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Effect of *Mimosa Pudica* cured extract against high fructose diet induced type 2 diabetes in rats

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ABSTRACT. The study evaluated the effects of *Mimusa pudica* (*M. pudica*) leaf extract on type 2 diabetes in rats fed high fructose diet (HFD). Rats were fed either control diet or HFD for 14 days, following which the diet was fortified with *M. Pudica* at a dose of 500 mg/kg BW. After 8 weeks, HFD caused deleterious metabolic effects, including increased body weight, hyperglycemia, hyperinsulinemia, dyslipidemia and liver dysfunction. Further, rats fed HFD alone showed increased activities of hepatocellular enzymes in plasma and lipid deposition in liver. Treatment with *M. pudica* significantly reduced the body weight, improved insulin sensitivity, managed the dyslipidemia and reduced liver damage towards normal. Histopathology of the liver confirmed the changes induced by HFD and the *M. pudiac* treatment significantly reversed towards normality. These data suggest that *M. pudica* treatment improve insulin sensitivity and attenuates fat accumulation in liver.

1. INTRODUCTION

Fructose is widely used as sweetener in food processing. It is sweeter than glucose (over than 2-folds) and it is transformed into lipids in the fastest pathway among all carbohydrates and therefore blamed for serious atherogenic effect (Shapiro et al., 2008). The consumption of fructose in diet has increased worldwide in the past two decades. This increase is largely because of an augmentation in the consumption of soft drinks and many other beverages high in fructose and the consumption of foods such as breakfast cereals, baked goods, condiments and desserts sweetened with sucrose and high fructose corn syrup (Elliott et al., 2002).

Exposure of liver to such large quantities of fructose leads to rapid stimulation of lipogenesis with the accumulation of triglycerides (TGs), which contributes, in turn, to reduced insulin sensitivity and hepatic insulin resistance / glucose intolerance (Moore, Cherrington, Mann, & Davis, 2000). Animal studies have shown that high fructose diet-fed rats display hepatic insulin resistance and altered lipid metabolism due to hepatic lipid accumulation as a result of the burden of fructose metabolism (Kelley et al., 2004). This cluster of disorders is similar to those observed in human multimetabolic syndrome or syndrome X or insulin resistance syndrome, which is observed in prediabetic patients that progresses to type 2 diabetes mellitus and cardiovascular diseases (Reaven and Banting, 1988).

Recently, plant foods have been used for prevention of diabetes mellitus because of the likelihood of high compliance and because they are largely free from side effects (Dimo et al., 2001). *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. Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root

contains tannins (Bhakuni et al, 1969). *Mimosa pudica* is used for its anti-hyperglycemic, antidiarrhoeal, anti-convulsant and cytotoxic properties (Rajendran et al., 2009).

As *Mimosa pudica* plant species have been traditionally claimed for the treatment of diabetes; hence, in the present study, an attempt has been made to screen the herbal extract of *Mimosa pudica* leaves, for the insulin signaling molecules in high fructose diet (HFD) induced type 2 diabetes in rats, to prove its claim in folklore practice

2. MATERIALS AND METHODS

2.1. Animals

Male albino rats, weighing about 160 - 180 g, were purchased and maintained under standard experimental conditions (temperature 27 ± 2 °C; relative humidity 60 ± 5% and 12 h light/dark cycle) and they were fed with pelleted diet manufactured by Hindustan Lever Ltd, and had free access to water *ad libitum*. All the experiments comply with the recommendations and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.2. Preparation of plant extract

The leaves of *M. pudica* were collected form Muthupet, Thiruvarur district, Tamilnadu, during the months of December to February. The collected leaves were shade dried, powdered. One kilogram of the dried plant material was exhaustively extracted with 2 L methanol. The extract was filtered and distilled on a water bath. The crude extract was used as a drug.

2.3. Preparation of diet

Standard pellet diet: The standard diet comprised of protein-21.1%, fat-5.1%, carbohydrates-60.0%, fiber-3.9%, minerals-7.9%, vitamins-2.0%. High fat diet: HFD was prepared by mixing fructose (66 %) with standard pellet diet. All measures were taken to ensure uniform mixing of the additives of the diet before kneading using a little water.

