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Hepatoprotective activity of *Mimosa pudica* leaves against Carbontetrachloride induced toxicity

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ABSTRACT

The methanolic extract of leaves of *Mimosa pudica* at the dose of 200mg/kg body weight per oral was studied for the hepatoprotective effect using Carbontetrachloride induced liver damage in wistar albino rats. Methanolic extract showed significant ($p<0.05$) hepatoprotective effect by lowering the serum levels of various biochemical parameters such as serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvates transaminase (SGPT), alkaline phospatase (ALP), total bilirubin (TBL), total cholesterol (CHL) and by increasing the levels of total protein (TPTN) and albumin (ALB), in the selected model. These biochemical observations were inturn confirmed by histopathological examinations of liver sections and are comparable with the standard hepatoprotective drug Silymarin (100mg/kg body weight i.p.) which served as a positive control. The overall experimental results suggests that the biologically active phytoconstituents such as flavonoids, glycosides alkaloids present in the methanolic extract of plant *Mimosa pudica*, may be responsible for the significant hepatoprotective activity and the results justify the use of *Mimosa pudica* as a hepatoprotective agent.

Keywords: *Mimosa pudica*; Hepatoprotective activity; Methanolic extract; Carbontetrachloride.

INTRODUCTION

Mimosa pudica (Mimosaceae) known as chue Mue, is a stout stragling prostrate shrubby plant with the compound leaves which gets sensitive on touching, spinous stipules and globose pinkish flower heads, grows as weed in almost all parts of the country (Ghani, 2003). Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins (Ghani, 2003). *Mimosa pudica* is used for its anti-hyperglycemic (Uma maheswari, 2007), anti-diarrhoeal (Balakrishnan, et al., 2006), anti-convulsant (Bum, et al., 2004) and cytotoxic properties (Sadia Afreen Chowdhury, et al., 2008).

The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in the treatment of sore gum and is used as a blood purifier (Ghani, 2003). In ayurvedic and unani system of medicine, this plant has been used in diseases arising from corrupted blood and bile, billious fever, piles, jaundice, leprosy, ulcers, small pox. The present studies were performed to assess the hepatoprotective activity in rats against Carbontetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorders.

MATERIALS AND METHODS

***Plant material*:** The leaves of *Mimosa pudica* were procured from the Thailavaram (near SRM University) in the month of Febuary-2009. The plant was identified by Dr D Narashiman. Centre for Floristic Research, Department of botany Plantbiology & Plantbiotechnology, Madras Christian College, Tambaram, Chennai, Tamilnadu, India. The voucher specimen (023/02/09) was deposited at the Department of Pharmacognosy & Phytochemistry M.S.A.J College of Pharmacy, medavakkam high road, Chennai-119, Tamilnadu, India.

Preparation of extract: The coarsely powdered leaves (300g) of *Mimosa pudica* was extracted to exhaustion in a soxhlet apparatus at 50^{o}C with 500ml of methanol. The extract was filtered through a cotton plug, followed by whatman filter paper (no.1) and then concentrated by using a rotary evaporator at a low temperature (40-60^{o}C) and reduced pressure to provide methanolic extractive of 8.20g.

Preliminary phytochemical analysis: The methanolic extract was then subjected to preliminary phytochemical (Harbone, 1984) analysis to assess the presence of various phytoconstituents, it revealed the presence of flavonoids, alkaloids and glycosides. Preliminary Thin layer chromatography studies also confirmed these constituents (Wagner and Blatt, 1996)

Animals: Wistar albino rats weighing 175-225g of either sex maintained under standard husbandary conditions (temp 23±2^{o}C, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, India 2009.

Toxicity studies: Acute toxicity study was performed for methanolic extract according to the acute toxic classic method as per OECD guidelines (Ecobichon, 1997). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose of 300mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,200 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

Carbontetrachloride *induced hepatotoxicity:* Rats were divided into four groups of six animals each. The rats of control group (I) received three doses of 5% gumacacia mucilage (1ml/kg, per oral.) at 12 hour intervals (0 hour, 12 hour and 24 hour). The rats of Carbontetrachloride group (II) received three doses of vehicle at 12 hour intervals and a single dose of Carbontetrachloride (1.25ml/kg i.p.) diluted in liquid paraffin (1:1) 30 minutes after the administration of 1st dose of vehicle. The rats of standard group (III) received three doses of Silymarin (100mg/kg) at0 hour, 12 hour and 24 hour.

