

Original Research Article

Assessment of thrombolytic activity of selected species from Lamiaceae family growing naturally in Kurdistan Region\Iraq using In-vitro model

ABSTRACT

Aims: The present study was aimed to assess most commonly used medicinal plant belong to **Lamiaceae** family for their thrombolytic activity.

Place and duration of the study: Pharmacy College\ Hawler Medical University, **June**, 2015.

Methodology: Dried leaf part ethanolic extracts of selected species have been evaluated for their thrombolytic activity using fresh human blood specimen at concentrations of 200, 400, 600, 800 and 1000 **µg**/ml, streptokinase drug and distilled water were incorporated in the study as control positive and negative, respectively.

Results: showed significant thrombolytic activity of leaf extracts at all tested concentrations in comparison with control negative distilled water ($P < 0.01$), highest clot lysis activity were recorded for *Salvia officinalis* leaf extract (68.43 ± 1.457 %) and minimum activity were exhibited by *Rosmarinus officinalis* leaf extract (49.27 ± 3.256 %). *Salvia officinalis* and *Thymus vulgaris* expressed significant thrombolytic activity in comparison with control positive streptokinase ($P < 0.05$) at highest tested dose concentration (1000 **µg**/ml).

Conclusion: Selected species from Lamiaceae plant family can open a venue for production of thrombolytic drugs derived from natural source with greatest activity and lowest adverse effects.

Key words: Thrombolytic drugs; *Salvia officinalis*; *Thymus vulgaris*; *Rosmarinus officinalis*

1. INTRODUCTION

World health organization (WHO) have estimated that more than **80%** of world population in developing countries depend on herbal remedies for maintaining their health [1]. The nature **is** considered as repository of medicinal agents from ancient times, since herbal remedies **are** believed to be safe as they are derived from natural source [2]. **Blood clot (thrombus) causes extensive diseases of cardiovascular system. Thrombolytic agents such as urokinase (UK), streptokinase (SK), tissue plasminogen activator (t-PA) are used to dissolve the formed clot, but they are accompanying by series side effects like haemorrhage and anaphylactic reactions [3-6].** Outstanding attempts were continued for discovering natural derived drugs form either animal or plant source with thrombolytic or antithrombotic activity [7-10].

Lamiaceae plant family is one of the most important plant family including about 400 **geneses**, in which about 40% of them **are** aromatic plants [11]. Lamiaceae plant **families** known for their

volatile oil contents, also they contain many other phytochemicals responsible for their therapeutic activities such as flavonoids and polyphenolic acids. The most commonly used species of this family are: *Salvia officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris*. These plants are chiefly used as antimicrobial, antioxidant. In digestive system ailments they are applied as spasmolytic and carminative. Different species of Lamiaceae contain various amounts of polyphenolic acid, which in turn affect the therapeutic activities of each species [12].

The aim of present study was to assess the thrombolytic activity of selected species from Lamiaceae family which are most commonly used by the local community for various purposes either culinary or medicinal.

2. MATERIALS AND METHODS

2.1. Plant collection

Leaf part of *Salvia officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris* were collected in different places of Erbil city, Kurdistan Region\Iraq have been identified by Department of Pharmacognosy, College of Pharmacy \ Hawler Medical University. Leaves were dried in shade and kept in closed air tight container under 21-23 ° C.

2.2. Plant material extraction

Powdered plant leaves were extracted separately using ethanol (75%) according to method described by Alupuli et al. using ultrasonic assisted extractor machine[13]. The extracts were concentrated and dried under vacuum. The dried extracts were reconstituted using distilled water forming 1mg/ml concentration used as stock solution, extracts were prepared in a serial dilution concentrations of 200, 400, 600, 800 and 1000 µg/mL using distilled water, the solutions were kept overnight. The insoluble material were removed by filtration using 0.22-µm syringe filter, the clear solution have been assessed for thrombolytic activity [14,15].

2.3. Streptokinase (SK) solution preparation

Commercially available lyophilized Streptokinase vials of 1500000 I.U (Abbott), was reconstituted using distilled water, mixed properly. This suspension was used as a standard stock solution from which 100 µl (30,000 I.U) was used for *in-vitro* thrombolytic assay [14,15].

2.4. Blood specimen

Ten ml of fresh blood sample were drawn from healthy human volunteers (n=5) without a history of taking anti-inflammatory and contraceptive for a minimum duration 7-10 days. A volume of (0.5 mL) of blood sample was transferred to ten pre-weighed properly labelled eppendorf tubes in a aseptic condition [14,15].

2.5. Thrombolytic assay

Thrombolytic assay were carried out according to described method by Dagainawala et al. and Sweta et al. [14,15]. Each labelled filled eppendorf tube was allowed to stand at 37 ° C for 45 min for clot formation. After clot formation the serum was withdrawn without disturbing the formed clot using syringe and the tubes were reweighed. The clot weight was estimated from the following equation:

$$\text{Weight of clot} = \text{weight of clot filled tube} - \text{weight of empty tube}$$

Aliquot of 100 µl of each concentration of leaves extracts separately have been added to each tube, and incubated at 37 ° C for 90 min. After incubation, released fluid from the clot lysis were removed using syringe without disturbing the clot and the tubes were weighed again. Positive control (Streptokinase drug, SK) and negative control (distilled water) were included in the study. The deviation in clot weight were expressed as percentage of clot lysis according to the following equation:

$$\% \text{ clot lysis} = (\text{weight of released clot} / \text{weight of clot}) \times 100$$

2.6. Statistical analysis

All data were estimated from triplicate procedure work, mean values $\pm$ standard deviation were determined. Comparison between control and tested samples were performed using two way ANOVA method using graph pad prism 6 considering ($P < 0.05$) statistically significant.

