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Hypoglycemic and hypolipidemic effect of *Meyna spinosa* leaves in high fat diet–alloxan induced type 2 diabetic rats

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Abstract

This study investigated the antidiabetic and hypolipedemic action of *Meyna spinosa* leaf fractions. Effect of methanol, ethyl acetate and petroleum ether fraction (75 and 150 mg/kg p.o.) from *M. spinosa* leaf methanol extract was evaluated in high fat diet-alloxan induced type 2 diabetic rats after 21 days treatment. Glibenclimide (5 mg/kg), methanol and ethyl acetate fraction at 150 mg/kg exhibited 57.7, 63.4 and 53.8% reduction in serum glucose level after 21 days. Fractions demonstrated significant decrease in triglycerides, total cholesterol, low density lipid, very low density lipid, α-amylase level, and increase in body weight, high density lipid level in diabetic rats. Fractions showed significant ($p<0.05$) hypoglycemic effect in glucose loaded animals but not in normal rats. Petroleum ether fraction did not produced significant effect. Result validated the claim made by folk medicinal uses and confirmed the antidiabetic potential of methanol and ethyl acetate fraction of *M. spinosa*.

Introduction

Type 2 or non insulin dependent diabetes account for about 95% of all diagnosed cases of diabetes mellitus, and has a significant impact on the health, excellence and expectancy of life (Dewanjee et al., 2009). Though several oral hypoglycemic agents are available, but most of these drugs possess different side effects. Thus, the hunt for a new therapeutic agent devoid of adverse effect originating from plants would be of interest (Kannur et al., 2006). *Meyna spinosa* Roxb. (Rubiaceae) has been used in the treatment of hepatic disorder, gastrointestinal problems, skin infection, and as refrigerant, and abortifacient (Bora and Kumar, 2003; Dea et al., 2009; Mitra and Mukherjee, 2009). The boiled fruit extract is used to treat diabetes in Manipur, while fruit and leaf juice are recommended to cure diabetes by the tribes of Tripura, India (Khan and Yadava, 2010; Sen et al., 2011). Scientific literature suggests that *M. spinosa* fruits extracts possess antimicrobial activity (Buragohain, 2008). Methanol extract of *M. spinosa* also possess antifungal activity (Goswami et al., 2006). The present study aim to investigate the *in vivo* antidiabetic, hypolipedemic potential of fractions of *Meyna spinosa* leaf against high fat diet-alloxan induced type 2 diabetes.

Materials and Methods

Plant materials

Leaves of *Meyna spinosa* Roxb. were collected from Tripura, India and identified by Dr. BK Datta, Department of Botany, Tripura University, Tripura, India (Voucher specimenno: TU/BOT/HEB/SS23072011a).

Preparation of extract and fractionation

The fresh leaves were air dried under shade, pulverized

into coarse powder (500 g) and extracted with methanol (2.5 L) using Soxhlet apparatus for 18 hours. The extract was concentrated to dryness under reduced pressure to obtain the methanol extract (15.2% w/w). The methanol extract (20 g) was pre-absorbed onto silica gel (60-120 mesh, SD Fine Ltd., Mumbai) and fractionated with column chromatographic technique using the following solvents in the order of increasing polarity viz petroleum ether, ethyl acetate and methanol. The yields afforded petroleum ether (PFMS, 8.3% w/w), ethyl acetate (EFMS, 34.5% w/w), and methanol (MFMS, 49.0% w/w) fractions.

Experimental animals

Healthy Albino mice (20-30 g) were used for acute toxicity study and Wistar rats (150-200 g) were used for the antidiabetic activity. Animals were maintained under controlled environmental conditions. The study was approved by the Institutional Animal Ethical Committee (Reg. No. 1305/ac/09/CPCSEA).

Acute toxicity study

The fixed dose (OCED Guideline no. 423, Annexure 2d) method of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was used for toxicity studies. The tested fractions were administrated orally.

