ABSTRACT

**Background** *Curcuma longa* is a plant whose rhizomes are used commonly as a spice, and the plant cultivated widely in the Indian subcontinent and many other countries of the world. The rhizomes reportedly possess glucose lowering and analgesic properties. The leaves of the plant are discarded; it was of interest to determine the antihyperglycemic and analgesic properties of the leaves. **Methods** Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT). Antinociceptive 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 whole plant 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 dose-dependently reduced blood glucose levels by 12.9, 21.7, 24.7, and 30.8%, 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 35.4%. In antinociceptive activity tests, the extract at doses of 50, 100, 200 and 400 mg per kg body weight...
weight reduced the number of abdominal constrictions by 24.1, 27.6, 31.0, and 34.5%, respectively. A standard pain relieving (antinociceptive) drug, aspirin, reduced the number of writhings by 31.0 and 51.7%, respectively, when administered at doses of 200 and 400 mg per kg body weight. **CONCLUSION** Antihyperglycemic and antinociceptive activities have not previously been reported for *Curcuma longa* leaves. The leaves can be a readily available mean for lowering blood sugar and for alleviating pain.

**Key Words** Antihyperglycemic, *Curcuma longa*, OGTT, antinociceptive, Zingiberaceae.

**INTRODUCTION**

*Curcuma longa* is a herbaceous perennial plant belonging to the Zingiberaceae family. In English it is known as ‘turmeric’, and in Bangladesh it is known as ‘holud’. The rhizomes of the plant are boiled, dried, powdered and used as a spice in various dishes of the country. The plant is widely cultivated in Bangladesh and the rest of the countries of the Indian sub-continent, and indeed in many other countries of the world. The plant and especially the rhizome are considered to be of ethnomedicinal significance. Rhizomes are used to treat menstrual disorders in Kerala, India. [1] Rhizomes are used to alleviate pain and for flu and nasal congestion in northwest Pakistan. [2] In the northern part of Nara Desert, Pakistan, the rhizomes are used for pain, inflamed joints, and tonsillitis. [3] The rhizomes and leaves are used as tonic, stimulant and blood purifier in the valley districts of Manipur, India. [4] The Malayali tribes of Yercaud Hills, Southern Eastern Ghats, Salem District, India use rhizomes to treat beetle bite. [5] In Gaurnadi Upazila of Barisal district, Bangladesh, the rhizomes are used to treat skin and gum diseases by folk medicinal practitioners. [6] The Bede community residing by the Turag River in Dhaka district, Bangladesh use rhizomes of the plant to treat abscesses and skin infections. [7] Folk medicinal healers of Sylhet Division, Bangladesh use the rhizomes for helminthiasis and itches. [8] Pharmacological activity studies have revealed that rhizomes of the plant possess considerable antihyperglycemic and analgesic activities. Significant lowering of blood sugar has been reported in streptozotocin (STZ)-diabetic rats when freeze-dried rhizome powder was orally administered following dissolving in milk. [9] Oral administration of rhizomes to healthy human subjects resulted in increased insulin secretion. [10] A water-soluble peptide, turmerin, has been isolated from rhizomes with antihyperglycemic properties as demonstrated by inhibition of α-amylase and α-glucosidase activities. [11] Human pancreatic α-amylase inhibitory activity has also been demonstrated for bisdemethoxycurcumin, present in rhizomes. [12] Curcumin, another compound present in
rhizomes, has also been found to be beneficial in Type 2 diabetes.\[^{13}\] The protective effect of crude \textit{C. Longa} drug and its methanolic extract has been reported in alloxan-diabetic rabbits.\[^{14}\] Treatment with curcumin has been found to attenuate pain and enhance functional recovery after incision.\[^{15}\] Modulation of pain by curcumin has also been reported for different pain models in mice.\[^{16}\] Although rhizomes of \textit{C. Longa} and various components present in the rhizomes have reportedly beneficial effects in diabetes and pain, the leaves of the plant have not been studied yet to the best of our knowledge. Since the plant is a perennial plant, the leaves can form a continuous and much more affordable source for possible antihyperglycemic and analgesic drugs. The objective of the present study was therefore to conduct antihyperglycemic (through oral glucose tolerance test, OGTT) and analgesic (through acetic acid-induced pain model) activity experiments with methanolic extract of leaves of the plant in Swiss albino mice.

