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EVALUATION OF ORAL GLUCOSE TOLERANCE EFFICACY OF VARIOUS SOLVENT EXTRACTS OF *MYRISTICA FRAGRANS* SEEDS

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ABSTRACT

Background. *Myristica fragrans* Houtt. (Myristicaceae) is a tropical evergreen tree, whose seeds are well known and used in cuisine of a number of countries as a spice. As part of our ongoing screening studies to examine oral glucose tolerance efficacy of various plant and plant part extracts, it was of interest to evaluate the oral glucose tolerance efficacy of various solvent extracts of seeds of the plant. **Methods.** Oral glucose tolerance test (OGTT) was done to evaluate glucose tolerance. **Results.** In oral glucose tolerance tests, methanol extract of seeds lowered blood glucose levels in glucose-loaded mice by 58.8 and 61.4%, respectively, at doses of 200 and 400 mg per kg body weight. Petroleum ether fraction of seeds at the same two doses reduced blood glucose levels by 62.0 and 63.6%. n-Hexane fraction at the above two doses reduced blood glucose by 58.5 and 60.7%. Finally, chloroform fraction at the same two doses reduced blood glucose vlevels by 64.0 and 65.1%. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 63.3% at a dose of 10 mg per kg. **Conclusion.** Various solvent extracts of *Myristica fragrans* seeds may prove useful as blood glucose lowering agents in diabetic patients and also in discovery of novel blood glucose lowering drugs.

KEYWORDS: Antihyperglycemic, *Myristica fragrans*, OGTT, diabetes.

BACKGROUND

Diabetes is a disorder, which has been known since ancient times. For instance, in the major Indian system of traditional medicine, the disorder was known as madhu meha or sweet urine.[1] In recent years, practically all countries of the world are witnessing an increase in the number of diabetic patients and patients with impaired glucose metabolism or pre-diabetes, both being characterized by elevated blood glucose levels. Globally, an estimated 422 million adults were living with diabetes in 2014 compared to 108 million in 1980.[2] Diabetes caused 1.5 million deaths in 2012. Another 2.2 million deaths occurred due to diabetes-induced complications like cardiovascular disorders.[2] Sadly, although the disease has been known from ancient times, neither traditional nor allopathic treatment can cure the disease.

As such, treatment of diabetes has primarily been limited to taking medications including insulin injection to lower elevated blood glucose levels. This is not a feasible method in countries like Bangladesh with a high diabetic population, but limited means of diabetic patients to afford appropriate diabetic medications. Food in the form of vegetables or spices can possibly be a way for diabetic patients to reduce blood glucose levels and to maintain glucose homeostasis. We had been screening for such glucose lowering plants for several years.[3-20] *Myristica fragrans* Houtt. (Myristicaceae) is a tropical evergreen tree, whose seeds are well known and used in cuisine of a number of countries as a spice. Hypoglycemic and antidiabetic activities of the seeds have been seen in normoglycemic and alloxan diabetic rats.[21] The seeds have also been shown to alleviate oxidative stress in alloxan diabetic rats.[22] The seeds are widely available in Bangladesh and are affordable and used in a number of dishes as spice. It was the objective of the present study to evaluate the glucose tolerance efficacy of various solvent extracts of *Myristica fragrans* seeds in glucose-loaded mice through oral glucose tolerance test (OGTT).

METHODS

Plant material collection

Myristica fragrans seeds were collected from an herbal shop at Dhaka. The seeds were washed properly and then air dried for several days. The seeds were then grounded into a coarse powder using high capacity grinding machine.

Preparation of methanolic extract of grounded seeds

For preparation of methanol extract, 800g of the powder was extracted with 2.5 liters of methanol over 15 days with occasional stirring and shaking. The mixture was then filtered

and methanol in the filtrate evaporated using a Rota evaporator. The final weight of the methanolic extract was 160g.

Solvent-solvent partitioning

10g of crude methanolic extract was dissolved in 10% aqueous methanol and sequentially extracted with petroleum ether, n-hexane and finally with chloroform.[23]

Chemicals and Drugs

Glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade. Glucometer and strips were purchased from Lazz Pharma, Bangladesh.

