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HYPOLIPIDEMIC EFFECT OF ETHANOLIC EXTRACT OF *MIMOSA PUDICA* LEAVES ON DYSLIPIDEMIA FOLLOWING HEPATIC INJURY INDUCED BY CARBON TETRACHLORIDE IN ALBINO RATS

Ayan Purkayastha *, Pinaki Chakravarty and Babul Dewan

Department of Pharmacology, Silchar Medical College and Hospital, Silchar, Assam, India.

Keywords:

Hypolipidemic, *Mimosa pudica*, dyslipidemia, carbon tetrachloride, hepatic injury, albino rats.

Correspondence to Author:
Dr. Ayan Purkayastha

Demonstrator,
Department of Pharmacology,
Silchar Medical College and Hospital, Silchar, Assam - 788014, India.

Email: ayanp007@gmail.com

ABSTRACT: Background: The *Mimosa pudica*, is well known across the world for its different pharmacological activities such as anti diabetic, antitoxin,antioxidant and wound healing activities.In the present study we investigated the hypolipidemic effects of ethanolic extract of leaves of *Mimosa pudica* linn against dyslipidemia following carbon tetrachloride (CCl_4) induced hepatic injury in albino rats. **Methods:** Liver damage was induced in rats by treating them with CCl_4 (1ml/kg /bw), administered subcutaneously, on alternate days for one week. Several biochemical parameters like triglyceride (TG),total cholesterol (TC), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) were taken into consideration to assess dyslipidemic changes .The effects of ethanolic extract of *Mimosa pudica* (at a dose of 400mg/kg) was observed on the following parameters. In addition to biochemical parameters liver weight and histological sections were also studied. The statistical analysis of data was done by using one way ANOVA followed by Dunnet's t test. **Results:** Administration of ethanolic extract of *Mimosa pudica* significantly prevented carbon tetrachloride induced elevation of serum TG, TC, VLDL and LDL levels. Histological examination of the liver section revealed improved hepatic architecture, after administration of *Mimosa pudica*. **Conclusions:** Our study demonstrates hypolipidemic effect of *Mimosa pudica* in dyslipidemia following carbon tetrachloride induced liver injury.

INTRODUCTION: The liver is an important organ in the body that plays a vital role in metabolism and elimination of foreign substances.[1] The liver is at a high risk of getting diseased by various foreign compounds because all these foreign compounds are eliminated by the liver. [2, 3]

Herbal medicine is based on the principle that plants contain some natural substances that can promote health and cure illness.[4] The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds.[2]

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Carbon tetrachloride (CCl_4) is a well recognized xenobiotic, liver and kidney being its principle target organs. CCl_4 is activated in the liver to a highly reactive trichloromethyl radical which initiates free radical mediated lipid peroxidation of the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane, which result in accumulation of lipid derived oxidants causing liver injury.[5] Features of CCl_4 induced hepatic injury include fatty degeneration of liver, raised liver enzymes and lipid levels and haemorrhagic necrosis of liver cell.

A fatty liver state is very often accompanied by dyslipidemia. Though the reason is not exactly known however it is thought to be due to but it is likely related to hepatic overproduction of the very low-density lipoprotein particles and dysregulated clearance of lipoproteins from the circulation.[6] Medicinal plants are considered to be an important

source of antioxidant compounds and the therapeutic benefit of many medicinal plants is to their antioxidant properties.[7]

Mimosa pudica also known as Chuimui [8] or Lajwanti in hindi has been used in traditional medicine for its wound healing, [9] analgesic and anti-inflammatory [10], anticonvulsant, [11] antidiarrhoeal, [12] antioxidant, [13] hypolipidemic, [14] antiulcer [15] activities. *M. pudica* leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, alkaloids, phenols, tannins, saponins and coumarins.[16]

The present study was performed to assess the hypolipidemic activity in rats against dyslipidemia following carbon tetrachloride induced hepatic injury.

