ABSTRACT

Background: Leucas aspera and Zingiber officinale are plant species commonly available in Bangladesh. Aerial parts of the first plant is consumed by the local people following cooking for alleviating pain, while the rhizomes of the second are both consumed and applied topically for blood glucose lowering and pain alleviating effects, respectively. It was thus of interest to evaluate the antihyperglycemic and antinociceptive potentials of a combination of Leucas aspera aerial parts and Zingiber officinale rhizomes. Methods: Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT) in mice. Antinociceptive activity was determined through reductions in number of writhings caused by intraperitoneally injected acetic acid-induced abdominal pain in mice. Results: Administration of methanol extract of a 1:1 (w/w) combination of aerial parts of Leucas aspera and rhizomes of Zingiber officinale led to significant and dose dependent reductions of blood glucose in mice in OGTT. At the highest dose of the combination (400 mg each of the two extracts per kg body weight) blood glucose was decreased by 54.0% versus control mice. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg, decreased blood glucose by 61.2%. In antinociceptive activity tests, the combination of the two extracts at the highest dose tested (400 mg each extract per kg) reduced abdominal writhings by 65.4%. A standard antinociceptive drug, aspirin reduced abdominal writhings by 50.0%, when administered at a dose of 400 mg per kg. Conclusion: The two extract combination demonstrated excellent antihyperglycemic and antinociceptive properties.
KEYWORDS: Antihyperglycemic, Leucas aspera, Zingiber officinale, antinociceptive, OGTT

BACKGROUND

Diabetes mellitus is characterized by elevated blood glucose levels. The disease cannot be cured totally and progresses with time to more complications like cardiovascular, eye, and kidney disorders. Existing allopathic drugs like metformin or glibenclamide (both administered orally), as well as insulin injections can lower blood glucose but are costly and not properly available to the rural people of Bangladesh. The disease is reaching endemic proportions in Bangladesh and the average cost for treatment of merely diabetes without added complications is beyond the capability of most people.[1,2]

Pain is also a common feature of most Bangladesh people because of the hard labor involved in agricultural work, construction work or work like manually pulling rickshaws or vans with heavy loads. Acute pain also arises from other factors like sprains or even minor injuries. In addition, diseases like cancer, Chikungunya or arthritis are common in Bangladesh and these diseases can cause chronic pain.[3,5] Pain is usually treated with drugs like aspirin or paracetamol; chronic and severe pain may need opioid drugs for treatment.

Metformin is contraindicated in renal diseases or renal dysfunction; the adverse effects of metformin with other antidiabetic drugs like glimepiride or glibenclamide have been noted.[6] Pain relieving drugs like aspirin, paracetamol, or opioids can with prolonged use or overdosage lead to complications like gastric ulceration, hepatotoxicity or addiction, respectively. As such, new drugs are necessary, which can combine efficacy with affordability, availability, and preferably no adverse effects.

Since plants have always proved to be good sources for effective new drugs.[7] we had been investigating various floral species of Bangladesh individually or in combination for their blood glucose lowering and/or pain-relieving capabilities.[8,33] Leucas aspera (Willd.) Link (Lamiaceae) is a common plant in Bangladesh (local name – dondo kolosh), sometimes cultivated and consumed following cooking to alleviate pain, but more often found growing in the wild. We have previously reported on antinociceptive activity of Leucas whole plants,[34] and antihyperglycemic activity of Leucas leaf and stems.[35] Zingiber officinale Roscoe (Zingiberaceae family, local name – ada) is commonly cultivated in Bangladesh both for its medicinal value and use as a spice. In a previous report, we have shown that an herbal formulation containing rhizomes of Zingiber officinale and cloves of Allium sativum can
improve oral glucose tolerance in mice.\textsuperscript{[36]} Antiinflammatory and analgesic properties have been observed with ethanol extract of rhizomes of \textit{Z. officinale}.\textsuperscript{[37]} As such, it was the objective of the present study to determine whether the combination of two commonly available and affordable plant parts in Bangladesh can lower blood glucose levels in glucose-challenged mice (oral glucose tolerance test or OGTT) and also alleviate pain (in acetic acid induced writhing test).

**METHODS**

**Plant material collection**

Rhizomes of \textit{Z. officinale} and aerial parts of \textit{L. aspera} were collected from a local market in Chandra, Dhaka city, Bangladesh. The rhizomes were peeled and were thoroughly dried and finely powdered prior to extraction with methanol. Aerial parts of \textit{L. aspera} were dried in the shade and finely powdered prior to extraction.

**Preparation of methanolic extract of plant parts**

Methanolic extraction was carried out separately for rhizomes of \textit{Z. officinale} and aerial parts of \textit{L. aspera}. Briefly, 100g of dried powdered plant part was extracted with 500 ml methanol at room temperature with constant stirring for 48 hours. The suspension was then filtered, and methanol evaporated at 50°C. Extracts were stored at -20°C.

**Chemicals and Drugs**

Glibenclamide, glucose and aspirin were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

**Animals**

Swiss albino mice, which weighed between 15-18g 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 (OGTT) for evaluation of antihyperglycemic activity**

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999).\textsuperscript{[38]} 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 a 1:1 (w/w) combination of the two extracts (MEZO + MELA – extracts of rhizomes of Z. officinale and aerial parts of L. aspera, respectively) at doses of 50, 100, 200, and 400 mg each extract per kg body weight, respectively. 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.

Percent lowering of blood glucose level = \((1 – \frac{W_e}{W_c}) \times 100\), where \(W_e\) and \(W_c\) represents the blood glucose concentration in glibenclamide or (MEZO + MELA) administered mice (Groups 2-6), and control mice (Group 1), respectively.

