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Thrombolytic, Cytotoxic and Antidiabetic Effects of *Paederia foetida* L. Leaf Extract

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Authors' contributions

Author AMAA carried out the study design, data collection, data interpretation, manuscript preparation and research grant collection. Authors MMI, MAR and MAH participated in experiments, data collection, literature search and manuscript preparation. Author MAR has provided assistance in taxonomical identification and data analysis. Author MAR also aided in data interpretation and literature search. All authors read and approved the final version of the manuscript.

Original Research Article

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ABSTRACT

Aims: This research investigated the thrombolytic, cytotoxic and antidiabetic effects of *Paederia foetida* leaf methanol extract.
Place and Duration of Study: Department of Genetic Engineering and Biotechnology and Department of Biochemistry and Molecular Biology, University of Chittagong, Bangladesh, between April to December, 2012.
Methodology: An *in-vitro* thrombolytic model was used to check the thrombolytic effect of *Paederia foetida* extract using streptokinase as a positive control. Cytotoxic effect was measured by Brine shrimp lethality bioassay. Antidiabetic effect of the extract was conducted in alloxan induced diabetic model of Swiss albino mice. Data were analyzed by one way ANOVA using statistical package for social science (SPSS) software.
Results: In *in-vitro* thrombolysis, *Paederia foetida* extract (100µl) lysed 21.40 ± 1.39 %,

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Streptokinase (positive control) 81.42 ± 0.88 % and water (negative control) 4.63 ± 0.31% of the blood clots. These clot lysis values were statistically different ($p<0.05$) from each other. In Brine shrimp bioassay, lethal concentration (LC_{50}) of *Paederia foetida* extract was found 65.31 µg/mL which was statistically significant compared to Vincristine sulfate (positive control, LC_{50}=0.76 µg/mL). The extract showed a significant and dose-dependent decrease in blood glucose level in alloxan induced diabetic mice. The effect of the extract was comparable to the reference antidiabetic drug metformin (150 mg/kg BW).
Conclusion: The results demonstrated a moderate thrombolytic, cytotoxic and antidiabetic effect of *Paederia foetida* leaf extract to be analyzed further for prospective pharmaceutical preparation.

Keywords: Paederia foetida; cytotoxic; thrombolytic; antidiabetic, alloxan; albino mice.

1. INTRODUCTION

Paederia foetida L. belonging to Rubiaceae family, locally known as Gandhabhaduli or Gandhal, is an aggressive competitive vine with a fetid smell growing high into the canopy of trees in a variety of habitats [1]. *P. foetida* is usually found in Bangladesh, India, Japan, Malaysia, Myanmar, Nepal, Thailand, Vietnam, southern part of Bhutan, Combodia and China [2]. It occurs as a non-native species in Hawaii and Southwestern United states [3,4]. In Bangladesh, *P. foetida* is mainly found in hilly areas of Chittagong and Sylhet [5].

Different parts of the plant are locally used as natural healing agent of several diseases. The leaves are cooked and taken as a remedy for indigestion and loose motion. Juice of the root is useful in piles, inflammation of the spleen and pain in the chest and liver [6]. Ethanol extract of the whole plant possesses wide range of good antibacterial and mild antifungal properties [7]. Anti-inflammatory effect of the plant extract is widely studied. Whole plant extract showed anti-inflammatory activity stronger than acetylsalicyclic acid and weaker than hydrocortisone [8]. Anti-inflammatory effect of methanol extract [9,10] and whole plant extract [8] is also reported. Alcoholic extract of the plant has also been found to have anti-arthritic activity on rats and mice [11].

Scientists also reported antioxidant effect [12] of fresh and dried extract and antidiarrheal activity of 90% ethanol extract of *P. foetida* in castor oil induced mice models [13]. A preliminary observation on the effects of *P. foetida* on gastrointestinal helminthes in bovines was also documented [14]. *P. foetida* extract was also investigated for its inhibiting effect of molecular interactions between nuclear factors and target DNA sequences mimicking NF-kappa B binding sites [15]. However numbers of biological effects of this plant are yet to be studied. As part of our continuous phytopharmacological work, we investigated the thrombolytic, cytotoxic and glucose lowering effect of *P. foetida* leaf extract in animal model.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Methanol (95%) from Sigma-Aldrich, USA and Streptokinase (SK) from Dongkook Pharma Co. Ltd. South Korea were purchased to use in this study. Metformin hydrochloride was kindly donated by Square Pharmaceuticals Ltd. Pabna, Bangladesh. Alloxan was procured

from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Fructose was purchased from VWR International Ltd. England. All chemicals and reagents used in the study were of analytical grade.