METHODS:

Preparation of extract: [17]

Mimosa pudica Linn leaves were collected from a field near Basic Science Building, Silchar Medical College and Hospital. The plant sample was verified by Mrs. Purnima Dutta Choudhury, M.Sc, Associate Professor, Department of Botany, Cachar College, Silchar.

Absolute alcohol (99.9% v/v) was used for the extraction process as almost all the components of *Mimosa pudica* is soluble in ethanol solvent. The leaves were shade dried and powdered. The powder was subjected to extraction with absolute alcohol in Soxhlet Apparatus at 45^{o} C for a period of 4 hours. The extract was collected and filtered using Whatman No 1 filter paper and the filtrate was then subjected to evaporation under reduced pressure until a soft semi solid mass was obtained. The sample was stored in the refrigerator as a stock solution dissolved in liquid paraffin in 1:2 ratio (sample **:** liquid paraffin) in an airtight container at a temperature of below 10^{o}C.

Preliminary phytochemical analysis:

The ethanolic extract was then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents, it revealed the presence of flavonoids, alkaloids and glycosides.

Experimental Animals:

Wistar albino rats of either sex weighing between 150-200 grams were procured from M/s Chakraborty Enterprise, Kolkata. The animals were acclimatized under laboratory conditions.

The animals were housed under standard conditions of temperature (25±2°C) and relative humidity (30%–70%) with a 12:12 light-dark cycle. They were fed with standard laboratory food. Water was allowed *ad libitum* under strict hygienic conditions.

Experimental induction of hepatotoxicity:

Liver toxicity was induced in rats by administrating carbon tetrachloride (CCl4) subcutaneously (sc), in a suspension of liquid paraffin (LP; 1: 2 v/v) at a dose of 1 ml/kg body weight, on alternate days, for one week.[18]

Experimental Methodology [19]

In the experimental design the albino rats were assigned into three groups of ten animals each.

Group A was maintained as control which received liquid paraffin (3ml/kg) only by subcutaneous route on alternate days for one week.
Group B was maintained as induced group which received CCl4 subcutaneously in a suspension of liquid paraffin in 1: 2 v/v at the dose of 1 ml of CCl4/kg body weight on alternate days for one week.

Group C served as test group and received ethanolic extract of the leaves of *Mimosa pudica* (400mg/kg, p.o) daily and CCl4 (1ml/kg, s.c in a suspension of liquid paraffin in a ratio 1:2 v/v) on alternate days for one week.

On the eighth day blood was withdrawn by retro orbital puncture and serum separated by centrifugation for estimation of biochemical parameters like triglyceride (TG), total cholesterol (TC), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).

Histology:

The animals were sacrificed under mild ether anaesthesia and the liver was removed from each animal after dissection. The liver tissue was excised

from the animals, washed with the normal saline to remove blood, fixed in 10% buffered neutral formalin for 12 hours and processed for paraffin embedding. Sections of 5µm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series, stained with alum haematoxylin and eosin, cleared in xylene and cover slipped in DPX. Histological examination was done under microscope. [20] Some of the slides were subjected to Periodic Acid Schiff (PAS) staining as fat droplets stain negative to PAS stain.

Statistical Analysis of Data:

The statistical analysis of data was done by using one way ANOVA followed by Dunnet's t test. Values were expressed as Mean ± SD (n= 10). The tests were considered to be significant when $p< 0.05$ and highly significant when $p< 0.01$.

RESULTS:

Acute toxicity studies:

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

Hypolipidemic activity:

The results of carbon tetrachloride induced dyslipidemia are shown in **Table 1**. In the carbon tetrachloride treated group, the significant dyslipidemic changes are indicated by the elevated levels of TG, TC, VLDL and LDL. The test drug of ethanolic extract at the dose of 400mg/kg body weight p.o showed a significant decrease in the elevated levels of TG, TC, VLDL and LDL. However, satisfactory results could not be obtained in case of serum HDL levels.

