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# Callus extract of *Ipomoea mauritiana* show analgesic and antihyperglycemic activity in Swiss albino mice

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**ABSTRACT**

Callus can be a viable alternative to obtain important phytochemicals and analyze crude extract for pharmacological activities rather than going the cumbersome way of collecting and destroying possibly endangered plants. In this study, callus was produced using nodal explant of *Ipomoea mauritiana*, and methanol extract of dried and powdered callus was evaluated for its analgesic and antihyperglycemic potential. The extract, when administered to Swiss albino mice at doses of 50, 100, 200 and 400 mg per kg significantly reduced the number of writhings in mice produced by intraperitoneal administration of acetic acid by 23.3, 33.3, 43.3, and 53.3%, respectively. A standard analgesic drug, aspirin, at doses of 200 and 400 mg per kg, reduced the number of writhings by 40.0 and 46.7%, respectively. In oral glucose tolerance tests conducted with glucose-loaded mice, the extract at doses of 50, 100, 200 and 400 mg per kg significantly reduced blood glucose levels by 35.1, 42.5, 53.6, and 58.8%. In comparison, a standard antihyperglycemic drug, glibenclamide, at a dose of 10 mg per kg reduced blood glucose levels by 60.7%. Our study indicates that methanol extract of callus of *I. mauritiana* can be used to alleviate pain and high blood sugar levels.

## INTRODUCTION

*Ipomoea mauritiana* Jacq. (Convolvulaceae) is a vinous plant known in English as giant potato and in Bangladesh as bhui kumra. It can be found in many parts of the world. The plant has ethnomedicinal importance. Tubers are taken orally to alleviate spinal cord pain and to increase milk in nursing mothers by the Garo tribal community of Madhupur in Tangail district, Bangladesh (Jahan *et al*., 2013). In Naogaon district, Bangladesh, folk medicinal practitioners use tubers to treat tuberculosis and to increase appetite (Anzumi *et al*., 2014). In two Mouzas of Kurigram district, Bangladesh, folk medicinal practitioners use the leaves of the plant to treat leucorrhea and diabetes with obesity (Azad *et al*., 2014). In Bagerhat district, banglades, tubers are used as nutritive, diuretic, to improve voice, to increase strength, as sexual stimulant, to increase sperm count and lactation, to treat skin assuming a different colour as in jaundice, as blood purifier and for treatment of biliary disorders and burning sensations in the body (Walid *et al*., 2013). Folk medicinal practitioners of Shitol Para village in Jhalokati district, Bangladesh use leaves, stems and roots of the plant to treat infrequent urination, to reduce excessive bile secretion, to increase strength, to increase lactation in nursing mothers and to treat pain in bones and gastric pain (Rahmatullah *et al*., 2010a). Medicinal plants of Bangladesh are comparatively undocumented and not studied in any detail as to their pharmacological potentials. Since diabetes and pain are very common afflictions in Bangladesh, we had been systematically screening medicinal plants of the country as to their pain alleviating and antihyperglycemic properties (Morshed *et al*., 2010; Rahmatullah *et al*., 2010b; Ahmed *et al*., 2011; Shahreen *et al*., 2012; Haque *et al*., 2013; Rahmatullah *et al*., 2013a,b; Ghosh *et al*., 2014; Hossain *et al*., 2014; Jahan *et al*., 2014; Rahman *et al*., 2014; Tazin *et al*., 2014). Folk medicinal and other types of traditional medicinal practitioners use medicinal plants in their formulations but most often disregard the conservation status of the plants. Moreover, if roots, tubers or rhizomes of a medicinal plant are necessary in traditional medicinal formulations, the whole plant is uprooted thus destroying the plant.

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This is done with scant regard to the plant's re-cultivation, thus making such plants becoming rapidly endangered. One way out of this impasse is to conduct pharmacological studies on calluses produced from nodal explants of the plant. If calluses can be seen to give the desired pharmacological effect or have the requisite phytochemical(s), they can serve the purpose of various plant parts including underground parts, and such calluses can be obtained within a relatively small space in the laboratory or industry and so negating the uprooting of plants and as a result endangering them. Traditional practitioners can substitute whole plants for calluses. Callus culture and concomitant pharmacological studies are rapidly gaining attention of scientists. *In vitro* callus and *in vivo* leaf extract of *Gymnema sylvestre* has been shown to stimulate β-cells regeneration and anti-diabetic activity in Wistar rats (Ahmed *et al*., 2010). Gymnemic acid, the active antidiabetic principle of *Gymnema sylvestre* has been obtained from calluses (Ali Ahmed *et al*., 2009). Antidiabetic activity has been seen in callus cultures of onion (Kelkar *et al*., 2001). The objective of the present study was to determine the analgesic and antihyperglycemic potential of methanol extract of calluses produced from nodal explants of *I. mauritiana*.

## MATERIALS AND METHODS

### Plant material collection

Explants of *I. mauritiana* were collected during November 2013 from Natore, Bangladesh. Calluses were produced from March to July 2014 using procedures as described before (Islam *et al*., 2014). The age of calluses was 40 days.

