British Journal of Pharmaceutical Research
4(11): 1400-1406, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Thrombolytic Effect of Seven Different Bangladeshi Plants

Mohammed Abdullah Jainul[1], Shofiul Azam[1*], Amin Chowdhury[1], Mohammad Mamun Ur Rashid[1], Abdullah Al Mamun[1] and Hasibul Quader Chowdhury[1]

[1]Department of Pharmacy, International Islamic University Chittagong, 154/A College Road, Chawkbazar, Chittagong-4203, Bangladesh.

Authors' contributions

Each and every authors in this project were very co-operative and without a single contribution it was impossible. Author AJ looks after our financial site as well as designed this project. The corresponding authors SA and AC designed and handled the laboratory procedure. Three others contributors were collected the plants from their source and complete extraction procedure. All authors approved this manuscript and helped to finish its writing.

Original Research Article

Received 12th September 2013
Accepted 28th February 2014
Published 31st May 2014

ABSTRACT

Aim: From time past till date, proper documentation of the use of medicinal plants are helpful in drug development and research. The objective of this study is to evaluate several plant extracts of Bangladesh for their thrombolytic activity.
Study Design: There are seven different plants from unlike families were studied in this primary research work. They were collected between August-September (2012) and the thrombolytic effect of their extractions was investigated. Streptokinase was used as standard to compare with and evaluate the significance of each result.
Result: Among all studied plants Gardenia coronaria showed most promising result of 49.61±0.866% of lysis, whereas streptokinase exhibited a lysis of 75.36±0.964%. The extracts of *Hedychium thyrsiforme* and *Artocarpus chaplasha* showed also promising activity with 48.39±1.813% and 43.69±0.906% of thrombolytic effect, respectively.
Conclusion: All the plants used in this study showed promising thrombolytic activity compared to standard. Proper phytochemical characterization and isolation of the constituent compounds responsible for this activity may be further investigated and could be a future source of lead thrombolytic compounds.

**Corresponding author: Email: shofiul_azam@hotmail.com;*

Keywords: Percent of lysis; anti-coagulation; clot; thrombolytic.

1. INTRODUCTION

Blood clotting is one of the major problems during blood circulation. Lodge formation by thrombi into blood vessel, block the flow of blood, deprive tissue in that location and cause lacking of oxygen supply to different part of the body. This can result in a serious destruction in that area creating tissue necrosis. Clot generally formed due to fibrinogen or thrombin and lysed by plasmin that activates plasminogen. Drugs used in such kind of problems restore blood supply, limit necrosis and improve prognosis [1].

Thrombic diseases like myocardial or cerebral infarction are due to the formation of thrombus in blood vessels [2-3]. Thrombolytic agents such as tissue plasminogen activator (tPA), urokinase and streptokinase are used to dissolve previously formed clot in the vessel [4-6]. However, these drugs have some limitations including hemorrhagic disorder, severe anaphylaxis etc. Sometimes, due to result of immunogenicity, different treatments with streptokinase in patients are restricted [7]. Because of these limitations the aforementioned drugs need to be replaced by new agents or should be developed in different way. Attempts to develop and improve these drugs are ongoing [8-11].

Since ancient times, herbal preparations have been used for the treatment of several diseases. Herbal products are often thought to be safe because they originated from natural sources [5]. Epidemiologic studies proved that there are many foods having either anti-thrombotic properties or the ability to reduce risk of thrombosis. Some of the significant reports have been observed in this regard [12].

Agents from plant sources supposed to be none or less antigenic are also available at cheaper rate. Considerable efforts have been noticed in the discovery and development of natural drugs from both herb and animal sources that have anti-platelet [13,14], anti-coagulant [4,15,16] and thrombolytic activity [17]. Based on the folkloric usages, this study was carried out in order to investigate the thrombolytic activity of the selected Bangladeshi plants. Epidemiologic studies indicate that many of these drugs are used as thrombolytic agents directly or similarly to our study. To mention some, *Millettia pachycarpa* is used in Chinese medicine as a blood tonic and also being able to reduce LDL [18-20] and *Gardenia coronaria* is important for its rheumatoid and mild to moderate pain healing properties including bronchitis [21]. A patent drug for the treatment of diabetes obtained from the extract of *Neolamarckia cadamba* [22] is also prominent for treatment of fever and as liver tonic.

