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Research Article

HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT OF *MIMOSA PUDICA* ROOTS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

Suneetha B*[1], Pavan Kumar P[1], Prasad KVSRG[3], Vidyadhara S[1] and Sambasiva Rao KRS[2]

[1]Department of Pharmacology, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, India
[2]Department of Biotechnology, Acharya Nagarjuna University, Guntur, India
[3]Department of Pharmacology, Sri Padmavathi Mahila Visva Vidyalayam, Tirupathi, India

***Corresponding author e-mail**: balusu_sunitha2001@yahoo.com

ABSTRACT

In the present study, methanolic root extract of *Mimosa pudica* (*M. pudica*) (200 and 400 mg/kg, *p.o.*) was used to screen the hepatoprotective activity. Biochemical parameters like serum glutamate Oxaloacetate transaminase (SGOT), serum glutamate Pyruvate Transaminase (SGPT) and serum bilirubin were measured. The activity of tissue antioxidant enzymes namely lipid peroxidation, catalase, reduced glutathione and histopathological evaluation of liver sections were also done. Carbon tetrachloride administration in rats elevated the levels of SGPT, SGOT, cholesterol and bilirubin. Administration of the methanolic root extract of *the Mimosa pedicure* at a dose (400mg/kg) significantly (P<0.01) prevented this increase. The activity of anti-oxidant enzymes like catalase and reduced gltathione was decreased and malondialdehyde content was increased in carbon tetrachloride (CCl_4)-treated group. The enzyme levels of catalase and reduced GSH were significantly (P<0.01) increased and malondialdehyde content significantly ($p < 0.001$) decreased in the group treated with *M. pudica* at a dose of 400mg/kg. Histopathological studies revealed that the concurrent administration of carbon tetrachloride with the *M. pudica* extract exhibited protection of the liver tissue. The study has confirmed the hepatoprotective activity of methanolic extract of *M. pudica*, which may be attributed to its antioxidant property.

Keywords: *Mimosa pudica*, Hepato protective, Silymarin, SGOT, SGPT, Bilirubin and Antioxidant

INTRODUCTION

Liver, an important organ actively involved in many metabolic functions and is a frequent target for a number of toxicants[1]. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases an excessive amount of oxidants which in turn cause injury to hepatic cells. Activation of some enzymes in the cytochrome P_{450} system such as CYP2EI also leads to oxidative stress. Injury of hepatocyte and bile duct cells lead to accumulation of bile acids inside the liver. This promotes further liver damage[2]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown[3]. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Natural remedies from medicinal plants are

considered to be a safe and effective alternate treatment for hepatotoxicity.

The plant selected for the present study is *Mimosa* which belongs to the taxonomic group Magnoliopsida and family *Mimosaseae*. In Latin it is called as *Mimosa pudica* Linn also known as chue Mue.It is native to South America and Central America. It has been introduced to many other regions and is regarded as an invasive species. It is short lived and evergreen. It is a stout shrubby plant with the compound leaves which get sensitive to touching, spinous stipules and globes pinkish flower heads, grow as a weed in almost all parts of the country. Leaves and stems **o**f the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and roots contain tannins[4]. *Mimosa pudica* leaves are used for its antihyperglcemic, anti ulcer[5], antidiarroheal, anticonvulsant, cytoxic and hepatoprotective properties. In Ayurvedic and Unani medicine, *Mimosa pudica* root is used to treat bilious fevers, piles, jaundice, leprosy, dysentery, vaginal and uterine complaints, inflammations, are burning sensation, fatigue, asthma, leucoderma, and blood diseases[6].

No systemic pharmacological studies were performed on roots so far to assess the hepatoprotective activity in rats against carbon tetra chloride induced hepatotoxicity; hence an attempt was made in the present study.

MATERIALS AND METHODS

Plant material:

The plant material of *Mimosa pudica* roots has been collected from the Tirumala hills, Tirupathi. The plant was authenticated by S.R.K Prasad, Taxonomist, Department of Botany, J K C College, and Guntur.

