Hepatoprotective Effect of *Mimosa pudica* Leaves Ethanoic Extract in CCl_4-Induced Hepatotoxicity

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Abstract

The aim of the current study was to evaluate the hepatoprotective potential of Mimosa pudica leaves ethanolic extract against carbon tetrachloride (CCl4)-induced hepatic damage in Wistar rats. Mimosa pudica is famous for its anticancer alkaloid, mimosine, along with several valuable secondary metabolites like tannins, steroids, flavonoids, triterpenes, and glycosyl flavones along with pharmacological properties like antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, antinociceptive, anticonvulsant, antidepressant, antidiarrheal, hypolipidemic activities, diuretic, antiparasitic, antimalarial, and hypoglycemic have been attributed to different parts of Mimosa pudica. Administration of carbon tetrachloride in rats significantly increased the levels of biochemical parameters of liver damage; bilirubin, cholesterol, alanine transaminase (ALT), alkaline phosphate, albumin, aspartate transaminase (AST), urea, and creatinine. Test

animals were subjected to different concentrations of ethanolic extract of Mimosa pudica leaves (500mg/kg. 750mg/kg and 1000mg/kg) through oral administration.

Out of three doses, 500mg/kg showed better results in reducing the elevated levels of hepatic parameters and showed hepatocellular regeneration in CCl4-induced injured rats as compared with the CCl4 group alone. While higher doses (750mg/kg and 1000mg/kg) also showed significant results but less than 500mg/ml dose. Histopathological examination of the liver also indicated the protective effect of Mimosa pudica on injured liver tissues with improved architecture. The results of this study strongly suggest that mimosa pudica leaves extract has hepatoprotective and healing properties.

Keywords:

Hepatoprotective, *Mimosa Pudica*, Ethanoic Extract, CCl_4, Hepatotoxicity.

1. Introduction

The liver is the largest gland of the human body, accounts for 2.5 percent of total body weight, and performs significant roles in the metabolism of lipids, minerals, vitamins, and amino acids (Sheila and Dooley, 2001). Owing to its function in the metabolism of harmful components, the liver is at a higher risk of getting infections and diseases (Purkayastha et al., 2016). Injuries in liver cells are mostly caused by chemotherapeutic agents, antibiotics, carbon tetrachloride (CCl4), alcohol intake, and microbial action. Carbon Tetrachloride is considered to be a potent carcinogen and has been extensively used in laboratory experiments for causing injury in experimental rats. It is a heavy and clear liquid primarily used in the refrigerator until it was known to be hepatotoxic and nephrotoxic for humans. It causes liver damage which results in hepatotoxicity (Naz et al., 2014). Toxicity of the liver that is caused by CCl4 by any means (inhalation, oral or subcutaneous) results in necrosis, liver cirrhosis, fatty degeneration, and fibrosis which ultimately causes hepatocellular carcinomas (Fahad et al., 2018). Impairment of protective cellular mechanisms is associated with hepatotoxicity. Carbon tetrachloride acts through enzymatic changes in the endoplasmic reticulum by P-450 cytochrome-dependent mixed oxidase (CYP2EI) then breaks into small pieces of trichloromethyl peroxyl (CCl302) and trichloromethyl (CCl3) free radicals. These radicals combine with lipids and cellular proteins in

favorable aerobic conditions that lead to membrane lipid peroxidation and eventually to cell necrosis (Leelaprakash et al., 2011).

Many old natural remedies originating from plant sources have been used for a very long time for treating hepatic injuries. Medicinal plants contain bioactive compounds that are beneficial for various human complications. According to World Health Organization (WHO), 80% population of Africa and Asia uses plants for primary health care (Ahmed et al., 2014). Plant components have been widely used for liver disease and are frequently explored for the regenerative and protective actions on the injured liver (Bhawna and Kumar, 2009; Gul et al., 2021). *Mimosa pudica* belongs to Family Fabaceae or Leguminosae. It is commonly known as the legume, pea, or bean family and is a large and economically important family of flowering plants (Mohammad et al., 2016). It also grows in sub-tropics and tropics, in Nigeria as a weed distributed commonly in the moist wasteland. *Mimosa* is typically a short plant with its branches growing very close to the ground (Asumpta et al., 2018).

