

A PRELIMINARY ANTIHYPERGLYCEMIC AND ANTINOCICEPTIVE ACTIVITY EVALUATION OF AMORPHOPHALLUS CAMPANULATUS CORMS

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ABSTRACT

Objective: The objective of the present study was to evaluate the antihyperglycemic and antinociceptive potential of methanol extract of *Amorphophallus campanulatus* corms in Swiss albino mice.

Methods: Antihyperglycemic activity was determined through oral glucose tolerance tests in glucose-loaded mice. Antinociceptive activity was determined through intraperitoneally administered acetic acid induced pain model in mice.

Results: The extract, when administered to mice at doses of 50, 100, 200 and 400 mg per kg body weight, dose-dependently reduced blood glucose levels in glucose-loaded mice, respectively, by 28.8, 29.1, 35.3, and 37.4%. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight reduced blood glucose level by 40.7%. Thus the extract, at the highest dose tested, showed a near equivalent antihyperglycemic potency to that of glibenclamide. At the afore-mentioned four doses, the extract reduced the number of abdominal constrictions induced by intraperitoneal administration of acetic acid in mice by 30.4, 33.3, 42.4, and 45.5%, respectively. By comparison, a standard antinociceptive drug, aspirin, when administered to mice at doses of 200 and 400 mg per kg body weight, reduced the number of abdominal constrictions by 27.3 and 36.4%, respectively, demonstrating that the extract, even at the lowest dose, was more potent than the lower dose of aspirin.

Conclusion: The results suggest that corms of the plant possess constituents with antihyperglycemic and antinociceptive activities, and which merits further isolation and identification.

Keywords: *Amorphophallus campanulatus*, Araceae, Antihyperglycemic, Antinociceptive.

INTRODUCTION

Amorphophallus campanulatus (Roxb.) Blume. (Family: Araceae; synonym: *Amorphophallus paeoniifolius*), otherwise known as the Elephant Foot Yam or the White spot Giant Arum in English, is a crop of Southeast Asian origin and can be found in both the wild and cultivated form in Bangladesh, India, Sri Lanka, the Philippines, Malaysia, and Indonesia. In Bangladesh, it is locally known as 'ol'. In Indian traditional medicinal systems of Ayurveda, Unani, and Siddha, the corm (tuber) is prescribed for bronchitis, asthma, abdominal pain, emesis, dysentery, enlargement of spleen, piles, elephantiasis, diseases due to vitiated blood, rheumatic swellings, and prostatic hyperplasia. The corms contain betulinic acid, tricontane, lupeol, stigmasterol, β -sitosterol and its palmitate [1].

Tubers of the plant have reported analgesic activity [2]. Antibacterial, antifungal and cytotoxic activities of amblyone isolated from the plant have been demonstrated [3]. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of tubers has also been reported [4].

Protective effects of tubers of the plant have been reported against thioacetamide induced oxidative stress in rats [5]. Chemopreventive effect of the tubers has been shown against aberrant crypt foci and cell proliferation in 1,2-dimethylhydrazine induced colon carcinogenesis [6]. The Murmu tribal community of Rajshahi district, Bangladesh use tubers of the plant to treat stomach pain [7]. The Garo tribal community of Bangladesh inhabiting the Madhupur forest region use tubers of the plant for treatment of rheumatic pain [8].

The antidiabetic effect of glucomannans with different molecular chains isolated from a related species plant, *Amorphophallus konjac* has been shown in experimental diabetic mice [9]. Three oligosaccharide fraction have also been isolated from roots of *A. konjac* with antidiabetic effects in streptozotocin-induced diabetes model of isolated islets.

The observed hypoglycemic effects have been attributed to free radical attenuation and lowering of risk of islet damage from nitric oxide radical [10]. A combination of traditional medicinal

uses of *A. campanulatus* tubers against pain, and the antidiabetic effects of oligosaccharides isolated from a related species, *A. konjac*, suggested that *A. campanulatus* tubers be also tested for antihyperglycemic and antinociceptive activity. The objective of the present preliminary study was to evaluate the antihyperglycemic and antinociceptive potential of methanol extract of tubers of *A. campanulatus* through oral glucose tolerance tests (OGTT) and acetic acid-induced pain model in mice.

