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Hypoglycaemic and Antidiabetic Activities of Seeds of *Myristica fragrans* in Normoglycaemic and Alloxan-induced Diabetic Rats

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Abstract : The present study was designed to investigate the hypoglycaemic and antidiabetic activity of seeds of *Myristica fragrans* in normoglycaemic and alloxan- induced diabetic rats. The petroleum ether (60-80º C) extract of *Myristica fragrans* (PEMF) was administered orally in normal fasted, glucose fed (1.5 g/kg, *p.o.*) and alloxan (120 mg/kg, *s.c.*)- induced diabetic rats (n=5). The blood glucose levels were estimated using glucometer (One Touch, Johnson and Johnson). In addition, changes in body weight, organ (liver, kidney and pancreas) weight, serum lipid profile and blood parameter (haemoglobin, erythrocytes and differential leukocytes) assessed after two weeks in the extract treated diabetic rats.

It has been found that, oral pre-treatment with PEMF at dose of 200 mg/kg induced a significant ($P<0.05$) decrease in blood glucose level, i) from 56.5±3.19 (0 h) to 49.75±2.05 mg% (4 h) in normoglycaemic rats, ii) from 145.75±9.65 to 81.5±4.03 mg% in oral glucose tolerance test (OGTT) at ½ h compared to control glucose fed rats, iii) from 305.8±12.49 to 276.6±6.11 mg% after single dose treatment and from 326.25±7.05 to 268.0±9.6 mg% in alloxan- induced diabetic rats after daily treatment of PEMF for two weeks. After two weeks daily administration of PEMF, diabetic treated rats showed improvement in body weight, organ (liver and pancreas) weight, lipid profiles and haemoglobin content as compared to diabetic control rats. Thus, the present data indicates that *Myristica fragrans* possesses potential as an antidiabetic and warrants the need for further studies to elucidate its mode of action.

Key words : *Myristica fragrans*, Hypoglycaemic, Alloxan

Introduction

Diabetes mellitus is the important disease involving the endocrine pancreas. Its manifestations include disordered metabolism and inappropriate hypoglycaemia (Karam, 1995). Plants have been the major source of drug for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems in the world. The importance of antidiabetic plants in the development of economic and effective treatment for diabetes, currently estimated to affect over 30 million people worldwide, has been recognized by the World Health Organization (WHO Technical Report Series, 1985).

Myristica fragrans Houttuyn (Myristicaceae) commonly known as nutmeg, is a dioecious or occasionally monoecious evergreen, aromatic tree. It is a native of Moluccas, now cultivated in many tropical

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countries of both hemispheres. In India it is grown in Tamil Nadu, Kerala, Assam and other states. Nutmeg is popular as a spice and also possesses various therapeutic properties. *M. fragrans* is used for both culinary and medicinal purposes. *M. fragrans* is used for treating diarrhea, mouth sores, and insomnia (Van and Cox, 1994). The essential oil of Nutmeg is used externally for rhumatism and internally as a carminative (Oliver, 1986). Compounds isolated from the seeds of this plant have been reported to possess strong platelet anti-aggregatory activity (Rasheed *et al*, 1984; Venton *et al*, 1991; Janssens *et al*, 1990). Nutmeg contains essential oils, many of which have been reported to possess analgesic and anti-inflammatory properties (Santos *et al*, 1997; Olajide *et al*, 1999). Also, nutmeg prevents hypercholesterolemia and atherosclerosis in (Sharma *et al*, 1995). The ethanolic extract of the nutmeg significantly produced hypolipidaemia in (Ram *et al*, 1996).

The aim of this study was to investigate the hypoglycaemic and antidiabetic effect of petroleum ether extracts of *M. fragrans* in normoglycaemic and alloxan-induced diabetic rats.

Materials and Methods

Preparation of extracts

The seeds of *Myristica fragrans* (MF) were obtained from the local market and were taxonomically authenticated by Dr. AK Singhai, Pharmacognosy Division of Dept. of Pharm. Sci., Dr. HS Gour University, Sagar. A voucher specimen of the plant (34254) was deposited in the Botanical Survey of India, Pune. The fruits were powdered and subjected to extraction with petroleum ether (60-80º C, PEMF). The percentage yield of PEMF was found to be 18.9 % w/w.

Experimental animals

Wistar rats weighing 200-250 g of either sex were used. Animals were housed in groups of five per cage at a temperature of 25º ± 1º C and relative humidity of 45-55% and acclimatized for one week after their arrival. The Institutional Animals Ethics Committee approved the protocol of the study.

