

# ***Bacopa monnieri*: An evaluation of antihyperglycemic and antinociceptive potential of methanolic extract of whole plants**

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**Abstract:** Antihyperglycemic and antinociceptive activity studies were carried out with methanolic extract of whole plants of *Bacopa monnieri*, respectively, through oral glucose tolerance test and gastric pain model induced by acetic acid in Swiss albino mice. In OGTT (oral glucose tolerance tests) conducted with glucose-challenged mice, the extract, administered at four doses of 50, 100, 200 and 400mg per kg body weight, dose-dependently and significantly inhibited the increase in serum glucose concentrations, respectively, by 33.3, 34.2, 42.1 and 44.2%. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10mg per kg body weight, inhibited increase in serum glucose concentration by 50.7%. From the results, it can be concluded that the methanolic extract of the plant possess significant antihyperglycemic potential. In antinociceptive activity tests, administration of the extract at the afore-mentioned four doses also significantly and dose-dependently reduced the number of acetic acid-induced gastric constrictions in mice. The percent inhibitions in gastric constrictions were, respectively, 43.4, 46.6, 50.0, and 53.4 at the above four doses. A reference antinociceptive drug, aspirin, when administered at a dose of 200 mg per kg body weight, reduced the number of gastric constrictions by 40.0%. Thus the extract at even the lowest dose of 50 mg, demonstrated antinociceptive activity better than that of aspirin, and which activity was much more than aspirin at the other three higher doses tested. The results demonstrate that the plant can be an excellent candidate for further studies towards isolation of antihyperglycemic and pain-killing compounds.

**Keywords:** *Bacopa monnieri*, antihyperglycemic, antinociceptive, OGTT.

## **INTRODUCTION**

*Bacopa monnieri* (L.) Penn. (Family: Scrophulariaceae) is a perennial herb found growing in the wild in wetlands of Bangladesh. The plant is known in Bengali as 'brahmi shak', and in English as water hyssop. Whole plants are used by folk medicinal practitioners primarily for improvement in memory and to prevent memory loss, but also used to lower high sugar levels in diabetic patients and to alleviate pain (Ghosh *et al.*, 2008; Subhan *et al.*, 2010). The plant has also uses in the Ayurvedic system of traditional medicine to prevent memory loss and to improve cognitive functions. A recent study has shown the efficacy of the plant in schizophrenia (Sarkar *et al.*, 2012).

The plant has been shown to attenuate neurotoxicity induced by acrylamide, and this attenuation has been attributed to elevated function of antioxidants (Shinomol *et al.*, 2013). In colchicines-induced dementia in rats, administration of the plant resulted in neuroprotective effects (Saini *et al.*, 2012). Other pharmacological activities have also been reported for the plant and several phytochemical constituents isolated from the plant. Ethanolic extract of the plant reportedly demonstrated hepatoprotective activity in rats against nitrobenzene-induced liver damage (Menon *et al.*, 2010). Wound healing activity has been reported for a compound,

namely bacoside-A, isolated from the plant (Sharath *et al.*, 2010). The oxidative damages observed in brain and kidney of diabetic rats has been shown to be reversed following administration of the plant extract (Kapoor *et al.*, 2009). Ethanolic extract of the plant has been shown to demonstrate anti-tumor properties (Kumar *et al.*, 1998). A depression in cardiac activity has been shown for crude ethanolic extract of the plant when evaluated against coronary flow, heart rate, and contractility of left ventricle (Rashid *et al.*, 1990).

Ethanol extracts of aerial parts of the plant have been shown to reverse weight loss and decrease blood glucose levels in alloxan-diabetic rats, as well as demonstrate antioxidant activity. The observed antihyperglycemic effect was attributed to increases in peripheral glucose utilization (Ghosh *et al.*, 2008). Bacosine, isolated from ethanolic extract of the plant reportedly showed antihyperglycemic activity in alloxan-induced diabetic rats (Ghosh *et al.*, 2011). A hydroethanolic extract of the plant showed antinociceptive activity in the acetic acid-induced writhing as well as hot plate test in mice (Subhan *et al.*, 2010). n-Butanol extract of the plant has also been shown to give an antinociceptive effect in acetic acid-induced writhing and hot plate tests (Abbas *et al.*, 2011). Ethanol extract of whole plant has also been reported to give antinociceptive effect in acetic acid-induced writhing model (Afjalus *et al.*, 2012). The analgesic effect of bacosides found in the plant has been reviewed (Rauf *et al.*, 2013).

