A PRELIMINARY STUDY ON SIGNIFICANT ANTIHYPERGLYCEMIC ACTIVITY AS DETERMINED THROUGH ORAL GLUCOSE TOLERANCE TESTS OF THREE COMMON PLANTS BELONGING TO THE BRASSICACEAE FAMILY

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ABSTRACT

Background. Evaluation of antihyperglycemic properties through oral glucose tolerance tests in glucose loaded mice (Swiss albino) using three available plants from Brassicaceae family that are used as vegetables in Bangladesh as well as most parts of the world. Methods. Different doses of the extracts were orally administered to glucose loaded mice to investigate antihyperglycemic activity. Results. Methanolic extract of all the plants tested showed significant antihyperglycemic activity. The more interesting part of this work is that antihyperglycemic activity of these extracts was retained at a significant level even after they were boiled. Conclusion. Our investigation strongly suggests that both boiled and non-boiled form of these vegetables can be potential replacement of antidiabetic drugs for diabetic patients to control blood glucose level.

KEYWORDS: Diabetes, antihyperglycemic, oral glucose tolerance test, Brassicaceae.

INTRODUCTION

Diabetes is a group of metabolic disorders where regulation of blood glucose level fails as a result of insufficient production and secretion of insulin from the body due to an autoimmune
destruction of pancreatic β-cells (type 1 diabetes).[11] Besides, a combination of impaired insulin resistance and insulin secretion (type 2 diabetes) also play pivotal role behind the progression of this disease.[2-4] Both diabetic conditions are characterized by high blood sugar level, unusual thirst, frequent urination, extreme hunger and loss of weight, blurred vision, nausea and vomiting, extreme weakness and tiredness, irritability, mood changes, etc.[5-6] According to the estimates of the World Health Organization (WHO), 347 million people worldwide have diabetes, and an estimated 3.4 million people died from the complications of high blood sugar in 2004, and that diabetes deaths would double between 2005 to 2030.[7] Multiple subcutaneous injections of insulin and regular monitoring of blood glucose levels are essential for type 1 diabetes patients and some type 2 diabetes patients.[8] However, such self-administration is uncomfortable and painful, and requires substantial patient compliance. More importantly, conventional treatment, where glucose sensing and drug therapy are not directly coupled, known as open-loop insulin delivery, does not tightly regulate glucose levels in patients.[9, 10] Lack of tight control of blood glucose levels further develop a group of heterogeneous diseases including limb amputation, blindness, retinopathy, neuropathy, kidney failure, and fatal hypoglycemia.[11-18] Therefore, multiple therapeutic approaches have to be considered for the diabetic patients. Insulin injections are administered to patients having lack of insulin where as in case of those where cells do not respond to insulin, many different drugs are prescribed. Although several therapies are in use for treatment to manage hyperglycemia, certain limitations like development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc have created a barrier for obtaining better therapeutic responses.[19-20]

Medicinal plants are being looked up once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose-lowering agent. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy.[21] Though a large number of medicinal plants are reported to have hypoglycemic activity, only one report has been published to show the antihyperglycemic activity of Brassica oleracea (var capitata) from the Brassicaceae family.[22] This is the first time we have evaluated antihyperglycemic activity of three vegetables of different varieties belonging to this family namely Brassica oleracea L. var botrytis (cauliflower), Brassica oleracea L. var capitata
Cauliflower is known to contain $\alpha$-amyrin, $\beta$-amyrin, $\beta$-sitosterol, ferulic acid, and quercetin.$^{[23]}$ These components have been reported to give antihyperglycemic effects.$^{[24-35]}$ Cabbage is known to contain $\beta$-sitosterol, chlorogenic acid, ferulic acid, kaempferol, and quercetin.$^{[23]}$ Chlorogenic acid reportedly inhibited key enzymes linked to type 2 diabetes in vitro.$^{[36]}$ Kaempferol has been identified as one of the antidiabetic components in Cassia alata leaves.$^{[37]}$ Kaempferol and quercetin have been reported in kohlrabi.$^{[23]}$ Thus taken together, the three experimental Brassicaceae family plants contain a number of compounds, which have been variously reported to give antidiabetic or blood sugar lowering effects. Thus it was of interest to determine whether antihyperglycemic activity was present in plant part extracts, more so because of the presence of a number of compounds with reported antihyperglycemic effects. Since the plants or plant parts are edible and consumed after cooking (which necessitates boiling), it was also of interest to evaluate the antihyperglycemic properties of the plant parts both before and after boiling, because then cooked and edible plant parts can become a convenient mean to alleviate high blood glucose levels.