Chemicals and drugs

Alloxan (S D Fine- Chem, India), insulin (USV Ltd, India) and glibenclamide (Sun Pharma, India) were used in this study. Other chemicals used were of analytical grade obtained from Qualigens, India. Diagnostic kits (Bio Labs, India) were procured from local supplier.

Administration of PEMF and blood glucose estimation

The PEMF suspended in PEG200 (0.1 ml) and calculated volumes in doses of 50, 100 and 200 mg/kg body weight and glibenclamide (0.40 mg/kg), were administered orally in normal fasted rats.

For blood glucose determination, blood was obtained by snipping tail with sharp razor (Aydin *et al*, 1995). The blood glucose concentration was determined by using One Touch glucometer (Johnson and Johnson, India) at 2, 4 and 6 hrs after treatment.

Single dose treatment

Groups of five rats each were allocated to control (vehicle treated), standard (glibenclamide/ insulin) and PEMF treatment. A single dose of the extracts was administered to each group of rats. The hypoglycaemic activity was evaluated using the following models,

Normoglycaemic rats

The rats (n= 5) were fasted overnight and their fasting blood glucose levels (0 hrs)

were determined. The glibenclamide and PEMF (50, 100 and 200 mg/kg, *p.o.*) groups were given their respective drugs and extracts, whereas control group received only the vehicle. Blood was taken out by tail snipping at 2, 4 and 6 h after the treatment and blood glucose was estimated using glucometer.

Oral glucose tolerance test

After overnight fasting, all animals (n= 5) in control group received glucose (1.5 g/ kg, *p.o.*) and blood glucose was estimated at 0, ½, 1, 2 h later using glucometer. PEMF was administered two hours prior to glucose.

Alloxan-induced diabetic rats

Animals (n= 5) were allowed to fast overnight and injected with freshly prepared aqueous solution of alloxan monohydrate (120 mg/kg, *s.c.*). After a week, rats with marked hyperglycaemia (fasting blood glucose > 250 mg%) were selected for drug treatments.

The blood samples were collected from diabetic control group at 0, 2,4 and 6 hrs and blood glucose was determined using glucometer. PEMF (200 mg/kg) was given orally and two hours after dose administration blood glucose was determined.

Repeated dose treatment

PEMF (200 mg/kg *p.o.*) was administered daily for two weeks in the alloxan-induced diabetic (blood glucose> 250 mg %) rats. After two weeks treatment, blood glucose was determined two hours after last dose using glucometer. In addition, changes in body weight, organ (liver, kidney and pancreas) weight, serum lipid profile and blood parameter (haemoglobin, erythrocytes and differential leukocytes) assessed after two weeks in the extract treated diabetic rats. All assays were carried out using diagnostic (Biolabs, Mumbai) kits.

Statistical analysis

All the data presented as mean ± SEM. One- way ANOVA followed by Dunnett's test was employed to calculate the statistical significance in case of multiple comparisons with control group. For dependent variables (before and after the treatment) paired Students t- test was used. Statistical significance was tested at 5% level.

Results

Effect of PEMF after single dose treatment

Effect on Normoglycaemic rats

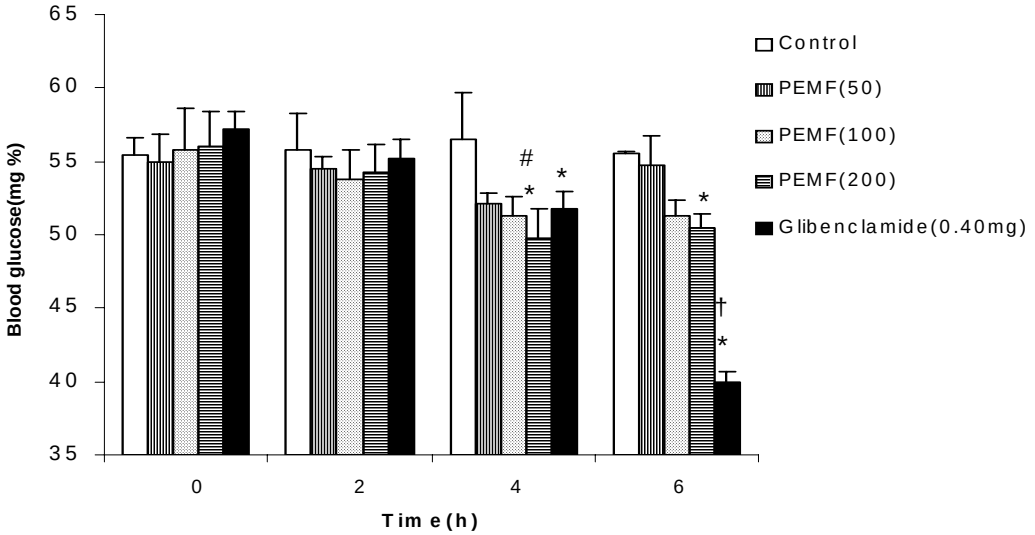
The mean blood glucose concentration of PEMF at various time intervals is shown in figure1. PEMF (200 mg/kg) decreased the mean blood glucose level after 4 h of treatment from 56.00 ± 2.38 to 49.75 ± 2.05 mg %. Glibenclamide decreased blood glucose level from 57.20 ± 1.20 to 40.00 ± 0.70 mg % after 6 hrs.