2.2 Plant Collection and Taxonomical Identification

The fresh and fully mature leaves of *Paederia foetida* were collected from hilly area of Khagrachari (Chittagong Hill Tracts) and Chittagong University campus during the month of January 2012. Plant species was identified and taxonomically authenticated by Dr. Sheikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen of the plant has been deposited in the Institutional Herbarium with the accession number GEBt-03.

2.3 Preparation of Extract

The fresh leaves of *P. foetida* were washed with distilled water immediately after collection and air dried for 14 days at room temperature. The collected leaves were chopped into small pieces and ground into coarse powder with a mechanical grinder (Miyako 3 in one blender, China) and stored in an airtight container. Dried 246 g powder was soaked into 750 mL 95% pure methanol for 7 days at room temperature (25 ± 1)°C with occasional stirring. After 7 days, methanol extract was filtered with Whatman No.1 filter paper. The extract was concentrated under reduced pressure below 50°C through Rotary evaporator (RE 200, Bibby Sterling, UK). The concentrated extract was collected in a Petri dish and allowed to air dry for complete evaporation of methanol. Blackish-green semisolid extract (19.15 g, yield 7.78% w/w) was preserved at 4°C until further use.

2.4 Determination of Thrombolytic Activity

2.4.1 Streptokinase (SK)

To the commercially available lyophilized SK vial of 15,00,000 I.U., 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μl (30,000 I.U.) was used for *in-vitro* thrombolysis.

2.4.2 Specimen

With all aseptic condition 5 mL of whole blood was drawn from healthy pre-informed human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Institutional Ethics Committee of the University of Chittagong, Bangladesh, AEIUP-03/2013). A 500 μl of blood was transferred to each of the twenty previously weighed eppendorf tubes to form clots [16].

2.4.3 Herbal preparation

A 100 mg extract of *P. foetida* was suspended in 10 mL distilled water and shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-micron syringe filter. A portion of this aqueous preparation (100 μl) was added to the eppendorf tubes containing the clots to check thrombolytic activity [16].

2.4.4 Clot lysis

Ten ml venous blood drawn from healthy volunteers was distributed in twenty different pre-weighed sterile eppendorf tubes (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight [16].

Clot weight = weight of clot containing tube –weight of tube alone

To each eppendorf tube containing pre-weighed clot, 100 µl of aqueous extract of *P. foetida* was added. As a positive control, 100 µl of Streptokinase solution and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated three times with the blood samples of volunteers.

$$\text{Percentage of clotlysis} = \frac{\text{Weight of clot after lysis}}{\text{Weight of clot after lysis}} \times 100$$

2.5 Cytotoxic Activity Assay

Cytotoxic activity of *P. foetida* extract was determined by Brine Shrimp Lethality Bioassay as described by [17] and [18].

2.5.1 Hatching of brine shrimp egg

The cysts (eggs) of the Brine shrimps were decapsulated in 200 ppm chlorine water for 15-20 min. Then the cysts were washed thoroughly with fresh water till all the chlorine gas escapes and cysts were then taken in a conical hatching container and filtered (clean) seawater was added. Soaking density was 1-2 g of cysts per liter of seawater. Moderate aeration was supplied from the bottom of the container at the rate of 10 to 20 liter of air per minute. The container was kept under illumination using a white lamp for 48 h for the eggs to hatch into shrimp larvae (nauplii). The nauplii were then harvested in a beaker.

2.5.2 Sample preparation and cytotoxicity assay

Freshly hatched 10 shrimp larvae (nauplii) were added to 5 ml of sea water in 15 test tubes containing 12.5 to 1000 µg/mL solution of extracts, prepared from 50 mg of crude extract (dissolved in DMSO) through serial dilution [19]. Negative control had 10 nauplii in 5 mL sea water containing 50µl DMSO solution. Each concentration was tested in triplicate. The test tubes were maintained under illumination. After 24 hrs have elapsed, survivors were counted with the aid of a 3X magnifying glass. The percentage of mortality was plotted against the logarithm of concentration. The concentration that would kill 50% of the nauplii (LC_{50}) was determined from linear regression equation using Microsoft Excel 2007.

2.6 Antidiabetic Activity Assay

The antidiabetic effect of *P. foetida* was investigated by the established method of [20] and [21] with slight modifications introduced by [22].

