AN INITIAL REPORT ON THE ANTIHYPERGLYCEMIC AND ANTINOCICEPTIVE POTENTIAL OF LABLAB PURPUREUS BEANS

Mousumi Ahmed, Ummay Kawchur Trisha, Shupti Rani Shaha, Amit Kumar Dey, Mohammed Rahmatullah*

Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh.

ABSTRACT

Background. *Lablab purpureus* is an important bean crop of Bangladesh for its edible fruits and seeds. The objective of this study was to scientifically analyze the antihyperglycemic and antinociceptive properties of methanol extract of beans (fruits containing seeds) of the plant. Methods. Oral glucose tolerance test (OGTT) was used to determine antihyperglycemic activity. Antinociceptive activity was determined by observed decreases in abdominal constrictions in intraperitoneally administered acetic acid-induced pain model in mice. Results. Administration of methanol extract of beans led to dose-dependent and significant reductions in blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, the extract reduced blood glucose levels by 16.4, 39.1, 40.1, and 54.8%, 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 53.8%. In antinociceptive activity tests, the extract at the above four doses reduced the number of abdominal constrictions by 32.3, 45.2, 54.8, and 58.1, respectively. A standard pain relieving (antinociceptive) drug, aspirin, reduced the number of writhings by 48.4 and 61.3%, respectively, when administered at doses of 200 and 400 mg per kg body weight. Conclusion. To our knowledge, this is the first report on oral glucose tolerance and antinociceptive activity evaluation of fruits of the plant. Since the plant is widely cultivated in Bangladesh, the fruits can be used as a source for lowering blood sugar in diabetic patients and for alleviating pain.
KEY WORDS: Antihyperglycemic, Lablab purpureus, OGTT, antinociceptive, Fabaceae.

BACKGROUND

Lablab purpureus (L.) Sweet (Fabaceae), also known as Dolichos lablab L. is an annual vinous plant grown in Bangladesh for its edible fruits and seeds (collectively known as beans). In English, the plant is known as hyacinth bean, dolichos bean, seim bean, lablab bean, Egyptian kidney bean, Indian bean, chicharo and Australian pea. In Bangladesh, the plant is known as seim. The fruits are cooked and eaten in the unripe form, and it is considered as a prized vegetable in Bangladesh.

The beans as well as other parts of the plant have ethnomedicinal uses in Bangladesh and elsewhere. The folk medicinal practitioners of Bogra district, Bangladesh use leaves of the plant to treat skin diseases.[1] The Santal tribe residing in Thakurgaon District of Bangladesh uses the leaves to treat tonsillitis.[2] The Thadou tribe of Manipur, India, eats boiled fruits to cure common cold.[3] The Garo tribe living in Netrakona District, Bangladesh eats the seeds to increase sperm count.[4] The local inhabitants of Bahraich district in Uttar Pradesh India take the cooked beans in vegetable form for control of diabetes.[5] The beans are cooked with fruits of Momordica charantia in fruit extract of Syzygium cumini and a little water and taken for diabetes by villagers of Sivagangai district, Tamil Nadu, India.[6]

Alcoholic extract of leaves of the plant has been shown to possess hypoglycemic activity.[7] Antiinflammatory, antioxidant and cytotoxic activity has been noted for methanolic extract of the beans.[8] Antioxidant activity of beans has been attributed to tannins present in beans.[9] A plant belonging to the same genus, Dolichos biflorus has been shown to lower fasting blood sugar levels when administered to streptozotocin (STZ)-induced diabetic rats.[10] Extract of seed of D. biflorus has been shown to have mild analgesic activity.[11] Triterpenoids present in ethanol extract of another related species, Dolichos falcata, reportedly inhibited the production of pro-inflammatory cytokines in monosodium urate-induced gouty-arthritic rats.[12]

