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The hypoglycemic effect of the semi-purified fractions of *Mukia maderspatana* Linn in streptozotocin-induced diabetic rats

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Abstract

The present study was to investigate the hypoglycemic effect of the semi-purified fractions of an ethanolic extract of *Mukia maderspatana Linn* in streptozotocin- induced diabetic male Wistar albino rats. The hypoglycemic effect of chloroform, butanol and aqueous fractions were assessed by the oral glucose tolerance test at a dose of 100 mg/kg-body weight in streptozotocin -diabetic rats (55 mg/kg i.p.). In the long-term study, the diabetic rats were randomly divided into four groups and treated orally by gavage with vehicle, chloroform fraction (100 mg/kg body weight), butanol fraction (100 mg/kg body weight), and glibenclamide (10 mg/kg body weight) respectively twice a day for 21 days. Hepatic glucose-6-phosphatase activity, glycogen content and thiobarbituric acid reacting substances were also estimated.The data revealed that the chloroform and butanol fractions produced significant blood glucose lowering effect in the diabetic rats and however aqueous fraction did not produce significant change. On day 7, 14 and 21, chloroform and butanol fractions, like the reference drug, glibencalmide, lowered the fasting blood glucose concentration significantly ($P < 0.01$, $P < 0.001$) when compared with the diabetic rats. Hepatic glucose-6-phosphatase activity was significantly lower ($P < 0.001$) in fractions compared to that in diabetic rats. However, there was no change in glycogen content in fractions compared to the diabetic rats. Thiobarbituric acid reacting substances in fractions treated diabetic rats were significantly lower ($P < 0.001$, $P < 0.01$) than in diabetic rats.These results indicate that fractions (chloroform and butanol) were potent in the amelioration of hyperglycemia in STZ-diabetic rats and are a potential source for the isolation of new orally active agent(s) for anti-diabetic therapy.

INTRODUCTION

Diabetes mellitus in humans is a manifestation of metabolic disturbances due to the dietary intake of excess carbohydrates and lipids [1]. Hyperglycemia and hyperlipidemia are important risk factors in the development of cardiovascular disease and metabolic disorders [2].Various chemicals have been used to induce diabetes in rodents, particularly streptozotocin (STZ), which has been extensively used in diabetes research. The development of hyperglycemia, following STZ injection is primarily due to the direct pancreatic beta cell destruction, and resulting insulin deficiency [3]. Currently, the antidiabetic drugs in use for long term therapy are found to be associated with various toxicities owing to which the developmental process in antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects [4]. Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents [5].

Mukia maderaspatana Linn, (Family: Cucurbitaceae) is an annual monoecious herb, densely covered with white hairs. It is found throughout India ascending up to 1800m in the hills. Folklore medicine claims that it is a good diuretic, stomachic [6], gentle aperient, antipyretic, and antiflatulent [7], antiasthmatic, and antibronchitis besides its use in vertigo and biliousness [8]. It is used in Ayurveda for various therapeutic purposes such as relief of toothache or flatulence, and as an expectorant and a sudorific. Certain traditional medical practitioners also use the leaf-tea of this plant for alleviation of jaundice [9-10]. Decoctions of leaves of this plant have been used by Siddha practitioners in Tamil Nadu for the treatment of hypertension [11]. This plant leaf extract has also been shown to have hepatoprotective [12-13] and immunomodulatory effects [14-15] and antiarthritic activity properties [16]. However, no study has been studied with the fractions towards mechanism of actions in diabetes.

The present study was undertaken with the aim of evaluating the hypoglycemic effect of the semi-purified fractions of ethanolic extract of *Mukia maderspatana* on blood glucose levels in STZ induced diabetic rats.

MATERIALS AND METHODS

Preparation of extract and fractions

The whole plant of *Mukia maderaspatana* Linn (MMe) 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 Phytopharmacy and Phytomedicine, JSS College of Pharmacy, Udhagamandalam.

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 (R-205, Buchi Laboratory equipment's, Flawil, Switzerland) under reduced pressure and controlled temperature (40-50°C). The extracts were stored in a refrigerator at 4°C for further studies. The clear supernatant was partitioned between chloroform and water to obtain aqueous fraction and chloroform fraction. The aqueous fraction was further partitioned by n- butanol to obtain n-butanol

fraction. Each fraction was concentrated at 40°C, using a rotavapor and freeze-dried to yield about 10 g of aqueous fraction (AF), 25 g of chloroform fraction (CF) and 12 g of butanol fraction (BuF). The freeze-dried powder of AF, BuF, and CF were used at dose of 100 mg/kg in both oral glucose tolerance test (OGTT) and long-term study.