2. MATERIALS AND METHODS

2.1 Collection and Identification

The plants were collected between August-September ‘12from Banderban hill tracts, Chittagong. They were identified by a taxonomist of The University of Chittagong and also confirmed by the experts of Botany department of Forest Research Institute Bangladesh, Chittagong branch.

2.2 Extraction

Two hundred gram of air dried plant parts like leave, rhizome, barks etc. were extracted in 500ml methanol and allowed to stand for a week at 25±2ºC. The crude suspension of plant extract was concentrated sequentially after filtration through Buchner funnel.

2.3 Specimen Collection for Thrombolytic Assay

Blood samples were collected from 24 healthy volunteers with no drug history since last four to five days. Three millimitre blood samples were withdrawn from each volunteers with their permission and 0.5ml of each sample was collected in alpine tube and marked alphabetically.

2.4 Preparation of Extract and Standard Suspension

Ten milligram of crude extract was taken to a vial and dispersed in water. The suspension was vibrated vigorously 10-15min to obtain finely dispersed particles. Then all suspensions were allowed to stand for 10-12hr before application. 30,000 IU Streptokinase (Streptase®, by Sanofi-Aventis) was used as standard.

2.5 Assay for Thrombolytic Analysis

Each alpine tube was incubated for around 45 minute at 37ºC, allowing after incubation blood cells to be precipitated and serum to be floated above. Then the serum was withdrawn from each tube carefully without disturbing the clot and weights are noted. Early prepared extract and standard suspensions were introduced to coagulated blood specimen and were allowed to stand for 90mins at 37ºC. After final heating liquefied bloods were carefully withdrawn and weighed again. From the weight variation, the percent of clot lysis was calculated.

2.6 Statistical Analysis

The % of lysis was calculated by the following equation-

$$wt.of\ tube\ and\ clot - wt.of\ tube = wt.of\ clot$$

$$wt.of\ tube\ and\ clot - wt.after\ lysis = wt.of\ lysis$$

$$\frac{wt.of\ lysis}{wt.of\ clot} X\ 100 = \%\, of\ lysis$$

Using common average equation final results were prepared and also their student ‘t’-test values were calculated to find probability unit (p-value) for measuring significance or rejection possibility. BioStat-2009 was used for statistical analysis.

3. RESULTS

In addition of 100µl of Streptokinase (30,000IU) as positive control and same amount of distilled water as negative control, both obtained thrombolytic percentages were 75.36% and

7.75% respectively Table 1. Among the studied plants *Gardenia coronaria, Hedychium thyrsiforme, Millettia pachycarpa, Artocarpus chaplash* and *Neolamarckia cadamba* resulted in 49.61%, 48.39%, 38.11%, 43.69% and 40.88% of lysis effect, respectively. The graphical representation in the Fig. 1 demonstrates clear knowledge about the result we obtained in the study.

Table 1. Thrombolytic activity of the studied plants expressed as percent of lysis

Name	Family	Percent of lysis*	'T'- test
Hedychium thyrsiforme	Zingiberaceae	48.39±1.813	26.696
Millettia pachycarpa	Fabaceae	38.11±1.135	33.579
Maesa indica	Primulaceae	31.64±1.360	23.274
Oroxylum indicum	Bignoniaceae	24.98±1.364	18.320
Artocarpus chaplasha	Moraceae	43.69±0.906	48.206
Neolamarckia cadamba	Rubiaceae	40.88±1.311	31.195
Gardenia coronaria	Rubiaceae	49.61±0.866	57.320
Streptokinase		75.36±0.964	78.218

