ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.5.L1-5

International Journal of Lifescience and Pharma Research

Research Article

Biochemistry for better diagnosis and therapy

A Study on Thrombolytic and Cytotoxic Activity of Methanolic Extract of *Zingiber Officinale*

P. Manju[1*] and Dr. A. Pushpa[2]**

[1]* *Department of Biochemistry, Rathnavel Subramaniam college of Arts and Science, Coimbatore, Tamilnadu, India.*

[2] ** *Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore-641043, Tamilnadu, India.*

Abstract: Medicinal plants have been well documented for their medicinal purposes for thousands of years and traditional medicines are still a major part of habitual treatments of different maladies in different parts of the world. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. Among the medicinal plants *Zingiberofficinale* (Roscoe Zingiberaceae) used locally as alleviates coughs in children. From its origin in Southeast Asia and its spread to Europe, it has a long history of use as herbal medicine to treat a variety of ailments, including vomiting, pain, indigestion, and cold-induced syndromes. The present study was carried out to investigate thrombolytic and cytotoxic activity of methanolic extract of *Zingiber officinale* rhizome. The cytotoxic activity was determined by using brine shrimp lethality bioassay and thrombolytic activity was determined in accordance with clot disruption method .In the thrombolytic activity the methanolic extracts showed clot lysis of 30.13%. The standard of thrombolytic activity , Streptokinase showed clot lysis of 58.38%. For Cytotoxic activity, the methanolic extract showed LC_{50} value of 30.64 µg/ml and the reference standard potassium dichromate showed LC_{50} value of 310 µg/ml. Our study revealed that the methanolic extract of *Zingiber officinale* (Roscoe Zingiberaceae) possess cytotoxic and thrombolytic activity. The potential of these activities may be due to the presence of most of the phytochemicals which supports previous claims and validate its uses as an expected folk medicine.

Key Words: Methanol, Streptokinase, Thrombolytic activity, cytotoxic activity, *Zingiber officinale*

***Corresponding Author**

P. Manju , Department of Biochemistry, Rathnavel Subramaniam college of Arts and Science, Coimbatore, Tamilnadu, India.

Recieved On 08 April 2020
Revised On 28 May 2020
Accepted On 01 August 2020
Published On 01 December 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation P. Manju and Dr. A. Pushpa , A Study On Thrombolytic and Cytotoxic Activity Of Methanolic Extract of Zingiber Officinale.(2020).Int. J. Life Sci. Pharma Res.10(5), 1-5 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.5.L1-5

1. INTRODUCTION

Medicinal plants are a reservoir of biologically active compounds with therapeutic properties that over time have been reported and used by diverse groups of people for treatment of various diseases. Traditional medicine has been in existence since time immemorial and has been well accepted and utilized by the people throughout history[1]. Since ancient times, plants have been an exemplary source of medicines. Plant-derived medicinal products have attracted the attention of scientists around the world for many years due to their minimum side effects and positive effects on human health. In the pharmaceutical landscape, plants with a long history of use in ethno medicine can be a rich source of substances for the treatment of various ailments and infectious diseases. Medicinal plants are considered as a repository of numerous types of bioactive compounds possessing varied therapeutic properties. The vast array of therapeutic effects associated with medicinal plants includes anti-inflammatory, antiviral, antitumor, antimalarial, antithrombotic activity and analgesic properties. Ginger (*Zingiber officinale*) or ginger root is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom, and galangal. Ginger (*Zingiber officinale*Rosc.) is a creeping perennial on a thick tuberous rhizome, which spreads underground. A number of active constituents and medicinal properties have been reported during the last decade. The present work provides a comprehensive account of important medicinal properties of this versatile herb. Thrombosis is the formation or presence of a blood clot in a blood vessel. Mainly there are two types of thrombosis based on the site of clot formation and these are venous thrombosis and Arterial thrombosis. Thrombolytic therapy uses drugs called thrombolytic agents, such as alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots[2]. Drugs from plant origin are relied by 80% of the world's population. In India, the use of herbal drugs is an important component of the traditional system of medicine. The current study demonstrated for the first time that *Zingiber officinale* methanolic extract has cytotoxic effect against Brine shrimp larva. This may be a promising finding for future antithrombolytic and in the prevention of thrombosis using natural products. Therefore, the aim of the present study is to investigate the thrombolytic activity of methanolic extracts of *Zingiber officinale* that might explore the remedial potential of this plant to a great extent.

