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IN VITRO STUDY ON THROMBOLYTIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *VALERIANA JATAMANSI* AND *ORIGANUM VULGARE* LEAVES

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ABSTRACT

The present study was investigated to find out the thrombolytic activity of hydroalcoholic extract of *Valeriana Jatamansi* and *Origanum Vulgare* leaves by using *in vitro* method along with streptokinase as a standard. The study clearly indicated that the extracts possess good thrombolytic activity. In our study, both the plants extract was found to have significant ($p<0.001$) thrombolytic activity at a dose of 100, 200 and 400 mg/ml with a maximum effect of 42.4±3.9 % and 31.5 ± 3.8 % while the standard streptokinase showed 53.4 ± 3.9 %. This finding demonstrates for the first time that the leaves extract of *Valeriana Jatamansi* and *Origanum Vulgare* has admirable thrombolytic effect.

Key words: *Valeriana jatamansi*, *Origanum Vulgare*, Thrombolytic.

INTRODUCTION

Thrombosis is defined as the formation of a blood clot inside a blood vessel and it reduces blood flow in the circulatory system [1]. Thrombosis divided into two types i.e., venous thrombosis and arterial thrombosis. Venous thrombosis is defined as the formation of a blood clot within a vein. Thrombolytic agents are defined as the agent that dissolved the blood clot which occluded blood vessels. Some examples of thrombolytic agents are streptokinase, urokinase, alteplase etc [2, 3].

Streptokinase is a metabolic product of β-haemolytic streptococci [4]. Streptokinase is a potent activator of the fibrinolytic enzyme and has proteolytic activity [4, 5]. Streptokinase has converting plasminogen to plasmin [2]. Streptokinase is made up of 414 amino acid residues. There are three domains of streptokinase such as α, β and γ domain [6]..

Valeriana jatamansi is a perennial herb and tetraploid species belonging to family Valerianaceae. *Valeriana jatamansi* is commonly named as Tagar, Sugandhawal, Jatamansi. It is widely distributed in Western Himalayan, Kashmir, Garhwal, Khasi hills and Bhutan at the heights of 2500-3000 meters [7]. The herb attains the height up to 40-50cm with a thick horizontal root stick. The leaves are 2.5-7.5 cm long. The flowers are unisexual, pinkish white in colour and 2-7 cm long. Leaves are persistent, long petioled, deeply cordate - ovate and toothed or sinuate. The fruits are oblong, compressed and hairy. The rhizomes are irregular in shape and quite characteristic and bitter in taste [7]. The major chemical constituents of *Valeriana jatamansi* are valerenic acid (sesquiterpenoids), valepotriates (iridiod esters), alkaloids, baldrinal, homobaldrinal, amino acid, phenolic acid, flavonoids, valerosidatum, chlorogenic acid, caffeic acid and fatty acid [8]. The reported activities of *Valeriana jatamansi* are antioxidant [9], anti-inflammatory [10], anti-diarrhoeal [11] and antimicrobial [10]. Further, the reported activities of *Valeriana jatamansi* are antioxidant due to the presence of phenolic compounds [9], anti-inflammatory due to the presence of volatile oils [10], anxiolytic activity due to the presence of valtrate [12], anti-diarrhoeal due to the presence of flavonoids [11] and antimicrobial due to the presence of volatile oils [10]. The traditional uses of *Valeriana jatamansi* are Liver protection, sleep improvement, skin disease, obesity, wound healing, antispasmodic and snake poisoning [13].

Origanum vulgare is a perennial herb which is commonly known as sathra, belonging to family Lamiaceae. It is distributed throughout Asia, Europe, North America and India [14]. The plant is about 20-80 cm long and having opposite leaves. The flowering season of *Origanum vulgare* is from June to September [15]. The major chemical constituents of *Origanum vulgare* are Origanol A

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& B, ursolic acid, β-sitosterol, triacontanol, protocatechuic acid, apigenin, luteolin, quercetin, flavonoids, diosmetin and rosmarinic acid [16]. Reported activities of *Origanum vulgare* are antiurolithic activity due to the presence of saponins [17], due to the presence of phenolic compounds. Further, *Origanum vulgare* reported to have antioxidant [18], antimicrobial activity [19] and anticancer activity due to the presence of phenols and flavonoids [14]. Moreover, the plant showed anti-inflammatory [20] and hepatoprotective activity [21]. The traditional uses of *Origanum vulgare* are fever, diarrhoea, indigestion, toothache, jaundice, vomiting, muscle and joint pain, sores and swelling [16]. The main objective of this study was to investigate the thrombolytic activity of hydroalcoholic extract of leaves of *Valeriana Jatamansi* and *Origanum Vulgare*.