Carbontetrachloride was administered (1.25ml/kg i.p.) 30 minutes after the first dose of silymarin. While the rats of test group (IV) received three doses of test extract at the dose of 200mg/kg body weight per oral at 0 hour, 12 hour and 24 hour. Carbontetrachloride was administered (1.25ml/kg i.p.) 30 minutes after the first dose of silymarin test extract (Rao and Mishra, 1997) After 36 hour of administration of carbontetrachloride, blood was collected and serum was separated and used for determination biochemical parameters.

Assessment of liver function: Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International federation of Clinical Chemistry in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malated dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the pyruvated formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALP) was estimated by method described by (Comb and Bowers, 1972) involving hydrolysis of p-nitrophenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly proportional to ALP activity; while total bilirubin (TBL) by (Jendrassik and Grof, 1938) which ivolves the reaction of bilirubin with diazotized sulphanilic acid to form an azo compound, the colour of which is measured at 546 nm. Total cholesterol (CHL) was determined by CHOD-PAP Method (Wrichmond, 1973) in which the free cholesterol is hydrolysed by cholesterol oxidase to cholestenone-4-en-3-one and hydrogen peroxide. Hydrogen peroxide by the action of peroxidase liberates oxygen which reacts with 4-amino antipyrine and phenol to form red coloured compound which is measured at 500nm. Total protein (TPTN) was estimated by Biuret method (Peters, 1968) where proteins produce a violet colour complex with copper ions in an alkaline solution. The absorbance of the colour complex is directly proportional to the protein in the sample, while the albumin (ALB) was estimated by BCG (Webster, 1974) involving formation of blue-green complex with bromocresol green at slightly acidic pH which is measured photometrically.

Histopathological studies: The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, then embeded in paraffin using conventional methods (Galighor and Kozloff, 1976) and cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis: The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance (Gennaro, 1995) was carried out and the individual comparisons of the group mean values were done using Dunnet's test (Dunnet, 1964).

RESULTS

Acute toxicity studies: Methanolic extract of *Mimosa pudica* did not produce any toxic symptoms or mortality upto the dose level of 2000mg/kg body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

Hepatoprotective activity: The results of Carbontetrachloride induced hepato-toxicity were shown in table-1. In the Carbontetrachloride control group, the significant acute hepato cellular damage, and biliary obstruction was indicated by the elevated level of SGPT, SGOT, ALP, TBL and CHL and decreased levels of TPTN and ALB. But the group which received the test drug of methanolic extract at the dose of 200mg/kg body weight p.o showed a significant decrease in the elevated levels of SGPT, SGOT, ALP, TBL and CHL and significant increase in the reduced levels of TPTN and ALB and these biochemical parameters are comparable with the standard silymarin hepatoprotective drug. Therefore, the silymarin and the methanolic extract restored the altered level of enzymes significantly ($P<0.05$).

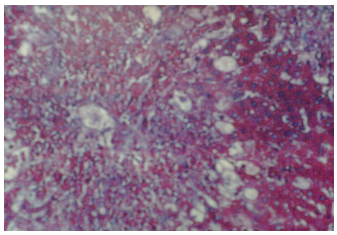
Table-1: Effect of *Mimosa pudica* on Carbontetrachloride induced hepatotoxicity in rat's enzymes.

Parameters	**Group I (control)**	**Group II** (Carbon tetrachloride $_{group}$)	**Group III** (Standard 100mg/kg)	**Group IV** (Test extract 200mg/kg)
SGPT (IU/L)	128.17±5.09	283.3±30.1	125±11.8*	132.1±15.152*
SGOT (IU/L)	105.5±2.8	242.1±36.4	103.8±10.1*	99.5±9.5*
ALP(IU/L)	200.3±5.2	424.5±47.1	194.3±6.8*	204.8±49.4*
TBL(mg/dl)	1.16±0.1	2.96±0.36	1.35±0.23*	1.50±0.29*
CHL(mg/dl)	101.3±2.4	2131.8±27	106.1±6.9*	111.3±10.48*
TPTN(gm/dl)	7.10±0.25	2.26±0.39	6.53±0.42**	6.95±0.53*
ALB(gm/dl)	3.98±0.14	1.06±0.28	4.35±0.21**	3.95±0.36*

