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ANTIHYPERGLYCEMIC ACTIVITY STUDIES WITH METHANOLIC EXTRACT OF WHOLE PLANTS OF *PIPER SYLVATICUM*

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ABSTRACT

Background: *Piper sylvaticum*, known as pipul in Bangladesh and Mountain Long Pepper in English is considered a medicinal plant by traditional medicinal practitioners in various countries. The objective of the present study was to evaluate whether methanolic extract of the whole plant (MEPS) possess antihyperglycemic activity. **Methods:** Oral glucose tolerance test (OGTT) was done to evaluate antihyperglycemic efficacy. **Results:** In oral glucose tolerance tests, MEPS dose-dependently and significantly reduced blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, MEPS lowered blood glucose levels by 25.9, 31.4, 40.4, and 49.4%, respectively, compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide reduced blood glucose levels by 29.7% at a dose of 10 mg per kg. **Conclusion:** The whole plant can be an effective means for lowering blood glucose in persons with elevated blood glucose levels like people with diabetes.

KEYWORDS: Antihyperglycemic, *Piper sylvaticum*, OGTT, diabetes.

BACKGROUND

Piper sylvaticum Roxb. (Piperaceae), known as pipul in Bangladesh and Mountain Long Pepper in English, is an herbaceous climber with light green foliage. It is found in wet areas of forest and prefers semi-shade for growth. In the Ayurvedic system of Indian medicine, the roots of *Piper sylvaticum* are used for their laxative, anthelmintic, and carminative properties, as well as to treat bronchitis, diseases of the spleen, and tumors.[1] Tribal people of Jalpaiguri district, West Bengal, India use the fruits to treat coughs and colds.[2] As a number of *Piper*

genera species have been found to alleviate diabetes-induced high blood glucose or diabetes-induced disorders,[3-5] it was of interest to determine the antihyperglycemic efficacy of methanol extract of *Piper sylvaticum* whole plants (MEPS).

Elevation of blood glucose concentration is a hallmark of diabetes mellitus, which is fast approaching almost endemic proportions throughout the world,[6] possibly because of changes in lifestyle and food habits with a more sedentary lifestyle and increased consumption of refined sugar and foods containing refined sugar. Existing antidiabetic drugs cannot cure the disease but can prove helpful in reducing blood glucose and diabetes-induced complications. However, these drugs are costly and neither available nor affordable to large segments of particularly the rural and urban slum population of Bangladesh. New and more efficacious antidiabetic drugs are necessary, which should be affordable, available and generate none or lesser adverse side-effects. Plants have since time immemorial always proved to be a good source of new drugs. Towards discovery of possible antidiabetic drugs from plants, we had been screening various plants of Bangladesh for their blood glucose lowering efficacies for a number of years.[7-27] The objective of the present study was to evaluate the antihyperglycemic activity of methanol extract of whole plants of *Piper sylvaticum* through oral glucose tolerance test (OGTT) in mice.

METHODS

Plant material collection

Whole plants of *Piper sylvaticum* were collected from Narsinghdi in Dhaka district, Bangladesh. The plant was identified at the University of Development Alternative by a competent botanist and voucher specimens were deposited with the Medicinal Plant Collection Wing of the University of Development Alternative.

Preparation of methanolic extract of Piper sylvaticum whole plants (MEPS)

For preparation of methanol extract of whole plants of *Piper sylvaticum* (MEPS), whole plants were thoroughly cut into small pieces, dried in the shade, and pulverized into a fine powder. 50g of the powder was extracted with 250 ml methanol over 48 hours with frequent stirring. Methanol was evaporated at 40-50^{o}C and the extract was dissolved in Tween 20 prior to administration to mice by gavaging. The final weight of the extract was 2.4g. The extract was maintained in small aliquots at -20^{o}C till use, and care was taken not to freeze-thaw the extract vials repeatedly.

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 18-20g 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 Committee of University of Development Alternative, Dhaka, Bangladesh. Care was taken that the animals did not suffer from any unnecessary pain during the acclimatization period.

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 [28] with minor modifications. Briefly, fasted mice were grouped into six groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 20 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received, respectively, MEPS at doses of 50, 100, 200 and 400 mg per kg body weight. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier [18, 19], all mice were orally administered 4g glucose per kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart following previously published procedures.[18,19] Blood glucose levels were measured with a glucometer. 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 MEPS administered mice (Groups 2-6), and control mice (Group 1), respectively. Gavaging was done carefully such that injuries do not happen, and no mice fatalities occurred during gavaging. Mice were handled carefully throughout the experiment so that they did not get

subjected to any unnecessary pain. All institutional and international ethical principles for handling of animals during experiments were followed.

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.[15]

RESULTS

In oral glucose tolerance tests, MEPS dose-dependently and significantly reduced blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, MEPS lowered blood glucose levels by 25.9, 31.4, 40.4, and 49.4%, respectively, compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide reduced blood glucose levels by 29.7% at a dose of 10 mg per kg. The results are shown in Table 1 and suggest that even at a dose of 100 mg per kg body weight, MEPS was comparable to glibenclamide in antihyperglycemic activity. However, some signs of hemolysis were observed while collecting blood from mice, which were MEPS administered.

Table 1: Lowering action of MEPS on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	6.88 ± 0.23	-
Glibenclamide	10 mg	4.84 ± 0.32	29.7*
(MEPS)	50 mg	5.10 ± 0.13	25.9*
(MEPS)	100 mg	4.72 ± 0.21	31.4*
(MEPS)	200 mg	4.10 ± 0.48	40.4*
(MEPS)	400 mg	3.48 ± 0.31	49.4*

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

DISCUSSION

The exact identification of component(s) responsible for the observed antihyperglycemic activity was not done in the present study. However, considering the extent of the antihyperglycemic effect observed, the plant appears to be a very promising candidate for such studies. On the other hand, toxicity studies are necessary before administration of the crude extract. It is possible that any toxic component(s) present in the crude extract may be

different from any antidiabetic component(s) in the crude extract, so isolation and administration of the pure antidiabetic component(s) may be safe and effective. It is to be noted that in general *Piper* genera plants have proved to be safe and a number of them like *Piper longum*, *Piper nigrum* and *Piper chaba* are used in Bangladesh and other countries as spices. However, *Piper sarmentosum* is known to increase serum potassium levels [29], although the plant is used in traditional medicine for lowering of elevated blood glucose in diabetic patients.

CONCLUSION

The results suggest that methanolic extract of whole plants of *Piper sylvaticum* 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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