Original Article

Hypolipidemic Activity of Chloroform Extract of *Mimosa pudica* Leaves

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Abstract

Mimosa pudica Lin., known as chue Mue, is a stout straggling prostrate shrubby plant, with spinous stipules and globose pinkish flower heads, and grows as weed in almost all parts of the country. It is traditionally used for its various properties and hence in the present study, chloroform extract of *Mimosa pudica* leaves has been screened for its hypolipidemic activity. Hypolipidemic activity is screened by inducing hyperlipidemia with the help of atherogenic diet in wistar albino rats and serum levels of various biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol were determined. Atherogenic index shows the measure of the atherogenic potential of the drugs. Chloroform extract showed significant ($p < 0.05$) hypolipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard drug Atorvastatin. Chloroform extract exhibited significant atherogenic index and percentage protection against hyperlipidemia. These biochemical observations were in turn confirmed by histopathological examinations of aorta, liver and kidney sections and are comparable with the standard hypolipidemic drug Atorvastatin. Preliminary phytochemical analysis revealed the presence of phytoconstituents such as steroids, flavonoids, glycosides, alkaloids, phenolic compounds which is further confirmed by the thin layer chromatography, High Performance Thin Layer Chromatography (HPTLC). The overall experimental results suggests that the biologically active phytoconstituents such as flavonoids, glycosides alkaloids present in the chloroform extract of *Mimosa pudica*, may be responsible for the significant hypolipidemic activity and the results justify the use of *Mimosa pudica* as a significant hypolipidemic agent.

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Introduction

Mimosa pudica (Mimosaceae) known as chue Mue, is a stout straggling prostrate shrubby plant with the compound leaves sensitive to touch. It has spinous stipules and globose pinkish flower heads and grows as weed in almost all parts of the country [(1)]. Leaves and stem of the plant have been reported to contain an alkaloid mimosine; leaves also contain mucilage and the root contains tannins [(1)]. *Mimosa pudica* is used for its anti-hyperglycemic [(2)], anti-diarrhoeal [(3)], anti-convulsant [(4)], cytotoxic [(5)] and hepatoprotective properties [(6)].

The plant also contains turgorins leaves.

The leaves and roots are used in the 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 [1]. In Ayurvedic and Unani system of medicine, this plant has been used in diseases arising from corrupted blood and bile, heart disorders, billious fever, piles, jaundice, leprosy, ulcers and small pox.

Dyslipidemia contributes to atherosclerosis, a disease in which fatty deposits called 'plaque' build up in the arteries over time. If plaque narrows the arteries, there is high likelihood to suffer from heart disease, heart attack, peripheral artery disease (reduced blood flow in the limbs, usually the legs), and stroke. People with diabetes are more likely to develop atherosclerosis, heart disease, poor circulation, and stroke than those who do not have diabetes.

Many people with diabetes have conditions called 'risk factors' that contribute to atherosclerosis and its complications. These include high blood pressure, excess weight, and high blood glucose levels. Dyslipidemia further raises the risk of atherosclerosis in people with diabetes. Dyslipidemia affects people with type 2 diabetes more often than those with type 1 diabetes. The most common dyslipidemia in diabetes is the combination of high triglycerides and low HDL levels. People with diabetes may also have elevated LDL cholesterol.

Among the drugs available to treat dyslipidemia, statins are often the first choice for lowering total and LDL cholesterol levels. Other drugs that lower cholesterol include cholesterol- absorption blockers, bile acid sequestrants, and nicotinic acid. These may be used in combination if a single drug is not effective in reaching target levels. Fibrates and extended-release niacin may be used to lower triglycerides or raise HDL cholesterol levels [7].

Hyperglycemia and dyslipidemia are significant and independent risk factors for the vascular complications and suggested to cause cardiovascular pathologic changes in diabetic states through the following molecular mechanisms: formation and accumulation of advanced glycation products, increased oxidetive stress, activation of protein kinase C pathway, increased activity of hexosamine pathway, and vascular inflammation and the impairment of insulin action in the vascular tissues [8].

