ORAL GLUCOSE TOLERANCE TESTS IN MICE WITH A POLYHERBAL FORMULATION DIABETO-MLF

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ABSTRACT

Background. Diabetes is a serious disease leading often to fatal conclusions. The disease and its related complications are fast becoming major health concerns among the population throughout the world. The objective of this study was to evaluate the antihyperglycemic activity of a polyherbal formulation, Diabeto-MLF.

Methods. Oral glucose tolerance test (OGTT) was used to determine antihyperglycemic activity of Diabeto-MLF, which consisted of methanol extract of leaves of Malva verticillata, and seeds of Lathyrus sativus and Foeniculum vulgare in a 1:1:1 (w/w/w) ratio.

Results. Administration of Diabeto-MLF led to dose-dependent reductions in blood glucose levels in glucose-loaded mice. At doses of 100, 200 and 400 mg per kg body weight, the extract reduced blood glucose levels by 36.3, 39.2, and 40.8%, respectively compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 47.1%. Conclusion. The results suggest that Diabeto-MLF can be used as a potential drug to reduce blood glucose levels in diabetic patients.

KEYWORDS: Antihyperglycemic, Malva verticillata, Lathyrus sativus, Foeniculum vulgare

INTRODUCTION

Diabetes mellitus is a disease where the body is unable to convert glucose to energy. The disease is characterized by blurred vision, unusual thirst, frequent urination, high levels of blood glucose, passing of glucose in urine, and slow-healing cuts among other symptoms. More than 220 million people in the world had diabetes in the world in 2004 according to...
World Health Organization (WHO). Since then, the progress of the disease has accelerated and the disease has reached endemic proportions in many countries of the world, including Bangladesh. There is no allopathic cure for diabetes, and the disease very often leads to other complicated factors like diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, and cardiovascular disorders.

Allopathic drugs can provide symptomatic relief through inducing the pancreas to produce more insulin, the lack of which or resistance to which being the major factors for diabetes to occur in the first place. However, these drugs are costly and not accessible or affordable to the majority of population living at or below poverty level incomes in underdeveloped countries. Moreover, many diabetic patients need daily injections of insulin, which is cumbersome and also costly. As a result, there is a serious need for new antidiabetic medications, which can be easily afforded by the poorer sections of the population.

Plants have always formed a source of many important allopathic medicines. *Malva verticillata* L. (Malvaceae) is known in English as Cluster mallow and in Bengali as Napa shak. The leaves of the plant are edible and used in Bangladesh cuisine as a vegetable dish. *Lathyrus sativus* L. (Fabaceae) is a leguminous crop cultivated in Bangladesh for its seeds. In English it is known as Grass pea and in Bengali as Kheshari dal. *Foeniculum vulgare* Mill. (Apiaceae) is cultivated in Bangladesh for its seeds and is known in English as Sweet fennel and in Bengali as Mouri. The seeds are used in various dishes of Bangladesh as a spice. The seeds are also chewed to remove foul odor from mouth or to leave a sweet taste in the mouth. All three plant parts are available in Bangladesh and affordable to the general population.

We had been conducting pharmacological studies on various medicinal plants as well as other aromatic crops and spices of Bangladesh.[1-20] The objective of the present study was to evaluate the potential of a polyherbal preparation, namely Diabeto-MLF, to lower blood glucose levels in glucose-loaded mice in oral glucose tolerance tests (OGTT). Diabeto-MLF was prepared by mixing in an equal weight to weight ratio (1:1:1), methanol extracts of leaves of *Malva verticillata*, and seeds of *Lathyrus sativus* and *Foeniculum vulgare*. 
METHODS

Plant material collection

_Vigna radiata_ leaves and seeds of _Lathyrus sativus_ and _Foeniculum vulgare_ were collected between January and May, 2015 from a local market in Dhaka city. The three plant parts were separately dried and powdered prior to methanol extraction.

