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ANTIHYPERGLYCEMIC AND ANTINOCICEPTIVE ACTIVITY EVALUATION OF 'KHOYER' PREPARED FROM BOILING THE WOOD OF *ACACIA CATECHU* IN WATER

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Abstract

'Khoyer' is prepared by boiling the wood of *Acacia catechu* in water and then evaporating the resultant brew. The resultant hard material is powdered and chewed with betel leaves and lime with or without tobacco by a large number of the people of Bangladesh as an addictive psycho-stimulating and euphoria-inducing formulation. There are folk medicinal claims that khoyer helps in the relief of pain and is also useful to diabetic patients to maintain normal sugar levels. Thus far no scientific studies have evaluated the antihyperglycemic and antinociceptive effects of khoyer. The present study was carried out to evaluate the possible glucose tolerance efficacy of methanolic extracts of khoyer using glucose-induced hyperglycemic mice, and antinociceptive effects with acetic acid-induced gastric pain models in mice. In antihyperglycemic activity tests, the extract at different doses was administered one hour prior to glucose administration and blood glucose level was measured after two hours of glucose administration (p.o.) using glucose oxidase method. The statistical data indicated the significant oral hypoglycemic activity on glucose-loaded mice at all doses of the extracts tested. Maximum anti-hyperglycemic activity was shown at 400 mg extract per kg body weight, which was less than that of a standard drug, glibenclamide (10 mg/kg body weight). In antinociceptive activity tests, the extract also demonstrated a dose-dependent significant reduction in the number of writhing induced in mice through intraperitoneal administration of acetic acid. Maximum antinociceptive activity was observed at a dose of 400 mg extract per kg body weight, which was greater than that of a standard antinociceptive drug, aspirin, when administered at a dose of 400 mg per kg body weight. The results validate the folk medicinal use of the plant for reduction of blood sugar in diabetic patients, as well as the folk medicinal use for alleviation of pain.

Key words: *Acacia catechu*, antihyperglycemic, antinociceptive, khoyer

Introduction

Khoyer (English: catechu) is prepared by boiling the wood of *Acacia catechu* (L.f.) Willd. (Mimosaceae) in water. This is followed by evaporating the resultant brew to dryness. It is a brownish powder and is commonly available in wayside shops of Bangladesh in both urban and rural areas. Large segments of the people of Bangladesh consume khoyer on a regular basis along with leaves of *Piper betle* L. (Piperaceae, local name: paan) and lime with or without tobacco as an addictive psycho-stimulating and euphoria-inducing formulation. Khoyer by itself is considered an astringent and has been used as such in the Indian sub-continent since ancient times in Ayurvedic medicine (Khare, 2007). Folk medicinal practitioners in Bangladesh claim that the benefits of khoyer also include alleviation of pain and lowering of blood sugar levels in diabetic persons. The plant, khoyer, is further used traditionally for treatment of gastrointestinal disorders like diarrhoea, dysentery, colitis, and ulcers, as well as haemorrhoids and skin eruptions (Rajendra *et al*., 2011).

Antioxidant activity has been described for methanol extract of heartwood of *A. catechu* (Guleria *et al*., 2011). Immunomodulatory activity has been described of aqueous extract of the whole plant (Ismail and Asad, 2009). Antiamnesic and antioxidant effect of catechin derived from the plant has been reported in normal, aged and scopolamine challenged cognitive deficit mice (Biradar *et al*., 2012). Gastric antiulcer activity has been demonstrated for aqueous extract of heartwood of the plant (Rajendra *et al*., 2011). Other activities reported for extract of whole plant are anti-pyretic, anti-diarrhoeal, hypotensive, hepatoprotective, anti-mycotic and anti-inflammatory activities (Sham *et al*., 1984; Ray and Thokchom, 2006; Burnett *et al*., 2007; Nagaraja *et al*., 2008). Seeds reportedly showed hypoglycemic activity in normal albino rats (Singh *et al*., 1976).

Catechu has been reported to contain catechuic acid, catechutannic acid, acacatechin, catechu red, quercetin, catechin, epicatechin, phlebotannin, quercitrin and fisetin. It also contains cyanodol, tannins and polyphenols (Rajendra *et al*., 2011;

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Lakshmi *et al*., 2011). Since catechu (khoyer) is used for treatment of diabetes and pain by the folk medicinal practitioners of Bangladesh, the objective of the present study was to evaluate the antihyperglycemic activity of methanolic extract of the plant using glucose-loaded mice in oral glucose tolerance tests (OGTT), and antinociceptive activity in acetic acid-induced gastric writing pain model in mice.