Preparation of Extract:

The plant roots were washed thoroughly and shade dried. The dried roots were subjected to size reduction to a coarse powder by using a dry grinder and passed through sieve. The powdered form was subjected to Soxhlet extraction with methanol as menstrum. Then the methanolic liquid extract was distilled to remove the excess methanol under vacuum. The extract obtained by distillation process stored in a refrigerator at 4-8^{0}C.

Preliminary Phytochemical screening:

The MEM roots were subjected to various qualitative tests for the identification of various plant constituents present in this species[7].

In vitro antioxidant studies: The antioxidant activities of MEM roots were determined by using Diphenyl picryl hydrazyl (DPPH) radical scavenging, Nitric oxide (NO) radical scavenging methods.

Nitric oxide (NO) radical scavenging activity[8]:

Sodium nitroprusside (10 µM) in phosphate buffer pH 7.7 was incubated with 25, 50, 75, 100 and 125µg concentrations of the drug dissolved in a suitable solvent (methanol) and tubes were incubated at 25ºC for 120 minutes. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess reagent. As a positive control ascorbic acid was used. The absorbance of the chromophore was measured at 546nm. Results were expressed as means of triplicates and percentage scavenging activity was calculated as follows.

DPPH radical scavenging activity[9]:

Solutions of test drug at different concentrations of 25, 50, 75, 100 and 125 µg were added to 100 µM DPPH in ethanol and tubes were kept at an ambient temperature for 20 minutes and absorbance were measured at 517 nm. Positive control ascorbic acid was used. Results were expressed as means of triplicates and were calculated using the same formula as described above.

Animals:

Healthy adult male Wistar albino rats between 2 and 3 months of age weighing about 150-200g were used for the study. The animals were housed in polypropylene cages, maintained under standard laboratory conditions. They were feed with standard rat pellet diet and water ad libitum.

Acute toxicity studies:

Normal healthy rats were divided into five groups of six animals each. Group-1 animals were treated with distilled water (2ml/kg/p.o) and group-2 to group-5 animals received 50, 100, 200, 400, 800mg/kg,p.o of fresh MEM roots respectively. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. Then intermittently and at the end of 24 hours, the number of deaths noted to calculate LD_{50}[10].

Evaluation of Hepatoprotective activity:

Hepatotoxicity induced by single intra peritoneal injection of carbon tetrachloride: liquid paraffin in 1:1 ratio. Animals were divided into five groups consisting of six animals in each group. The first group of animals was received only vehicle and served as normal group. A second group of animals received CCl_4 (0.125ml/kg) and served as a positive control group. A third group of animals received

silymarin (10mg/kg) p.o. once daily and served as standard. The fourth group received MEM roots (200mg/kg) P.O. once daily. The fifth group received MEM roots (400mg/kg) p.o. once daily. The drug treatment was started 5 days prior to CCl_4 administration and continued till the end of the experiment[11].

Estimation of Biochemical parameters:
After 48 h, following CCl_4 administration, blood samples were collected from the retro orbital plexus and serum was separated for analyzing SGOT[12] (Serum glutamate oxaloacetate transaminase) and SGPT[12] (Serum glutamate pyruvate transaminase) and serum total bilirubin[13].

Histopathological studies:
At the end of the study, animals were decapitated and cut open to excise the liver. The liver was immediately removed and a small piece was placed in 10% formalin for histopathological assessment.

In vivo antioxidant studies:

Preparation of post mitochondrial supernatant (PMS):
The liver was per fused with ice cold saline (0.9% sodium chloride) and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 rpm for 5 minutes at 4^{o}C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 rpm for 20 minutes at 4^{o}C to get the PMS which was used to estimate the catalase, lipid per oxidation and reduced glutathione activity.

Estimation of lipid per oxidation (LPO) from PMS[14]:
0.5 ml of PMS was taken and to it 0.5 ml of tris hydrogen chloride buffer was added and incubated at 37^{o}C for 2h and then 1ml of ice cold trichloro acetic acid was added, centrifuged at 1000rpm for 10 minutes. From the above, 1ml of distilled water was added. Absorbance was measured at 532 nm by using a spectrophotometer. Blank was prepared without tissue homogeneous.

