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Investigation of Anti-hyperglycemic, Anti-allergic activities of ethanolic extract from *Brownlowia tersa* (L.) stem

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Abstract
Brownlowia tersa (L.) (Family: Malvaceae), has been used traditionally to treat inflammation, pain, diarrhea, allergic reaction etc. The aim of the recent study is to investigate the ethanolic extract of *Brownlowia tersa* (L.) stem for its anti-hyperglycemic and anti-allergic activity as well as to identify the phytochemicals constituents. Blood glucose level in mice, in anti-hyperglycemic activity, evaluated by OGTT (oral glucose tolerance test) considerably reduced by the extract at both dose 250 and 500 mg/kg b. wt. The extract also decreased significantly the allergic-like symptoms like sneezing, scratching, and nasal score induced by TDI in mice model. The count of WBC (total and differential) in both blood and Bronchoalveolar lavage (BAL) fluid were also reduced in the extract treated group. In qualitative phytochemical investigation, existence of different bioactive phytochemicals like alkaloids, flavonoids, steroids and tannins were observed. So the investigation suggests the anti-hyperglycemic and anti-allergic activity of *B. tersa* stem extract due to the availability of one or more active metabolites in it.

Keywords: *Brownlowia tersa* (L.), anti-hyperglycemic activity, anti-allergic activity.

Introduction
Hyperglycemia is defined as high blood glucose level, which is a diagnostic of having a metabolic disorder called diabetes [1]. Vascular complications are associated with the rising epidemic of type 2 diabetes and worldwide one of the dominant causes of death. The World Health Organization (WHO) recommends oral glucose tolerance test (OGTT) to diagnose diabetes. The oral glucose tolerance test (OGTT) is able to measure the glucose utilization ability of body as well as identify diabetes and pre-diabetes [2]. An oral glucose tolerance test in mice was employed to determine anti-hyperglycemic potential of the crude extract.
Allergy is nowadays vary common health complication worldwide and allergens, such as food, dirt, pollen, feather of animal, etc. are the main cause of allergic reaction [3]. When a particular allergen enters into the body results in activation of the body's immune system to exhibit an allergic reaction in a person with hypersensitivity to that allergen. Even a normally harmless foreign substance can cause allergic reactions. The body contains an immune system called an antibody (immunoglobulin E or IgE) to fight against these substances. Allergy can be diagnosed by depending on a case of symptoms due to exposure to an allergen and detecting the IgE level. Blood specific testing or skin prick testing can be used to detect allergen-specific IgE [4].
Phytochemical constituents of the medicinal plants are helpful for healing and curing various health disorders. Phytochemicals are present normally in the medicinal plant's leaves, fruits, stem, bark, and roots that can defense and protect from various diseases. These phytochemicals are mainly the secondary metabolites in the plants. Such as alkaloids, steroids, flavonoids, glycoside, saponin, tannins, terpenoids, phenolic compounds etc., which acts as responsible compounds for relief of different diseases [5].
Brownlowia tersa (L.) is a mangrove shrub from the Malvaceae family. In Bangladesh, the plant is locally known as Sundori lata [6] which is a shrub. The leaf stalk is approximately 1-2 cm long. The fruit is like woody capsule or nut and 15 mm long. Traditionally the plant has been used for a long time to cure diarrhea, dysentery etc. [7]. Leaf has been indicated to show anti-inflammatory, antioxidant, analgesic, antinociceptive, antidiarrheal and anti-diabetic and anti-allergic activities [8].

The ethanolic extract of *B. tersa* contains various phytochemicals (carbohydrates, reducing sugars, saponins, glycosides, flavonoids etc.) [9]. This present study was designed to find out whether the stem possess the antidiabetic and antiallergic therapeutic potential or not.

Materials and methods

Extract preparation

The stem of *Brownlowia tersa* (L.) accumulated from Khulna region, Bangladesh and was recognized by authority at Bangladesh National Herbarium, Mirpur, Dhaka through submitting a voucher specimen (voucher specimen no. DACB-43594). The dried stems were crushed into powder of which 400 g was macerated in 2.0 L ethanol (95%). Filtration was done after fourteen days and after drying the filtrate we got a viscous material (Yield = 2.75%). The ethanol extract of *Brownlowia tersa* (L.) stems was named as EEBT.