Animals

Healthy Wistar rats weighing 180-220 g, were procured from the animal house, J.S.S. College of Pharmacy, Udhagamandalam, India. Six animals were used in each group (n=6). 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 course of the experimental period and room temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. JSSCP/IAEC/PH.D/PH.COLOGY-01/2009-10).

Induction of experimental diabetes mellitus

The overnight fasted Wistar rats were made diabetic with streptozotocin (STZ) (55 mg/kg, i.p). The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and maintained on ice prior to use; the injection volume was 1 mL/kg [17]. Diabetes was confirmed in the STZ-treated rats by measuring the fasting blood glucose concentration 72 h post injection. The Wistar rats with blood glucose level above 300 mg/dl were considered to be diabetic and were used in the experiment [18]. Animals had free access to food and water after the STZ injection.

Experimental procedure

The OGTT in STZ -diabetic rats using the semi-purified fractions of Mme

Prior to an oral glucose tolerance test (OGTT), rats were fasted for 16 h. The animals were grouped as follows: group I served as control (distilled water), group II served as diabetics (distilled water), III served as glibenclamide at a dose of 10mg/kg and groups IV, V and VI served as three different fractions of MMe viz. AF, BuF, and CF each at a dose of 100 mg/kg and were orally administered to groups of 6 rats each. Thirty minutes later, glucose (3 g/kg) was orally administered to each rat with a feeding syringe [19]. Blood samples were collected from the tail vein by tail milking , 0 (just before the oral administration of glucose), 30, 60, 90, and 120 min after glucose load for the assay of glucose [20] [Glucose assay kit, E.Merck.,India].

Repeated administration of CF and BuF in STZ-diabetic rats

One week after STZ induction of diabetes in male Wistar rats, the fasting blood glucose levels were measured. The hyperglycemic rats (blood glucose level > 300 mg/dl) were divided on day zero into four groups (n=6). The animals were grouped as follows: group I served as control (distilled water), group II served as diabetics (distilled water), group III served as glibenclamide at a dose of 10 mg/kg and groups IV and V served as two different fractions of MMe viz. BuF, and CF each at a dose of 100 mg/kg. The fasting blood glucose level was measured on day zero at 9.00 am. Distilled water, BuF (100 mg/kg), CF (100 mg/kg) and glibenclamide (10 mg/kg) were then administered orally twice a day to diabetic control, treatment and positive control groups respectively for 3 weeks. On 21st day, after 16 h fasting, the rats were decapitated and the blood was collected for estimation of the fasting blood glucose. The organs such as liver and kidney were isolated, weighed and stored at -70 °C for the assay of hepatic glucose-6-phosphatase, glycogen in liver and thiobarbituric acid reactive substances (TBARS) in both liver and kidney.

The hepatic glucose-6-phosphatase activity was assayed by the method of Baginski et al., (1974) [21].

The liver glycogen content was determined by the enzymatic method of Murat and Serfaty (1974) [22]. The determination of thiobarbituric acid reactive substances (TBARS) values was performed by the method of Uchiyama and Mihara (1978) [23-24].

STATISTICALANALYSIS

The results are presented as means ± SEM with n =6. The changes in body weight, food and water intakes during the 21-day treatment period were compared by two-way Analysis of Variance (ANOVA). The data on blood glucose, hepatic glucose-6-phosphatase, hepatic glycogen content and TBARS were analysed by one-way Analysis of Variance (ANOVA) followed by Bonferroni multiple comparison test. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

In the OGTT (Table 1), BuF and CF (100 mg/kg BW) caused a significant ($P < 0.001$) hypoglycemic effect within 60 minutes after oral administration to streptozotocin-diabetic rats. The aqueous fraction (AF) did not cause any reduction in blood glucose level at any time point. The single high dose STZ-induced diabetic rat is one of the animal models of human IDDM or type I diabetes mellitus. In this model, diabetes arises from irreversible destruction of the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion. In this type I model of diabetes, the insulin is markedly depleted, but not absent

.

The present study revealed that the semi-purified fractions of the ethanolic extract of *Mukia maderspatana* such as CF and BuF have potent hypoglycaemic property when given for 3 weeks to STZ-diabetic rats. Similar effects were reported for other hypoglycemic agents such as tungstate and vanadate [25-26].