Estimation of reduced glutathione (GSH) from PMS[15]:
1.0 ml of PMS (10%) was precipitated with 1.0ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 hour and then subjected to centrifugation at 1200 rpm for 15 minutes at 4°C. The assay mixture contained 0.1 ml filtered aliquot and 2.7 ml phosphate buffer (0.1 M, pH7.4) in a total volume of 3.0ml. The yellow color developed was read immediately at 412 nm on a spectrophotometer using the same formula as described above. Blank was prepared without tissue homogenate.

Estimation of catalase (CAT) from PMS[16]
The assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml PMS (10%) in a final volume of 3.0 ml. Changes in the absence were recorded at 240 nm. Catalase activity was calculated in terms of k minutes^{-1}.

Statistical Analysis:
All the data were represented as means ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) test for multiple comparisons followed by Turkey-Kramer test. The statistical significance was set accordingly.

RESULTS

Preliminary Phytochemical screening:
Preliminary phytochemical screening of MEM roots revealed the presence of alkaloids, tannins and flavonoids.

Acute toxicity studies:
All groups treated with methanolic extract showed no discernible behavioral changes up to 500mg/kg by oral route. No mortality was observed at this dose during 72 h observation period.

In vitro antioxidant studies:
The MEM roots showed praising free radical scavenging activity of DPPH and NO induced release of free radicals. The free radical scavenging activity was seen in a concentration dependent manner and the percentage of inhibition was given in Table-1.

Biochemical Parameters:
The effect of MEM roots on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in Table-2. The CCl_4 treated group showed a significant increase in serum SGOT, serum SGPT, serum total bilirubin, indicated the liver injury caused by CCl_4. Animals treated with low dose of MEM roots (200mg/kg) did not show any significant effect, whereas animals treated with high dose of MEM roots (400mg/kg) showed a significant increase in serum SGOT, serum SGPT and serum total bilirubin.

In vivo Antioxidant Studies:
Animals treated with CCl_4 showed a significant decrease in catalase (0.059 ± 0.77), GSH (0.004 ± 0.03), increased MDA levels (0.004 ± 0.3). The

group treated with MEM roots (200mg/kg, p.o, once daily) showed an increase in catalase values (0.126 ± 0.65), GSH values (0.01 ± 0.02), but no significant decrease in MDA levels (0.037 ± 0.45). Whereas group treated with high dose of MEM roots (400mg/kg) showed a significant increase in the catalase values (0.155 ± 2.55), GSH values (0.009 ± 0.14) and also a significant decrease in MDA levels (0.0008 ± 0.12). All these results were compared with a group of animals treated with a positive control group and results are given Table-3.

Histopathological Studies:

The hepatoprotective effect of MEM roots was further confirmed by histopathological examination of liver samples from the respective groups. The Histological architecture of CCl_4 treated liver sections showed in figure-1.

Table 1: ***In vitro*** **Antioxidant Studies**

S.No	Concentration of test drug (µM)	Percentage of inhibition	
		NO Scavenging	DPPH
1.	25	20%	23.6%
2.	50	23%	37.2%
3.	75	43%	47%
4.	100	55%	59%
5.	125	61.4%	86.3%

