

Antidiabetic and thrombolytic effects of ethanolic extract of *Spilanthes paniculata* leaves

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Abstract: Experimental studies explored the antidiabetic and thrombolytic effect of several *Spilanthes* species in various animal models, but previously no study was conducted to establish the antidiabetic and thrombolytic potentiality of *Spilanthes paniculata*. The present study investigate the antidiabetic and thrombolytic effects of ethanolic extract of *Spilanthes paniculata* leaves with the intention to find the drug for diabetes and thrombosis management from natural sources. The hypoglycemic effect of the extracts was tested in normal and alloxan-induced diabetic mice. Blood glucose level was measured according to glucose oxidase method. The thrombolytic activity was assessed by using human erythrocyte and the results were compared with standard streptokinase (SK). In the present research, the ethanolic extract of *Spilanthes paniculata* reduces the blood glucose level at a dose and time dependant manner. It was observed that the plant possess significant antidiabetic activity ($P < 0.05$) at higher dose (450 mg/kg body weight) when compared with standard drug glibenclamide. The extract, at a dose of 150, 300 and 450 mg/kg body weight showed glucose reduction from 23.37 ± 1.80 , 20.1 ± 2.60 and 17.13 ± 1.36 initial levels to 11.07 ± 1.98 , 10.1 ± 0.26 and 8.3 ± 0.15 mmol/L after 8 hours respectively. In this study, the ethanolic extract of *Spilanthes paniculata* showed moderate clot lysis activity. The clot lysis activity of control, standard (streptokinase) and ethanolic extract of *Spilanthes paniculata* was 2.65%, 93.35% and 46.78% respectively. This study explored that ethanolic extract of *Spilanthes paniculata* leaves has potential antidiabetic and moderate thrombolytic activity.

Keyword: *Spilanthes paniculata*, Antidiabetic, Alloxan, Streptokinase, Thrombolytic

1. Introduction

Spilanthes paniculata (*S. paniculata*) is an important medicinal plant with rich source of therapeutic and medicinal constituents. The genus *Spilanthes* (Asteraceae) contains 30 species and 9 additional intraspecific taxa that are cardinally distributed in the tropical and subtropical regions around the world [1]. This species is famous as a folklore remedy for toothache and for throat and gum infections, earning it the English nickname, the “toothache plant”. The larvicidal activity against Anopheles mosquitoes of *S. paniculata* indicating a possible role for *Spilanthes* is not just the treatment but also prevention of malaria [2]. *Spilanthes* comprises a number of biologically active compounds, [3] of which the most studied have been the alkylamides [4].

Isolated alkylamides from *Spilanthes* have demonstrated activity against mosquito larvae. There are no published reports of antiplasmodial activity of isolated *Spilanthes* alkylamides, however alkylamides from other plants have shown such activity [5]. Roots of *S. paniculata* release more than 90% of N, P and K within 150 day. In poorly managed stirring cultivation systems, *S. paniculata* can play an important role in soil nutrient enrichment [6]. Root decoction is used as purgative, Leaf decoction is used as diuretic and lithotriptic, Whole plant is used in treatment of dysentery [7].

Diabetes mellitus (DM) also known as simply diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (World Health Organization, 2014). In this condition hyperglycemia, or the

accumulation of glucose (sugar) occurs in the bloodstream [8]. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications involve diabetic ketoacidosis and nonketotic hyperosmolar coma [9]. According to International Diabetes Federation, DM affects nearly 10% of the world population. Many drugs are currently available for the management of diabetes but most of them are expensive and have potential side effects e.g. Adjunctive exenatide causes hypoglycemia and obesity [10]. That's why; screening of plants for hypoglycemic activity will be of enormous implication in this circumstance.

Thrombosis is the formation of a blood clot (thrombus) inside a blood vessel, obstructing the flow of blood through the circulatory system [11]. Thrombolysis is the complex process of in vivo clot dissolution [12], involving the interaction of clot components with the surrounding plasma. Atherothrombotic diseases like myocardial or cerebral infarction may lead to death [13] owing to the development of thrombus that causes hindrance in the passage of vessels [14], even, in critical conditions, patients may die due to embolism [15-16]. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (TPA) are commonly used thrombolytic agents [17]. Thrombolytic agents which are available still have significant shortcomings, including the need for large doses to be sufficiently effective, limited fibrin specificity and bleeding tendency owing to their shortcomings. So there is a try to develop improved recombinant variants of these drugs [18].

