Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(3): 129-131
Received: 19-03-2016
Accepted: 20-04-2016

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In vitro thrombolytic potentials of methanolic extract of *Vigna unguiculata* Linn (seed)

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Abstract
Inquisition with methanolic extract of *Vigna unguiculata* Linn (seeds) was carried out to determine the thrombolytic potential of this plant. Plant seeds were extracted with methanol at room temperature. Then concentrated methanolic extract five different concentration (2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, 10mg/ml) from concentrated methanolic extract was used to evaluate thrombolytic potential of *Vigna unguiculata* seeds. An easy & rapid methodology (In-vitro Thrombolytic model) was applied to find out their thrombolytic potential where streptokinase and distilled water were employed as positive and negative controls respectively. The plant showed significant clot lysis, i.e. concentrations 12.01 ± 1.50, 16.48 ± 2.31,24.88 ± 1.49,31.24 ± 0.68,40.33 ± 3.64 at 2mg/dl, 4mg/ml, 6mg/ml, 8mg/ml, 10mg/ml respectively, while the standard (streptokinase) and negative control (distilled water) showed 58.41 ± 3.71 and 2.56±1.23% clot lysis respectively. It is clear that *Vigna unguiculata* Linn (seed) methanolic extract showed thrombolytic activity significantly while comparing with standard. Our present studied suggest that further studies needed to be carried out to get ultimate conclusion of this studies.

Keywords: Inquisition, *Vigna unguiculata*, Thrombolytic potential, positive and negative control.

Introduction
The history of human beings used medicinal plants to treat diverse diseases goes back to thousands of years ago [1]. Though now a days synthetic drugs greatly abolish the roles of medicinal plant in the advent of modern or allopathic medicine, even now a number of modern drug discoveries have been based on medicinal plants used by indigenous people [2]. It has been reported that about 64% of the total world population is using traditional medicine to satisfy their health-care needs [3]. All this précised information encourage me to study with new plant to determine different pharmacological activity of different plant. Here, I carried out my study with *Vigna unguiculata* Linn (seeds). Vigna unguiculata Linn.is medicinal plant belonging to the family Fabaceae or Leguminosae, having lot of pharmacological properties. But there were very few research works on this plant, especially in Bangladesh. My present study is carried out to determine the in-vitro thrombolytic potentials of methanolic extract of Vigna unguiculata Linn (seeds).

Present study suggest that blood clot formation has been a severe problem of blood circulation [4]. Thrombus or embolus causes the Blocking of blood vessel thus depriving blood and oxygen supply to tissues and yield tissue necrosis [5, 6]. Thrombin formed blood clot from fibrinogen and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). The purpose of a fibrinolytic drug is to dissolve thrombin in acutely occluded coronary arteries thereby to restore blood supply to ischemic myocardium, to limit necrosis and to improve prognosis [7]. Now a days, Tissue plasminogen activator, urokinase, streptokinase (SK), etc. are used as thrombolytic agents to liquefy the already deposited clots in the blood vessels [8-10]. Though, these drugs have definite limitations which cause severe and sometimes fatal disorders including hemorrhage, severe anaphylactic reaction, lacked specificity, etc. Moreover, consequently of immunogenicity multiple treatments with SK in a specified patient are restricted [11]. Agents from plant source are likely to be less antigenic and inexpensive. That is why Significant efforts have been focused towards the finding and progress of natural products from various plant and animal sources which have antiplatelet [12, 13], anticoagulant[14-16], antithrombotic and thrombolytic activities [17].

Collection and Extraction

For this present investigation *Vigna unguiculata* Linn (seeds), were collected from Noakhali, Bangladesh on April, 2015. After collection seeds were thoroughly washed with water. The plant was identified by expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. 500 g of the dried and powdered sample was soaked in 1000ml of 99.8% methanol. After 15 days the solution was filtered using filter cloth and Whatman® filter paper No. 1. The resulting filtrates were then evaporated in rotary evaporator below 40 °C to dryness and thus a concentrated semisolid mass of the extract was obtained.

Streptokinase (SK)

Commercially available lyophilized stac (Streptokinase) vial (Incepta Pharmaceutical Ltd) of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for in vitro thrombolysis.

Specimen

Five different test solutions were used to evaluate the thrombolytic activity of the plant extract. The plant extract was dissolved in methanol and shaken vigorously on a vortex mixer to prepare different concentrations (2, 4, 6, 8 and 10 mg/ml respectively) of the test sample. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 µl of the methanolic preparations of the plant were added to the micro-centrifuge tube containing the clots to check thrombolytic activity [6, 2].

