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ANTIDIABETIC ACTIVITY AND POTENTIAL MECHANISM OF *MUKIA MADERASPATANA* LINN. IN RATS INDUCED BY HIGH FAT DIET AND LOW DOSE STZ

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ABSTRACT: To evaluate the anti-diabetic effects of the *Mukia maderaspatana* Linn. (MML), and to explore the possible mechanism. High-fat diet and STZ (35 mg/kg) induced diabetic rats were administered with MML at two dose levels (200 and 400 mg/kg/day, p.o.) for 21 days. Fasting blood glucose, lipid and lipoprotein levels such as triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and glucose tolerance were tested to evaluate its anti-diabetic effects. Moreover, the preliminary study of MML on the antioxidant activity was performed. The MML possessed anti-diabetic activities as shown by the decreased serum levels of fast blood glucose (FBG), TG, TC, LDL-C, and VLDL-C, as well as increased serum levels of HDL-C. MML also improved the oral glucose tolerance test (OGTT) to a certain degree. These benefits were also associated with increased catalase (CAT) superoxide dismutase (SOD) and decreased malondialdehyde (MDA) in serum. The experimental results highlighted the hypoglycemic and hypolipidemic properties of the MML on diabetes and its complications, possibly through a strong antioxidant activity.

INTRODUCTION: Type 2 diabetes mellitus is one of the most common metabolic disorders and the world prevalence of diabetes among adults is 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030 [1]. Hyperglycemia and hyperlipidemia, as the most common features of diabetes mellitus, contribute to the development of microvascular and macrovascular complications of diabetes, which cause the morbidity and mortality of diabetes [2].

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Treatment involves diet control, exercise, and the use of insulin and/or oral hypoglycemic drugs. However, they usually have decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness [3]. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are recognized as a wonderful source for medicines [4]. World Health Organization (WHO) has emphasized strongly on the rational use of traditional and natural indigenous medicines, for treating diabetes mellitus [5]. In contrast, hundreds of traditional folk medicines have demonstrated the potential for the treatment of diabetes with less tolerability and side effects. Thus, there is an increasing need to search for more natural antidiabetic agents from traditional medicine.

Mukia maderaspatana Linn., (Family: Cucurbitaceae) is an annual monoecious herb, densely covered with white hairs. It is found throughout India ascending to 1800 m in the hills. Folklore medicine claims that it is a good diuretic, stomachic, gentle aperient, antipyretic and antiflatulent [6], antiasthmatic, and antibronchitis besides its use in vertigo [7].

Certain traditional medical practitioners also use the leaf-tea of this plant for the alleviation of jaundice [8]. Decoctions of leaves of this plant have been used by Siddha practitioners in Tamil Nadu for the treatment of hypertension [9]. This plant leaf extract has also been shown to have hepatoprotective [10-11] and immunomodulatory effects [12] antiarthritic activity properties, hypoglycemic [13] and enzyme inhibitory activity [14]. However, no study has been studied towards the mechanism of actions in diabetes in high-fat diet model.

The present study was undertaken to evaluate anti-diabetic effects of the *Mukia maderaspatana* Linn (MML), and to explore the possible mechanism in high fat and low dose STZ model.

MATERIALS AND METHODS:

Plant Material Collection, Identification, and Crude Extract Preparation: The whole plant of *Mukia maderaspatana* Linn (MML) were collected from Doddabetta, Nilgiris, Tamilnadu and authenticated by Dr. S. Rajan, Ph.D. Field Botanist, Survey of Medicinal Plants & Collection Unit, Emerald, Nilgiris. A voucher specimen (JSSCPDP/ 2008/167) has been deposited at the Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy, Udhagamandalam, Tamilnadu.

The plant material was air dried, coarsely powdered and extracted separately with ethanol (95%) in a soxhlet extractor for 24 h. The extract was concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50 ºC). The extracts were stored in a refrigerator at 4 ºC for further studies.