In spite of, there are a numbers of publications on several biological and pharmacological activities of *Spilanthes* species there is no literature is currently available to substantiate antidiabetic and thrombolytic effects of ethanolic extract of *Spilanthes paniculata* leaves. Therefore the present study was designed to investigate the Antidiabetic and thrombolytic effects of ethanolic extract of *Spilanthes paniculata* leaves.

2. Materials and Methods

2.1. Collection and Identification

For this investigation *Spilanthes paniculata* Linn. was collected from Noakhali district and Mirpur-6, Dhaka, Bangladesh. The plants were identified by expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Accession number- DACB: 37656.

2.2. Preparation of Plant Extracts

The collected plant parts (leaves) were separated from undesirable materials or plants or plant parts. They were sun-dried for one week. The dried and powdered materials (200 g) were soaked in 1000 ml of 90% ethanol for about 15 days at room temperature with occasional stirring. After 15 days the solution was filtered using cotton filter and Whatman filter paper. The filtrate obtained was evaporated under ceiling fan

and in a water- bath until dried. It rendered a gummy concentrates and were designated as crude extract of Ethanol.

2.3. Chemicals

Alloxan (Hydrate, Lobachemie PVT. LTD.), Glibenclamide (DIBENOL Tablet, Square Pharmaceuticals Ltd, Bangladesh), Saline water, Streptokinase (standard). 1500000 IU (Durakinase) was purchased from local market.

2.4. Animals Used for Experiment

Male Albino mice (30-35 gm) maintained under standard laboratory condition (temp $22 \pm 2^{\circ}\text{C}$ relative humidity $55 \pm 5\%$ and 12 h light: dark cycle) were used for the study. Animals were fed with standard laboratory food and water during study period. The protocol of the study was approved by the institutional animal ethics committee.

2.5. Antidiabetic Activity Test

2.5.1. Induction of Experimental Diabetes

The animals were weighed and randomly divided into six groups consisting of three mice in each group. The animals were fasted for 16-18 h with free access to water prior to the induction of diabetes. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) and it takes 48 hr [19]. Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, mice were treated with 20% glucose solution orally after 6 h. To prevent hypoglycemia the mice were then kept for the next 24 h on 5% glucose solution bottles in their cages [20].

2.5.2. Experimental Design

After induction of diabetes the diabetic animals were randomly divided into different group as follows:

Group I: Kept as normal group.

Group II: Alloxan Monohydrate was used to induce diabetes mellitus in mice. After 24 hrs of fasting a single dose (150 mg/ kg body weight) of 2% alloxan monohydrate in saline was injected intraperitoneally.

Group III: Alloxan induced diabetes mice treated with glibenclamide orally for 8 hours.

Group IV. A: Alloxan induced diabetes mice treated with leaf extract (150 mg/kg body weight) orally for 8 hours.

Group IV. B: Alloxan induced diabetes mice treated with leaf extract (300 mg/kg body weight) orally for 8 hours.

Group IV. C: Alloxan induced diabetes mice treated with leaf extract (450 mg/kg body weight) orally for 8 hours.

2.5.3. Determination of Blood Glucose Level

Blood glucose level of each group was measured during fasting condition by using Glucometer (Ez Smart 168, Tyson Bioresearch, Inc. Chu-Nan, Taiwan) and Glucose oxidase-peroxidase reactive strips (Tyson Bioresearch, Inc.). To measure the blood glucose level, tail tip of experimental animals were cut with a sharp blade. Little amount of blood was collected and exposed to the touch of glucose test strips.

Within few seconds blood glucose level was visualized in the glucometer. Standard and test samples were administered orally to the experimental animals with the help of Tuberculin syringe with ball shaped end. Then again the blood glucose level was measured after 2nd, 4th, 6th and 8th hr to observe the antidiabetic effect. The result of antidiabetic effects of the test samples were compared to control and standard groups.

