Antithrombolytic and Antidiabetic Activity of Methanolic Extract of *Paederia foetida*

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ABSTRACT

Present study was carried out to investigate the antidiabetic and antithrombolytic properties of methanolic extracts of the whole plant of *Paederia foetida*. Alloxan-induced male Sprague Dawley (SD) rats were given the whole plant extract of methanol by gastric gavage at doses of 250, 500, 1000 mg/kg body wt. and the results of reduction of blood glucose levels were compared with negative control (Tween 80 solution) & positive control (Glibenclamide). Methanolic extracts of the whole plant of *P. foetida* has moderate antidiabetic activity. At the 3rd hour, the dose of 250, 500 and 1000 mg/kg decreased the blood glucose levels by 31.6%, 28% and 30.3%, respectively, while the reference drug (glibenclamide, 2 mg/kg) decreased the blood glucose level by 70%. 100 μl extract of *P. foetida* exhibited the highest thrombolytic activity (among three samples) with clot lysis value of 23.82% whereas standard streptokinase (positive control) and water (negative control) demonstrated clot lysis value of 45.85% and 2.81%, respectively.

Key words: *paederia foetida*, antithrombolytic activity, antidiabetic activity, methanol extract

INTRODUCTION

*Paederia foetida* belonging to family *Rubiaceae*[^1] is one of 30 species in the genus *Paederia*. The origin of this plant is considered to be Eastern and southern Asian. It is usually found in different parts of India like Assam, Bihar and Orissa and also in Bangladesh. It possesses perennial twining vine from woody rootstock; stems to 7 m (23 ft) or more, climbing, orprostrate and rooting at the nodes. Leaves are opposite (rarely in whorls of 3), with conspicuous stipules. Petioles are commonly to 6 cm (2.4 in) long; blades entire, oval to linear-lanceolate, 2-11 cm (1-4.3 in) long, hairy or glabrous, often lobed at base. The leaves and stems have disagreeable odor, especially when crushed. Flowers are small, grayish pink or lilac, in broad or long, “leafy” curving clusters, terminal or at leaf axils. Corolla are densely hairy, tubular with 5 (usually) spreading lobes. Fruits are shiny brown, nearly globose capsule, to 0.7 cm (0.3 in) wide, with 2 black roundish seeds, often dotted with white raphides. Many traditional plants are used for thrombolytic and antidiabetic activity throughout the world.[^2] Plant drugs[^3] and herbal formulations[^4][^5][^6] are frequently considered to be less toxic and freer from side effects than the synthetic one.

Streptokinase, an antigenic thrombolytic agent, is used for the treatment of acute myocardial infarction. In most infarct patients, it reduces mortality as effectively as the nonantigenic alteplase while having the advantages of being much less expensive. Tissue-type Plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or streptokinase type activators. All available thrombolytic agents still have significant shortcomings. In some cases, large doses are required to be maximally effective, have limited fibrin specificity and a significant associated bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs.[^7][^8][^9][^10][^11] The present study was done to measure the thrombolytic activity of methanol extract of the whole plant of *P. foetida* by using streptokinase as a reference standard.

Diabetes is a serious metabolic disorder with micro and macro vascular complications which causes significant morbidity and mortality. In the developing world, the diabetes epidemic is accelerating with an increased...
Recently, some reports described that type-2 diabetes was being diagnosed even in children and adolescents. The latest WHO Global Burden of Disease estimates the worldwide burden of diabetes in adults to be around 173 million in the year 2002 and around two-thirds of these live in developing countries. So, now-a-days, it has become a growing public health concern worldwide causing severe and costly complications including blindness, cardiac and kidney diseases. Current therapies do little in preventing complications although they provide good glycemic control. Besides this, these drugs are also associated with side effects. Thus, it is necessary to continue research for new and, if possible, more efficacious drugs. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important. No study has been carried out on the antidiabetic activity of *Paederia foetida* leaves. Therefore, the current study focuses on the antidiabetic potentiality of methanol extracts of the whole plant of *P. foetida*.

**MATERIALS AND METHOD**

**Plant materials**

The whole plant of *Paederia foetida* was collected from Ishurdi, Pabna and was taxonomically identified with the help of the National Herbarium of Bangladesh. Accession Number of the plant is 34418. The whole plants were cut into small pieces and then sun dried for seven days. The dried plants were then ground into coarse powder with the help of an attrition type of a grinder.

**Extraction and isolation**

Extraction of dried and powdered plant of *P. foetida* was done by the extraction process by using methanol as a solvent. The air-dried and pulverized plant material was extracted with methanol. After that the fractions were evaporated by roto-dryer to dryness at low temperature (40-50 °C). Crude methanol extract was subjected to evaluate thrombolytic and antidiabetic activity.

**Experimental animal**

Male Sprague Dawley rats, aged 5-6 weeks, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (icddr,b). Animals were maintained under standard environmental conditions (temperature: 24.0 ± 1.0 °C), relative humidity: 55-65% and 12 h light/dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to the experiments. Principles of laboratory animal care guidelines (NIH publication number 85-23, revised 1985) were followed.

