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ANTIHYPERGLYCEMIC AND ANTINOCICEPTIVE ACTIVITY TESTS WITH BETA VULGARIS L. SSP. VULGARIS ROOTS: A PRELIMINARY REPORT

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ABSTRACT

Background: *Beta vulgaris* ssp. *vulgaris* is cultivated throughout Bangladesh for its edible leaves and roots. Poor rural people consume the leaves, while the more costly roots are consumed by the more affluent urban population. The objective of this study was to scientifically analyze the antihyperglycemic and antinociceptive properties of methanol extract of roots (beet root) of the plant. **Methods:** Oral glucose tolerance test (OGTT) was used to determine antihyperglycemic activity. Antinociceptive activity was determined by observed decreases in abdominal writhings in intraperitoneally administered acetic acid-induced pain model in mice. **Results:** Administration of methanol extract of roots 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 17.5, 33.2, 40.2, 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 35.5, 41.9, 45.2, and 48.4%, 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 roots of the plant. Since the plant is widely available in Bangladesh, the roots can be a source for lowering blood sugar in diabetic patients and for alleviating pain.

KEYWORDS: Antihyperglycemic, *Beta vulgaris*, OGTT, antinociceptive, Chenopodiaceae

INTRODUCTION

Beta vulgaris ssp. *vulgaris* L. (Chenopodiaceae, occasionally included in the Amaranthaceae family) is known in English as garden beet, and in Bangladesh as beet. Beet is cultivated throughout Bangladesh in the winter months of November to February. The urban poor usually consume the leaves in the cooked form as a vegetable, while the more affluent urban people consume the roots in the raw form in salads. The root is dark red in color both outside and inside.

A number of reports exist on the antidiabetic properties of *B. vulgaris* var. *cicla* (chard), which is consumed in Turkey by diabetic patients to reduce blood sugar, and extract of which has been shown to decrease nonenzymatic glycosylation of skin proteins and decrease blood glucose when fed to streptozotocin (STZ)-induced diabetic rats [1].In a study conducted with STZ-diabetic rats, administration of chard extract led to increase in the number of B cells of Langerhans islets, suggesting regeneration of B cells [2]. In STZ-diabetic rats, administration of chard extract led to reversal of STZ-induced elevated serum urea and creatinine levels, and also normalized the STZ-induced significant kidney tissue degenerative damages [3]. In STZ-induced diabetic female rats, higher levels of aorta and heart tissue lipid peroxidation and reduced levels of tissue glutathione, as well as increased level of blood glucose were noted, these were reversed following administration of chard extract [4]. In STZ-induced diabetic rats, blood glucose levels, serum alanine aspartate transaminase, alkaline phosphatase activities, total lipids, sialic and uric acid levels, liver lipid peroxidation (LPO), and nonenzymatic glycosylation (NEG) levels increased, while blood glutathione, body weight, and liver glutathione (GSH) levels decreased. Administration of chard extract led to reversal of these changes, demonstrating a beneficial effect of the extract on the liver in diabetic rats [5]. One study showed that sugar beet (*Beta vulgaris* ssp. *saccharifera*) pectin (SBP) and polydextrose (PDX) did not have positive effects on fasting or postprandial plasma glucose concentrations or serum lipid profile in human subjects with abnormal glucose metabolism [6]. The ethanolic extract of sugar beet plant has been shown to possess an anti-inflammatory

effect against acute (xylene-induced ear oedema) and chronic (cotton-pellet granuloma) inflammation [7]. In comparison with chard, reports are almost non-existent on the pharmacological properties of garden beet. However, garden beet (*Beta vulgaris* ssp. *vulgaris*) root juice has been shown to lower blood pressure in men when consumed as part of a normal diet in free-living healthy adults [8]. Ethanolic extract of garden beet root has been shown to give hepatoprotective action against carbon tetrachloride-induced hepatoxicity in rats [9].

We had been screening various common Bangladesh plants for their antihyperglycemic and antinociceptive properties [10-13]. Considering that other sub-species of *Beta vulgaris* (like chard and sugar beet) has been reported to demonstrate antidiabetic and anti-inflammatory properties, 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 garden beet root (root of *Beta vulgaris* subsp. *vulgaris*) towards evaluating the antihyperglycemic and antinociceptive potential of the extract.

Methods

Plant Material Collection

Beet roots were collected during September 2013 from Mohammadpur in Dhaka city, Bangladesh and taxonomically identified at the Bangladesh National Herbarium (Accession Number 39,204).

Preparation of Methanolic Extract of Roots

Roots were cut into small pieces, air-dried in the shade, and 200g of dried and powdered roots were extracted with methanol (w:v ratio of 1:5, final weight of the extract 42g).

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 15-21 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.

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) [14] 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 beet root extract (MEBV) 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 [15]. 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 MEBV administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive Activity Evaluation through Abdominal Writhing Test

Antinociceptive activity of MEBV was examined as previously described [16]. 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 MEBV 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 MEBV, 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 [11], 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 = $(1 – W_e/W_c) X 100$

Where W_e and W_c represents the number of writhings in aspirin or MEBV administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute Toxicity Test

Acute toxicity test was conducted as previously described [17]. 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 MEBV 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 [13].

