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ANTI-HYPERGLYCAEMIC EFFECTS OF AQUEOUS PREPARATION OF *MEYNA SPINOSA* ROXB. FRUIT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Oinam Joychandra Singh, Laishram Babycha * and Laishram Surbala

Department of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences, Porompat, Imphal East - 795005, Manipur, India.

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Correspondence to Author:
Laishram Babycha

Associate Professor,
Department of Pharmacology,
Jawaharlal Nehru Institute of Medical Sciences, Porompat, Imphal East - 795005, Manipur, India.

E-mail: laishrambabycha@gmail.com

ABSTRACT: The unripe fruit of *Meyna spinosa / Laxiflora* is used for the treatment of diabetes by traditional healers. The effect of the aqueous extract of the fruit is investigated on the normal and elevated blood glucose level of the experimental animal rats. The blood glucose level of the rats is elevated with the administration of I.P streptozotocin. The estimation of the blood glucose level is done by using a kit based on the glucose oxidase method. The rat has a blood glucose level above 280mg/dl is considered for the normal and anti-hyperglycemic study. The doses of the extract are selected as 200, 300mg/kg, and the route of administration is oral. The effect of the extract on the normal and elevated glucose level of the rat is compared with that of the standard drugs- glibenclamide and metformin. The effect of the extract on normal blood glucose level is not significant when compared with that of glibenclamide, whereas the effect of the extract on elevated blood glucose level is significant when compared with that of metformin. The dose of the extract 200mg/kg is not having any significant effect on the elevated blood glucose level. The dose of the extract 300mg per kg is administered daily for nine days, and the effectiveness of the extract 300mg per kg is observed two hours after the oral feeding of the extract on the 9th day. The result shows that the extract seems to act as an anti- hyperglycaemic agent. Therefore, a detailed study for the definitive data evidence in respect of therapeutic utilization may be necessary.

INTRODUCTION: Natural products, particularly of plant origin, are the main source for discovering promising lead candidates and play an imperative role in the upcoming drug development programs. Ease of availability, low cost, and least side effects make plant-based preparations the main key player of all available therapies, especially in rural areas [1, 2].

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The ethnobotanical information suggests that about 880 plants are believed to possess hypoglycaemic activity, and the hypoglycaemic properties of more than 400 plants have been reported in scientific papers [3]. The antidiabetic profile of many medicinal plants found in the Indian subcontinent has been evaluated for their hypoglycemic activity till date [4].

Plants are known to exhibit hypoglycemic, hypolipidemic, and antioxidant activity due to the presence of flavonoids, ellagic acids, phenolic acids, phytosterols, gallotannins, and other related polyphenols [5]. They also reduce cholesterol, tri-glyceride, and alkaline phosphatase levels along with increased total protein contents [6].

However, all the plants used traditionally for the treatment of diabetes are not scientifically studied about their potency, mode of action, and safety.

Meyna spinosa Roxb., an angiosperm in the family Rubiaceae,, is an important medicinal plant. It is a small spiny tree or large shrub and is distributed in India, Nepal, Bangladesh, China, Myanmar, Thailand Cambodia, Laos, and Vietnam. The leaves are used for the preparation of pickle and singju (spicy salad). The dried fruits are directly consumed. Traditionally, fruit and bark of *Meyna spinosa*has have been used in the treatment of a hepatic disorder, skin infection, headache, peptic ulceration, intestinal worm, dysentery, indigestion, and painful urination [7]. The boiled extract of unripe fruit is used for the treatment of diabetes in Manipur, India, and the fruit and leave juice is used by the tribe of Tripura, India for the treatment of diabetes [8, 9, 10]. In spite of its huge ethnomedicinal use, very few pharmacological investigations have been carried out *viz.* antioxidant, antimicrobial, cytotoxic, hepatoprotective, and nephroprotective activity. The methanol and ethyl acetate fractions fractionated from methanolic extract of leaves of *Meyna spinosa* reduced the elevated blood glucose level [11].

