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Oral Glucose Tolerance and Antinociceptive Activity Evaluation of *Couroupita guianensis* Fruits

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ABSTRACT

The oral glucose tolerance and antinociceptive potential of *Couroupita guianensis* fruits (pulp plus seeds without fruit skin) were investigated. In oral glucose tolerance tests conducted with glucose-loaded Swiss albino mice, methanolic extract of fruits significantly and dose-dependently reduced blood glucose concentrations. At extract doses of 50, 100, 200 and 400 mg per kg body weight mice, the percent lowering of blood glucose by the extract was, respectively, 18.9, 33.0, 44.4, and 47.8. A standard antihyperglycemic drug, glibenclamide, when administered to glucose-loaded mice, reduced blood glucose level by 49.2%. 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 (writhings) in mice caused by the gastric pain, respectively, by 22.2, 37.0, 40.7, and 44.4%. 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 44.4 and 55.6%, respectively. The results thus demonstrate also significant antinociceptive potential of fruits of the plant, which was equivalent to 200 mg aspirin at the highest dose of the extract (400 mg) tested.

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INTRODUCTION

Couroupita guianensis Aubl. belongs to the family Lecythydaceae and is native to Central and South America. In English it is known as the Cannonball tree and in Bengali as Naglingam. The tree is not plentiful in Bangladesh but can be found in large numbers in the northeastern part of the country and as isolated trees in other parts.

Ethnomedicinal uses have been reported for various parts of the tree. The native Amazonian groups from the Nanay River (Peru) use roots of the tree for treatment of malaria (Ruiz *et al.*, 2011). The Yanadi tribes in Seshachalam Biosphere Reserve Forest of Chittoor District, Andhra Pradesh, India use leaves of the plant to vitalize hair (Ganesh and Sudarsanam, 2013). The tribal people of Kailasagirikona Forest Range of Chittoor District, Andhra Pradesh, India, use leaves of the plant for the same purpose (Pratap *et al.*, 2009). The folk medicinal practitioners of Gaurnadi Upazila, Barisal District, Bangladesh use leaves and barks of the plant to treat snake bite (Biswas *et al.*, 2011).

Antifungal activity has been reported for leaves and barks of the plant (Duraipandiyan and Ignacimuthu, 2011). Antimicrobial activity has also been reported for leaf extracts (Kavitha *et al.*, 2011). Antimicrobial and antimycobacterial activity has been reported for chloroform extract of fruits (Al-Dhabi *et al.*, 2012). Antinociceptive activity has been reported for leaf extract of the plant (Pinheiro *et al.*, 2010). Antiinflammatory activity has been reported for ethanol extract and various fractions of leaves (Pinheiro *et al.*, 2013). Hydroalcoholic extract of leaf extract also reportedly showed protective effect against oxygen reactive species and skin fibroblast stimulation (Martínez *et al.*, 2012). Antioxidant and anticancer activities of isatin (1H-indole-2,3-dione) has been reported, which was isolated from flowers of the plant (Premanathan *et al.*, 2012).

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Ongoing studies by our research group have centered on ethnomedicinal surveys followed by screening of the plants obtained for antihyperglycemic, antinociceptive and cytotoxic activities (Rahmatullah *et al.*, 2009a-c; Anwar *et al.*, 2010; Jahan *et al.*, 2010; Rahman *et al.*, 2010; Rahmatullah *et al.*, 2010a-h; Shoha *et al.*, 2010; Ali *et al.*, 2011; Barman *et al.*, 2011; Hossan *et al.*, 2011; Jahan *et al.*, 2011; Rahman *et al.*, 2011; Rahmatullah *et al.*, 2011a,b; Sutradhar *et al.*, 2011; Ahmed *et al.*, 2012; Arefin *et al.*, 2012; Haque *et al.*, 2012; Sathi *et al.*, 2012; Rahmatullah *et al.*, 2012a-d; Haque *et al.*, 2013). As part of the screening process to locate plants with antihyperglycemic and antinociceptive properties, this study was conducted to evaluate the antihyperglycemic (as conducted through oral glucose tolerance tests) and antinociceptive (as conducted through acetic acid induced abdominal pain) properties of methanolic extract of fruits (pulp and seeds but without fruit skin) of *Couroupita guianensis* in Swiss albino mice.

MATERIALS AND METHODS

Fruits of *Couroupita guianensis* were collected from Lawachora Forest Reserve in Sylhet district, Bangladesh during November, 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 38,710). The fruit pulp and seeds (after taking off the fruit skin) of *Couroupita guianensis* were air-dried in the shade, grounded into a fine powder and 100g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 22g.

