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Effect Of Tecoma stans Leaves For Its Preventive Role On Experimentally Induced Liver Toxicity

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Abstract: Liver plays a vital role in metabolism and excretion. Any injury to it or impairment of its function may lead to many implications on one's health. Liver ailments need to be treated with utmost care. Management of liver diseases is still a challenge to modern medicine. The allopathic medicine has little to offer for the alleviation of hepatic ailments whereas the most important representatives are of phytoconstituents. **Objective:** To assess the hepatoprotective activity of ethanolic extract of *Tecoma stans leaf against* CCl4 and thioacetamide induced liver injury in rats followed by *in-vivo* antioxidant property. **Methodology:** The acute liver damage in albino rats was induced by a single dose of thioacetamide (100mg/kg s.c) and CCl_4 at dose of 2ml/kg. on 2nd and 3rd day. The hepatoprotective activity was monitored biochemically by like SGPT, SGOT, ALP, total bilirubin and direct bilirubin, LPO and GSH were estimated in addition to the measurement of liver weight, liver volume. The histopathological changes of liver samples were compared with that of control. **Results:** All the biochemical markers, liver weight, liver volume, lipid peroxidation (LPO) of hepatic injury were elevated and reduced Glutathione (GSH) upon Thioacetamide and CCL_4 challenge and they were brought down to near normal levels by pretreatment with 250 mg/kg and 500 mg/kg of test extract in both the models. The elevated levels of liver weight, liver volume and destructed liver architecture were normalized by the treatment with test extract. **Conclusion:** The results are indicating that test extract possesses hepatoprotective property against Thioacetamide and CCL_4 induced hepatic damage. The study was concluded as plant possesses *invivo* antioxidant and hepatoprotective property. The antioxidant and hepatoprotective property may be attributing to the phenol and flavonoids content of the plant.
Keywords: *Tecoma stans*, Thioacetamide, CCL_4, Lipid peroxidation, hepatoprotective.

Introduction

The liver is the prime organ concerned with various states of metabolic and physiologic homeostatis of the body[1]. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being[2]. Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated[3]. However there are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders. Therefore, many folk remedies from plant origin are tested for its potential hepatoprotection in animal models.

The plant *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[4]. It is recommended in diabetes mellitus, bacterial infections[5-7], 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[8, 9]. 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[10, 11].

The present study was performed to assess the hepatoprotective activity of Ethanolic extract of *Tecoma stans* leaves in rats against Thioacetamide and CCL_4 as hepatotoxin to prove its claim in folklore practice against liver disorders.

Materials And Methods

Plant material: *Tecoma stans* leaves were collected from Sree Bettada Malleswara temple near to Harapanahalli. The plant was identified and authenticated by Prof. K. Prabhu, Head, Dept of Pharmacognosy, S.C.S. College of pharmacy, Harapanahalli-583131. A herbarium **specimen No. SCSCOP.Ph.Col Herb.No. 012/2006-2007** was preserved in our college museum.

Preparation of extract: The dried powder of leaves were defatted with petroleum ether, chloroform and then extracted with 70% ethanol using soxhlet apparatus. The extracts was concentrated under reduced pressure using rota flash evaporator and stored in airtight container in refrigerator below 10ºC.

Chemicals: Thioacetamide and CCL_4 is obtained from SD fine chemicals, Mumbai, Silymarin 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.

Determination of oral acute toxicity: The acute toxicity was determined on albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose method of OECD guideline No.420 given by CPCSEA[12] was adopted for toxicity studies. Ethical clearance for handling the animals was obtained from the IAEC prior to the beginning of the project work, the registration no. is as mentioned above.

Experimental Design:

Healthy albino Wistar rats were randomly assigned to 5 different groups having six animals in each group in all the models.

a) Thioacetamide induced hepatotoxicity in rats[13]

Group 1(normal control) and 2 (positive control) received saline 1ml/kg p.o for 9days. Group 3 received Silymarin 100 mg/kg p.o., for 9 days, group 4 and 5 received 70% EETSL 250 and 500mg/kg b.w p.o for 9 days respectively. On 7th day, 30 min after administration of scheduled doses to corresponding groups, the animals of groups 2, 3, 4 and 5 Thioacetamide (100 mg/kg, s.c) was administered which was prepared in distilled water (2% solution). The animals were sacrificed 48 hr. after the administration of Thioacetamide under mild ether anaesthesia. The liver sample was dissected out, blotted off blood, washed with saline for wet liver weight, liver volume, GSH estimation as per the method of Ellman *et al*, lipid peroxidation according to the method of Ohkawa *et al* and stored it in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically. The blood so collected was centrifuged immediately to get clear serum and was subjected to various biochemical studies like SGPT, SGOT, ALP, direct and total bilirubin.

