

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, April, 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 mcg\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 concentration in comparison with control negative distilled water (p value < 0.01), highest clot lysis activity were recorded for the *Salvia officinalis* leaf extract (68.43333±1.457 %) and minimum activity were exhibited by *Rosmarinus officinalis* leaf extract (49.27333±3.256 %). *Salvia officinalis* and *Thymus vulgaris* expressed significant thrombolytic activity in comparison with control positive streptokinase (p value < 0.05) at highest tested dose concentration (1000 mcg\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 considered as repository of medicinal agents from ancient times, since herbal remedies believed to be safe as they are derived from natural source [2]. Blood clot (thrombus) caused extensive diseases of cardiovascular system, thrombolytic agents such as urokinase (UK), streptokinase (SK), tissue plasminogen activator (t-PA) used to dissolve the formed clot, but they are accompanied by series side effects like haemorrhage and anaphylactic reactions [3]. 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 species, in which about 40% of them are aromatic plants [11]. Lamiaceae plant family known for their volatile oil contents, also they contains many other phytochemicals responsible for their therapeutical activities such as flavonoid and polyphenolic acids. The most commonly used species of this family are: *Salvia*

officinalis, *Rosmarinus officinalis* and *Thymus vulgaris*. These plants were chiefly used as antimicrobial, antioxidant and digestive system ailments as spasmolytic and carminative. Different species of Lamiaceae have variant polyphenolic acid quantities, which in turn affecting of 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 variant purposes either culinary or medicinal.

2. MATERIALS AND METHODS

2.1. Plant collection

Leaf part of *Salvia officinalis* (SO), *Rosmarinus officinalis* (RO) and *Thymus vulgaris* (TV) 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, 2009 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 mcg/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 100µL (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. 0.5 ml of blood sample were 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 2006 and Sweta et al, 2007 [14,15]. Each labelled filled eppendorf tube were 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 periods the released fluid from the clot lysis were removed using syringe without disturbing the clot and the tubes were weighed again. Control positive and control negative of Streptokinase drug (SK) and distilled water were included in the study, respectively. The deviation in clot weight were expressed as percentage of clot lysis according to the following equation:

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

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 value $\geq$.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 mcg/ml) for all tested plants (Table.1.) and (Figure.1.) . The significant thrombolytic activity were exhibited by the *Salvia officinalis* extracts in comparison with control negative [distilled water] (P value $<$.001) (Table.2.). A significant mild to moderate activity were expressed by the leaf extracts of *Salvia officinalis* and *Thymus vulgaris* in comparison to the positive control after incubation for 90 min at 37 °C at (1000 mcg/ml) concentration [P value $\geq$.05]. In general all tested concentration showed activity ranging from (19.52667 $\pm$ 0.784 %) for *Rosmarinus officinalis* at (200 mcg/ml) to the highest activity (68.43333 $\pm$ 1.457 %) for *Salvia officinalis* at (1000 mcg/ml). The thrombolytic activity of leaf extracts can be arranged as (*Salvia officinalis* $>$ *Thymus vulgaris* $>$ *Rosmarinus officinalis*). From literatures the rosmarinic acid phenolic acid is a characteristic phytochemical constituent of Labiata family plant which is responsible for thrombolytic activity of the plants [15]. Reported quantity of rosmarinic acid in tested plant were as follow: in *Salvia officinalis* (39.3 $\pm$ 0.5 mg/g) was highest followed by *Thymus vulgaris* (23.5 $\pm$ 0.5 mg/g) and *Rosmarinus 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* at different concentration ranges:

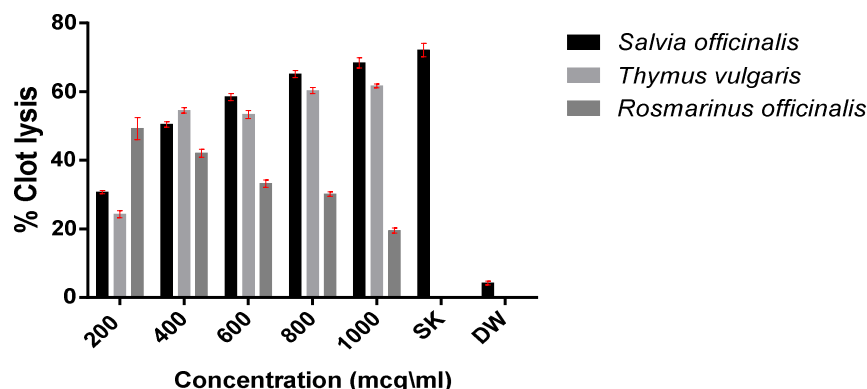
Concentration	% Clot lysis (mean $\pm$ SD)($n=3$)		
	<i>Salvia officinalis</i>	<i>Thymus vulgaris</i>	<i>Rosmarinus officinalis</i>
200 mcg/ml	30.740 $\pm$ 0.505	24.300 $\pm$ 1.000	19.52667 $\pm$ 0.784
400 mcg/ml	50.49334 $\pm$ 0.83	54.56667 $\pm$ 0.814	30.17333 $\pm$ 0.660
600 mcg/ml	58.48667 $\pm$ 1.020	53.36666 $\pm$ 1.102	33.20333 $\pm$ 1.105
800 mcg/ml	65.200 $\pm$ 1.000	60.33334 $\pm$ 0.902	42.070 $\pm$ 1.145
1000 mcg/ml	68.43333 $\pm$ 1.457	61.66667 $\pm$ 0.569	49.27333 $\pm$ 3.256

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Table.2: Thrombolytic activity of negative and positive control:

Control	% Clot lysis (mean $\pm$ SD) ($n=3$)
Distilled water (negative control)	72.16367 $\pm$ 1.945
SK (positive control)	4.2452 $\pm$ 0.526

105



106

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

109 4. CONCLUSION

110 In a **continues** venue for developing drugs from natural source for protection of cardiovascular system
 111 with maximum efficacy and lower side effects, **we concluded** that the tested selected species from
 112 Lamiacea plant family can play an important role in developing thrombolytic drugs derived from
 113 nature specially *Salvia officinalis* leaf.

114 CONSENT:

115 Author declared that written informed consent was taken from patient  publication  study.

116 ETHICAL APPROVAL:

117 Author declare that all experiment were examined under approval of Pharmacy College ethics
 118 committee.

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