Materials and Methods

Collection of plant material

Khoyer was collected in October of 2011 from a khoyer manufacturing shop in Dhaka district, Bangladesh. When inquired about the processing method, the manufacturer mentioned that the wood of *A. catechu* was boiled in 5 volumes of water for 2 hours. The water was then strained to separate wood pieces from the water, and then the resultant water was further boiled to dryness to yield khoyer. The khoyer was then powdered and sold. The khoyer manufacturer produced plant specimen of *A. catechu*. The plant was identified by the Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 36,069) and sample specimens have been kept over there.

Preparation of the test samples

Khoyer was pulverised into a fine powder and was mixed with methanol at a ratio of 1:6 (w/v). After 48 hours, the mixture was filtered and filtrate was collected. Filtrate was evaporated to dryness (approximate yield 13%) using rotary evaporator. Extracts were suspended in 1% Tween 80 in water prior to administration.

Animals

Swiss albino mice (male), weighing 18-22g bred in the animal house of ICDDR,B (International Centre for Diarrhoeal Disease and Research, Bangladesh) were used for the present experiments. All the animals were acclimatised one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2^0C, and 12 hrs light-dark cycles). The animals were fed with standard diet from ICDDR,B and had free access to water. The study was approved by the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh.

Anti-hyperglycemic activity test

Antihyperglycemic activity of the extract was studied through the glucose tolerance test method. Glucose tolerance test was performed following the procedure as described by Joy and Kuttan (1999) with slight modifications (Rahman *et al*., 2011; Ahmed *et al*., 2011). In brief, fasted mice were divided into six groups of six mice each. Each group received a particular treatment, like Group 1 served as control and received vehicle (1% Tween 80 in water, 10 ml per kg body weight), while Group 2 received standard drug (glibenclamide, 10 mg per kg body weight). Groups 3-6 received methanolic extract of khoyer at four different doses of 50, 100, 200 and 400 mg extract per kg body weight, respectively. Each mouse was weighed properly and the doses of the test samples, standard drug, and control materials were adjusted accordingly. Test samples, control, and glibenclamide were given orally. After one hour, all mice were orally treated with 2 g per kg body weight of glucose. Blood samples were collected two hours after glucose administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method (Venkatesh *et al*., 2004).

Antinociceptive activity test

Antinociceptive activity of the methanol extract was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, for methanolic extract of khoyer, mice were divided into six groups of six mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered with the standard antinociceptive drug aspirin at a dose of 400 mg per kg body weight. Groups 3-6 were administered with methanolic extract of khoyer at doses of 50, 100, 200 and 400 mg per kg body weight, respectively.

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.

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Results and Discussion

The methanolic extract of khoyer exhibited dose-dependent and significant antihyperglycemic effect when administered to glucose-loaded mice in oral glucose tolerance tests. Briefly, the reductions in serum glucose levels were, respectively, 28.6, 35.9, 37.0 and 37.7% at extract doses of 50, 100, 200 and 400 mg per kg body weight of mice. The results are shown in Table 1. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered to mice at 10 mg per kg body weight, lowered serum glucose level by 48.6%. Thus, even at a dose of 100 mg extract per kg body weight, the extract exhibited glucose lowering properties, which though less than that of glibenclamide, still were substantial enough to merit further scientific studies with the extract towards isolation of responsible bio-active constituents. The results validate the folk medicinal use of khoyer in diabetic patients to lower blood sugar levels.

Table 1: Effect of crude methanol extract of khoyer on serum glucose level in hyperglycemic mice following 120 minutes of glucose loading

Treatment	Dose (mg/kg body weight)	Serum glucose level (mmol/litre)	% lowering of serum glucose level
Control	10 ml	7.25 ± 0.62	-
Glibenclamide	10 mg	3.73 ± 0.37*	48.6
Khoyer	50 mg	5.18 ± 0.23*	28.6
Khoyer	100 mg	4.65 ± 0.48*	35.9
Khoyer	200 mg	4.57 ± 0.47*	37.0
Khoyer	400 mg	4.52 ± 0.28*	37.7

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

Table 2: Antinociceptive effect of crude methanol extract of khoyer in acetic acid-induced gastric pain model mice

