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PHYTOCHEMICAL EVALUATION AND *IN-VITRO* THROMBOLYTIC ACTIVITY OFSALVIA HISPANICA

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ABSTRACT

The present study was designed to investigate the in-vitro thrombolytic activity of various extracts of seeds of Salvia hispanica by Clot lysis method. In this study, Streptokinase was used as a positive control and the human blood was taken as test sample. The mean % of clot lysis for streptokinase was found to be 66.8%. Similarly the methanolic, ethanolic, aqueous and acetonic extracts of salvia hispanica exerted 48.5%, 35.8%, 33.3%, 27.7% lysis of the blood clot in thrombolytic activity test respectively. From our findings it was observed that all the extracts of Salvia hispanica revealed remarkable thrombolytic activity.

INTRODUCTION

Thrombus development in the circulatory system may occur due to failure of hemostasis and can causes vascular blockage leading to serious consequences in thrombolytic diseases such as acute myocardial or cerebral infarction which may cause death [1]. Thrombolytic drugs are used to dissolve blood clots in a procedure termed as thrombolysis [2]. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) are commonly used thrombolytic agents to dissolve blood clots [3]. Continuous investigation in this area can provide new insights and promote progress towards the development of the ideal thrombolytic agents which are characterized by maximal coronary arterial thrombolysis with minimal bleeding [4]. The selective thrombolytic drugs which belongs to third generation such as monoteplase, tenecteplase, reteplase etc. result in a greater angiographic potency in patients with acute myocardial infarction, although so far, mortality rates have been similar to those of new drugs that have been studied in large-scale trials .In recent years, it is observed that the heart diseases are increasing to a great extent and side effects of synthetic drugs are becoming an ever increasing therapeutic problem. Almost all the available thrombolytic agents still have significant shortcomings [5]. According to one of thereports, approximately, 30% of the pharmaceuticals are prepared from plants worldwide[6] and are considered to be less toxic and free from side effects than the synthetic one [7]. Hence, it isneeded to find out the safe, less or no side effect, effective herbal drugs because natural products of higher plants may give a new source of thrombolytic agent [8].The present study is undertaken to investigate the phytochemical and in-vitro pharmacological profile of various extracts of Salvia hispanica and to verify the potential of plants with scientific approach for thrombolytic activity.

MATERIALS AND METHODS

Plant Material: The seeds of Salvia hispanica were obtained from Central Market of Telangana State and were authenticated.

Preparation of Extracts: The seeds were cleaned and dried in shade. It was then subjected to size reduction by mechanical grinder. The powdered material was subjected to soxhlet extraction with ethanol, methanol, distilled water and acetone; the extracts obtained were dried and stored at 0-4^{0}C and used for In-vitro studies.

PHYTOCHEMICAL SCREENING:

Phyto-chemical screening of various extracts of Salvia hispanica was carried out using standard Phyto-chemical methods for quantitative identification of phyto-chemical constituents [9].

DRUGS & CHEMICALS:

Commercially available Lyophilised streptokinase vial (15, 00,000 IU) was purchased from Apollo pharmacy, Hyderabad.

***IN VITRO* STUDY MODEL: CLOT LYSIS**

Preparation of Extract Solution for Thrombolytic activity: 100 mg of Methanolic, Ethanolic, Aqueous and Acetonic extracts were suspended in 10ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was then filtered through a 0.22 micron syringe filter. The filtered solution was ready for in vitro evaluation of thrombolytic activity.

Preparation of Streptokinase (SK) Solution: To the lyophilized streptokinase vial of 1,500,000 I.U., 5 ml distilled water was added and mixed properly. From this stock solution, 100 µl of streptokinase (30,000 I.U) was used for in vitro thrombolytic activity

Specimen of Thrombolytic Test: About5ml blood was collected from healthy human volunteers without a history of anticoagulant therapy or oral contraceptive. From 5ml, 1 ml of blood was transferred to each of the ten previously weighed microcentrifuge tubes to form clots.