**Antinociceptive activity**

Antinociceptive activity of a 1:1 (w/w) combination of MEZO and MELA was examined using previously described procedures.\[^{[40]}\] Briefly, mice were divided into six groups of five mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered the standard antinociceptive drug aspirin at a dose of 400 mg per kg body weight. Groups 3-6 were administered (MEZO + MELA) at doses of 50, 100, 200 and 400 mg each extract per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight, which leads to pain and writhings. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period the number of abdominal constrictions (writhings) was counted for 10 min. The following formula was used for calculation of percent inhibition of the number of writhings in aspirin and (MEZO + MELA) administered animals compared to control mice,

\[
\text{Percent inhibition} = (1 – \frac{W_e}{W_c}) \times 100
\]

where \(W_e\) and \(W_c\) represents the number of writhings in aspirin or (MEZO + MELA) administered mice (Groups 2-6), 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.[41]

Phytochemical screening of the extracts
Preliminary phytochemical screening of the two extracts, MELA and MEZO, were conducted as described previously.[42]

RESULTS
(MEZO + MELA), when administered at doses of 50, 100, 200 and 400 mg each extract showed significant and dose-dependent reductions in blood glucose levels in glucose-administered mice. At the afore-mentioned four doses, the percent reductions in blood glucose levels were, respectively, 32.3, 45.0, 51.2, and 54.0%, respectively. Glibenclamide, a standard antihyperglycemic drug, when administered at a dose of 10 mg per kg body weight, lowered blood glucose by 61.2%. The results are shown in Table 1, and suggest that the two extract combination has substantial blood glucose lowering capacity.

At doses of 50, 100, 200 and 400 mg extract each of MEZO and MELA per kg body weight, the combination reduced dose-dependently and significantly the number of writhings by 26.9, 34.6, 53.8, and 65.4%, respectively versus the 50.0% reduction in the number of writhings obtained with aspirin at 400 mg per kg. The results are shown in Table 2 and demonstrate that the extract combination possess more potent antinociceptive activity than aspirin.

Phytochemical analysis of MEZO indicated the presence of saponins, alkaloids and flavonoids. Tannins, alkaloids and flavonoids were present in MELA.

Table 1: Effect of (MEZO + MELA) 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>5.82 ± 0.25</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.26 ± 0.18</td>
<td>61.2*</td>
</tr>
<tr>
<td>(MEZO + MELA)</td>
<td>50 mg each</td>
<td>3.94 ± 0.39</td>
<td>32.3*</td>
</tr>
<tr>
<td>(MEZO + MELA)</td>
<td>100 mg each</td>
<td>3.20 ± 0.54</td>
<td>45.0*</td>
</tr>
<tr>
<td>(MEZO + MELA)</td>
<td>200 mg each</td>
<td>2.84 ± 0.37</td>
<td>51.2*</td>
</tr>
<tr>
<td>(MEZO + MELA)</td>
<td>400 mg each</td>
<td>2.68 ± 0.27</td>
<td>54.0*</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: Antinociceptive effect of (MEZO + MELA) in the acetic acid-induced pain model in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>5.2 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>400 mg</td>
<td>2.6 ± 0.24</td>
<td>50.0*</td>
</tr>
<tr>
<td>MEZO + MELA (Group 3)</td>
<td>50 mg</td>
<td>3.8 ± 0.37</td>
<td>26.9*</td>
</tr>
<tr>
<td>MEZO + MELA (Group 4)</td>
<td>100 mg</td>
<td>3.4 ± 0.40</td>
<td>34.6*</td>
</tr>
<tr>
<td>MEZO + MELA (Group 5)</td>
<td>200 mg</td>
<td>2.4 ± 0.24</td>
<td>53.8*</td>
</tr>
<tr>
<td>MEZO + MELA (Group 6)</td>
<td>400 mg</td>
<td>1.8 ± 0.49</td>
<td>65.4*</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**

Diabetes is a disease, which in a large number of cases needs continuous monitoring of blood glucose and adjustments in drug doses or units of insulin injected. Blood glucose can be conveniently monitored at home with a glucometer, but such equipment plus glucose measuring strips are costly, needs some training to be used properly, and considered to be inconvenient by many people because of pricking with a needle. As a result oral drugs which are safe (less toxic), and more affordable and readily available will be preferred by most people and certainly the rural people of countries like Bangladesh, people who are not able to afford allopathic drugs. In the present instance, since both plant parts are consumed, it is very much possible that simple consumption of plant parts can lead to the desired effects of lowering elevated blood glucose levels and alleviation pain. However, this possibility needs to be investigated.

From these practical considerations, the combination of MEZO and MELA has all the advantages of affordability, availability and possibly no toxicity. Both plants are readily available in Bangladesh and aerial parts of *Leucas aspera* and rhizomes of *Zingiber officinale* are consumed without any reported problems of adverse effects.

6-gingerol, a phytocomponent of *Zingiber officinale*, can be responsible for the observed antinociceptive effect of MEZO.\(^{[43]}\) Both 6-gingerol and 8-gingerol have been reported to increase glucose uptake and so can be beneficial during diabetes.\(^{[44]}\) Alkaloids and flavonoids
present in Leucas aspera may be responsible for the observed antihyperglycemic and antinociceptive effects as has been previously reported with other plants.\[45,46\]

**CONCLUSION**

The results suggest that methanolic extract of aerial parts of *Leucas aspera* and rhizomes of *Zingiber officinale* can be used for lowering blood glucose and alleviate pain when used in combination.

**Conflicts of interest**

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

**REFERENCES**