Effect on glucose tolerance test

Glucose administration to normal rats caused significant ($p < 0.05$) rise in mean blood glucose level, ½ h after administration. This rise was from 56.75 ± 2.25 to 145.75 ± 9.65 mg %. Two hours prior administration of PEMF and glibenclamide significantly controlled the rise in blood glucose from 56.75 ± 1.75 to 81.50 ± 4.03 mg % and 58.50 ± 2.21 to 80.25 ± 2.28 mg % respectively after ½ h (figure 2).

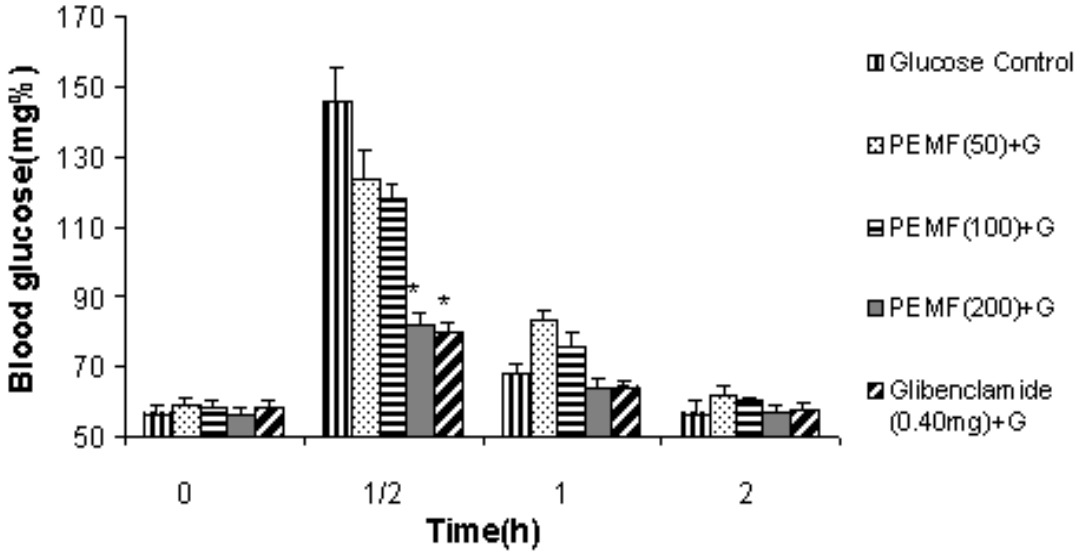
Effect on alloxan-induced diabetic rats

Figure 3 showed the antihyperglycaemic effect of PEMF on the fasting blood glucose levels in diabetic rats. PEMF and



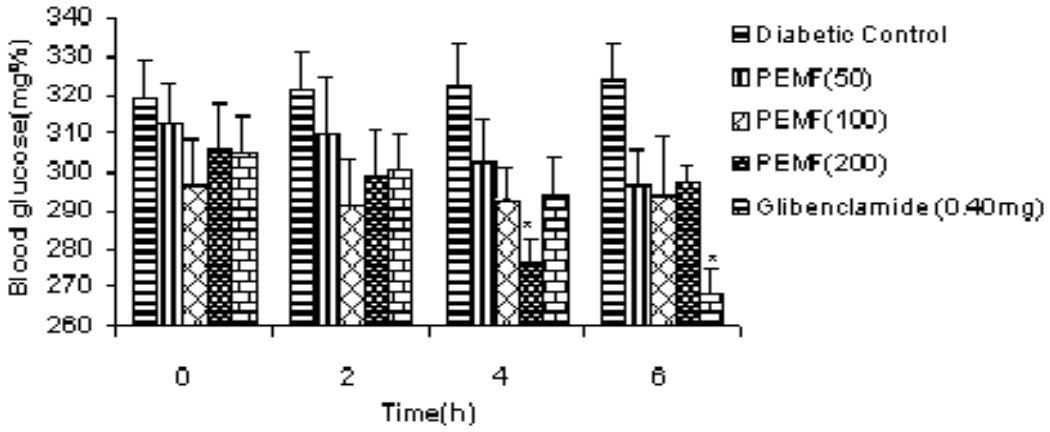
*n= 5 in each group, values are mean ± SEM ; * p< 0.05 compared to control group (ANOVA followed by Dunnett's test); # p< 0.05 compared to initial value (0 h) (paired t test); † p< 0.001 compared to initial value (0 h) (paired t test)*

