

Antihyperglycemic and antinociceptive activities of methanolic extract of *Euphorbia thymifolia* L. whole plants



Mohammed Rahmatullah, Sumaiyah Kanij Hasan, Zulfiqar Ali, Shahnaz Rahman, Rownak Jahan

Department of Biotechnology and Genetic Engineering, Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka 1205, Bangladesh

OBJECTIVE: To study the antihyperglycemic and antinociceptive activities of methanolic extract of the whole plant of *Euphorbia thymifolia* L., a plant used in folk medicine of Bangladesh for treatment of diabetes and pain.

METHODS: Antihyperglycemic activity studies were conducted in glucose-loaded mice by oral glucose tolerance tests. Mice were given various doses of the extract, followed by glucose (2 g/kg body weight), 1 h after administration of the extract. Serum glucose levels were measured 2 h after glucose administration. Antinociceptive activity studies were conducted in intraperitoneally acetic acid-injected mice through measurement of reductions in abdominal writhing times caused by acetic acid-induced gastric pain. Following a period of 1 h after oral administration of various doses of the extract, all mice received intraperitoneal injection of 1% acetic acid at a dose of 10 mL/kg body weight. To ensure bioavailability of acetic acid, a period of 5 min was given to each animal following which period the number of writhings was counted for 10 min.

RESULTS: The extract caused a significant dose-dependent reduction in serum glucose levels in mice, when administered at doses of 50, 100, 200 and 400 mg/kg body weight as compared to the control animals ($P < 0.05$). The highest reduction of serum glucose (60.5%) was observed at a dose of 400 mg/kg. In comparison, a standard antihyperglycemic drug glibenclamide, when administered at a dose of 10 mg/kg body weight, lowered serum glucose levels by 48.6%. The extract also demonstrated a significant dose-dependent antinociceptive activity compared to control animals ($P < 0.05$). At a dose of 400 mg/kg body weight, the number of abdominal writhings was inhibited by 40.9% as compared to 49.0% inhibition obtained with a standard antinociceptive drug aspirin, administered at a dose of 200 mg/kg

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body weight.

CONCLUSION: The significant antihyperglycemic and antinociceptive activities demonstrated by the extract validate the use of *E. thymifolia* in folk medicine of Bangladesh for treatment of diabetes and pain, and merit further scientific studies leading to discovery of efficacious drugs.

KEYWORDS: *Euphorbia*; plant extracts; hypoglycemic agents; analgesics; mice

Euphorbia thymifolia L. (Euphorbiaceae) is found in wild and neglected areas of Bangladesh. The plant is used in folk medicinal system of the country for treatment of hypertension, heart diseases, helminthiasis and leucorrhoea. It is also occasionally used for treatment of diabetes and pain. The plant is bitter in taste and used in traditional Indian medicine for constipation, helminthiasis, ringworm, skin diseases and leprosy^[1]. The leaves and seeds are used for bowel disturbances in children and are considered laxative^[2]. Antibacterial activity has been reported for the plant^[3]. Flavonoids extracted from the aerial parts of the plant reportedly showed high cytotoxicity on human epidermoid cancer cells and moderate antiviral activities^[4]. Antioxidant and antiviral activities have also been reported for methanol, chloroform, ethyl acetate, *n*-butanol and aqueous extracts of the plant as well as several isolated pure compounds like rugosin B, 3-*O*-galloyl-4, 6-(*S*)-HHDP-D-glucose and 1, 3, 4, 6-tetra-*O*-galloyl-K-beta-D-glucose^[5]. Ethyl acetate fraction of the plant reportedly exhibited anti-herpes simplex virus-2 activity *in vitro*^[6].

