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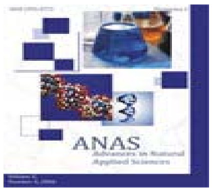


# Antihyperglycemic and Antinociceptive Activity of Methanolic Extract of *Luffa acutangula* Fruits

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ABSTRACT

The methanolic extract of *Luffa acutangula* fruits was evaluated for its antihyperglycemic and antinociceptive potentials in Swiss albino mice. Antihyperglycemic activity was evaluated through oral glucose tolerance tests in glucose-loaded mice, while antinociceptive potential was evaluated in gastric pain model mice, where pain was induced through intraperitoneal administration of acetic acid, resulting in pain and concomitant abdominal constrictions. In antihyperglycemic activity tests conducted with glucose-loaded Swiss albino mice, methanolic extract of fruits significantly and dose-dependently reduced blood sugar concentrations. At extract doses of 100, 200 and 400 mg per kg body weight mice, the percent lowering of blood sugar by the extract was, respectively, 38.5, 39.6, and 41.8. The results were both dose-dependent and statistically significant. At a lower extract dose of 50 mg per kg body weight, the extract reduced blood sugar concentrations by 13.1%, but the results were not statistically significant. A standard antihyperglycemic drug, glibenclamide, when administered to glucose-loaded mice at a dose of 10 mg per kg body weight, reduced blood sugar levels by 41.3%. The results demonstrate that the methanolic extract possesses antihyperglycemic potential. In antinociceptive activity tests conducted with intraperitoneally administered acetic acid-induced gastric pain model in mice, the extract at the afore-mentioned four doses dose-dependently reduced the number of abdominal constrictions in mice caused by the gastric pain, respectively, by 46.7, 50.0, 53.3, and 63.3%. The results were statistically significant at all doses of the extract. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 36.7 and 56.7%, respectively. The results thus demonstrate also significant antinociceptive potential of fruits of the plant, which was greater than the standard drug, aspirin (200 mg per kg body weight), at all the doses of the extract tested. The results suggest that phytochemicals present in fruits deserve further scientific attention towards possible discovery of antihyperglycemic and pain-alleviating drugs.



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## INTRODUCTION

*Luffa acutangula* (L.) Roxb. (Family: Cucurbitaceae, English: ridged gourd, local name: jhinga) ranges from central and eastern Asia to southeastern Asia, and is commercially grown for its edible unripe fruits, which are cooked and eaten as vegetable in Bangladesh. Tribal areas of North Maharashtra, India use fruits of the plant, which are taken as very fine powder through the nose for one week to protect from jaundice (Badgujar and Patil, 2008). Folk medicinal practitioners of Bangladesh use the whole plant against jaundice, tetanus, vomiting, insanity and itches (Rahmatullah *et al*., 2012e).

Fruit extract of the plant has been shown to have potent antibacterial and antifungal activities, which were more potent than leaf extract (Dandge *et al*., 2012). Acutosides A-G, oleanane-type triterpene saponins have been reported from the plant (Nagao *et al*., 1991). A novel ribosome inactivating peptide, luffangulin, has been reported from seeds (Wang and Ng, 2002). Hepatoprotective activity of the plant has been reported against carbon tetrachloride and rifampicin induced liver toxicity in rats (Jadhav *et al*., 2010). Protective effect of plant extract has also been seen on gastric ulceration in non-insulin dependent diabetes mellitus (NIDDM) rats

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(Pimple *et al*., 2012). The ameliorative effect of plant extract has also been shown in doxorubicin-induced cardiac and nephrotoxicity in mice (Jadhav *et al*., 2013).

Antioxidant, anti-inflammatory and analgesic potential of ethanolic extract of seeds of the plant has been shown in mice and rats, where antiinflammatory effects of extract was demonstrated through carragennan-induced rat paw edema, and analgesic effects demonstrated by tail flick and tail immersion methods (Gill *et al*., 2011). Methanol extract of leaves has been reported to possess hypoglycemic but not analgesic properties (Quanico *et al*., 2008).