Animals: Healthy Wistar rats weighing 180-220 g, were procured from the animal house, J.S.S. College of Pharmacy, Udhagamandalam, India. The animal house was well ventilated, and animals had 12 ± 1 h day and night schedule. The animals were housed in large spacious hygienic cages during the experimental period, and the room temperature was maintained at 25 ± 1 °C. The animals were fed with standard rat feed and water *ad libitum*. The guidelines of the committee for control and supervision of experiments on animals (CPCSEA), Chennai, Govt. of India were followed, and prior permission was sought from the institutional animal ethics committee for conducting this study.

Chemicals: The kits for measurement of fasting blood glucose (FBG), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), lipids and lipoprotein profiles were purchased from Merck and Randox Co Ltd. STZ was bought from Sigma Co. (USA). All solvents used in this study were of analytical reagent grade.

Experimental Model and Treatment: The rats were allocated into dietary regimens by feeding HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) *ad libitum*, respectively, for the initial period of 2 weeks [15]. The composition and preparation of HFD were as described elsewhere [16]. After the 2 weeks of dietary manipulation, the group of rats fed by HFD was injected intraperitoneally (i.p.) with a low dose of STZ (35 mg/kg), while the respective control rats were given vehicle citrate buffer (pH 4.4) in a dose volume of 1 mL/kg, i.p. The fasting blood glucose was measured 3 days after the STZ injection. The rats with the FBG of more than 300 mg/dL were considered diabetic and selected for further pharmacological studies. The rats were allowed to continue to feed on their respective diets until the end of the study. The rats were divided into five groups as follows

Group NC – normal control rats

Group DC – diabetic control rats

Group RG – diabetic rats treated with rosiglitazone (2 mg/kg, p.o)

Group MML I – diabetic rats treated with MML (200 mg/kg, p.o.) and

Group MML II – diabetic rats treated with MML (400 mg/kg, p.o).

All the treatment groups were administered orally for 21 days. Blood samples were collected 2 h after administration from the rats fasted for 12 h previously, and serum glucose levels were estimated. OGTT was performed the day before rats were sacrificed. At the end of the experiment, blood samples were collected from the eyes (venous pool) and centrifuged at 2900 × g for 10 min to separate the plasma from the whole blood and stored at -80 ºC until assayed.

Oral Glucose Tolerance Test (OGTT): The day before sacrificed, rats underwent an oral glucose tolerance test after an overnight fast. Different doses of MML were administered 60 min before oral glucose load (2.0 g/kg). The blood samples were collected from each group just before glucose administration (0 min) and at 30, 60, 120, and 180 min after glucose administration. Plasma glucose concentrations were determined by the glucose oxidase method.

Biochemical Assays: Glucose levels were estimated by commercially available glucose kits based on the glucose oxidase method. TC, TG, HDL-C, LDL-C, and VLDL-C were measured using commercial assay kits according to the manufacturer's directions. The contents of MDA, the activity of CAT and SOD were determined by commercially available kits according to the manufacturer's directions.

Statistical Analysis: Results are presented as mean ± SEM., and the comparison between groups was performed by two way, and one-way ANOVA followed by Bonferroni's multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS:

Effect of MML on Blood Glucose Levels in Diabetic Rats: The effects of MML on the fasting blood glucose levels of diabetic rats are summarized in **Table 1**. After 2 weeks of high-fat diet, intraperitoneal injection of STZ (35 mg/kg) led to an over fourfold elevation of the blood glucose level ($p<0.001$). After 21 days of daily treatment with MML, I and MML II caused significant reduction ($p<0.001$) in the blood glucose levels by 39 and 53% respectively when compared to the diabetic control group.

Effects of MML on Glucose Tolerance in Diabetic Rats: Results of the glucose tolerance test conducted on diabetic rats fed with MML are shown in **Table 2**. Treatment with MML at both the dose levels showed significant reduction ($p<0.001$) in the blood glucose level at 90 min after oral administration to diabetic rats and produced a maximum reduction in blood glucose by 4 and 5%, respectively in 180 min.