As *Mimosa pudica* plant species have been traditionally claimed for the treatment of atherosclerosis; hence, in the present study, an attempt has been made to screen the herbal extract that is chloroform extract of *Mimosa pudica* leaves, for the hypolipidemic activity to prove its claim in folklore practice.

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 plant biology and Plant biotechnology, Madras Christian College, Tambaram, Chennai, Tamilnadu, India. The voucher specimen (023/02/09) was deposited at the Department of Pharmacognosy and Phytochemistry M. S. A. J College of Pharmacy, India, for future reference. Care was taken to collect the healthy and young leaves of *Mimosa pudica*.

Preparation of extract

The coarsely powdered leaves (300 *g*) of *Mimosa pudica* was extracted to exhaustion in a soxhlet apparatus [9] at 50 ^{o}C with 500 *ml* of chloroform. 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 chloroform extractive of 8.20 *g*.

Preliminary phytochemical analysis

The chloroform extract of *Mimosa pudica* leaves was then subjected to preliminary phytochemical [10] analysis to assess the pre-

sence of various phytoconstituents; it revealed the presence of flavonoids, alkaloids and glycosides. Preliminary thin layer chromategraphy studies also confirmed these constituents [11].

HPTLC analysis

Fifteen *µl* of chloroform extract of *Mimosa pudica* Lin., was spotted on pre-coated silica gel TLC plate of dimension (10 x 6 *cm*) (E. Merck) after activation at 105 *°C*. Then the spotted plate was developed in a pre-saturated chamber containing the solvent system of Toluene: Ethylacetate (3:1), as the mobile phase conditions for separation. Developed plate was air dried and scanned under UV 254 *nm* using Camag densitometer and the chromatogram was recorded. Then the plate was sprayed with spray reagent 1% vanillin sulphuric acid and heated at 105 *°C* in hot air oven for 5 to 10 *min* to develop the color of the spots. After color development, the plate was again scanned under visible range at 550 *nm* and the chromatogram was recorded.

$$\text{RF (Retention factor)} = \frac{\text{Migration distance of substance}}{\text{Migration distance of solvent}}$$

Animals

Wistar albino rats weighing 175-225 *g* of either sex maintained under standard husbandry conditions (temp 23 ± 2 ^{o}C, relative humidity 55 ± 10% and 12 *hr* 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 were approved by the institutional animal ethics committee, India 2009.

Toxicity studies

Acute toxicity study was performed for chloroform extract according to the acute toxic classic method as per OECD guidelines [12]. 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 300 *mg/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 & 2000 *mg/kg* body weight. The animals were observed for toxic symptoms for 72 *hrs*.

Hypolipidemic Activtty [13]

The animals were divided into four groups with six animals in each group. In order to render the rat's hyperlipidemia, they were given an atherogenic diet comprising of corn flour base, milk powder, butter, salt, ground nut oil, sucrose, and vitamin mixture. In addition 400 *mg* of cholesterol powder/ *kg* body weight was dissolved in coconut oil and administered orally for 45 days.

Group I was considered as control which received 0.5% sodium carboxy methyl cellulose; Group II was considered as atherogenic group and received the atherogenic diet; Group III was considered as test group and received the test extract that is chloroform extract of *Mimosa pudica* at the dose of 200 *mg/kg* body weight per oral along with the atherogenic diet and Group IV was considered as standard group which received the standard drug Atorvastatin (dose of 1.2 *mg/kg* body weight per oral) along with the atherogenic diet.

At the end of 45th day, blood serum was withdrawn from the retro orbital plexus after overnight fasting for the study of biochemical parameters. Serum was estimated for the total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol. Atherogenic Index (AI), which is a measure of the atherogenic potential of an agent, was calculated using the following formula and the results were tabulated.

$$\text{Atherogenic Index} = \frac{\text{Total serum triglyceride}}{\text{Total serum HDL-C}}$$

$$\text{\% Protection} = \frac{\text{AI of control- AI of treated group}}{\text{AI of control}} \times 100$$

Histopathological studies

At the end of the treatment period, animals from all the four groups were sacrificed and aorta, liver, kidney were dissected out, washed, 5 *μm* thick section slides were prepared and stained with haemotoxylin – eosin and examined by light microscopy.

