A PRELIMINARY REPORT ON ANTIHYPERGLYCEMIC AND ANALGESIC PROPERTIES OF METHANOL EXTRACT OF 

BRASSICA OLERACEA L. VAR. ITALICA SPROUTS

Md. Yousuf Hasan1, Rahat Al-Mahamud1, Shahnaz Rahman1, Ishtiaq Ahmad2, Mohammed Rahmatullah2*

1Department of Biotechnology & Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh.
2Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh.

ABSTRACT

Background. Brassica oleracea var. italic (broccoli) is cultivated in Bangladesh for its edible sprout, which is known to be rich in several bio-active components and has therapeutic potential for treatment of cancer, diabetes and inflammatory diseases. It was of interest to determine the antihyperglycemic and analgesic properties of the sprouts 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 broccoli sprouts led to dose-dependent reductions in blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, the extract reduced blood glucose levels by 12.9, 29.6, 35.9, and 40.4%, 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 45.6%. In analgesic activity tests, the extract at doses of 50, 100, 200 and 400 mg per kg body weight significantly and dose-dependently reduced the number of abdominal constrictions by 14.8, 29.6, 37.0, and 51.9%, respectively. A standard pain relieving (analgesic) drug, aspirin, reduced the number of writhings by 37.0 and 55.6%, respectively, when administered at doses of 200 and 400 mg per kg body weight.
Conclusion. Broccoli sprouts can be beneficial in lowering blood glucose and for alleviating pain.

KEY WORDS: Antihyperglycemic, Brassica oleracea, analgesic, Brassicaceae, broccoli.

BACKGROUND
Brassica oleracea var. italica (broccoli) is an edible plant belonging to the Brassicaceae family and which is primarily cultivated for its sprouts, which are cooked or steamed and eaten in the form of vegetable in Bangladesh. The sprouts are known to contain a number of important bio-active compounds like glucoraphin, sulforaphane, and isothiocyanates making the sprouts a potential source of phytochemicals for treatment of cancer, diabetes, inflammatory diseases, and neural disorders. Consumption of sulforaphane has been reported to be associated with a lower risk of myocardial infarction.

Sulforaphane has been shown to prevent the development of cardiomyopathy in type 2 diabetic mice. Sulforaphane reportedly could prevent nephropathy, diabetes-induced fibrosis, and vascular complications. Consumption of broccoli sprouts has been associated with improvement in insulin resistance in type 2 diabetic patients. In a randomized double-blind placebo-controlled clinical trial, broccoli sprouts powder reportedly improved serum triglyceride and oxidized LDL/LDL-cholesterol ratio in type 2 diabetic patients and reduced oxidative stress in type 2 diabetes. The antioxidative effect and protective potential against diabetes of the broccoli flower has been investigated both in vitro and in vivo.

Since broccoli sprouts have been reported to possess a number of phytochemicals of possible therapeutic importance, it was of interest to evaluate the antihyperglycemic and analgesic activities of the sprouts. It is noteworthy in this regard that we had been systematically evaluating medicinal and other plants of Bangladesh for their antihyperglycemic and analgesic potential. The objective of the present study was to determine the antihyperglycemic and analgesic effects of methanol extract of broccoli sprouts in Swiss albino mice, respectively, through oral glucose tolerance tests and acetic acid-induced pain model.

METHODS
Plant material collection
Broccoli sprouts were collected during April 2014 from a local market in Dhaka city, Bangladesh. The sprouts were taxonomically identified at the Bangladesh National Herbarium (Accession Number 34,921).

Preparation of methanolic extract of sprouts
Sprouts were thoroughly dried in the shade and 100g of dried and powdered sprouts were extracted with methanol (w:v ratio of 1:5, final weight of the extract 10.5g).

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) 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 broccoli sprout extract (MEBS) 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. 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 MEBS administered mice (Groups 2-6), and control mice (Group 1), respectively.