Chemicals and Reagents

HPLC grade ethanol was obtained from Merck (Darmstadt, Germany). Toluene 2, 4-diisocyanate (TDI) from Wako Chemical, Tokyo, Japan. Paracetamol was and the standard drug cetirizine were collected from Square Pharmaceutical Ltd., Dhaka, Bangladesh and Glibenclamide was obtained from Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh.

Phytochemical screening

To identify different chemical constituent's phytochemical screening of the crude extract was done qualitatively [10].

Experimental animal

Swiss albino mice aged 4-7 weeks (25-35 g) were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. They were nursed in the laboratory of Pharmacy Discipline, Khulna University, Bangladesh following standard guidelines for handling and caring of mice provided by the Animal Ethics Committee, Khulna University. (Approval reference number was KU/PHARM/AEC/15/06/32)

Acute toxicity study

Acute toxicity study was done using standard method (11). In the first phase, 20 mice were separated into 4 groups and 5 mice were in every category. Groups 1, 2, 3 and 4 animals were given 500 mg/kg, 1000 mg/kg, 2000 mg/kg, and 3000 mg/kg body weight of the extract, respectively. After 7 days of adaptation each mice was given a single dose. Another group of 5 mice was set up as a control group (5th group) and animals of this group received vehicle only. After administration of extract, we observed mice during the first thirty min to find the sign of toxicity or mortality and then at every 24 h for 14 days.

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was done following the procedure previously stated by Joy and Kuttan with small changes (12). After twelve hours of fasting 24 mice were allotted randomly and divided into four groups. Group-I, group-II, group-III and group-IV were administered 2% aqueous solution of tween 80, glibenclamide 10 mg/kg body weight and the extract at 250 and 500 mg/kg body weight, respectively. After 30 minutes, 10% glucose solution (2 g/kg body weight) was administered to all groups. Blood sample was collected by piercing the tail of mice and the blood glucose level was checked by through the glucometer (Accu-check Active) and was manifested in millimole per liter (mmol/L).

Anti-allergic activity test

Experimental procedure

Experimental animals were separated in five groups named six mice in every group. Group-I (control) was sensitized with ethyl acetate (10µl) bilaterally on each nasal vestibule and treated with 2% tween 80 in water orally. Group-II denoted as TDI control and was sensitized with 10µl of 5% TDI solution in ethyl acetate on the nasal vestibules and treated with 2% tween-80 in water. Group-III considered as standard group and was sensitized with TDI and treated with cetirizine (20 mg/kg body weight) orally. Group-IV and Group-V were given extract at dose 250 mg/kg and 500 mg/kg b. wt. respectively orally and TDI bilaterally on the nasal vestibules. This sensitization procedure was once a day for five recurrent days, then reduplicated after a 2-days gap. After 9 days of the 2nd sensitization, 10 µl of 5% TDI solution was again applied to instigate nasal allergic reaction [13].

Assessment of allergy-like symptoms

After provocation animals were separated in different cases and nasal allergic reaction like number of sneezes, scratch and nasal score were observed for ten minutes. Nasal score was calculated as described previously [13].

Blood sample collection and analysis

After 24 hr of provocation, mice were anesthetized and blood samples were gathered from the cervical vein following previous method of Mahajan *et al.* [14]. Then, the blood was collected into a heparinized tube which was used for total and differential count of WBC. Blood slides were prepared and stained with Leishman reagent to do differential analysis. Slides were dried and examined through microscope.

BAL fluid collection and analysis

The BAL (Broncho Alveolar Lavage) fluid was collected after 24 hours of provocation following the procedure described by Olsen *et al*. with little changes [15]. The BAL fluid was collected from Trachea by using 0.9% Sodium Chloride solution. Then the fluid was centrifuged and sediment was used to prepare the slide for WBC count.

Statistical analysis

Every data was statistically evaluated using Student's *t*-test and visualized graphically in Microsoft Excel 2013. Findings are presented as mean ± SEM and $p < 0.05$ was deliberated as limit for statistical significance.

