The Journal of Phytopharmacology 2015; 4(2): 87-89
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(2): 87-89
March- April

Md. Ibrahim Tarek
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazer, Chittagong-4203, Bangladesh

Abul Hasanat
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazer, Chittagong-4203, Bangladesh

Mohammad Shah Hafez Kabir
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazer, Chittagong-4203, Bangladesh

Tanvir Ahmad Chowdhury
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazer, Chittagong-4203, Bangladesh

Md. Mizanur Rahman
Department of Pharmacy, North South University (NSU), Dhaka, Bangladesh

Dr. Mir Ezharul Hossain
Professor, Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazer, Chittagong-4203, Bangladesh

Correspondence:
Abul Hasanat
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazer, Chittagong-4203, Bangladesh

In-vitro thrombolytic and cytotoxic activity of methanolic extract of *Syzygium operculatum* leaves

Md. Ibrahim Tarek, Abul Hasanat, Mohammad Shah Hafez Kabir, Tanvir Ahmad Chowdhury, Md. Mizanur Rahman, Dr. Mir Ezharul Hossain*

Abstract

The existing study was made to investigate the thrombolytic exercise and cytotoxic potential with the methanol extract of *Syzygium operculatum* leaves. The cytotoxicity had been assessed while using brine shrimp lethality bioassay and also thrombolytic impact with individual blood. The brine shrimp lethality bioassay was employed to evaluate cytotoxicity (LC_{50}=272.82µg/ml) compared to vincristine associated with sulphate (LC_{50}=0.512µg/ml). It had been also assessed as thrombolytic agent compared to streptokinase. It's got Significant thrombolytic exercise (36. 28%) compared to standard streptokinase (75.09%).

Keywords: Thrombolytic, Cytotoxic, Clot lysis, *Syzygium operculatum*.

Introduction

Thrombosis is the development or vicinity of blood coagulation in a vein. Basically two sorts of thrombosis based by the site of clump arrangement and these are venous thrombosis and arterial thrombosis. The coagulation itself is termed a thrombus.[1,2] medicinal plants assume a predominant part in the treatment of mixtures of human ailments from the dusk of the human development.[3] The Parliamentary Health Select Committee heard in 2005 that the yearly rate of death because of thrombosis was 25,000, with no less than half of these being healing center gained.[4] Fixation on cutting edge restorative framework leads individuals to an option methodology to enhance and keep up great wellbeing is increased tremendously by utilizing therapeutic herb over a century ago. A number of the current day's vital medications and prepared prescriptions are of plant starting point.[5] Restorative plants contain distinctive remedial specialists which may have thrombolytic action, cytotoxic impact and so on. Working with distinctive restorative plants concentrate demonstrated that they can lyses thrombus as streptokinase.[6] A portion of the plant concentrates additionally builds lethality of the cell because of their known cytotoxic impact. Cytotoxicity is performed for assessing the level of lethality. Various novel antitumor and pesticidal common items have been disengaged utilizing this bioassay.[7] Streptokinase (SK) fits in with a gathering of drugs known as fibrinolytics, and edifices of streptokinase with human plasminogen can hydrolytically enact other unbound plasminogen by actuating through bond cleavage to deliver plasmin.SK is utilized as a compelling and cheap thrombolysis drug at times of myocardial dead tissue (heart assault) and aspiratory embolism.

Syzygium operculatum (Family-Myrtaceae) this is found in moist forests. It is found along streams within early serial rain forest habitat, often originating on stream banks after land clearance for cultivation tree up to 12 m high. leaves sub-opposite, simple, elliptic or obovate, entire, acute, cuncate at base, leathery, up to 13 x 6 cm. Inflorescence an axillary cymose panich;bracts and bracteoles small,ovate,acute. Calyx tube funnel-shaped lobes orbicular. Corolla four, orbicular, early caducous. Stamens many, inserted on the rim of the cup filaments free, up to 6 mm long anthers ovate or oblong.

