ORAL GLUCOSE TOLERANCE AND ANTINOCICEPTIVE ACTIVITY
EVALUATION OF METHANOLIC EXTRACT OF VIGNA
UNGICULATA SSP. UNGICULATA BEANS

Tasneem Quader Tazin¹, Jannatul Ferdous Rumi¹, Shahnaz Rahman², Abdullah Al-Nahain¹, Rownak Jahan², Mohammed Rahmatullah¹*

¹Department of Pharmacy, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh.
²Department of Biotechnology & Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh.

ABSTRACT

Background. Vigna unguiculata ssp. unguiculata is a popular vegetable in Bangladesh with beans of the plant forming the edible portion. The objective of this study was to scientifically analyze the antihyperglycemic and antinociceptive properties of methanol extract of beans 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 24.8, 32.2, 42.0, and 51.7%, 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 55.2%. In antinociceptive activity tests, the extract at the above four doses reduced the number of abdominal constrictions by 30.0, 33.3, 36.7, and 43.3%, respectively. A standard pain relieving (antinociceptive) drug, aspirin, reduced the number of writhings by 40.0 and 56.7%, 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 beans of this species. Since the plant is widely available in Bangladesh, the beans can be a good source for lowering blood sugar in diabetic patients and for alleviating pain.

**Key words**: Antihyperglycemic, *Vigna unguiculata*, OGTT, antinociceptive, Fabaceae.

**Background**

*Vigna unguiculata* ssp. *unguiculata* (L.) Walp. is a vinous plant widely cultivated in Bangladesh for its edible beans, which are cooked and consumed as vegetable. In English, it is known as cowpea; in Bangladesh, it is known as ‘barbati’. Various sub-species of *V. unguiculata* beans are consumed throughout the world, including Bangladesh.

The plant is considered to have ethnomedicinal importance. Seeds of the plant are taken in Kerala, India, for menstrual disorders. [1] The seeds are also used for menstrual disorders in Northern Telangana, India. [2] The roots are used against syphilis, gonorrhea, and sexually transmitted diseases in Nebbi district, Uganda. [3]

A new triterpenoid saponin, 3-O-[α-L-rhamnopyranosyl-(1-->4)-β-D- galactopyranosyl-(1-->4)-β-D-glucuronopyranosyl]-soyasapogenol B was isolated along with cycloartenol, stigmasterol, 3-O-acetyloleanolic acid, and sitosterol 3-β-D-glucoside from a methanolic extract of the seeds of *Vigna unguiculata* subsp. *unguiculata*. [4] From the pods of *Vigna unguiculata* spp. *sesquipedalis*, various anthocyanins have been reported, which include delphinidin-3-O-glucoside, cyanidin-3-O-sambubioside, cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, and peonidin-3-O-glucoside; seed coats reportedly contained delphinidin-3-O-galactoside, cyanidin-3-O-galactoside, petunidin-3-O-glucoside, and malvidin-3-O-glucoside. [5] Phenolic compounds like neochlorogenic acid, chlorogenic acid and caffeic acids with antioxidant capacities have been reported from methanolic extract of seeds of *V. unguiculata* (ssp. not mentioned). [6] Catechin-O-glucoside and (epi)afzelechin has also been reported from beans of the plant. [7] Ethyl acetate fraction of leaves showed antioxidant and anti-atherogenic potential in ameliorating cholesterol-induced atherosclerosis. [8]

Various important pharmacological activities have been reported on other species belonging to the *Vigna* genus, although any such reports are non-existent (except for one [8]) for *V. unguiculata*. Crude methanolic extract of black gram (*Vigna mungo*) and green gram (*Vigna radiata*) has been shown to inhibit cyclooxygenase (COX)-2 *in vitro*, thus demonstrating
analgesic and anti-inflammatory potential of the extract. A hot water extract of beans from Vigna angularis demonstrated antidiabetic potential by inhibiting α-glucosidase and α-amylase activities in mice and diabetic rats. Seed coats of V. angularis containing polyphenols showed protective action on the renal cortex in streptozotocin (STZ)-induced diabetic rats. The hypoglycemic effect of hot water extract of beans of V. angularis has been demonstrated in spontaneously diabetic KK-A(y) mice. Extract of Vigna nakashimae significantly suppressed postprandial hyperglycemia and blood glycated hemoglobin in db/db mice.

We had been screening various common Bangladesh plants for their antihyperglycemic and antinociceptive properties. Considering that other Vigna species have reported antihyperglycemic and antinociceptive potential, and that various sub-species of V. unguiculata have reported bioactive compounds with reported antihyperglycemic and antinociceptive activities, it was of interest to conduct oral glucose tolerance test (OGTT) and acetic acid-induced gastric pain model test with methanol extract of V. unguiculata ssp. unguiculata beans towards evaluating the antihyperglycemic and antinociceptive potential of the extract.

METHODS

Plant material collection

V. unguiculata ssp. unguiculata beans were collected during October 2013 from Savar in Dhaka city, Bangladesh and taxonomically identified at the Bangladesh National Herbarium (Accession Number 39,072).