Thrombolytic activity

In vitro clot lysis activity of *Vigna unguiculata* was carried out according to the method of Prasad et al., 2007 with minor modifications. 7 ml of venous blood was drawn from healthy volunteers (n = 3) and transferred to different pre weighed sterilized micro-centrifuge tube (1 ml/tube). The micro-centrifuged tubes were exposed to incubation at 37°C for 45 minutes. After the formation of clot, serum was completely discarded 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). Each micro-centrifuge tube containing clot was appropriately labeled and 100 µl of the plant extract with various concentrations (2, 4, 6, 8 and 10 mg/ml respectively) was added to the tubes accordingly. As a positive control, 100 µl of streptokinase and as a negative non-thrombolytic control, 100 µl of sterilized distilled water were distinctly added to the control tubes numbered. After that the tubes were incubated again at 37°C for 90 minutes and observed for clot lysis. After The following incubation, the obtained fluid was discarded from the tubes and they were again weighed to observe the difference in weight after clot disruption. Finally, difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the under beneath equation.

% of clot lysis = (wt. of released clot /clot wt.) × 100%

Results

As a part of discovery of cardio protective drugs from natural sources extractives of *Vigna unguiculata* were assessed for thrombolytic activity and the results are presented in Table 1

Table 1: Thrombolytic activity of methanolic extract of *Vigna unguiculata* Linn (seeds)

Treatment	% Clot lysis (Mean ± SEM)	P-value when compared to negative control (water)
Extract (2 mg/ml)	12.01 ± 1.50*	0.0238
Extract (4 mg/ml	16.48 ± 2.31*	0.0203
Extract (6 mg/ml)	24.88 ± 1.49**	0.0050
Extract (8 mg/ml)	31.24 ± 0.68***	0.0007
Extract (10 mg/ml)	40.33 ± 3.64**	0.0054
Streptokinase	58.41 ± 3.71**	0.0032

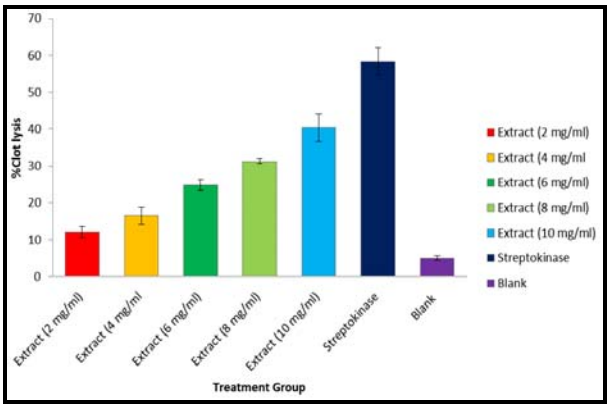

Fig 1: Thrombolytic activity of methanolic extract of *Vigna unguiculata* Linn (seeds)

Discussion

As a part of discovery of cardio protective drugs from natural resource of the extract of *Vigna unguiculata* for thrombolytic activity and the results are presented in Table 1. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 58.41% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot (4.94%). When clots were treated with 100 µl each of different concentrations (2, 4, 6, 8 & 10 mg/ml respectively) of the test sample significant clot lysis activity, i.e.,12.01%, 16.48%, 24.88%, 31,24% and 40.33% respectively, was observed and when compared with the negative control (water) the mean of percentage (%) of clot lysis was found most significant in case of 8mg/ml concentration, % lysis was more significant in case of 6mg/ml, 10mg/ml, and significant in case of 2mg/ml, 4mg/ml. Percentage of clot lysis after treatment with different concentrations of the methanolic extract and proper controls is shown in Figure. The aim of the present study was to find if the herbal preparation of *Vigna unguiculata* clot lysis potentiality or not. The evaluation of the positive control (streptokinase) with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. Encouraged by the result of the positive control, we compared five different concentrations of the test sample in the same manner with the negative control and observed

significant thrombolytic activity. Since phytochemical analysis showed that the crude extract contains tannin & alkaloid; it could be predicted that these phytochemicals may be responsible for its clot lysis activity. So, further study may carry on this seeds of cowpea to build up an acceptable report in the Thrombolytic field.

Conclusion

In order to conclude this study, based on the above findings it can be cleared that this plant seeds may have significant implications in the Thrombolytic field. In addition, this study also indicate the possibility of developing novel drugs in thrombolytic field from *Vigna unguiculata* Linn (seed). Further studies are needed to carry out to isolate and characterize the compounds responsible for blood clot lysis.

Acknowledgement

Authors are really grateful to Incepta Pharmaceuticals limited for their standard anticoagulant agent during the study carried out. The authors are also grateful to all the teachers and staffs of the Department of Pharmacy, Noakhali Science and Technology University for their support and co-operation.

Conflict of interest

The authors declare no conflict of interest with this study

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