**METHODS**

**Plant Material Collection**

Leaves of \textit{C. Longa} were collected during October 2013 from Santosh in Tangail district, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,737).

**Preparation of Methanolic Extract of Leaves**

Leaves were cut into small pieces, air-dried in the shade, and 200g of dried and powdered leaves were extracted with methanol (w:v ratio of 1:5, final weight of the extract 9g).

**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)\cite{17} 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 leaf extract (MECL) 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 2 g 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.\cite{18} The percent lowering of blood glucose levels were calculated according to the formula described below.

**Percent Lowering Of Blood Glucose Level** = \(1 - \frac{W_e}{W_c}\) X 100,

where \(W_e\) and \(W_c\) represents the blood glucose concentration in glibenclamide or MECL administered mice (Groups 2-6), and control mice (Group 1), respectively.

**Analgesic Activity Evaluation Through Abdominal Writhing Test**

Analgesic activity of MECL was examined as previously described.\cite{19} 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 antinociceptive drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MECL 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 MECL, 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\cite{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.

**Percent Inhibition** = \(1 - \frac{W_e}{W_c}\) X 100

where \(W_e\) and \(W_c\) represents the number of writhings in aspirin or MECL administered mice (Groups 2-7), and control mice (Group 1), respectively.
Acute Toxicity Test
Acute toxicity test was conducted as previously described. [21] 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 MECL 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. [22]

Preliminary Phytochemical Screening
Preliminary phytochemical analysis of MECL for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. [23]

RESULTS
Toxicity Evaluation
The crude extract 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.

Preliminary Screening Of Phytochemicals
Various tests conducted for presence of phytochemicals in MECL indicated the presence of alkaloids, flavonoids, saponins, and tannins.

Antihyperglycemic Activity Evaluation Results
When administered at doses of 50, 100, 200 and 400 mg per kg body weight, MECL caused dose-dependent reductions in blood glucose levels in glucose-loaded mice. At the aforementioned four doses, the percent reductions in blood glucose levels were, respectively, 12.9, 21.7, 24.7, and 30.8%. However, the results were significant only at the higher three doses of the extract. In comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose levels by 35.4%. The results are shown in Table 1 and suggest that the extract possessed antihyperglycemic
properties, which although not so potent as glibenclamide, may still prove to be beneficial for reducing blood glucose levels in diabetic patients.

**Analgesic Activity Evaluation Results**

MECL, when administered at doses of 50, 100, 200 and 400 mg per kg body weight, caused dose-dependent and significant reductions in the number of abdominal constrictions (writhings) caused by intraperitoneal administration of acetic acid in mice. At these four doses, the number of abdominal constrictions was reduced, respectively, by 24.1, 27.6, 31.0, and 34.5%. A standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, caused, respectively, a 31.0 and 51.7% fall in the number of constrictions. The results are shown in Table 2 and show that at the highest dose of the extract, it was more potent than 200 mg aspirin. Thus MECL can be used as an alternative to aspirin by people suffering from pain.