b) CCL_4 induced nephrotoxicity in rats[14]

Group-I received liquid paraffin (1ml/kg) s.c., on 2nd and 3rd day. Group-II, III, IV and V received CCl_4: liquid paraffin (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd day, after 30 min of vehicle, 100mg/kg silymarin, 250 mg/kg and 500 mg/kg EETSL administration. Animals were sacrificed on the 6th day under mild ether

anesthesia. Blood samples were collected for evaluating the serum biochemical parameters as said above. The liver samples were dissected out, blotted off blood, washed with saline and used to estimate the wet liver weight, liver volume, GSH estimation and lipid peroxidation estimation and stored it in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

Histopathology[15]:

Pieces of liver from each group in all the 2 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 hepatotoxicity such as extensive fatty change, ballooning of hepatocytes 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:

Effects of 70% EETSL on Thioacetamide and CCL_4 induced hepatotoxicity

Thioacetamide and CCL_4 treated rats showed a significant increase in serum marker enzymes like SGOT, SGPT, ALP, total bilirubin, direct bilirubin and increased wet liver weight, liver volume and also there is marked depletion of tissue GSH levels and increased lipid peroxidation levels when compared with control. Silymarin and 70% ethanol extract pretreated rats showed significantly ($P<0.001$) decreased levels of serum marker enzymes, wet liver weight, liver volume, restoration of tissue GSH and inhibition of lipid peroxidation levels when compared with the hepatotoxicants treated rats. The effects are statistically significant in a dose dependent manner. The results are summarized in table 1 to 4.

Histopathological Studies in Thioacetamide and CCL_4 induced hepatotoxicity

Fig. 1 and 6 shows the light micrograph of control liver showing hepatic globular structure, central vein, portal tract and kupffer cells look normal. The hepato-toxicants induced rat liver showed the extensive fatty change and ballooning of hepatocytes, more around central vein (Fig.2 and 7). Pretreatment with Silymarin, 70% EETSL 250 and 500mg/kg demonstrated marked improvement with mild fatty change, with no congestion (Fig. 3, 4, 5, 8, 9 and 10) as compared to hepatotoxicants administered groups.

Discussion

The aim of current study was to evaluate protective effects of *Tecoma stans* leaves extracts on Thioacetamide and CCl_4 mediated liver damage in rats. Liver toxicity caused by Thioacetamide and CCl_4 is perhaps widely used animal model for the assessment of hepatoprotective agent[16]. Thioacetamide is a potent hepatotoxin, the hepatotoxicity induced by liver is due to the production of its metabolite thioacetamide-5-oxide, which directly acts on various cellular component and destroy it. In addition thioacetamide is also metabolised by CYP 450 $2E_1$ isoenzymes rendering sulfone and sulfoxy derivative, which are apparently responsible for inactivation of structural proteins and enyzmes[17].CCl_4 is the inactive metabolite which is biotransformed by cytochrome P-450 systems to produce the trichloromethyl free radical ($CCl_3\bullet$) that causes lipid peroxidation and thereby produce liver damage[18-20].

The dose dependent reduction of Thioacetamide and CCl_4 induced increased plasma activities of SGPT, SGOT, ALP, total and direct bilirubin levels in animals pre treated with 70 % EETSL demonstrated their ability to restore the normal functional status of the toxic liver, and also to protection against subsequent Thioacetamide and CCl_4 liver damage.