Effect of fractions on normoglycemic plus glucose-hyperglycemic model (NG-OGTT)

To access the effect of fractions on combined NG-OGTT model, blood samples were collected from overnight fasted rats, followed by the administration of vehicle, glibenclimide (5 mg/kg), MFMS, EFMS, and PFMS at a dose of 75 and 150 mg/kg, *p.o.* Serum glucose level was determined after ½, 1, and 2 hours after drug administration. The rats were orally loaded with 2 g/kg glucose after the last measurement (at 2 hours) and the serum glucose levels were continued to determine after 2½, 3, 4 and 6 hours by using a commercially available kit (Agapee Diagnostic Ltd, Kerala) (Orhan et al., 2005).

Induction of type 2 diabetes

High fat diet was administered continuously for 10 days to healthy rats. On 11th day freshly prepared alloxan solution in physiological saline (120 mg/kg) injected into the caudal vein of overnight fasted rats. Fifteen minute later 0.4 U insulin was injected (*i.p.*); 2.5 and 5.0 hours later, 25% glucose (10 mL/kg, *p.o.*) administered to decrease animal mortality rate caused by the hypoglycemia and hyperglycemia in rats with injection of alloxan. Twenty four hour after first alloxan injection, alloxan (100 mg/kg body weight) was injected again into the caudal vein of rats. Insulin (0.4 U) was intraperitoneally injected 15 min later to rats, then 25% glucose (10 mL/kg) given orally 2.5 and 5.0 hours later respectively. The blood glucose was tested and animals with fasting blood glucose more than 200 mg/dL were used for the experiments (Shu et al., 2009).

Antidiabetic and hypolipedemic evaluation of fractions

Healthy and diabetic animals were divided into nine groups in fix sex ratio (3 males and 3 females). Groups I and II served as normal control and diabetic untreated control respectively. Group III, diabetic rat treated orally with 5 mg/kg/day glibenclamide. Group IV-VII, diabetic rat treated with the MFMS (75 and 150 mg/kg/day, *p.o.*), EFMS (75 and 150 mg/kg/day, *p.o.*) and PFMS (75 and 150 mg/kg/day, *p.o.*) for 21 days respectively. Serum glucose levels and body weight were measured on day 0, 7, 14 and 21 of the study.

Initial and final total cholesterol (TC), triglyceride (TG), high density lipids (HDL), low density lipids (LDL), very low density lipids (VLDL), and α–amylase level in serum were determined using a commercially available kit (Agapee Diagnostic Ltd, Kerala).

Statistical analysis

Results are expressed as mean ± SEM, (n = 6). Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Tukey tests. A level of $p<0.05$ was used as the criterion for statistical significance.

Results

Fractions did not show any mortality at the dose of 2,000 mg/kg. Therefore, 2000 mg/kg dose was considered as ALD_{50} cut off the dose under Globally Harmonised Classification System (GHS) category 5 (safe dose).

Fractions did not show any significant change in serum glucose level, while glibenclamide decreases serum glucose levels in normoglycemic rats (between 14.3 and 44%). However, methanol and ethyl acetate fraction prevented drastic increase of serum glucose after the glucose loading, and decrease the serum glucose level below normal levels after 360 min (Table I).

MFMS and EFMS exhibited significant hypoglycaemic activity in diabetic rats, while no significant effect observed for PFMS (Table II). After 21 days of treatment with MFMS (75 and 150 mg/kg/day), there was 34.9 and 63.4% decrease ($p<0.001$) in serum glucose levels, while EFMS at a dose of 75 and 150 mg/kg results 30.7 and 53.8% inhibition of serum glucose level. At the end of 21 days treatment standard drug, MFMS, and EFMS significantly increases body weight ($p<0.05$, 0.01) (Table II).

A significant decrease in serum TG, TC, LDL and VLDL levels, and increase in HDL level were observed after the treatment with glibenclamide, MFMS, and EFMS.