It helps to stop bleeding and accelerates the wound healing process, also helpful in treating gynecological disorders, skin healing, neurological disorders, diarrhea, and bleeding piles. All parts of the plant have been useful in treating various diseases. It can also potentially cure baldness, depression, amnesia, irritability, mental stress, jaundice, fatigue, asthma, insomnia, and premenstrual syndrome. Additionally, it has also been used in modern medicine but in prescribed amounts, especially for pregnant women (Ghani et al., 2003). The phytochemical analysis of *Mimosa pudica* leaves showed the presence of bioactive compounds such as phenols, tannins, and saponins (Gandhiraja et al., 2009). Keeping in view all the health-beneficial effects, this study was conducted to evaluate the hepatoprotective properties of different concentrations of *Mimosa pudica* leaves in Wister rats against carbon tetrachloride-induced toxicity to prove its practice against liver injuries.

2. Materials and Methods

Ethical Approval

Animal ethics: The experiments were performed according to the regulations about the care and use of laboratory animals published by the US National Institute of Health (NIH Publication No. 85 to 23, revised 1985) and approved by the Institutional Ethical Committee, the University of

Lahore, Pakistan (Approval no; IMBB/UOL/20/416). All the methods were performed following the relevant guidelines and regulations.

The experiment was initiated and completed at Animal House of the Institute of Molecular Biology & Biotechnology, the University of Lahore.

Experimental Design

The 70% ethanol and olive oil acquired from the University of Lahore were used as vehicle controls for plant extract and CCl4, respectively. For the current *in vivo* study, a limited amount of CCl4 (400µg/ml) was used combined with a 1:1 ratio of olive oil to induce liver toxicity that was equal to a working dose of 0.4ml/kg. This dose was administered intraperitoneally in experimental rats twice a week for up to three weeks. Twenty male healthy rats with uniform approximate age and weight were purchased from the Animal House of the University of Lahore. Each rat weight 200-270 grams was chosen and kept in separate cages. Rats were allowed to acclimatize for one week under controlled conditions before the initiation of the experiment. They were provided with identical standard food, and water and kept at a proper temperature of 25 ±2°C with a humid environment of 30-70% under a 12:12 dark-light cycle. Rats were divided into five Groups each comprising four rats namely: A, B, C, D, and E as under:

Group A: Negative control (NC) group (untreated group), rats were given standard feed and tap water.

Group B: Positive control (PC) group, rats were given 0.4ml/kg CCL_4 + 0.4ml/kg of olive oil.

Group C: Rats were given standard dose of CCL_4 + plant extract 500 mg/kg.

Group D: Rats were given standard dose of CCL_4 + plant extract 750 mg/kg

Group E: Rats were given with standard dose of CCL_4 + plant extract 1000 mg/kg.

Preparation of Plant Extract

Plant preparation was performed according to our recently published article (Abid et al., 2020; Maqbool et al., 2019). Briefly, the plants of *Mimosa pudica* were purchased from a plant nursery in Lahore, Pakistan and its leaves were separated and washed with water to remove any dust.

Fresh leaves were allowed to dry under shade for a few days and then made in a powdered form. The dried plant sample was grinned and sieved to remove impurities. The solution was prepared by using conical flasks and cylinders. Ethanol 70% was used as a solvent and then the powdered form of leaves weighing 100 grams was dissolved in 1000 ml (1Liter) of ethanol for 1-2 days at room temperature. To obtain a final volume of 100 mL of the solution, it was filtered by using filter paper and then evaporated by using a rotatory evaporator under reduced pressure. The solution became thick then a thick paste was stored in falcon tubes for further use. This form of the extract was used for dose preparations, 500, 750, and 1000 mg, respectively.

Plant ethics: the use of plants in the present study complies with international, national and our institutional guidelines.

Blood Sample Collection and Histopathological Study

After the completion of twenty-one days of treatment, the experimental animals were given normal anesthesia before slaughtering. A combination of ketamine and xylazine was used as anesthetic agent in the current study. The doses used were; 100mg/kg body weight for ketamine and 5mg/kg body weight for xylazine.