Experimental Design:

Test animals were fed initially, before the study, with standard diets. Then these were assigned to one of four groups with 6 rats in each group: Group 1 received standard pellet diet for 6 weeks. Group 2 received *M. pudica* (100 mg/kg BW) for last 2 weeks. Group 3 received HFD for 6 weeks. Group 4 received HFD for first 4 weeks then oral administration of *M. pudic* (100 mg/kg BW) along with HFD for next 2 weeks.

Group I	:	Normal untreated rats.
Group II	:	Normal rats administered *M. pudica* 100 mg/kg BW orally twice a day for last 2 weeks.
Group III	:	Fructose induced diabetic rats.
Group IV	:	*M. pudica* was administered 100 mg/kg BW orally twice a day for last 2 weeks after the induction of diabetes.

Body weight and food intake of mice were measured weekly once. At the end of the experimental period, rat was sacrificed by cervical dislocation. Blood was collected by cutting the jugular vein into heparinised glass tubes. Plasma was obtained from blood samples after centrifugation (1500×g for 10 min) and stored at 4 °C until analysis. After collecting the blood, the liver was removed, rinsed with physiological saline and used for the various biochemical parameters.

2.4. Biochemical Estimation

Glucose was estimated by the method of Trinder using a reagent kit (Trinder, 1969). The insulin in the rat plasma was measured by the method of Burgi et al. (1988). Glycogen content was determined as described by Morales et al. (1975). Plasma and liver total cholesterol, triglycerides

and free fatty acids were estimated by the methods of Siedel et al. (1983), Foster and Dunn (1973) and Falholt et al. (1973), respectively. Plasma high density lipoprotein-C was estimated by the method of Warnick et al. (1985). The activities of serum AST, ALT, and GGT were assayed by the method of Reitman and Frankel (1957) and Rosalki and Rau (1972).

2.5. Histopathology of liver tissue

Liver tissue was quickly removed after euthanasia, fixed in 10% buffered formalin for 48 h, dehydrated by passing successively in different concentrations of ethanol–water, cleaned in xylene, embedded in paraffin and sectioned (5–6 μm thickness) using a microtome. Sections were stained with hematoxylin and eosin (H&E) dye.

2.6. Statistical analysis

Values are given as means ± S.D. for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at $p \leq 0.05$.

3. RESULTS

Figure 1 show the body weight of normal and experimental rats. The HFD caused a significant increase in body weight in comparison with the normal group. The *M. pudica* treatment caused a significant reduction in body weight comparison with the HFD induced diabetic rats.

Table 1 illustrates the plasma glucose, insulin and glycogen level (liver and skeletal muscle) in normal and experimental rats. The levels of plasma glucose and insulin increased significantly and glycogen level (liver and skeletal muscle) decreased significantly in the HFD group in comparison with the normal group. *M. pudica* treatment showed a significant reduction in plasma glucose and insulin while glycogen level (liver and skeletal muscle) was significantly increased.

Table 2 depicts the effect of *M. pudica* on serum lipid profile in normal and experimental groups. A significant increase in total cholesterol (TC), TG and free fatty acid (FFA) with concomitant decrease in high density lipoprotein (HDL) was seen in HFD fed groups as compared to normal group. Treatment with *M. pudica* significantly decreased TC, TG and FFA while HDL level increased significantly.

Table 3 represents the levels of lipid profiles in liver of normal and experimental groups. HFFD fed rats showed significant increase in TC, TG, FFA and PL as compared to control group. Treatment with *M. pudica* significantly decreased TC, TG, FFA and PL level as compared to HFFD fed rats.

Table 4 shows liver weight and activities of aspartate transaminaseAST, ALT and GGT in the liver of control and experimental rats. The liver weight was significantly higher in HFFD fed mice when compared to normal rats. However, increased liver weight observed in HFFD fed mice was significantly brought down to near-control levels in *M. pudica* administered HFFD fed animals. The activities of AST, ALT and GGT in the liver were significantly elevated in the HFFD fed rats as compared to the normal rats. Administration with *M. pudica* decreased the activities of all the above enzymes in the liver as compared to the HFFD fed rats.

4. DISCUSSION

Fructose is a potent reducing sugar that promotes the formation of toxic advanced glycation end-products. Excessive fructose consumption may be responsible in part for the increasing prevalence of obesity, diabetes mellitus, and non-alcoholic fatty liver disease characterized by an impaired glucose tolerance test. In the present study clearly shows, high fructose feeding resulted in significant increase in the body weight, fasting hyperglycemia, hypertriglyceridemia, hyperinsulinemia, glucose intolerance and accumulation lipids in liver leading to the development of insulin resistance.

