WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 5, 1621-1627. Research Article ISSN 2277– 7105

EVALUATION OF THROMBOLYTIC ACTIVITY IN JUSTICIA TRAQUEBARIENSIS

Varuva Ashok Kumar* and C. Girish

Department of Pharmacology, SVU College of Pharmaceutical Sciences(S,V University), Tirupathi, 517502-India.

Article Received on
11 March 2021,
Revised on 01 April 2021,
Accepted on 22 April 2021
DOI: 10.20959/wjpr20215-20410

***Corresponding Author**
Varuva Ashok Kumar
Department of Pharmacology, SVU College of Pharmaceutical Sciences(S,V University), Tirupathi, 517502-India.

ABSTRACT

Thrombolysis could be a treatment to dissolve dangerous clots in blood vessels, improve blood flow, and forestall harm to tissue and organs.[1] It's informally mentioned as clot busting for this proof. Considering this, present study was designed to investigate thrombolytic activity of the hexane and ethanol extract of Justicia traquebariensis leaves. The ethanol extract was found to have significant thrombolytic activity **((38.02±0.08)** compared to the effect of Streptokinase **(66.98±0.11%)** used as positive control and water **(3.14±0.31%)** used as a negative control. Preliminary phytochemical screening of the extract showed the presence of carbohydrates, flavonoids, Quinones and Coumarins were present in leaves extract of Justicia traquebariensis one of which has thrombolytic properties.

KEYORDS: Thrombolysis, Justicia traquebariensis, Phytochemical screening, streptokinase.

INTRODUCTION

Formation of blood is clot and method is occlusion that obstructs the flow of blood through cardiovascular system. Body uses platelets and protein to make blood as opening move of repairing method when injury.[1] These square measure several drug that square measure accustomed dissolve a clot and to treat heart failure, stroke, deep vein occlusion and occlusion of peripheral artery like enzymes, S-enzyme etc.[2] Circulatory platelets square measure aggregate to the positioning of injury and become the most important elements for clot development. Occlusion may be a vital stage for blood vessel un wellness related to infarct and stroke chargeable for sizable morbidity and mortality. Moreover for cancer patients, thrombosis is that the second leading reason for death.[3] For treatment of these

unwellness, clot buster agents like tissue proteolytic enzyme (t-PA), peptidase (UK), enzyme (SK), square measure used. In India among the pharmaceutical, Britain and SK square measure wide used.[4,5] They need high risk of hemorrhage[6] and severe hypersensitivity reactions. Moreover, numerous treatment with SK is restricted because of immunogenicity.[7] Developing of improved recombinant variants of those medication is worrying becaue of inconvenience of clot buster medication.[8-13] Plants square measure the wide supply of bioactive principles and medication and ancients medication is one in all the first health care system in several developing countries.[14,15] In infarct (heart attack),[16] and embolism, SK is employed as lysis medication.[17]

MATIRIAL AND METHODS

1. Collection of Plant Matirial

The fresh leaves of Justicia traquebariensis were collected from region of Seshachala forest, Tirumala. The material was taxonomically identified, confirmed and authenticated by Dr.K.Madhava Shetty, Department of Botany, S.V. University Tirupati. The collected leaves were shade dried and the dried material was crushed to coarse powder with mechanical grinder. The powder was stored in air-tight container which was used for extraction.

2. Preparation of Extracts; Collected plants were dried at room temperature and ground to make a fine powder. 20gm of plant powder was well dissolved in 100ml of solvents (Hexane and ethanol) . The Suspension was filtered by using filter paper of pore size 0.2µm. The filtrate was then air dried, and extracts were collected in sterile vials of further use.[18]

3. Phytochemical Test; The phytochemical test of these extract was performed using the method adopted by Harborne[19] and Sofowora.[20]

3.1. Test for Carbohydrates (Molisch's Test): To 2 ml of plant extract, 1ml of Molisch's reagent and a few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

3.2. Test for Tannins (Ferric Chloride Test): To 1 ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or Greenish black indicates the presence of tannins.

3.3. Test for Saponins (Frothe's Test): To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of a 1 cm layer of foam indicates the presence of saponins.

3.4. Test for Flavonoids (Shinoda Test): To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

3.5. Test for Alkaloids (Mayer's Test): To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagents were added. The presence of green color or white precipitate indicates the presence of alkaloids.

3.6. Test for Quinines: To 1ml of extract, 1ml of concentrated sulfuric acid was added. Formation of red color indicates the presence of Quinones.

