ANTIHYPERTYLEMIC, ANALGESIC ACTIVITY, AND ACUTE TOXICITY STUDIES WITH METHANOL EXTRACT OF FOeniculum vulgare SEEDS

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ABSTRACT

Background. Foeniculum vulgare, also known as sweet fennel is an aromatic 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 50, 100, 200 and 400 mg per kg body weight, the extract dose-dependently reduced blood glucose levels by 25.0, 42.4, 47.6, and 52.1%, 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 52.4%. In analgesic activity tests, the extract at doses of 50, 100, 200 and 400 mg per kg body weight significantly reduced the number of abdominal constrictions by 29.6, 37.0, 40.7, 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, Foeniculum vulgare, analgesic, Apiaceae.
BACKGROUND

*Foeniculum vulgare* Mill. (Apiaceae), known in English as ‘sweet fennel’ and in Bengali as ‘mouri’ is an aromatic plant commonly grown in Bangladesh primarily for its seeds which are used for both culinary and medicinal purposes. The plant belongs to the carrot family of plants. Antioxidant properties have been reported for various parts of the plant.[1,2] Antidiabetic antihyperlipidemic and hepatoprotective effect has been reported for a polyherbal formulation containing the plant.[3] Antiinflammatory, analgesic and antioxidant activities have been reported for fruits of the plant.[4] Seeds of the plant have been found to be effective in relieving pain during dysmenorrheal.[5] The fruits and their constituents have been shown to inhibit 5-lipoxygenase activity.[6]

The plant is used to treat diabetes in traditional medicines of Portugal and Sudan.[1,2] Some other reported ethnomedicinal uses of the plant include use of leaves for skin diseases by the Yanadi tribals of Sriharikota Island, Andhra Pradesh, India.[7] use of fruits for indigestion and flatulence by tribals of North Bengal plains, India.[8] and use for leaves for nosebleeds by the people of Bale Mountains National Park, Southeastern Ethiopia.[9] We had been screening various plants (including plant parts) of Bangladesh for their antihyperglycemic and analgesic potential.[10-21] Diabetes and pain are common problems in Bangladesh and indeed throughout the world, and a readily affordable and available means of treatment with plants can save the rural population of Bangladesh, who lack access to modern treatment facilities or who cannot afford allopathic treatment from much financial costs. The objective of the present study was to evaluate the antihyperglycemic and analgesic potential of methanol extract of *Foeniculum vulgare* seeds through oral glucose tolerance tests and acetic acid induced intraperitoneal pain methods, respectively, in mice.

METHODS

**Plant material collection**

Seeds of *F. vulgare* 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 3g).
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 14-18g 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 (MEFV) 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 – \(W_e/W_c\)) X 100,
where \(W_e\) and \(W_c\) represents the blood glucose concentration in glibenclamide or MEFV administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic activity evaluation through abdominal writhing test
Analgesic activity of MEFV was examined as previously described.[24] 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 MEFV 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 MEFV, 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.\textsuperscript{[25]} 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.

Percent inhibition = \( \frac{1 - \text{W}_e/\text{W}_c}{} \times 100 \),

where \( \text{W}_e \) and \( \text{W}_c \) represents the number of writhings in aspirin or MEFV administered mice (Groups 2-7), and control mice (Group 1), respectively.

**Acute toxicity test**

Acute toxicity test was conducted as previously described.\textsuperscript{[26]} 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 MEFV 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.\textsuperscript{[17]}

**RESULTS**

**Toxicity evaluation**

The crude extract (MEFV) 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), MEFV at doses of 50, 100, 200 and 400 mg/kg caused, respectively, 25.0, 42.4, 47.6, and 52.1% reductions in blood glucose levels. The results were both dose-dependent and statistically significant. Glibenclamide (a standard antihyperglycemic drug), when administered at a dose of 10 mg/kg lowered blood glucose by 52.4%, which was equivalent with the highest dose of the extract. The results suggest that the
extract at the highest dose tested possess strong antihyperglycemic activity. The results are shown in Table 1.

**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 MEFV. At doses of 50, 100, 200 and 400 mg per kg body weight, MEFV was observed to reduce the number of constrictions, respectively, by 29.6, 37.0, 40.7, and 44.4%. 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 100 mg/kg MEFV showed higher analgesic activity than 200 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 *F. vulgare* seeds (MEFV) 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>5.76 ± 0.34</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.74 ± 0.25</td>
<td>52.4*</td>
</tr>
<tr>
<td>(MEFV)</td>
<td>50 mg</td>
<td>4.32 ± 0.29</td>
<td>25.0*</td>
</tr>
<tr>
<td>(MEFV)</td>
<td>100 mg</td>
<td>3.32 ± 0.49</td>
<td>42.4*</td>
</tr>
<tr>
<td>(MEFV)</td>
<td>200 mg</td>
<td>3.02 ± 0.58</td>
<td>47.6*</td>
</tr>
<tr>
<td>(MEFV)</td>
<td>400 mg</td>
<td>2.76 ± 0.21</td>
<td>52.1*</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 *F. vulgare* seeds (MEFV) 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>(MEFV)</td>
<td>50 mg</td>
<td>3.8 ± 0.49</td>
<td>29.6*</td>
</tr>
<tr>
<td>(MEFV)</td>
<td>100 mg</td>
<td>3.4 ± 0.40</td>
<td>37.0*</td>
</tr>
<tr>
<td>(MEFV)</td>
<td>200 mg</td>
<td>3.2 ± 0.49</td>
<td>40.7*</td>
</tr>
<tr>
<td>(MEFV)</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.

DISCUSSION
The seeds of *F. vulgare* are in use in traditional medicines of Portugal and Sudan for treatment of diabetes.\(^{1,2}\) Our experimental results validate the traditional uses and suggest that necessary research needs to be conducted on isolation and identification of relevant antihyperglycemic phytochemicals from the seeds as well as elucidating their mechanism of action.

Acetic acid induced pain can be due to increased production of prostaglandins and prostacyclins mediated by cyclooxygenases and lipoxygenases.\(^{[24]}\) Thus any phytochemical constituent(s) present in the extract which can inhibit prostaglandin and prostacyclin production through inhibition of cyclooxygenases and lipoxygenases will be able to alleviate such pain. Interestingly, ethanol extract of fruits of the plant has been shown to inhibit 5-lipoxygenase, which has been attributed to presence in the extract of several terpene derivatives including γ-terpinene and fenchone as well as the phenylpropanoid, trans-anethole.\(^{[6]}\) Thus these phytochemicals may account for the observed analgesic activity, if also present in the seeds. Currently, work is being undertaken in our laboratory to isolate the responsible phytochemicals in *F. vulgare* for analgesic effects and identify them.

CONCLUSION
The results suggest that methanolic extract of *F. vulgare* seeds can be used for lowering blood glucose and for alleviating pain. Since the seeds are widely available in Bangladesh, they may be used by the rural low income people who are suffering from diabetes or chronic pain for which they cannot afford or lack access to modern medicines. Moreover, the seeds present a possibility for discovery of new antihyperglycemic and analgesic drugs.

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

REFERENCES