2.6.1 Animal and diet

Thirty, 6-7 weeks old Swiss Albino mice, average body weight 25-30 g, were purchased from Bangladesh Council of Scientific and Industrial Research (BCSIR). They were randomly divided into six groups 5 in each group; Group 1: Normal untreated mice, Group 2: Diabetic control mice are given 1 mL of aqueous solution, Group 3: Diabetic mice + Metformin (Standard 150 mg/kg bw) in 1 mL of aqueous solution, Group 4: Diabetic mice + PFE (100 mg/kg bw) in 1 mL of aqueous solution, Group 5: Diabetic mice + PFE (300 mg/kg bw) in 1 mL of aqueous solution and Group 6: Diabetic mice + PFE (500 mg/kg bw) in 1 mL of aqueous solution. Mice were housed in colony cages having dimension of (28 × 22 × 13 cm3). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions (temperature: 25 ± 1°C, relative humidity: 55-65% and 12 hr light/12 hr dark cycle) having proper ventilation in the room. The mice were fed normal pellet-diets purchased commercially from the vendors and water *ad libitum*. The newly bought mice were acclimatized for a week before commencing the experiment.

2.6.2 Induction of diabetes

To induce diabetes, one week fructose-fed mice (5% fructose, previously fasted for 16 h) were intraperitoneally (i.p) injected with alloxan (100 mg/kg, 1 mL/kg) dissolved in 0.1 M citrate buffer, pH 4.5. Animals with fasting blood glucose level over15 mmol/L, 7-8 days after Alloxan administration, were considered diabetic and used for further experimentation. Control mice were injected with the equivalent volume of citrate buffer alone [23].

2.7 Statistical Analysis

Data were expressed as Mean ± SD. Statistical analysis for animal experiments was carried out using One-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison tests using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The results obtained were compared with the vehicle control group. Values with P = 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Thrombolytic effect of *P. foetida* extract

In *in-vitro* thrombolytic assay, *P. foetida* extract lysed the clot 21.40 ± 1.39%. This percentage of clot lysis was statistically different from the maximum clot lysis 81.42 ± 0.88% for the positive control streptokinase (SK) and 4.63 ± 0.31% for negative control (Fig. 1). However, the combined effect (32.25 ± 0.64%) of SK and *P. foetida* was close to that of *P. foetida* alone and the effect was comparable to SK. The mean difference in clot lysis percentage between positive and negative control was also very significant (p value < 0.0009) (Table 1).

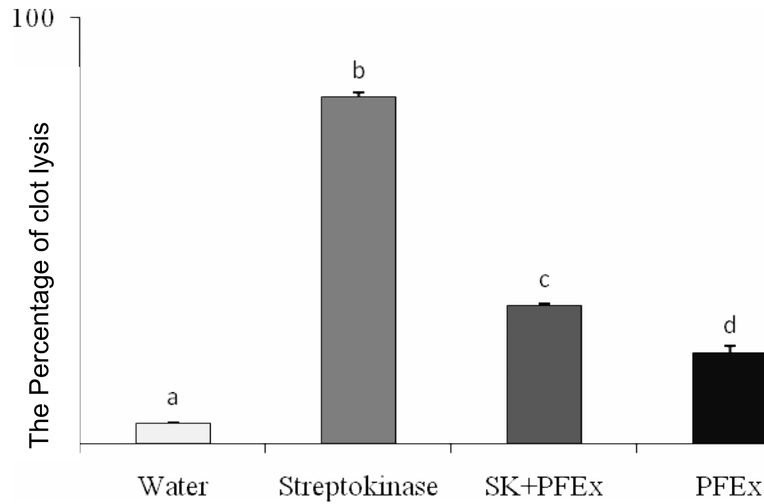

Fig. 1. Comparative % of *in vitro* clot lysis by Streptokinase, *P. foetida,* streptokinase with *Paederia* extract and distilled water. Data are shown as Mean ± SD of ten volunteers. Values with superscript letters on the bar graph are significant ($p < 0.05$) to each other. Data were analyzed by one-way ANOVA followed by Dunnet's test (SPSS, Version 18.0) for multiple comparisons

Table 1. Effect of *Paederia foetida* extract and streptokinase on *in vitro* clot lysis

Treatment	Mean ± SEM (Percentage of clot lysis)	P value when compared to negative control (water)
Streptokinase	81.42 ± 0.88^a	< 0.0009
PFEx	21.40 ± 1.39^b	< 0.0001

Data for ten healthy volunteers were analyzed as Mean ± SD (standard deviation), PFEx denotes Paederia foetida Extract. Values with superscript letters ^{a} and ^{b} are significant ($p < 0.05$) to each other. Data were analyzed by one-way ANOVA followed by Dunnet's test (SPSS, Version 18.0) for multiple comparisons

3.1.2 Cytotoxic effect of *P. foetida* extract

Paederia foetida extract administration showed a dose dependent mortality of brine shrimp nauplii (Fig. 2). The maximum mortality 100% brine shrimp was observed at the highest concentration of extract. However, the highest mortality was achieved by the lower concentration of reference cytotoxic agent vincristine sulfate. Linear regression analysis (Fig. 3) of the mortality data showed that the LC_{50} value of *P. foetida* leaf extract and vincristine sulfate was 65.31 and 0.76 µg/ mL respectively (Table 2).