3.7. Test for Glycosides (Molisch's Test): To 2ml of plant extract, 3ml of chloroforms and 10% ammonia solution was added. Formation of pink color indicates the presence of glycosides.

3.8. Test for Cardiac Glycosides (Keller-Kiliani Test): To 0.5ml of extract, 2ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under layered with 1ml of concentrated sulfuric acid. The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

3.9. Test for Terpenoids (Salkowski Test): To 0.5ml of extract , 2ml of chloroform was added and concentrated sulfuric acid is added carefully. Formation of red-brown color at the interface indicates the presence of terpenoids.

3.10. Test for Triterpenoids: To 1.5 ml of extract, 1ml of Liebman-Buchard reagent was added. Formation of blue or green color indicates the presence of triterpenoids.

3.11. Test for Phenols (Ferric Chloride Test): To 1ml of the extract, 2ml of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates the presence of phenols.

3.12. Test for Coumarins: To 1ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates the presence of coumarins

3.13. Steroids and Phytosteroids (Libermann- Burched Test): To 1ml of plant extract equal volume of chloroform is added and subjected with a few drops of the concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of the bluish- brown ring indicates the presence of phytosterols.

3.14. Phlobatannins: To 1ml of plant extract a few drops of 2% HCL was added the appearance of red color precipitate indicates the presence of phlobatannins.

3.15. Anthraquinones (Borntrager's Test): To 1ml of plant extact, a few drops of 10% ammonia solution were added, appearance pink color precipitate indicates the presence of anthraquinones.

4. DRUGS AND CHEMICALS

Streptokinase was purchased form local market made by popular pharmaceutical Ltd, India.

5. THROMBOLYTIC ACTIVITY TEST[21]

The blood was drawn from healthy human volunteers (n=3) while not a history of oral contraceptive or anticoagulants therapy and 1.0 ml of venous blood was transferred to the previously weighed small centrifuge tubes and incubated at 37° for 45mints and was allowed to clot. The Thrombolytic agent activity of extracted was evaluated by using streptokinase (SK) as the standard substance. The plant extract was suspended in 10ml of distilled water and was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22 micron syringe filter. After clot formation, the serum was completely removed without disturbing the clot and every tube containing the clot was once more weighed to see the clot weight.

Clot weight = weight of clot containing tube – weight of tubealone

The ethical clearance for the experiment was obtained from the institutional ethical review committee and was performed by following the safe handling protocol. To each micro centrifuge tube with the pre-weighed clot, 100 µl aqueous solution of crude extract was added separately. Then, 100 µl of streptokinase (30,000 IU) and 100 µl of distilled water were separately added 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 released 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 as shown below;

% of clot lysis = (weight of clot after release of fluid / clot weight) X 100

RESULT AND DISCUSSION

Results of phytochemical screening

Table 1: Results of phytochemical Screening of Justicia traquebariensis.

S.NO	Phytochemical Tests	Test performed	Hexane Extract	Ethanol Extract
1	Carbohydrates	Molisch's test	+	+
2	Tannin	Ferric chloride test	-	+
3	Saponin	Frothe's test	-	-
4	Flavonoids	Shinoda test	+	+
5	Alkaloids	Mayer's test	-	-
6	Quinones	-	+	+
7	Glycosides	Molisch's test	-	-
8	Cardiac glycosides	Keller- Kiliani test	-	+
9	Terpenoids	Salkowski test	-	-
10	Phenols	Ferric chloride test	-	+
11	Coumarins	-	+	+
12	Steroids	Libermann- Burchard test	-	-
13	Phlobotanins	-	-	-
14	Anthraquinones	Borntrager's test	-	-

NOTE; (+) = Indicates the presence and (-) = Indicates the absence.

Thrombolytic activity test

Table 2: Thrombolytic activity (in terms of % clot lysis) of Justicia traquebariensis.

S.N	Sample	% of clot lysis
1	Blank	3.4±0.31
2	Streptokinase	66.98±0.11
3	Justicia traquebariensis extract from hexane (100mg)	25.23±0.04
4	Justicia traquebariensis extract from hexane(150mg)	36.12±0.09
5	Justicia traquebariensis extract from ethanol (100mg)	26.41±0.06
6	Justicia traquebariensis extract from ethanol (150mg)	38.02±0.08

(SK= Streptokinase, JT=Justicia traquebariensis, Blank = Water) Data are expressed as percentage ± SEM and ANOVA statistical significance indicates ***P<0.001

DISCUSSION

Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37ºC, showed 66.98±0.11% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of clot 3.4± 03.1%.