**METHODS**

**Materials**

Methanol and DMSO were bought from Merck (Germany), glucose and glibenclamide were from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

**Collection of plants**

Brassica oleracea L. var botrytis (cauliflower), Brassica oleracea L. var capitata (cabbage) and Brassica oleracea L. var gongyloides (kohlrabi) were bought from a local market in Dhaka, Bangladesh. The plants were identified at the Bangladesh National Herbarium, with Accession Numbers of 39514, 39513, and 38099 for cauliflower, cabbage, and kohlrabi, respectively. The edible parts of the plants, i.e. inflorescence of botrytis, leaf of capitata and swollen stem of gongyloides were cut into small pieces, subjected to boiling or kept non-boiled, and dried in the shade with proper protection from contamination from environmental pollutants and dust. All collected parts were converted into powder following grinding and subjected to extraction with methanol.

**Preparation of the extract**

Collected fresh vegetables were separated into two groups- one for boiling and the other for non-boiled. The vegetables were cut down into suitable pieces and boiled using hot steam for
twenty minutes. After completion of boiling it was allowed to cool down at room temperature and finally dried in the shade. To prepare the extract of *Brassica oleracea* L. var *botrytis*, finely ground 100 gm boiled and non-boiled powder was independently dissolved in 500 ml of methanol and allowed to soak for 48 hours. After completion of soaking, methanol was evaporated at 40°C using rotary evaporator. Following the same procedure, extract of the other two vegetables were prepared. All prepared extracts were preserved for further investigation and characterization.

**Animals**
Swiss albino mice, which weighed between 18-22g, 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 in the laboratory 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 test**
Following our previous study, glucose tolerance activity was carried out. For each extract, the mice were divided into six groups where each group contained 5 mice. Among all groups, one was for control, one for standard and remain four were for different doses of the extract. Glibenclamide (10 mg/kg of body weight) was administered as standard and 50, 100, 200 and 400 mg/kg body weight of individual extract was orally administered to mice 1 hour prior to administration of glucose (2g/kg of body weight). After glucose administration to all groups, all mice were allowed to rest for another two hours. Finally all mice were sacrificed and blood was collected to evaluate blood glucose level through puncturing heart and following glucose oxidase method, glucose levels in blood were computed. The results were expressed as mean ± SEM. The percent lowering of blood glucose levels were calculated according to the formula described below.

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

where $W_e$ and $W_c$ represents the blood glucose concentration in glibenclamide or extract administered mice, and control mice (Group 1), respectively.

**RESULTS**

**Product yield**
Yield of the product from each extract of boiled plant part is presented in Table 1. It can be seen that extract of boiled exhibits better yield than non-boiled (Table 2).
**Oral glucose tolerance test**

The blood glucose level of normal mice was 5.94 while it was 2.52 in case of glibenclamide, which was administered as standard. After administering methanolic extract (boiled) of inflorescence of *botrytis* variety of *Brassica oleracea* (MECF), the blood glucose levels were 4.74 ± 0.31, 3.86 ± 0.36, 2.46 ± 0.22 and 2.2 ± 0.2, respectively at doses of 50, 100, 200 and 400 mg/kg body weight respectively. On the other hand after administration of non-boiled methanol extract at the same doses, blood glucose levels were 4.42 ± 0.22, 3.6 ± 0.25, 3.02 ± 0.23, and 2.56 ± 0.21 (Table 3). Blood glucose levels were 4.66 ± 0.28, 3.84 ± 0.25, 3.36 ± 0.32, and 2.9 ± 0.15 for boiled leaf extract of *capitata* variety (MECA) while these were 4.4 ± 0.12, 3.1 ± 0.41, 3.5 ± 0.33, and 2.74 ± 0.26 for non-boiled extract (Table 4). On the other hand, after administration of swollen stem of boiled extract of *gongyloides* variety (MEKR), blood glucose levels were 3.98 ± 0.31, 3.96 ± 0.43, 3.36 ± 0.21, and 2.82 ± 0.26 and 5.02 ± 0.2, 4.04 ± 0.23, 3.64 ± 0.23, and 2.86 ± 0.24 for non-boiled extract (Table 5).