Statistical analysis

Results were presented as mean±SD. The significance of difference among the groups was assessed using one way analysis of variance (ANOVA) followed by Dunnet's test. $P < 0.05$ was considered significant.

Results

The preliminary phytochemical screening revealed the presence of phytoconstituents such as glycosides, alkaloids, flavonoids and phenolic compound in the chloroform extract of *Mimosa pudica* Lin., leaves. TLC and HPTLC analysis also confirmed these phytoconstituents. HPTLC profile is shown in table 1, the TLC plate for the chloroform extract is shown in figure 1. The HPTLC finger print for chloroform extract showed 6 peaks at the wavelength 260 *nm* and at 550 *nm*, the chloroform extract showed 7 peaks. The total height and the area of the peaks are shown in table 1.

Acute toxicity studies

Chloroform extract of *Mimosa pudica* did not produce any toxic symptoms or mortality up to 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

A marked increase in the level of serum cholesterol, triglycerides, LDL and VLDL were found in the animals which received atherogenic diet and HDL levels were decreased. Administrations of chloroform extract at the dose of 400 *mg/kg* shows significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard Atorvastatin, and are almost near the levels of normal control.

Table 1. HPTLC finger print data for chloroform extract of M*imosa pudica*

Extract	Wavelength (*nm*)	No. of peaks	Total height	Total area
Chloroform	260	6	782.8	32750.1
	550	7	13670	85680.4

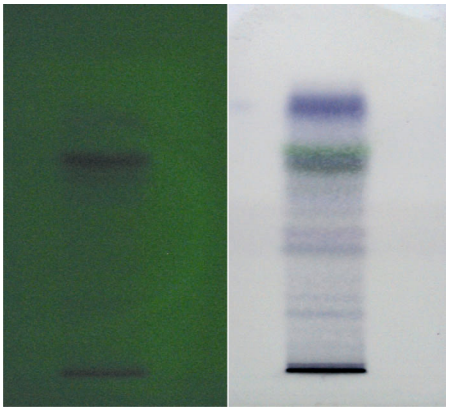

Figure 1. TLC plates for chloroform extract of *Mimosa pudica*

A significant percentage reduction of serum cholesterol, triglyceride, LDL, VLDL and percentage increase in HDL in test extract was also comparable with the standard drug. A potent hypolipidemic effect of chloroform extract was evident by a significant reduction in the level of serum cholesterol, LDL, VLDL and triglycerides in the cholesterol treated animals and also marked increase in the HDL level (Table 2).

The Atherogenic Index was considerably decreased in the plant extract group which was also comparable with the standard group Atorvastatin against hyperlipidemia. The percentage of protection against the hyperlipidemia in the plant extract treated group was 63.3%, where as the standard group protection is 68%, which further confirms the significant protective effect of the plant extract against hyperlipidemia (Table 3).

The histopathological section of aorta of atherodiet fed animals shows marked atheromatous thickening in the intima. The atheromatous inflammatory changes were absent in normal control group and standard drug treated group (Figure 2). Liver section of atherodiet fed animals shows marked peri-

Table 2. Effect of *Mimosa pudica* on biochemical parameters

Treatment groups	Total cholesterol (*mg/ dl*)	Triglycerides (*mg/ dl*)	HDL (*mg/ dl*)	LDL (*mg/ dl*)	VLDL (*mg/ dl*)
Control I	101.4 ± 0.16	121.2 ± 0.82	36.3 ± 0.07	80.9 ± 0.5	19.4 ± 0.16
Atherodiet II	242.4 ± 0.31	160.8 ± 0.12	25.3 ± 0.06	135.2 ± 0.13	30.6 ± 0.05
Chloroform Extract III	112.6 ± 0.17**	112.6 ± 0.42***	34.1 ± 0.11***	82.9 ± 0.15**	18.9 ± 0.09**
Standard Atorvastatin IV	107.1 ± 0.08***	107.3 ± 0.03***	32.2 ± 0.07***	85.0 ± 0.25***	20.6 ± 0.10***