Treatment	Dose (mg/kg body weight)	Mean number of writhing	% inhibition
Control	10 ml	5.50 ± 0.85	-
Aspirin	400 mg	2.17 ± 0.98*	60.5
Khoyer	50 mg	2.67 ± 1.05*	51.5
Khoyer	100 mg	2.33 ± 0.84*	57.6
Khoyer	200 mg	2.00 ± 0.93*	63.6
Khoyer	400 mg	1.67 ± 0.92*	69.6

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); $^{*}P < 0.05$; significant compared to control

In antinociceptive activity tests, the extract also demonstrated dose-dependent and significant reductions in the number of writhing induced by intraperitoneal administration of acetic acid in mice. At doses of 50, 100, 200 and 400 mg extract per kg body weight, the percent reductions in the number of writhing were, respectively, 51.5, 57.6, 63.6, and 69.6%. The percent reduction in the number of writhing at the highest dose of the extract tested compares favourably with that of a standard antinociceptive drug, aspirin, when administered at a dose of 400 mg per kg body weight, which reduced the number of writhing by 60.5%. The results are shown in Table 2. The results demonstrate that at least at the two highest doses of the extract, namely 200 and 400 mg per kg body weight, the extract possessed greater pain-relieving effects than aspirin. Thus, the folk medicinal use of the extract for treatment of pain was also found to be validated in the present study.

The observed glucose lowering effect by the crude extract may occur through several possible mechanisms. The extract may inhibit glucose absorption in gut (Bhowmik *et al*., 2009). Alternately, the extract may potentiate the pancreatic secretion of insulin or increase the glucose uptake (Farjou *et al*., 1987; Nyunai *et al*., 2009). Although the present study did not identify the chemical component(s) responsible for the observed antihyperglycemic effect, it may be noted that quercetin is one of the components reported to be present in khoyer (Rajendra *et al*., 2011). Methanol extract of *Moringa oleifera* pods containing quercetin and kaempferol has been reported to have antidiabetic and antioxidant effects when tested in streptozotocin-induced diabetic albino rats (Gupta *et al*., 2012). Quercetin was also one of the components found in cell cultures of *Morus nigra* exhibiting hypoglycemic efficacy (Abd El-Mawla *et al*., 2011). Quercetin was also one of the major compounds found in rice bean (*Vigna umbellata*), when tested for antioxidant capacity and antidiabetic potential (Yao *et al*., 2012).

Both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Production of prostaglandins [mainly prostacyclines (PGI_2) and prostaglandin- (PG-E)] has been shown to be responsible for excitation of Aδ-nerve fibres, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 1993). As such, the antinociceptive activity exhibited by crude methanolic extract of khoyer may be due to the extract's ability to

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block synthesis of prostaglandins, which may be effected through inhibition of cyclooxygenase and/or lipooxygenase activities. 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 (Sulaiman *et al*., 2008). It is noteworthy in this regard that cyclooxygenase and lipoxygenase enzyme inhibitory activity has been reported for *A. catechu* whole plant extract (Burnett *et al*., 2007), although the bio-active components responsible for this activity are yet to be elucidated. It is noteworthy in this aspect that quercitrin has been reported to be present in khoyer (Rajendra *et al*., 2011). Antinociceptive activity as demonstrated by inhibition of acetic acid-induced visceral pain in mice has been reported for *Bauhinia microstachya* leaf extract as well as the isolated active component from leaf, quercitrin (Gadotti *et al*., 2005). Quercitrin also reportedly demonstrated significant anti-inflammatory activity against 12-O-tetradecanoyl-13-acetate-induced mouse ear edema (Erdemoglu *et al*., 2008). Thus, quercitrin, which is present in khoyer, can account for the observed antinociceptive activity, but this remains to be established.

Diabetes mellitus is a disease currently affecting millions of people worldwide and with no known total cure in allopathic medicine. Moreover, the disease can quickly progress to cardiovascular disorders, diabetic retinopathy, and diabetic nephropathy. As such, a remedy obtained from khoyer, which even if it cannot cure the disease at least is effective in normalising the high blood sugar levels as seen in diabetic patients, can be a boon to human beings. Pain also afflicts millions of people every day in the world and can arise from various factors. Common pain killing medicines like aspirin and paracetamol suffer from adverse effects like causing gastric ulceration and hepatic damage through prolonged use or over-dosage. As such, a simple but efficacious pain killing drug from khoyer can also alleviate the sufferings of millions of people. Studies are underway in our laboratory to find out the responsible constituent(s) in khoyer for its observed antihyperglycemic and antinociceptive activities.

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