Results

Screening of phytochemical constituents

Phytochemical screening of the crude ethanol extract of *Brownlowia tersa (*L.) stem exhibited the presence of tannins, flavonoids, phenolic compounds, alkaloids, glycosides, saponins, and terpenoids and steroids.

Acute toxicity study

In this test, any fatality or adverse reactions were not observed in the exploratory time at the dose up to 3000 mg/kg body weight. The results suggest that the LD50 of the extract is above 3000 mg/kg body weight. This finding reports that the t extract is therapeutically safe.

Measurement of anti-hyperglycemic action

In oral glucose tolerance test, the ethanolic stems extract with the dose of 250 mg/kg and 500 mg/kg body weight represented considerable reduction in blood glucose in respect with control mice.

Table 1: Effect of *B. tersa* stem extract on oral glucose tolerance.

Treatment group	Fasting stage (mMol/L)	30min (mMol/L)	90 min (mMol/L)	150 min (mMol/L)
Control	4.38±0.48	14.04±0.12	8.42±0.34	6.06±0.31
Standard (10 mg/kg)	3.8±0.29	4.72±0.24***	3.76±0.40***	3.12±0.29***
Extract (250 mg/kg)	4.3±0.21	7.62±0.35***	6.84±0.31*	4.8±0.38*
Extract (500 mg/kg)	4.64±0.38	8.78±0.35***	7.16±0.38*	4.6±0.26*

Values are represented as mean ± SEM (n=6). *p<.05 vs. control, ** p<.001 vs. control, ***p<.0001 vs. control

Anti-allergy test

Assessment of allergy-like symptoms

Use of TDI (Intranasal) provocked nasal allergic reaction such as sneezing, watery rhinorrhea, redness, swelling and scratching. After sensitization of mice through TDI, the total number of sneezes, scratches and nasal score were measured. Oral administration of ethanolic extract of *B. tersa* stem reduced significantly the number of sneezes, scratches and the nasal score. In case of scratch and nasal score at a dose of 500 mg/kg, efficiency of extract was similar with the antihistamine but the efficacy was less than the antihistamine in case of sneezes at both doses of the extract (Table 2).

Table 2: Measurement of allergic symptoms in mice.

Group	No. Of sneezes	No. Of Scratch	Nasal score
Control	0.5±0.34	12±2.1	0
TDI control	32.5±4.4$^{\#}$	286.3333±23.21$^{\#}$	2.95±0.28**
Standard	11.5±1.78*	114.33±12.73***	0.533±0.15*
Test-I (250 mg/kg)	21.17±1.25*	189.5±20.66*	2.33±0.33*
Test-I (500 mg/kg)	14.17±1.25*	136±10.68**	1.15±0.412*

Values are represented as mean ± SEM (n=6). $^{\#}$p<0.001 vs. control,
$^{***}p$< 0.0001 vs. TDI control, $^{**}p$<0.001 vs. TDI control, $^{*}p$<0.05 vs. TDI control

Assessment of blood sample

The experiment found that the total number of leukocytes, lymphocytes, neutrophils, eosinophils and monocytes went up in the blood sample of TDI-control mice. However, administration of extract (orally) at 250 and 500 mg/kg body weight significantly lowered the total count of WBC comparing to TDI-control.

Table 3: Effect of extract on WBC counts in blood

Group	× 10^3 cells /ml					
	TC (WBC)	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
Control	8.33±0.15	5.367±0.17	2.47±0.12	0.37±0.08	0.026±0.02	0.12±0.054
TDI control	11.18±0.12$^{\#}$	7.14±0.11 $^{\#}$	3.72±0.10 $^{\#}$	1.03±0.02 $^{\#}$	0.18±0.05$^{\#}$	0.02±0.02
Standard	8.97±0.25 ***	4.72±0.15 ***	2.81±0.06 ***	0.41±0.05 ***	0.04±0.02*	0.09±0.02 *
Test-I (250 mg/kg)	9.13±0.15 ***	5.70±0.16 ***	2.66±0.09	0.69±0.06 **	0.09±0.05	0±0
Test-II (500 mg/kg)	5.46±0.07 ***	2.97±0.09 ***	2.30±0.04 ***	0.18±0.037***	0.01±0.01*	0±0

Values are represented as mean ± SEM (n=6). #p< .05 vs. Control. ***p< .0001 vs. TDI control, **p< .001 vs. TDI control *p< .05 vs. TDI control.