Table: 2 Effect of MEM roots on Biochemical parameters

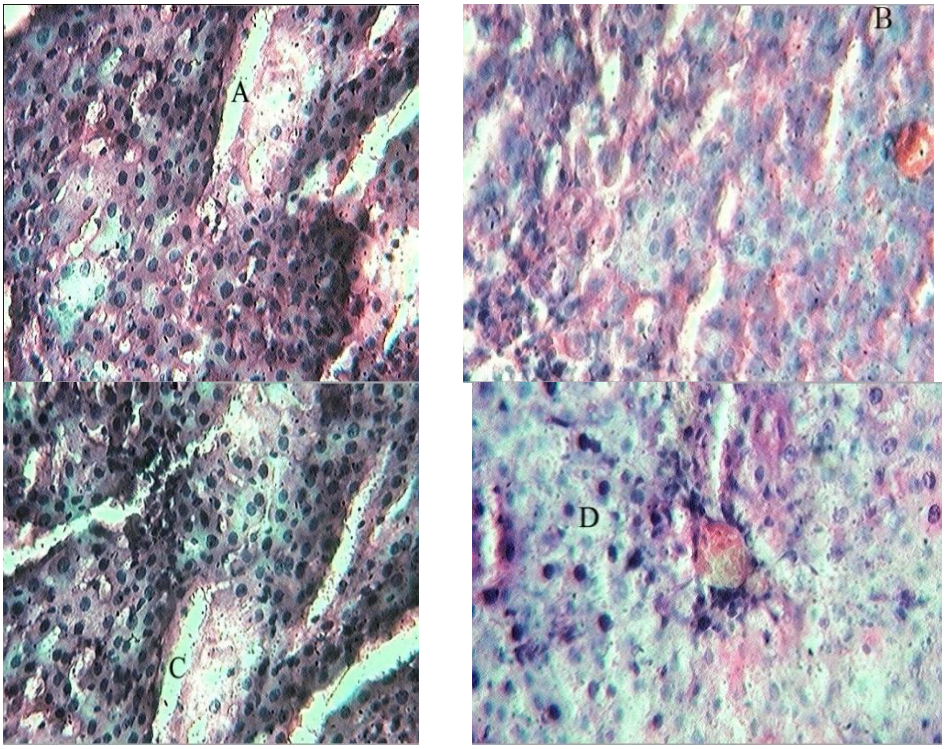
GROUPS	SERUM SGOT (IU/L)	SERUM SGPT (IU/L)	SERUM TOTAL BILIRUBIN(IU/L)
	Mean ±SEM	Mean ±SEM	Mean ±SEM
Normal	12.384±63.62	11.74±44.44	0.125±1.128
Posittive control group (ccl_4 0.125ml i.p.)	24.21±169.33[a]	24.36±214.97[a]	0.039±1.99[a]
Standard group(Silymarin 10mg/kg)	9.205±72.53 [b]	17.23±68.44 [b]	0.048±1.328[b]
Test group-I (Methanolic root extract of Mimosa pudica 200mg/kg)	11.259±147.56[b]	15.66±136.4 [b]	0.267±1.685[c]
Test group-II (Methanolic root extract of Mimosa pudica 400mg/kg)	10.02±65.86[b]	15.68±66.47[b]	0.136±1.173[c]

Values are expressed as Mean ±SEM of 6 animals in each group.
a=P<0.001 Considered statistically significant as compared to normal group
b=P<0.01Considered statistically significant as compared to positive control group
c= P<0.05Considered statistically significant as compared to positive control group

Table 3: *In vivo* Antioxidant Studies

GROUPS	Catalase	Reduced glutathione(GSH)	Lipid peroxidation (LPO)
	Mean ±SEM	Mean ±SEM	Mean ±SEM
Normal	0.365±2.55	0.007±.04	0.009±.09
Positive control group (Ccl_4 0.125ml i.p.)	0.059±.77[a]	0.004±.03[a]	0.004±.34[a]
Standard group (Silymarin 10mg/kg)	0.491±3.01[b]	0.005±.03[b]	0.015±.25[b]
Test group-I(MEM roots 200mg/kg)	0.126±.65[ns]	0.010±.02[ns]	0.037±.45[c]
Test group-II(MEM roots 400mg/kg)	0.155±2.55[ns]	0.009±.14[b]	0.0008±.12[d]

Values are expressed as mean ±SEM for six animals in each group.
a=P<0.001 Considered statistically significant as compared to normal group
b=P<0.01 Considered statistically significant as compared to positive control group
c= P<0.05 Considered statistically significant as compared to positive control group
d= P<0.001 Considered statistically significant as compared to positive control group
ns=non-significant