**mean±SEM each result have p<0.0001, that means statistically significantly beneficial as do standard*

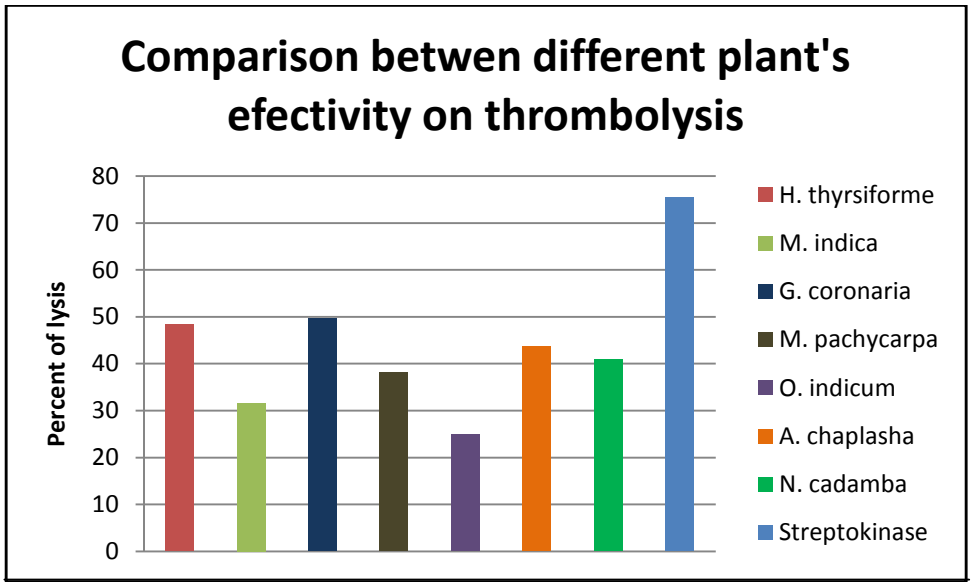

Fig. 1. Graphical representation of studied plant's potency to lyses thrombus in comparison to positive control streptokinase

4. DISCUSSION

There are several thrombolytic drugs obtained from various sources. Some are modified or need further modification to increase efficacy or specificity using the recombinant technology. Many of them have been reported for their adverse effect, while some others are found to be deadly due enhancing bleeding to patient [23-25]. Scientists are always concern of these effects, most of which are solved either by optical is omerization of drug structure, or finding new chemical of same family or mode of action.

Although beneficial effects of thrombolytic therapy are established long before and it's satisfactory [26], search for better and effective therapy is ongoing. The present study with these plants is a part of searching for better anti-coagulant agent. The percent of clot lysis by *G. coronaria* (leaf) and *H. thyrsiforme* (rhizome) was found to be 49.61±0.87% and 48.39±1.81%, respectively, which is substantial compared to standard Streptokinase with 75.36±0.96% of clot lysis effect. These two plants also found to have promising anti-oxidant activity with good reducing capacity and scavenging power, resulting in IC_{50} value of 47.19µg/ml and 55.15µg/ml, respectively. On the other hand *A. chaplasha* (bark) and *N. cadamba* (leaf) also gave good results with 43.69±0.91% and 40.88±1.31% of anti-coagulation effect, respectively.

5. CONCLUSION

This was a preliminary study on these seven plants to screen out their pharmacological action as thrombolytic agents. The importance of this study is that for the first time is reported thrombolytic activity for the studied plants which are proposed for further investigation to identify and characterize their bioactive constituents responsible for this activity that could be a future source of lead thrombolytic compounds.

CONSENT

All persons in this study were willingly volunteered and no one was forced to do this. Before withdrawal of blood sample from them they were alert about the procedure. We also maintained and ensured the personal hygiene and safety procedure before starting with them, thus they feel safe with us. So there is no chance of violation with patient consent or ethical rules in case of using human volunteers.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Laurence DR, Bennett PN. Clinical Pharmacology. 7th Edition. 1992;483.
2. Davies MJ, Thomas AC. Plaque fissuring: The cause of acute myocardial infarction, sudden is chaemic death and crescendo angina. Br. Heart J. 1985;53:363-373.
3. DeWood MA, Spores J, Notske R, Mouser LT, Burroughs R, Golden MS, Lang HT. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. N. Engl. J. Med. 1980;303:897-902.
4. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of *Fagonia Arabica* (Dhamasa) on *In vitro* thrombolysis. BMC Complementary and Alternative Medicine. 2007;7:36.
5. Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits *In vivo* platelet activity and thrombosis in stenosed canine coronary arteries. Circulation. 1995;91:1182-88.

