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Research Article

ANTIDIABETIC AND CYTOTOXIC ACTIVITIES OF METHANOLIC EXTRACT OF *TABERNAEMONTANA DIVARICATA* (L.) LEAVES IN ALLOXAN INDUCED MICE

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ABSTRACT

Tabernaemontana divaricata a common garden plant in tropical countries has been used as a traditional medicine. The present investigation was designed to evaluate the antidiabetic activity of the methanol extract of leaves of *T. divaricata* on alloxen indueced diabetic mouse model. The extract was given intraperitonially at different doses (300 and 400 mg/kg body weight) and the blood glucose levels were measured at 0, 2, 6, 12 and 16 hours of the study period. The antihyperglycemic effect of the extract was compared with metformin, a standard drug. The extract of 400 mg/kg reduced maximum blood glucose level at 12th hour of the treatment period from 14.19±0.47 to 6.86±0.41 mmol/L. The extract was also subjected to Brine shrimp lethality bioassay. LC_{50} value of the extract was 27.87 µg/ml. So, the present results showed that *T. divaricata* has potential (P<0.05) antidiabetic activity and low cytotoxicity as compared to standard drugs.

Key words: Antidiabetic, Alloxan, Cytotoxicity, *Tabernaemontana divaricata*

INTRODUCTION

Diabetes is a chronic metabolic disease that changes the way the body uses glucose for energy. It is characterized by high levels of blood glucose resulting from defects in insulin production. The overall prevalence of diabetes is approximately 10 percent of the population, of which 90 percent is type 2. There are estimated 246 million people worldwide sufferings from diabetes [1]. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 [2]. It is predicted by 2030, India, China and the United States will have the largest number of people with diabetes [3]. The long term manifestation of this disease can result in the development of vascular disorders such as retinopathy, nephropathy, neuropathy, and angiopathy [4]. In conventional therapy, Type 1 diabetes is treated with exogenous insulin and Type 2 with synthetic oral hypoglycemic agents as monotherapy or in combination to achieve better glycemic regulation. The synthetic oral hypoglycemic agents can produce serious side effects. In addition they are not considered safe for use during pregnancy [5]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated [6]. So as a part of the ongoing research works to find out noble antidiabetic agents, here we study the leaves of the *T. divaricata*.

Tabernaemontana divaricata (Linn.) Roem. & Schult is an evergreen ornamental plant known as Tagar belongs to the family Apocynaceae distributed throughout Bangladesh and other parts of the South-East Asia. The phytochemistry and a number of chemical constituents such as alkaloids, terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes from the leaves, stems, and roots have been reported previously [7,8,9,10].The most common medicinal use of crude *T. divaricata* extract involves its antimicrobial action against infectious diseases such as syphilis, leprosy, and gonorrhoea, as well as its antiparasitic action against worms, dysentery, diarrhoea, and malaria [11]. It is also used as tonic to the brains, liver and spleen. The pharmacological properties of *T. divaricata* are antioxidant, anti-infection [12], anti-ulcer [13], analgesia [14]and the enhancement of cholinergic activity in both peripheral and central nervous systems [15]. To the best of our knowledge, no scientific data regarding the antidiabetic effect of *T. divaricata* leaves. Thus the present study was undertaken to evaluate the hypoglycemic effect of methanolic extract of *T. divaricata* leaves.

MATERIALS AND METHODS

Plant material

The leaves of *T. divaricata* were collected from Chittagong, Bangladesh during the month of April and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Preparation of Extract

The leaves were dried under shade and ground. The ground leaves (200 gm) was soaked in sufficient amount of methanol for one week then filtered through a cotton plug followed by Whitman filter paper number 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Experimental Animals

Male Swiss Albino mice (20-25gm) were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12 hours light and dark cycle. Animals were allowed free access to drinking water and pellet diet, collected from ICDDRB Dhaka. Mice were acclimatized for 7 days.

Drugs and Chemicals

Following is the list of chemicals used. Alloxan Monohydrate (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), Metformin Hydrochloride (Square Pharmaceuticals Ltd., Pabna, Bangladesh), Methanol. All other chemicals and reagent used were of analytical grade.