**Table 1: Yield of the extract of different varieties of *Brassica oleracea* (boiled).**

<table>
<thead>
<tr>
<th>Name of Variety</th>
<th>Used Part</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrytis</td>
<td>Inflorescence</td>
<td>15.0</td>
</tr>
<tr>
<td>Capitata</td>
<td>Leaf</td>
<td>15.851</td>
</tr>
<tr>
<td>Gongyloides</td>
<td>Swollen stem</td>
<td>9.938</td>
</tr>
</tbody>
</table>

**Table 2: Yield of the extract of different varieties of *Brassica oleracea* (non-boiled).**

<table>
<thead>
<tr>
<th>Name of Variety</th>
<th>Used Part</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrytis</td>
<td>Inflorescence</td>
<td>4.4</td>
</tr>
<tr>
<td>Capitata</td>
<td>Leaf</td>
<td>5.494</td>
</tr>
<tr>
<td>Gongyloides</td>
<td>Swollen stem</td>
<td>5.376</td>
</tr>
</tbody>
</table>

**Table 3: Effect of crude methanol extract of cauliflower (MECF) 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>5.69 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.76 ± 0.10</td>
<td>51.5*</td>
</tr>
<tr>
<td>(MECF) boiled</td>
<td>50 mg</td>
<td>4.74 ± 0.31</td>
<td>16.7*</td>
</tr>
<tr>
<td>(MECF) boiled</td>
<td>100 mg</td>
<td>3.86 ± 0.33</td>
<td>32.2*</td>
</tr>
<tr>
<td>(MECF) boiled</td>
<td>200 mg</td>
<td>2.46 ± 0.22</td>
<td>56.8*</td>
</tr>
<tr>
<td>(MECF) boiled</td>
<td>400 mg</td>
<td>2.20 ± 0.09</td>
<td>61.3*</td>
</tr>
<tr>
<td>(MECF) non-boiled</td>
<td>50 mg</td>
<td>4.42 ± 0.12</td>
<td>22.3*</td>
</tr>
<tr>
<td>(MECF) non-boiled</td>
<td>100 mg</td>
<td>3.60 ± 0.25</td>
<td>36.7*</td>
</tr>
<tr>
<td>(MECF) non-boiled</td>
<td>200 mg</td>
<td>3.02 ± 0.23</td>
<td>46.9*</td>
</tr>
<tr>
<td>(MECF) non-boiled</td>
<td>400 mg</td>
<td>2.56 ± 0.21</td>
<td>55.0*</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 4: Effect of crude methanol extract of cabbage (MECA) 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>5.69 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.76 ± 0.10</td>
<td>51.5*</td>
</tr>
<tr>
<td>(MECA) boiled</td>
<td>50 mg</td>
<td>4.66 ± 0.28</td>
<td>18.1*</td>
</tr>
<tr>
<td>(MECA) boiled</td>
<td>100 mg</td>
<td>3.84 ± 0.25</td>
<td>32.5*</td>
</tr>
<tr>
<td>(MECA) boiled</td>
<td>200 mg</td>
<td>3.36 ± 0.32</td>
<td>40.9*</td>
</tr>
<tr>
<td>(MECA) boiled</td>
<td>400 mg</td>
<td>2.90 ± 0.15</td>
<td>49.0*</td>
</tr>
<tr>
<td>(MECA) non-boiled</td>
<td>50 mg</td>
<td>4.40 ± 0.12</td>
<td>22.7*</td>
</tr>
<tr>
<td>(MECA) non-boiled</td>
<td>100 mg</td>
<td>4.10 ± 0.41</td>
<td>27.9*</td>
</tr>
<tr>
<td>(MECA) non-boiled</td>
<td>200 mg</td>
<td>3.50 ± 0.33</td>
<td>38.5*</td>
</tr>
<tr>
<td>(MECA) non-boiled</td>
<td>400 mg</td>
<td>2.74 ± 0.26</td>
<td>51.8*</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.

Table 5: Effect of crude methanol extract of kohlrabi (MEKR) 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>5.69 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.76 ± 0.10</td>
<td>51.5*</td>
</tr>
<tr>
<td>(MEKR) boiled</td>
<td>50 mg</td>
<td>3.98 ± 0.31</td>
<td>30.1*</td>
</tr>
<tr>
<td>(MEKR) boiled</td>
<td>100 mg</td>
<td>3.96 ± 0.43</td>
<td>30.4*</td>
</tr>
<tr>
<td>(MEKR) boiled</td>
<td>200 mg</td>
<td>3.36 ± 0.21</td>
<td>40.9*</td>
</tr>
<tr>
<td>(MEKR) boiled</td>
<td>400 mg</td>
<td>2.82 ± 0.26</td>
<td>50.4*</td>
</tr>
<tr>
<td>(MEKR) non-boiled</td>
<td>50 mg</td>
<td>5.02 ± 0.20</td>
<td>11.8*</td>
</tr>
<tr>
<td>(MEKR) non-boiled</td>
<td>100 mg</td>
<td>4.04 ± 0.23</td>
<td>29.0*</td>
</tr>
<tr>
<td>(MEKR) non-boiled</td>
<td>200 mg</td>
<td>3.64 ± 0.23</td>
<td>36.0*</td>
</tr>
<tr>
<td>(MEKR) non-boiled</td>
<td>400 mg</td>
<td>2.86 ± 0.24</td>
<td>49.7*</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

Though a large number of medicinal plants have been reported to control or reduce blood glucose levels, antihyperglycemic activity of vegetables belonging to Brassicaceae family are yet to be well documented. Only one group has recently reported the hypoglycemic activity
of one variety of *Brassica oleracea* using only non-boiled extract.\(^{[22]}\) But we have found that the boiled extract also possessed significant antihyperglycemic activity among these three experimented *Brassica oleracea* varieties, which to our knowledge, is the first time of such activity reported in the three plant parts used in the present study.