Diabetes is a debilitating disease affecting millions of people worldwide which is projected to rise because of changes in lifestyle. Pain, resulting from various causes, also affects millions of people worldwide on a daily basis. Modern allopathic medicine has no known cure for diabetes. Although there are effective medicines for pain like aspirin or paracetamol, these medicines can cause side effects like gastric ulceration and hepatic damages. The folk medicinal practitioners of Bangladesh (Kavirajes) claim to have effective treatments for both diabetes and pain without resultant toxicity as occurring with allopathic pain killers. We had

been screening the various anti-diabetic and antinociceptive medicinal plants used by the Kavirajes for some time to determine their actual efficacies for the purposes that they are used for^[7-10]. The objective of the present study was to evaluate the antihyperglycemic and antinociceptive activities of methanolic extract of *E. thymifolia* whole plants.

1 Materials and methods

1.1 Plant materials and extraction Whole plants of *E. thymifolia* were collected from Dhaka district, Bangladesh in November 2010. The plant was taxonomically identified by the Bangladesh National Herbarium at Dhaka (accession number: 35394). The whole plants were air-dried in the shade for 120 h, grounded into a fine powder, and were extracted with methanol at a ratio of 1 : 6.25 (weight/volume). The initial weight of dried powder used for extraction was 80 g; the final weight of the methanol extract obtained from the dried powder was 5.96 g (yield = 7.45%).

1.2 Chemicals and drugs Glibenclamide, aspirin and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

1.3 Animals In the present study, Swiss albino mice (male), which weighed between 17 to 22 g were used. The animals were obtained from the International Center for Diarrheal Disease Research, Bangladesh. All animals were kept under ambient temperature with 12 h light followed by 12 h dark. The animals were acclimatized for 3 d prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh.



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1.4 Antihyperglycemic activity Glucose tolerance property of the methanol extract of the whole plant was determined as per the procedure previously described by Joy *et al*^[11] with minor modifications. In brief, fasted mice were grouped into six groups with six mice in each group. The various groups received different treatments. Group 1 received vehicle (1% Tween 80 in water, 10 mL/kg body weight) and served as the normal control; group 2 received standard drug (glibenclamide, 10 mg/kg body weight); groups 3 to 6 received methanol extract of *E. thymifolia* whole plant at doses of 50, 100, 200 and 400 mg/kg body weight, respectively. Doses were prepared by thoroughly suspending methanol extract in 1% Tween 80 in water. Each mouse was weighed and dose-adjusted accordingly prior to administration of the vehicle, standard drug, and test samples. All substances were orally administered. Following a period of 1 h, all mice were orally administered 2 g/kg glucose. Blood samples were collected 2 h after the glucose administration by puncturing heart of the mice. Serum glucose levels were measured by glucose oxidase method^[12].

1.5 Antinociceptive activity Antinociceptive activity of the methanol extract of *E. thymifolia* whole plant was examined by using previously described procedures^[13]. Briefly, mice were divided into seven groups with six mice in each group. Group 1 served as the normal control and was administered vehicle only; groups 2 and 3 were orally administered standard antinociceptive drug aspirin at doses of 200 and 400 mg/kg body weight, respectively. Groups 4 to 7 were administered methanolic extract of *E. thymifolia* whole plant at doses of 50, 100, 200 and 400 mg/kg body weight, respectively. Following a period of 1 h after oral administration of the standard drug or extracts, all mice received intraperitoneal injection of 1% acetic acid at a dose of 10 mL/kg body weight. To ensure bioavailability of acetic acid, a period of 5 min was given to each animal following which period the number of writhings was counted for 10 min.

1.6 Statistical analysis Results were expressed as mean ± standard error of mean. Statistical analysis was performed using one-way analysis of variance by a statistical package SPSS version 15.0. To determine the significant differences

between different groups, LSD-*t* test was done. $P < 0.05$ implied statistical significance.