**Thrombolytic activity**

5 ml of blood sample was collected from a healthy volunteer and was distributed into five separate pre-weighed (W) microcentrifuge tubes. The tubes were centrifuged at 2500 rpm for five minutes and then incubated for 45 minutes at 37 °C. After clotting of blood, serum was decanted and removed. Then weight of clotted blood (ΔW) was taken by subtracting the pre-weight (W₂) from the weight of the clot containing tube (W₁) as: \( ΔW = W₁ - W₂ \). Then 100 μl extract of *P. foetida* was added to the clot containing tube. Similarly 100 μl of streptokinase was added to clot of standard tube and 100 μl of water was added to clot of blank tube, which were used as positive and negative control, respectively. Then all test tubes were incubated at 37 °C for 90 minutes and weighed again for getting the weight variation among the pre-weight and final weight (W₃) that was achieved for clot lyses (thrombolysis). Average value of weight loss was calculated in percentage (%) of clot lysis which was calculated with the following formula:

\[
\text{% of clot lysis} = \left( \frac{W₁ - W₃}{W₂} \right) \times 100\%
\]

**Anti-diabetic study**

Alloxan monohydrate (Sigma-Aldrich, USA) solution of 10 mg/ml was prepared in 0.1M ice-cold citrate buffer (pH 4.5) and then administered to the rats within 5 mins at a dose of 50 mg/kg bodyweight intraperitonially. The fasting blood sugar levels of each of the rats were checked everyday with an autoanalyzer (Glucometer, Bioland G-423 S) glucose kit. After 8 days, animals with fasting blood sugar levels of 250 mg/dl and above were considered to be diabetic and were used for the study. All the selected rats were divided into five groups of five rats each. Group I served as the negative control and received tween-80 solution (solvent used to dissolve the extract) at the rate of 10 ml/kg body weight. Groups II, III & IV received the *P. foetida* extract at the dose of 250, 500 and 1000 mg/kg respectively while group V served as the positive control and received the standard reference drug glibenclamide (5 mg tablet of Daonil from Sanofi-Aventis) 5 mg/kg all by gastric gavage. The blood glucose levels of the rats were measured at 0, 1, 2 and 3 h after administration of drug and extracts. Blood samples were collected by tail snip and the blood glucose measured with an autoanalyzer (Glucometer, Bioland G-423 S) glucose kit. At the end of the experiment, percentage reduction of the glucose levels of the rats at the 3rd hour was calculated using the formula below:

\[
\text{Percentage Reduction} = \left( \frac{BGL\text{ at 0 hr} - BGL\text{ at 3rd hr}}{BGL\text{ at 3rd hr}} \right) \times 100\%
\]

\( BGL = \text{Blood Glucose Level} \)
**RESULTS**

**Thrombolytic activity**

The percentage of weight loss of clot after application of the extract solution was taken as the functional indication of thrombolytic activity. The study was implemented on one healthy volunteer with five blood samples (Table 1).

**Antidiabetic Activity of Diabetic Induced Rats**

The effect of the methanolic extracts of the whole plant of *P. foetida* on the fasting blood glucose levels of alloxan-induced diabetic rats is presented in Table 2.

**DISCUSSION**

The comparison of positive and negative control as shown in Table 1 clearly stated that clot dissolution doesn’t occur when water was added to the clot. On the basis of our study, compared to the value of standard (45.85%), the average % of clot lyses was 20.82 ± 2.71 which is approximately half of the standard. So, it can be said that the methanolic extract of the whole plant has moderate thrombolytic activity.

The result showed that there was no significant change in the blood glucose levels of rats in group I that received tween-80 solutions (negative control). The methanolic extract of *P. foetida* in all the doses used, including the reference drug, caused a time dependent and significant (*p* < 0.001) reduction of the blood glucose levels of the alloxan-induced diabetic rats when compared to the negative control group. The highest activity of *P. foetida* extract in this experiment was observed at the dose of 250 mg/kg while the reference drug glibenclamide (5 mg/kg) had a superior activity.

**REFERENCES**


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**Table 1: Thrombolytic activity of methanolic extract of *Paederia foetida***

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Weight of empty test tubes, W₁</th>
<th>Weight of blood clot test tubes, W₂</th>
<th>Weight of release clot test tubes, W₃</th>
<th>% of clot lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS-1</td>
<td>6.0279</td>
<td>6.1539</td>
<td>6.1286</td>
<td>20.08</td>
</tr>
<tr>
<td>PFS-2</td>
<td>6.0450</td>
<td>6.3203</td>
<td>6.2547</td>
<td>23.82</td>
</tr>
<tr>
<td>PFS-3</td>
<td>6.0599</td>
<td>6.2470</td>
<td>6.2123</td>
<td>18.55</td>
</tr>
<tr>
<td>Water</td>
<td>6.0560</td>
<td>6.1425</td>
<td>6.1027</td>
<td>45.85</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>6.0321</td>
<td>6.2416</td>
<td>6.2357</td>
<td>2.81</td>
</tr>
</tbody>
</table>

*PFS = *Paederia foetida* sample

**Table 2: Effect of *Paederia foetida* on the fasting blood glucose levels of alloxan-induced diabetic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Fasting Blood Glucose Level (mg/ml)</th>
<th>% reduction at the 3rd hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Alloxan ± Tween80 (1%)</td>
<td>387 ± 5.1</td>
<td>398.5 ± 4.3</td>
</tr>
<tr>
<td>II</td>
<td>Alloxan ± PFE (250 mg/kg)</td>
<td>365.8 ± 5.3</td>
<td>222.3 ± 3.9</td>
</tr>
<tr>
<td>III</td>
<td>Alloxan ± PFE (500 mg/kg)</td>
<td>389.7 ± 4.8</td>
<td>337.6 ± 3.3</td>
</tr>
<tr>
<td>IV</td>
<td>Alloxan ± PFE (1000 mg/kg)</td>
<td>355.2 ± 4.7</td>
<td>313.6 ± 3.4</td>
</tr>
<tr>
<td>V</td>
<td>Alloxan ± Glibenclamide (5 mg/kg)</td>
<td>344.3 ± 1.0</td>
<td>172.7 ± 1.1</td>
</tr>
</tbody>
</table>

*p < 0.001 was considered statistically significant, PFE = *P. foetida* extract