Values expressed as mean ± SEM. Levels of significance - Group II compared with Group I, III and IV. *** $p < 0.01$, *** $p < 0.001$ and *** $p < 0.0001$

Table 3. Atherogenic Index of *Mimosa pudica*

Treatment groups	Athergonic Index (A.I)	% Protection
Control I	3.34	-
Atherodiet II	6.36	-
Chloroform Extract III	2.33	63.3%
Standard Atorvastatin IV	2.25	68%

venular inflammatory fatty changes, which are comparatively mild in chloroform extract (Figure 3). Kidney section of atherodiet fed animals shows marked congestion and hyaline droplet formation in the tubules. While in standard and chloroform extract treated animals there was mild congestion, the architecture of the kidney was not altered markedly (Figure 4). From this, it the anti-atherosclerotic effect of *Mimosa pudica* is confirmed.

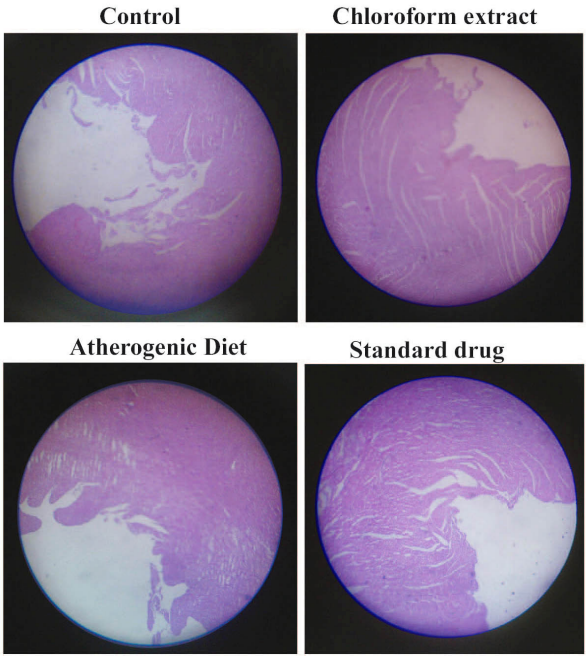

Figure 2. Histopathological sections of aorta (Atherogenic- atheromatous thickening intima)

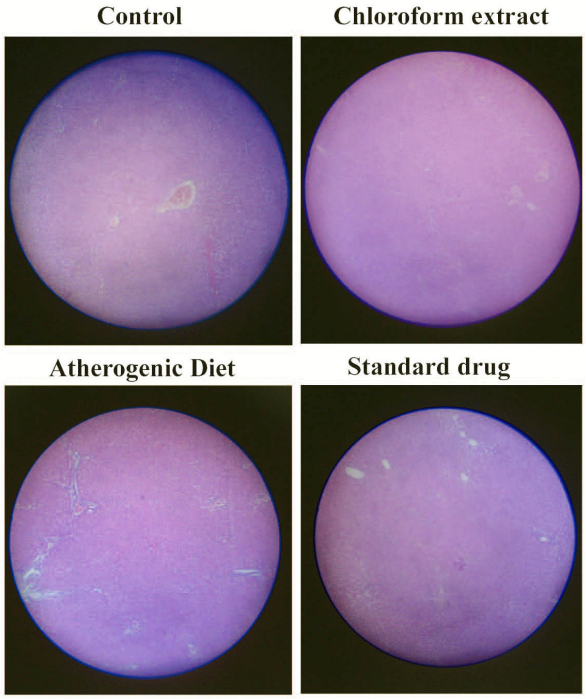

Figure 3. Histopathological sections of liver (Atherogenic- perivenular inflammatory change, fatty lobular infiltration)