Chronic fructose feeding in experimental animals is reported to produce glucose tolerance and increase in body weight associated with hyperinsulineamia and loss of normal in vivo sensitivity to

insulin (Rizkalla et al., 1993; Tordoff and Alleva, 1990). Our results are consistent with previous studies which found that consumption of high-fructose diets markedly induces an increase in body weight, glycemia associated with hyperinsulinemia and, consequently, a reduction of glycogen level in tissues. In the present study *M. pudica* treatment significantly reduced the body weight, plasma glucose and insulin level and the improvement in glycogen level. The likely advantage of drugs ameliorating hyperinsulineamia is likely to have greater therapeutic potential as drug.

The dyslipidemia observed in fructose fed rats was reflected by significantly enhanced plasma TG, TC and FFA with significantly decreased HDL-C. The blood lipid levels are also probably a major determinant of cardiovascular disease (Shalam et al., 2006). Many extensive studies have examined the ability of natural compounds to induce hypolipidaemia in normal animal models or fructose induced obese animal model without side effects. High levels of plasma triacylglycerols are a well-established consequence of dietary fructose intake (Vos et al., 1996). *M. Pudica* treatment produced significant decrease in serum TG, TC and FFA while there was a significant increase in HDL-C.

Levels of total lipid and those of cholesterol, TG and FFA were significantly elevated in liver. Fatty liver is a serious risk factor for the development of liver injury. Results of the histological changes in HFD rats, such as widespread deposition of lipid droplets are consistent with the result of the biochemical analysis. Evidence of lipid accumulation in liver exposed to HFD and in rats drinking fructose-sweetened beverages (Aragno et al., 2009) has been reported. Fructose is highly lipogeneic and the HFFD diet used in this study may have resulted in the increased delivery of fatty acids through the portal circulation resulting in fatty liver. Treatment with *M. pudica* substantially reduced the elevated levels of hepatic lipids in liver.

Hepatic damage in fructose-fed rats was evident from the increased plasma transaminase, as well as the decline in hepatic function. Increased AST, ALT and GGT activity is considered as markers of increased metabolic activity (Shalam et al., 2006). This biochemical changes reflect hepatocellular damage in fructose-fed rats. The additive effect of *M. pudica* decreased the AST and ALP activities towards normality showing the hepato protective effect. Reduction in AST activity therefore suggests decreased metabolic activity in *M. pudica* administered rats. Moreover drug being Hepatoprotective may also have a role in attenuating hepatic changes brought about by high fructose feeding.

5. CONCLUSION

In conclusion, *M. pudica* treatment has the potential to reverse the inability of increase in the body weight, hyperglycemia, hypertriglyceridemia, hyperinsulinemia, glucose intolerance and accumulation lipids in liver of rats receiving fructose-rich chow. From this point of view, *M. pudica* could become a promising category of therapeutic agents for in the treatment for fructose induced type 2 diabetes.

Table 1. Effect of *M. pudica* in the levels of plasma glucose, insulin and tissue glycogen in normal and experimental rats

Treatment	Glucose (mg/dl)	Insulin (µU/ml)	Liver Glycogen (mg/g tissue)	Muscle Glycogen (mg/g tissue)
Normal	83.87 ± 2.58^{a}	15.42 ± 1.46^{a}	20.14 ± 0.74^{a}	6.78 ± 0.56^{a}
Normal + *M. pudica*	86.31 ± 2.47^{a}	32.54 ± 3.63^{a}	19.65 ± 0.70^{a}	6.54 ± 0.48^{a}
HFD	286.49 ± 7.88^{b}	25.64 ± 2.76^{b}	8.91 ± 0.43^{b}	3.24 ± 0.24^{b}
HFD + *M. pudica*	96.49 ± 2.73^{c}	17.63 ± 1.96^{c}	21.43 ± 0.54^{c}	5.48 ± 0.28^{c}

Values that have a different superscript letter (a, b, c) differ significantly with each other ($p < 0.05$, DMRT).

