ANTIHYPERTERMIC AND ANALGESIC ACTIVITY
EVALUATION OF A COMBINATION OF BRASSICA ALBA,
CORIANDRUM SATIVUM AND FOENICULUM VULGARE SEEDS

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ABSTRACT

Background: We have previously evaluated the antihyperglycemic potential of seeds of Brassica alba and Foeniculum vulgare separately. It was of interest to determine whether a combination of the two plant seeds along with seeds of Coriandrum sativum demonstrates antihyperglycemic and analgesic effects and so can be useful in lowering blood glucose and pain. Methods: Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT) in mice. Analgesic activity was determined through intraperitoneal acetic acid induced pain model in mice. Results: Administration of a combination of the three seed extracts (1:1:1, w/w) at doses of 100, 200 and 400 mg/kg led to, respectively, 1.0, 28.5 and 49.7% reductions in blood glucose levels. 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 demonstrate that a formulation containing extracts from seeds of the plants can give blood glucose lowering effects, which at the highest dose tested of the combination, is equivalent to that of glibenclamide. At the afore-mentioned three
doses, the combination lowered acetic acid induced abdominal constrictions (writhings) in mice, respectively, by 21.4, 39.3 and 46.4%. In comparison, a standard analgesic drug, aspirin, when administered at a dose of 200 mg per kg body weight, lowered the number of writhings by 42.9%, demonstrating that the polyherbal extract was more effective in reducing pain than aspirin. **Conclusion:** A polyherbal formulation containing extracts of Brassica alba, Coriandrum sativum and Foeniculum vulgare seeds is effective in lowering blood glucose levels and alleviating pain.

**KEYWORDS:** Antihyperglycemic, analgesic, Brassica alba, Coriandrum sativum, Foeniculum vulgare.

**BACKGROUND**

Brassica alba Rabenh., is an annual plant belonging to the Brassicaceae family and cultivated in Bangladesh for culinary uses of its seeds, and is known as ‘white mustard’ in English and ‘shada shorisha’ in Bengali. Coriandrum sativum L. (Umbelliferae) is also widely cultivated in Bangladesh and its seeds are used as a spice in various dishes as well as a digestive. The plant is known as ‘coriander’ in English and ‘dhonia’ in Bengali. Foeniculum vulgare Mill. (Umbelliferae), known in English as ‘sweet fennel’ and in Bengali as ‘mouri’ is cultivated in Bangladesh for its seeds which are used for both culinary and medicinal purposes as well as to remove mouth odor.

Ethnomedicinal use reports are available for Coriandrum sativum. The people of Bellary district, Karnataka, India use the plant for treatment of asthma.[1] In experimental studies, a combination of methanol extract of Cuminum cyminum and Coriandrum sativum seeds have been shown to lower blood glucose and alleviate pain in mice.[2] Seed extract has been shown to produce hypoglycemic effect in Type 2 (NIDDM) diabetic patients.[3] Methanol extract of leaves has been reported to show antidiabetic activity in alloxan-induced diabetic rats.[4] Antidiabetic activity of fruits (seeds) has also been noted in streptozotocin-induced diabetic rats.[5]

Methanolic extract of Brassica alba seeds reportedly showed antihyperglycemic and pain alleviating activity in mice models.[6] Antihyperglycemic and analgesic activities have also been noted with Foeniculum vulgare seeds methanolic extract.[7] Antidiabetic activity of aqueous extract of the plant has been seen in streptozotocin-induced diabetic rats.[8] Ethnomedicinally, Foeniculum vulgare seed is used to treat indigestion and flatulence by the
people of North Bengal plain, India. The tribals of Sriharikota Island, Andhra Pradesh, India use seed paste applied to scalp for cooling effect on body.

Diabetes and pain are common afflictions suffered on a daily basis by millions of people throughout the world. Treatment of diabetes is difficult for particularly rural people of Bangladesh who lack access to modern health-care facilities. Over-dosage of common pain killing drugs like aspirin or paracetamol can lead to gastric ulceration and liver toxicity, respectively. As such, we had been experimenting with common medicinal plants (including spices) of Bangladesh in both monoherbal and polyherbal formulations to produce a low-cost and available treatment of these two disorders and which can avoid the side effects of allopathic drugs. The objective of the present study was to evaluate the antihyperglycemic and analgesic activity of a methanol extract of a combination of Brassica alba, Coriandrum sativum, and Foeniculum vulgare seeds.

METHODS

Plant material collection

Seeds of all three plants were collected during May, 2015 from Dhaka district, Bangladesh.