Table I

Effects of the *M. spinosa* fractions on blood glucose levels in normal and glucose-loaded hyperglycaemic rats

Test sample	Dose (mg/kg)	Serum glucose concentration (mg/dL) [%inhibition]							
		0 min	30 min	60 min	120 min (glucose load)	150 min	180 min	240 min	360 min
Control	-	66.1 (2.9)	70.2 (3.2)	72.4 (3.3)	75.1 (2.7)	150.8 (5.0)	137.4 (4.4)	98.6 (4.2)	77.9 (3.2)
Glibenclimide	5	68.3 (3.1)	60.2 (2.1) [14.3]	56.1 (3.1) [22.5]^{a}	42.1 (2.0) [44.0]†	106.9 (4.0) [29.2]^{a}	86.8 (2.5) [36.8]‡	60.2 (3.1) [38.9]‡	53.6 (2.9) [31.2]‡
Methanol fraction	75	68.5 (2.7)	72.2 (3.0)	69.9 (3.2)	75.6 (3.2)	137.0 (4.1)	117.5 (3.9) [14.5]^{a}	88.8 (3.7)	80.7 (3.0)
Methanol fraction	150	64.2 (2.4)	68.3 (3.9)	70.0 (4.5)	69.2 (2.5)	119.5 (5.4) [20.8]^{a}	89.3 (2.9) [35.0]‡	65.8 (4.1) [33.3]‡	51.0 (2.2) [34.5]‡
Ethyl acetate fraction	75	69.7 (2.3)	71.2 (3.3)	67.5 (3.0)	74.0 (3.5)	144.4 (5.0)	120.0 (3.4) [12.6]	95.1 (3.9)	87.6 (3.3)
Ethyl acetate fraction	150	69.1 (2.1)	63.8 (1.9)	70.1 (3.8)	65.6 (2.7)	126.3 (5.4) [24.6]†	102.3 (2.6) [25.5]^{a}	77.3 (4.2) [21.6]†	62.3 (2.2) [20.0]^{a}
Petroleum ether fraction	75	71.2 (2.4)	72.1 (3.4)	68.7 (3.2)	73.8 (3.1)	148.0 (5.0)	135.0 (3.0)	107.0 (3.0)	92.0 (2.8)
Petroleum ether fraction	150	67.1 (2.8)	71.3 (2.9)	64.4 (3.0)	67.9 (2.2)	144.3 (3.1)	131.2 (2.4)	105.3 (3.4)	87.7 (2.5)

Values are given as mean ± (SEM). n = 6; Only inhibitory effect on blood glucose concentration above 10% is shown in bracket [ ] $^{a}p<0.05$, †$p<0.01$, ‡$p<0.001$ when test drug treated group compared with control animals

Higher dose of ethyl acetate fraction showed similar hypolipedemic activity compare to standard, but methanol fraction (150 mg/kg) produced better effect than glibenclamide (Table III). Diabetic control rats had significantly higher serum amylase activity compared to normal control. The enzyme level was reduced ($p<0.05$, 0.01) following the treatment with glibenclamide, MFMS and EFMS (Table III).

Discussion

Despite the fact that substantial progress has been made in the management of diabetes mellitus, but the search for plant based products mainly from folk/traditional medicinal system for the control of diabetes mellitus continues with great expectation (Kannur et al., 2006). Oral glucose tolerance test is acknowledged for indirect assessment of *in vivo* peripheral insulin action and insulin resistance (Liou et al., 2002). Fractions did not have any effect on normal animal, but prevent drastic increase in serum glucose level after glucose administered. Hence the fraction may have the potential to improve insulin sensitivity.

Alloxan has been observed to cause necrosis of β-cells and induce hyperglycemia. Intraperitoneal injection of alloxan after administration of high fat diet causes type 2 diabetes (Shu et al., 2009). MFMS, EFMS significantly decreased the fasting serum glucose in diabetic animals. Alloxan causes characteristic loss in body weight in diabetic rats which is due to improved muscle wasting and due to loss of tissue protein (Nammi et al., 2003; Jain et al., 2010), and a similar phenomenon was observed in the present study also. Treatment with MFMS and EFMS was found effective, which may due to the protective effect of fractions in controlling muscle wasting, i.e. reversal of gluconeogenesis and may also be due to proper glycemic control of fractions may responsible for this effect.

The sulphonylureas cause hypoglycaemia by increasing the secretion of insulin and mainly active in mild alloxan-induced diabetes (Al-Shamaony et al., 1994). Fractions of *M. spinosa* had similar activity like that of glibenclamide in diabetic rats which could be due to improving the metabolism of glucose, and increasing insulin secretion from the β-cells.

