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

**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.

**Acute toxicity study**

The fixed dose (OCED Guideline No. 423) 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 alloroan 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 alloroan. Twenty four hour after first alloroan injection, alloroan (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 hypolipidemic 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 ALD50 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. Higher dose of ethyl acetate fraction showed similar hypolipidemic 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 administration. 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 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.

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 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

Table I

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose (mg/kg)</th>
<th>Serum glucose concentration (mg/dl) [% inhibition]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>66.1</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>5</td>
<td>68.3</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>75</td>
<td>68.5</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>150</td>
<td>64.2</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>75</td>
<td>69.7</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>150</td>
<td>69.1</td>
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<td>Petroleum ether fraction</td>
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<td>71.2</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>150</td>
<td>67.1</td>
</tr>
</tbody>
</table>

Values are given as mean ± (SEM). n = 6; Only inhibitory effect on blood glucose concentration above 10% is shown in bracket [    ] p<0.05, † p<0.01, * p<0.001 when test drug treated group compared with control animals.
or treat the complications of diabetes. The acini produce \( \alpha \)-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 methanol fraction contain alkaloids, glycosides, triterpenoids, tannins and flavonoids. 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 \( \alpha \) amylase activity.

### Conclusion

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.

### Financial Support

Self-funded

### Ethical Issue

The study was approved by the Institutional Animal Ethical Committee (Reg. No. 1305/ac/09/CPCEA), OCED Guideline No. 423 method of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCEA) was used for toxicity studies.

### Conflict of Interest

Authors declare no conflict of interest

### References


Buragohain J. Phytochemical and antimicrobial investigation

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**Table II**

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

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose (mg/kg)</th>
<th>Body weight (g)</th>
<th>Serum glucose concentration (mg/dL) [% inhibition]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>7 day</td>
<td>14 day</td>
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<tr>
<td>Normal control</td>
<td>-</td>
<td>180.2</td>
<td>(12.1)</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>-</td>
<td>170.4</td>
<td>(10.2)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>5</td>
<td>166.3</td>
<td>(11.1)</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>75</td>
<td>172.1</td>
<td>(13.9)</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>150</td>
<td>167.9</td>
<td>(14.1)</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>75</td>
<td>168.3</td>
<td>(10.1)</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>150</td>
<td>164.6</td>
<td>(11.7)</td>
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<tr>
<td>Petroleum ether fraction</td>
<td>75</td>
<td>163.2</td>
<td>(12.0)</td>
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<tr>
<td>Petroleum ether fraction</td>
<td>150</td>
<td>170.3</td>
<td>(13.7)</td>
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Values are given as mean ± (SEM); n = 6; Only inhibitory effect on blood glucose concentration above 10% is shown in the bracket [ ] 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.
<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose (mg/kg)</th>
<th>TG (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>α-amylase (U/L)</th>
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</thead>
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<tr>
<td>Normal control</td>
<td>95.6 (6.1)</td>
<td>98.0 (6.0)</td>
<td>48.3 (4.1)</td>
<td>52.1 (4.9)</td>
<td>14.5 (2.2)</td>
<td>16.0 (2.6)</td>
<td>19.1 (2.0)</td>
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<tr>
<td>Diabetic control</td>
<td>150.4 (8.0)</td>
<td>159.4 (8.0)</td>
<td>179.4 (10.3)</td>
<td>186.6 (11.0)</td>
<td>75.3 (3.8)</td>
<td>91.0 (6.6)</td>
<td>30.1 (3.2)</td>
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<tr>
<td>Glibenclamide</td>
<td>5</td>
<td>147.4 (8.8)</td>
<td>109.8 (5.3)</td>
<td>170.0 (8.8)</td>
<td>79.4 (7.0)</td>
<td>74.2 (3.9)</td>
<td>35.3 (3.2)</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>75</td>
<td>155.3 (9.9)</td>
<td>128.0 (6.4)</td>
<td>181.2 (12.7)</td>
<td>121.5 (7.1)</td>
<td>78.1 (4.0)</td>
<td>52.1 (4.0)</td>
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<tr>
<td>Ethyl acetate fraction</td>
<td>75</td>
<td>144.9 (9.2)</td>
<td>131.0 (8.0)</td>
<td>169.5 (9.6)</td>
<td>118.3 (7.0)</td>
<td>74.8 (3.9)</td>
<td>50.3 (4.1)</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>75</td>
<td>151.6 (7.8)</td>
<td>153.2 (8.5)</td>
<td>175.7 (10.9)</td>
<td>182.3 (10.4)</td>
<td>79.0 (3.3)</td>
<td>83.2 (6.3)</td>
</tr>
</tbody>
</table>

Values are given as mean ± (SEM); n = 6; *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.

Table III

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


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