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Evaluation of anxiolytic and hypoglycemic activity of methanolic extract of *Ixora cuneifolia* in an animal model

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ABSTRACT

Objective: To investigate anxiolytic and hypoglycemic activity of *Ixora cuneifolia* (Family: Rubiaceae) in an experimental animal model.
Methods: Anxiolytic test was performed by elevated plus maze (EPM) and hole-board method. Hypoglycemic activity was measured in glucose-loaded Swiss albino mice by glucose tolerance test.
Results: The methanol extract of *Ixora cuneifolia* exhibited dose-dependent and statistically significant ($P < 0.05$) anxiolytic activity at doses of 200 and 400 mg/kg. Reduction of glucose level was observed with the highest dose 400 mg/kg of the extract in glucose tolerance test.
Conclusions: The better anxiolytic and hypoglycemic activities in the present study could be due to the presence of different chemical constituents like alkaloids, flavonoids, saponin, phenols and tannins in this methanolic extract.

1. Introduction

Nowadays, anxiety is the most common psychiatric illness which is considered as a diffuse, unpleasant, elusive sense of apprehension[1] with the common autonomic symptoms like headache, perspiration, palpitations, tightness in the chest, and mild stomach distress[2]. From ancient times many medicinal plants like passion flower, *Melissa*, *Valerian* were used around the world for the management of anxiety ailments[3]. On the other hand, diabetes is a pandemic disease characterized by hyperglycemia (elevated blood glucose level). The approximate mortality in people with diabetes is around twofold of that in people without diabetes[4]. A published survey reported 37 medicinal plants belonging to 25 families that are used in the management of diabetes in Bangladesh to avoid the adverse drug reactions of oral hypoglycemic drugs[5].

Ixora cuneifolia (*I. cuneifolia*) (Family: Rubiaceae) is an evergreen shrub, widely distributed throughout the regions of Bangladesh, India, and other Southeast Asian countries[6]. It is commonly known by the name of Kyamoachuie and Chi Shing Da Keu in Marma and Khumi communities[7]. Ethnic groups of different regions use the leaves, flowers, roots, stem and fruits of this plant for different traditional treatments of fever, abdominal pain, diarrhea, menstrual problem, tonsillitis and vomiting[8,9]. Despite the traditional use of this plant, some studies have validated its anthelmintic, antidiarrheal, thrombolytic, analgesic, anti-inflammatory and antioxidant properties[7,10].

Since the studies exploring the medicinal and pharmacological value of this plant are limited, the present study was conducted to find out some major pharmacological activities *e.g.*, anti-diabetic and anxiolytic activity of the methanolic extract of *I. cuneifolia* in mice model.

2. Materials and methods

2.1. Drugs and chemicals

The standard drugs diazepam and glibenclamide were procured from Square Pharmaceuticals Ltd., Bangladesh and tween 80 and 99% methanol were purchased from KM Scientific Ltd., Bangladesh.

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The experimental protocols were approved by the Ethical Review Committee of Noakhali Science and Technology University, and were performed in accordance with guidelines.

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2.2. Plant material

The fresh part of *I. cuneifolia* was collected from hilly forests of Balipara, Thanchi Upzilla, Bandarban in January, 2015. It was recognized and validated by an expert taxonomist of Bangladesh working in National Herbarium of Bangladesh, Mirpur, Dhaka, where a voucher specimen (accession number of 37790) was deposited for forthcoming reference.

2.3. Preparation of plant extract

The plant parts were washed gently, cut into small pieces and air dried at room temperature for 3 weeks under shade and protected from sunlight. The plant parts were pounded into coarse powder with the help of a suitable grinder. About 250 g of powdered material was added into a clean, flat-bottomed glass container and soaked in 1500 mL of 99% methanol (Merck, Germany) and preserved for 15 days with irregular shaking and stirring. The extract was then filtered using filter cloth followed by Whatman No. 42 filter paper. The obtained filtrate was concentrated using rotary evaporator (IKA, Germany) followed by drying at room temperature until dried. The resulting semi granular leftover mass was designated as crude extract and preserved at 4 ˚C until further examination.

2.4. Phytochemical screening

The fresh crude methanolic extract was subjected to qualitative phytochemical screening for the presence of carbohydrate, alkaloids, phenol, flavonoids, saponins, glycosides, proteins, fixed oils and fat which have definite medicinal and pharmacological importance by using standard procedures previously described[11,12].