Procedure for Thrombolytic test: The blood was collected from healthy human volunteers (n=5) without a history of anticoagulant therapy or oral contraceptive and 1.0 ml of blood was transferred to the previously weighed micro centrifuge tubes and incubated at 37° C for 45 min and was allowed to clot. The thrombolytic activity of extracts was evaluated by using streptokinase (SK) as the standard substance (positive control). 100 mg of extracts from different solvent was suspended in 10 ml of distilled water and kept overnight. Then the supernatant was decanted and filtered through a 0.22 micron syringe filter. After formation of clot, the serum was removed completely without disturbing the clot and each tube containing the clot was weighed again to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each micro centrifuge tube with the pre-weighed clot, 100 µl solutions of various extracts were added separately. Then, 100 µl of streptokinase (30,000 IU) and distilled water were added separately to the positive and negative control tubes, respectively. All tubes were then incubated at 37° C for 90 min and observed for lysis of clot, if any. After incubation, the fluid 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 -

% clot lysis: (weight of clot before lysis/ weight of clot after lysis) x 100

GROUP CLASSIFICATION

Sl. No	Groups	Concentration (µg/ml)
I	Normal Control (Distilled water)	100 µg/ml
II	Streptokinase (Standard)	100 µg/ml
III	Methanolic extract of salvia hispanica	100 µg/ml
IV	Ethanolic extract of salvia hispanica	100 µg/ml
V	Aqueous extract of salvia hispanica	100 µg/ml
VI	Acetonic extract of salvia hispanica	100 µg/ml

Figure no 1: Extracts after clot lysis

Figure no 2: Phyto-chemical evaluation of Extracts

RESULTS

YIELD CALCULATION

Calculate the % dry weight as follows:

$$\% \text{ dry weight} = \frac{wt\ of\ dry\ sample}{wt.of\ sample} \times 100$$

- Ethanolic Extract of Salvia hispanica: % dry weight= 5.8/50 x 100 =11.6 %
- Methanolic Extract of Salvia hispanica: %dry weight =5.4/50x100 =10.8%
- Aqueous Extract of Salvia hispanica: % dry weight = 15/50 x100 =30 %
- Acetone Extract of Salvia hispanica: %dry weight = 11.1/50 x 100 =22.2 %

PHYTOCHEMICAL EVALUATION

The extract of Salvia hispanica was subjected to preliminary phyto-chemical screening for the presence of different phytoconstituents with their respective reagents and the results are summarized below. The presence of Carbohydrates was confirmed by Fehling's test, Benedict test, Seliwanoff's test, Tollen'sphloroglucinol test. The presence of protein & amino acids were confirmed by Millon's test, Ninhydrin test, Tyrosin test & Cysteine test. While; alkaloids by Dragendroff's test, Mayer's test, Hager's test & Wagner's test. The presence of Steroids by Salkowski reaction, Liebermann burchard reaction & Tannins by 5% FeCl3 solution. While of Glycoside by Legal's test, Keller killani test &Flavanoids by lead acetate test.

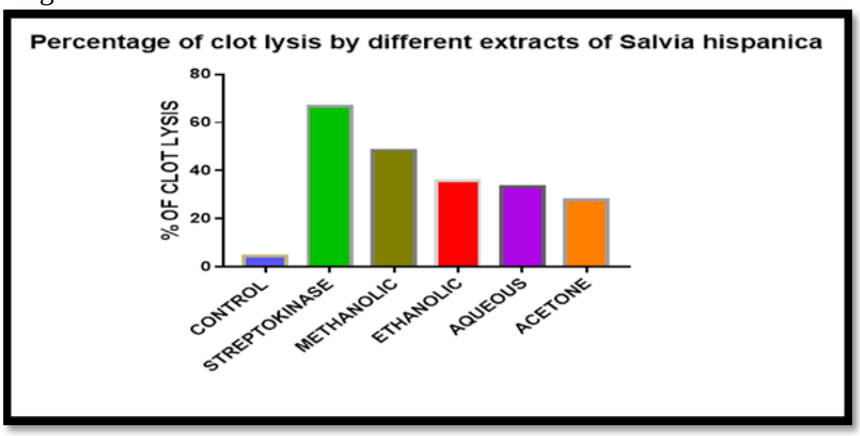

Figure no 3: Graphical representation of percentage of clot lysis byvarious extracts of Salvia hispanica