2.6. Thrombolytic Activity

In vitro thrombolytic activity of the leaves of was carried out according to the method of Prasad et al. [21] with minor modification. With ethical considerations, and aseptic precaution, 7 ml of venous blood was drawn from healthy volunteers (n =5) having no history of smoking, taking lipid lowering drugs, oral contraceptive or anticoagulant therapy and transferred to different pre weighed sterile micro-centrifuge tube (1 ml/tube). The micro-centrifuged tubes were subjected to incubation at 37°C for 45 min. After the formation of clot, serum was completely removed from the tubes (carried out without disturbing the clot formed) and each tube having clot was again weighed to determine the weight of the clot (clot weight = weight of clot containing tube – weight of tube alone).

To each micro-centrifuge tube containing pre-weighed clot, 100 µl solution of different extracts (n-hexane, ethyl acetate, chloroform and methanolic extract), concentration 1 mg/mL, were added accordingly. As a positive control, 100 µl of streptokinase and as a negative non thrombolytic control, 100 µl of sterilized distilled water were separately added to the control tubes numbered. Then all the tubes were incubated again at 37°C for 90 min and observed for clot lysis. After incubation, the obtained fluid was removed from the tubes

and they were again weighed to observe the difference in weight after clot disruption. At last, difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the underneath equation.

$$\% \text{ of clot lysis} = (\text{wt. of released clot} / \text{clot wt.}) \times 100$$

3. Results

3.1. Antidiabetic Activity

The crude ethanolic extract of leaves of *Spilanthes paniculata* was subjected to mice to determine the antidiabetic activity. Glibenclamide was used as the standard at a dose of 10 mg/kg body weight and sample extract was used as study sample at a dose of 150 mg/kg, 300 mg/kg and 450 mg/kg body weight in this study.

In standard group (Glibenclamide 10 mg/kg treated), blood glucose level fall about 23.07%, 17.2%, 15.37% and 13% and 11.27% at the zero, 2nd, 4th, 6th and 8th hour respectively. In the group of crude ethanolic extract of leaves of *Spilanthes paniculata* at concentration 150 mg/kg blood glucose level fall 17.13%, 13.17%, 13.03% and 16.33% and 16.83% at the zero, 2nd, 4th, 6th and 8th hour respectively. When the concentration was 300 mg/kg blood glucose level fall about 20.1%, 21.17%, 13.57% and 8.3%, 9.1% at the same time interval. At the zero, 2nd, 4th, 6th and 8th hour the blood glucose level fall about 23.37%, 21.27%, 17.7% and 15% 21.07% respectively, when the concentration was 450 mg/kg. The plant sample showed promising antidiabetic activity and further bioactivity guided investigation can be done to find out potent antidiabetic compounds.

Table 1. Antidiabetic activities of normal control, diabetic control, std. group (Glibenclamide 10 mg/kg), CEELSP at different concentration and time interval

Time (hr)	Control	Diabetic Group (Alloxan 150 mg/kg)	Std. Group (Glibenclamide 10 mg/kg)	Leaves Extract (150 mg/kg)	Leaves Extract (300 mg/kg)	Leaves Extract (450 mg/kg)
0	7.67±0.65	30.03±1.75	23.07±6.86	23.37±1.80	20.1±2.60	17.13±1.36 **
2	7.43±0.64	31.03±1.78	17.2±5.27	21.27±4.19	21.17±5.95	13.17±1.46 **
4	7.63±0.64	30.7±1.78	15.37±4.26	17.7±1.57	13.57±2.64	13.03±3.62 **
6	7.67±0.44	30.5±1.88	13±3.55	15±0.55	12.3±1.15	9.1±0.26 **
8	7.53±0.23	30.37±1.14	11.27±2.68	11.07±1.98	10.1±0.26	8.3±0.15 **

CEELSP= Crude ethanolic extract of leaves of *Spilanthes Paniculata*, **P<0.05 indicate statistically significant difference in comparison to standard drug glibenclamide.