TABLE 1: EFFECTS OF MML ON BLOOD GLUCOSE LEVELS IN DIABETIC RATS

Group	Blood glucose level (mg/dL)			
	0 day	7th day	14th day	21th day
NC	89.50 ± 0.84	90.67 ± 0.67	90.83 ± 0.87	90.33 ± 1.05
DC	314.33 ± 1.17	364.33 ± 1.17$^{###}$	394.33 ± 1.20$^{###}$	405.66 ± 1.52$^{###}$
RG	331.50 ± 1.08	248.16 ± 3.41***	181.16 ± 1.51***	141.83 ± 1.04***
MML I	312.33 ± 0.91	272.66 ± 0.99***	213.33 ± 1.33***	188.16 ± 1.04***
MML II	328.16 ± 1.16	252.66 ± 1.08***	183.16 ± 1.16***	154.50 ± 1.31***

Values are expressed as mean± SEM (n = 6). $^{###}p<0.001$ compared with normal control, *** $p<0.001$, compared with diabetic control. Two way ANOVA followed by Bonferroni's multiple comparison tests.

TABLE 2: EFFECTS OF MML ON GLUCOSE TOLERANCE IN DIABETIC RATS

Group	Blood glucose levels (mg/dL)				
	0 min	30 min	60 min	120 min	180 min
NC	89.50 ± 0.84	92.50 ± 1.02	95.83 ± 1.16	96.83 ± 1.01	95.50 ± 0.92
DC	314.33 ± 1.17	324 ± 1.29	344.33 ± 1.11$^{###}$	364.66 ± 0.98$^{###}$	371.66 ± 0.95$^{###}$
RG	331.50 ± 1.08	346.16 ± 1.42	337.50 ± 1.54***	327.33 ± 0.84***	317 ± 1.12***
MML I	312.33 ± 0.91	321.33 ± 0.74	313.66 ± 5.15***	314 ± 1.15***	308 ± 1.06***
MML II	328.16 ± 1.16	348.16 ±1.16	343.16 ± 1.16***	338.16 ± 1.16***	323.66 ± 1.28***

Values are expressed as mean± SEM (n = 6). $^{###}p<0.001$ compared with normal control, *** $p<0.001$ compared with diabetic control. Two way ANOVA followed by Bonferroni's multiple comparison tests.

Effects of MML on Lipids and Lipoprotein in Diabetic Rats: As shown in **Table 3** the diabetic animals showed a significant increase in the level of TC, TG, LDL, and VLDL cholesterol and a decrease in the level of HDL cholesterol in serum when compared to the normal animals ($p<0.001$). The levels of TG, TC, LDL, and VLDL significantly decreases ($p<0.001$) whereas HDL significantly increases ($p<0.001$) in both the dose levels of MML I and MML II when compared to the diabetic control group.

TABLE 3: EFFECTS OF MML ON LIPIDS AND LIPOPROTEINS IN DIABETIC RATS

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
NC	88.17 ± 0.65	79.83 ± 1.04	51.50 ± 1.11	18.50 ± 1.23	15.97 ± 0.20
DC	174.7 ± 1.67$^{###}$	187 ±0 .21$^{###}$	28.17 ± 0.65$^{###}$	46.17 ± 0.65$^{###}$	37.40 ± 0.24$^{###}$
RG	106.3 ± 1.20***	104.3 ± 0.80***	42.83 ± 0.74***	25 ± 0.36***	20.87 ± 0.16***
MML I	129 ± 0.61***	125.3 ± 1.23***	39.17 ± 0.83***	29.50 ±0.95***	25.07 ± 0.24***
MML II	116.2 ± 0.65***	102.3 ± 0.95***	45 ± 0.81***	21.50 ± 0.80***	20.73 ± 0.24***

Values are expressed as mean ± SEM (n = 6). $^{###}p<0.001$ compared with normal control, *** $p<0.001$ compared with diabetic control. One way ANOVA followed by Bonferroni's multiple comparison tests.