Preparation of methanolic extract of beans

Beans (fruitpods containing seeds) were cut into small pieces, air-dried in the shade, and 150g of dried and powdered roots were extracted with methanol (w:v ratio of 1:5, final weight of the extract 10.43g).

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 15-19 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.

**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) [18] 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 bean extract (MEVU) 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. [19] 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 MEVU administered mice (Groups 2-6), and control mice (Group 1), respectively.

**Antinociceptive activity evaluation through abdominal writhing test**

Antinociceptive activity of MEVU was examined as previously described. [20] 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 MEVU 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 MEVU, 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 [15], 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 = \((1 – \frac{W_e}{W_c}) \times 100\)
where \( W_e \) and \( W_c \) represents the number of writhings in aspirin or MEVU 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 MEVU 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. [17]

**Preliminary phytochemical screening**
Preliminary phytochemical analysis of MEVU for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. [22]

**RESULTS**

**Preliminary screening of phytochemicals**
Various tests conducted for presence of phytochemicals in MEVU indicated the presence of alkaloids, flavonoids, and tannins.

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

**Antihyperglycemic activity evaluation results**
MEVU, 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 mice. At these four doses, the percent lowering of blood glucose levels were, respectively, 24.8, 32.2, 42.0, and 51.7. 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 by 55.2%. The results are shown in Table 1 and indicate that at the highest dose tested, the extract contains substantial antihyperglycemic activity and as such could be used for lowering blood glucose in hyperglycemic patients.
Antinociceptive activity evaluation results

Dose-dependent and significant reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MEVU. At doses of 50, 100, 200 and 400 mg per kg body weight, MEVU reduced the number of constrictions, respectively, by 30.0, 33.3, 36.7, and 43.3%. 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 40.0 and 56.7%, respectively. Thus, at the highest dose of the extract, MEVU showed antinociceptive activity better than that of 200 mg per kg aspirin. The results are shown in Table 2 and suggest that the extract possesses antinociceptive properties.

Table 1: Effect of crude methanol extract of V. unguiculata ssp. unguiculata beans (MEVU) 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>5.72 ± 0.31</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.56 ± 0.20</td>
<td>55.2*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>50 mg</td>
<td>4.30 ± 0.33</td>
<td>24.8*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>100 mg</td>
<td>3.88 ± 0.17</td>
<td>32.2*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>200 mg</td>
<td>3.32 ± 0.26</td>
<td>42.0*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>400 mg</td>
<td>2.76 ± 0.19</td>
<td>51.7*</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 V. unguiculata ssp. unguiculata beans (MEVU) 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>6.0 ± 0.55</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>3.6 ± 0.40</td>
<td>40.0*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.6 ± 0.51</td>
<td>56.7*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>50 mg</td>
<td>4.2 ± 0.66</td>
<td>30.0*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>100 mg</td>
<td>4.0 ± 0.32</td>
<td>33.3*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>200 mg</td>
<td>3.8 ± 0.97</td>
<td>36.7*</td>
</tr>
<tr>
<td>(MEVU)</td>
<td>400 mg</td>
<td>3.4 ± 0.51</td>
<td>43.3*</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
Cycloartenol, stigmasterol, 3-O-acetyloleanolic acid, and sitosterol 3-β-D-glucoside has been reported from a methanolic extract of the seeds of Vigna unguiculata subsp. unguiculata. [4] Ethanol extract of banana pseudostems reportedly significantly suppressed the maltose/glucose-induced postprandial plasma glucose elevation and wielded an antihyperglycemic effect in normal and alloxan-induced diabetic rats. The extract was found to contain high amounts of β-sitosterol and stigmasterol. [23] The methanolic extract of Tournefortia hartwegiana has been shown to exert its antidiabetic effect by suppressing carbohydrate absorption from intestine, and thereby reducing the post-prandial increase of blood glucose. The extract was found to contain stigmasterol among other compounds. [24] β-Sitosterol-3-β-D-glucoside has been reported to be the active antidiabetic agent of Centaurea seridis var. maritima; it has been suggested that the compound exerts its action on intact pancreatic β-cells through stimulating insulin secretion. [25] Cycloartenol has been reported for its anti-inflammatory effects. [26] Taken together, the various components reportedly present in V. unguiculata ssp. unguiculata can account for the observed antihyperglycemic and antinociceptive activities as observed in the present study.

It is surprising that this ethnomedicinal plant has previously been neglected regarding the pharmacological potential of the plant or plant parts. It is expected that this study will lead to further scientific efforts in determining the full phytochemical constituents of the plant and their bioactivities.

CONCLUSION
The results suggest that V. unguiculata ssp. unguiculata can be used for lowering of blood sugar and alleviation of 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.
Authors’ contributions
TQT, JFR, and SR collected the beans, did the extraction, and performed the experiments under the supervision of RJ and MR. MR wrote the manuscript draft, which was read and edited by all authors. All authors read and approved the final version of the manuscript.

REFERENCES