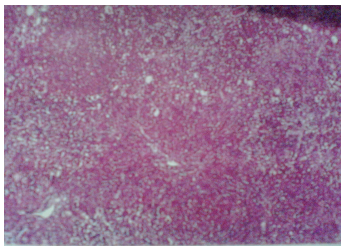
- Values in Mean ±SEM, N=6,
- *significant reduction compared to Carbontetrachloride ($P<0.05$),
- **significant increase compared to Carbontetrachloride ($P<0.05$).

Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein (Fig 1a). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in Carbontetrachloride intoxicated liver (Fig 1b). The liver sections of the rat treated with 200mg/kg bodyweight p.o of methanolic extract of *Mimosa pudica* followed by Carbontetrachloride intoxication (Fig 1c) showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard Silymarin (Fig 1d), supplementing the protective effect of the test drug and the standard hepatoprotective drug.

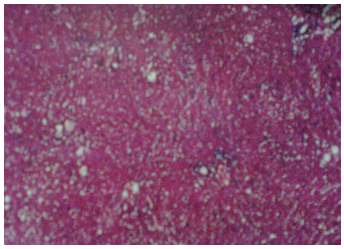
Figure-1
Hispathological sections of liver.



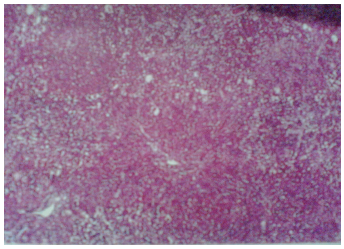
1a – Contol



1b- Carbontetrachloride group.



1c- standard silymarin group.



1d- Test extract of *Memosa pudica* group

DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats, against Carbontetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorder. The changes associated with Carbontetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis (Venukaumar and Latha, 2002). Carbontetrachloride is a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} haemostasis and finally result in cell death (Recknag, et al., 1989).

Animals of Group II (received Carbontetrachloride) significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of Group III and IV (received Carbontetrachloride plus 200mg/kg body weight of test extract and standard drug Silymarin 100mg/kg body weight) showed a significant increase in body weight and food consumption when compared to Carbontetrachloride group animals. These findings suggested the extract administered has significantly neutralized the toxic effects of Carbontetrachloride and helped in regeneration of hepatocytes (Farooq, et al., 1997).

Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage (Mitea, et al., 1998). The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of antihepatotoxic effects of the extract.

The levels of total protein and albumin were reduced due to the Carbontetrachloride-induced hepatotoxicity. The reduction is attricbuted to the initial damage produced and localized in the endoplasmic reticulam which result in the loss of P-450 leading to fatty liver (Recknagal, 1967). Inhibitin of bile acids synthesis from cholesterol which is synthesis in liver or derived from plasma lipids, leading to increase in cholesterol levels were also resulted due to Carbontetrachloride intoxication suppression of cholesterol levels by the extract suggest the bile acid synthesis inhibition was reversed. Reduction in the levels of SGPT and SGOT towards the normal value is an indication of regeneration process. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbontetrachloride. The protein albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. This hepato protective effect exhibited by the methanolic extract of *Mimosa pudica* at the dose level of 200mg/kg body weight was comparable with the standard drug, A Silymarin.

Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in Carbontetrachloride group, whereas in the liver sections of the rat treated with the methanolic extract and intoxicated with Carbontetrachloride the normal cellular architecture was retained and it in comparable with the standard Silymarin group, hence confirming the significant hepato protective effect of extract of *Mimosa pudica* at the dose of 200mg/kg body weight.

In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as flavonoids, alkaloids and glycosides which are present in the methanolic extract could be considered as, responisible for the significant hepatoprotective activity. In conclusion, it can be said that the methanolic extract of *Mimosa pudica* exhibited a hepato protective effect against Carbontetrachloride induced hepatotoxicity. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant.

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