eISSN: 2231-0541 CAS CODEN: PHARN8 An EMBASE, EMCare Covered Journal
PHARMANEST
An International Journal of Advances in Pharmaceutical Sciences

Volume 6|Issue 2|March-April 2015|Pages 2828-2830

Original Research Article

PHYTOCHEMICAL INVESTIGATION AND *IN -VITRO* THROMBOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF *FERULA ASAFOETIDA* L. EXUDATE.

SUSHAMA PATIL, SARIKA GAIKWAD, NIKITA DESHMUKH, ASHISH JAIN

Shri. D. D. Vispute College of Pharmacy and Research Center, New Panvel, Maharashtra.

Author for Correspondence: sushamapatil24@gmail.com

ABSTRACT

Investigation with the crude methanolic extract of *Ferula asafoetida* L. exudate carried out to evaluate its possible thrombolysis activity. Myocardial or cerebral infractions are the serious consequences in atherothrombotic diseases leading to death and the side effects produced by consecutive use of thrombolytic agent like urokinase and streptokinase to treat these diseases has become a global concern. The crude methanolic extract was found to have significant thrombolytic activity at a dose of 800 µL (Concentration-10 mg/ml) with amaximum effect comparable with Streptokinase as a positive control and water as a negative control. Activity of the crude drug may be attributed to the components like ferulic acid, coumarins and sulphur containing compounds.

Key Words: Asafoetida, methanolic extract, Streptokinase, Clot lysis.

INTRODUCTION

A blood clot (thrombus) developed in the circulatory system can leads to various circulatory, cardiovascular diseases and death of human being. A healthy hemostatic systemsuppresses the development of blood clots in normal circulation, but in presence of any vascular injury it react excessively in order to prevent blood loss.

This ultimately left the patient with stroke, pulmonary embolism, deep vein thrombosis and acute myocardial infraction. [1]

Fibrinolytic therapy is the most suitable treatment for acute myocardial infarction (MI), a condition that kills more patients worldwide than any other.Thrombolyticagentsare drugs which convert plasminogen to plasmin that then degrades fibrin, a major structure component of the thrombus; thus, the more appropriate approach is fibrinolytic therapy. The relatively nonspecific agents are streptokinase, anistreplase, and urokinase.

The newer (or second-generation) plasminogen activators are either recombinant tissue-type plasminogen activator (rt- PA) which includes alteplase and duteplase or several variants of tissue type plasminogen activator which includes reteplase, tenecteplase and lanoteplase. [2]

Because of the shortcomings of the available nonspecific thrombolytic drugs, attempts are underway to develop, a new improved recombinant variants of these drugs [3, 4]. There is no doubtthat, newly developed selective thrombin inhibitors and antiplatelet agents are more potent, however, their safety remains a big question. There is need of continued investigation in this area which can provide new insights about these newer agents and promote progress toward the development ofthe ideal thrombolytic therapy. Recently several third generation thrombolytic agents have been developed. In comparison to second generation agents (altreplase), third generation thrombolytic agents like monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase, and staphylokinaseare having greater potency rates, however, mortality rates are same for both classes [3,4]. Since ancient times, herbs and their preparations have beenused for the treatment of several diseases by human. Herbal products are often perceived as safe because they are natural in origin. Herbs showing thrombolytic activity have been studied and some significant observations have been reported. Epidemiologic studies showed that the herbs with experimentally proved anti-thrombotic effect could reduce risk of thrombosis and further consequences [4, 5].

Asafoetida is an oleo-gum-resin obtained from the exudates of the Iranian endemic medicinal plant, *Ferula asafetida* L., Family Umbelliferae. This species (*Ferula asa-foetida*) is often considered to be the main source of asafoetida, however other *Ferula* species, such as *Ferula rubricaulis*, *Ferula rigidula*, *Ferula alliacea* and *Ferula narthex*are also the sources of asafoetida. Asafoetida has been used as a spice and a folk phytomedicine for centuries. It is traditionally used for the treatment of different diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza.

Several fractions of asafetida such as gum fraction (25%, including glucose, galactose,l-arabinose, rhamnose and glucuronic acid), resin fraction (40–64%, including ferulic acid esters: 60%, free ferulic acid: 1.3%, and coumarin derivatives e.g. umbelliferone) and oil fraction (3–17%, including sulphur-containing compounds, and various monoterpenes) have been separated [6].

Recentely various pharmacological and biological activities of asafoetida have been studied, such as antioxidant [7],antiviral [8], antifungal [9], chemopreventive [10], anti-diabetic [11], antispasmodic [12], hypotensive [12], and molluscicidal activity[13].