It is thus surprising that studies are yet to be carried out on the antihyperglycemic and antinociceptive effects of L. purpureus beans considering the ethnomedicinal and other pharmacological activity reports on this plant and other related species. It is also noteworthy in this context that mineral, flavonoid and fatty acids have been reported in ten diverse L. purpureus varieties with health benefits and antioxidant properties.[13] and that the beans,
because of their clinically proven nutraceutical and pharmaceutical traits have been suggested for use as medicinal food.\textsuperscript{[14]} We had been screening various common Bangladesh plants for their antihyperglycemic and antinociceptive properties.\textsuperscript{[15-18]} As such, the objective of the present study was to conduct oral glucose tolerance test (OGTT) and acetic acid-induced gastric pain model test with methanol extract of beans (fruits containing seeds) of \textit{L. purpureus} towards evaluating the antihyperglycemic and antinociceptive potential of the extract.

**METHODS**

\textit{Plant material collection}

Beans were collected during September 2013 from a local market in Dhaka city, Bangladesh and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,574).

\textit{Preparation of methanolic extract of beans}

Beans were cut into small pieces, air-dried in the shade, and 200g of dried and powdered beans were extracted with methanol (w:v ratio of 1:5, final weight of the extract 24.25g).

\textit{Chemicals and Drugs}

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

\textit{Animals}

Swiss albino mice, which weighed between 17-21 g 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.

\textit{Oral glucose tolerance tests for evaluation of antihyperglycemic activity}

Baseline blood glucose levels were measured in a group of 12h-fasted mice (n = 5). Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999).\textsuperscript{[19]} with minor modifications. Briefly, fasted mice (12h fasting) 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 bean extract (MELP) 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. The percent lowering of blood glucose levels were calculated according to the formula described below.

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

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

**Antinociceptive activity evaluation through abdominal writhing test**

Antinociceptive activity of MELP was examined as previously described. 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 MELP 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 MELP, 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 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 MELP administered mice (Groups 2-7), and control mice (Group 1), respectively.

**Acute toxicity test**

Acute toxicity test was conducted as previously described. 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 MELP 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.[18]

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

Foaming test for saponin.
300 mg of extract was boiled with 5 ml of distilled water and filtered. 3 ml of distilled water was added to the filtrate followed by vigorous shaking for several minutes. Any frothing, which persisted on warming was taken as an indication of the presence of saponin.

Ferric chloride test for tannin.
500 mg of extract was mixed with 10 ml of distilled water and then filtered. Several drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Appearance of a blue-black precipitate was taken as an indication for the presence of tannin.

Mayer’s test for alkaloid
1 ml of extract was added to 2 ml of Mayer’s reagent, which was freshly prepared by dissolving 1.36g mercuric chloride and 5g potassium iodide in 100 ml distilled water. Appearance of a cream-colored precipitate was taken as an indication for presence of alkaloid.

Shinoda test for flavonoid
5 drops of diluted HCl were added to 0.5 ml of extract and then a small piece of magnesium was added to the mixture. Appearance of a reddish pink coloration was taken as an indication for the presence of flavonoid.
RESULTS

Preliminary screening of phytochemicals
Various tests conducted for presence of phytochemicals in MELP indicated the presence of alkaloids and flavonoids.

Toxicity evaluation
The crude extract did not show any toxicity in mice even at the highest dose tested.

Antihyperglycemic activity evaluation results
Fasted mice had baseline blood glucose levels of 3.93 ± 0.13 mmol/l, which increased to 5.98 ± 0.20 mmol/l following glucose-loading. MELP, when administered at doses of 50, 100, 200 and 400 mg per kg body weight, dose-dependently and significantly reduced the levels of blood glucose in glucose-loaded mice. At these four doses, the percent lowering of blood glucose levels were, respectively, 16.4, 39.1, 40.1, and 54.8. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered to mice at a dose of 10 mg per kg body weight, reduced blood glucose levels in glucose-loaded mice by 53.8%. The results are shown in Table 1 and indicate that the extract at the highest dose was comparable to that of glibenclamide and as such could be used for lowering blood glucose in hyperglycemic patients.

