Antidiabetic and Cytotoxic Activities of Methanolic Extract of *Tabernaemontana divaricata* (L.) Flowers

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**Abstract**

The research work was designed to investigate the antidiabetic activity of the methanol extract of flowers of *Tabernaemontana divaricata* on alloxan induced diabetic mouse model. The extract was given intraperitonially at a single dose of 200 mg/kg and 300 mg/kg body weight and the blood glucose levels were measured at 0, 1, 3, 5, 10 and 24 hours of the study period. The antihyperglycemic effect of the extract was compared with metformin, a standard drug. The dose of 300 mg/kg was found to be more effective dose to reduce maximum blood glucose level at 10th hour of the treatment period from 14.15± 0.42 to 8.81± 0.27 mg/dl whereas maximum result was obtained for metformin at the same time from 14.04±0.36 to 6.13±0.19 mg/dl. The extract was also subjected to Brine shrimp lethality bioassay. LC₅₀ value of the extract was 84.03 µg/ml and for vincristin sulphate, it was 10.58 µg/ml. So, the present results suggest that *Tabernaemontana divaricata* possess antidiabetic activity in mice with alloxan induced diabetes and low cytotoxicity that may provide new molecules for the treatment of diabetes.

**Key words:**

Diabetes, Alloxan, *Tabernaemontana divaricata*, Cytotoxicity

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**Introduction**

Diabetes is one of the most prevalent and devastating chronic non-communicable diseases...
having serious health, economic and social consequences [1]. Diabetes mellitus is characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein [2]. According to World Health Organization (WHO) projections, the prevalence of diabetes is likely to increase 35% by 2020 [3]. Currently the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 worldwide. India, China and the United States estimated to have the highest numbers of people with diabetes in 2000 and 2030. Statistical projection about Bangladesh suggests that the number of diabetics will rise from 3.2 million in 2000 to 11.1 million in the year 2030, the seventh highest number of diabetics in the world [4]. This disease can lead to serious, long-term complications, including kidney damage or failure, blindness, heart disease, stroke, high blood pressure, neuropathy, and amputations [5]. Sedentary lifestyle, degree of obesity, changes in food consumption, aging, and other concomitant medical conditions have been implicated in this increasing prevalence in the past two decades.

Type 2 diabetes is treated with insulin and oral hypoglycemic agents that are capable of reducing blood sugar level belong to sulfonylureas, biguanides, glitazones and alpha-glycosidase inhibitors. However, the use of antidiabetic agent is limited due to their adverse effects including hypoglycaemic coma and disturbances of liver and kidney functions; even they are not suitable for use during pregnancy except insulin [6,7].

*Tabernaemontana divaricata* (Linn.) Roem. & Schult commonly known as Tagar belongs to the family Apocynaceae is a beautifully shaped evergreen shrub which blooms in spring but flowers may appear sporadically all year and distributed throughout Bangladesh and other parts of South-East Asia. The phytochemistry and a number of chemical constituents from the leaves, stems, and roots have been reported previously. Constituents studied include alkaloids, and non-alkaloid constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes [8-11]. In folklore practice it is used to treat fever and diarrhea. The plant is also used as tonic to the brains, liver and spleen [12]. It is reported that plant extract possesses antinociceptive [13], antimicrobial, antioxidant [14], anti-inflammatory [15] and reversible acetylcholinesterase inhibition [16] activities. To the best of our knowledge, no scientific data regarding the antidiabetic effect of *T. divaricata* flowers. Thus the present study was undertaken to evaluate the hypoglycemic effect of methanol extract of *T. divaricata* flowers.

**MATERIALS AND METHODS**

**Plant material**

*T. divaricata* flowers were collected from the local areas of Chittagong, Bangladesh during the month of April 2011 and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

**Preparation of Extract**

The flowers were dried under shade and ground. The ground flowers (150 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 about 28-32 gm, 4-6 weeks 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 ICDDR,B Dhaka. Mice were acclimatized for 7 days.

**Drugs**

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 saline (154mM NaCl) just prior to injection. Diabetes was induced by injecting it at a dose of 100 mg/kg b. wt., intraperitoneally after overnight fasting. After 48 hours, fasting blood glucose levels of 13 to 16 mmol/L were separated and included in the study.

**Antidiabetic Activity**

In the experiment, a total 25 male Swiss Albino mice were used and divided randomly into five groups with each group containing five mice. Group I served as a control which received vehicle alone.

- **Group I:** Normal control
- **Group II:** Diabetic control
- **Group III:** Standard control
- **Group IV:** Treatment control (200 mg/kg)
- **Group V:** Treatment control (400 mg/kg)

Group II – V received a single dose of alloxan (100 mg/kg i.p.) after overnight fasting. Group-I received only dimethyl sulfoxide (DMSO) as normal control group and Group-II was diabetic control group, which did not receive either metformin, or flower extract. Metformin (150 mg/kg b. wt.) was injected intraperitoneally to Group III and extract at a dose of 200 mg/kg b. wt. and 300 mg/kg b. wt. were injected to Group IV and Group V respectively. Metformin and extract both were dissolved in DMSO vehicle. Blood samples were then analyzed for blood glucose content at 0, 2, 6, 12, 16 and 24 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](http://www.graphpad.com)).