Table No 1: Phytochemical Investigation of Salvia Hispanica Extract

S.No	Name of The Compound	Ethanol Extract	Methanol Extract	Aqueous Extract	Acetone Extract
1.	CARBOHYDRATES Molisch test (General test) Test for reducing sugars Fehling's test Benedict test Test for non-reducing sugars Iodine test Tannic acid test	 ++ - ++ +++	 ++	 +++	 ++
2.	PROTEINS Biuret test	+++	-	++	++
3.	AMINOACIDS Ninhydrin	++	-	+++	+
4.	ALKALOIDS Dragendroff's test Mayer 's test Hager's test Wagner's test	 +++ +++ +++ +++	 +++ +++ +++ +++	 ++ ++ ++ ++	 ++ ++ ++ ++
5.	STEROIDS Salkowski reaction Libermannburchard reaction	 +++ +++	 - -	 - -	 - -
6.	TANNINS 5% $FeCl_3$ solution Bromine water Acetic acid solution	 - - -	 - - -	 - - -	 - - -
7.	GLYCOSIDES Legal's test Keller killani test	 ++ -	 ++ -	 + -	 - -
8.	FLAVANOIDS	+++	+++	-	-

+ = slightly present; ++ = moderately present; +++ = highly present; - = absent

Table No 2: % Clot Lysis By Different Extracts Of Salvia Hispanica

Fraction	Weight of empty vial (w1) g	Weight of vial with clot (w2) g	Weight of clot before lysis (w2-w1) g	Weight of vial with clot after lysis (w3) g	Weight of clot lysis (w2-w3) g	% of clot lysis
Control	0.872	1.238	0.366	1.222	0.016	4.371584699
Streptokinase	0.868	1.35	0.482	1.028	0.322	66.80497925
Methanolic	0.862	1.574	0.712	1.228	0.346	48.59550562
Ethanolic	0.866	1.412	0.546	1.216	0.196	35.8974359
Aqueous	0.874	1.378	0.504	1.21	0.168	33.33333333
Acetone	0.87	1.418	0.548	1.266	0.152	27.73722628

DISCUSSION

In the present study the seeds of Salvia hispanica were dried, powdered and then extracted with methanol, ethanol, distilled water and acetone by soxhlet extraction method. The extracts were subjected to preliminary phytochemical screening. The phytochemical results showed the presence of Carbohydrate, Proteins, Alkaloid, Amino acids, Steroids, Glycosides and Flavanoids. Addition of 100µl Streptokinase to the clots along with 90 minutes incubation at 37ºC showed 66.6% clot lysis. On the other hand, clots when treated with 100µl of sterile distilled water (negative control) showed only negligible clot lysis which was only 4.3%. When clots were treated with methanolic extract of Salvia hispanica, the percentage of clot lysis was found to be 48.59 %. Meanwhile the ethanolic extract also showed considerable results, with the percentage of clot lysis being 35.89%. The aqueous extract and acetone extract of salvia hispanica when tested in similar conditions, the percentage of clot lysis were found to be 33.3% and 27.73% respectively. Therefore, it was observed that the various extracts of Salvia hispanica have thrombolytic activity and significant activity was shown by the methanolic activity.

CONCLUSION

The results of this study confirmed that the seed extracts of Salvia Hispanica shows significant thrombolytic activity. The Methanolic extract of Salvia hispanica has significant in vitro thrombolytic activity when compared with other extracts of salvia hispanica. However, further studies would be necessary to evaluate the contribution of other substances for the activities showed and elucidate the exact mechanism behind these observed activities, to find out more specific medicinal and pharmaceuticals potentials as well as to isolate and characterize the specific active ingredients responsible for the presented activities. In conclusion, further study is needed to investigate the in vivo thrombolytic activity, and the component(s), and mechanism for clot lysis by Salvia hispanica.

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