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Isolated phytochemicals reported from the plant include cucurbitacins, namely bacobitacin A-D, and phenylethanoid glycosides, namely monnieraside I and III along with plantioside B (Bhandari *et al.*, 2007); triterpene glycosides and their analogues, namely bacopasides VI-VIII, bacopasides I and II, and bacopasaponin C, the latter three compounds having antidepressant activities (Zhou *et al.*, 2007); bacosterol glycoside (Bhandari *et al.*, 2006); and triterpenoid glycosides and saponins (Sivaramakrishna *et al.*, 2005).

We had been investigating the antihyperglycemic and antinociceptive potential of medicinal plants used in folk medicinal system of Bangladesh to lower high blood sugar levels and for alleviation of pain for several years (Rahman *et al.*, 2010; Alam *et al.*, 2011; Reza *et al.*, 2011; Khatun *et al.*, 2012). Such evaluation leads not only to scientific validation of folk medicinal uses, but also serves as a pointer for further scientific studies leading towards isolation and identification of the responsible phytochemical constituent(s), which in turn can lead to discovery of potentially more efficacious drugs. Diabetes is rapidly spreading throughout the world because of changes in human beings' life style and food habits, and pain in one form or another occurs to human beings worldwide on a regular basis. As such, the potential of new medicinal plants (among other substances) for alleviation of high blood sugar levels and pain needs to be continuously evaluated in scientific strivings for better and possibly cheaper and more accessible drugs. The aim of this present study was to evaluate the antihyperglycemic and antinociceptive potential of methanol extract of *B. monnieri*.

## MATERIALS AND METHODS

### *Plant material collection*

Whole plants of *B. monnieri* were collected in February 2013 from Pabna district, Bangladesh. The plant was identified at the Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 38,322) and voucher specimens deposited with the Herbarium. Authenticity of the plant sample was verified from specimens preserved at the Herbarium and from the Plant List [<http://www.thepantlist.org/>], which provides up-to-date information on floral species.

### *Preparation of methanolic extract of whole plants*

Whole plants were air-dried in the shade and powdered. Methanol extraction of powdered whole plants was done with 80g dried plant and with a powder to methanol ratio of 1:5 (w/v). Extraction was conducted over 48 hours. After 48 hours, the mixture was filtered and the filtrate collected and evaporated to dryness. The final weight of the extract was 11g. The extract was suspended in 1% Tween 80 in water prior to administration in mice.

### *Chemicals and drugs*

Glucose, glibenclamide and aspirin were obtained from Square Pharmaceuticals Ltd., Bangladesh. Glacial acetic acid was from Sigma Chemicals, USA. All other chemicals used were of analytical grade.

### *Animals*

Swiss albino mice weighing between 22-27g were obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh. The animals were acclimatized for one-week prior to experiment. The Institutional Animal Ethical Committee of the University of Development Alternative approved the study.

### *Oral glucose tolerance tests for evaluation of antihyperglycemic activity*

Oral glucose tolerance test, using glucose-loaded mice, as previously described by Joy and Kuttan (1999) was used to evaluate antihyperglycemic activity. In brief, fasted mice were divided into groups of six mice each. Vehicle in the form of 1% Tween 80 in water (10 ml per kg body weight) was administered orally to Group 1. A standard drug, glibenclamide at a dose of 10mg per kg body weight, was administered orally to Group 2. Groups 3-6 were administered orally the crude methanol extract at four doses of 50, 100, 200 and 400mg per kg body weight, respectively. Following weighing of individual mouse, dose per mouse was individually adjusted. Mice were given a 60 min period following administration of vehicle, glibenclamide or extract and then each mouse was orally administered 2g glucose per kg body weight. Blood samples were collected 120 min following glucose administration by puncturing heart. Concentrations of glucose in serum were measured following the procedure of Venkatesh *et al.* (2004), which uses the glucose oxidase method.

### *Acetic acid-induced constriction method*

Previously described procedures (Shanmugasundaram and Venkataraman, 2005) were used for evaluating antinociceptive activity of methanol extract. Abdominal pain as demonstrated by constrictions (gastric writhings) was induced in mice through intraperitoneal injection of acetic acid (1% acetic acid, 10 ml per kg body weight). Mice were divided into six groups, each group consisting of six mice. Group 1 mice served as control and were administered vehicle (1% Tween 80 in water, 10 mg per kg body weight). Group 2 was administered a standard antinociceptive drug, aspirin, at a dose of 200 mg per kg body weight. Groups 3-6 were administered extract orally, respectively, at 50, 100, 200 and 400 mg per kg body weight, 30 min prior to acetic acid administration. To ensure bio-availability of acetic acid, five minutes was given to each mouse. The number of constrictions induced by gastric pain in mice was then measured for the next 10 minutes.