2.5. Experimental animals

Adult Swiss albino mice (20–30 g) of either sex, aged 4–5 weeks, were procured from the animal house of Jahangirnagar University, Bangladesh. They were kept under standard laboratory conditions [room temperature (25 ± 2) ˚C, relative humidity 55%–65%, and 12 h light-dark cycle] and fed with ICDDR, B standard rodent food and water (*ad libitum*). The experimental protocols were approved by the Ethical Review Committee of Noakhali Science and Technology University, and were performed in accordance with guidelines. The animals were divided into four groups, each containing five mice. Mice were fasted for 18 h before the experiment and later treated through the following methods.

2.6. Anxiolytic activity test

2.6.1. Elevated plus maze (EPM) test

The apparatus consisted of two opposing open (50 × 10 cm) and two opposing closed arms (50 × 10 × 30 cm). The apparatus was kept to a height of 40 cm above floor level. The mice of four groups treated with distilled water (10 mL/kg, *p.o.*), plant extracts (200 and 400 mg/kg, *i.p.*) and diazepam (1 mg/kg, *i.p.*) respectively were individually placed in the middle of the EPM for a period of 5 min for free exploration. The time spent in both the open and closed arms was recorded for 5 min. The numbers of entries into the open and closed arms were counted during the test. An entry was defined as having all four paws within the arm[13].

2.6.2. Hole board test

The hole board test was adopted in this test[14]. The hole board was made of a wooden box (40 × 40 × 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. One hour after treatment with distilled water (10 mL/kg *p.o.*), plant extract (200 and 400 mg/kg *i.p.*) and diazepam (1 mg/kg *i.p.*), each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes. The number of head dips during a period of 5 min was recorded for individual mouse[15].

2.7. Hypoglycemic activity

This experiment was performed according to a previously reported method with minor modifications[16]. Briefly, the blood samples were collected from all groups of mice respectively. Then Groups 1–4 received normal saline 10 mL/kg, standard hypoglycemic drug (glibenclamide) 10 mg/kg, methanolic extract 200 and 400 mg/kg body weight, respectively. After 2 h blood samples were again collected from each mice of each group. Serum glucose level will be determined by means of glucometer.

The glucose tolerance test (GTT) was also performed for comparing the value of normal mice with glucose loaded mice according to the method of Joy and Kuttan[17] with minor modification[18]. In GTT all the mice were divided in the same manner as above. All treatments were orally adminstered. After one hour all mice were loaded with glucose of 3 g/kg body weight. Blood glucose levels were measured by using glucometer.

2.8. Statistical analysis

Results are expressed as mean ± SEM. The data were analysed statistically using One-way ANOVA, followed by Dunnett's *t*-test. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Phytochemical screening

The qualitative phytochemical screening showed the presence of flavonoids, saponins, alkaloid, cardiac glycoside, carbohydrate, phenol, fixed oil and fat in the methanolic extract of *I. cuneifolia* (Table 1).

3.2. Anxiolytic activity

In EPM test, *I. cuneifolia* at both doses significantly ($P < 0.05$) increased time spent and number of entries in open arms as compared to control (Table 2). On the other hand, in hole board test the dose of 400 mg/kg of the plant extract significantly ($P < 0.05$)

increased the number of head dipping without changing locomotion in the hole board test as compared to control group (Table 3).

Table 1
Phytochemical screening of methanolic extract of *I. cuneifolia.*

Phytochemical	Type of test	Result
Alkaloid	Wagner's test	+
	Hager's test	++
	Meyar's test	++
Cardiac glycoside	Legal's test	+
	Balget test	++
Carbohydrate	Molisch's test	+
	Benedict's test	+
Saponins		+
Phenol		+
Phytosterol	Libermann Burchard's test	-
Flavinoid	General test	+
	Specific test	++
Protein	Xanthoproteic test	-
	Ninhydrin test	-
Fixed oil and fat	Copper acetate test	+

+: Presence; ++: Significant presence; -: Absence.

Table 2
Results of EPM test.

Treatment	Time spend in open arm (s)	Time spend in closed arm (s)	No. of entries into open arm	No. of entries into closed arm
Control (10 mL/kg)	20.00 ± 1.14	263.00 ± 1.36	3.60 ± 0.51	11.20 ± 0.53
Diazepam (1 mg/kg)	59.80 ± 1.66*	230.00 ± 1.52*	3.40 ± 0.51	5.00 ± 0.70*
Extract (200 mg/kg)	69.40 ± 1.36*	213.00 ± 1.07*	7.00 ± 0.70*	6.40 ± 0.51*
Extract (400 mg/kg)	78.40 ± 1.21*	215.00 ± 1.36*	7.20 ± 0.73*	13.80 ± 0.58*

All values are expressed as mean ± SEM (n = 5). *: $P < 0.05$ compared with control.