This present study reports the antihyperglycemic effect of different varieties of *Brassica oleracea* even after boiling compared to non-boiled. Table 1 shows that yield of the product using same amount of initial materials were always higher from the boiled extract, which might be due to breakdown of complex constituents, which following breakdown were more soluble in methanol.

From the conducted oral glucose tolerance activity experiment, it is clearly seen that depending on doses, methanol extract of all varieties exhibited significant glucose lowering activity. At low doses (50 and 100 mg/kg of body weight), boiled extract of *botrytis* species did not lower blood glucose level to a higher extent compared to non-boiled extract, whereas at high doses (200 and 400 mg/kg of body weight) the boiled extract exhibited more significant glucose lowering level, which was almost similar to a standard antihyperglycemic drug, glibenclamide (Table 3). This may be due to higher amount of phytochemicals in this extract possessing antihyperglycemic activity. Earlier we mentioned that yield of the boiled extract was almost three times higher than the boiled extract but during glucose tolerance test the dose of the extract was same. Therefore, compared to same doses, the boiled extract contained more antihyperglycemic phytochemical(s) than non-boiled.

Table 4 displays that with increasing concentration, methanolic extract of *capitata* (cabbage) exhibited higher glucose lowering activity. Similar results were also obtained with *gongyloides* variety (Table 5). All of the experimental varieties contain quercetin which is well documented to have hypoglycemic activity.\(^{[39]}\) Besides, the variety *capitata* contains δ-sitosterol, ferulic acid, kaempferol and chlorogenic-acid, *botrytis* contains kaempferol. The boiling point and melting point of these antihyperglycemic phytochemicals are high. Therefore after boiling at experimental temperature, structures of these phytochemicals should be stable and as such retain the hypoglycemic activity. According to one study, quercetin exhibited hypoglycemic effect probably by increasing insulin secretion.\(^{[40]}\) It has been concluded that kaempferol and quercetin potentially act at multiple targets to ameliorate hyperglycemia, including by acting as partial agonists of glitazone receptor.\(^{[41]}\) As kaempferol is also one of the major phytochemicals in our investigated varieties, this can
potentially give the observed antihyperglycemic affects. It is interesting to note that among all varieties, *botrytis* exhibited better glucose lowering activity which may be due to containing more amount of active phytochemicals possessing antihyperglycemic properties. On the other hand as this variety contains both quercetin and kaempferol, a synergistic effect may have played a major role to reduce blood glucose level.

Low cost and readily affordable substitutes for antidiabetic drugs (including insulin injections) are a sore necessity for controlling high blood glucose levels in diabetic patients (particularly patients who are poor and/or live in remote areas). Naturally available plants including vegetables and spices can fulfill this role if they display blood glucose lowering activity. As such, we had been experimenting with various plants and plant parts (both wild as well as cultivated) for their blood glucose lowering effects.\(^{[42-66]}\) Antidiabetic activity of plant parts, if they can be consumed directly or following cooking (that is, where boiling takes place) can lead to further ease of partaking antidiabetic plants, and so we had also been experimenting with antidiabetic activity of boiled plants or plant parts. This is the first report of its kind of antihyperglycemic activity in both boiled and non-boiled plant parts of the three Brassicaceae family plants tested. Although the exact compound(s) responsible for the anitihyperglycemic effects have not been tested, a number of compounds reported to be present in these plants may be responsible for the effects. Further work is undergoing in our laboratory to identify the compound(s) responsible for the antihyperglycemic effects.

**CONCLUSION**

Our study clearly demonstrated antihyperglycemic potential of different varieties of *Brassica oleracea*. Therefore, these vegetables can play a role to control blood glucose level of diabetic patients. Moreover, since boiling did not destroy antihyperglycemic effects, the vegetables can be consumed in both raw (as in the form of salad) as well as boiled form.

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

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Authors’ contributions
MA, EI, MEH, PRD, MTI, AAN, SR collected the plant materials, did the extraction, and performed the experiments under the supervision of RJ and MR. MR, AAN and RJ wrote the manuscript draft, which was read and edited by all authors. All authors read and approved the final version of the manuscript.

REFERENCES