Discussion

The present studies were performed to assess the hypolipidemic activity and to prove its claim in folklore practice against various disorders. Probucol, a hypolipidemic drug is a potent lipophilic antioxidant and the ability to inhibit atherosclerosis has been attributed to its antioxidant properties [14]. Probucol lowers the level of cholesterol in the bloodstream by increasing the rate of LDL catabolism. Additionally, probucol may inhibit cholesterol synthesis and delay cholesterol absorption. Probucol is a powerful antioxidant, which inhibits the oxidation of cholesterol in LDLs; this slows the formation of foam cells, which contribute to atherosclerotic plaques. Similar-

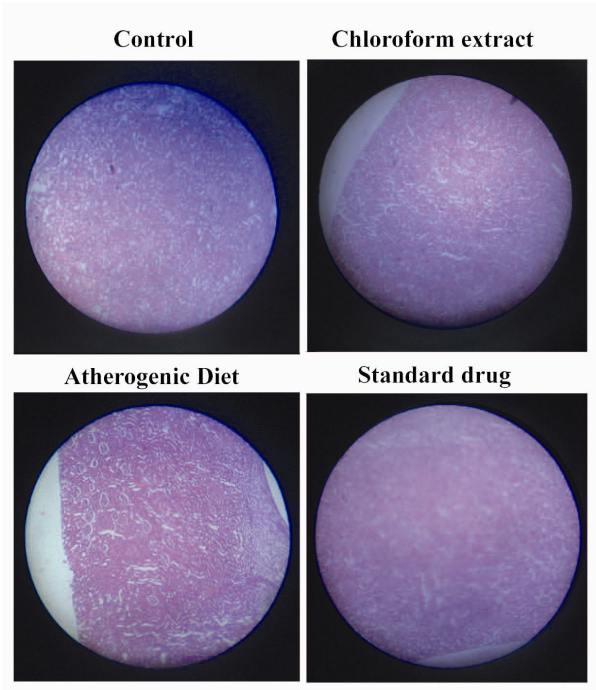

Figure 4. Histopathological sections of kidney (Atherogenic- congestion and hyaline droplet formation)

ly, flavonoids present in the plant *Mimosa pudica* may be responsible for its hypolipidemic action and as already reported significant antioxidant activity of chloroform extract further confirms its significant hypolipidemic activity [15].

In addition, the past decade saw a series of remarkable studies that suggested oxidative systems; particularly oxidation of LDL is a risk factor and plays a role at several steps of atherosclerosis [16, 17]. A decrease in oxidative stress and protection of LDL from oxidation might therefore be a strategy with great promise for prevention of atherosclerosis associated cardiovascular disease [18]. The intense interest in this area stems in part from the generally low toxicity of antioxidants and the hope that treatment with antioxidants might be additive with cholesterol lowering regimes.

It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of cholesterol and LDL levels achieved by administration of chloroform extract, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about. The observed hypolipidemic activity may be further confirmed by the previous studies of the different types of plant extracts [19, 20].

Histopathological findings also supported the protective role of *Mimosa pudica* chloroform extract in preventing atherogenic diet induced hepatic, liver and kidney steatosis. Chloroform extract showed significant ($p < 0.05$) hypolipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard drug Atorvastatin. Chloroform extract exhibited significant Atherogenic Index and percentage protection against hyperlipidemia.

Conclusion

In accordance with these results, it may be confirmed that due to the presence of phytoconstituents such as flavonoids, alkaloids and glycosides in the chloroform extract, it could be responsible for the observed significant hypolipidemic activity. In conclusion, it can be said that the chloroform extract of *Mimosa pudica* exhibited a significant hypolipidemic effect at the dose of 200 *mg/kg* body weight. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hypolipidemic efficacy of this valuable medicinal plant and further studies are required to establish the efficacy of the *Mimosa Pudica* as a hypolipidemic drug.

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