Till date, no investigation on the anti-diabetic property of the fruit of *Meyna spinosa* is carried out, though the fruit is traditionally being used by people to reduce elevated blood glucose. Therefore, the present experimental study was designed to investigate the effect of the unripe fruit of *Meyna spinosa* Roxb. on normal blood glucose levels and elevated blood glucose levels in experimental rats.

MATERIALS AND METHODS:

Chemicals: Streptozotocin, glibenclamide, and metformin were purchased from Sigma (MO, USA). The rest of the chemicals and solvents used were of analytical grade.

Plant Material: The unripe fruit of *Meyna spinosa* (FMS) was collected in the month of September – October from Andro, Manipur, India (24.7253° N, 94.0440° E). The specimen was identified by the Plant Systematics and Conservation Laboratory, Medicinal, Aromatic and Horticultural Plant Resources Division, Institute of Bioresources and Sustainable Development, Manipur, India. A voucher specimen (IBSD/M-253) was deposited to the IBSD herbarium.

Preparation of Unripe Fruit of *Meyna spinosa* (FMS) Extract: The fruit was chopped into pieces and air-dried at room temperature. The dried fruit was crushed to powder, and 200 g of the powder of the fruit was exhaustively extracted in distilled water using the Soxhlet apparatus. The filtrate was then concentrated *in-vacuo* using a vacuum rotary evaporator (EYELA, Japan) and finally freeze-dried (Thermo, Modulyod) to yield 50 g of FMS extract. The dried extract was stored in a porcelain jar at 4 ºC for future use.

Experimental Animals: The experimental protocol was approved by the Institutional Animal Ethics Committee of the Regional Institute of Medical Sciences, Imphal, Manipur (IAEC No: 1560/GO/a/ 12/CPCSEA). Wistar rats of both sex, about the same age group (60 days) and weighing about 125-200 g were used in the present study. The animals were acclimatized for one week with three rats per cage and maintained at a temperature of 22 ± 2 °C. The animals were allowed free access to standard diet and water *ad libitum* during the entire period of the experiment. Precaution for avoiding of coprophagy was taken.

Sample Preparation for Oral Feeding: The solution of the FMS extract, glibenclamide and metformin was prepared by suspending the sample in 2% gum acacia in distilled water.

Acute Toxicity Test: Acute oral toxicity of the FMS extract was carried out as per OECD/OCED guidelines 2008 [12], on Wistar rats. Animals were administered orally with FMS extract at 2000 mg/kg body weight.

Observations were done at least once during the first 30 min after dosing, periodically during the first 24 h, and daily for a total period of 14 days.

Hypoglycaemic Study in Normal Fasted Rats: The hypoglycaemic effect of the FMS extract on the normal fasted rats was performed according to Devesh Kumar Kushawaha *et al.* [13] Briefly, after 1 week of acclimatization, the fasted rats were randomly divided into four groups (6 animals per group) as described below.

Group I (no drug control)	2% gum acacia in distilled water
Group II (test dose I)	FMS 1 (200 mg/kg b.w p.o.)
Group III (test dose II)	FMS 2 (300 mg/kg b.w p.o.)
Group IV (standard drug)	Glibenclamide (0.5 mg/kg b.w.p.o.)

The blood glucose level was measured using blood samples collected from the tail bleeds by applying blood onto a glucose analyzer (Accu-Chek, Aviva, Mumbai, India), just before and after 30, 60, and 120 min of FMS extract/drug feeding.

Effect of FMS on Elevated Blood Glucose: The anti-hyperglycaemic effect of the FMS extract on the normal blood sugar level was performed according to Kadali S. L. D. V. R. M. *et al*. [14]

Briefly, after 1 week of acclimatization, animals were treated with streptozotocin (50 mg/kgb.w., i.p.) prepared in 0.9% saline while the control group received an equal volume of the vehicle. After 48 h of streptozotocin injection, the fasting blood glucose level was estimated by tapping blood from the tail. Rats having blood glucose level ≥ 280 mg/dl were considered diabetic and selected to undergo treatment for 7 days as described below:

Group I (Normal control, non-induced)	2% gum acacia in distilled water
Group II (hyperglycaemic control)	FMS 1 (200 mg/kg b.w p.o.)
Group III (hyperglycaemic + standard drug)	FMS 2 (300 mg/kg b.w p.o.)
Group IV (hyperglycaemic induced +test dose I)	Glibenclamide (0.5 mg/kg b.w.p.o.)
Group V (hyperglycaemic induced + test dose II)	FMS extract 2 (300 mg/kg/day b.w. p.o.)