Preparation of methanolic extract of aerial parts

The seeds were separately thoroughly dried in the shade and 100g of dried and powdered seeds of each plant were extracted separately with methanol (w:v ratio of 1:5, final weight of the extract of the seeds of Brassica alba, Coriandrum sativum, and Foeniculum vulgare being 6.489, 8.306, and 8.567g, respectively.. The extracts were stored at -20°C till use. Before use, the extracts were combined in a 1:1:1 (w/w) ratio to form a polyherbal formulation tentatively named as MEBCF (methanolic extract of Brassica alba, Coriandrum sativum and Foeniculum vulgare seeds).

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 15-19g 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)[38] with minor modifications. Briefly, fasted mice were grouped into five 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 3-5 received MEBCF at doses of 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.[39] The percent lowering of blood glucose levels were calculated according to the formula described below.

\[
\text{Percent lowering of blood glucose level} = (1 - \frac{W_e}{W_c}) \times 100, \\
\text{Where } W_e \text{ and } W_c \text{ represents the blood glucose concentration in glibenclamide or MEBCF administered mice (Groups 2-5) and control mice (Group 1), respectively.}
\]

**Analgesic activity evaluation through abdominal writhing test**

Analgesic activity of MEBCF was examined as previously described.[40] Mice were divided into five groups of five mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered the standard analgesic drug aspirin at a dose of 200 mg per kg body weight, respectively. Groups 3-5 were administered MEBCF at doses of 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MEBCF, 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[41], 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 MEBCF 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.\(^{[21]}\)

**RESULTS**

**Antihyperglycemic activity evaluation results**
Administration of a combination of the three seed extracts (1:1:1, w/w) or MEBCF at doses of 100, 200 and 400 mg/kg led to, respectively, 1.0, 28.5, and 49.7% dose-dependent reductions in blood glucose levels. The result at the lowest dose of MEBCF was not statistically significant but the two highest doses were. 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 demonstrate that the formulation, MEBCF containing extracts from seeds of the plants can give blood glucose lowering effects, which at the highest dose tested of the combination, is equivalent to that of glibenclamide.

**Analgesic activity evaluation results**
In analgesic activity tests, the extract at doses of 100, 200 and 400 mg per kg body weight dose-dependently reduced the number of abdominal constrictions by 21.4, 39.3 and 46.4%, respectively. The results at the lowest dose of MEBCF was not statistically significant but the the higher two doses gave statistically significant results compared to control mice. A standard pain relieving (analgesic) drug, aspirin, reduced the number of writhings by 42.9% when administered at a dose of 200 mg per kg body weight. The results are shown in Table 2 and demonstrate that MEBCF at the highest dose had better pain alleviating effect than that of aspirin.

**Table: 1 Effect of MEBCF 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</td>
<td>10 mg</td>
<td>2.92 ± 0.29</td>
<td>51.0*</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 MEBCF 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>5.6 ± 0.51</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>3.2 ± 0.58</td>
<td>42.9*</td>
</tr>
<tr>
<td>(MEBCF)</td>
<td>100 mg</td>
<td>4.4 ± 0.60</td>
<td>21.4</td>
</tr>
<tr>
<td>(MEBCF)</td>
<td>200 mg</td>
<td>3.4 ± 0.68</td>
<td>39.3*</td>
</tr>
<tr>
<td>(MEBCF)</td>
<td>400 mg</td>
<td>3.0 ± 0.32</td>
<td>46.4*</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.

DISCUSSION

The seeds of Foeniculum vulgare are in use in traditional medicines of Portugal and Sudan for treatment of diabetes. Our experimental results validate the traditional uses and also are in agreement with previous reports of the antihyperglycemic and analgesic activities of the plant seeds. Brassica alba seeds have also been previously shown to give antihyperglycemic and analgesic activity in mice. In separate experiments (data not shown), seed extracts of Brassica alba, Coriandrum sativum and Foeniculum vulgare were seen in the present study to lower blood glucose levels, respectively, by 20.8, 19.8 and 27.9%, when used singly at a dose of 200 mg per kg body weight. Thus the combination proved to be more effective in lowering blood glucose levels for at a combined extract dose of 200 mg per kg body weight, blood glucose levels in glucose-loaded mice were lowered by 28.5%.

MEBCF also proved effective in relieving pain. It is to be noted that all three plant seeds are used as spices in various culinary dishes of Bangladesh and are cultivated and so readily available. Thus they can form an affordable and readily available substitute for lowering blood glucose levels in hyperglycemia and for lowering pain without the necessity of visiting allopathic doctors, which may not be convenient to the rural people of remote areas of Bangladesh.
CONCLUSION
The results suggest that the methanolic extract of seeds of Brassica alba, Coriandrum sativum, and Foeniculum vulgare used in combination (MEBCF) 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