Table 2. Comparative LC_{50} values of *P. foetida* extract and reference drug Vincristine sulfate

Sl No	Sample	LC_{50}
1	PFEx	65.31µg/mL
2	VS	0.76 µg/mL

**PFEx denotes P. foetida Extract*

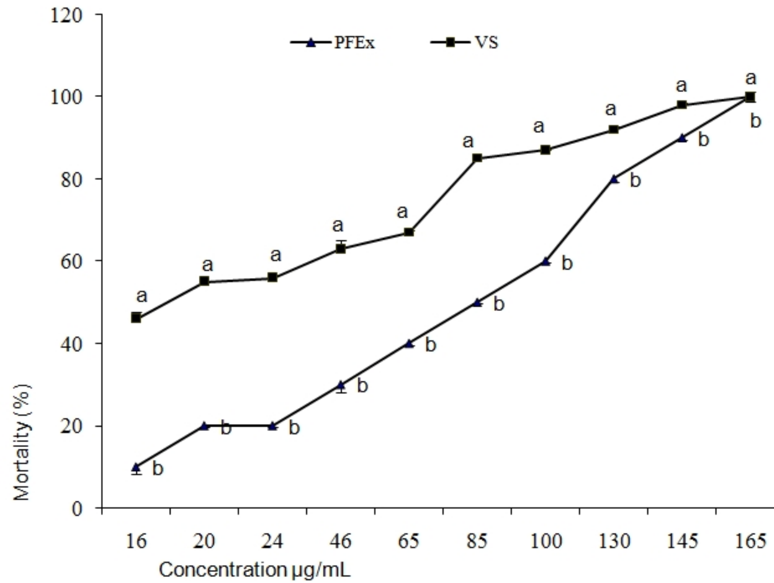

Fig. 2. Comparative cytotoxic effects of *P. foetida* extract and vincristine sulfate. Data are shown as Mean ± SD of twenty nauplii in each group. Values with superscript letters are significant (p < 0.05) to each other. Data were analyzed by one-way ANOVA followed by Dunnet's test (SPSS, Version 18.0) for multiple comparisons

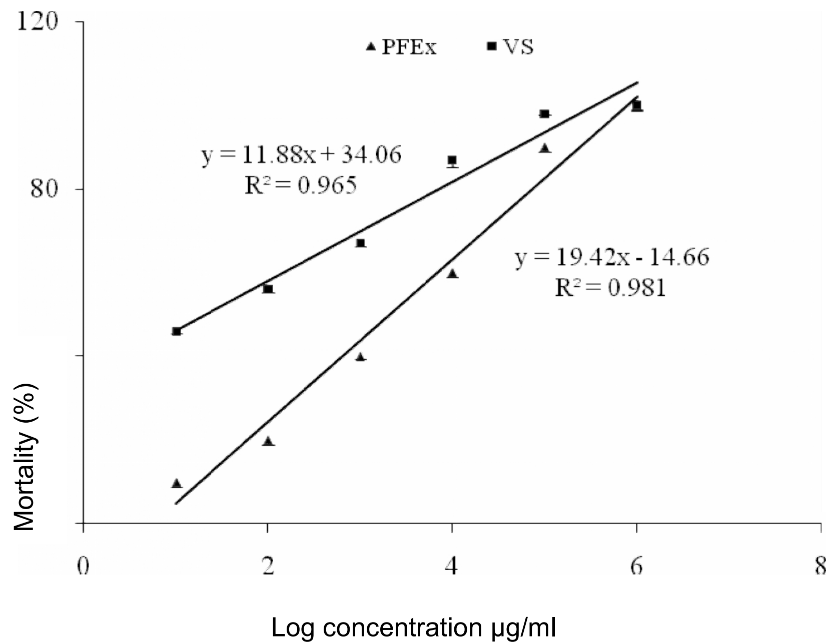

Fig. 3. Linear regression analysis of brine shrimps mortality to determine the LC_{50} value of *P. foetida* extract and vincristine sulfate. Data are shown as Mean ± SD of twenty nauplii in each group. Values with superscript letters are significant (p < 0.05) to each other. Data were analyzed by one-way ANOVA followed by Dunnet's test (SPSS, Version 18.0) for multiple comparisons