Table 2. Percentage of fall of blood glucose level by crude ethanolic extract of leaves of *Spilanthes Paniculata*

Group	% of fall of blood glucose level			
	After 2 nd hour	After 4 th hour	After 6 th hour	After 8 th hour
Std. Group (Glibenclamide 10 nmg/kg)	17.25%	15.37%	13%	11.27%
CEELSP (150 mg/kg)	13.17%	13.03%	16.33%	16.83%
CEELSP (300 mg/kg)	21.17%	13.57%	8.3%	9.1%
CEELSP (450 mg/kg)	21.27%	17.7%	15%	21.07%

%= Percentage, CEELSP= Crude ethanolic extract of *Spilanthes paniculata*

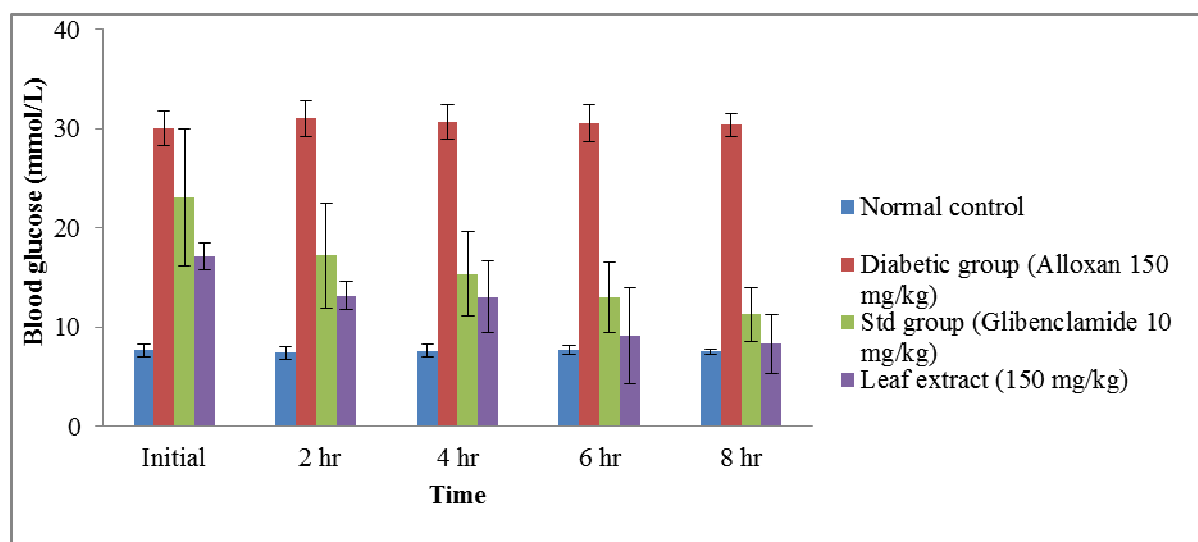


Figure 1. Antidiabetic activity of crude ethanolic extract (150 mg/kg) of *Spilanthes paniculata*