Table 1. Effect of 70% EETSL on liver weight, liver volume and biochemical markers in thioacetamide induced hepatotoxicity

Treatment	Liver		Biochemical parameters Mean ï SEM				
	Volume (ml/100g)	Weight (g/100g)	SGOT U/L	SGPT U/L	ALP IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
Negative control (1ml vehicle)	6.2±0.14	6.19±0.27	167.20±3.57	111.06±2.56	149.67±2.73	0.85±0.02	0.24±0.016
Thioacetamide (positive control) (100mg/kg s.c.)	4.66±0.21	6.37±0.21	125.66±0.98	129.83±0.47	199.66±2.44	1.78±0.03	0.45±0.02
Thioacetamide + Silymarin (100mg/kg, s.c. + 100mg/kg, p.o.)	6.02±0.11***	5.97±0.17**	26.8±1.15***	32.65±0.74***	94.00±2.70***	0.86±0.06***	0.32±0.02**
Thioacetamide+70% EETSL (100mg/kg s.c.+ 250mg/kg p.o.)	5.80±0.18***	4.90±0.28**	36.44±3.35***	46.21 ± 4.10***	101.00±2.40***	1.16±0.09***	0.38±0.03***
Thioacetamide+70% EETSL (100mg/kg s.c.+ 500mg/kg p.o.)	4.80±0.17*	4.85±0.35**	30.40±4.50***	41.8±4.03***	90.20±7.17***	0.90±0.10***	0.36±0.04**

Values are the mean ± S.E.M. of six rats/ treatment.
Significance **P <0.01 and *** P<0.001, compared to thioacetamide treatment.

Table 2. Effect of 70% EETSL on tissue GSH and *in-vivo* lipid peroxidation levels in thioacetamide induced hepatotoxicity

Treatment	Absorbance Mean ï SEM	% Increase	Absorbance Mean ï SEM	% Inhibition
Negative Control (1ml vehicle)	0.85 ± 0.05	--	0.25 ± 0.01	--
Positive Control Thioacetamide (100 mg/kg s.c.)	0.19± 0.01	--	0.39 ± 0.016	--
Thioacetamide + Standard (Silymarin) (100 mg/kg s.c. + 100 mg/kg p.o.)	0.78 ± 0.04***	310.52%	0.18 ± 0.004***	53.84%
Thioacetamide + 70% EETSL (100 mg/kg s.c. + 250 mg/kg p.o.)	0.59 ± 0.02***	210.52%	0.27 ± 0.002***	44.44%
Thioacetamide + 70% EETSL (100 mg/kg s.c. + 500 mg/kg p.o.)	0.65 ± 0.019***	242.10%	0.23 ± 0.006***	41.02%

Values are the mean ± S.E.M. of six rats/ treatment., Significance *** P<0.001, compared to thioacetamide treatment.

Table 3. Effects of 70% EETSL on wet liver weight, liver volume and biochemical markers in CCl_4 induced hepatotoxicity

Treatment	Liver		Biochemical parameters Mean ï SEM				
	Volume (ml/100g)	weight (gm/100g)	SGOT U/L	SGPT U/L	ALP IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
Negative Control (1ml vehicle)	6.52 ±0.09	6.25±0.09	138.81±9.65	109.75±2.49	134.83±06.14	0.83±0.028	0.17±0.012
Positive Control CCl_4 + Liq. Paraffin (1:1) (2 ml/kg s.c.)	8.3±0.12	8.69±0.18	328.68±4.94	166.22±5.80	324.80±5.15	2.36±0.098	1.5±0.00
CCl_4+ Standard (Silymarin) (2 ml/kg s.c.+100 mg/kg p.o.)	6.65±0.047*	6.33±0.01**	141.17±1.24***	90.08±1.03**	215.32±3.45***	0.92±0.02***	0.31±0.01***
CCl_4 + 70% EETSL (2 ml/kg s.c.+250 mg/kg p.o.)	6.25±0.57**	7.35±0.74*	288.28±30.18***	117.43±10.27**	203.73±14.44***	1.41±0.07***	1.15±0.03***
CCl_4 + 70% EETSL (2 ml/kg s.c+500 mg/kg. p.o.)	6.08±0.55**	6.40±0.57**	281.05±38.59***	108.80±12.73**	190.36±17.15**	1.18±0.09***	0.56±0.02***

Values are the mean ± S.E.M. of six rats/ treatment.
Significance *P<0.05, **P <0.01 and *** P<0.001, compared to CCl_4 treatment

Table 4. Effect of 70% EETSL on tissue GSH and *in-vivo* lipid peroxidation levels in CCl_4 induced hepatotoxicity