Table 2. Effect of M. pudica in the levels of plasma TC, TG, HDL and FFA in normal and experimental rats

Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/g tissue)	FFA (mg/g tissue)
Normal	80.06 ± 3.65a	59.20 ± 3.96^{a}	47.12 ± 3.13^{a}	58.27 ± 5.54^{a}
Normal + *M. pudica*	74.96 ± 2.46^{a}	54.46 ± 2.73^{a}	44.29 ± 4.40^{a}	56.27 ± 7.62^{a}
HFD	152.25 ± 7.79^{b}	164.15 ± 9.54^{b}	28.60 ± 2.80^{b}	110.13 ± 11.02^{b}
HFD + *M. pudica*	88.22 ± 6.18^{f}	82.74 ± 6.49^{d}	43.12 ± 3.78^{c}	90.66 ± 7.35^{d}

Values that have a different superscript letter (a, b, c) differ significantly with each other ($p < 0.05$, DMRT).

Table 3. Effect of *M. pudica* in the levels of liver TC, TG, HDL and FFA in normal and experimental rats

Treatment	TC (mg/g tissue)	TG (mg/g tissue)	FFA (mg/g tissue)	PL (mg/g tissue)
Normal	4.06 ± 0.37^{a}	3.83 ± 0.34^{a}	8.00 ± 0.43^{a}	22.34 ± 1.75^{a}
Normal + *M. pudica*	3.93 ± 0.23^{a}	3.61 ± 0.32^{ae}	7.86 ± 0.48^{a}	20.53 ± 2.05^{a}
HFD	6.16 ± 0.26^{b}	7.11 ± 0.40^{b}	14.80 ± 0.83^{b}	47.56 ± 3.76^{b}
HFD + *M. pudica*	5.41 ± 0.33^{c}	6.27 ± 0.53^{c}	13.06 ± 0.78^{c}	36.18 ± 3.46^{c}

Values that have a different superscript letter (a, b, c) differ significantly with each other ($p < 0.05$, DMRT).

Table 4. Effect of *M. pudica* in liver weight and activities of ALT, AST and GCT in normal and experimental rats

Treatment	Liver weight (g)	ALT (IU/L)	ALT (IU/L)	GGT (IU/L)
Normal	4.06 ± 0.37^{a}	28.23 ± 2.35^{ad}	28.23 ± 2.35^{ad}	17.07 ± 1.36^{a}
Normal + *M. pudica*	3.93 ± 0.23^{a}	26.48 ± 2.46^{a}	26.48 ± 2.46^{a}	6.52 ± 1.20^{a}
HFD	6.16 ± 0.26^{b}	57.30 ± 5.50^{b}	57.30 ± 5.50^{b}	29.06 ± 2.14^{b}
HFD + *M. pudica*	5.41 ± 0.33^{c}	35.29 ± 3.45^{c}	35.29 ± 3.45^{c}	20.09 ± 2.01^{c}

Values that have a different superscript letter (a, b, c) differ significantly with each other ($p < 0.05$, DMRT).