Blood was drawn from the heart to test the biochemical parameters. The liver of each rat was removed with help of scissors, and forceps, and preserved in formalin 10% for Histopathological Tests for a few hours then fatty changes, necrosis, ballooning degeneration, and infiltration of kupffer cells and lymphocytes were observed under 100x

Biochemical test parameters

These animals were tested for hepatoprotective effects through an analysis of biochemical parameters in the blood. Several enzymes which are used widely for testing liver functioning and abnormality were analyzed including Albumin, Total bilirubin (BIL), Alanine aminotransferase (ALT), Alkaline phosphate (ALP), Total protein (TP), Aspartate aminotransferase (AST), Globulin and A/G ratio, Urea, Cholesterol, and creatinine were determined by using automated techniques.

Statistical analysis

Data were interpreted by using Graph pad prism 8.0.1 and analyzed by One-way Analysis of Variance (ANOVA) and two-way ANOVA to make the comparison within the groups and between the groups. The tests values <0.05 were considered statistically significant

3. Results

Acute toxicity studies

Ethanolic extract of *Mimosa pudica* did not cause any toxicity in animals up-to a dose of 1000mg/kg, no animals died, nor were any health adverse effects seen during the whole process. so extract was considered to be safe for further use and experimentations.

Effect of different treatment groups on liver weight

Animals in the induced group (group B only treated with CCl4) lost weight and showed some signs of weakness after treatment as compared with the normal group 1. After before the slaughter group 3, which received 500mg/kg of the extract showed a huge sign of recovery and had a normal weight compared to the control group. Moreover, groups 4, and 5 also showed normal weight but less than group 3. (Table 1)

Hepatoprotective activity of Mimosa Pudica

In control groups, the levels of bilirubin, ALT, AST, alkaline phosphate, albumin, A/G ratio, urea, and creatinine, elevated after treatment with CCl4 thus showing the effectiveness of carbon tetrachloride in inducing hepatotoxicity in animals except for total proteins. Group 3 which received ethanoic extract at the dose of 500mg/kg showed a significant decrease in the elevated levels of ALT, AST, bilirubin, cholesterol, albumin, alkaline phosphate, A/G ratio, urea, and creatinine as these parameters are compared with the control group. Group 4 treated with 750mg/kg and group 5 treated with a high dose of 1000mg/kg showed non-significant results (Table 1).

Table 1. Is showing the results of biochemical parameters of all groups in Wistar rats. (Blood test)

	Control	CCl4 group	CCl4+500mg/ml	CCl4+750mg/ml	CCl4+1000mg/ml	P value
Cholestrol	69.5± 0.70	104.5±24.79	80.50±10.40	63.25±12.68	69.75±10.68	0.032
Bilirubin	0.10±0.00	0.165±0.05	0.10±0.00	0.125±0.050	0.15±0.57	0.04

SGPT (U/L)	41.0±7.07	75.0±22.31	34.7±10.87	45.0±6.21	39.5±4.04	0.001
SGOT (U/L)	272.0±42.42	341.0±63.67	283.5±45.31	287.0±29.1	297.7±23.6	0.002
Alkaline Phosphate (U/L)	147.0±22.62	388.2±109.00	280.2±240.2	233.5±127.8	248.5±218.4	0.001
Total Proteins (g/dl)	7.35±0.21	6.10±0.21	7.02±0.27	6.65±0.23	6.50±0.27	0.001
Albumin (g/dl)	3.00±0.21	3.12±0.26	3.05±0.38	3.00±0.33	3.07±0.27	0.04
Globulin (g/dl)	4.35±0.21	3.07±0.26	3.97±0.20	3.57±0.95	3.52±0.27	0.02
A/G ratio %	0.685±0.35	0.88±0.10	0.76±012	0.80±0.11	0.81±0.11	0.001
Urea (mg/dl)	76.00±16.97	74.5±3.69	69.0±4.96	70.2±1.70	720.0±41.4	0.02
Creatinine (mg/dl)	0.750±0.070	0.800±0.08	0.775±0.050	0.780±0.577	0.800±0.129	0.05
Weight	100	69.5	92.5	87	85	0.027

Administration of Mimosa Pudica extract enhanced liver tissue regeneration in rats