Marked increase in TG, TC, LDL, and VLDL in diabetic rats was observed. The most frequent lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Al-Shamaony et al., 1994; Shirwaikar et al., 2005). Hypertriglyceridemia is coupled with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and insulin intolerance (Shirwaikar et al., 2005). In our study, administration of MFMS and EFMS significantly ($p<0.05$) improved these parameters. Decreased of cholesterogenesis and fatty acid synthesis may responsible for the observed

Table II

Effects of the *M. spinosa* fractions on body weight and serum glucose level in NIDDM rats

Test sample	Dose (mg/kg)	Body weight (g)				Serum glucose concentration (mg/dL) [% inhibition]			
		0 day	7 day	14 day	21 day	0 day	7 day	14 day	21 day
Normal control	-	180.2 (12.1)	185.3 (13.8)	189.9 (14.2)	193.2 (16.1)	65.8 (3.9)	70.5 (4.0)	67.3 (3.9)	73.6 (4.2)
Diabetic control	-	170.4 (10.2)	156.9 (13.3)	143.3 (11.4)†	145.4 (14.9)†	230.1 (7.4)‡	242.1 (8.9)‡	252.8 (10.1)‡	258.0 (10.1)‡
Glibenclimide	5	166.3 (11.1)	164.3 (10.4)	170.1 (13.3)†	175.3 (10.8)†	251.3 (8.1)	182.0 (8.0)† [24.8]	131.4 (7.2)‡ [48.0]	109.0 (4.9)‡ [57.7]
Methanol fraction	75	172.1 (13.9)	163.4 (10.5)	165.2 (16.0)†	167.3 (14.1)[a]	240.3 (9.4)	210.2 (8.4)[a] [13.2]	198.1 (9.2)† [21.7]	168.1 (8.3)‡ [34.9]
Methanol fraction	150	167.9 (14.1)	163.4 (13.0)	168.2 (14.1)[a]	172.2 (15.1)†	244.9 (9.1)	173.0 (7.0)† [28.5]	127.1 (8.2)‡ [49.7]	94.6 (3.1)‡ [63.4]
Ethyl acetate fraction	75	168.3 (10.1)	166.3 (12.5)	162.6 (12.7)†	161.3 (12.2)[a]	238.1 (8.8)	222.0 (7.8)	208.8 (8.0)[a] [17.4]	178.8 (7.0)† [30.7]
Ethyl acetate fraction	150	164.6 (11.7)	160.6 (9.9)	165.7 (12.8)[a]	169.3 (13.3)†	246.9 (9.4)	172.0 (6.1)† [29.0]	138.3 (7.0)‡ [45.3]	119.2 (3.5)‡ [53.8]
Petroleum ether fraction	75	163.2 (12.0)	150.6 (11.5)	145.3 (11.2)	140.5 (13.3)	245.8 (8.2)	254.9 (11.7)	260.3 (7.1)	267.2 (11.1)
Petroleum ether fraction	150	170.3 (13.7)	157.1 (15.3)	150.1 (13.9)	143.3 (12.9)	231.0 (7.2)	227.4 (8.9)	224.8 (9.9) [11.1]	215.7 (9.9)[a] [16.4]

Values are given as mean ± (SEM); n = 6; Only inhibitory effect on blood glucose concentration above 10% is shown in the bracket [ ] [a]p<0.05, †p<0.01, ‡p<0.001 when test drug treated group compared with diabetic control group and diabetic control group compared with normal control group

hypolipidemic effect. Significant lowering of TC, LDL and raise in HDL is a very enviable biochemical state for avoidance of atherosclerosis and ischemic conditions (Shirwaikar et al., 2005; Akpan et al., 2012). The result indicated fractions have the potential to prevent or treat the complications of diabetes. The acini produce α-amylase enzyme that involved in the digestion of polysaccharide in diet. Diabetic cause damage to acini cells results leakage of the enzyme into blood (Akpan et al., 2012). MFMS and EFMS may prevent cellular damage thus decrease the enzyme level in serum. Preliminary phytochemical analysis suggested that methanol extract contain alkaloids, glycosides, triterpenoids, tannins and flavonoids. Therefore, presence of any of these compounds in fraction may be responsible for observed biological activities. These statistical data indicated MFMS possess highest hypoglycemic effect is likely due to the increase in insulin secretion or ameliorating insulin sensitivity or due to the inhibition of α amylase activity.