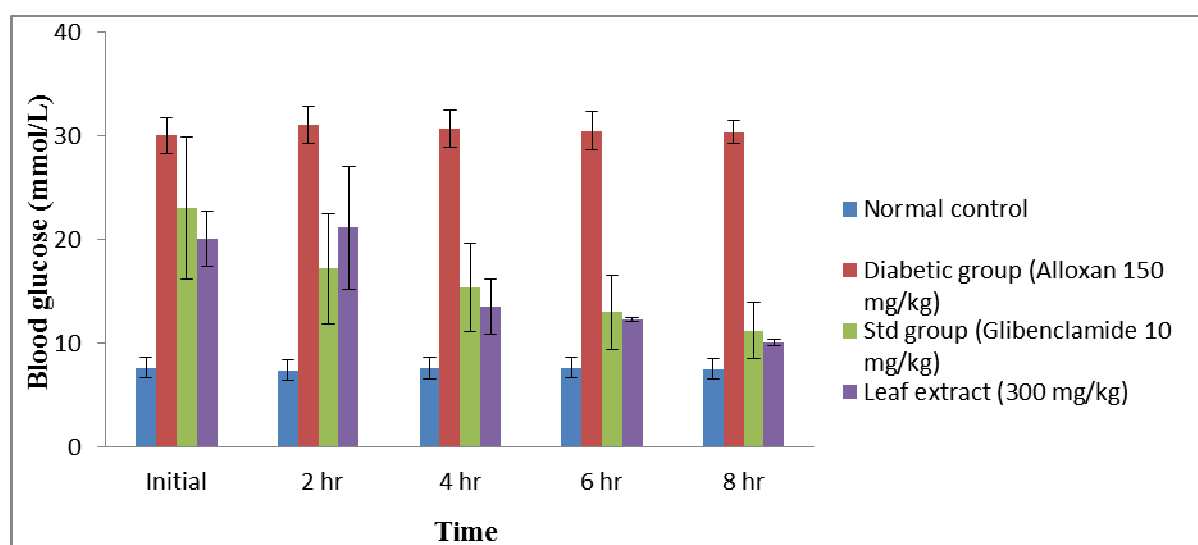


Figure 2. Antidiabetic activity of crude ethanolic extract (300 mg/kg) of *Spilanthes paniculata*

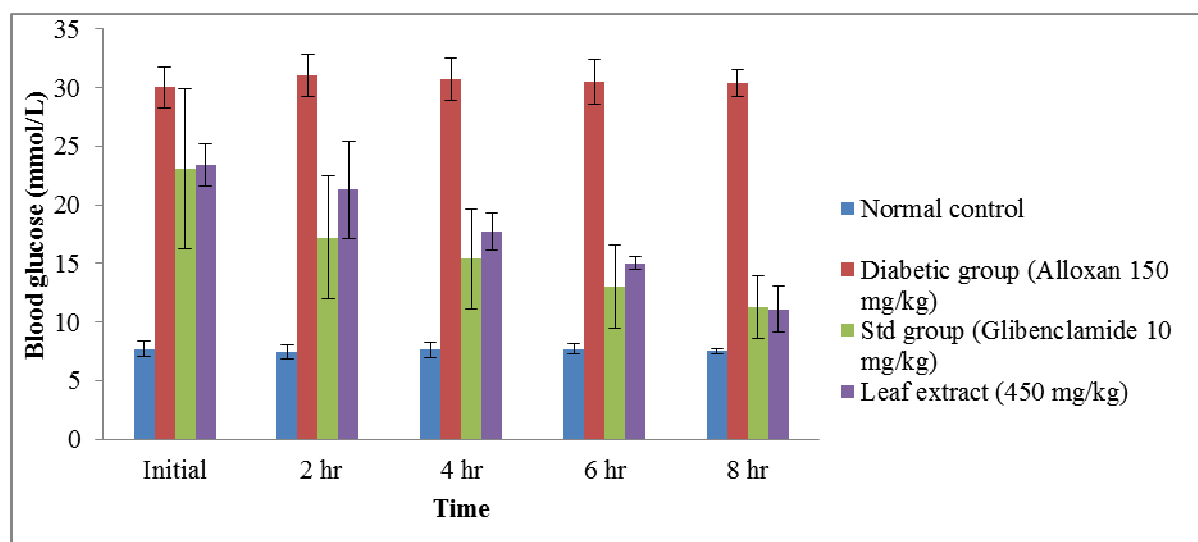


Figure 3. Antidiabetic activity of crude ethanolic extract (450 mg/kg) of *Spilanthes paniculata*

3.2. Thrombolytic Activity

The present study was conducted to find out the cardio protective drugs from natural sources. So, the ethanolic leaves extract of *Spilanthes paniculata* were assessed for thrombolytic activity and the results are presented in table 3.