Histological sections of the liver of the negative control group showed no sign of inflammation or necrosis. Distinct central vein and hepatic cells were seen (Figure1 A). The appearance of necrosis and disarrangement of hepatic cells can be observed in the group treated with carbon tetrachloride (PC) (Figure 1B). The liver section of a rat treated with 500mg/kg of *Mimosa pudica* extract (Figure 1C) showed less vacuole formation and absence of necrosis with overall less visible inflammation. The group treated with 750mg/kg (Figure 1D) of extracts showed cytoplasmic degeneration and some aggregations of inflammatory cells were present. Peri-portal fibrosis and focal and central vein dilation were evident with no inflammation, hydropic degeneration, fibrosis, necrosis, atypia, or malignancy. In figure 1E, mild activation of Kupffer cells and multi-necrotic foci filled with hemorrhage demonstrating the presence of infiltrative cells was seen.

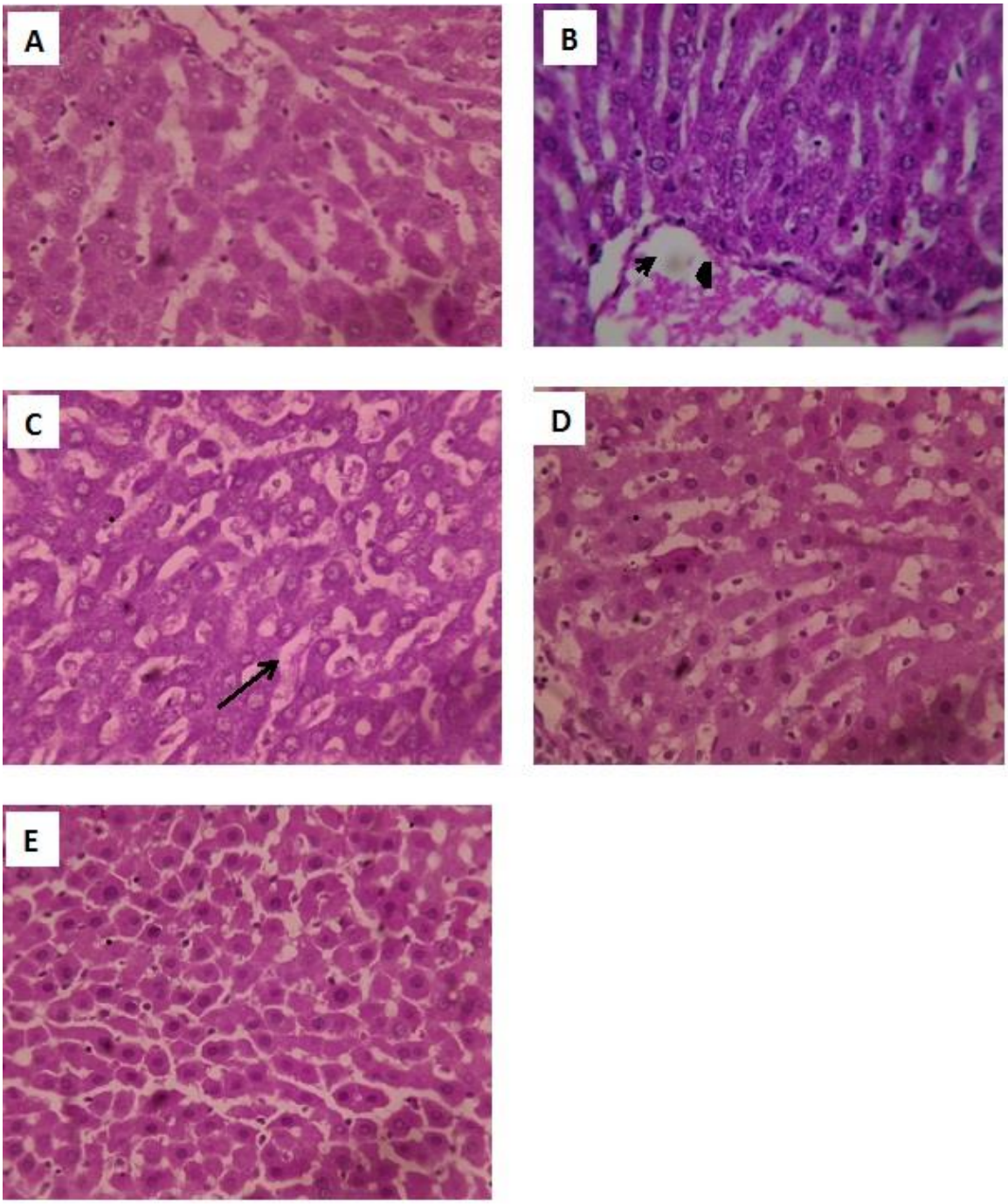

Figure 1: (A) Photomicrograph of liver from Group A showing normal hepatocellular architecture at100x **(B)** Photomicrograph of liver from Group B showing features of hepatic injury at100x denoted by arrow and arrow head **(C)** Photomicrograph of liver from Group C showing features of regeneration (denoted by arrow) and repair at 100x **(D)** Photomicrograph of

liver from Group D showing features of regeneration and repair at 100x **(E)** Photomicrograph of liver from Group E showing features of regeneration and repair at 100x

4. Discussion

This study was undertaken to evaluate the hepatoprotective effect of *Mimosa pudica* leaves extract against CCl4-induced liver toxicity in rats. The animals were divided into five different groups for the study and three different concentrations of ethanoic *Mimosa pudica* extract were given to the investigative groups. Since the liver is considered to be highly susceptible to toxic agents so for studying the effect of various reagents, liver enzymes are of great help (Vaishwanar and Kowale, 1976; Kalsoom et al., 2022) and it is the focus of our current study. Herbal medicines contain naturally occurring bioactive compounds that can cure illness and give health benefits with minimum side effects. Many important active elements are found in plants like tannins, flavonoids, alkaloids, and phenolic compounds. Research evidence confirms that *Mimosa pudica* has antioxidant properties and can be used as a hepatoprotective agent (Joseph et al., 2016). Glycosides and flavonol glycone are potent antioxidants that are present in the leaves of *Mimosa pudica* that can offer hepatoprotection and prevent disease (Roginsky, 2003; Hadi et al., 2023).