Antinociceptive activity evaluation results
Significant dose-dependent reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MELP. At doses of 50, 100, 200 and 400 mg per kg body weight, MELP reduced the number of constrictions, respectively, by 32.3, 45.2, 54.8, and 58.1%. A standard antinociceptive drug, aspirin, when administered to experimental animals at doses of 200 and 400 mg per kg body weight, reduced the number of abdominal constrictions by 48.4 and 61.3%, respectively. Thus, the three highest doses of the extract were comparable to or better than that of 200 mg per kg aspirin. The results are shown in Table 2 and suggest that the extract possess antinociceptive properties.
Table 1: Effect of crude methanol extract of *L. purpureus* beans (MELP) 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>5.98 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.76 ± 0.25</td>
<td>53.8*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>50 mg</td>
<td>5.00 ± 0.35</td>
<td>16.4*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>100 mg</td>
<td>3.64 ± 0.26</td>
<td>39.1*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>200 mg</td>
<td>3.58 ± 0.24</td>
<td>40.1*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>400 mg</td>
<td>2.70 ± 0.20</td>
<td>54.8*</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 *L. purpureus* beans (MELP) 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>6.2 ± 0.58</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>3.2 ± 0.37</td>
<td>48.4*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.4 ± 0.51</td>
<td>61.3*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>50 mg</td>
<td>4.2 ± 0.58</td>
<td>32.3*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>100 mg</td>
<td>3.4 ± 0.51</td>
<td>45.2*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>200 mg</td>
<td>2.8 ± 0.58</td>
<td>54.8*</td>
</tr>
<tr>
<td>(MELP)</td>
<td>400 mg</td>
<td>2.6 ± 0.68</td>
<td>58.1*</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**

Alkaloids and flavonoids were found to be present in preliminary phytochemical analysis of the beans in our laboratory. *L. purpureus* is a legume crop. Previous studies have shown that alkaloids present in another legume crop, *Lupinus mutabilis*, are responsible for its hypoglycemic effects in human subjects with Type 2 diabetes; notably, the hypoglycemic effect persisted even after cooking.[24] It has been shown that four different legume crops (*Vigna unguiculata ssp. dekindtiana var. dekindtiana*, *V. unguiculata ssp. unguiculata*, *Sphenostylis stenocarpa*, and *Vigna subterranean*) lowered plasma glucose levels in alloxan-diabetic rats, even though they could not reverse diabetes-induced nephropathy.[25] The effect of *L. purpureus* beans when administered in diet remains to be studied. Flavonoids were the
main components present in extract of leaves of *Bromelia plumieri* exhibiting hypoglycemic effects in STZ-nicotinamide-induced diabetic rats.\[26\]

Alkaloids and flavonoids have also been implicated in producing antinociceptive effects; the antinociceptive and antiinflammatory activities observed with *Trigonella foenum-graecum* methanolic seed extract in formalin and carrageenan-induced paw edema tests, respectively, have been attributed to presence of alkaloids and flavonoids in the extract.\[27\] Alkaloids and flavonoids have been hypothesized behind the antinociceptive and anti-inflammatory activities observed with methanol extract of flowers of *Alangium salvifolium*.\[28\]

Our results suggest that despite the ethnomedicinal importance of *L. purpureus* in Bangladesh and elsewhere, the plant has been thus far neglected in scientific studies. Further studies need to be conducted to validate its other ethnomedicinal uses.

**CONCLUSION**

The present results validate the ethnomedicinal uses of beans for control of diabetes. The results suggest that bean extract can be used for lowering of blood sugar and alleviation of pain. It is of interest to determine whether cooked beans taken in diet can still produce the antihyperglycemic and antinociceptive effects.

**Conflicts of interest**

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

**ACKNOWLEDGEMENTS**

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**REFERENCES**