3.1.3 Antidiabetic effect of *P. foetida* extract

Metformin hydrochloride and *P. foetida* extract (PFE) was intraperitoneally administered in fructose fed alloxan induced diabetic mice whereas normal control and diabetic control group fed only normal diet. PFE-300 mg/kg and PFE-500 mg/kg showed significant ($p < 0.05$) fasting glucose lowering (FBG) effect of diabetic mice compared to normal control and diabetic control mice (Fig. 4). The extract was found to reduce FBG by 42.37% and 56.32% respectively from 0 to 120 min of treatment period. The activity was comparable to with the standard drug metformin HCl at a dose of 150 mg/kg body weight which reduced FBG 79.37%. The extract and reference drug caused a time dependent and significant ($p<0.01$and $p<0.001$) reduction of the fasting blood glucose in alloxan-induced diabetic mouse compared to the negative control group (Fig. 5).

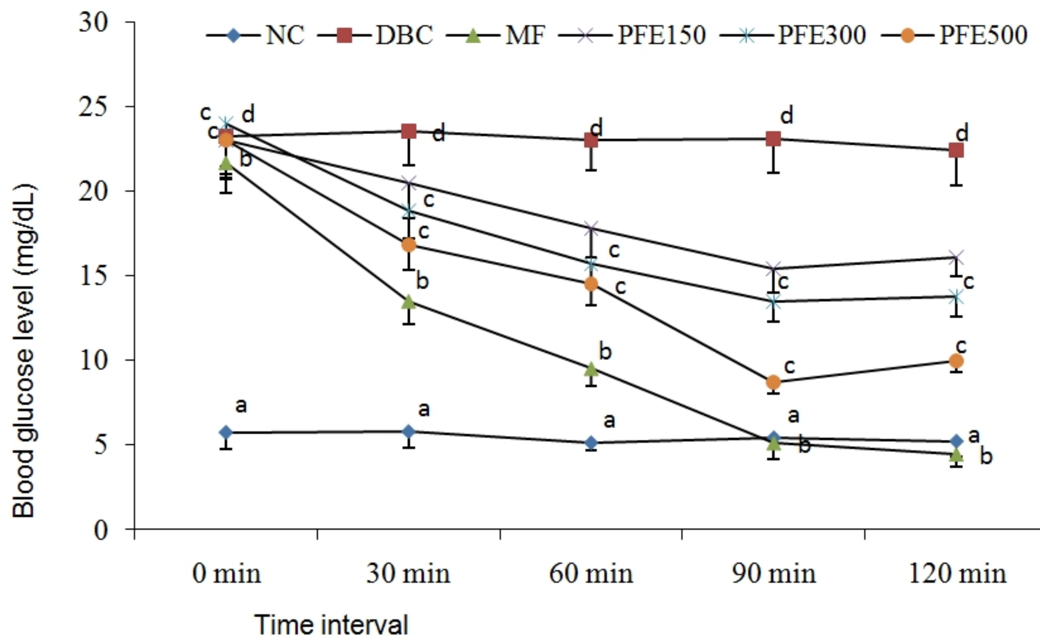

Fig. 4. Effect of *P. foetida* extract (PFE) on glucose tolerance for alloxan induced mice in different time intervals. Data are shown as Mean ± SD of five animals in each group. Values with superscript letters are significant (*p* < 0.05) to each other. Data were analyzed by one-way ANOVA followed by Dunnet's test (SPSS, Version 18.0) for multiple comparisons