Table 4: Effect of extract on WBC counts in BAL fluid

Group	× 10^3 cells /ml					
	TC (WBC)	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
Control	10.58±.1562	7.20±.1821	2.31±.04	.87±.05	0.17±.07	0.04±.03
TDI control	12±.16#	7.81±.37	3.02±.24#	1.10±.04#	0.05±.03	0.02±.02
Standard	9.48±.18***	5.89±.06*	2.92±.03	0.77±.04**	0.23±.18	0.39±.24
Test-I (250 mg/kg)	10.8±.07***	6.66±.319*	2.83±.07*	0.84±.04	0.21±.09***	0.04±04
Test-II (500 mg/kg)	4.92±.17***	2.59±.11***	2.15±.11*	0.12±.01***	0.22±.18	0.009±0.01

Values are represented as mean ± SEM (n=6). #p< .05 vs. control, ***p<.0001 vs. TDI control, **p< .001 vs. TDI control, *p< .05 vs. TDI control

Discussion

Plants contained different types of effective secondary metabolites that used in therapeutic purpose from ancient age and also open opportunities for synthesis of new drug leads [16]. The phytochemical screening showed that the ethanolic extract of *B. tersa* have several secondary metabolites like reducing sugar, tannins, flavonoids, alkaloids and steroids and they have medicinal values and physiological activity [17]. The presence of these active metabolites in the *B. tersa* (L.) stem may be the basis of pharmacological activity of the extract. In an oral glucose tolerance test, *B. tersa* stem extract showed significant anti-hyperglycemic activity. At the doses of 250 mg/kg and 500 mg/kg body weight lowered considerably the blood glucose level at different time intervals comparing to control (Table 1).

After a meal, the amount glucose in the circulation is increased. The rate of exogenous delivery can be greater than two time of the post absorptive endogenous glucose generation [18]. Exogenous glucose absorption suppresses endogenous glucose generation, and increases the use of

glucose by liver, muscle, and fat tissue. As a result, exogenous glucose is accumulated, and the concentration plasma glucose backs to probable stravation state [19].

Flavonoids are phenolic compounds which are widely found on plants. These compounds have reported to have biological activities such as hypoglycemic effects [20]. Isolating alkaloids from several medicinal plants, exerted a broad range of antidiabetic activities through different mechanisms (21). Hypoglycemic effects of tannin containing extract was proved in an investigation on streptozotocin (STZ) induced hyperglycemic mice [22].

However, at the dose of 250 mg/kg and 500 mg/kg, the extract decreased significantly the allergic reactions such as sneezing, scratching, and nasal score (Table 2). In both doses the WBC count significantly decreased in blood and BAL fluid. At 500 mg/kg, the extract decreased more rapidly the WBC count in blood and BAL fluid. Cross-linking the allergens perform IgE tied to the high-proneness receptor FcεRI on mast cells, allergic symptoms are triggered. Mast cells aware the immune system to local infection [23]. The action of anti-allergic agents is to prevent the relief of mediators of inflammation or inhibit the functions [24].

Flavonoids are natural products that have antioxidant, anti-inflammatory, anti-allergic functions, and immune-modulating effects used as complementary and alternative medicine (25). The anti-hyperglycemic and anti-allergic effects of *B. tersa* may be because of the availability of bioactive phytochemicals such as flavonoids, alkaloids, and tannins.

Conclusion

To sum up, the ethanolic extract of *B. tersa* stem contains many important phytochemicals such as flavonoids, steroids, alkaloids, and tannins. The extract represented significant anti-hyperglycemic activity. The extract also considerably decreased the allergic ractions like sneezing, scratching, and nasal score. The total and differential count of WBC of both blood and BAL fluid were also reduced (Table 3 and 4). So the extract may contain anti-allergic action. More investigations are necessary to identify the effective principles liable for anti-hyperglycemic and anti-allergic activities

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