Materials and Methods

Plant Collection and Identification

The matured plant leaves was collected from the Chittagong division of Bangladesh. Then it was identified by taxonomist Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, The University of Chittagong. Voucher specimens, collection id: CTG 120, for S. operculatum kept in the Department of Pharmacy, International Islamic University Chittagong, Chawkbazer, Chittagong-4203, Bangladesh for further reference.

Extracts preparation

The collected plant was washed thoroughly with water and air dried for a week at 35 to 40°C and pulverized in an electric grinder (NOWAKE,Japan). The obtained powder was successively added to methanol with vigorous shaking at 55 to 60°C temperature. The extracts were made to dry by using a rotary evaporator under reduced pressure. Extract sample preserved at 4°C as sample no. SOL1, in Department of Pharmacy, IIUC, for further reference.

Chemicals and drugs

Lyophilized streptokinase vial (1500000 IU) was purchased from Square Pharmaceuticals Ltd, dimethyl sulfoxide (DMSO) and Vincristine sulfate (2 mg/vial; Techno Drugs Limited Bangladesh). All other chemicals and reagents were of analytical grade.

Sample preparation

The crude extract was suspended in 10 ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper. The solution was then ready for in vitro evaluation of clot lysis activity.

Streptokinase (SK) solution preparation

To the commercially available lyophilized SK vial (Square Pharmaceuticals Ltd) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000I.U) was used for in vitro thrombolysis.

Specimen

Whole blood (2 ml) was drawn from healthy human volunteers (n =10) without a history of oral contraceptive or anticoagulant therapy. 500µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots. Different countries volunteers are used for collection of sample (Nigeria, Somalia, and Nepal). Blood collection and preservation were conducted by Sharif (Pathologist)

Thrombolytic assay

Experiments for clot lysis were carried as reported earlier Sweta *et al.*[6] Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile eppendorf tube (500 µl/ml) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (clot weight = weight of the clot containing tube – weight of tube alone). Each eppendorf tube containing clot was properly labeled and 100µl of plant extract was added to the tubes. 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. The difference in weight taken before and after clot lysis was expressed as percentage of clot lysis. Streptokinase and water were used as positive and negative control, respectively. The experiment was repeated several times with the blood samples of different volunteers. % clot lysis= (Weight of the lysis clot /Weight of clot before lysis) × 100.

Brine shrimp lethality bioassay

For the preparation of sea water 38 g of sodium chloride was weighed, dissolved in distilled water to make a 1 liter solution and then filtered off to get clear solution. This simulated sea water was used for hatching of brine shrimp. The shrimp were allowed for two days to hatch and mature as nauplii (larvae). In a small beaker, measured amount of the sample was accurately weighed and dissolved in DMSO (Dimethyl sulfoxide) to give a final concentration of 10 mg/ml (10µg/µl). From the test tube containing brine shrimp nauplii, 10 test tubes were taken for the sample where each contained 5ml of seawater and 10 nauplii. These test tubes were marked from 1to8for the sample. To these test tubes different concentrations (800 µg/ml, 500 µg/ml, 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.525 µg/ml and 12.5 µg/ml) of the sample were added. Then the samples were subjected to brine shrimp lethality evaluation.[7] In this case, only 50µl DMSO was added in 5 ml sea water containing 10 nauplii. No extract was added to prepare control solution. Measured amount of the Vincristine sulphate (Techno Drugs Ltd., Bangladesh) was used dissolved in DMSO to get an initial concentration of o.512 µg/ml. 8 test tubes for the standard sample were taken where each contained 5 ml of seawater and 10 naupli.

Statistical analysis

The data was analyzed statistically using ANOVA followed by student't' test with GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). $P < 0.05$ - 0.01 were considered as statistically significant.