**Table 1:** Effect of methanol extract of *B. monnieri* whole plants on serum glucose level in hyperglycemic mice

Treatment	Dose (mg/kg body weight)	Serum glucose level (mmol/l)	% of inhibition
Control (Group 1)	10 ml	5.82±0.25	-
Glibenclamide (Group 2)	10 mg	2.87±0.60	50.7*
<i>B. monnieri</i> (Group 3)	50 mg	3.88±0.31	33.3*
<i>B. monnieri</i> (Group 4)	100 mg	3.83±0.20	34.2*
<i>B. monnieri</i> (Group 5)	200 mg	3.37±0.28	42.1*
<i>B. monnieri</i> (Group 6)	400 mg	3.25±0.12	44.2*

All administrations were made orally. Values represented as mean ±SEM, (n=6); \**P*<0.05; significant compared to hyperglycemic control animals.

**Table 2:** Antinociceptive effect of methanol extract of *B. monnieri* whole plants in acetic acid-induced gastric pain model mice

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control (Group 1)	10 ml	5.00±0.52	-
Aspirin (Group 2)	200 mg	3.00±0.73	40.0*
<i>B. monnieri</i> (Group 3)	50 mg	2.83±0.48	43.4*
<i>B. monnieri</i> (Group 4)	100 mg	2.67±0.67	46.6*
<i>B. monnieri</i> (Group 5)	200 mg	2.50±0.62	50.0*
<i>B. monnieri</i> (Group 6)	400 mg	2.33±0.56	53.4*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); \**P*<0.05; significant compared to control.

#### Acute toxicity test

Acute toxicity test was conducted as previously described (Ganapaty *et al.*, 2002). Mice were divided into nine groups, each group consisting of six animals. Group 1 was given 1% Tween 80 in normal saline (2ml per kg body weight). The other eight groups (Groups 2-9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000mg of crude methanol extract per kg body weight. 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

As previously described (Reza *et al.*, 2011; Khatun *et al.*, 2012), Student's *t*-test was used to compare any significant differences between control and experimental animals. *P*<0.05, was considered significant as compared to control.

#### RESULTS

##### *The extract did not show any toxic effects in acute toxicity tests.*

In antihyperglycemic activity evaluation tests, the extract when administered at doses of 50, 100, 200 and 400mg per kg body weight significantly and dose-dependently reduced the concentration of glucose in glucose-loaded mice. At the afore-mentioned four doses, the percent inhibitions in rise of serum glucose concentrations as compared to control mice (Group 1) were, respectively, 33.3, 34.2, 42.1, and 44.2. A standard antihyperglycemic

drug, glibenclamide, by comparison inhibited the rise in serum glucose concentration by 50.7%, when administered at a dose of 10mg per kg body weight. The results are shown in table 1. Thus the inhibitions in rise of serum glucose concentration was nearly comparable between the highest dose of extract tested (400 mg) and glibenclamide.

In antinociceptive activity evaluation tests, the extract at the afore-mentioned four doses also dose-dependently and significantly reduced the number of acetic acid-induced gastric constrictions in mice. At the four doses of the extract tested, the percent reductions in gastric constrictions were, respectively, 43.4, 46.6, 50.0 and 53.4. By comparison, a standard antinociceptive drug, aspirin, when administered at a dose of 200mg per kg body weight decreased the number of gastric constrictions (writhings) by 40.0%. The results show that even at an extract dose of 50mg per kg body weight, the extract attenuated the number of gastric constrictions better than that of aspirin. The results are shown in table 2.

#### DISCUSSION

In the present study, the phytochemical(s) responsible for the observed antihyperglycemic and antinociceptive activities have not been isolated or identified. Further work is now ongoing in our laboratory to identify the responsible phytochemicals. However, it may be noted in this regard, that a triterpene, namely bacosine, isolated from ethanolic extract of the plant reportedly showed antihyperglycemic activity in alloxan-induced diabetic

rats (Ghosh *et al.*, 2011). Bacopasides, isolated from the plant has been reported to be an emerging class of therapeutics for management of particularly chronic pains (Rauf *et al.*, 2013). Bacosine has also been reported for demonstrating analgesic effects (Vohora *et al.*, 1997). Thus these compounds may be responsible for the observed antihyperglycemic and antinociceptive effects as observed in the present study.

## CONCLUSION

The methanol extract of whole plant of *B. monnieri* demonstrated significant antihyperglycemic activity in oral glucose tolerance tests and significant antinociceptive activity in acetic acid-induced writhing tests in mice. The results validate the traditional uses of the plant for lowering blood glucose levels and for alleviating pain.

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