Towards documenting medicinal plants of Bangladesh with antidiabetic, antinociceptive, and anticancer activities, our research group has been conducting ethnomedicinal surveys (Rahmatullah *et al*., 2009a-c; Rahmatullah *et al*., 2010a-g; Rahmatullah *et al*., 2011a,b; Rahmatullah *et al*., 2012a-d) followed by screening of the plants obtained for antihyperglycemic, antinociceptive and cytotoxic activities (Anwar *et al*., 2010; Jahan *et al*., 2010; Khan *et al*., 2010; Mannan *et al*., 2010; Rahman *et al*., 2010; Rahmatullah *et al*., 2010h; Shoha *et al*., 2010; Ali *et al*., 2011; Barman *et al*., 2011; Hossan *et al*., 2011; Jahan *et al*., 2011; Rahman *et al*., 2011; Sutradhar *et al*., 2011; Ahmed *et al*., 2012; Arefin *et al*., 2012; Haque *et al*., 2012; Sathi *et al*., 2012). As part of the screening process to locate plants or plant parts with antihyperglycemic and antinociceptive properties, this study was conducted to evaluate the above two properties of methanolic extract of fruits of *Luffa acutangula* in Swiss albino mice. Antihyperglycemic activity of methanolic fruit extract was evaluated through oral glucose tolerance test (OGTT) in glucose-loaded mice, while antinociceptive activity was evaluated in intraperitoneally administered acetic acid mice. Intraperitoneal administration of acetic acid causes pain, which is manifested by abdominal constrictions (writhings). Any decrease in the number of writhings was taken as an indicator of pain alleviation.

## MATERIALS AND METHODS

Fruits of *Luffa acutangula* were collected from Jessore district, Bangladesh during May 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 38,318). The sliced and air-dried fruits of *Luffa acutangula* were grounded into a fine powder and 100g of the powder was extracted with 500 ml methanol for 48 hours. The extract was evaporated to dryness at $40^{o}$C. The final weight of the extract was 8.00g.

*Chemicals:*

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

*Animals:*

In the present study, Swiss albino mice (male), which weighed between 12-15g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh.

*Antihyperglycemic activity:*

Glucose tolerance property of methanol extract of *Luffa acutangula* fruits was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into six groups of six 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 methanol extract of *Luffa acutangula* fruits (LAME) at doses of 50, 100, 200 and 400 mg per kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. 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 (Venkatesh *et al*., 2004).

*Antinociceptive activity:*

Antinociceptive activity of the methanol extract of *Luffa acutangula* fruits was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, 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 a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic fruit extract of *Luffa acutangula* (LAME) 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 extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bio-availability of acetic acid, following which period the number of abdominal constrictions (writhings) was counted for 10 min.

*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.

## RESULTS AND DISCUSSION

In antihyperglycemic activity tests conducted with glucose-loaded Swiss albino mice, methanolic extract of fruits significantly and dose-dependently reduced blood sugar concentrations. At extract doses of 100, 200 and 400 mg per kg body weight mice, the percent lowering of blood sugar by the extract was, respectively, 38.5, 39.6, and 41.8. The results were both dose-dependent and statistically significant. At a lower extract dose of 50 mg per kg body weight, the extract reduced blood sugar concentrations by 13.1%, but the results were not statistically significant. A standard antihyperglycemic drug, glibenclamide, when administered to glucose-loaded mice at a dose of 10 mg per kg body weight, reduced blood sugar levels by 41.3%. The results are shown in Table 1. The results demonstrate that the methanolic extract (LAME) possesses antihyperglycemic potential.

**Table 1:** Effect of methanol extract of *Luffa acutangula* fruits 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 (Group 1) | 10 ml | 5.50 ± 0.31 | - |
| Glibenclamide (Group 2) | 10 mg | 3.23 ± 0.14 | 41.3* |
| LAME (Group 3) | 50 mg | 4.78 ± 0.38 | 13.1 |
| LAME (Group 4) | 100 mg | 3.38 ± 0.48 | 38.5* |
| LAME (Group 5) | 200 mg | 3.32 ± 0.32 | 39.6* |
| LAME (Group 6) | 400 mg | 3.20 ± 0.21 | 41.8* |

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

The observed lowering of blood sugar following administration of the extract can be the resultant effect of a number of mechanisms. Glucose absorption in gut may be inhibited by phytochemical(s) present within the fruits. Such a mechanism has been postulated for *Mangifera indica* L. (Anacardiaceae) stem-barks (Bhowmik *et al*., 2009). Alternately, any bio-active compound or compounds present in the extract may lower blood sugar either through potentiating the pancreatic secretion of insulin or increasing the glucose uptake, as has been observed in studies with *Artemisia* extract and extract of *Ageratum conyzoides* L. (Asteraceae), respectively (Farjou *et al*., 1987; Nyunai *et al*., 2009). Another possible mechanism can possibly be increase of peripheral glucose consumption induced by the extract, as has been seen with ethanolic extract of *Sapindus trifoliatus* L. (Sapindaceae) (Sahoo *et al*., 2010). In either of these mechanisms or a combination of these mechanisms, the resultant effect will be reduction of sugar levels in the blood.

