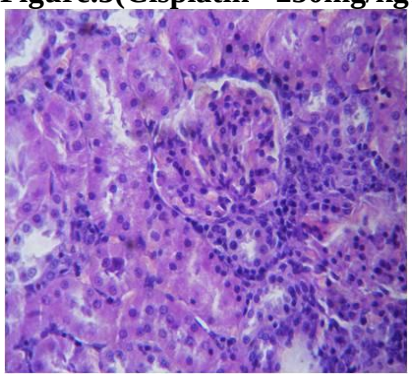

Figure. 5(vehicle)

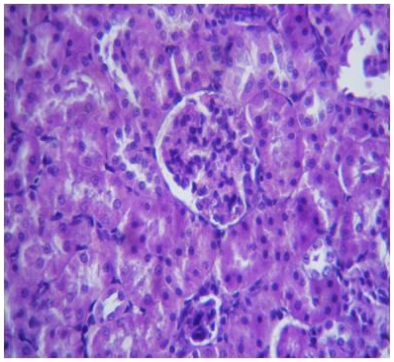

Figure. 6 (Gentamicin)

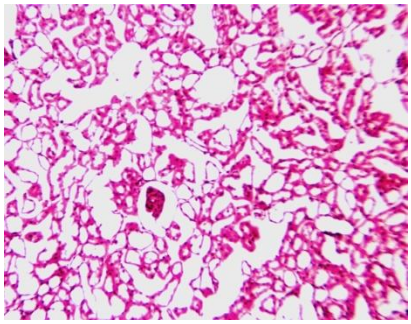

Figure.7(Gentamicin+250mg/kg70%EETSL)

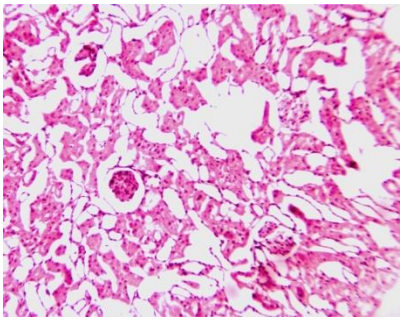

Figure.8(Gentamicin+500mg/kg70%EETSL)

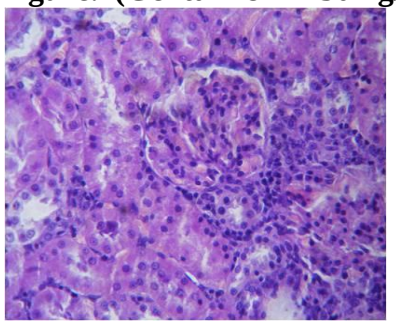

Figure.9(vehicle)

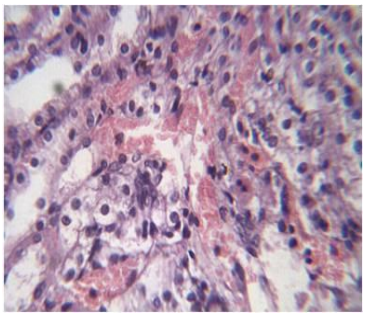

Figure.10(Paracetamol)

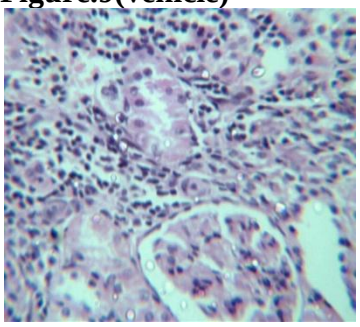

Figure11(Paracetamol+250mg/kg70%EETSL)

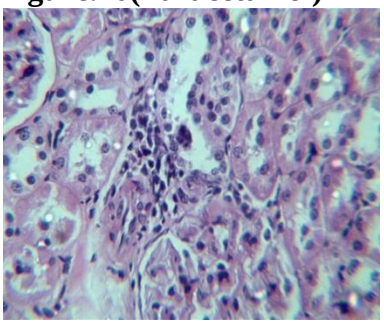

Figure12(Paracetamol+500mg/kg70%EETSL)

Kidneys are involved in the excretion of various xenobiotics pollutants, toxins and hence they are prone to liberate high quantities of free radicals. Therefore this organ is prone to be destroyed by such highly reactive free radicals[18]. Nephrotoxicity occurs as a disturbance in renal function due to various drug interactions and inadequate elimination of chemicals. This may limit the clinical usefulness of many therapeutic agents[19].

In the current investigation, the 70% EETSL was subjected to nephroprotective activity by against various nephrotoxicants such as cisplatin, gentamicin and paracetamol in rats. Biochemical markers of kidney function like blood urea, serum creatinine levels, body weight, kidney weight, tissue GSH and lipid peroxidation were considered for assessing the nephroprotective properties. Cisplatin administration enhanced the serum markers like blood urea and serum creatinine. These findings are in conformity with the earlier reports. Co-administration of 70% EETSL reduced the elevated biochemical marker levels to a significant extent. Hence it may be inferred that 70% EETSL possess nephroprotective activity against

cisplatin challenge. The evidences indicate that cisplatin induced nephrotoxicity via oxidative stress which increases the generation of free radicals and increase lipid peroxidation. Even there are reports that cisplatin inhibits the activities of antioxidant enzymes in rat kidneys suggesting the cytotoxicity of it and this may be due to generation of reactive oxygen species[20, 21].

Gentamicin administration exhibited a marked decrease in body weight, tissue GSH level and increased kidney weight and lipid peroxidation levels which is supported by a significant increase in serum markers like blood urea and serum creatinine. Co-administration of test extract normalized the blood urea, serum creatinine, tissue lipid peroxidation level and prevented the reduced tissue GSH level. There are reports that gentamicin induced nephrotoxicity is due to generation of superoxide, hydroxyl radical and hydrogen peroxide free radicals[15.] Similarly the paracetamol increased the blood urea and serum creatinine levels in addition to enhanced tissue lipid peroxidation and decreased tissue GSH levels. Co-administration of test extract reversed all the above mentioned parameters of nephrotoxicity to the near normal levels. Since the paracetamol induced nephrotoxicity was reported to be via NAPQI radical, the nephroprotective activity of test extract in this model is also attributed to the antioxidant activity of the plant[22].

The nephro protective property of the extract is further confirmed by significant improvement of the kidney architecture by reversal the glomerular congestion, interstium with inflammatory cells, tubular necrosis, peritubular necrosis and presence of caspe suggesting massive total necrosis over cisplatin, paracetamol and gentamicin group. Further our earlier studies revealed that the test extract possesses antioxidant principles like phenolic and flavonoids. Therefore it may be concluded that nephroprotective activity observed in the study due to antioxidant principles.

CONCLUSION

It may conclude that the leaves of the *Tecoma stans* possesses profound nephroprotective activity by preventing alterations in marker enzyme activity and cellular damage due to its antioxidant potential. Therefore these leaves may be used along with cisplatin cancer treatment to prevent organ toxicity.

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