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 13-19g 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 University of Development Alternative, Dhaka, Bangladesh.

Antihyperglycemic activity:

Glucose tolerance property of methanol extract of *Couroupita guianensis* fruits (MECG) 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 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 methanol extract of *Couroupita guianensis* fruits 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).

The percent lowering of blood glucose level was calculated as follows.

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 extract administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive activity:

Antinociceptive activity of the methanol extract of *Couroupita guianensis* fruits (MECG) 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 methanol extract of *Couroupita guianensis* fruits (MECG) 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 writhings was counted for 10 min.

The following formula was used for calculation of percent inhibition of the number of writhings in aspirin and MECG administered animals compared to control mice,

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

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

Statistical analysis:

Experimental values are expressed as mean \pm 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 oral glucose tolerance tests conducted with glucose-loaded Swiss albino mice, methanolic extract of fruits significantly and dose-dependently reduced blood glucose concentrations. At extract doses of 50, 100, 200 and 400 mg per kg body weight mice, the percent lowering of blood glucose by the extract was, respectively, 18.9, 33.0, 44.4, and 47.8. A standard antihyperglycemic drug, glibenclamide, when administered to glucose-loaded mice, reduced blood glucose level by 49.2%. The results demonstrate that the methanolic extract possesses antihyperglycemic potential. The highest dose of the extract was comparable to that of the standard drug, glibenclamide, in its effectiveness in lowering blood glucose. The results are shown in Table 1, and suggest that the extract contains antihyperglycemic constituents.

Table 1: Effect of methanol extract of *Couroupita guianensis* fruits (MECG) 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	5.94 \pm 0.33	-
Glibenclamide (Group 2)	10 mg	3.02 \pm 0.16	49.2*
MECG	50 mg	4.82 \pm 0.08	18.9*
MECG	100 mg	3.98 \pm 0.19	33.0*
MECG	200 mg	3.30 \pm 0.32	44.4*
MECG	400 mg	3.10 \pm 0.13	47.8*

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

Table 2: Antinociceptive effect of crude methanol extract of *Couroupita guianensis* fruits (MECG) 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	5.40 \pm 0.24	-
Aspirin (Group 2)	200 mg	3.00 \pm 0.55	44.4*
Aspirin (Group 3)	400 mg	2.40 \pm 0.40	55.6*
MECG	50 mg	4.20 \pm 0.37	22.2*
MECG	100 mg	3.40 \pm 0.24	37.0*
MECG	200 mg	3.20 \pm 0.49	40.7*
MECG	400 mg	3.00 \pm 0.32	44.4*

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

Intraperitoneal administration of acetic acid to mice induces pain, which is manifested by abdominal constrictions or writhings. 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 (writhings) in mice caused by the gastric pain, respectively, by 22.2, 37.0, 40.7, and 44.4%. 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 44.4 and 55.6%, respectively. The results thus demonstrate also significant antinociceptive potential of fruits of the plant, which was equivalent to 200 mg aspirin at the highest dose of the extract (400 mg) tested. The results suggest that the extract also contains phytochemical constituents capable of relieving pain.

Fruit pulps of *Couroupita guianensis* are known to contain glycosides, tannins, and alkaloids, although their exact identification has not been mentioned (Shah *et al.*, 2012). However, such phytochemical constituents from other plants have been reported for both antihyperglycemic as well as antinociceptive properties. Antihyperglycemic activity has been observed with stem bark extract of *Tamarindus indica* in alloxan-diabetic and normoglycemic rats; phytochemical screening revealed the presence of carbohydrates, glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids, and triterpenes (Yerima *et al.*, 2014). The inhibitory effect of *Azadirachta indica* leaf extract on alpha-amylase and alpha-glucosidase activities has been attributed to the presence of phytochemicals such as flavonoids, tannins and saponins (Kazeem *et al.*, 2013). The methanolic extract of *Teucrium stocksianum* has been shown to have antinociceptive activity; phytochemicals present in the extract included flavonoids, tannins, and glycosides (Shah *et al.*, 2014). Antinociceptive activity has also been

observed with methanolic extract of *Muntingia calabura* leaves; phytochemicals screening revealed the presence of saponins, flavonoids, tannins and triterpenes (Zakaria *et al.*, 2014). The antinociceptive and anti-inflammatory actions of *Alangium salvifolium* flower extract in mice has been attributed to the presence of alkaloids and flavonoids in the extract (Zahan *et al.*, 2013).

Although the exact phytocomponents and their mechanism of action has not been elucidated in this preliminary study, the strong antihyperglycemic and antinociceptive effects of MECG warrant further studies and such are being presently undertaken in our laboratory.

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