3. RESULTS AND DISCUSSION

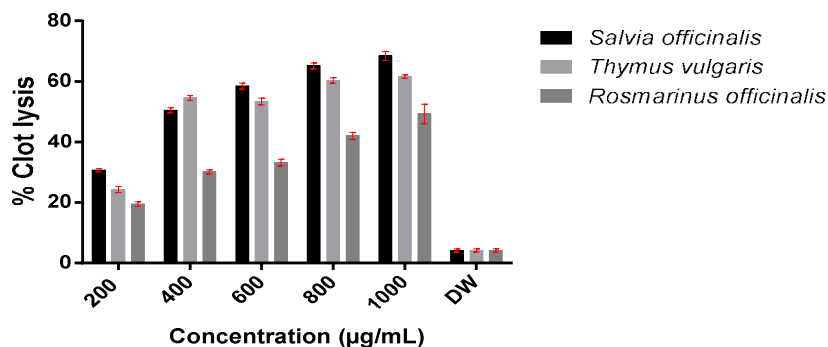
In-vitro thrombolytic activity assessment for all extracts showed dose dependent manner, highest activity where expressed at greatest concentration of 1000 $\mu\text{g/mL}$ for all tested plants (Table 1) and (Figure 1). The significant thrombolytic activities were exhibited by the leaf extracts in comparison with control negative ($P < 0.001$) (Table 2). A significant mild to moderate activity were expressed by the leaf extracts of *S. officinalis* and *T. vulgaris* in comparison to the positive control after incubation for 90 min at 37 $^{\circ}\text{C}$ at (1000 $\mu\text{g/mL}$) concentration ($P < 0.05$). In general all tested concentrations were showed activity ranging from (19.53 $\pm$ 0.784 %) for *R. officinalis* at (200 $\mu\text{g/mL}$) to the highest activity (68.43 $\pm$ 1.457 %) for *S. officinalis* at (1000 $\mu\text{g/mL}$). The thrombolytic activity of leaf extracts can be arranged as (*S. officinalis* > *T. vulgaris* > *R. officinalis*). From literatures the rosmarinic acid, is a characteristic phytochemical constituent of Lamiaceae family plant which is responsible for thrombolytic activity of the plants [15]. It was reported that the quantities of rosmarinic acid in tested plants were as follow: in *S. officinalis* (39.3 $\pm$ 0.5 mg/g) was highest followed by *T. vulgaris* (23.5 $\pm$ 0.5 mg/g) and *R. officinalis* (7.2 $\pm$ 0.1 mg/g) at third rank [16], which was explained the demonstrated activity expressed by the tested plants.

Table 1: Thrombolytic activity of *Salvia officinalis*, *Thymus vulgaris* and *Rosmarinus officinalis*

Concentration ($\mu\text{g/mL}$)	% Clot lysis (mean $\pm$ SD)(n=3)		
	<i>Salvia officinalis</i>	<i>Thymus vulgaris</i>	<i>Rosmarinus officinalis</i>
200	30.74 $\pm$ 0.505	24.30 $\pm$ 1.000	19.53 $\pm$ 0.784
400	50.49 $\pm$ 0.83	54.57 $\pm$ 0.814	30.17 $\pm$ 0.660
600	58.49 $\pm$ 1.020	53.37 $\pm$ 1.102	33.20 $\pm$ 1.105
800	65.20 $\pm$ 1.000	60.33 $\pm$ 0.902	42.07 $\pm$ 1.145
1000	68.43 $\pm$ 1.457	61.67 $\pm$ 0.569	49.27 $\pm$ 3.256

Table 2: Thrombolytic activity of negative and positive control:

Control	% Clot lysis (mean $\pm$ SD) (n=3)
Distilled water (negative control)	72.16 $\pm$ 1.945
Streptokinase SK (positive control)	4.25 $\pm$ 0.526



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110 **Fig.1. Concentration gradient clot lysis % of *Salvia officinalis*, *Thymus vulgaris* and**
 111 ***Rosmarinus officinalis* along with control positive streptokinase (SK) and control**
 112 **negative (DW)**

113 4. CONCLUSION

114 In a continuous venue for developing drugs from natural source for protection of
 115 cardiovascular system with maximum efficacy and lower side effects, it is concluded that the
 116 tested selected species from Lamiaceae plant family can play an important role in developing
 117 thrombolytic drugs derived from nature specially *Salvia officinalis* leaf.

118 CONSENT:

119 Author declared that written informed consent was taken from patients for publication of this
 120 study.

121 ETHICAL APPROVAL:

122 Author declares that all experiments were examined under approval of Pharmacy College
 123 ethics committee.

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