2 Results

2.1 Antihyperglycemic activity In oral glucose tolerance test conducted with glucose-loaded mice, the methanolic extract demonstrated a significant dose-dependent antihyperglycemic activity. Following administration of the standard antihyperglycemic drug glibenclamide, serum glucose concentration was significantly reduced by 48.6% ($P < 0.05$). All treatments with various doses of the extract led to significant reductions in serum glucose levels as compared to the control group ($P < 0.05$). The extract, when administered at doses of 50, 100, 200 and 400 mg/kg body weight reduced serum glucose concentrations by 30.8%, 42.0%, 47.6%, and 60.5%, respectively. Even at a dose of 200 mg/kg, the result can be favorably compared with that of the glibenclamide group. The results are shown in Table 1.

2.2 Antinociceptive activity The extract also showed dose-dependent reductions in the number of writhings in mice induced by acetic acid intraperitoneal administration in the antinociceptive activity test. The results were significant for all doses of extract-administered mice, when compared to the control group ($P < 0.05$). At the highest dose of the extract tested (400 mg/kg), the number of writhings was reduced by 40.9%. In contrast, a standard antinociceptive drug aspirin reduced the number of writhings by 49.0% and 67.3%, respectively, when administered at doses of 200 and 400 mg/kg body weight. The results are shown in Table 2.

3 Discussion

Antihyperglycemic and antinociceptive activities of the extract were measured in two separate groups of animals. This is the standard procedure as reported in scientific literature, for the two procedures for measurement of the two activities are different. If using the same set of experiment on the same group of animals then there could be complications in the results obtained. For instance, the pain created in mice by intraperitoneal administration of acetic acid and the consequent writhing times may affect the levels of blood glucose in the experimental animals which may be erroneously

Table 1 Antihyperglycemic activity evaluation of the methanolic extract of whole plant of *Euphorbia thymifolia* in mice
(Mean ± standard error of mean)

Group	n	Serum glucose level (mg/L)	Lowering of serum glucose level (%)
Control (10 mL/kg 1% Tween 80, p.o.)	6	1 288.3 ± 42.3	—
Glibenclamide (10 mg/kg, p.o.)	6	662.2 ± 79.2*	48.6
<i>Euphorbia thymifolia</i> extract (50 mg/kg, p.o.)	6	891.9 ± 79.2*△	30.8
<i>Euphorbia thymifolia</i> extract (100 mg/kg, p.o.)	6	747.7 ± 101.3*△	42.0
<i>Euphorbia thymifolia</i> extract (200 mg/kg, p.o.)	6	675.7 ± 86.0*	47.6
<i>Euphorbia thymifolia</i> extract (400 mg/kg, p.o.)	6	509.0 ± 47.5*	60.5

* $P < 0.05$, vs control group; △ $P < 0.05$, vs glibenclamide group. p.o.: per oral.

Table 2 Antinociceptive activity evaluation of the methanolic extract of whole plant of *Euphorbia thymifolia* in mice (Mean±standard error of mean)

Group	n	Number of writhings	Inhibition (%)
Control (10 mL/kg 1% Tween 80, p.o.)	6	8.17±0.79	—
Aspirin (200 mg/kg, p.o.)	6	4.17±0.65*	49.0
Aspirin (400 mg/kg, p.o.)	6	2.67±0.88*	67.3
<i>Euphorbia thymifolia</i> extract (50 mg/kg, p.o.)	6	5.50±1.38*▲	32.7
<i>Euphorbia thymifolia</i> extract (100 mg/kg, p.o.)	6	5.33±1.05*▲	34.8
<i>Euphorbia thymifolia</i> extract (200 mg/kg, p.o.)	6	5.00±0.37*	38.8
<i>Euphorbia thymifolia</i> extract (400 mg/kg, p.o.)	6	4.83±0.40*	40.9

* P<0.05, vs control group; ▲P<0.05, vs aspirin group (400 mg/kg). p.o.: per oral.

attributed to the extract. However, the extract had both antihyperglycemic and antinociceptive activities (as separately determined) which can be utilized in treatment of pain in diabetic animals as well as used separately for treatment of diabetes or pain. Notably, this applies to the extract only. If antihyperglycemic and antinociceptive activities are due to different phytochemical constituents of the extract which is possible, then administration of isolated responsible phytochemicals will demonstrate only one type of activity.