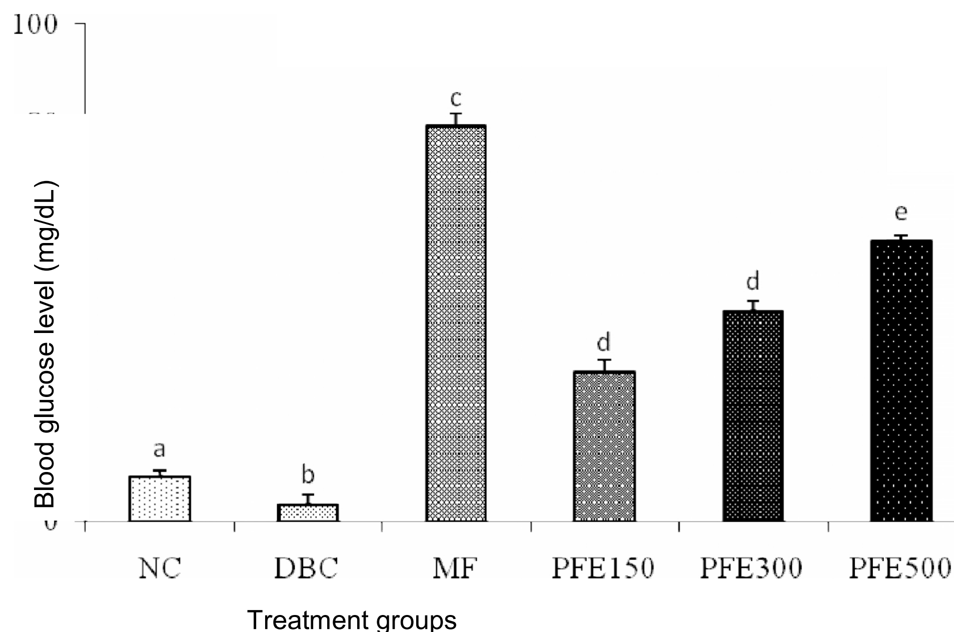

Fig. 5. Fasting blood glucose (FBG) levels in different treatment groups. Data are shown as Mean ± SD of five animals in each group. Values with superscript letters on bar graph are significant (*p* < 0.05) to each other. Data were analyzed by one-way ANOVA followed by Dunnet's test (SPSS, Version 18.0) for multiple comparisons

3.2 Discussion

Streptokinase is a novel thrombolytic agent used as a positive control to compare the clot lysis effects of *P. foetida* extract [24] which is researched first time for such effect. Although there are numbers of plants have already been recorded for their thrombolytic potentials [16]. Our study revealed a moderate but significant role of the extract in thrombolysis compared to Streptokinase. The effect is improved once the extract is combined with streptokinase to administrate indicating that the activity of plant extract could be ameliorated in combination with synthetic drugs. The clot lysis mechanism could be explained through the investigation of [25]. He established that bacterial contaminants of plants bind plasminogen which is easily activated to plasmin leading to fibrinolysis. Staphylokinase and streptokinase are bacterial plasminogen activators which act as cofactor molecules to enhance the substrate presentation to the enzyme through exosite formation. Staphylokinase destroys the extracellular matrix and fibrin fibers that hold cells together and activates plasminogen to dissolve clots [26,27]. This could be complied with the reason how *P. foetida* extract dissolves the clot as it is evidenced to have antibacterial effect against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri* [28]. Apart from this, researchers reported that clot lysis effects are exerted by the richness of alkaloids, flavonoids, tannins and terpenoids in the extract [29,30]. Similar secondary metabolites present in *P. foetida* extract, therefore, might contribute to the exerted thrombolytic effects.

The cytotoxic activity of crude extract of *P. foetida* was investigated *in vitro* Brine shrimp bioassay. Brine shrimp lethality bioassay is an indicator of cytotoxicity, pesticidal effects and various pharmacologic actions [31]. The results of our study coincided with the findings of

[32] who described that *in vitro* test is highly correlated with *in vivo* tests and it is a useful alternative model for predicting toxicity in plant extracts. In this assay, rate of mortality of nauplii was found to be a dose dependent phenomenon. LC_{50} value of the leaf extract was found 65.31µg/mL which was significant compared to that (0.76 µg/mL) of Vincristine sulfate, a standard cytotoxic agent. Results indicate that the leaf extract may have potential pharmaceutical actions [33] to be processed further to formulation. As it is postulated that crude extracts resulting in LC_{50} values less than 250 µg/mL is usually considered significantly active and potential for further investigation [34]. The reason underlying the cytotoxic effect of the extract might be due to the natural metabolites especially the flavonoids existed in the extract. Previous studies demonstrated that the most of the natural flavonoids exert cytotoxic effects [35-37]. The plants may therefore be a potential source for the discovery of new cytotoxic compounds.

Alloxan induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents [38]. In our study, *P. foetida* extract displayed a dose dependent glucose lowering effect on alloxan induced diabetic mouse model. Among the doses, PFE-300 mg/kg and PFE-500 mg/kg showed a very significant ($P<0.01$) antidiabetic effect. The activity was comparable to the activity of the standard drug metformin HCl at a dose of 150 mg/kg BW. However, there was no significant change in the fasting blood glucose level of normal control group that received only citrate buffer solution indicating the impact of *P. foetida* extract. Such antidiabetic effect might be due to the presence of glycosides and steroids in the leaf extract because these secondary metabolites are documented to show antidiabetic effect [39]. Diabetes mellitus is a condition where strong oxidative stress is involved to produce free radicals. Flavonoids and tannins, among the secondary metabolites, possess potent antioxidant activity to deactivate the free radicals through different mechanisms and thus reducing the diabetic condition produced through alloxan injection [40-42].

