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Der Pharmacia Lettre, 2010: 2 (1) 261-264 (http://scholarsresearchlibrary.com/archive.html)



Hepatoprotective effects of 50% ethanolic extract of *Mimosa* pudica against CCl₄ induced hepatotoxicity in rats

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Abstract

The hepatoprotective effect of 50% ethanolic extract of *Mimosa pudica* L (Fabaceae) by carbon tetrachloride (CCl₄) induced liver damage in rats. The 50% ethanolic extract of *M. pudica* was studied for their hepatoprotective effects on CCl₄ induced liver damage on Wistar albino rats. The degree of protection was measured by physical changes (liver weight), biochemical (SGPT, SGOT, ALP, total bilirubin, albumin and decreases in total protein). Pretreatment with extract significantly prevented the physical, biochemical changes induced by CCl₄ in the liver. The effects of extract of *M. pudica* were comparable to that of standard drug, silymarin. These results indicate that the *M. pudica* could be useful in preventing chemically induced acute liver injury. From this study, it can be concluded that the 50% ethanol extracts of *M. pudica* possesses significant hepatoprotective activity.

Keywords: Carbon tetrachloride, hepatoprotective, M. pudica.

Introduction

Mimosa pudica is identified in Ayurvedic literature as Lajjalu. In contemporary medicine, *Mimosa pudica* is being investigated for its potential to yield novel chemotherapeutic compounds. It contains an alkaloid called mimosine which has been found to have potent antiproliferative and apoptotic effects. The aerial parts of *M. pudica* have been extensively studied for their hepatoprotective activity. The present pharmacological investigations focus on evaluation of the efficacy of aqueous and ethanol extracts of *M. pudica* for its protection against carbon tetrachloride induced hepatotoxicity in rats.

Materials and methods Plant material

Leaves of *Mimosa pudica* was collected in and around shevaran hills, Salem, Tamilnadu India in the month of September 2009 and identification done by Professor (Dr) P. Jayaraman, Plant Anatomy research Centre, Chennai, India. The voucher specimen of M.

pudica (MP/88/09) has been preserved in our laboratory for further collection and reference. The leaves were dried under shade, powdered with a mechanical grinder and pass through sieve no 40 and were extracted with 50% ethanolic 48 h using soxhlet apparatus. The solvent was removed from the extract under reduced pressure by using rotary vacuum evaporator.

Phytochemiclal analysis

The 50% ethanolic extract of *M. pudica* was subjected to identify the presence of various phytoconstituents viz. alkaloids (Dragendroffs test), steroids and terpenoids (Leibermann Burchard test), tannin and phenolic compounds (ferric chloride test), flavonoids (Shinoda test), amino acids (Ninhydrin test), etc. by usual methods prescribed in standard texts [1, 2]

Experimental animals

Wistar albino rats (150-200 g) used in the present studies. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use. The animals were received the drug by oral gavages tube. The laboratory conditions duly undertaken by registered veterinary practitioner.

Chemicals

All the chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. The standard drug silymarin was obtained as gift sample from Micro Lbs, India. Standard kits for SGOT, SGPT, ALP and bilirubin were obtained from Span Diagnostics Ltd., India.

Toxicity studies

Healthy Wistar albino rats of either sex weighing 150-200 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Co-operation and Development guidelines 423 (OECD, 1996). A total of three animals were used which received a single oral dose of (2000 mg/kg) of 50% ethanolic extract. After administration of extract the food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for period of 3 days.

CCl₄ induced hepatotoxicity rat

The rats were divided into seven groups of 6 animals (n = 6) in each [3]

Group I: Received water (5 ml/kg. p.o) for 9 days once daily, and served as normal control.

Group II: Received water (5 ml/kg. p.o) for 9 days once daily and carbon tetra chloride (CCl₄)1 ml/kg in 50% v/v olive oil, s.c. on 7^{th} day.

Group III: Received standard drug silymarin (25 mg/kg. p.o.) for 9 days once daily and carbon tetra chloride (CCl_4) 1 ml/kg in 50% v/v olive oil, s.c. on 7th day.

Group IV and V: Received 50% methanolic extract of *M. pudica* (200 and 400 mg/kg) 9 days once daily and carbon tetra chloride (CCl₄)1 ml/kg in 50% v/v olive oil, s.c. on 7^{th} day.