CF a caused a significant ($P < 0.001$) time-dependent hypoglycemic effect (Fig 1) after twice-daily oral administration at a dose of 100 mg/kg for 7, 14 and 21 days. BuF also showed a significant ($P < 0.001$) hypoglycemic property on day 7 as well as on day 14 and 21 compared to the diabetic rats.

Glucose-6-phosphatase activity in the liver (Table 2) was significantly reduced ($P < 0.001$) in both fractions (CF and BuF) and glibenclamide-treated groups when compared to the diabetic rats. Glucose-6-phosphatase catalyzes the final step in glucose production by the liver and kidney. Streptozotocin increases the expression of glucose-6-phosphatase mRNA, which contributes to the increased glucose-6-phosphatase activity in diabetes mellitus [27]. Similarly, 60 % ethanolic extract of *Coccinia indica* and 95 % ethanolic extract of *Momordica charantia* extracts were found to lower blood glucose by depressing its synthesis, on the one-hand, through depression of the key gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-bisphosphatase and on the other by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme G6PDH [28].

However, there was no significant difference in the level of hepatic glycogen content (Table 2) in CF, BuF and also glibenclamide treated rats compared to diabetic rats although the

Table1. Minimal Inhibitory Concentration of *Cymbopogon* Species

S.No.	Treatment	Blood glucose level (mg/dL)				
		0 min	30 min	60 min	90 min	120 min
1	Control (Distilled water)	89.33 ± 0.88	93.06 ± 0.96	97.5 ± 1.45	99.66 ± 1.45	95.5 ± 0.61
2	Diabetic (Distilled water)	305.33 ± 3.67###	316.33 ± 4.30###	325.66 ± 5.38###	333 ± 4.88###	340.83 ± 4.65###
2	Diabetic – Glibenclamide (10 mg/kg)	301.83 ± 1.10	310.16 ± 1.10	302.66 ± 1.66***	294.16 ± 1.68***	284.16 ± 1.61***
3	Diabetic – AF (100 mg/kg)	307.16 ± 0.74	312.16 ± 1.04	318.83 ± 1.55	325.833 ± 1.51	331.± 1.46
4	Diabetic – CF (100 mg/kg)	308.33 ± 2.15	316.66 ± 1.20	310.83 ± 1.57***	306.33 ± 1.40***	301.16 ± 1.01***
5	Diabetic – BuF (100 mg/kg)	308.50 ± 3.13	319.50 ± 2.51	314.50 ± 1.87***	309.66 ± 1.20***	305.66 ± 1.76***

All value are expressed as mean SEM (n=6).
P 0.001, compared with control, ***P 0.001, compared to diabetic.
One-way ANOVA followed by Bonferroni multiple comparison test.
AF- aqueous fraction, CF- chloroform fraction, BuF- n-butanol fraction

Table1. Minimal Inhibitory Concentration of *Cymbopogon* Species

S.No.	Treatment	G-6-PD (mmol/min/mg)	Liver glycogen content (mg/g wet tissue)	TBARS (nmol of MDA/mg of tissue)	
				Liver	Kidney
1	Control (Distilled water)	0.284 ± 0.004	23.5 ± 0.76	9.50 ± 0.42	8.17 ± 0.47
2	Diabetic (Distilled water)	0.505 ± 0.007###	13.67 ± 0.21###	23.2 ± 0.47###	20.7 ± 0.42###
3	Diabetic + Glibenclamide (10 mg/kg)	0.348 ± 0.004***	14.83 ± 0.30	12.2 ± 0.30***	10.2 ± 0.30***
4	Diabetic + CF (100 mg/kg)	0.402 ± 0.005***	14.33 ± 0.33	18.0 ± 0.365**	16.0 ± 0.36**
5	Diabetic + BuF (100 mg/kg)	0.410 ± 0.003***	14.83 ± 0.70	17.3 ± 0.33**	15.3 ± 0.33**

All value are expressed as mean SEM (n=6).
P 0.001, compared with control, ***P 0.001, **P 0.001 compared to diabetics.
One-way ANOVA followed by Bonferroni multiple comparison test.
CF- chloroform fraction, BuF- n-butanol fraction

hepatic glycogen content tended to be higher in the BuF treated group when compared to the diabetic rats. Similarly Vanadate compounds have been shown to inhibit hepatic G-6-Pase activity there by reducing blood glucose levels in non-obese diabetic (NOD) mice. However no significant difference was found in the hepatic glycogen stores of the treatment groups compared to control [29].