MATERIALS AND METHODS

Plant material collection and extraction

Corms of *A. campanulatus* were collected from Dhaka district, Bangladesh during November 2012. The corms were taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 37,905). The thinly sliced air-dried corms of *A. campanulatus* were 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 1.7g.

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 15-18 g 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 *A. campanulatus* corms (ACCE) was determined through oral glucose tolerance tests as per the procedure previously described by Joy and Kuttan [11] 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 ACCE 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 as described by Venkatesh et al [12]. The following formula was used for calculation of percent inhibition of blood glucose levels in glibenclamide and ACCE administered animals compared to control mice,

$$\text{Percent inhibition} = (1 - G_e/G_c) \times 100$$

where G_e and G_c represents glucose levels in glibenclamide or ACCE administered mice (Groups 2-6), and control mice (Group 1), respectively.

Antinociceptive activity

Antinociceptive activity of ACCE was examined using previously described procedures [13]. Briefly, mice were divided into seven groups of six 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 ACCE 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. Intraperitoneal administration of acetic acid to a mouse results in pain, which is manifested by the number of abdominal constrictions of the mouse. A period of 5 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. The following formula was used for calculation of percent inhibition of the number of writhings in aspirin and ACCE administered animals compared to control mice,

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

where W_e and W_c represents the number of writhings in aspirin or ACCE 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

ACCE, when administered at doses of 50, 100, 200 and 400 mg per kg body weight dose-dependently and significantly reduced blood glucose levels compared to control mice. At these four doses, ACCE inhibited rise in blood glucose levels by 28.8, 29.1, 35.3, and 37.4%, respectively. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, inhibited rise in blood glucose levels by 40.7%. Thus ACCE at the highest dose tested demonstrated nearly comparable antihyperglycemic activity as glibenclamide. The results are shown in Table 1. This is the first report on antihyperglycemic studies of leaves of *A. campanulatus* to our knowledge. The results suggest presence of possible antihyperglycemic phytochemical component(s) in ACCE.

Table 1: Effect of methanol extract of *A. campanulatus* corms 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	6.95 \pm 0.92	-
Glibenclamide (Group 2)	10 mg	4.12 \pm 0.19	40.7*
ACCE (Group 3)	50 mg	4.95 \pm 0.61	28.8*
ACCE (Group 4)	100 mg	4.93 \pm 0.32	29.1*
ACCE (Group 5)	200 mg	4.50 \pm 0.19	35.3*
ACCE (Group 6)	400 mg	4.35 \pm 0.46	37.4*

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

ACCE, when also administered at doses of 50, 100, 200 and 400 mg per kg body weight demonstrated considerable antinociceptive activity. At these four doses, the percent inhibitions in the number of writhings were, respectively, 30.4, 33.3, 42.4, and 45.5. In comparison, a standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 27.3 and 36.4%, respectively. The results are presented in Table 2, and suggest that ACCE contains constituent(s) with stronger antinociceptive activity than aspirin.

Table 2: Antinociceptive effect of crude methanol extract of *A. campanulatus* corms in acetic acid-induced pain model in mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	5.50 \pm 0.34	-
Aspirin (Group 2)	200 mg	4.00 \pm 0.63	27.3*
Aspirin (Group 3)	400 mg	3.50 \pm 0.76	36.4*
ACCE (Group 4)	50 mg	3.83 \pm 0.48	30.4*
ACCE (Group 5)	100 mg	3.67 \pm 0.61	33.3*
ACCE (Group 6)	200 mg	3.17 \pm 0.60	42.4*
ACCE (Group 7)	400 mg	3.00 \pm 0.58	45.5*