Statistical Analysis: Results were expressed as mean ± standard deviation (SD). Data were analyzed using Graph pad Instat 3 software. Comparison between different groups was done by One-Way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. p-value less than 0.05 was considered statistically significant.

RESULTS:

Acute Toxicity Study: The FMS extract did not show any sign and symptom of toxicity with the dose up to 2000 mg/kg. But subjective toxicity (headache, nausea) study could not be observed.

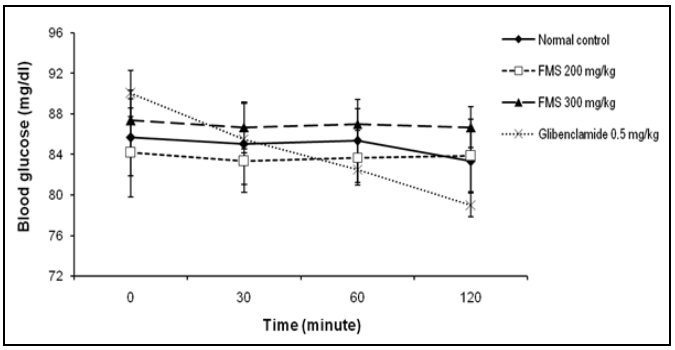

FIG. 1: FMS EXTRACT DOES NOT INDUCE HYPO-GLYCAEMIC EFFECT ON NORMAL RATS. Normal rats were fed with either MS (200 and 300 mg/kg b.w.) or glibenclamide (0.5 mg/kg b.w.) and blood glucose level was measured just before and after 30, 60 and 120 m of FMS/drug feeding. Data is expressed as mean ± SD (n=6); ***p<0.001 is significant when compared with the corresponding values at 0 h, as analyzed by one-way ANOVA supplemented with Tukey-Kramer multiple comparisons test.

Effect of FMS Extract on Blood Glucose in Normal Rats: To study the effect of FMS on normal blood glucose level *in-vivo*, fasted normal rats were orally fed with two different doses of FMS extract (200 and 300 mg/kg), and blood glucose level was determined at different time intervals *viz.* 30 min, 60 min and 120 min after sample/drug administration. The results revealed that, unlike glibenclamide which significantly reduced normal blood glucose level (*** p<0.001), FMS did not reduce the blood glucose level of normal rats **Fig. 1**.

Effect of FMS Extract on Elevated Blood Glucose in Rats: To study the anti-hyperglycaemic effects of FMS on blood glucose level *in-vivo*, hyperglycaemia was induced to normal rats with streptozotocin. These hyperglycaemic rats were administered orally with two different doses of FMS extract (200 and 300 mg/kg). It was observed that the blood glucose of FMS extract-treated hyperglycaemic rats was significantly reduced on the 9th day compared to hyperglycaemic control rats in both the tested concentrations (**Table 1**; p<0.001). Further, different time intervals study revealed that like metformin, FMS extract significantly reduced the blood glucose of hyper-glycaemic rats after 2 h of its administration **Table 2**. FMS was able to lower the hyperglycaemic effect on the rats by 37% (FMS1) and 53% (FMS2) of hyperglycaemic control on day 9.

TABLE 1: EFFECT OF FMS EXTRACT ON BLOOD GLUCOSE LEVEL OF STREPTOZOTOCIN INDUCED HYPERGLYCAEMIC RATS

Group	Blood glucose (mg/dl)			
	Dose (mg/kg)	1st day	3rd day	9th day
Normal control	0	87.33±3.9	87.5±5	83.33±3***
Hyperglycaemic control	0	87.66±4.2	303.5±17.8	326.83±11
Metformin	350	90.66±4.1	281.83±16.5	136.83±1***
FMS 1	200	85±5.2	286.33±15.7	207.33±7***
FMS 2	300	91.5±2.4	288.16±14.6	152±6***

