ANTINOCICEPTIVE AND ANTI-HYPERGLYCEMIC ACTIVITY OF METHANOL LEAF EXTRACT OF *CYPERUS SCARIOSUS*

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ABSTRACT

The objective of the present study was to investigate the antinociceptive and anti-hyperglycemic activity of methanolic leaf extract of *Cyperus scariosus*. Antinociceptive activity was determined using a model of acetic acid-induced gastric pain in mice and anti-hyperglycemic activity through glucose tolerance test using glucose loaded mice. In writhing assays induced by acetic acid, the methanolic leaf extract showed dose dependent significant pain inhibition compared to control. The maximum writhing inhibition (46.62%) was found at a dose of 200 mg/kg body weight which was less than that of the positive control, aspirin (56.74%), when used at the same dose. Anti-hyperglycemic activity of the extract was also found to be significant in mice loaded with glucose at doses of 200 and 400 mg/kg body weight. Maximum tolerance (42.86%) was showed at 400 mg extract/kg body weight, which compared favorably with that of glibenclamide at 10 mg/kg body weight (57.62%). In summary, the methanol extract of *C. scariosus* leaves has had beneficial effects as a pain reliever and also in reducing the elevated blood glucose level of hyperglycemic mice.

Keywords: *Cyperus scariosus*, antinociceptive activity, anti-hyperglycemic.

INTRODUCTION

Cyperus scariosus R. Br. (Family: Cyperaceae) is a delicate grass, available in different places of Bangladesh (local name – choila) and in eastern and southern parts of Indo-Pak subcontinent (Kirtikar and Basu, 1918). The brown colored plant rhizomes has a folkloric reputation as cordial, tonic, enunenagogue, vermifuge, diuretic, diaphoretic and desiccant (Kirtikar and Basu, 1918; Watt, 1972; Said, 1982). It is prescribed by the local traditional medicine practitioners to treat a variety of diseases including diarrhoea, epilepsy, gonorrhea, syphilis and liver damage (Kirtikar and Basu, 1918; Watt, 1972; Said, 1982). The essential oil obtained after steam distillation of rhizomes and roots of the plant has its value in perfumery (Kahol, 1987), and is also known to possess antibacterial (Laharia and Rao, 1979), antifungal (Desmukh and Jain, 1985) as well as plant growth-regulating properties (Kalsi *et al*., 1980). It also has hypotensive and spasmolytic properties as well (Gilani, *et al*., 1994). People in southern region of Bangladesh use leaf juice as a preliminary measure against pain and diabetes (M. Rahmatullah, personal communication).

Phytochemical studies have revealed the presence of sesquiterpenes (Nerali and Chakravarti, 1969; Nerali, *et al*., 1970), steroidal saponin (Bhatt, *et al*., 1982), aurone (Bhatt, *et al*., 1984) and substituted hydrocarbons (Neville, *et al*. 1968; Uppal, 1984). Though the existing informations confer the importance of this plant in traditional medicine, the antinociceptive and anti-hyperglycemic potential is lacking any scientific evidences. In the present investigation, methanol extract of the plant leaves was tested against aspirin and glibenclamide to validate its folkloric use in pain inhibition and in diabetes, respectively.

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MATERIALS AND METHODS

Plant material and extraction

The leaves of *C. scariosus* R. Br. were collected from different places of Khulna, Bangladesh in December, 2009. The plant was taxonomically identified by Mr. Manzur-ul-Kadir Mia, ex-Principal Scientific Officer and Curator of Bangladesh National Herbarium at Dhaka (Voucher specimen No.34,418). The dried leaves of *C. scariosus* were grounded into a fine powder and were extracted twice with methanol each for 24 hrs. The extract was evaporated to dryness using rotary evaporator and freeze dryer.

Chemicals and drugs

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

Animals

In the present study, Swiss albino male mice, which weighed between 25-30g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). Prior to experiments, all the animals were acclimatized for one week. The study was conducted following approval by the Institutional

Animal Ethical Committee of University of Development Alternatives, Dhaka, Bangladesh.

Acetic acid-induced writhing method

Antinociceptive activity of methanol extract of *C. scariosus* leaves was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005) with minor modifications. Mice were separated into five groups of seven mice each. Group-I served as control and was administered (1% Tween 80 in water, 10 mg/kg body weight). Aspirin was administered to Group-II mice at a dose of 200 mg/kg body weight. Groups-III to V received 50, 100 and 200 mg/kg body weight of the extract, respectively orally 30 min before acetic acid injection. In all groups, pain was induced through intraperitoneal administration of 1% acetic acid at a dose of 10 ml/kg body weight. A period of 5 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.

Anti-hyperglycemic activity

Glucose tolerance property of *C. scariosus* leaves was determined following the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into six groups of seven mice each. Prior to administration of vehicle, standard drug, and test samples, each mouse was weighed and doses adjusted accordingly. All substances were orally administered. Group-I received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, group-II received standard drug (glibenclamide, 10 mg/kg body weight) and the other four groups (III-VI) received the methanol extract of *C. scariosus* leaves at four different doses. Following a period of one hour, all mice were orally administered glucose at a dose of 2 g/kg of body weight. Blood samples were collected two hours after the glucose administration through puncturing heart. Serum glucose levels were measured by glucose oxidase method (Venkatesh *et al*., 2004).