Preparation of methanolic extract of seeds

54g of powdered _Malva verticillata_ leaves were extracted with 270 ml methanol (final weight of the extract was 2.99g). 100g of powdered _Lathyrus sativus_ seeds were extracted with 500 ml methanol (final weight of the extract was 8.44g). 100g of _Foeniculum vulgare_ seeds were extracted with 500 ml methanol (final weight of the extract was 6.78g). The three extracts were mixed in a 1:1:1 (weight/weight/weight) ratio to prepare Diabeto-MLF.

Chemicals and Drugs

Glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

Swiss albino mice, which weighed between 12-17 g 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. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Oral glucose tolerance tests for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999)[21] with minor modifications. Briefly, fasted mice (12h fasting) were grouped into eight 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 3-5 received, respectively extracts of _Malva verticillata_, _Lathyrus sativus_, and _Foeniculum vulgare_ at doses of 400 mg per kg body weight. Groups 6-8 received, respectively, Diabeto-MLF at doses of 100, 200 and 400 mg per kg body weight. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose
administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method.[22] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = \( (1 - \frac{W_e}{W_c}) \times 100 \),

Where \( W_e \) and \( W_c \) represents the blood glucose concentration in glibenclamide or various extracts and Diabeto-MLF administered mice (Groups 2-8), and control mice (Group 1), respectively.

**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.[9]

**RESULTS**

Administration of Diabeto-MLF led to dose-dependent reductions in blood glucose levels in glucose-loaded mice. At doses of 100, 200 and 400 mg per kg body weight, the extract reduced blood glucose levels by 36.3, 39.2, and 40.8%, respectively compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 47.1%. Individual methanol extracts of leaves of *Malva verticillata* (MEMV), seeds of *Lathyrus sativus* (MELS), and seeds of *Foeniculum vulgare* (MEFV), when administered to mice at doses of 400 mg per kg body weight, reduced blood glucose levels, respectively, by 26.1, 29.6, and 32.8%. The results are shown in Table 1 and suggest that the combination of the three extracts, namely Diabeto-MLF possess good blood glucose lowering capability and can be used as such by diabetic patients. It also appears from the results that the three extracts are acting synergistically.

<p>| Table 1: Effect of MEMV, MELS, MEFV, and Diabeto-MLF 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            | 6.28 ± 0.30     | -               |
| Glibenclamide   | 10 mg            | 3.32 ± 0.17     | 47.1*           |
| (MEMV)          | 400 mg           | 4.64 ± 0.11     | 26.1*           |
| (MELS)          | 400 mg           | 4.42 ± 0.14     | 29.6*           |
| (MEFV)          | 400 mg           | 4.22 ± 0.11     | 32.8*           |</p>
<table>
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<th>(Diabeto-MLF)</th>
<th>100 mg</th>
<th>4.00 ± 0.04</th>
<th>36.3*</th>
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<tr>
<td>(Diabeto-MLF)</td>
<td>200 mg</td>
<td>3.82 ± 0.16</td>
<td>39.2*</td>
</tr>
<tr>
<td>(Diabeto-MLF)</td>
<td>400 mg</td>
<td>3.72 ± 0.33</td>
<td>40.8*</td>
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All administrations were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to hyperglycemic control animals.

**DISCUSSION**

The seeds of *Malva verticillata* have been reported previously to produce hypoglycemic effects.[23] Antihyperglycemic effect of leaves of the plant is reported here, to our knowledge, for the first time. Antioxidant potential and inhibition of alpha-amylase and alpha-glucosidase enzyme activities have been reported for *Lathyrus sativus* seeds.[24] The antidiabetic activity of *Foeniculum vulgare* seed aqueous extract has also been reported in streptozotocin-induced diabetic rats.[25] Thus the previous reports along with the present results suggest that Diabeto-MLF can be a good antidiabetic agent and prove useful for diabetic patients and people with impaired glucose metabolism and who are not able to afford costlier antidiabetic allopathic drugs.

**CONCLUSION**

The results suggest that Diabeto-MLF can be used to lower blood glucose levels in diabetic patients and glucose metabolism impaired people.

**CONFLICTS OF INTEREST**

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

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**REFERENCES**