Figure 1: Effect of various doses of PEMF on blood glucose in normal fasted rats



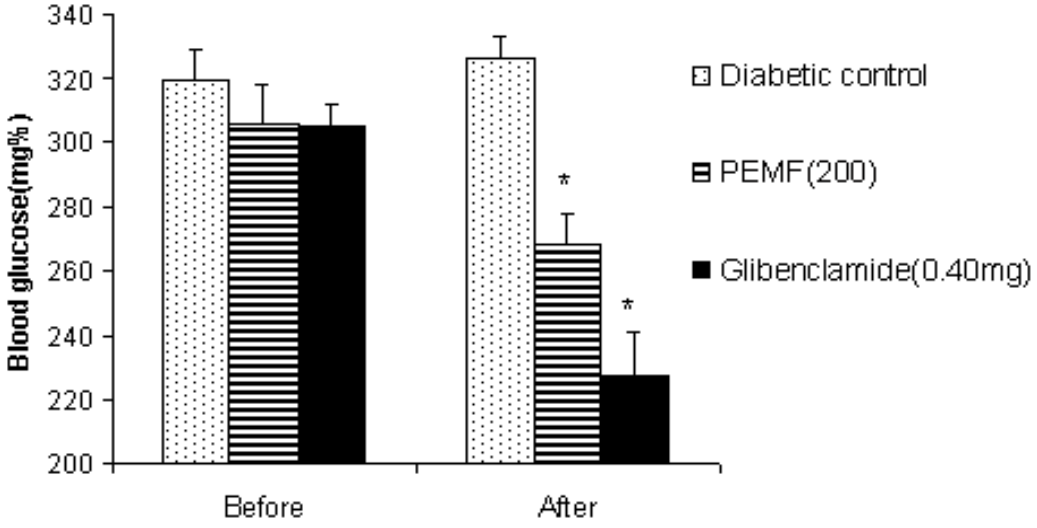
*n= 5 in each group, values are mean ± SEM; * p< 0.05 compared to Glucose control group (ANOVA followed by Dunnett's test)*

Figure 2: Effect of PEMF on glucose tolerance test



*n= 5 in each group, values are mean ± SEM; * p< 0.05 compared to Diabetic control group (ANOVA followed by Dunnett's test)*

Figure 3: Effect of single dose treatment of PEMF on alloxan- induced diabetic rats



*n= 5 in each group, values are mean ± SEM; * p< 0.05 compared to Diabetic group (ANOVA followed by Dunnett's test)*

Figure 4: Effect of repeated dose treatment of PEMF on alloxan- induced diabetic rats

glibenclamide significantly ($p < 0.05$) decreased blood glucose level from 305.80 ± 12.49 to 276.60 ± 6.11 mg % and 305.00 ± 9.25 to 268.20 ± 6.92 mg %.

Effect of PEMF after repeated dose on diabetic rats

Repeated dose treatment of PEMF and glibenclamide for two weeks, produced significant ($p < 0.05$) decrease in blood glucose level of alloxan- induced diabetic rats. PEMF and glibenclamide decreased blood glucose from 305.80 ± 12.49 to 268.00 ± 9.60 mg%, and 305.00 ± 9.25 to 227.75 ± 13.07 mg % respectively after two weeks treatment (figure 4). After two weeks daily administration of PEMF, diabetic treated rats showed improvement in body weight, organ (liver and pancreas) weight and haemoglobin content as compared to diabetic control rats. The biochemical assay showed that serum cholesterol, triglyceride and alkaline phosphatase levels were significantly higher while serum HDL-cholesterol and total protein levels were significantly decreased in diabetic group as compared to normal rats. Table 1 demonstrates the effect of PEMF and glibenclamide on biochemical parameters. The serum cholesterol, triglyceride was decreased and albumin was increased by PEMF and glibenclamide compared to diabetic control group.