Acute toxicity study

Acute toxicity test was carried out as previously described (Ganapaty *et al*., 2002). Animals were divided into nine groups consisting of six animals per group. The control group was given 1% Tween 80 in normal saline (2 ml/kg body weight). The other groups received 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg/kg of the methanol extract of leaves. Animals were kept under close observation for the next 8 hours and were maintained up to 14 days for any mortality to occur.

STATISTICAL ANALYSIS

Student's *t*-test was used to analyze any significant differences between control and experimental groups. $P < 0.05$, was considered significant as compared to control.

RESULTS

Antinociceptive activity

In the acetic acid-induced writhing model mice, administration of methanol extract of leaf of *C. scariosus* showed significant dose-dependent writhing inhibition. For the methanol extract of leaves, the maximum inhibition of writhing (46.62%) was obtained at the dose of 200 mg extract/kg body weight ($p<0.01$), whereas the standard antinociceptive drug, aspirin caused 56.74 % ($p<0.001$) writhing inhibition at the same dose (table 1).

Anti-hyperglycemic effect

The results from the investigation suggested that the methanol extract exhibited dose-dependent and significant anti-hyperglycemic activity in glucose-induced hyper-glycemic mice. The extract showed very little effect at lower doses but at higher doses showed significant effect compared to control. The maximum inhibition effect was found with the dose of 400 mg extract/kg body weight (46.86%), which was close to that of the standard drug glibenclamide (57.62%) at 10 mg/kg body weight dose (Table 2).

Acute toxicity study

Mortality was not observed with any of the extract doses till the end of the observation period of 14 days.

DISCUSSION

Investigation was carried out in the present study to explore the effect of methanol extract of *C. scariosus* leaf as antinociceptive using acetic acid-induced writhing model in mice, and anti-hyperglycemic using glucose loaded mice model. Both central and peripheral analgesia can be suitably detected with the acetic acid-induced writhing test (Shanmugasundaram & Venkataraman, 2005). Intraperitoneal administration of acetic acid (1%) leads to pain and inflammation mainly through production of prostaglandins (mainly prostacyclines (PGI_2) and prostaglandin-E (PG-E)), which have been reported to be responsible for excitation of the Aδ-nerve fibers, leading to sensation of pain (Reynolds, 1982; Rang & Dale, 1993). Therefore any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis. Centrally acting analgesics are most sensitive to hot plate and tail flick tests involving opioid μ and κ receptors (Abbott and Young, 1988; Furst *et al*., 1988), which tests were not done in the present study. However, the results obtained in the present study with the acetic acid writhing model does not preclude both peripheral and central analgesic effects, a conclusion also supported by previous report (Shanmugasundaram & Venkataraman, 2005). The major conclusion is that leaf extract of *C. scariosus* caused reduction of the number of abdominal constrictions as well as stretching of hind limbs induced by the intraperitoneal injection of acetic

Table 1: Antinociceptive effect of crude methanol extract of *Cyperus scariosus* leaves in the acetic acid-induced gastric pain model mice.

Groups	Dose (mg/kg body weight)	Mean no. of writhing	Inhibition (%)
Control (vehicle)	10 ml	24.67 ± 1.36	-
Aspirin	200 mg	10.67 ± 0.95	56.74***
C. scariosus	50 mg	20.83 ± 1.3	15.56*
C. scariosus	100 mg	17.33 ± 0.88	29.75**
C. scariosus	200 mg	13.17 ± 1.01	46.62**

All administrations were made orally. Values represented as mean ± SEM, (n=7); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, significant compared to control.

Table 2: Effect of methanol extract of *Cyperus scariosus* leaf on serum glucose level in hyperglycemic mice.

Treatment	Dose (mg/kg body weight)	Serum Glucose level (mg/dl)	% of inhibition
Control	10 ml	105 ± 7.73	
Glibenclamide	10 mg	44.5 ± 4.33	57.62*
C. scariosus	50 mg	93.34 ± 3.86	11.1
C. scariosus	100 mg	90.36 ± 6.68	13.94
C. scariosus	200 mg	68.08 ± 4.95	35.2*
C. scariosus	400 mg	60 ± 5.76	42.86*

All administrations were made orally. Values represented as mean ± SEM, (n=7); * $P < 0.01$; significant compared to hyperglycemic control animals.

acid in a dose-dependent manner, which suggest that methanol extract of *C. scariosus* leaf possess significant antinociceptive properties (table-1).

The result from the glucose loaded hyperglycemic mice model showed that the methanol extract of *C. scariosus* leaves at the doses of 200 mg/kg body weight and 400 mg/kg body weight significantly inhibited the rise of glycemia. This observation suggests that the extract may act by potentiating the pancreatic secretion or increasing the glucose uptake (Nyunai *et al*., 2009; Farjou *et al*., 1987) or inhibiting glucose absorption for gut (Bhowmik *et al*., 2009). Further studies are undergoing in our laboratory to isolate the active constituents and elucidate the exact mechanism for its anti-hyperglycemic and anti-nociceptive activity.

In conclusion, the obtained results provide pharmacological evidence and validate the folkloric use of the leaves as an antinociceptive and anti-hyperglycemic agent. However, further studies are necessary to find out the active phytochemicals as well as the exact mechanisms of antinociceptive and hypoglycemic action of *C. scariosus.* Isolation of active compounds and elucidation of exact mechanism may contribute to develop drugs for both pain and diabetes management.

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