Animals

Swiss albino mice, which weighed between 30-35g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were acclimatized for three days prior to actual experiments. During this time, the animals were fed with mice chow (supplied by ICDDR,B) and water *ad libitum*. The study was conducted following approval by the Institutional Animal Ethical Committees of the University of Development Alternative and the University of Dhaka, Dhaka, Bangladesh.

Oral glucose tolerance tests for evaluation of antihyperglycemic activity

Oral glucose tolerance tests (OGTT) were carried out as per the procedure previously described by Joy and Kuttan[24] with minor modifications. Briefly, fasted mice were grouped into ten groups of five mice each. The various groups received different treatments like Group 1 received vehicle and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3 and 4 received, respectively, methanolic extract at doses of 200 and 400 mg per kg body weight. Groups 5 and 6 received, respectively, 200 and 400 mg petroleum ether extract per kg body weight. Groups 7 and 8 received, respectively, 200 and 400 mg n-hexane extract per kg body weight. Groups 9 and 10 received, respectively, 200 and 400 mg chloroform extract per kg body weight. All substances were orally administered by gavaging. The amount of vehicle administered was same in both control and experimental mice. Following a period of 30 minutes, all mice were orally administered 2g glucose per kg of body weight. Blood glucose levels were measured

with a glucometer after 150 minutes of glucose loading. The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 – W_e/W_c)$ X 100,

Where W_e and W_c represents the blood glucose concentration in glibenclamide or various extract(s) administered mice (Groups 2-10), and control mice (Group 1), respectively.

Statistical analysis

Experimental values are expressed as mean ± SEM. The results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's test using SPSS ver. 17. A value of $P< 0.05$ was considered to be statistically significant.

RESULTS

In oral glucose tolerance tests, methanol extract of seeds lowered blood glucose levels in glucose-loaded mice by 58.8 and 61.4%, respectively, at doses of 200 and 400 mg per kg body weight. Petroleum ether fraction of seeds at the same two doses reduced blood glucose levels by 62.0 and 63.6%. n-Hexane fraction at the above two doses reduced blood glucose by 58.5 and 60.7%. Finally, chloroform fraction at the same two doses reduced blood glucose vlevels by 64.0 and 65.1%. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 63.3% at a dose of 10 mg per kg. The results are shown in Table 1 and suggest that the chloroform fraction contains the more potent agent(s) giving better glucose tolerance activity.

Table 1: Evaluation of oral glucose tolerance activity of various extracts of *Myristica fragrans* seeds.

Group	Dose	Blood glucose level (mmol/l) Mean ± SEM	% lowering of blood glucose level (After 150 minutes)
1) Control		12.68 ± 0.25	-
2) Standard (Glibenclamide)	10 mg/ Kg	4.66 ± 0.05	63.25*
3) Crude Methanolic Extract	200 mg/ Kg	5.22 ± 0.11	58.83*
4) Crude Methanolic Extract	400 mg/ Kg	4.90 ± 0.22	61.36*
5) Petroleum Ether fraction	200 mg/ Kg	4.82 ± 0.14	61.99*
6) Petroleum Ether fraction	400 mg/ Kg	4.62 ± 0.04	63.56*
7) n-Hexane fraction	200 mg/ Kg	5.26 ± 0.24	58.52*
8) n-Hexane fraction	400 mg/ Kg	4.98 ± 0.09	60.73*
9) Chloroform fraction	200 mg/ Kg	4.56 ± 0.10	64.04*
10) Chloroform fraction	400 mg/ Kg	4.42 ± 0.11	65.14*

All administrations were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to hyperglycemic control animals.

DISCUSSION

It appears that various extracts of seeds of *Myristica fragrans* have the ability to improve glucose tolerance in glucose-challenged mice. As such, it would be interesting to do further studies and determine the responsible bioactive constituent(s). Seeds are known to contain epicatechin.[25] Epicatechin can reduce blood glucose levels in diabetic patients.[26] From that view point, it is possible that the observed effects may be due to epicatechin.

CONCLUSION

The results suggest that various extracts of seeds of *Myristica fragrans* can be used for lowering of blood glucose.

Conflicts of interest

The author(s) declare that they have no competing interests.

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