Induction of Diabetes

Alloxan was first weighed individually for each animal according to its weight and then solubilized with 0.2 ml sterile saline (154mM NaCl) just prior to injection. Diabetes was induced by injecting it at a dose of 100 mg/kg b. wt., intraperitonially after overnight fasting. After 48 hours, fasting blood glucose levels of 13 to 15 mmol/L were separated and included in the study. The leaves extracts were administered intraperitoneally after dissolving in dimethyl sulfoxide (DMSO) vehicle at doses of 300 and 400 mg/kg b. w. Standard drug, metformin hydrochloride was injected in the same route at the dose of 150 mg/ kg b. w.

Antidiabetic Effect

In the experiment, a total 25 Swiss Albino mice about 20-25 gm; 4-6 weeks were used and divided randomly into four groups (five mice in each group). Treatment was done for 16 hrs as follows

Group A: Normal control
Group B: Diabetic control
Group C: Standard control
Group D: Methanolic Extract (300 mg/kg)
Group E: Methanolic Extract (400 mg/kg)

Group B–E received a single dose of alloxan (100 mg/kg i.p.) after overnight fasting. Group-A received only DMSO as normal control group and Group-B was diabetic control group, which did not receive either metformin, or plant extract. Metformin and extract were injected intraperitoneally to Group-C, Group-D and Group-E respectively after 48 hours of alloxan injection. Blood samples were then analyzed for blood glucose content at 0, 2, 6, 12 and 16 hours respectively using a glucometer kit (Accu-Check active, Roche Diagnostic GmbH, Mannheim, Germany).

Statistical Analysis

The experimental data are presented as the means ± SEM. The differences between the groups were considered as significant at **P*<0.05 by student's T-test and Tukey's test using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

BRINE SHRIMP LETHALITY BIOASSAY

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [16, 17]. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The dried cyst of the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with strong aeration for 48 hours day/dark cycles to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method [16]. The test sample (extract) were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 12.5, 25, 50, 100, 200 and 400 µg/ml. A vial containing 50 µL DMSO diluted to 5 mL was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental and control vials. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \frac{N_t}{N_0} \times 100$$

Where, N_t = Number of killed nauplii after 24 hrs of incubation,

N_0 =Number of total nauplii transferred i.e 10.

The LC_{50} (Median lethal concentration) was then determined using Probit analysis.

RESULTS

Antidiabetic Effect

The effect of single intraperitoneal injection of methanol extract of *T. divaricata* leaves on blood glucose levels in normal and diabetic mice are shown in Table 1 and Figure 1. Following a 48 hours post alloxan injection, all diabetic mice exhibited hyperglycemia, which ranged between 13 and 15 mmol/L while normal control mice showed a normal blood sugar level of about 6 mmol/L. After treatment, the blood glucose levels were decreased both in positive control and test control groups. Maximum reduction of 52.43% of blood glucose level was observed for the extract of 400 mg/kg at 12th hour of the 16 hours experimental period and it was comparable with standard drug metformin which showed maximum reduction of 64.14% blood glucose level at the dose of 150 mg/kg. So, the extract showed significant antihyperglycemic activity in alloxan induced diabetic model.

Table 1: Antidiabetic effect of leaves extract of *T. divaricata* on diabetic mice

Group	Treatment	Blood glucose level (mmol/L)				
		0 hr	2 hr	6 hr	12 hr	16 hr
A	Normal Control	6.44±0.17	6.20±0.28	6.31±0.42	6.03±0.74	6.22±0.28
B	Diabetic Control (Vehicle)	14.61±0.40	14.53±1.07	14.35±0.26	14.42±0.53	13.99±0.45
C	Metformin (150 mg/kg)	14.01±0.34	10.88±0.16*	6.77±0.35*	5.17±0.52*	6.24±0.43*
D	Methanolic Extract (300 mg/kg)	13.92±.027	11.28±1.02	8.76±0.54*	7.30±0.46*	9.13±0.37*
E	Methanolic Extract (400 mg/kg)	14.19±0.47	12.19±0.31*	8.10±0.40*	6.86±0.41*	8.35±1.14

The results are expressed as mean ± SEM (Standard Mean Error), n = 5. *P<0.05 indicates significant activity comparing with diabetic control group.