**CYTOTOXICITY USING BRINE SHRIMP LETHALITY BIOASSAY**

The cytotoxicity assay was performed on brine shrimp (*Artemia salina*) nauplii using Meyer method [17]. 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 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 vials and control vial. After 24 hrs, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula: % 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 flowers on blood glucose levels in normal and diabetic mice are shown in Table 1 and Figure 1. Following a 24 hours post alloxan injection, all diabetic mice exhibited hyperglycemia, which ranged between 13 and 16 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 blood glucose level was observed for the extract of 200 mg/kg and 300 mg/kg b.wt at 10\(^{th}\) hour of the 24 hours experimental period and it was comparable with standard drug metformin which showed maximum reduction of blood glucose level at the dose of 150 mg/kg. So, the extract showed considerable antihyperglycemic activity in alloxan induced diabetic model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time in hour</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Standard Control (Metformin 150 mg/kg)</th>
<th>Treatment Control (Extract 200 mg/kg)</th>
<th>Treatment Control (Extract 300 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6.24±0.14</td>
<td>14.52±0.33</td>
<td>14.04±0.36</td>
<td>14.32±0.69</td>
<td>14.15±0.42</td>
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<tr>
<td></td>
<td>1</td>
<td>6.19±0.26</td>
<td>14.16±0.63</td>
<td>11.34±0.48*</td>
<td>14.07±0.22*</td>
<td>13.22±0.44*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.23±0.38</td>
<td>14.21±0.26</td>
<td>9.34±0.33*</td>
<td>12.64±0.27*</td>
<td>11.48±0.47*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.29±0.30</td>
<td>14.22±0.50</td>
<td>7.49±0.48*</td>
<td>11.10±050*</td>
<td>10.46±0.40*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.26±0.24</td>
<td>13.90±0.52</td>
<td>6.13±0.19*</td>
<td>9.13±0.37*</td>
<td>8.81±0.27*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>6.18±0.35</td>
<td>13.63±0.24</td>
<td>7.81±0.64</td>
<td>11.34±0.60</td>
<td>10.67±0.48</td>
</tr>
</tbody>
</table>

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

Brine Shrimp Lethality Bioassay

Brine shrimp lethality results of the methanol crude extract of \( T. \) divaricata flowers is shown in Figure 2 and calculated LC\(_{50}\) value is recorded in Table 2. The LC\(_{50}\) value of the crude extract was 84.03 µg/ml, has low toxicity compared to Vincristin Sulphate served as the positive control for this brine shrimp lethality assay and its LC\(_{50}\) value was 10.58 µg/ml. No mortality was found in the control group, using DMSO and sea water.

Table 1: Antidiabetic effect of flowers extract of \( T. \) divaricata on alloxan induced diabetic mice

Figure 1: Effect of flowers of \( T. \) divaricata in lowering fasting blood glucose
Figure 2: Determination of LC50 values for extract of flowers of T. divaricata from linear correlation between log concentrations versus Probit value

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

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Log C</th>
<th>Total</th>
<th>Alive</th>
<th>Death</th>
<th>% Mortality</th>
<th>Probit</th>
<th>LC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>1.09691</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>84.03</td>
</tr>
<tr>
<td>25</td>
<td>1.39794</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>10</td>
<td>3.72</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1.69897</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>30</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>60</td>
<td>5.25</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>2.30103</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>80</td>
<td>5.84</td>
<td></td>
</tr>
<tr>
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<td>10</td>
<td>0</td>
<td>10</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSIONS

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species [18]. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction.

A multitude of herbs spices and other plant materials have been used for the treatment of diabetes throughout the world. Approximately 25 percent of modern drugs used in the United States have been derived from plant origins [19]. So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.

Our present study revealed that methanolic extract of T. divaricata flowers has considerable effect in lowering fasting blood glucose level in alloxan induced diabetic mice. Metformin showed maximum reduction of blood glucose level at tenth hour and at the same time maximum reduction was obtained for extract of 300 mg/kg in alloxan.
induced 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 flowers 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.

CONCLUSION
Methanolic extract of *T. divaricata* flowers exhibited hypoglycemic activity in alloxan induced diabetic mice and also showed low cytotoxicity on brine shrimp nauplii. More investigations must be carried out to evaluate the precise active substance(s) and mechanism of action of *T. divaricata* with antidiabetic effect. The long term toxic effect and its protective effects on the pancreas should also be elucidated.

REFERENCES
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