**Table 1: Effect Of Crude Methanol Extract Of C. Longa Leaves (MECL) On Blood Glucose Level In Hyperglycemic Mice Following 120 Minutes Of Glucose Loading.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(Mg/KgBodyWeight)</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>5.26 ± 0.15</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>3.40 ± 0.30</td>
<td>35.4*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>50 mg</td>
<td>4.58 ± 0.42</td>
<td>12.9</td>
</tr>
<tr>
<td>(MECL)</td>
<td>100 mg</td>
<td>4.12 ± 0.31</td>
<td>21.7*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>200 mg</td>
<td>3.96 ± 0.25</td>
<td>24.7*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>400 mg</td>
<td>3.64 ± 0.14</td>
<td>30.8*</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 2: Antinociceptive Effect Of Crude Methanol Extract Of C. Longa Leaves (MECL) 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>5.8 ± 0.37</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>4.0 ± 0.84</td>
<td>31.0*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.8 ± 0.37</td>
<td>51.7*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>50 mg</td>
<td>4.4 ± 0.24</td>
<td>24.1*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>100 mg</td>
<td>4.2 ± 0.20</td>
<td>27.6*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>200 mg</td>
<td>4.0 ± 0.63</td>
<td>31.0*</td>
</tr>
<tr>
<td>(MECL)</td>
<td>400 mg</td>
<td>3.8 ± 0.73</td>
<td>34.5*</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
Curcuma genera plants appear from the published scientific literature to be good candidates for potent analgesic drugs. Analgesic properties of different parts of *Curcuma zedoaria* have been reported. [24] Analgesic and antiinflammatory properties of ethanolic extract of *Curcuma mangga* rhizomes have also been demonstrated. [25] O-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid has been reported to be present in leaves of *C. Longa*. [26] Antiinflammatory and analgesic properties of protocatechuic acid have been shown in different rat and mice models of pain and inflammation. [27] Antioxidative and antidiabetic effect of protocatechuic acid has also been shown in STZ-diabetic rats. [28] Thus this compound can be responsible for the observed antihyperglycemic and analgesic effects as observed in the present study. Syringic acid, extracted from *Herba dendrobii*, reportedly inhibited diabetic cataract pathogenesis by inhibiting aldose reductase activity, [29] and thus is another potential candidate for the observed antihyperglycemic effects. Antiinflammatory and analgesic effects have also been observed with vanillic acid in Swiss mice and male Wistar rats. [30] Preliminary phytochemical analysis of MECL showed the presence of alkaloids, flavonoids, saponins and tannins. Various reports have mentioned that compounds belonging to these four groups or extracts containing one or more of these four groups possess antihyperglycemic and/or analgesic effects. For instance ethanol extract of *Sida cordifolia* roots showed analgesic activity. Phytochemical analysis of the ethanolic extract indicated the presence of reducing sugar, alkaloids, steroids and saponins. [31] Crude extracts of *Holoptelea integrifolia* have been shown to possess antihyperglycemic and analgesic activities. Phytochemical analysis of the extracts showed the presence of terpenoids, sterols, saponins, tannins, proteins, carbohydrates, alkaloids, phenols, flavonoids, glycosides, and quinines. [32] Ethanolic extract of whole plant of *Tridax procumbens* reportedly showed hypoglycemic effect in STZ-diabetic rats. Alkaloids, flavonoids and saponins were present in the extract. [33] Aqueous extract of *Vernonia condensata* leaves has been reported to exhibit antinociceptive activity in writhing tests; the extract was found to contain alkaloids, flavonoids, and saponins. [34] Diabetes is reaching endemic proportions in the world and pain is an affliction which affects human beings in an acute or chronic manner very commonly. An abundant source of blood glucose lowering components or pain alleviating components like *C. Longa* leaves can prove to be beneficial not only for diabetic patients but also people who suffer from acute pain arising from injury or chronic pain as occurs during rheumatoid arthritis or cancer. *C. Longa* is easily available and its leaves merit scientific attention for identifying the responsible bioactive components. Not only these components may prove to novel
compounds, but the extract by itself can form the basis of a crude and affordable drug for patients with diabetes or painful conditions.

CONCLUSION
The results suggest that methanolic extract of *C. Longa* can be used for lowering of blood glucose and for alleviating pain.

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

ACKNOWLEDGEMENTS
This work was funded through internal funding of the University of Development Alternative.

REFERENCES