Table 3
Effect of methanol extract of *I. cuneifolia* in hole board test.

Treatment	Number of head dipping
Control (10 mL/kg)	36.80 ± 0.73
Diazepam (1 mg/kg)	55.40 ± 1.20
Plant extract (200 mg/kg)	61.60 ± 0.67
Plant extract (400 mg/kg)	71.60 ± 1.07*

All values are expressed as mean ± SEM (n = 5). *: $P < 0.05$ compared with control.

3.3. Hypoglycemic activity

The results of hypoglycemic study are summarized in Table 4. Administration of extract to normal mice showed dose-dependent reduction in glucose level by 48.54% and 49.21% at 200 and 400 mg/kg body weight, respectively, which was significant compared to control; whereas glibenclamide at a dose of 10 mg/kg body weight reduced blood glucose level by 55.50%. Again in GTT the glucose loaded mice showed dose dependent and statistically significant reductions in the blood glucose level. The extract at 200 and 400 mg/kg body weight showed significant reduction in blood glucose level by 29.70% and 37.29%, respectively, while standard drug glibenclamide showed 45.54% reduction in the blood glucose level.

4. Discussion

In the present study, the phytochemical screening confirmed the presence of flavonoids, saponins, alkaloid, cardiac glycoside, phenol, fixed oil and fat, and carbohydrate. The presence of those phytochemicals in the plant may be the basis for management of various diseases[19].

EPM is considered as useful model used in evaluation of anxiolytic effect of drug. Anxiolytic agents increase the entry and time spent in open arm of the EPM[20]. Normally mice always prefer to avoid open areas and stay in darker areas. The unpleasant emotion or fright due to height bring about anxiety in mice when mice are positioned on the test apparatus. In the present study, the extract at 400 mg/kg exhibited noteworthy increase ($P < 0.05$) in time spent in open arm, which clearly discloses the anxiolytic potential of the plant extract. The hole board test was also used to evaluate the anxiolytic effect. This test easily measured the response of an animal to a weird environment, and different behaviors can be smoothly observed[21]. Standard drug diazepam significantly increased the number of head dipping in hole board test, and similarly, the plant extract at both doses (200 and 400 mg/kg) significantly increased the number of head dipping in comparison to control having no change in locomotion in the hole board. These results reveal the significant anxiolytic activity of methanolic extract of *I. cuneifolia*.

The medicinal plants containing saponins, tannins, sterols, and flavonoids have showed anxiolytic activity as previously reported[21-23]. Consequently, the perceived anxiolytic potential of the extract might be due to the binding of its constituents probably of flavonoids and saponins to the GABAA-BZDs complex. It was also described that many flavonoids were found as ligands for the GABA-A receptors in the central nervous system and to exert their action by opening activated GABA chloride channel. In hypoglycemic study and GTT, methanol extract of *I. cuneifolia* at both doses (200 and 400 mg/kg) significantly reduced glucose level compared to control. Studies suggest that phenols are responsible for anti-hyperglycemic activity[24] and the flavonoids are responsible for potentiating insulin secretion[25]. Thus hypoglycemic activity of *I. cuneifolia* may be attributed to the presence of these secondary metabolites which can act synergistically or independently. This type of mechanism is also been mentioned for the root of *Helicteres isora*[26].

Table 4
Hypoglycemic effect of *I. cuneifolia* on normal and glucose loaded mice

Treatment	Glucose level in normal mice (mmol/L)		Inhibition of glucose level (%)	Glucose level in glucose loaded mice (mmol/L)	Inhibition of glucose level (%)
	Before treatment	After treatment			
Control (10 mL/kg)	2.33 ± 0.17	2.20 ± 0.15	-	6.06 ± 0.08	-
Standard (10 mg/kg)	4.63 ± 0.33	2.06 ± 0.12	55.50	3.30 ± 0.15*	45.54
Extract (200 mg/kg)	6.86 ± 0.20	3.53 ± 0.20*	48.54	4.26 ± 0.12*	29.70
Extract (400 mg/kg)	3.80 ± 0.11	1.93 ± 0.08	49.21	3.80 ± 0.05*	37.29

All values are expressed as mean ± SEM (n = 5). *: $P < 0.05$ compared with control.

Finally, the experimental outcomes obtained in the laboratory tests may suggest an effective traditional use of this plant as an anxiolytic, and hypoglycemic agent. Hence, further study is required to identify which constituents are actually responsible for those effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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