Treatment	Absorbance Mean ï SEM	% Increase	Absorbance Mean ï SEM	% Inhibition
Negative Control (1ml vehicle)	0.940 ± 0.012	--	0.065 ± 0.016	--
Positive Control CCl_4 + Liq. Paraffin (1:1) (2 ml/kg s.c.)	0.760 ± 0.05	--	0.40 ± 0.04	--
CCl_4 + Standard (Silymarin) (2 ml/kg s.c.+ 100 mg/kg p.o.)	0.890 ± 0.017***	17.10%	0.092 ± 0.003***	77.0%
CCl_4 + 70% EETSL (2 ml/kg s.c. + 250 mg/kg p.o.)	0.820 ± 0.007*	7.89%	0.33 ± 0.002 ns	17.5%
CCl_4 + 70% EETSL (2 ml/kg s.c. + 500 mg/kg p.o.)	0.84 ± 0.005**	10.52%	0.29 ± 0.009**	27.5%

Values are the mean ± S.E.M. of six rats/ treatment.
Significance ***P<0.001, compared to CCl_4 treatment.

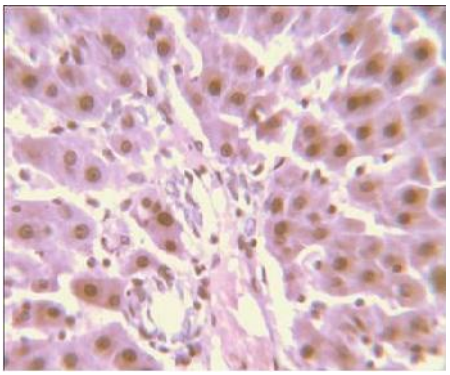

Fig.1 Negative Control (1ml vehicle)

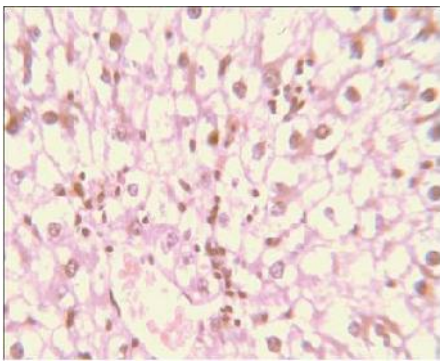

Fig.2 Positive Control Thioacetamide (100 mg/kg s.c.)

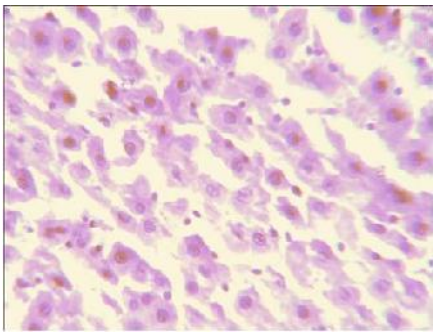

Fig.3 Thioacetamide (100 mg/kg s.c.) Standard (Silymarin 100 mg/kg p.o.)

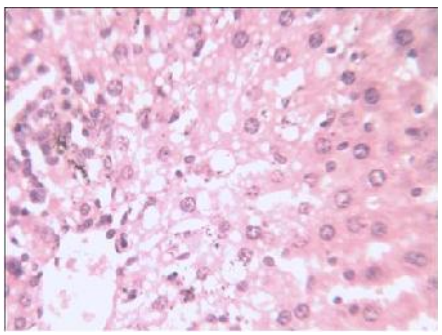

Fig.4 Thioacetamide(100 mg/kg s.c.) + 70% EETSL 250 mg/kg p.o.)

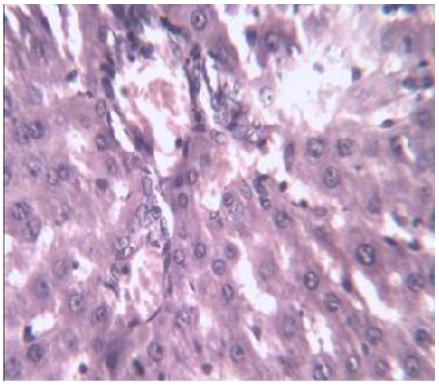

Fig. 5 Thioacetamide(100 mg/kg s.c.)
+ 70% EETSL 500 mg/kg p.o.)