Effects of different treatment groups on weight of liver:

In the induced group i.e Group B there is an increase in the weight of liver as compared to control group i.e Group A and Group C, the group that received ethanolic extract of the leaves of *Mimosa pudica* (400mg/kg). See **Table 2.**

Histological findings:

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 carbon tetrachloride intoxicated liver (**Fig. 1b**). The liver sections of the rat treated with 400mg/kg body weight p.o of ethanolic extract of *Mimosa pudica* (**Fig. 1c**) showed less vacuole formation and absence of necrosis. Some of the slides were subjected to Periodic Acid Schiff (PAS) staining as fat droplets stain negative to PAS stain. This was done to rule out glycogen droplets (**Fig. 1d**).

Table 1: Comparison of effects of different treatment groups on lipid parameters in carbon tetrachloride induced hepatic injury in albino rats.

Groups	Biochemical Parameters (Mean ± SD)				
	TG (mg/dl)	TC (mg/dl)	VLDL(mg/dl)	LDL (mg/dl)	HDL (mg/dl)
GROUP A (Control)	90.3 ± 6.2	95.7 ± 7.07	24.7 ± 2.9	33.6 ± 2.5	38.7 ± 4.7
GROUP B (Induced)	190.4 ± 11.7 **	200.7 ± 12.2 **	29.2 ± 3.3 *	59.2 ± 3.2 **	32.8 ± 3.7**
GROUP C (Test)	96.8 ± 5.2ns	98.7 ± 5.2ns	26.9 ± 4.1ns	37.4 ± 2.7*	34.3 ± 3.8*

Values are mean ± SD (n = 10), data were analyzed using one way ANOVA and Dunnet's t test. Where, ** represents highly significant at $p < 0.01$, * represents significant at $p < 0.05$ and ns represents non significant during comparison of induced group B & test group C with control group A.

TABLE 2: COMPARISON OF EFFECTS OF DIFFERENT TREATMENT GROUPS ON THE WEIGHT OF LIVER

Groups	Weight of liver (gram)
Group A (Control)	4.262±0.380
Group B (Induced)	5.035±0.285**
Group C (Test)	4.415±0.312*

Values are mean ± SD (n= 10), data were analyzed using one way ANOVA and Dunnet's t test, Where, ** represents highly significant at $p < 0.01$(Comparison of Group B with Group A).Also, * represents significant at $p < 0.05$ (Comparison of Group C with Group B)