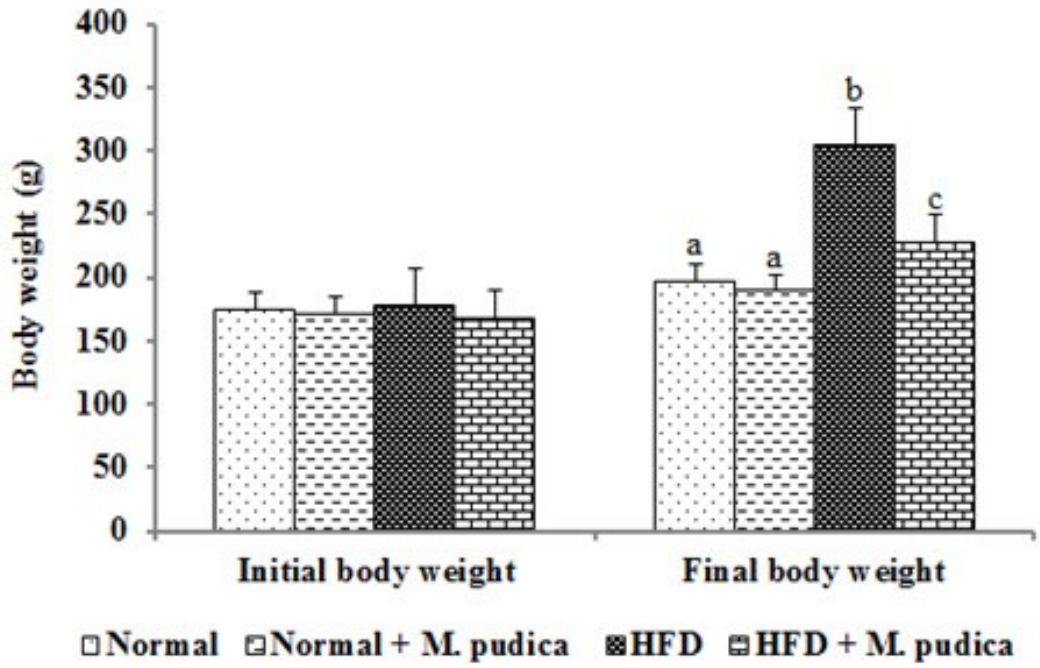

Figure 1. Effect of *M. pudica* on body weight of normal and experimental rats
Values that have a different superscript letter (a, b, c) differ significantly with each other ($p < 0.05$, DMRT).

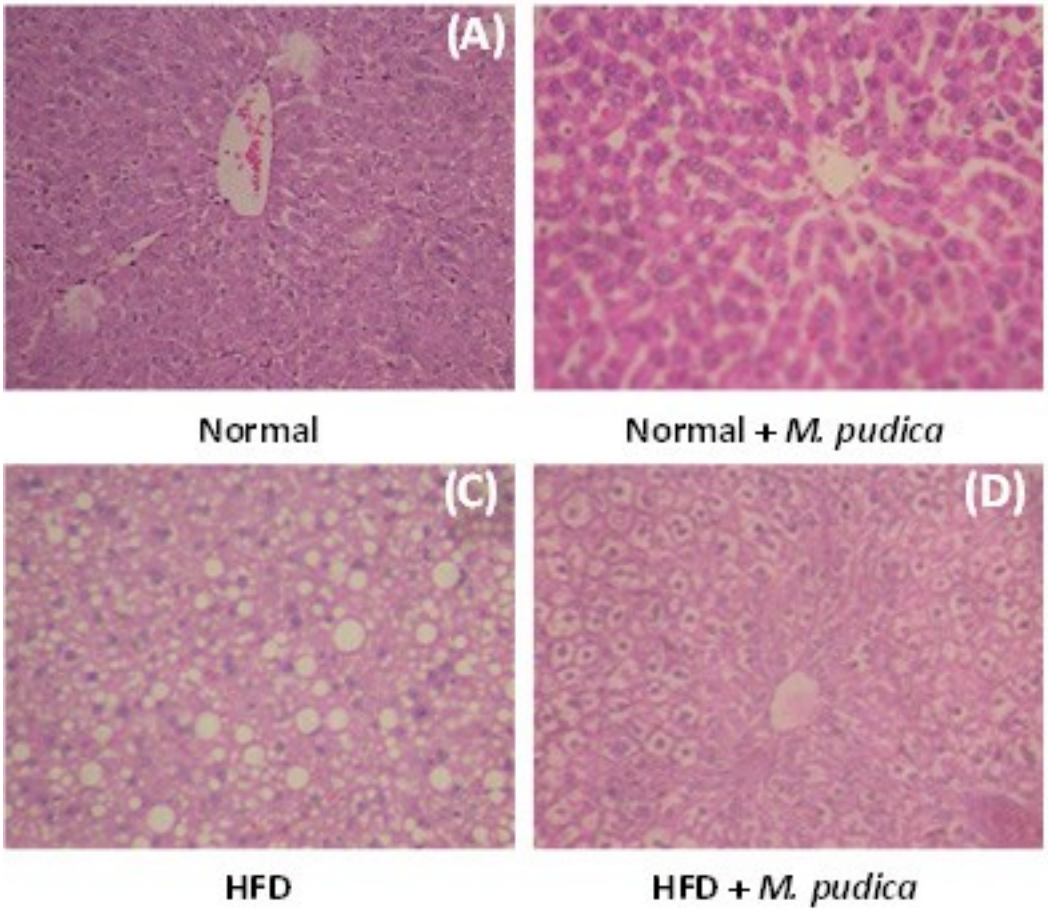

Figure 2. Photomicrographs of liver sections (H and E, 20X)
(A) Normal: shows normal architecture. (B) Normal + *M. pudica*: shows normal appearance. (C) HFD: micro and macro vesicular fatty change with mild inflammation. (D) HFD + *M. pudica*: retains normal hepatic architecture.

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