Results

In vitro clot lysis study: 100 µl SK as a positive control (1500000 I.U.) was added to the clots along with 90 minutes of incubation at 37°C showed 75±0.09% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (4.19±0.12%). The in vitro thrombolytic activity study revealed that *Syzygium operculatum* showed 36.28±0.43 % clot lysis. The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. % Clot lysis obtained after treating clots with different concentration of the sample was shown in (Figure 1). Brine shrimp lethality bioassay: In brine shrimp lethality bioassay, the methanolic extract of *Syzygium operculatum* showed positive result in comparison with the positive control vincristine sulphate. By plotting the log of concentration (log C) versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration (LC_{50}) was determined to check the toxic level of the extract. The crude extract of *Syzygium operculatum* showed significant cytotoxic activity against brine shrimp nauplii and LC_{50} value was 272.82 µg/ml (Figure 2). DMSO was used as a negative control to validate the test method.

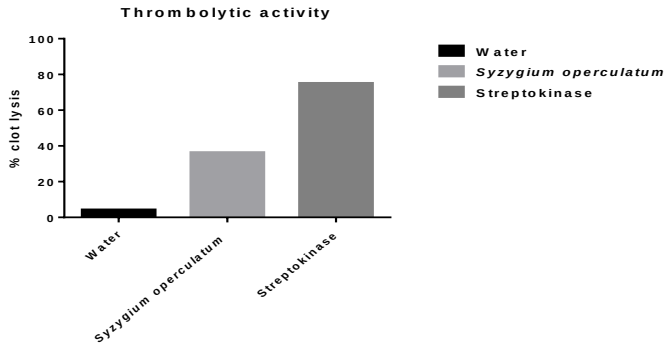

Figure 1: Clot lysis by Streptokinase, Syzygium operculatum and water

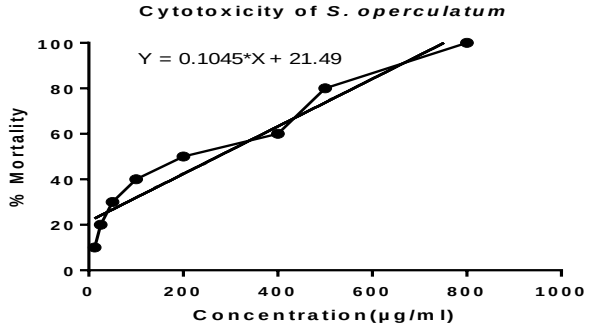

Figure 2: Determination of LC_{50} value for extract of *Syzygium operculatum* from linear correlation between logc versus % of mortality

Discussion

This study assessed the Shrimp lethality and thrombolytic movement bioassay of methanolic concentrate of *Syzygium operculatum*. Herbal arrangements have been utilized since old times for the treatment of diseases. Phytopharmacological and phytochemical assessment lead to medication disclosure. Around 30% of the pharmaceuticals are readied from plants around the world .[8,9] Various studies have been led by different analysts to figure out the herbs and characteristic nourishment sources and their supplements having thrombolytic (anticoagulant and antiplatelet)[10] effect and there is confirmation that expending such sustenance prompts counteractive action of coronary occasions and stroke.[11] Albeit there are a few thrombolytic medications including those got by recombinant DNA innovation, however reactions identified with some of these medications that prompt further confusions have been reported. brine shrimp lethality bioassay is a simple and straight forward seat top screening system for anticipating vital pharmacological exercises like catalyst hindrance, particle channel obstruction, antimicrobial and cytotoxic movement.[12] The concentrate demonstrated LC_{50} at a low fixation with quick reaction demonstrating that the concentrate is fundamentally powerful. In a perfect world, any operators valuable in the treat men to of disease ought not to be lethal to typical cell. In any case, in all actuality, anticancer operators are frequently poisonous to typical cells, especially towards quickly developing cells. It is important to test this concentrate in low focus to assess its strength furthermore against different growth cell lines as ordinary cell lines to defend the possibility to further research this plant for anticancer movement. Further examination is obliged to discover the dependable compound(s) for the cytotoxic action watched for *Syzygium operculatum*.[13] In the thrombolytic bioassay result recommended that the concentrate demonstrated exceptionally intense movement. The plant can be assessed for further research for thrombolytic movement to a particular illness.

