ORAL GLUCOSE TOLERANCE AND ANALGESIC STUDIES WITH METHANOL EXTRACT OF BRASSICA ALBA SEEDS

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ABSTRACT

Background. Brassica alba, also known as white mustard is an annual plant cultivated in Bangladesh for culinary and medicinal uses. It was of interest to determine the antihyperglycemic and analgesic properties of the seeds of the plant. Methods. Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT). Analgesic activity was determined by observed decreases in abdominal constrictions (writhings) in intraperitoneally administered acetic acid-induced pain model in mice. Results. Administration of methanol extract of seeds led to significant 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 dose-dependently reduced blood glucose levels by 20.1, 32.4, and 39.5%, 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 45.3%. In analgesic activity tests, the extract at doses of 100, 200 and 400 mg per kg body weight significantly reduced the number of abdominal constrictions by 29.6, 33.3, and 44.4%, respectively. A standard pain relieving (analgesic) drug, aspirin, reduced the number of writhings by 33.3 and 51.9%, respectively, when administered at doses of 200 and 400 mg per kg body weight. Conclusion. The seeds can be beneficial in lowering blood glucose and for alleviating pain.

KEY WORDS: Antihyperglycemic, Brassica alba, analgesic, Brassicaceae.
BACKGROUND

*Brassica alba* Rabenh. also known as *Sinapis alba* is an annual plant belonging to the Brassicaceae family. It is grown in Bangladesh primarily for culinary and medicinal uses. The plant is known as ‘white mustard’ in English and ‘shada shorisha’ in Bengali. Not much has been reported on the pharmacological properties of the plant or its various parts. However, it has been reported that sinalbin and $\alpha$-sitosterol, two components present in seeds significantly inhibited mice prostatic hyperplasia induced by testosterone propionate.$^{[1]}$

Although any antihyperglycemic or analgesic properties are yet to be reported for *Brassica alba* seeds, a related species *Brassica juncea* or mustard has been studied more regarding particularly its antidiabetic properties. The antioxidant effects of isorhamnetin 3,7-di-O-$\alpha$-D-glucopyranoside isolated from mustard leaf has been observed in rats with streptozotocin-induced diabetes.$^{[2]}$ The protective effects of mustard leaf have been reported against diabetes-induced oxidative stress.$^{[3]}$ Hypoglycemic effect has been observed following administration of mustard seed aqueous extract in streptozotocin-induced diabetic male albino rats.$^{[4]}$ Antihyperglycemic effects have also been observed with extract of seeds of another related species, *Brassica nigra*, in streptozotocin-induced diabetic rats.$^{[5]}$ The extract has been shown to give an insulinotropic effect thus leading to improved glucose homeostasis.$^{[6]}$ Antinociceptive and antihyperglycemic activities of methanol extract of *Brassica juncea* leaves have also been described.$^{[7]}$

Diabetes is becoming almost endemic in Bangladesh possibly through a combination of changes in life style, food habits and presence of arsenic in drinking water and soil.$^{[8,9]}$ This is a disease for which allopathic medicine has only symptomatic but not total cure and which is rapidly affecting large sections of population throughout the world. Pain is another factor, which affects large numbers of people throughout the world on a daily basis, whether due to simple reasons like sprains or cuts and wounds or due to more complicated factors like cancer, gout, or rheumatoid arthritis. Rural people in Bangladesh often lack access to or cannot afford allopathic medicines. Towards providing them with affordable relief, we had been screening various plants (including plant parts) of Bangladesh for their antihyperglycemic and analgesic potential.$^{[10-21]}$ The objective of the present study was to evaluate the antihyperglycemic and analgesic potential of methanol extract of *Brassica alba* seeds through oral glucose tolerance tests and acetic acid-induced pain model in Swiss albino mice.
METHODS

Plant material collection
Seeds of *B. alba* were collected during August 2014 from a local market in Dhaka city.

Preparation of methanolic extract of seeds
Seeds were thoroughly dried and 100g of dried and powdered seeds were extracted with methanol (w:v ratio of 1:5, final weight of the extract 5.881g).

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

Animals
Swiss albino mice, which weighed between 12-15g 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)[22] 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 80 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 methanolic seed extract (MEBA) at doses of 50, 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 2g 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.[23] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = \((1 – W_e/W_c) \times 100\),
where *W_e* and *W_c* represents the blood glucose concentration in glibenclamide or MEBA administered mice (Groups 2-6), and control mice (Group 1), respectively.
Analgesic activity evaluation through abdominal writhing test

Analgesic activity of MEBA was examined as previously described. Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEBA at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MEBA, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

\[
\text{Percent inhibition} = (1 - \frac{W_e}{W_c}) \times 100,
\]

where \(W_e\) and \(W_c\) represents the number of writhings in aspirin or MEBA administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute toxicity test

Acute toxicity test was conducted as previously described. Mice were divided into nine groups, each group consisting of six animals. Group 1 was given 1% Tween 80 in normal saline (2 ml per kg body weight). The other eight groups (Groups 2-9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg of MEBA per kg body weight. All animals were closely observed for the next 8 hours to notice any behavioral changes or mortality and were kept under close observation for the next two weeks.