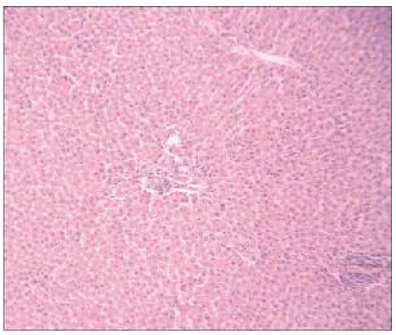

Fig.6 Negative Control
(1ml vehicle)

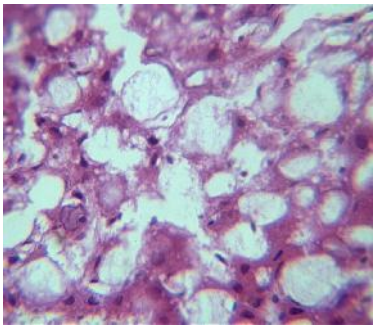

Fig.7 Positive Control
CCl_4 + Liq. Paraffin (1:1)(2 ml/kg s.c.)

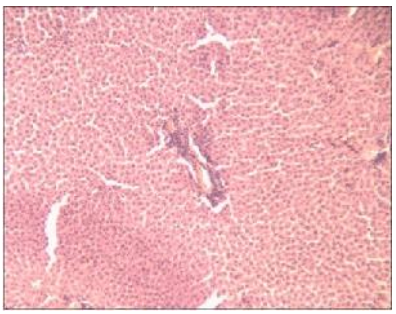

Fig.8 CCl_4 + Liq. Paraffin (1:1)(2 ml/kg s.c.)
Standard (Silymarin 100 mg/kg p.o.)

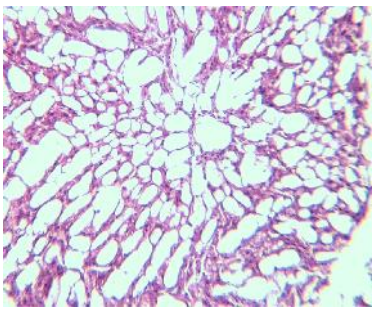

Fig.9 CCl_4 + Liq. Paraffin)(2 ml/kg s.c.)
+ 70% EETSL 250 mg/kg p.o.)

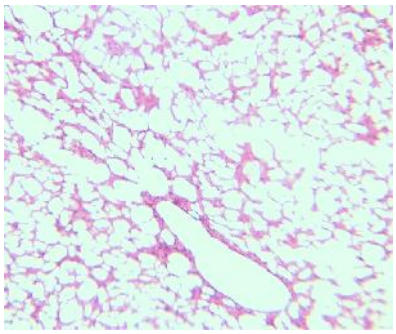

Fig.9 CCl_4 + Liq. Paraffin)(2 ml/kg s.c.)
+ 70% EETSL 500 mg/kg p.o.)

The development of hepatotoxicity induced by Thioacetamide and CCl_4 challenge was exacerbated following the depletion of glutathione. Therefore, in the current study glutathione content was measured to observe the preventive effects of EETSL in rats. The results are clearly demonstrated that Thioacetamide and CCl_4 intoxication has significantly reduce the glutathione level compared to normal control animals. Rats on pre treatment with EETSL have clearly restored the glutathione levels significantly in dose dependant fashion. The Thioacetamide and CCl_4 mediated liver injury was associated with marked increase in lipid peroxidation levels. This is one of the reliable measure of hepatotoxicity due to oxidative stress. From the results it is clearly indicated that Thioacetamide and CCl_4 intoxicated rats showed significant increase in the lipidperoxidation levels compared to normal control group. Rats on pre treatment with EETSL (250 and 500 mg/kg, doses) have clearly decrease the levels of lipid peroxidation significantly in dose related manner.

The results obtained from the present investigation suggest, leaves extract of the *Tecoma stans* possess significant preventive effects against Thioacetamide and CCl_4 damaged rat liver. Further, preliminary phytochemical investigation revealed that the test extract showed presence of flavonoids, tannins, alkaloids, saponins and glycosides. Thus, it revealed that the hepatoprotection offered by titled plant extract may be due to its flavonoid content.

Conclusion:

In conclusion, 70 % ethanolic extract of *Tecoma stans* leaves exhibited dose dependent significant hepatoprotective effect against Thioacetamide and CCl_4 induced hepatotoxicity in rats. This effect may be attributed to the polyphenolic constituents that are present in *Tecoma stans*.

Acknowledgements

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