BOILED BROCCOLI – A PANACEA FOR LOWERING BLOOD GLUCOSE AND ALLEVIATING PAIN?

Symun Naher¹, Nazmul Karim², Md. Mofidul Islam², Emad Hossain²,
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. italica (broccoli) is cultivated in Bangladesh for its edible sprout, which is consumed in the boiled or fried form. Broccoli is known to be rich in several phytochemicals, which may prove useful in treatment for treatment of cancer, diabetes and inflammatory diseases. It was of interest to determine the antihyperglycemic and analgesic properties of the boiled 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 boiled 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 21.8, 37.6, 39.6, and 44.0%, 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 51.0%. 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 22.2, 33.3, 44.4, 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:** Boiled broccoli sprouts can be beneficial in lowering blood glucose and for alleviating pain.

**KEYWORDS:** Antihyperglycemic, *Brassica oleracea*, analgesic, Brassicaceae, broccoli.

**BACKGROUND**

*Brassica oleracea* var. *italica* (broccoli) is an edible plant belonging to the Brassicaceae (also referred to as Cruciferae) family and which is primarily cultivated for its sprouts (inflorescences), which are cooked or boiled (steamed) and eaten in the form of vegetable in Bangladesh. The plant is rich in phytochemicals with potential benefits against cancer, diabetes, and other diseases. [1] We have previously shown that methanol extract of broccoli sprouts can reduce blood glucose levels in glucose-loaded mice and alleviate pain in mice following intraperitoneal administration of acetic acid. [2]

Diabetes and pain are common afflictions affecting people throughout the world. Towards mitigating these two afflictions, we had been experimenting with various medicinal plants of Bangladesh towards providing relief from these two afflictions through commonly available and affordable means in the form of the plants themselves or their crude extracts. [3-22] Broccoli is readily available in Bangladesh and taken in the boiled or cooked (which also involves boiling) form. Thus it was of interest to determine whether boiled broccoli sprouts also have the ability to reduce blood glucose and alleviate pain, for in that case, simple consumption of broccoli can be a viable alternative to more costly antidiabetic or pain relieving drugs and which drugs are mostly non-affordable or non-available to the less affluent population of the country.

**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*

The sprouts were cut down into suitable pieces and boiled using hot steam for twenty minutes. Sprouts were next 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 12g).
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) \[23\] 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 methanolic boiled 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. \[24\] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = (1 − We/Wc) X 100,
where We and Wc 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.\[25\] 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 \[^{[20]}\], 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. \[^{[11]}\]

RESULTS

Antihyperglycemic activity evaluation results

Administration of methanol extract of boiled 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 21.8, 37.6, 39.6, and 44.0%, 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 51.0%. The results are shown in Table 1 and suggest that boiled broccoli sprouts maintain their blood glucose reducing ability even after boiling.

Analgesic activity evaluation results

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 22.2, 33.3, 44.4, 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. Thus even at a lower dose of 200 mg per kg, methanol extract of boiled broccoli sprouts demonstrated better analgesic activity than 200 mg per kg aspirin. The results are shown in Table 2 and demonstrate that like the observed antihyperglycemic activity, boiled broccoli sprouts retain their analgesic activity following boiling.
Table 1: Effect of crude methanol extract of boiled 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>5.96 ± 0.49</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide (MEBS)</td>
<td>10 mg</td>
<td>2.92 ± 0.29</td>
<td>51.0*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>50 mg</td>
<td>4.66 ± 0.61</td>
<td>21.8*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>100 mg</td>
<td>3.72 ± 0.60</td>
<td>37.6*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>200 mg</td>
<td>3.60 ± 0.66</td>
<td>39.6*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>400 mg</td>
<td>3.34 ± 0.41</td>
<td>44.0*</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 boiled 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>4.2 ± 0.37</td>
<td>22.2*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>100 mg</td>
<td>3.6 ± 0.40</td>
<td>33.3*</td>
</tr>
<tr>
<td>(MEBS)</td>
<td>200 mg</td>
<td>3.0 ± 0.45</td>
<td>44.4*</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

Our observation of reduction of blood glucose levels and alleviation of pain by methanolic extract of boiled broccoli sprouts suggests that broccoli in the cooked form may prove to be a useful alternative to antihyperglycemic and analgesic drugs. If so, this would be beneficial to millions of diabetes patients and patients with impaired glucose metabolism, as well as people suffering from chronic or acute pain arising from simple causes like cuts and sprains to more complicated causes like cancer or rheumatoid arthritis.

It is to be noted in this regard that broccoli has been reported to contain sulforaphane, a compound shown to have antidiabetic potential in a number of studies.[27, 28] Hydrolysis of glucoraphanin, which is also found in broccoli sprouts, produces sulforaphane.[1] Broccoli
sprouts also contain kaempferol, a compound known for its antihyperglycemic and analgesic effects.\[29-32\] Thus these two compounds may be responsible for the observed two effects.

CONCLUSION
The results suggest that methanolic extract of boiled 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