The kidney TBARS in fractions and glibenclamide treated diabetic rats were significantly lower (Table 2, $P < 0.001$, $P < 0.01$) than in the diabetic rats. The liver TBARS in fractions and glibenclamide treated diabetic rats were also significantly lower ($P < 0.001$, $P < 0.01$) than in the diabetic rats. (Table 2) Hypoinsulinemia in diabetes increases the activity of fatty acyl coenzyme A oxidase, which initiates β-oxidation of fatty acids, resulting in lipid peroxidation [30]. Hence the significant change in the TBARS levels in the liver of CF and BuF-treated and glibenclamide treated diabetic rats could again reflect the resistance of the liver to the oxidative stress in the diabetic state.

However, the chemical nature of potential antihyperglycemic component (s) of CF and BuF remains to be established. The CF and BuF can be separated into different fractions by reversed phase HPLC and the hypoglycemic activity of each can be tested *in vivo* in streptozotocin diabetic rats or by *in vitro* glucose uptake studies. The molecular masses of the active fraction can be identified by mass spectrometry followed by nuclear magnetic resonance (NMR) spectrometric analysis to obtain the structural information about the active component(s).

CONCLUSION

The present study shows that the semi-purified fractions of the ethanolic extract of *Mukia maderspatana* have hypoglycemic action in STZ-diabetic rats. Hence further biochemical and pharmacological studies are being carried out to purify and isolate the active principle(s) in both fractions and to elucidate their mechanism(s) of action.

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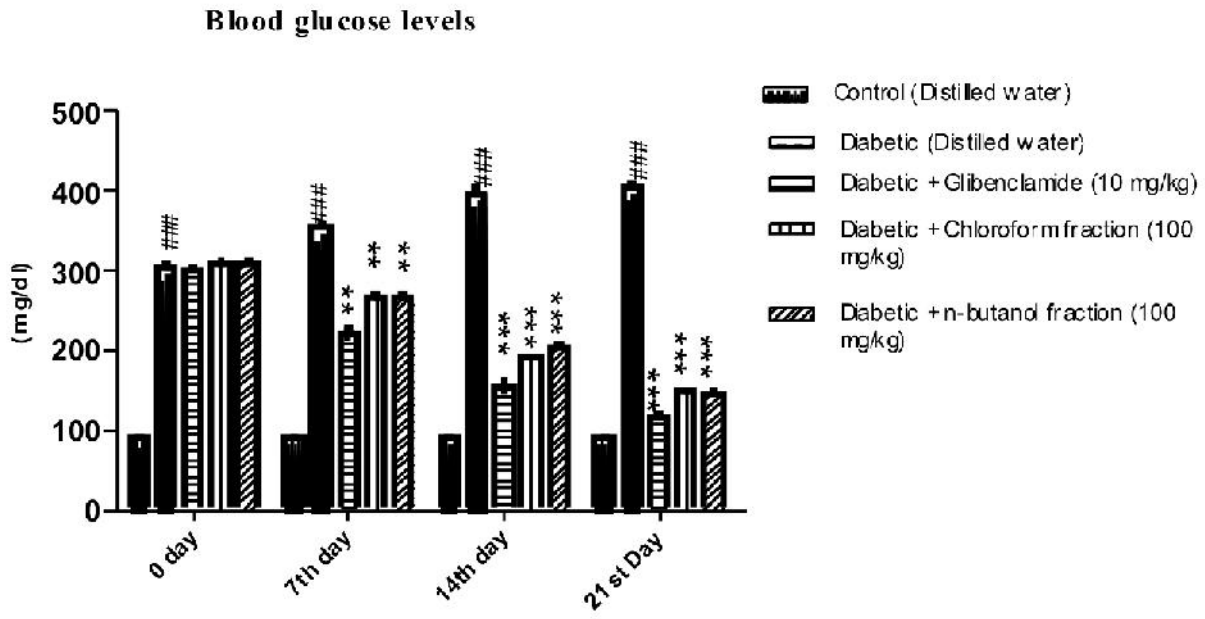

Figure: 1. Effect of Mukia maderspatana fractions on the blood glucose level in streptozotocin-diabetic rats

All value are expressed as mean SEM (n=6).
P 0.001, compared with control, *** P 0.001, **P 0.01 compared to control.
One-way ANOVA followed by Bonferroni multiple comparison test.

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