The plant has significant hepatoprotective abilities against CCL4 –induced damage in rat liver according to the biochemical parameters. The results suggested that the hepatoprotective effect of *Mimosa pudica* can be due to its ability to block the toxicant bioactivation and its potential antioxidant activity or by hunting the free radical and blocking the accumulation of lipid peroxidation (Kumar et al., 2015; Abid et al., 2020).

Carbon tetrachloride has been used widely as a hepatotoxic agent; it causes lipid peroxidation and causes liver damage. Due to the induction of CCL4, an increase in hepatic markers can be seen when compared with the normal group such as Bilirubin, Alkaline Phosphate (ALP), Alanine Transaminase, ALT, Urea, Creatinine, etc. These parameters play an important role in detecting liver damage and injury (Hadi et al., 2020). The activity of CCL4 give rise to cell damage and necrosis, treating the rats with *Mimosa pudica* helped in the reduction of the parameters. It is due to the activity of glycosides, alkaloids, tannins, and flavonoids. These components helped in enhancing the hepatoprotective activity of *Mimosa pudica* (Abid et al., 2022).

In the present study treatment with 500mg/kg of Mimosa pudica leaves significantly reversed the elevated levels of liver enzymes, the other two doses did not show significant results so it can be concluded that giving high doses of extract won't work on the toxicity just like taking too many medicines is harmful to a body. The working of the extract can also be demonstrated by weight lost and histology examination. The comparison between groups showed the working of *Mimosa pudica* leaves extract on toxicity as $p<0.05$ was indicated in four parameters. Treatment with *Mimosa pudica* leaves expressively declined the effects of CCl4 damage in the liver indicated by low levels of liver enzymes and renewal of hepatocellular architecture.

5. Conclusion

The present bio-chemical and histological results proved that *Mimosa pudica* leaves possess great potential to prevent liver damage against toxicity and could be used as an effective and cheap medicine against CCl_4 induced liver injuries.

Conflict of Interest

All the authors have no conflict of interest.

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