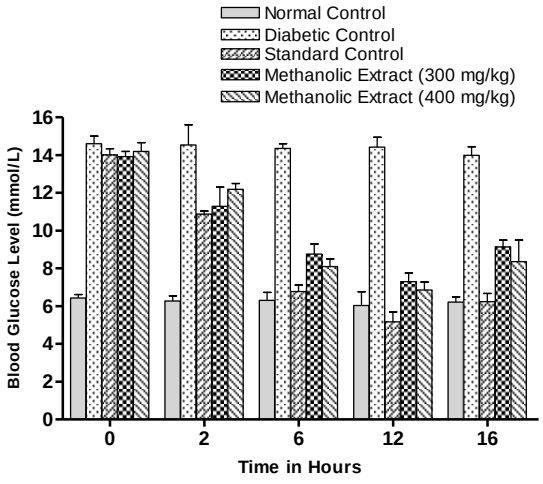

Figure 1: Effect of leaves of *T. divaricata* in lowering blood glucose level.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality results of the methanol crude extract of *T. divaricata* leaves is shown in Figure 2 and LC_{50} calculated value is recorded in Table 2. The crude extract showed positive result, indicating that the samples are biologically active. Crude extracts resulting in LC_{50} value of less than 1 mg/ml are considered as significantly active which suggest that the *T. divaricata* crude extract, with LC_{50} value of 27.87 µg/ml, has a very low toxicity. Vincristin sulphate served as the positive control for this brine shrimp lethality assay and its LC_{50} value was 10.51 µg/ml. No mortality was found in the control group, using DMSO and sea water.

DISCUSSIONS

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. According to World Health Organization, about 80% of the world's population presently uses phytotherapy for some aspect of primary health care system [18]. A large number of world's population who live in developing countries can not take the benefits of modern pharmaceuticals as those are very expensive. Hence, phytotherapy is still a popular means of primary healthcare for which people bear a little or no cost. Among the 120 active compounds currently isolated

from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [19]. Approximately 25 percent of modern drugs used in the United States have been derived from plant origins [18]. So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.

Our present study revealed that methanol leaves extract of *T. divaricata* has significant effect in lowering fasting blood glucose level in alloxan induced diabetic mice. Blood sugar levels were then raised slightly for both extract and metformin treated mice group till observation probably due to loss of their duration of action. So, the leaves extract has considerable hypoglycemic activity considering the blood sugar level in standard and diabetic control. In Brine shrimp lethality bioassay, the extract did not show considerable cytotoxicity comparing standard drug vincristine sulphate.

Table 2: Brine shrimp cytotoxicity of methanolic extract of *T. divaricata* leaves

Conc. (µg/ml)	Log C	Total	Alive	Death	% Mortality	Probit	LC_{50} (µg/ml)
12.5	1.09691	10	10	0	0	-	27.87
25	1.3979	10	8	2	20	4.16	
50	1.69897	10	5	5	50	5	
100	2	10	3	7	70	5052	
200	2.30103	10	1	9	90	6.28	
400	2.60206	10	0	10	100	-	

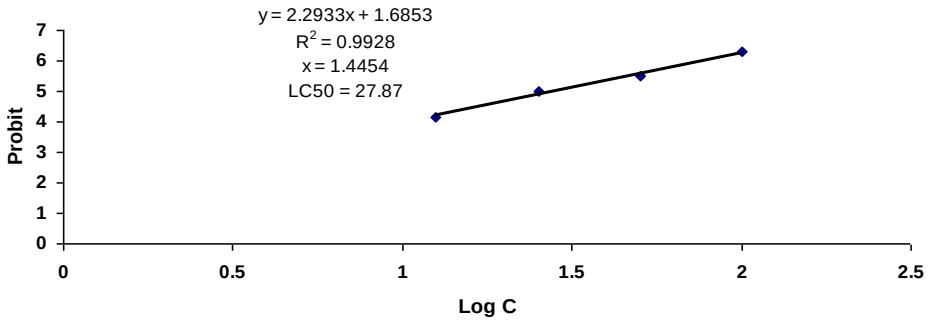

Figure 2: Determination of LC_{50} values for extract of leaves of *T. divaricata* from linear correlation between log concentrations versus Probit value

CONCLUSION

T. divaricata has traditionally been used to treat a number of diseases. Here experimental studies of leaves extract exhibited considerable antidiabetic activity and low cytotoxicity. So, further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for antidiabetic and cytotoxic activities and their mode of actions. The long term toxic effect and its protective effects on the pancreas should also be elucidated.

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