The results obtained in the present study are in agreement with some previous antidiabetic studies with fruit extract of *Luffa acutangula*. Antidiabetic and antihyperlipidemic effect of fruit extract has previously been reported for streptozotocin-induced NIDDM rats. The authors observed that methanolic extract of fruit had more antidiabetic activity than aqueous extract (Pimple *et al*., 2011). Methanolic extract of fruits of a Luffa genera, *Luffa cylindrica*, have also been shown to have hypoglycemic and antihyperglycemic effects in alloxan-induced diabetic rats (Hazra *et al*., 2011). Chloroform and alcoholic extracts of fruits of *Luffa acutangula* also reportedly decreased blood sugar levels in alloxan-induced diabetic Wistar rats (Patil *et al*., 2010).

In antinociceptive activity tests conducted with intraperitoneally administered acetic acid-induced gastric pain model in mice, LAME at the afore-mentioned four doses dose-dependently reduced the number of abdominal constrictions in mice caused by the gastric pain, respectively, by 46.7, 50.0, 53.3, and 63.3%. The results were statistically significant at all doses of the extract. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 36.7 and 56.7%, respectively. The results are shown in Table 2. The results thus demonstrate also significant antinociceptive potential of fruits of the plant, which was greater than the standard drug, aspirin (200 mg per kg body weight), at all the doses of the extract tested. At the highest dose of LAME (400 mg per kg body weight), the extract showed more antinociceptive activity than aspirin (400 mg per kg body weight). It may be concluded that the fruits of this plant possess considerable antinociceptive potential, which merits further scientific research towards isolation of bioactive constituents.

Analgesia may be of two types – central and peripheral, and both central and peripheral analgesia can be detected with the test of acetic acid-induced gastric pain, followed by measurement of the number of abdominal constrictions (Shanmugasundaram and Venkataraman, 2005). Increases in the expression of prostaglandins [mainly prostacyclines ($PGI_2$) and prostaglandin- (PG-E)] have been shown to be responsible for the sensation of pain (Reynolds, 1982; Rang and Dale, 2003). As such, the antinociceptive activity exhibited by crude methanolic extract of the fruits may be due to the extract's ability to inhibit further expression of prostaglandins, which may in turn be mediated through inhibition of cyclooxygenase and/or lipooxygenase activities, because these enzymes are responsible for biosynthesis of prostaglandins. Notably, a similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* Jack (Moraceae) aqueous extract in acetic acid-induced gastric pain model (Sulaiman *et al*., 2008).

**Table 2:** Antinociceptive effect of crude methanol extract of *Luffa acutangula* fruits in the acetic acid-induced gastric pain model mice.

| Treatment | Dose (mg/kg body weight) | Mean number of writhings | % inhibition |
|---|---|---|---|
| Control (Group 1) | 10 ml | 6.00 ± 0.84 | - |
| Aspirin (Group 2) | 200 mg | 3.80 ± 0.66 | 36.7* |
| Aspirin (Group 3) | 400 mg | 2.60 ± 1.08 | 56.7* |
| LAME (Group 4) | 50 mg | 3.20 ± 0.49 | 46.7* |
| LAME (Group 5) | 100 mg | 3.00 ± 0.45 | 50.0* |
| LAME (Group 6) | 200 mg | 2.80 ± 0.86 | 53.3* |
| LAME (Group 7) | 400 mg | 2.20 ± 0.86 | 63.3* |

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

To conclude, fruits of *Luffa acutangula* demonstrated significant antihyperglycemic and antinociceptive activities. Since diabetes and pain are common ailments suffered by human beings throughout the world, fruits of this plant need to be investigated in greater details towards discovery of possible efficacious components against these two ailments. Other Cucurbitaceae family plants have been reported for their antidiabetic effects. Some of the recent reports on antidiabetic or antihyperglycemic effects of Cucurbitaceae family plants or plant parts include hypoglycemic activity of lectin from *Trichosanthes kirilowi* (Li *et al*., 2012), fruits of *Momordica charantia* (Ooi *et al*., 2012), seed extracts of *Citrullus colocynthis* (Benariba *et al*., 2012), antihyperglycemic effect of *Coccinia indica* (Shibib *et al*., 2012), antihyperglycemic effect of *Trichosanthes dioica* (Rai *et al*., 2013), and antidiabetic effect of *Mukia maderaspatana* (Srilatha and Ananda, 2013). *Luffa acutangula* also belongs to the Cucurbitaceae family. Thus this family of plants may prove to be important in the quest for better antidiabetic drugs.

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