Assessment of hepatotoxicity

After 48 h of carbon tetrachloride administration, the blood was obtained from animals by puncturing retro orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. The serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT and SGPT [4], ALP [5], serum bilirubin [6] and serum protein [7].

After collection of blood samples, the animals were sacrificed under deep ether anesthesia and their livers were excised immediately and washed with ice cold saline and a 10% homogenate prepared in phosphate buffer (pH 7.0). The homogenate was centrifuged at 3000

rpm for 15 min at 4°C and the supernatant was used for the estimation glutathione [8] and lipid per oxidation [9].

Statistical Significance

The results of the study were expressed as mean \pm SEM, n = 6. ANOVA [10] was used to analyze and compare the data, followed by Dunnett's test for multiple comparisons.

Results

Preliminary phytochemical studies revealed the presence of various phytochemicals including alkaloids, glycosides, steroids, flavonoids, saponin, tannin and phenolic compounds, terpenoids, carbohydrates, gums and mucilage in 50% methanolic extract was found to be nontoxic up to a dose of 2000 mg/kg.

CCl₄ caused significant elevation of serum liver enzymes and bilirubin. Treatment with 50% methanolic extract of *M. pudica* (200 and 400 mg/kg) caused significant hepatoprotective effect was almost comparable to that of silymarin, the known hepatoprotective agent (Tables 1 and 2).

Table 1. Effect of 50% ethanolic extract of *Mimosa pudica* on biochemical parameters viz SGPT, SGOT, ALP in CCl₄ induced hepatotoxicty in rats

Treatment/Dose/ mg/kg	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Control	58.74 ± 7.740	106.28 ± 9.23	83.55 ± 6.52
CCl ₄ (1ml/kg i.p)	170.81 ± 7.55	223.72 ± 15.87	138.27 ± 7.97
Silymarin 100	68.87 ± 4.23**	135.68 ± 3.77**	89.36 ± 5.74**
M. pudica 200	92.46 ±4.73**	$158.54 \pm 8.00 **$	$109.07 \pm 8.33^*$
M.pudica 400	$64.09 \pm 3.76^{**}$	$126.76 \pm 1.70 **$	89.64 ± 5.82**

Values are expressed in Mean \pm SEM of 6 rats in each group. *p<0.01, **p<0.001 when compared with respective CCl₄ treated group.

Table 2.	Effect of 50%	6 ethanolic ex	tract of <i>Mi</i>	mosa pudic	a on bioc	hemical p	arameters
viz tota	l protein, alb	umin and tota	al biluribin	in CCl ₄ ind	luced hep	patotoxict	y in rats.

Treatment/ Dose/	Total protein (g/dL)	Albumin (g/dL)	Total bilurubin	
Mg/kg			(mg/dL)	
Control	6.51 ± 0.62	4.210 ± 0.450	0.54 ± 0.03	
CCl ₄ (1ml/kg i.p)	2.12 ± 0.54	1.070 ± 0.210	1.76 ± 0.09	
Silymarin 100	$7.29 \pm 0.30 **$	$5.020 \pm 0.140 **$	$1.03 \pm 0.10 **$	
M.pudica 200	$6.72 \pm 0.39 **$	$3.210 \pm 0.620 *$	$1.09 \pm 0.14 **$	
M.pudica 400	$8.34 \pm 0.43^{**}$	$4.600 \pm 0.090 **$	$0.61 \pm 0.11^{**}$	

Discussion

In the present study, 50% ethanolic extract at the doses of 200 and 400 mg/ kg caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization plasma membrane as well as repair of hepatic tissue damage caused by CCl_4 . On the other hand suppression of elevated ALP activities with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants [11].

Conclusion

It can be concluded that the 50% ethanolic extract of *Mimosa pudica* have significant hepatoprorective on CCl_4 induced hepatic damage in rats, as evidenced by the biochemical parameters. These results reveal that the hepatoprotective effect of extract of *M. pudica* may be due to its ability to block the bioactivation of toxicant and its potent antioxidants activity, and/or by scavenging the free radicals and inhibiting lipid peroxiditon. Further work is in progress to isolate and characterize the active principles in these extracts.

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