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of MEBV for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before [18].

Results

Preliminary Screening of Phytochemicals

Various tests conducted for presence of phytochemicals in MEBV indicated the presence of tannins, 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

MEBV, 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, 17.5, 33.2, 40.2, 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 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 MEBV. At doses of 50, 100, 200 and 400 mg per kg body weight, MEBV reduced the number of constrictions, respectively, by 35.5, 41.9, 45.2, and 48.4%. 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, at the highest dose of 400 mg of MEBV tested, the extract showed antinociceptive activity comparable to that of 200 mg per kg aspirin. The results are shown in Table 2 and suggest that the extract possess antinociceptive properties.

DISCUSSION

This is the first reported instance of antihyperglycemic (in the form of OGTT) and antinociceptive activity studies with garden beet root to our knowledge. This lack of studies is surprising, considering the antihyperglycemic effects observed with another *Beta vulgaris* subspecies, namely chard [1-5], and anti-inflammatory effects reported for sugar beet [7]. A number of bioactive compounds have been reported for garden beet root, which includes acetamide, adipic acid, □-spinasterylglucoside, betaine, betanidine, betanine, homogentisinic acid, melilotic acid, phytosterols, salicylic acid, and syringic acid [19]. Acetamide derivatives like 2-(Pyrrolidynyl)-N-(3- chlorophenyl) acetamide and 2-(Piperazinyl)-N-(4-methoxy phenyl) acetamide have been found to give potent hypoglycemic activities [20]. The common over the counter (OTC) antinociceptive drug, acetaminophen, can also be considered as a derivative of acetamide. Thus acetamide can be responsible for the observed antihyperglycemic and antinociceptive effects. Phytosterols like fucosterol and □-sitosterol have been mentioned as responsible agents for the observed antihyperglycemic activity of *Swietenia macrophylla* seed extracts [21]. □-Sitosterol has been reported from leaves of the plant [19], but is yet to be confirmed whether it is present in roots. Another OTC antinociceptive drug, aspirin, is acetyl salicylic acid, so salicylic acid present in roots can be responsible for the observed antinociceptive activity of the beet root extract (MEBV).

Preliminary phytochemical analysis of MEBV demonstrated the presence of alkaloids, flavanoids, and tannins. Although the exact phytochemical constituent(s) responsible for the observed antihyperglycemic and antinociceptive effects were not confirmed in the present study, a number of reports exists that alkaloids, flavonoids, and tannins have

antihyperglycemic and antinociceptive properties. For instance, antidiabetic effects of ethanolic extract of whole plant of *Tridax procumbens* (containing alkaloids, flavanoids and tannins) have been shown in STZ-induced diabetic rats [22]. Hypoglycemic effects have been seen with aqueous extract of *Persea americana* seeds (containing alkaloids, flavonoids, and tannins) in alloxan-induced diabetic rats [23]. Antinociceptive and antioxidant activities have been observed with ethanolic crude extract of leaves of *Ageratum conyzoides* from Bangladesh; phytochemical analysis of the crude extract revealed the presence of tannins, alkaloids, and flavonoids [24]. Phytochemical analysis of alcoholic extract of stem bark of *Erythrina indica* showing hypoglycemic effect in alloxan diabetic rats demonstrated the presence of alkaloids, flavonoids, phytosterols, and tannins [25]. The methanolic root bark extract of *Afzelia africana* showing antidiabetic activity in alloxan diabetic mice also showed the presence of alkaloids, flavonoids, tannins, and saponins [26]. Thus alkaloids, flavonoids and tannins can also be responsible for the observed antihyperglycemic and antinociceptive effects of MEBV besides acetamide, phytosterols, and salicylic acid reportedly present in garden beet roots. Presently, work is undergoing in our laboratory to identify the active constituent(s) and the mechanism(s) responsible behind the antihyperglycemic and antinociceptive effects.

Table-1: Effect of crude methanol extract of *B. vulgaris* ssp. *vulgaris* roots (MEBV) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.72 ± 0.31	-
Glibenclamide	10 mg	2.56 ± 0.20	55.2*
(MEBV)	50 mg	4.72 ± 0.34	17.5*
(MEBV)	100 mg	3.82 ± 0.23	33.2*
(MEBV)	200 mg	3.42 ± 0.16	40.2*
(MEBV)	400 mg	2.76 ± 0.17	51.7*

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 *B. vulgaris* ssp. *vulgaris* roots (MEBV) in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	6.2 ± 0.58	-
Aspirin	200 mg	3.2 ± 0.37	48.4*
Aspirin	400 mg	2.4 ± 0.51	61.3*

(MEBV)	50 mg	4.0 ± 0.32	35.5*
(MEBV)	100 mg	3.6 ± 0.51	41.9*
(MEBV)	200 mg	3.4 ± 0.51	45.2*
(MEBV)	400 mg	3.2 ± 0.73	48.4*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to control.

CONCLUSION

The results suggest that garden beet roots 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.

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Authors' Contributions

IM, HJ, and SR collected the roots, 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.

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