MATERIALS AND METHODS

Plant material

Leaves of *Origanum vulgare* and *Valeriana jatamansi* were collected and authenticated from Dr. Yashwant Singh Parmar University of Horticulare and Forestry, Solan (Naun) Himachal Pradesh-173230 by Dr. Bhupinder Dutt, Associate Professor (Forestry) vide book No. 3401, receipt no. 067, dated 31/8/2013.

Preparation of *Valeriana jatamansi* extract

Valeriana jatamansi were collected from Solan region, Himachal Pradesh. The leaves of the plant were washed with running tap water and then shade dried. Leaves were cut into small pieces and grinded into coarse powder using a blender then 50gm of coarse powder were defatted by using 140ml of petroleum ether and further the marc were extracted by using 90% ethanol (140ml) and water (10ml) in soxhlet apparatus. The extract was concentrated on water bath and this extract was stored in airtight container in cool place.

Preparation of *Origanum vulgare* extract

Origanum vulgare were collected from Solan region, Himachal Pradesh. The leaves of the plant were washed with running tap water and then shade dried. Leaves were cut into small pieces and grinded into coarse powder using a blender then 50gm of coarse powder were defatted by using 140ml of petroleum ether and further the marc were extracted by using 90% ethanol (140ml) and water (10ml) in soxhlet apparatus. The extract was concentrated on water bath and this extract was stored in airtight container in cool place.

Procedure for *in-vitro* thrombolytic activity

Venous blood was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy (n=8). 0.5ml of blood was transferred to each of the previously weighed micro-centrifuge tubes to form clots. To the commercially available lyophilized streptokinase vial (15, 00,000 I.U.) 5 ml phosphate buffered saline (PBS) was added and mixed properly. This suspension was used as a stock from which appropriate dilutions were made to observe the thrombolytic activity using the in- vitro model developed in our laboratory. 2 ml venous blood drawn from healthy volunteers was transferred in different pre weighed sterile micro centrifuge tube (500µl/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Each micro centrifuge tube containing clot was properly labelled, 100µl of streptokinase and distilled water was added to the tubes. To each micro centrifuge tube containing pre-weighed clot, different concentration of hydro-alcoholic extracts of test samples (100, 200 and 400 mg/ml) was added. Water was also added to one of the tubes containing clot and this serves as a negative thrombolytic control and 100µl of streptokinase as positive control. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained 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 was expressed as percentage of clot lysis and calculated using the following formula [22, 23].

% Clot lysis = weight of clot after lysis/weight of clot before lysis×100

RESULTS

Valeriana jatamansi at a dose of 100 mg/ml, 200mg/ml and 400mg/ml exhibit significant thrombolytic activity. Further, *Origanum vulgare* at a dose of 100 mg/ml, 200 mg/ml, and 400 mg/ml was also found to be significant thrombolytic activity, when compared to water control. Moreover, the standard drug Streptokinase showed 53.48% clot lysis activity when compared to water control. The above results are depicted in table 1 and presented in figure 1 and 2 respectively.

Table 1. Thrombolytic activity of test compounds and standard.
% clot lysis is represented as mean ± S.D. and * P < 0.05, significant compared to control

S. No.	Extract/Drug	Concentration (mg/ml)	% Clot Lysis
1.	Water Control	-	2.93±0.78
2.	Streptokinase (Standard)	15, 00, 000 I.U	53.48±3.9*
3.	*Valeriana jatamansi*	100	14.6±2.5*
		200	29.1±3.6*
		400	42.4±3.9*
4.	*Origanum vulgare*	100	23.9±2.3*
		200	27.7±3.4*
		400	41.5±3.8*

Figure 1. % Lysis of crude extract of *Valeriana jatamansi* (VJ) and Streptokinase by clot lysis activity. % clot lysis is represented as mean ± S.D. and * p < 0.0001, significant compared to control