Conclusion

We reasoned that *Syzygium operculatum* has got the potential as a possibility for future thrombolytic operators. It can likewise be explored as a conceivable wellspring of antitumor medications. This is just a preparatory study and to make last remark the concentrate ought to altogether research be phytochemically and pharmacologically to endeavor their restorative and pharmaceutical possibilities. This study may be useful for further research meets expectations.

Acknowledgements

We are grateful to the authority of International Islamic University for providing the facilities to conduct this research work.

Conflict of interest statement

We declare that we have no conflict of interest.

Author's contribution

This work was carried out in collaboration between all authors. Authors MIT and MMR collected the plant leaves and prepared the extract. MSHK and AH designed the study, wrote the protocol. Author MSHK performed the statistical analysis and Author AH wrote the first draft of the manuscript. MIT and TAC performed the experiment. Author DMEH managed the literature searches. All authors read and approved the final manuscript.

References

1. Furie B.and Furie B.C. Mechanisms of thrombus formation. The New England Journal of Medicine 2008;359:938–949.

2. Handin RI. "Chapter 53: bleeding and thrombosis". In Kasper DL, Braunwald E, Fauci AS et al. Harrison's Principles of Internal Medicine (16th ed.). New York, NY: McGraw-Hill, 2005.

3. Nostro A, Germano M.P., Angelo D.V., Marino A., and Cannatelli M.A. Extraction methods and bioauthography for evaluation of Medicinal plant antimicrobial activity. Letters in Applied Microbiology 2000;30(5):379-385.

4. Hunt B.J. Awareness and politics of venous thromboembolism in the United Kingdom. Arteriosclerosis Thrombosis Vascular Biology 2008;28:398-399.

5. Thomas S., Patil D.A., Patil A.G., and Naresh C. Pharmacognostic evaluation and physicochemical analysis of an *Averrhoa carambola* L. Fruit. Journal of Herbal Medicine and Toxicology 2008;2(2):51-54.

4. Sikri N., and Bardia A. A history of streptokinase use in acute myocardial infarction. Texas Heart Institute Journal 2007;34(3):318-327.

5. Meneveau N., Schiele F., and Vuillemenot A. Streptokinase vs. Alteplase in massive pulmonary embolism. A randomized trial assessing right heart haemodynamics and pulmonary vascular obstruction. European Heart Journal 1997;18:1141-1148.

6. Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MT, Hatim FD. Effect of *Fagonia arabica* (Dhamasa) on *in vitro* thrombolysis. BMC Complementary and Alternative Medicine 2007; 7:36.

7. Mayer B.N., Ferrigni N.R.., Putnam J.E., Jacobsen L.B., Nichols D.E., and McLaughlin J.L. Brine shrimp: a convenient bioassay for active plant constituents. Journal of Medicinal Plant Research 1982;45:31-34.

8. Carrubba A. Scalenghe R. Scent of mare nostrum-medicinal and aromatic plants (MAPs) in mediterranean soils. J Scie Food Agri. 2012;92(6):1150–1170.

9. Kumar D.J., Santhi R.J., Antioxidant and cytotoxic effects of hexane extract of *Morinda pubescens* leaves in human liver cancer cell line. Asian Pac J Trop Med. 2012;5(5):362–366.

10. Diaz, M.N., F. Balz, A.V. Joseph and F.K. John. Antioxidants and atherosclerotic heart disease. New Eng. J. Med.,1997:408-416.

11. Naderi G.A., Asgary S., Jafarian A., Askari N., Behagh A., Aghdam R.H.. Fibrinolytic effects of *Ginkgo biloba* extract. Exp Clin Cardiol 2005;(10):85-87.

12. Cardellina J.H, Fuller R..W., Gamble W.R., Westergaard C, Boswell J, Munro M.H.G., Currens M and Boyd M.P. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV inhibitory natural products extracts. In: Bohlin L, Bruhn J.G. (Eds), Bioassay Methods in Natural Product Research and Development. Kluwer Academic Publishers, Dordrecht, pp.1999: 25-36.

13. McLaughlin J.L., Rogers L.L., Anderson J.E.. The use of biological assays to evaluate botanicals. Drug Information Journal 1998;32:513-524.