4. CONCLUSION

The study suggested that the *P. foetida* leaf extract has moderate thrombolytic effect, strong cytotoxic and promising antidiabetic effects. Further studies are suggested to confirm the molecular mechanism involved in these effects. Isolation and identification of bioactive compounds are also suggested to assist the mechanistic evaluation.

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CONSENT

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

COMPETING INTEREST

Authors declare that there is no competing interest.

REFERENCES

1. Tandrima M, Biplab D, Arindam D, Avijit C, Binoy BG. Isolation of antimicrobially active compounds from the leaf of tribal edible plants of Tripura: *Momordica charantia* L. and *Paederia foetida* L. J Nat Prod Plant Resour. 2011;1(4):108-116.
2. Christian P. The genus *Paederia* L. A multidisciplinary study. Opera Botanica Belgica, Meise, Belgium; 1991.
3. Weber E. Invasive plant species of the world: a reference guide to environmental weeds. CABI Publishing Series, Oxford University Press; 2003.
4. Pemberton R W, Pratt PD. Biological control of invasive plants in the eastern United States. Department of Agriculture, Forest Service: 2002; 373-383. Accessed 12 August 2012.
   Available: http://www.invasive.org/eastern/biocontrol /27SkunkVine.html .
5. Ghani A. Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edition, Asiatic Society of Bangladesh; 2003.
6. Yusuf M, Wahab MA, Yousuf M, Chowdhury JU, Begum J. Some tribal medicinal plants of Chittagong hill tracts, Bangladesh. Bangladesh J Plant Tax. 2007;14(2):117-128.
7. Begum J, Yusuf M, Chowdhury JU, Khan S, Anwar MN. Antifungal Activity of Forty Higher Plants against Phytopathogenic Fungi. Bangladesh J Microbiol. 2007;24(1):76-78.
8. Rastogi RP, Mehrotra BN, Sinha S, Seth R. Central Drug Research Institute (India), Council of Scientific & Industrial Research, India; 1993.
9. De S, Ravishankar B, Bhavsar GC. Investigation of the anti-inflammatory effects of *Paederia foetida*. J Ethnopharmacol. 1994;43(1):31-8.
10. Srivastava MC, Tewari JP, Kant V. Anti-inflammatory activity of an indigenous plant-*Paederia foetida* (Gandhali). Indian J Med Sci. 1973;27(3):231-4.
11. Hossain MM, Ali MS, Saha A, Alimuzzaman M. Antinociceptive Activity of Whole Plant Extracts of *Paederia foetida*. Dhaka Univ. J Pharm Sci. 2006;5(1-2):67-69.
12. Osman H, Rahim AA, Isa NM, Bakhir NM. Antioxidant activity and phenolic content of Paederia foetida and *Syzygium aqueum*. Molecules. 2009;14(3):970-8.
13. Afroz S, Alamgir M, Khan MT, Jabbar S, Nahar N, Choudhuri MS. Antidiarrhoeal activity of the ethanol extract of *Paederia foetida Linn*. (Rubiaceae). J Ethnopharmacol. 2006;105(1-2):125-30.
14. Roychoudhury GK, Chakrabarty AK, Dutta B. A preliminary observation on the effects of *Paederia foetida* on gastro-intestinal helminths in bovines. Indian Vet J. 1970;47(9):767-9.