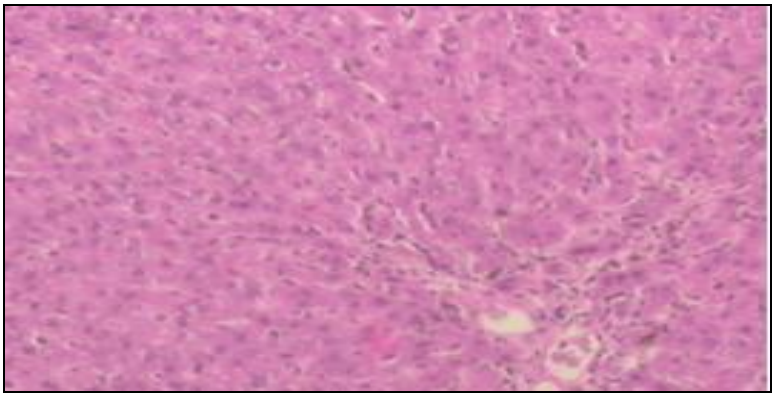

FIG.1A: PHOTOMICROGRAPH OF LIVER FROM GROUP A SHOWING NORMAL HEPATOCELLULAR ARCHITECTURE; H&E STAIN. VIEW: 10 X 40X

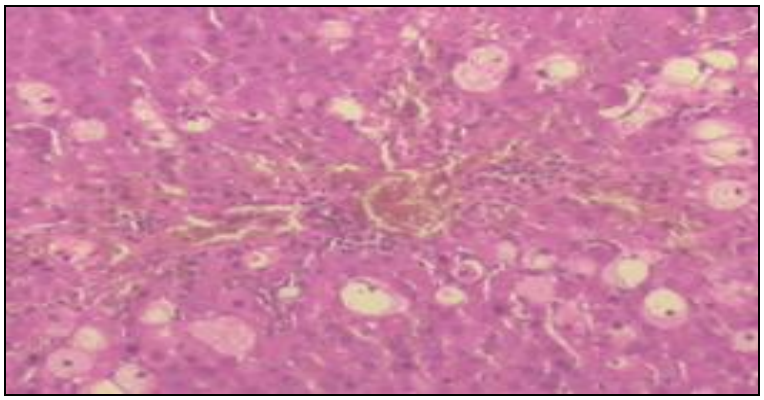

FIG.1B: PHOTOMICROGRAPH OF LIVER FROM GROUP B SHOWING FEATURES OF HEPATIC INJURY H&E STAIN. VIEW: 10 X 40X

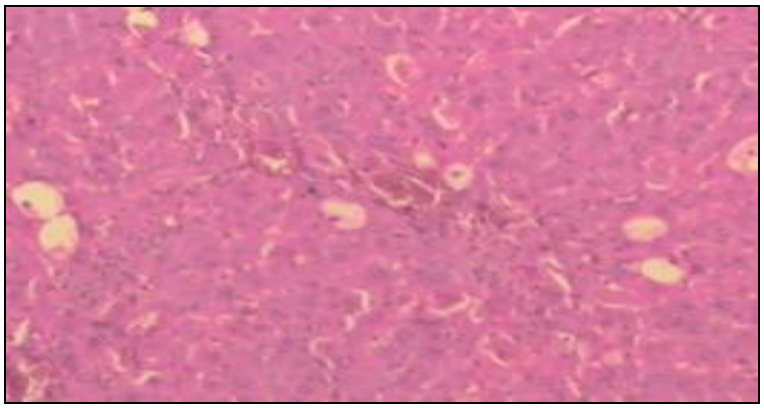

FIG. 1C: PHOTOMICROGRAPH OF LIVER FROM GROUP C SHOWING FEATURES OF REGENERATION AND REPAIR; H&E STAIN. VIEW: 10 X 40X

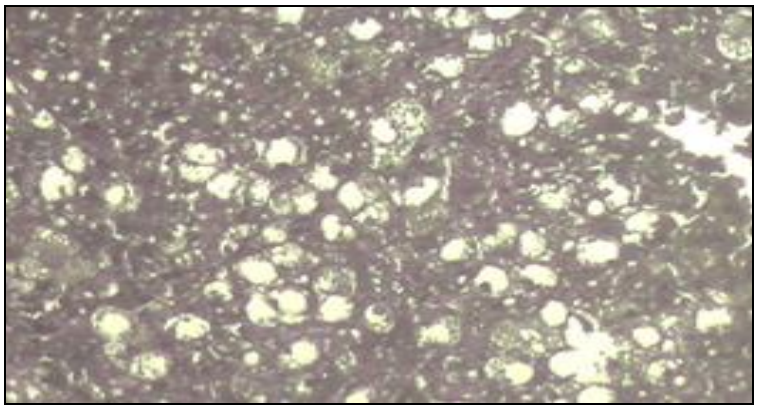

FIG. 1D: PHOTOMICROGRAPH OF LIVER FROM GROUP B SHOWING FEATURES OF HEPATIC INJURY. PAS STAIN.VIEW: 10 X 40X

DISCUSSION: In the living system liver is considered to be highly sensitive to toxic agents. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Herbal medicine is based on the principle that plants contain some natural substances that can promote health and cure illness.[4] The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. Upon literature review it was found that *Mimosa pudica* possessed antioxidant properties and could very well serve as a hepatoprotective agent. The ethanolic extract of the leaves possesses different phytoconstituents like alkaloids, flavonoids, tannins, saponins, coumarin, terpenoids and phenlols. Flavonal aglycone and their glycosides are potent antioxidants which can prevent degenerative diseases and dyslipidemia.[21]

