ABSTRACT

Background. *Nigella sativa* and *Brassica alba* are two spice plants cultivated in Bangladesh, albeit on a small scale. A number of spice plants of Bangladesh are known for their antihyperglycemic properties. It was therefore of interest to determine the antihyperglycemic potential of a polyherbal formulation containing seeds of both plants.

Methods. Oral glucose tolerance test (OGTT) was done to evaluate antihyperglycemic potential. Results. In oral glucose tolerance tests, methanol extract of seeds of *Nigella sativa* (MENS), administered at a dose of 400 mg per kg body weight reduced blood glucose by 32.2%. Methanol extract of seeds of *Brassica alba* (MEBA), administered at the same dose reduced blood glucose by 28.1%. Administration of a combination of MENS and MEBA (1:1, w/w), tentatively termed MENSBA, significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 29.5, 34.2 and 40.8%, respectively, at doses of 100, 200 and 400 mg each per kg body weight in mice. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 41.4% at a dose of 10 mg per kg.

Conclusion. MENSBA can potentially be an excellent source for blood glucose lowering in diabetic patients in the absence of antihyperglycemic drugs.


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BACKGROUND

*Nigella sativa* L. (Ranunculaceae family) is known in English as black cumin and locally in Bangladesh as kalo jira or kali jira. The seed of the plant (which grows to about 20-30 cm tall) is used as a spice. The plant is considered to be one of the most important medicinal plants in Unani and other traditional medicines, with seeds and other parts of the plant being used for diabetes, cancer, pain, and gastrointestinal disorders.[1] *Nigella sativa* is cultivated in Bangladesh for both culinary and medicinal uses. *Brassica alba* (L.) Rabenh. (Cruciferae family) is another spice plant cultivated in Bangladesh, primarily for culinary uses. The plant is known in English as white mustard and locally as shada shorisha. The seeds of the plant have traditional medicinal uses as appetizer, digestive and carminative.

*Nigella sativa* seeds have been reviewed as beneficial for not only controlling blood glucose but improving glucose tolerance and insulin production. The seeds have also been found to be beneficial in diabetes-induced complications.[2] We have previously reported the beneficial effects of a polyherbal formulation in improving glucose tolerance, one of the components of the formulation being *Nigella sativa* seeds.[3] Improved glucose tolerance with *Brassica alba* seeds has also been reported by us.[4]

Diabetes is becoming almost endemic in Bangladesh.[5] A large section of the population cannot afford diabetes treatment with allopathic drugs. Moreover, modern hospitals, clinics and doctors are not available to most of the population of the country. As such, locally readily available and affordable products are necessary to treat diabetes. To alleviate elevated blood glucose levels during impaired glucose metabolism with easily affordable and available drugs, we had been experimenting with various local plants and formulations for their blood glucose lowering effects.[6-18] The objective of the present study was to evaluate the oral glucose tolerance efficacy in glucose-loaded mice with methanol extract of a combination of *Nigella sativa* and *Brassica alba* seeds.

METHODS

Plant material collection

Seeds of *Nigella sativa* and *Brassica alba* were collected during September 2016 from a local market at Tongibari, Bikrampur, Bangladesh.
Preparation of methanolic extract of Nigella sativa and Brassica alba seeds

For preparation of methanol extract of seeds of *Nigella sativa* (MENS), seeds were at first thoroughly dried and pulverized into a fine powder. 100 g of the powder was extracted with 500 ml methanol over 48 hours. Methanol was evaporated at 50°C and the extract was dissolved in Tween 20 prior to administration to mice by gavaging. The final weight of the extract was 9.557 g. The same procedure was followed for methanol extraction of *Brassica alba* seeds. The final weight of the extract (MEBA) was 6.024 g.

**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 12-15 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. 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.

**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\(^{[19]}\) with minor modifications. Briefly, fasted mice were grouped into seven 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 and 4 received MENS and MEBA, respectively, at a dose of 400 mg per kg body weight. Groups 5-7 received, respectively, (MENS + MEBA) at doses of 100, 200 and 400 mg each extract per kg body weight. All substances were orally administered by gavaging. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier\(^{[9,15]}\), 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 following previously published procedures.\(^{[9,15]}\) 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 = \(1 - \frac{W_e}{W_c}\) X 100, where \(W_e\) and \(W_c\) represents the blood glucose concentration in glibenclamide or MENS, MEBA or (MENS + MEBA) administered mice (Groups 2-7), and control mice (Group 1), respectively. Gavaging was done carefully such that injuries do not happen, and no mice fatalities occurred during gavaging.

**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, methanol extract of seeds of *Nigella sativa* (MENS), administered at a dose of 400 mg per kg body weight, reduced blood glucose by 32.2%. Methanol extract of seeds of *Brassica alba* (MEBA), administered at the same dose, reduced blood glucose by 28.1%. Administration of a combination of MENS and MEBA (1:1, w/w), tentatively termed MENSBA, significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 29.5, 34.2 and 40.8%, respectively, at doses of 100, 200 and 400 mg each extract per kg body weight in mice. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 41.4% at a dose of 10 mg per kg. Thus the combination of the two seed extracts demonstrated synergistic action with results highly comparable to that of glibenclamide.

**Table 1: Effect of MENS, MEBA and (MENS + MEBA) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Blood glucose level (mmol/l)</th>
<th>% lowering of blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>5.84 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>3.42 ± 0.10</td>
<td>41.4*</td>
</tr>
<tr>
<td>(MENS)</td>
<td>400 mg</td>
<td>3.96 ± 0.09</td>
<td>32.2*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>400 mg</td>
<td>4.20 ± 0.11</td>
<td>28.1*</td>
</tr>
<tr>
<td>(MENS + MEBA)</td>
<td>(100 + 100) mg</td>
<td>4.12 ± 0.08</td>
<td>29.5*</td>
</tr>
<tr>
<td>(MENS + MEBA)</td>
<td>(200 + 200) mg</td>
<td>3.84 ± 0.08</td>
<td>34.2*</td>
</tr>
<tr>
<td>(MENS + MEBA)</td>
<td>(400 + 400) mg</td>
<td>3.46 ± 0.09</td>
<td>40.8*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=5); *\(P < 0.05;\) significant compared to hyperglycemic control animals.
DISCUSSION
Availability and affordability of modern medicines and doctors are two key factors in developing countries like Bangladesh. When modern medicines are expensive and difficult to procure, the only viable alternative is to look for efficacious substitutes. Plants usually form cheaper substitutes; in fact, many modern medicines have been discovered through observations of indigenous use of plants.\textsuperscript{[20]} From that viewpoint, the present polyherbal formulation may prove to be an effective means to improve glucose tolerance and so restore glucose homeostasis in diabetic patients and patients with impaired glucose metabolism.

CONCLUSION
The results suggest that a combination of methanolic extract of Nigella sativa and Brassica alba seeds can be used for lowering of blood glucose. Since the plants can be easily cultivated, the seeds can be readily available and affordable.

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

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Author’s contributions
ZA collected the plant materials, did the extraction, and performed the experiments with MNH under the supervision of MR. MR wrote the manuscript draft, which was read and edited by all authors. All authors read and approved the final version of the manuscript.

REFERENCES