2. MATERIALS AND METHODS

2.1 Screening of Plants for Thrombolytic Potential

Ten different rhizomes namely Glechoma hederacea, Alpinia galangal, Tanacetum vulgare, Zingiber officinale, Curcuma domestica,Equisetum hyemale, Curcuma longa, Polygonum cuspidatum, Curcuma amada and *Canna edulis were* collected from Coimbatore and screened for thrombolytic activity. *Zingiber officinale* showed the maximum thrombolytic activity and hence it was selected for the present investigation.

2.2 Collection and extraction of plant materials

The rhizome of *Zingiber officinale* was collected from the market of Coimbatore, India. The collected plant rhizome was authenticated by the Botanical Survey of India. TNAU, Coimbatore (BSI/SRC/5/23/2015/TECH/2088). The selected rhizhome sample was washed thoroughly with distilled water twice and shade dried at room temperature for 3 days . Then, about 200 g of dried rhizome was ground into fine powder by using a dry, clean mixer grinder and the powder was stored in separate sterile, polythene bags[3] . 10 % of polar extracts were prepared with powdered sample. For the preparation of extracts 10g of powdered sample were added to 100 ml of water and 100ml of methanol solvent and the containers were incubated at 40^0C for 24hrs in shaking incubator at 60-70rpm.After incubation the extracts again heated at 40^0C in water bath to get effective results. The extracts were filtered with Whatman No.1 filter paper and were used for further study. The methanolic extract of *Zingiber Officinale* was used for thrombolytic and cytotoxic studies.

2.3 Streptokinase (SK) solution preparation

Streptokinase (15,00,000 I.U.) a standard thrombolytic drug is used as a positive control, which is commercially available lyophilized sample (Polamin Werk GmbH, Hedrick, Germany). To the Streptokinase vial , 5ml of sterile distilled water was added and mixed properly . The suspension was used for *in vitro* thrombolytic study.

2.4 Specimen

Whole blood (5 ml) was drawn from healthy human volunteers(n = 25) without a history of oral contraceptive or anticoagulant therapy using a protocol approved by the Institutional Ethics Committee of Avinashilingam university, faculty of Science(AUW/IHEC/BC-17-18/XMT/01), 500µl of blood was transferred to each of the ten previously weighed eppendorf tubes to form clots. The clot lysis was carried out as per the method reported by Prasad *et al*., 2006 [4]. Venous blood drawn from healthy volunteers was transferred into different pre weighed sterile micro centrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube - weight of tube). To each micro centrifuge tube containing pre-weighed clot, 100 µl of methanolic extracts of *Zingiber officinale* rhizome was added. To the commercially available lyophilized streptokinase vial (Lupiflo, Lupin Limited, Mumbai, India) 2.5 ml of PBS was added and thoroughly mixed. This suspension was used as a stock from which 100 µl was added to the micro centrifuge tube as a positive control. For negative control, 100 µl of distilled water were added. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the fluid released 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. The experiment was repeated 25 times with the blood samples of twenty five different healthy volunteers.

The percentage of clot lysis was calculated by the following equation:

% of clot lysis= (Weight of released clot) /clot weight after clot disruption)x 100

2.5 Brine shrimp lethality bioassay

Brine shrimp (*Artemia salina*) were obtained by hatching brine shrimp eggs in artificial sea water (3.8% non-ionized sodium chloride solution) for 48 hours. About 200µl of the methanolic extracts of rhizome were added to 10 ml of brine shrimp solution with 20 nauplii each. The study was conducted with 1 – 10 mg/ml concentration of extract. These vials were maintained at room temperature for 24 hours under the light and surviving larvae were counted using a magnifying lens. Experiments were conducted along with potassium dichromate as positive control[5]. The mortality rate was calculated by the formula,

$$\% \text{ Mortality} = \frac{\text{No of dead nauplii}}{\text{Total no of nauplii}} \times 100$$

3. STATISTICAL ANALYSIS

Experimental results are shown as mean ± standard deviation (SD) of three replicates and the datas were analysed. The statistical analysis was carried out using one way ANOVA . The *p*-value of 0.05 or less was considered significant for all experiment.