Although there is some evidence for anticoagulant action of *Ferula asafetida* gum extract and ferulic acid [14, 15], there is no evidence of *in vitro* clot lysis activity of asafoetida as per our knowledge. Thus present study was performed as starting point for evaluation of phytoconstituents and *in vitro* thrombolytic activity of Asafoetida methanolic extract.

MATERIAL AND METHODS

Plant material

Asafoetida was collected from the local market of Mumbai, India and was authenticated by Dr. Ganesh Iyer, Department of life science, Ramnarayan Ruia College, Matunga, Mumbai.

Preparation of plant extract

100 gm of Asafoetida was refluxed with 100 % methanol for 3 hrs, in a distillation flask over a boiling water bath, cooled at room temperature and filtered. The marc was re-extracted twice by the same procedure and the extracts were mixed together, the solvent was removed by rotary evaporator under reduced pressure until constant weight comes, which gives rise to a semisolid mass of extract.

Phytochemical screening

The preliminary phytochemical analysis of methanol extract of asafetida was done according to the method described by Harbrone [16].

In vitro thrombolytic activity [17]

Specimen- 5 ml of venous blood was drawn from healthy human volunteers (n = 20) without a history of oral contraceptive or anticoagulant therapy and transferred to pre weighed microcentrifuge tubes (0.5 ml/tube).

Streptokinase - To the commercially available lyophilized streptokinase vial (15, 00,000 I.U.) 5 ml phosphate buffered saline(PBS) was added and mixed properly. This suspensionwas used as a stock from which appropriate dilutions were made to observe the thrombolytic activity.

Study design

Microcentrifuge tubes with blood were incubated at 37°C for 45 minutes. After the clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. These tubes were divided into 6 groups namely (6 tubes each group).

Group 1: Negative control group
Group 2:Methanolic extract of asafoetida 200 µL
Group 3:Methanolic extract of asafoetida 400 µL
Group 4:Methanolic extract of asafoetida 600 µL
Group 5:Methanolic extract of asafoetida 800 µL
Group 6: Positive control group

Procedure

A volume of 200,400,600,800 µL of methanol extract (10 mg/mL) was added to micro centrifuge tube of group 2, 3, 4, 5 respectively. As a positive control, 100 µL (5,000 I.U.) of streptokinase and as a negative control 100 µL of distilled water were added to tubes from group 1 and 6 respectively. All tubes were then incubated at 37 °C for 90 min and observedfor clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weights after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis.

Statistical analysis

The data were expressed as mean±S.E.M. Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's test using the Graph Pad software. *P*-value <0.05 was regarded as statistically significant.

RESULTS

The phytochemical screening indicates presence of tannins, alkaloid, glycosides, saponins, flavonoids, carbohydrates and proteins (Table:1). Result showed dose dependent thrombolytic activity of asafetida methanolic extract. At volume of 600 µL and 800 µL, % clot lysis was found to be 41% and 63% respectively (Figure: 1). Therefore, it is evident that the test extract has thrombolytic activity when compared with positive and negative control groups.

Table 1: Phytochemical constituents identified in the Asafoetida methanol extract.

Phytoconstituents	Methanol extract of asafoetida
Alkaloids	+
Tannins	+
Anthraquinones	-
Glycosides	+
Carbohydrates	+
Saponins	+
Flavonoids	+
Terpenoids	-
Proteins	+
Steroids	-

+: Present; -: Absent.

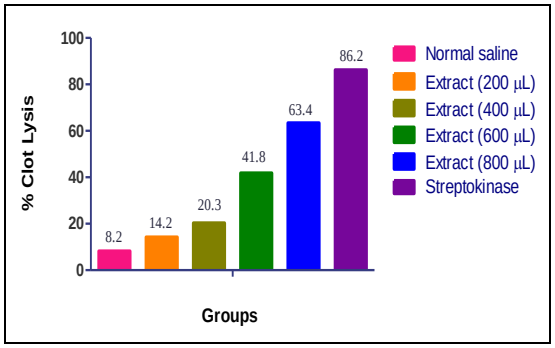

Figure 1: In vitro thrombolytic activity of methanolic extract of Asafoetida.