***p<0.001 when compared with the corresponding values of hyperglycaemic control.

TABLE 2: EFFECT OF FMS EXTRACT ON BLOOD GLUCOSE LEVEL OF STREPTOZOTOCIN INDUCED HYPERGLYCAEMIC RATS AT DIFFERENT TIME INTERVALS AFTER DRUG ADMINISTRATION ON 9th DAY

Group	Blood glucose (mg/dl)				
	Dose (mg/kg)	0 h	1/2 h	1h	2 h
Normal control	0	84.3±4.17	85±4	85.33±4	83.33±3
Hyperglycaemic control	0	327.66±12.4	326.5±12	327.5±13	326.83±11
Metformin	350	154.33±6.6	148.66±9.1	143.16±7.5	136.83±.1**
MS 1	200	223.83±6.9	220.66±6	215±5	207.33±7**
MS 2	300	173±8.7	167.5±9	161±8	152±6 **

**p<0.01 when compared with the corresponding values at 0 h.

DISCUSSION: Many investigators are now working on many herbs and plants which are having medicinal value and are usually consumed as food. *Meyna spinosa* is such a plant that is consumed as food and has many medicinal values. Traditionally, the boiled unripe fruit extract and juice of *Meyna spinosa* is used for the treatment of diabetes in Manipur, India, and Tripura, India, respectively. For the first time, the effect of the unripe fruit of *Meyna spinosa* Roxb. on normal blood glucose and elevated blood glucose was investigated on rats.

The unripe fruit of FMS was exhaustively extracted using distilled water. The FMS extract was then subjected to toxicity testing on normal rats by administering orally upto a dose of 2000 mg/kg for a period of 14 days. As expected, no visible signs and symptoms of toxicity were observed. Further, the effect of FMS on the blood glucose of normal rats was investigated.

Interestingly, FMS extract did not reduce the blood glucose level of normal rats, whereas gliben-clamide; an anti-hyperglycemic drug, significantly reduced the blood glucose level of normal rats. It is reported that glibenclamide at low-normal glucose exerted a disproportionate effect on insulin secretion, thus increasing the risks of hypoglycaemia [15]. This clearly indicates that FMS does not have hypoglycaemic properties on normal blood glucose level **Fig. 1**.

To test the anti hyperglycaemic property of FMS, hyperglycaemia was induced to normal rats by administering streptozotocin (50 mg/kg IP). Treatment of FMS extract at doses 200 and 300 mg/kg/day showed a significant reduction in the fasting blood glucose of hyperglycaemic rats on day 9 **Table 1**.

Further, it was also observed that FMS extract significantly decreased the elevated blood glucose after 2 h of its administration **Table 2**. The combined and concerted action of phytochemicals (*i.e.*, polyphenols, carotenoids, glucosinolates, lignans, *etc*.) gives the potentially beneficial properties of each plant [16]. And earlier studies reported that methanol and ethyl acetate fractions fractionated from methanolic extract of leaves of *Meyna spinosa* reduced the elevated blood glucose level and alpha-amylase activity and also improved lipid profile of high fat diet-alloxan induced-diabetic rats [11]. A phytochemical study showed that the fruit of *M. spinosa* contains phenoloic compounds, tannins, flavonoids, saponin, oleanolic acid and triterpenoid [17]. These fractions reportedly have various beneficial effects likeanti-diabetic, anti-oxidant, anti-tumour, anti-inflammatory, and anti-microbial effect [18].

CONCLUSION: The fruit of *Meyna spinosa* extract did not induce toxicity and hypoglycaemia on the normal blood glucose level of rats. FMS acts as an oral anti-hyperglycaemic agent by reducing

the elevated blood glucose level in rats. Further, our results also supported the claims made by the folk traditional users about the use of the fruit of *Meyna spinosa* in decreasing the elevated blood glucose. These results call for further chemical characterization of FMS extract for isolation of active anti-hyperglycaemic molecule to be used as a therapeutic agent for the treatment of diabetes.

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CONFLICTS OF INTEREST: The authors declare that they have no competing interests.

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