4. RESULTS

4.1 Thrombolytic activity

The extracts have the potential for thrombolytic activity. It was confirmed by comparing with positive and negative control. Streptokinase which act as a positive control showed 58.38% and water treated as a negative control exhibited 4.49% clot lysing ability. The clot was lysed by *Zingiber officinale* rhizome (30.13%) as shown in Table 1. It was noted unambiguously that among the samples analyzed for the thrombolytic activity, *Z. Officinale* rhizomes exerted a better activity. This could be attributed to the presence of high antioxidant capacity for thrombolytic diseases. Therefore, the rhizome of *Zingiber officinale* were chosen for the present study and subjected to further investigations.

Table 1 : Percent clot lysis of diverse plant sources

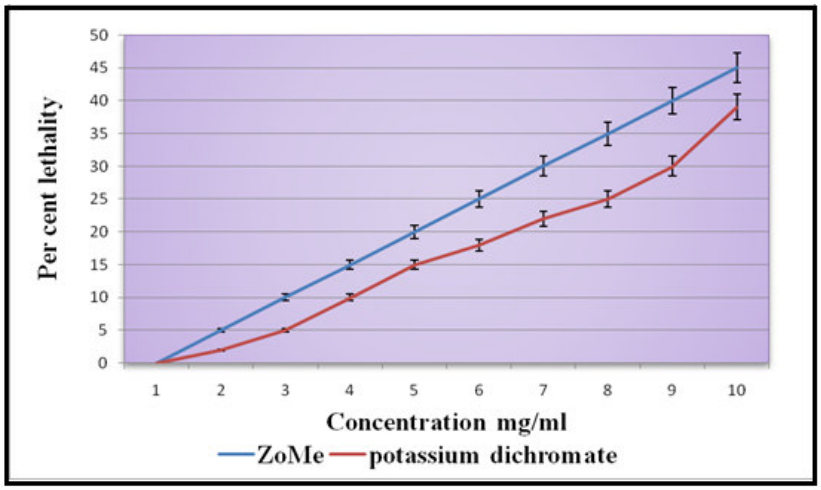
S.no	Plant sample	% clot lysis
1	*Glechoma hederacea*	2.84
2	*Alpinia galangal*	3.91
3	*Tanacetum vulgare*	5.26
4	*Zingiber officinale*	30.13
5	*Curcuma domestica*	9.67
6	*Equisetum hyemale*	11.79
7	*Curcuma longa*	20.33
8	*Polygonum cuspidatum*	13.81
9	*Curcuma amada*	29.20
10	*Canna edulis*	3.78
11	Streptokinase	58.38
12	Water	4.49

Values are mean of triplicates

4.2 Cytotoxic assay

Brine Shrimp lethality assay was performed for the methanolic extract of *Zingiber Officinale (ZoMe)* rhizhome to evaluate the preliminary toxicity against *Artemia salina* and the results showed the lethality of the shrimp at different concentrations. Potassium dichromate was used as a positive control and the results are represented in figure 1

.



Values are mean ± SD of three replicates p<0.02 when compared to control -

Fig 1: Brine shrimp lethality tests of Methanolic extract of Zingiber officinale (ZoMe)

From the graph it is stated that the methanolic extract of rhizome showed a significant cytotoxic activity against brine shrimp nauplii. The extract showed significant difference in lethality to *Artemia salina* at a very low concentration with very quick response indicating that the extract is significantly potent. The LC_{50} value of potassium dichromate 310 µg /ml proving the non toxic nature of the extract. The mortality rate was seen at a maximum concentration of 30 mg/ml .