Carbon tetrachloride, a widely used experimental hepatotoxicant, is biotransformed by cytochrome P – 450 systems to produce the trichloromethyl free radical (CCl3•) that causes lipid peroxidation and, thereby, produce liver damage. Carbon tetrachloride produces the dose dependent hepatotoxicity by directly affecting the liver, causing lipid peroxidation.[22]

Dyslipidemia is a key health concern in India and other developing countries, which paves the way to conditions like atherosclerosis, stroke etc which can be critical in the long run. Dyslipidemia, leads to disturbed homeostasis of fatty acids, triglycerides and lipoproteins.[23] Dyslipidemia leads to damages in different tissues,that can unbalance the cellular functions leading to several pathological conditions.

In the present study, treatment with ethanolic extract of *Mimosa pudica* (400 mg/kg p. o.) significantly reversed these elevated serum lipid markers, viz. – TG, TC, VLDL and LDL. In our study, 400mg/kg p. o. dose was highly effective against the dyslipidemia following hepatic injury caused by carbon tetrachloride. Amina EE et al, in their study described that serum TG, TC and LDL cholesterol levels were increased following CCl_4 treatment which was reversed by *Nigella sativa* seed extract.[19] In our study also, we found that serum TG, TC and LDL cholesterol levels were increased following CCl_4 treatment which was reversed by *Mimosa pudica* leaf extract. So, this is evident that *Mimosa pudica* leaf extract reversed dyslipidemic changes following CCl_4 induced hepatic injury.

Also, Hashemi JM, in his study observed that serum VLDL levels were raised in albino rats treated with CCl_4 and this was reversed by treatment with *Hibiscus Sabdariffa* Calyx extract.[24] In our study also, we observed that raised serum VLDL level in albino rats treated with CCl_4 was reversed following treatment with *Mimosa pudica* leaf extract.

Also, in our study, a comparative histopathological study of the liver from different groups, also shows the efficacy of *Mimosa pudica linn* in reversing fatty liver changes like increased fatty infiltration, signs of inflammation and necrosis, distorted architecture of hepatic cells.[25] Hence it can be considered that as fatty liver is associated with dyslipidemia, therefore a drug which can reverse fatty changes in the liver can also afford protection against dyslipidemia.

When we compared serum HDL levels amongst the three groups, we observed that, when the induced group (Group B) and the test group (Group C) were compared with control group (group A), they revealed highly significant ($p < 0.01$) and significant ($p < 0.05$) lowering of serum HDL. However we could not demonstrate any significant effect on HDL cholesterol levels of *Mimosa pudica* leaf extract as compared to the induced group. So, it is not evident how much beneficial effect *Mimosa* extract has on HDL levels. Hence, further detailed studies are anticipated in the future which could reflect more on this aspect.

When we observed the liver size, we found that Group B showed highly significant ($p < 0.01$) increase in liver size as compared to Group A. Eidi A *et al* in his study hepatoprotective effects of Pantothenic acid on carbon tetrachloride-induced toxicity in rats observed increase in weight of liver of albino rats treated with CCl_4.[26] Then when we compared between Group C and Group B we observed that Group C showed a significant

reduction in liver size as compared to group B. So it can be considered that treatment with *Mimosa pudica* leaf extract reduced liver size in CCl_4 treated patients.

Now there is drug called Probucol, a hypolipidemic drug which happens to be a powerful antioxidant and its capability to inhibit atherosclerosis has been ascribed to its antioxidant properties.[27] Similarly, flavonoids present in the plant *Mimosa pudica* may be responsible for its hypolipidemic action because of their antioxidant properties.

In conclusion, from all the above findings, our study demonstrates that the ethanolic extract of leaves of *Mimosa pudica* can be an effective treatment against dyslipidemia following hepatic injury and that future advanced research in this field might revolutionize the treatment of liver disorders.

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DECLARATIONS

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