The observed lowering of blood glucose level by the extract may be achieved through various individuals or a combination of mechanisms. The extract may have potentiated pancreatic secretion of insulin, increased glucose uptake from serum, or decreased glucose absorption from gut^[14-16]. The potent antihyperglycemic activity exhibited by the extract merits further studies for isolation of responsible phytochemical components and such studies are now being actively pursued in our laboratory.

Acetic acid-induced writhing test can detect both central and peripheral analgesia^[13]. Intra-peritoneal administration of acetic acid leads to occurrence of pain, which is caused mainly through increased production of prostacyclin and prostaglandin E leading to excitation of the A-delta nerve fibers and so causing sensation of pain^[17]. Therefore, any agent that lowers the number of abdominal constrictions will demonstrate analgesia by inhibition of prostaglandin synthesis. It is under investigation whether the extract contains components which can inhibit the synthesis of prostaglandins and so decrease the number of writhings induced by acetic acid in mice.

Taken together, both antihyperglycemic as well as antinociceptive effects observed in *E. thymifolia* extract validate the folk medicinal uses of this plant for treatment of diabetes and pain. Many allopathic drugs like aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine have been discovered through observing indigenous medicinal practices^[18]. It is expected that screening of folk medicinal plants of Bangladesh and scientific validation of their relevant pharmacological activities can open up new avenues for discovering more efficacious drugs and at the

same time spur the conservation efforts of the plant species because many of these medicinal plants are rapidly becoming endangered through over-exploitation, spread of human habitat, and destruction of forest areas.

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5 Competing interests

The authors declare that they have no competing interests.

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千根草甲醇提取物的抗高血糖及镇痛作用

Mohammed Rahmatullah, Sumaiyah Kanij Hasan, Zulfiquar Ali, Shahnaz Rahman, Rownak Jahan
Department of Biotechnology and Genetic Engineering, Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka 1205, Bangladesh

目的:本研究旨在证实孟加拉国传统药用植物千根草甲醇提取物的抗高血糖及镇痛作用。

方法:实验分为独立的两个部分。在研究千根草甲醇提取物抗高血糖作用的实验中,36只小鼠被分为6组,每组6只,分别给予口服不同剂量的千根草甲醇提取物、降血糖药格列本脲及10 mL/kg 1% Tween 80 溶剂,1 h后,予各组小鼠口服2 g/kg 葡萄糖,2 h后测取所有小鼠的血糖水平并进行比对。为观察千根草甲醇提取物的镇痛作用,另外42只小鼠被分为7组,每组6只,分别给予各组小鼠口服10 mL/kg 1% Tween 80 溶剂、不同剂量千根草甲醇提取物,或200和400 mg/kg 镇痛药阿司匹林1 h后,向所有小鼠腹腔内注射10 mL/kg 1% 醋酸诱发扭体反应。诱发扭体反应5 min后,每只小鼠观察10 min,记录扭体次数。

结果:分别口服50、100、200和400 mg/kg 千根草甲醇提取物的4组小鼠与对照组相比血糖水平显著降低($P < 0.05$)。当小鼠口服400 mg/kg 千根草甲醇提取物时,血糖水平下降最多。口服10 mg/kg 格列本脲的小鼠血糖水平下降48.6%。与对照组相比,甲醇提取物同样被证实具有显著的镇痛作用($P < 0.05$)。当服用剂量在400 mg/kg时,小鼠扭体次数抑制率达40.9%,而口服200 mg/kg 镇痛药阿司匹林时,小鼠扭体次数抑制率为49.0%。

结论:孟加拉国民间草药千根草在治疗糖尿病与疼痛方面有显著作用,其甲醇提取物抗高血糖及镇痛的作用值得进一步研究,以便开发出以此植物为基础的新型化合物与有效药。

关键词:大戟属; 植物提取物; 降血糖药; 镇痛药; 小鼠