6. Briggs WH, Folts JD and Osman HE. Administration of raw onion inhibits platelet-mediated thrombosis in dogs. J. Nutr. 2001;131:2619-22.
7. Leta GC, MouraoPAS and Tovar AMF. Human venous and arterial glycosaminoglycans have similar affinity for plasma low-density lipoproteins. Biochim. Biophys. Acta. 2002;586:243-53.
8. Basta G, Lupi C, Lazzerini G, Chiarelli P, L'Abbate A, Rovai D. Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An *In vitro* study on blood of normal subjects and patients with coronary artery disease. Thromb Haemost. 2004;91:1078-83.
9. Nicolini FA, Nichols WW, Mehta JL, Saldeen TG, Schofield R, Ross M, Player DW, Pohl GB, Mattsson C. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue plasminogen activator. J Am Coll Cardiol. 1992;20:228–35.
10. Marder VJ. Recombinant streptokinase–opportunity for an improved agent. Blood Coagul Fibrinolysis. 1993;4:1039–40.
11. Lijnen HR, Vanhoef B, DeCock F, Okada K, Ueshima S, Matsuo O. On the mechanism of fibrin-specific plasminogen activation by Staphylokinase. J Biol Chem. 1991;266:11826–32.
12. Wu DH, Shi GY, Chuang WJ, Hsu JM, Young KC, Chang CW. Coiled coil region of streptokinase gamma-domain is essential for plasminogen activation. J Biol Chem. 2001;276:15025–33.
13. Zhiguang L, Hongli W, Jiazeng L, Zhang G and Gao C. Basic and clinical study on the antithrombotic mechanism of glycoaminoglycan extracted from sea cucumber. Chin. Med. J. 2000;113:706-11.
14. Rajapakse N, Jung WK, Mendis E, Moon SH and Kim SK. A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. Life Sci. 2005;76: 2607-19.
15. Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. Nutrition. 2005;21:580-87.
16. Anwar AK, Ashfaq M, Nasveen MA. Pharmacognostic Studies of Selected Indigenous Plants of Pakistan, Pakistan Forest Institute, Peshawar NWFP, Pakistan. 1979;15-35.
17. Gillman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC, Castelli WP, Wolf PA. Protective effect of fruits and vegetables on development of stroke in men. JAMA. 1995;273:1113-17.
18. Zhang H. Observation on curative effect of Huteng Tang (Huzhang and Millettia Combination) in treating side effects caused by cancer chemotherapy. The Practical Journal of Integrated Chinese and Western Medicine.1996;9(3):137.
19. Dorsher P, Peng Z. Chinese medicinal herbs use in Managing cancer. In: (Cho WCS, ed) Supportive Cancer Care with Chinese Medicine. Springer, Netherlands. 2010;55-75.
20. Wu T, Fu J, Yang Y, Zhang L, Han J. The effects of phytosterols/stanols on blood lipid profiles: A systematic review with meta-analysis. Asia Pac J Clin Nutr. 2009;18(2): 179–186.
21. Yusuf M, Begum J, Hoque MN, Chowdhury JU. Medicinal plants of Bangladesh. BCSIR, Chittagong. 2009;794.
22. Patent of Kadamba. India.bigpatent.org/view/19618.
23. Boudjeltia KZ, Cauchie P, Remacle C, Guillaume M, Brohee D, Hubert JL, Vanhaeverbeek M. A new device for measurement of fibrin clot lysis: Application to the Euglobulin Clot Lysis Time. BMC Biotechnology. 2002;2:8.

24. Greenberg RK, Ouriel K, Srivastava S, Shortell C, Ivancev K, Waldman D, Illig K, Green R. Mechanical versus Chemical Thrombolysis: An *In vitro* differentiation of thrombolytic mechanisms. Journal of Vascular and Interventional Radiology. 2000;11:199-205.
25. Komorowicz E, Kolev K, Lerant I, Machovich R. Flow rate-modulated dissolution of fibrin with clot-embedded and circulating proteases. Circulation Research. 1998;82:1102-08.
26. Collen D. Fibrin-selective thrombolytic therapy for acute myocardial infarction. Circulation. 1996;93:857-65.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=526&id=14&aid=4751