The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study Justicia traquebariensis displayed highest thrombolytic activity (38.02±0.08).

CONCLUSION

In the context of the above results and discussion it can be said that the hexane and ethanol extract of Justicia traquebariensis, possesses mild thrombolytic activity compared to standard streptokinase. In conclusion, further study is needed to investigation the in vivo thrombolytic activity, and causative component(s), and mechanism for clot lysis by Justicia traquebariensis.

ACKNOWLEDGEMENT

I Wish to thank SVU College of pharmaceutical sciences (SV University), Tirupati, for their support to complete this research successfully.

REFERENCES

1. http;//en.wikipedia.org/wiki/Thrombosis(http;//www.newsmedical.net/health/what is Thrombosis.aspx)
2. file;//%20my%20project%20paper20%rima/Jan/CV%20Pharmacology%20%20Thrambolytic%20%Fibrinolytic%29%20Drugs.htm
3. Furie B and Furine BC: Mechanism of thrombus formation. New England Journal of Medicine, 2008; 359(9): 38-49.
4. Collen D; Coronary thrombolysis; streptokinase or recombinant tissue type Plasminogen activator? Annals of Internal Medicine, 1990; 112(7): 529-38.
5. Mucklow JC; Thrombolytic treatment. Streptokinase is more economical than alteplase British Medical Journal, 1995; 311(7018): 1506.
6. Rouf SA, Moo-Young M, and Chisti Y; Tissue-type plasminogen activator; characterstics, applications and production technology, Biotechnology Advance, 1996; 14(3): 239-66.
7. Jennings K; Antibodies to streptokinase, British Medical Journal, 1996; 312(7028): 3934.
8. Adams DS,et al.; A synthetic DNA encoding a modified human urokinase resistant to inhibition by serum plasminogen activator inhibition. Journal of Biological Chemistry, 1991; 266(13): 8476-82.
9. Lijnen HR, et. al; On the mechanism of fibrin-specific plasminogen activation by strephylokinase. Journal of Biological Cheemistry, 1991; 266(180): 11826-32.

10. Mader VJ; Recombinanat streptokinase; opportunity for an improved agents. Blood Coagulation and Fibrinolysis, 1993; 4(6): 1039-40.
11. Nicolini FA et.at; Sustained reflow in dogs with coronary thrombosis with K2P.a novel mutant of tissue-plasminogen activator. Journal of the American College of Cardiology, 1992; 20(1): 228-35.
12. Wu DH, et.,at; Coiled coil region of streptokinase gamma-domain is essential for plasminogen activation. Journal of Biological Chemistry, 2001; 276(18): 15025-33.
13. Farnsworth NR; Ethnopharmacology and future drug development; the North American experience. J of Ethnopharmacology, 1993; 38(2-3): 14552.
14. Houghton PJ; The role of plants in traditional medicine and current therapy. Journal of Alternative and Complementary Medicine, 1995; 1(2): 131-143.
15. Sikri N, Bardia A; "A history of streptokinase use in acute myocardial infraction" Tex Heart Inst. J., 2007; 34(3): 318-27.
16. Meneveau N, Schiele F, Vuillemenot A, et.at., "Streptokinase vs alteplase in massive pulmonary embolism. A randomized trail assessing right heart haemodynamics and pulmonary vascular obstruction "Eur. Heart J., July, 1997; 18(7): 11418.
17. Mundada L, Prorok, M. "Structure –Function Analysis of Streptokinases Amino Termius" Journal of Biological Chemistry, 2003; 278(3): 24421-24427.
18. Krishnamoorthi and Bai at.al; Phytochemical analysis and antioxidant property of justicia traquebariensis IJP, 2015; 2(5): 254-258.
19. Harborne JB; Phytochemical methods; A guide to modern technique of plant analysis. Chapman and Hall, New York, Edition 3rd, 1973; 29.
20. Sofowora A; Medicinal plants and Traditional medicinal in Africa. Sunshine house, Ibadan, Nigeria; Spectrum books Ltd; Screening plants for bioactive agents; Edition 2nd, 1993; 134-156.
21. Islam MA, at.al; Evaluation of Thrombolytic activity and Brine Shrimp Lethality Bioassy of Methanol Extract of steam of Tinospora crispa. Int .J. Pharm Res., 2013; 4(3): 1148-1153.