Table 3. Data analysis for thrombolytic activity of crude ethanolic root extract of *Spillenthus paniculata*

Name of sample	W ₁ (gm)	W ₂	W ₃ (gm)
Plant extract	5.97	8.92	7.54
Standard	6.07	6.23	6.08
Blank	6.16	9.56	9.47

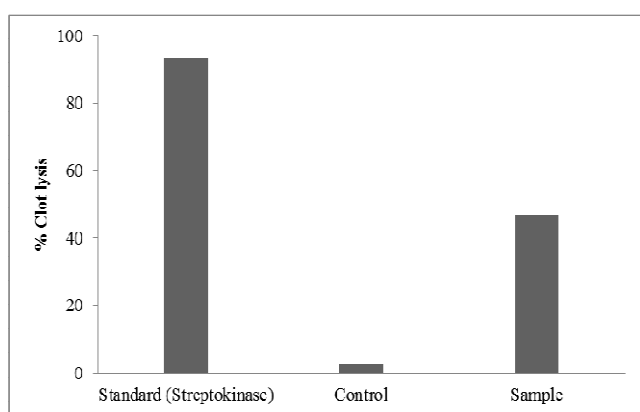


Figure 4. Thrombolytic activity of crude ethanolic root extract of *Spillenthus paniculata*

3.2.1. Calculation

$$\begin{aligned} \text{\% of clot lysis} &= (\text{wt of released clot} / \text{clot wt}) \times 100 \\ &= (W_2 - W_3 / W_2 - W_1) \times 100 \end{aligned}$$

Standard (Streptokinase):

$$\begin{aligned} \text{\% of clot lysis} &= (W_2 - W_3 / W_2 - W_1) \times 100 \\ &= \frac{6.23 - 6.08}{6.23 - 6.07} \times 100 \\ &= 93.35\% \end{aligned}$$

Blank:

$$\begin{aligned} \text{\% of clot lysis} &= (W_2 - W_3 / W_2 - W_1) \times 100 \\ &= \frac{9.56 - 9.47}{9.56 - 6.16} \times 100 \\ &= 2.65\% \end{aligned}$$

Sample:

$$\begin{aligned} \text{\% of clot lysis} &= (W_2 - W_3 / W_2 - W_1) \times 100 \\ &= \frac{8.92 - 7.54}{8.92 - 5.97} \times 100 \\ &= 46.78\% \end{aligned}$$

4. Discussion

Alloxan has been seen to cause a massive reduction of the β -cells of the islets of Langerhans which induce hyperglycemic [22]. A number of plants were found to possess hypoglycemic effects and the possible mechanism suggested for such hypoglycemic actions could be through the increased insulin secretion from β -cells of islets of Langerhans or its release from bound insulin. That is to say such hypoglycemic effects of plant extracts could also be due to their insulin like actions [23].

After the administration of plant extracts, dose-dependent reduction in blood glucose levels in mice was observed. There was a remarkable fall in the blood glucose levels at dose of 450 mg/kg compared to 300 mg/kg and 150 mg/kg for all the three plant extracts. Plant extract in a dose of 150 mg/kg body weight showed less significant effect in diabetic mice and lower the blood glucose to little extent while plant extract at a dose of 300 mg/kg body causes slightly higher reduction of blood glucose than at a dose of 150 mg/kg body weight but the reduction of blood glucose was statistically significant at a dose of 450 mg/kg body weight.

Streptokinase is a novel thrombolytic agent used as a positive control to compare the clot lysis effects of *Spilanthes paniculata* extract. Addition of 100 μ l Streptokinase (30,000 I.U.), standard to the clots along with 90 minutes of incubation at 37°C, showed 93.35% clot lysis. Clots when treated with 100 μ l sterile distilled water (control) showed only negligible clot lysis (2.65%). In this study, the ethanolic extract of *Spilanthes paniculata* revealed clot lysis activity of 46.78%.

5. Conclusion

Based on the results of our study it can be concluded that the ethanolic extract of *Spillenthus paniculata* leaves possess significant antidiabetic as well as moderate thrombolytic activity. The potential of the extract of *S. paniculata* as antidiabetic and thrombolytic activity may be due to the presence of various phytochemical constituents present in the crude plant extracts. All of the experiments were performed in multiple dose & further studies have to be carried out to identify the phyto-constituent responsible for the exact and detailed mechanism of action responsible for this activity.

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