Table 1: Effect of repeated dose treatment of PEMF on different blood parameters in alloxan induced diabetic rats

Sr. No.	Parameters	Normal Control	Diabetic Control	Diabetic + PEMF (200 mg/kg/day)	Diabetic + Glibenclamide (0.40 mg/kg/day)
1	**Body weight**	228.75±6.57	190±7.07	214.25±4.26*	215±2.88*
2	**Organ weight**				
	Liver	6.01±0.17	4.85±0.38	5.89±0.22*	5.90±0.30
	Kidney	0.62±0.04	0.54±0.02	0.58±0.02	0.59±0.02
	Pancreas	0.42±0.07	0.68±0.03	0.86±0.04*	0.85±0.02
3	**Lipid profile**				
	Total Cholesterol	108.58±8.96	171.26±8.89	142.31±2.2*	115.58±4.44*
	HDL-Cholesterol	40.23±0.83	35.47±0.37	38.97±0.38	39.1±1.63
	Triglyceride	147.96±6.9	192.37±5.45	153.9±3.93*	154.44±5.07*
4	**Other blood parameters**				
	Haemoglobine	14.7±0.73	11.2±0.33	13.37±0.24*	13.7±0.39*
	Erythrocytes	4.47±0.41	2.52±0.21	3.56±0.57	3.34±0.23*
	Leukocytes	8.7±0.50	6.7±0.62	7.4±1.03	8.44±0.26*
	Neutrophils	5.6±0.5	4.2±0.53	4.9±0.73	5.27±0.39
	Eiosinophils	0.1±0.02	0.2±0.04	0.1±0.04	0.11±0.02
	Lymphocytes	2.5±0.13	1.7±0.12	2.15±0.31	2.82±0.16*
	Monocytes	0.3±0.04	0.4±0.04	0.2±0.06	0.42±0.02

n= 5 in each group, values are mean±SEM

** P< 0.05 compared to diabetic control group (ANOVA followed by Dunnett's test)*

Discussion

The results obtained showed that the petroleum ether extract (PEMF) of *M. fragrans* decreased blood glucose levels in normal, glucose fed, and alloxan- induced diabetic rats, compared with their respective control groups. The single treatment of PEMF caused a significant decrease in blood glucose level in normal rats. The hypoglycaemic effect in normal rats may be due to the potentiation of insulin release from beta- cells similar to glibenclamide. Some medicinal plants with hypoglycaemic properties are known to increase circulating insulin level in normoglycaemic rats (Lamela *et al.*, 1985). Glibenclamide caused significantly more hypoglycaemia in comparison with the plant extract. It has been reported that sulphonyl urea compounds produce hypoglycaemia in normal animals by stimulating the pancreatic beta- cells to produce more insulin and increasing the glycogen deposition in the liver. It is, therefore, conceivable that hypoglycaemic principles in the extract exert effect probably by a mechanism similar to glibenclamide.

In glucose tolerance test, the oral administration of PEMF suppressed the increase in glucose level induced by glucose loading. Such an effect might be due to decrease in the rate of intestinal glucose absorption or by potentiation of pancreatic secretions or increasing the glucose uptake.

It is know that the factors influencing the glucose metabolism under various physiological conditions do influence lipid metabolism as well (Jenkins *et al.*, 1995). It has also been revealed that triglyceride accumulation increase considerably in diabetes mellitus (Iams and Wexler, 1977). Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic (Riyad *et al*., 1988; Tarfa *et al*., 1988; Sharma *et al*., 1996) and a significant increase in cholesterol and triglyceride observed in our experiment in accordance to these studies. Reduction in the body weight in diabetic animals as well as in humans is well known. In case of diabetes, the body weight will increase when normal glycaemic levels is achieved which is particularly seen in sulfonyl ureas or insulin. PEMF increases body weight in diabetic animals, which might be due to increased insulin secretion and better glyceamic control.

In conclusion, the results suggests that PEMF showed the hypoglycaemic activity in normal and diabetic rats, improved glucose tolerance suggests that the extracts may stimulate the beta-cells of the pancreas to release the insulin, which is similar to that of sulphonyl ureas. Thus, the present data indicates that *Myristica fragrans* possesses potential as an antidiabetic and warrants the need for further studies to elucidate its mode of action.

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