All administrations (aspirin and extract) were made orally. Values represented as mean \pm SEM, (n=6); * P < 0.05; significant compared to control. Isolation and identification of bio-active constituent(s) responsible for the observed antihyperglycemic and antinociceptive effects along with their mechanism of action were not done in this preliminary study and is currently being investigated in our laboratory. However, betulinic acid, tricontane, lupeol, stigmaterol, β -sitosterol and its palmitate has been reported to be present in corms [1]. Hypoglycemic effect of *Morus alba* root bark extract has been observed in streptozotocin (STZ)-induced diabetic rats; betulinic acid was one of the components present in the extract [14]. Betulinic acid has also been identified as a component in antidiabetic plants exhibiting β -glucosidase inhibitory activity [15]. The beneficial effects of betulinic acid as well as other pentacyclic triterpenoids in diabetes and diabetic complications have been reviewed [16]. Lupeol can be another potential component of *A. campanulatus* corms responsible for the observed antihyperglycemic activity. Lupeol has been reported to inhibit β -amylase activity, which can be effective in reducing blood glucose levels [17]. Methanolic extract of *Tournefortia hartwegiana* has been shown to reduce plasma glucose levels in normoglycemic and alloxan-induced diabetic rats through inhibition of β -glucosidase activity; lupeol and stigmaterol were among the active components [18]. Lupeol, isolated from *Solanum xanthocarpum*, was observed to suppress the progression of diabetes with decreases in glycated hemoglobin, serum glucose and nitric oxide, and increases in serum

insulin and antioxidant levels [19]. Lupeol has been shown to be one of the active constituents (along with aegelin) in the hypoglycemic and β -cells regenerative effects of *Aegle marmelos* bark extract in streptozotocin-induced diabetic rats [20].

Besides betulinic acid and lupeol, the antihyperglycemic activity of β -sitosterol (another component of *A. campanulatus* corms) has also been documented. Lupeol and β -sitosterol were among the bioactive constituents reported for antidiabetic activity of *Terminalia sericea*; their mechanism of action has been attributed to inhibition of α -amylase and β -glucosidase [21]. Although the mechanism of action was not determined, β -sitosterol has been shown as the active principle behind the hypoglycemic activity shown by extract of *Ipomoea digitata* tuber in streptozotocin-induced diabetic rats [22]. β -Sitosterol has also been shown to be the active principle in antihyperglycemic activity of *Swietenia macrophylla* seed extracts in normoglycemic rats undergoing glucose tolerance tests [23]. Thus betulinic acid, lupeol, and β -sitosterol, which are all present in corms of *A. campanulatus*, has the potential of acting synergistically and producing a strong antihyperglycemic effect, as has been observed in the present study with oral glucose tolerance tests.

The presence of lupeol, stigmasterol, and β -sitosterol in corms can also explain the observed antinociceptive activity of ACCE. Analgesic and anti-inflammatory effects of *Cassia siamea* stem bark extracts has been attributed (among other compounds) to these three components [24]. Lupeol (along with amyirin) has been implicated in analgesic effect exhibited by methanol extract from *Ligustrum morrisonense* leaves in rodents in acetic acid-induced writhing tests and carrageenan-induced inflammation model [25], and the possible mechanism of action has been attributed to inhibition of cyclooxygenase-2 activity. Lupeol (along with ursolic acid) has also been shown to be the responsible agents behind the analgesic activity shown by methanol extract of *Cissus repens* in mice in acetic acid-induced writhing responses and formalin-induced paw licking [26].

Prostaglandins are thought to be promulgators of pain (particularly increased synthesis of PGE₂), and as such, analgesics can act by reducing PGE₂ synthesis through inhibition of cyclooxygenase and/or lipoxygenase activities [27]. Although the exact mechanism for the observed antinociceptive effect was not determined in this preliminary study, it is possible that lupeol as well as other components present in the corms of *A. campanulatus* may be exerting antinociceptive effects through inhibition of cyclooxygenase activity.

It is to be noted that a similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* aqueous extract in acetic acid-induced gastric pain model [25, 28]. It is further to be noted that yet to be reported oligosaccharide compounds may also be present in corms of *A. campanulatus* similar to that in *A. konjac* [9, 10], and which compounds may also contribute to the observed antinociceptive effects. The actual component(s) responsible for the observed antihyperglycemic and antinociceptive effects are currently under investigation in our laboratory.

CONCLUSION

Antihyperglycemic and antinociceptive effects were observed with methanol extract of corms of *A. campanulatus*, respectively, in oral glucose tolerance tests and acetic acid-induced pain model in Swiss albino mice. The results validate the traditional medicinal uses of this plant against pain, and suggest that the corms may prove to be a useful source for isolation of antihyperglycemic and pain alleviating compounds.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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