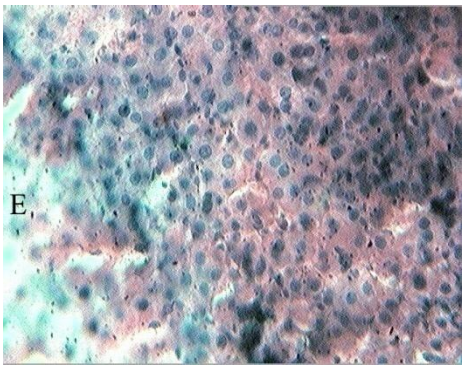


Figure: 1 Histopatholgical appearance of liver cells (A): Normal cells; (B): liver cells treated with CCl_4; (C): Liver cells of rats treated with CCl_4 and silymarin; (D): Liver cells treated with CCl_4 and methanolic root extract of *M. pudica* low dose (200mg/kg) (E): Liver cells treated with CCl_4 and methanolic root extract of M. *pudica* high dose (400mg/kg).

DISCUSSION

The present study indicated that the high dose of MEM roots (400mg/kg) provided significant protection against CCl_4 induced liver damage. The CCl_4 is biotransformed by the cytochrome P_{450} system to produce trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation[17]. Further it has been evident that several phyto constituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of lipid per oxidation induced by CCl_4. In the present study, the plant drug was found to be having phyto constituents like alkaloids, flavanoids and tannins. These phyto constituents were proved of having hepatoprotective activity earlier[18].

The serum SGOT and SGPT activities were used as a marker of liver damage. In the present study administration of high dose of MEM roots (400mg/kg, P.o, once daily) to CCl_4 treated rats showed a significant decrease in serum SGOT, SGPT, total bilirubin. While the low dose administration of the extract (200mg/kg, P.o, once daily) did not show any effect on serum SGOT, SGPT and total bilirubin. This administration of MEM roots showed significant and dose dependent hepatoprotective activity which was compared to the positive control group.

Oxidative stress induced due to the generation of free radicals and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis[19]. During cell membrane damage, various enzymes leak down to the circulatory fluid and their assessment in serum serves as markers in clinical studies. Scavenging of the free radicals is one of the major antioxidant mechanisms to inhibit the chain reaction of lipid peroxidation. Reduced lipid peroxidation was observed by a significant decrease in MDA level in groups pretreated with standard drug and high dose of extracts simultaneously with a significant elevation in GSH and CAT activities. In the present study, increase in catalase and reduced GSH activities and decrease in MDA levels were observed in rats treated with silymarin and MEM roots indicated that silymarin and MEM roots have an antioxidant property.

In vitro antioxidant studies were also carried out to assess the scavenging potential of *Mimosa pudica*. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses color stoichometrically depending on the number of electrons uptake[20].

From the results of the present study, it was postulated that the active principles in the root extract of *Mimosa pudica* have hydrogen donors thus scavenging the free radical. Nitric oxide (NO) is an important chemical mediator generated by the endothelial cells, neurons involved in the regulation of various physiological processes[21]. Oxygen reacts with the excess NO to generate nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25^0C was reduced by root extract of *Mimosa pudica*. In the present study, the methanolic root extract of *Mimosa pudica* exhibited greater inhibition than ascorbic acid in scavenging NO.

The Histopathological appearance of rat liver treated CCl_4 showed extensive inflammation around central veins, less apoptosis, microvesicular fatty changes and ballooning degeneration. This appearance indicated poor protection of the hepatocyte against the hepatotoxic agent. Liver tissue of rats treated with CCl_4 and high dose of methanolic root extract of *Mimosa pudica* (400mg/kg) showed mild to moderate inflammation. It displayed a good progress with the disappearance of fatty changes and necrosis. This appearance of hepatocytes indicated the effectiveness of root extract of *Mimosa pudica* to express the significant protective effect of the liver cells against CCl_4. From these results, it is evident that the high doses of MEM roots have hepatoprotective activity and antioxidant activity. These results thus support the claim of the use of this plant in hepatotoxicity conditions.

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