**Analgesic activity evaluation through abdominal writhing test**

Analgesic activity of MEBS was examined as previously described.[30] 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 MEBS 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 MEBS, 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.[31] 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 MEBS administered mice (Groups 2-7), 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.[18]

**RESULTS**

**Antihyperglycemic activity evaluation results**

In oral glucose tolerance tests (OGTT), MEBS at doses of 50, 100, 200 and 400 mg/kg caused, respectively, 12.9, 29.6, 35.9, and 40.4% reductions in blood glucose levels. The results were dose-dependent and statistically significant except for the 50 mg/kg dose of extract. Glibenclamide (a standard antihyperglycemic drug), when administered at a dose of 10 mg/kg lowered blood glucose by 45.6%. The results are shown in Table 1 and suggest that the extract possess antihyperglycemic activity, with the highest dose of the extract being almost comparable to glibenclamide.
**Analgesic activity evaluation results**

Dose-dependent and statistically significant reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MEBS. At doses of 50, 100, 200 and 400 mg per kg body weight, MEBS was observed to reduce the number of constrictions, respectively, by 14.8, 29.6, 37.0, and 51.9%. 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 37.0 and 55.6%, respectively. Thus, a dose of 200 mg/kg MEBS was equivalent to 200 mg/kg aspirin, while a dose of 400 mg/kg MEBS gave better analgesic activity than 200 mg/kg aspirin and was close to that obtained with 400 mg/kg aspirin. The results are shown in Table 2 and suggest that the extract possesses significant analgesic properties.

**Table 1: Effect of crude methanol extract of broccoli sprouts (MEBS) 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>5.74 ± 0.26</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>3.12 ± 0.33</td>
<td>45.6*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>50 mg</td>
<td>5.00 ± 0.54</td>
<td>12.9</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>100 mg</td>
<td>4.04 ± 0.56</td>
<td>29.6*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>200 mg</td>
<td>3.68 ± 0.23</td>
<td>35.9*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>400 mg</td>
<td>3.42 ± 0.26</td>
<td>40.4*</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 broccoli sprouts (MEBS) 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>3.4 ± 0.40</td>
<td>37.0*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.4 ± 0.60</td>
<td>55.6*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>50 mg</td>
<td>4.6 ± 0.24</td>
<td>14.8*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>100 mg</td>
<td>3.8 ± 0.37</td>
<td>29.6*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>200 mg</td>
<td>3.4 ± 0.24</td>
<td>37.0*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>400 mg</td>
<td>2.6 ± 0.24</td>
<td>51.9*</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.
DISCUSSION

The observed antihyperglycemic effects observed with MEBS can be due to presence of sulforaphane in the extract, which compound has been shown to have antidiabetic potential in a number of studies.[1,4,5] Sulforaphane is produced from hydrolysis of glucoraphanin, which is also found in broccoli sprouts.[1] Oral treatment with sulforaphane has been shown to give positive results in streptozotocin induced diabetic rats.[32] Interestingly, broccoli sprouts are also known to contain kaempferol.[33] Hydroalcoholic extract of aerial parts of Leandra lacunosa containing kaempferol has been shown to give a hypoglycemic effect in alloxan induced diabetic rats.[34] Water extract of aerial parts of Equisetum myriochaetum has been shown to give hypoglycemic activity in streptozotocin induced diabetic rats; the extract was found to contain kaempferol glucosides.[35]

Kaempferol may also be responsible for the observed analgesic effects. The compound is known to inhibit cyclooxygenase-2 (COX-2) expression induced by ultraviolet B (UVB).[36] Acetic acid induced pain is a result of increased production of prostaglandins and prostacyclics mediated by COX-2 and lipoxygenases.[30] The analgesic and anti-inflammatory activities of kaempferol and its glycosides have been reviewed.[37] Thus kaempferol may also be the responsible component in broccoli sprouts for the observed pharmacological activities and since the sprouts are edible there may be lesser probability of any toxic effects.

CONCLUSION

The results suggest that methanolic extract of broccoli sprouts 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