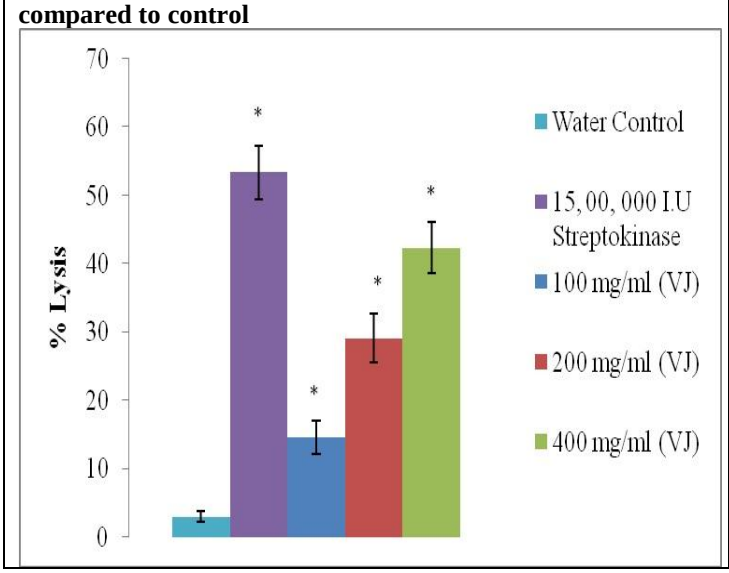
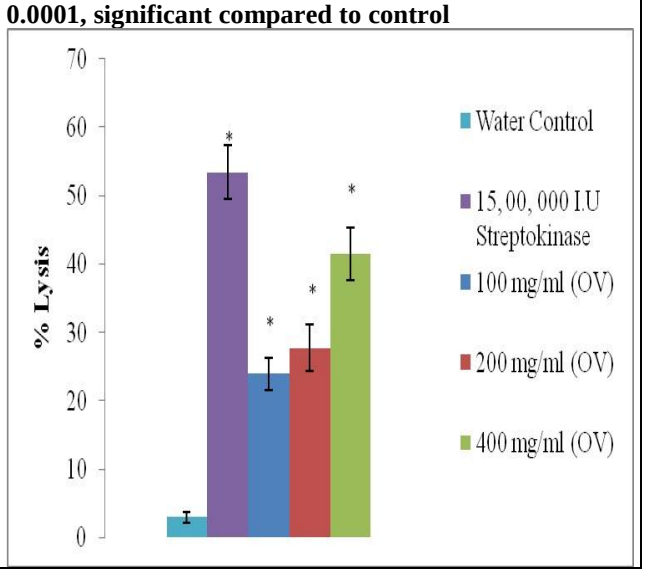


Figure 2. % Lysis of crude extract of *Origanum vulgare* (OV) and Streptokinase by clot lysis activity. % clot lysis is represented as mean ± S.D. and * p < 0.0001, significant compared to control



DISCUSSION

In our study hydroalcoholic extract of *Valeriana jatamansi* and *Origanum vulgare* at a dose of 100mg/ml, 200mg/ml and 400mg/ml showed Thrombolytic activity.

Thrombin plays an important role in the coagulation protease cascade and activates factor XIII and various co-factors which inhibit fibrinogen [24, 25]. Thrombolytic agents are those agents that dissolved the blood clot. Streptokinase is a potent activator of the fibrinolytic enzyme and has proteolytic activity [5] and it converts plasminogen to plasmin [6]. The hydro-alcoholic extracts of *Valeriana jatamansi* and *Origanum vulgare* have showed thrombolytic activity at different concentration i.e., 100, 200 and 400 mg/ml. Our study revealed that *Valeriana jatamansi* and *Origanum vulgare* having thrombolytic activity may be due to the presence of flavonoids, tannins and polyphenols components. The percentage lysis of *Valeriana jatamansi* at the doses of 100, 200 and 400 mg /ml was 14.6, 29.1 and 42.4% respectively and the percentage lysis of *Origanum vulgare* at the doses of, 200 and 400 ml/kg was 23.9, 27.7 and 41.5% respectively.

CONCLUSION

The clot lysis activity of *Valeriana jatamansi* and *Origanum vulgare* showed significant results when compared with standard streptokinase. Therefore, it has been concluded from the above mentioned results that the *Valeriana jatamansi* and *Origanum vulgare* exhibits thrombolytic activity.

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CONFLICT OF INTEREST

The Author(s) declare(s) that he has no conflicts of interest to disclose.

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