### Preparation of methanolic extract of calluses

Calluses were cut into small pieces, air-dried in the shade, and 25g of dried and powdered calluses were extracted with methanol (w:v ratio of 1:5, final weight of the extract 3.367g).

### 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.

### Analgesic activity evaluation through abdominal writhing test

Analgesic activity of methanolic extract of calluses (MEIMC) was examined as previously described (Shanmugasundaram and Venkataraman, 2005). 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 analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEIMC 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 MEIMC, 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 (Akter *et al*., 2014), 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.

$$\text{Percent inhibition} = (1 - W_e/W_c) \times 100$$

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

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

Oral glucose tolerance tests (OGTT) were carried out as per the procedure previously described by Joy and Kuttan (1999) 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 callus extract (MEIMC) 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 2g 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 (Venkatesh *et al*., 2004). The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 - W_e/W_c) \times 100$,

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

### 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 (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 MEIMC 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 (Hossain *et al*., 2014).

## RESULTS AND DISCUSSION

**Toxicity evaluation**

The crude extract (MEIMC) 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.

**Analgesic activity evaluation results**

MEIMC exhibited dose-dependent and significant analgesic activity in acetic acid-induced writhing tests. At doses of 50, 100, 200 and 400 mg per kg, administration of MEIMC led to respectively, 23.3, 33.3, 43.3, and 53.3% reductions in the number of writhings in experimental mice compared to control animals. A standard analgesic drug, aspirin, when administered to mice at doses of 200 and 400 mg per kg, led to, respectively, 40.0 and 46.7% reductions in the number of writhings. Thus, at the highest dose of 400 mg per kg, MEIMC administration showed better analgesic activity than 400 mg aspirin per kg, and a dose of 200 mg MEIMC showed better analgesic activity than 200 mg per kg aspirin. The results are shown in Table 1 and suggest that the methanolic crude extract of MEIMC can be used for analgesic purposes.

**Table 1:** Analgesic effect of crude methanol extract of *I. mauritiana* callus (MEIMC) in acetic acid-induced pain model mice.

| Treatment | Dose (mg/kg body weight) | Mean number of abdominal constrictions | % inhibition |
|---|---|---|---|
| Control | 10 ml | 6.0 ± 0.32 | - |
| Aspirin | 200 mg | 3.6 ± 0.24 | 40.0* |
| Aspirin | 400 mg | 3.2 ± 0.37 | 46.7* |
| (MEIMC) | 50 mg | 4.6 ± 0.51 | 23.3* |
| (MEIMC) | 100 mg | 4.0 ± 0.55 | 33.3* |
| (MEIMC) | 200 mg | 3.4 ± 0.40 | 43.3* |
| (MEIMC) | 400 mg | 2.8 ± 0.37 | 53.3* |

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

The analgesic activity of crude ethanol extract of whole plants of *I. mauritiana* has been previously reported (Monjur-Al-Hossain *et al*., 2013). Our results suggest that the crude callus extract can substitute for the whole plant regarding analgesic activity effects.

**Antihyperglycemic activity evaluation results**

In oral glucose tolerance tests, MEIMC when administered at doses of 50, 100, 200 and 400 mg per kg body weight, dose-dependently and significantly reduced the amount of blood glucose in experimental animals. At these four doses, MEIMC, respectively, decreased blood glucose levels by 35.1, 42.5, 53.6, and 58.8%. A standard antihyperglycemic drug, glibenclamide when administered at a dose of 10 mg per kg body weight, reduced blood glucose levels by 60.7%. Thus MEIMC at the highest dose tested showed comparable activity to glibenclamide. The results are shown in Table 2 and suggest that MEIMC can be used to reduce blood glucose levels in hyperglycemic subjects.

**Table 2:** Effect of crude methanol extract of *I. mauritiana* callus (MEIMC) 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 | 7.58 ± 0.22 | - |
| Glibenclamide | 10 mg | 2.98 ± 0.42 | 60.7* |
| (MEIMC) | 50 mg | 4.92 ± 0.83 | 35.1* |
| (MEIMC) | 100 mg | 4.36 ± 0.20 | 42.5* |
| (MEIMC) | 200 mg | 3.52 ± 0.40 | 53.6* |
| (MEIMC) | 400 mg | 3.12 ± 0.25 | 58.8* |

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

We have previously examined the blood glucose lowering ability of *I. mauritiana* tuber root powder when administered in diets of rats. Significant blood glucose lowering ability was observed when rat diet contained tuber root powder of the plant compared to control rats fed a diet without tuber root powder (Moushumi *et al*., 2010). The ability of MEIMC to lower blood glucose thus suggests that whatever antidiabetic phytoconstituent(s) are present in tuber root powder may also be present also in the calluses. However, the exact nature of the phytoconstituent(s) remains to be elucidated and is currently undergoing analysis in our laboratory. Interestingly, phenolic antioxidants have been reported in the plant (Sulaiman *et al*., 2014). Although their nature was been determined, such compounds if present in MEIMC may be responsible for the observed analgesic and antihyperglycemic effects.

## CONCLUSION

The results suggest that methanolic extract of *I. mauritiana* callus can be used for lowering of blood glucose and for alleviating pain.

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