15. Lampronti I, Khan MT, Bianchi N, Ather A, Borgatti M, Vizziello L, Fabbri E, Gambari R. Bangladeshi medicinal plant extracts inhibiting molecular interactions between nuclear factors and target DNA sequences mimicking NF-kappaB binding sites. Med Chem. 2005;1(4):327-33.
16. Prasad S, Rajpal SK, Jayant YD, Hemant JP, Girdhar MT, Hatim FD. Effect of *Fagonia arabica* Dhamasa. on *in vitro* thrombolysis. BMC Complement Altern Med. 2007;7:7-36.
17. McLaughin JL, Chnag CJ, Smith DL. Bench-Top Bioassays for the discovery of bioactive natural product. In: An update (Atta Ur- Rahman Ed), Studies in natural product Chemistry. Elsevier Science Publisher Amsterdam. 1991;9:101-103.
18. Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45:31-34.
19. Mannan A, Kawser JM, Ahmed AMA, Islam NN, Alam SMM, Emon MAEK, Gupta SD. Assessment of antibacterial, thrombolytic and cytotoxic potential of *Cassia alata* seed oil. J App Pharm Sci. 2011;1(9):56-59.
20. Trinder P, Barham D. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 1972;97(151):142-145.
21. Teuscher A, Richterich P. Enzymatic method of glucose. Schweizerische Medizinische Wochenschrift. 1971;101:345-390.
22. Helal M; Md SI, Salma P, Maizbha UA. Antimicrobial and Cytotoxic Activity of the Methanol Extract of *Paederia foetida* Linn. (Rubiaceae). J App Pharm Sci. 2012;2(1):77-80.
23. Gayathri M, Kannabiran K. Hypoglycemic activity of *Hemidesmus indicus* R. Br. on streptozotocin-induced diabetic rats. Int J Diab Dev Ctries. 2008;28(1):6-10.
24. Tillett WS, Garner RL. The fibrinolytic activity of hemolytic streptococci. Bacteriol Rev. 1938; 2(2):161–216.
25. Pantzar M, Ljungh A, Wadström T. Plasminogen binding and activation at the surface of *Helicobacter pylori* CCUG 17874. Infect Immun. 1998;66(10):4976-4980.
26. Parry MA, Zhang XC, Bode I. Molecular mechanisms of plasminogen activation: bacterial cofactors provide clues. Trends Biochem Sci. 2000;25(2):53-59.
27. Collen D: Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator? Ann Intern Med. 1990;1(12):529-538.
28. Uddin B, Nahar T, Khalil MI, Hossain S. In vitro antibacterial activity of the ethanol extract of *Paederia foetida* L. (Rubiaceae) leaves. Bangladesh J Life Sci. 2007;19(2):141-143.
29. Hoque N, Imam MZ, Akter S, Mazumder MEH, Hasan SMR, Ahmed J, Rana MS. Antioxidant and antihyperglycemic activities of methanolic extract of Glinus oppositifolius leaves. J App Pharm Sci. 2011;1(7): 50-53.
30. Dwivedi S. Terminalia arjuna Wight & Arn.-A useful drug for cardiovascular disorders. J Ethnopharmacol. 2007;114(2):114-129.
31. McLaughin JL, Chnag CJ, Smith DL. Bench-Top Bioassays for the discovery of bioactive natural product: In:An update Atta Ur- Rahman Ed., Studies in natural product Chemistry. Elsevier Science Publishers, Amsterdam; 1991.
32. Parra AL, Yhebra RS, Sardinas IG, Buela LI. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine. 2001;8(5):395–400.

33. Gupta A, Kumar R, Pal K, Singh V, Banerjee PK, Sawhney RC. Influence of Sea buckthorn (*Hippophae rhamnoides* L.) flavone on dermal wound healing in rats. Mol Cell Biochem. 2006;290:193–198.
34. Rieser MJ, Gu ZM, Fang XP, Zeng L, Wood KV, McLaughlin JL. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. J Nat Prod. 1996;59(2):100-108.
35. Choi SI, Jeong CS, Cho SY, Lee YS. Mechanism of apoptosis induced by apigenin in HepG2 human hepatoma cells: involvement of reactive oxygen species generated by NADPH oxidase. Arch Pharm Res. 2007;30:1328-1335.
36. Jeune M A, Kumi JD, Brown J. Anticancer activities of pomegranate extracts and genistein in human breast cancer cells. J Med Food. 2005;8:469-475.
37. Lim do Y, Jeong Y, Tyner AL, Park JH. Induction of cell cycle arrest and apoptosis in HT-29 human colon cancer cells by the dietary compound luteolin. Am J Physiol Gastrointest Liver Physiol. 2007;292:G66-75.
38. Szkudelski T. The mechanism of Alloxan and streptozotocin action in β cells of the rat's pancreas. Physiol Res. 2001;50(6):536-546.
39. Kato A, Miura T, Fukunaga T. Effects of steroidal glycosides on blood glucose in normal and diabetic mice. Biol Pharm Bull. 1995;18(1):167-168.
40. Dias AS, Porawski M, Alonso M, Marroni N, Collado PS, González-Gallego J. Quercetin decreases oxidative stress, NF-B activation, and iNOS over expression in liver of streptozotocin induced diabetic rats. J Nutr. 2005;135: 2299-2304.
41. Lukačínová A, Mojžiš J, Beňačka R, Keller J, Maguth T, Kurila P, Vaško L. Preventive Effects of Flavonoids on Alloxan-Induced Diabetes Mellitus in Rats. Acta Vet Brno. 2008; 77: 175-182.
42. Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Antidiabetes and Anti-obesity Activity of Lagerstroemia speciosa. Evid Based Compl Alt Med. 2007;4(4):401-407.

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