Effects of MML on Antioxidant Parameters in Diabetic Rats: Table 4 indicates that the MDA level has significantly increased ($p<0.001$) whereas the CAT and SOD activity has significantly decreased ($p<0.001$) in diabetic rats compared with normal control rats. MML I and MML II significantly increased ($p<0.001$) the CAT and SOD activity in serum in comparison to diabetic control rats and significantly decreased ($p<0.001$) the MDA level.

TABLE 4: EFFECTS OF MML ON ANTIOXIDANT PARAMETERS IN DIABETIC RATS

Group	CAT (IU/min/mg of tissue)	SOD (IU/min/mg of tissue)	TBARS (nmole of MDA/mg of tissue)
NC	15.39 ± 0.49	10.70 ± 0.49	11.50 ± 0.42
DC	6.33 ± 0.33$^{###}$	4.67 ± 0.33$^{###}$	24.20 ± 0.60$^{###}$
RG	12.30 ± 0.21***	8.33 ± 0.33***	14.20 ± 0.30***
MML I	10.80 ± 0.30***	7.08 ± 0.26***	18.70 ± 0.42***
MML II	12.20 ± 0.30***	9.08 ± 0.91***	14.70 ± 0.42***

Values are expressed as mean ± SEM (n = 6). $^{###}p<0.001$ compared with normal control, *** $p<0.001$ compared with diabetic control. One way ANOVA followed by Bonferroni's multiple comparison tests.

DISCUSSION: The antidiabetic effect of MML extract was investigated using the obese-diabetic rat model by high-fat feeding and streptozotocin. The rats fed with HFD can result in insulin-resistant mainly through Randle or glucose-fatty acid cycle [17]. Furthermore, although high-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes [17]. There is no significant variation in plasma insulin concentrations between diabetic and normal rats. However, because fasting blood glucose was significantly higher in diabetic rats, it suggested that insulin resistance has been developed in these animals. Therefore, this rat model exhibits hyperglycemia, hyperlipemia, and insulin resistance that would closely reflect the natural history and metabolic characteristics of humans, and it is further sensitive to pharmacological testing.

In our present findings, the antihyperglycemic effect of MML indicated that *Mukia maderspatana* could control hyperglycemia and also MML was also able to improve some lipid metabolites including TC, TG, HDL- and LDL cholesterol levels in diabetic rats. It is reported that diabetes is associated with profound alterations in lipid and lipoprotein profile [18]. Regulating of plasma or tissue lipid levels leads to a decrease in the risk of micro- or macrovascular disease and related complications [19].

Thus, this result suggested that MML would be helpful to the prevention of diabetic complications through improving dyslipidemia.

Hyperglycemia, the most important feature of diabetes mellitus, is in itself very dangerous for diabetic patients. It impairs the prooxidant/ antioxidant balance, reducing antioxidant levels and increasing free radicals [20], which can damage

the pancreatic beta-cells and induce insulin resistance. There is a close relationship between the increase of free radicals, blood glucose, and lipid peroxidation (LPO) in the progress of diabetes [21]. Diabetics usually exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes free radicals generation [22].

Oxygen free radicals could react with polyunsaturated fatty acids, which lead to LPO. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors [23]. As a by-product of lipid peroxidation, MDA reflects the degree of oxidation in the body.

CONCLUSION: The findings of our study revealed that the *Mukia Maderspatana* had the potential to attenuate the glucose metabolism disorder and nearly normalized the lipid metabolism. These benefits were associated with attenuation of oxidative stress. Further pharmacological and biochemical investigations are in progress confirm our results and to elucidate the detailed mechanisms which may be valuable in the treatment of dyslipidemia and atherosclerosis in diabetic patients.

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CONFLICT OF INTEREST: Nil

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