DISCUSSION AND CONCLUSION

Clot lysis may be the result of the combinatorial effect of the active compounds present orby the

individual compounds like ferulic acid, coumarins and sulphur compounds [18-20]. With further research on cell cytotoxicity and *in vivo* studies, this finding may have important implications in the treatment of cardiovascular diseases which is increasing at an alarming rate. Further advanced studies should be carried out for compound isolation and it is necessary to observe which compounds are actually responsible for the above mentioned effects. Since the drugs used for the cardiovascular diseases are not economical and not accessible to the greater section of the society, application of this study may be a boon for them.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are very much grateful to Shri D. D. Vispute College of Pharmacy and Research Center for timely providing all the research facilities and other logistic supports**.**

REFERENCES

[1] Banarjee UC, Yusuf CB. Streptokinase-a clinically useful thrombolytic agent. BiotechnolAdv2004Feb 22(4): 287-307.
[2] Ohman EM, Harrington RA, Cannon CP, Cairns JA, Kennedy JW. Intravenous thrombolysis in acute myocardial infarction. Chest 2001 Jan 119 (1):supplement253S.
[3] Marder VJ. Recombinant streptokinase – opportunity for an improved agent. Blood Coagul Fibrinolysis 1993 Dec 4 (6): 1039-40.
[4] Verstraete M. Third Generation thrombolytic drugs. Am J Med2000Jul 109(1): 52-8.
[5] BastaG, LupiC, Lazzerini G, Chiarelli P. Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An in vitro study on blood of normal subjects and patients with coronary artery disease. ThrombHaemost2004Jun 91 (6): 1078-83.
[6] Iranshahy M. Traditional uses,phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)—A review. J Ethnopharmacol 2011 Mar 134 (1): 1–10.
[7] Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. GrasasAceites 2009 Jul 60 (4): 405–12.
[8] Lee CL, Chiang LC, Cheng LH, Liaw CC, Abd El-Razek, MH, Chang FR, Wu YC. Influenza A (H1N1) antiviral and cytotoxic agents from *Ferula assa-foetida*.J Nat Prod 2009 Sep72 (9): 1568–72.
[9] Singh R. In vitro evaluation of aqueous and alcoholic extracts of spices for antifungal properties. Indian J Anim Sci 2007 Aug 77 (8): 675–7.
[10] Aruna K, Sivaramakrishnan VM. Anticarcinogenic effects of some Indian plant products. Food Chem Toxicol. 1992 Nov 30 (11): 953–6.
[11] Abu-Zaiton AS. Anti-diabetic activity of *Ferula assafoetida* extract in normal and alloxan-induced diabetic rats. Pak J Biol Sci 2010 Feb 13 (2): 97–100.
[12] Fatehi M, Farifteh F, Fatehi-Hassanabad Z. Antispasmodic and hypotensive effects of *Ferula asafoetida* gum extract. J Ethnopharmacol 2004 Apr 91 (2-3): 321–4.
[13] Kumar P, Singh DK. Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. Chemosphere 2006 Mar 63 (10): 1568–74.
[14] Leung AY, editor. Encyclopedia of Common Natural Ingredients used in Food, Drugs and Cosmetics. New York: Wiley;1980.
[15] Wang S, Gao Z, Chen X, Lian X, Zhu H, Zheng J, Sun L. The anticoagulant ability of ferulic acid and its applications for improving the blood compatibility of silk fibroin. Biomed Mater 2008 Dec 3 (4): 044106.
[16] Harborne JB, editor. Phytochemical methods-a guide to modern techniques of plant analysis. London, UK: Chapman and Hall; 1998.
[17] Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in vitro model to studyclot lysis activity of thrombolytic drugs. Thromb J 2006 Sep 4(14):1-4.
[18] Barone E, Calabrese V, Mancuso C. Ferulic acid and its therapeutic potential as a hormetin for age-related diseases. Biogerontology 2009 Apr 10 (2): 97-108.
[19] Parker KL, Brunton LL, Lazo JS. Goodman & Gilman's The Pharmacological basis of therapeutics: Blood coagulation and Anti-coagulant, thrombolytic and Anti-platelet drugs.11th ed. New Delhi: McGraw-Hill Professional; 2006.
[20] Bordia A,Verma SK, Srivastava KC. Effect of garlic (*Allium sativum*) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. Prostag Leukotr Ess 1998 Apr 58 (4): 257- 63.

HOW TO CITE THIS ARTICLE
Sushama Patil, Sarika Gaikwad, Nikita Deshmukh, Ashish Jain. (2015 March 1) Phytochemical Investigation and *In -Vitro* Thrombolytic Activity of Methanolic Extract of *Ferula Asafoetida* L. Exudate..*PHARMANEST, 6(2)*,2828-2830.
http://www.pharmanest.net