In conclusion, it can be stated that the MFMS, EFMS has beneficial effects, in reducing the elevated blood glucose level, alpha amylase activity and lipid profile of high fat diet-alloxan induced-diabetic rats. Thus, justifying the claim made by folk medicinal uses and can be viewed as potential source of new antidiabetic molecule.

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Table III

Effect of fraction of *M. spinosa* leaves on serum lipid profile and α-amylase in type 2 diabetic rats

Test sample	Dose (mg/kg)	TG (mg/dL)		TC (mg/dL)		LDL (mg/dL)		VLDL (mg/dL)		HDL (mg/dL)		α-amylase (U/L)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Normal control		95.6 (6.1)	98.0 (6.0)	48.3 (4.1)	52.1 (4.9)	14.5 (2.2)	16.0 (2.6)	19.1 (2.0)	19.6 (2.2)	26.4 (3.3)	28.0 (3.6)	206.0 (8.9)	211.4 (7.9)
Diabetic control		150.4 (8.0)‡	159.4 (8.0)‡	179.4 (10.3)‡	186.6 (11.0)‡	75.3 (3.8)‡	91.0 (6.6)‡	30.1 (3.2)†	31.9 (3.8)‡	22.3 (3.3)[a]	17.9 (3.2)†	288.6 (10.0)‡	296.3 (11.6)‡
Glibenclimide	5	147.4 (8.8)	109.8 (5.3)‡	170.0 (8.8)	79.4 (7.0)‡	74.2 (3.9)	35.3 (3.2)‡	29.5 (3.4)	22.0 (1.7)†	21.4 (3.0)	27.1 (4.0)‡	290.3 (10.0)	222.6 (9.0)‡
Methanol fraction	75	155.3 (9.9)	128.0 (6.4)†	181.2 (12.7)	121.5 (7.1)†	78.1 (4.0)	52.1 (4.0)‡	31.1 (3.0)	25.6 (2.3)†	22.8 (3.0)	25.3 (3.6)†	275.8 (9.4)	250.8 (8.3)[a]
	150	142.6 (7.1)	103.5 (5.9)‡	164.7 (8.6)	66.4 (6.8)‡	72.1 (5.0)	32.1 (3.3)‡	28.5 (2.9)	20.7 (1.9)†	20.4 (1.9)	27.0 (3.9)‡	282.7 (9.0)	228.7 (7.8)†
Ethyl acetate fraction	75	144.9 (9.2)	131.0 (8.0)†	169.5 (9.6)	118.3 (7.0)†	74.8 (3.9)	50.3 (4.1)‡	29.0 (2.6)	26.2 (3.2)†	21.1 (2.8)	25.9 (3.0)†	280.8 (8.7)	261.3 (8.1)[a]
	150	148.7 (8.3)	107.7 (5.9)‡	174.3 (10.9)	83.7 (6.1)‡	76.9 (4.1)	38.7 (3.0)‡	29.7 (3.3)	21.5 (3.6)	23.4 (3.3)	27.1 (3.3)†	272.1 (9.9)	134.0 (6.3)†
Petroleum ether fraction	75	151.6 (7.8)	153.2 (8.5)	175.7 (9.7)	182.3 (10.4)	79.0 (3.3)	83.2 (6.3)	30.3 (4.3)	30.7 (4.1)	19.4 (2.0)	16.8 (1.9)	281.8 (8.5)	179.0 (8.0)
	150	147.0 (8.4)	138.7 (7.5)[a]	167.4 (8.2)	140.5 (7.0)[a]	72.0 (4.6)	70.4 (6.0)[a]	29.4 (3.6)	27.7 (3.0)[a]	20.0 (2.7)	19.6 (2.3)	286.4 (10.2)	172.9 (8.5)

Values are given as mean ± (SEM); n = 6; [a]p<0.05, †p<0.01, ‡p<0.001 when test drug treated group compared with diabetic control group and diabetic control group compared with normal control group

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