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.

RESULTS

Toxicity evaluation

The crude extract (MEBA) did not show any toxicity in mice even at the highest dose tested. There were no changes in behavioral pattern and mortality was not observed.
Antihyperglycemic activity evaluation results
In oral glucose tolerance tests (OGTT), MEBA at doses of 50, 100, 200 and 400 mg/kg caused, respectively, 9.7, 20.1, 32.4, and 39.5% reductions in blood glucose levels. The results were dose-dependent and statistically significant except for the 50 mg/kg MEBA dose, which did not give statistically significant reduction in blood glucose levels. Glibenclamide (a standard antihyperglycemic drug), when administered at a dose of 10 mg/kg lowered blood glucose by 45.3%. The results are shown in Table 1 and suggest that the extract possess antihyperglycemic activity, indicating that the crude extract may be used for lowering blood glucose levels in hyperglycemic patients.

Analgesic activity evaluation results
Dose-dependent and significant reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MEBA. At doses of 50, 100, 200 and 400 mg per kg body weight, MEBA was observed to reduce the number of constrictions, respectively, by 22.2, 29.6, 33.3, and 44.4%. The percent reduction observed with 50 mg/kg MEBA was not statistically significant. A standard analgesic drug, aspirin, when administered to experimental animals at doses of 200 and 400 mg per kg body weight, reduced the number of constrictions by 33.3 and 51.9%, respectively. Thus, a dose of 200 mg/kg MEBA was equivalent to 200 mg/kg aspirin, while a dose of 400 mg/kg MEBA gave better analgesic activity than 200 mg/kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant analgesic properties.

DISCUSSION
Phenolic compounds are known to be present in large amounts in Brassica genera of vegetables.[27] These type of compounds may be responsible for the observed antihyperglycemic and analgesic effects observed with methanol extract of seeds of B. alba. Baccharis dracunculifolia methanol extract has been shown to enhance glucose-stimulated insulin secretion in pancreatic islets of monosodium glutamate induced-obesity model rats and which effect has been attributed to presence of phenols in the extract.[28] Thus phenolic group of compounds present in MEBA can be responsible for the antihyperglycemic effect; however, the exact nature and identification of the actual compounds remain to be elucidated. Phenolic compounds may also be responsible for the observed analgesic effects. Analgesic effect has been observed with methanol extract of pericarp of Pleiogynium solandri, which has been attributed to presence of phenolic compounds.[29] The analgesic and
antiinflammatory activities of *Opuntia elatior* fruits have also been attributed to phenolic compounds.\[30\] It remains to be seen whether phenolic or other groups of compounds were responsible for the analgesic effect observed in the present study with MEBA.

**Table 1:** Effect of crude methanol extract of *B. alba* seeds (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>6.18 ± 0.43</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>3.38 ± 0.26</td>
<td>45.3*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>50 mg</td>
<td>5.58 ± 0.37</td>
<td>9.7</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>100 mg</td>
<td>4.94 ± 0.24</td>
<td>20.1*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>200 mg</td>
<td>4.18 ± 0.13</td>
<td>32.4*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>400 mg</td>
<td>3.74 ± 0.27</td>
<td>39.5*</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.

**Table 2:** Analgesic effect of crude methanol extract of *B. alba* seeds (MEBA) in acetic acid-induced pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of abdominal constrictions</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>5.4 ± 0.24</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>3.6 ± 0.40</td>
<td>33.3*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.6 ± 0.51</td>
<td>51.9*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>50 mg</td>
<td>4.2 ± 0.66</td>
<td>22.2</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>100 mg</td>
<td>3.8 ± 0.37</td>
<td>29.6*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>200 mg</td>
<td>3.6 ± 0.40</td>
<td>33.3*</td>
</tr>
<tr>
<td>(MEBA)</td>
<td>400 mg</td>
<td>3.0 ± 0.32</td>
<td>44.4*</td>
</tr>
</tbody>
</table>

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

**CONCLUSION**

The results suggest that methanolic extract of *B. alba* seeds can be used for lowering blood glucose and for alleviating pain.

**CONFLICTS OF INTEREST**

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