5 DISCUSSION

Herbal-derived medicines have a long history of use for the prevention and treatment of human diseases. Ginger rhizome has been used extensively used in food industries and traditional medicine; consumers still showing increasing interests in this plant product. Recently, many pharmaceuticals currently approved by the Food and Drug Administration (FDA) have origins to plant sources[7]. A number of plants source especially several fruits and vegetables have been studied for their supplements having anticoagulant, antiplatelet and fibrinolytic activity and there is evidence that consuming such food leads to prevention of coronary events and stroke. Brine shrimp lethality assay has been extensively used as a simple and useful tool for preliminary screening of toxicity of synthesized compounds as well as physiologically active plant extracts. Our findings are similar to *Protium serratum rhizome,* that showed LC_{50} at 30.90 mg/ml[8], which revealed that the extract is pharmacologically active Concurrent findings were reported by Shahana Sharmin *et al.,* 2018[9] who have observed an increase in mortality in brine shrimp with the increase in concentration of methanolic extract of *Aporosawallichii* leaves. The brine shrimp bioassay results of *Z.officinale* clearly demonstrate the nontoxic effects of methanolic extracts which could be due to any of the secondary metabolites (alkaloids, flavonoid or saponins) of the plant. Platelets play a significant role in the development of arterial thrombosis as well as damage the regions of the endothelial surface (produced by reactive oxygen species). The stimulated platelets form platelets to platelets bonds, binds also to leucocytes carrying them into an intricate process of plaque development and progression. In thrombolytic assay, the comparison of positive control with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. When compared with the clot lysis percentage obtained through extract and water, an extremely significant thrombolytic activity was observed after treating the clots with methanol fraction of *Zingiber officinale* . Several studies revealed that *A. bilimbi,* possesses tannin, alkaloid, saponin[10] which could be participated for its clot lysis activity. Streptokinase activates plasminogen to dissolve clots, also destroys the extracellular matrix and fibrin fibers that hold cells together[11]. From our findings it is observed that rhizome extract of *Zingiber officinale* revealed remarkable thrombolytic activity. Therefore, steps should be taken to observe *in vivo* clot dissolving potential and to isolate active component(s) of the extract. Statistical analysis revealed that the rhizome extracts have a significant percentage of clot lysis when compared with positive and negative control. As the first generation drugs (SK and UK), found to cause side effects[12,13], plant-based thrombolytic drugs will improve the treatment of thrombosis. The methanolic leaf extract of *Euphorbia hirta* have maximum activity for lysing the clot. [14] Similar findings were observed in the methanolic extract of *Persicaria orientalis* ,that showed maximum cytotoxic activity [15] Therefore, *in-vivo* study of *Zingiber officinale* is further needed to be recognized as a thrombolytic agent. However, the extremely significant effect of *Zingiber officinale* demonstrates it to be the best thrombolytic component for further processing.

6 CONCLUSION

The present study was conducted to evaluate the methanolic extract of *Zingiber officinale* rhizomes by *in vitro* thrombolytic activity. The extract showed significant clot dissolution activity which may be due to activation of plasminogen. The above result suggests that the application of *Zingiber officinale* rhizomes may be accessible for the treatment of ischemic myocardium or thrombo embolic disorders. In conclusion, further study is needed to study the *in vivo* thrombolytic activity and the mechanism of clot lysis by *Zingiber officinale* .

7. AUTHORS CONTRIBUTION STATEMENT

Mrs.P. Manju and Dr.A.Pushpa designed the work . Mrs. Manju carried out the experiments under the guidance of Dr.A.Pushpa. Analysis and Intrepretation of data was done

by Mrs.P.Manju and verified by Dr.A.Pushpa.The manuscript was wrote by Mrs.P.Manju in consultation with Dr.A.Pushpa. Both the authors helped shaped the research ,analysis and the final manuscript.

8. ACKNOWLEDGEMENT

The authors wish to thank Rathnavel Subramaniam College of Arts and Science,Coimbatore -641 402 ,Avinashilingam University for Women,Coimbatore-641043 and Centre for Bio science and Nano science Research (CBNR),Coimbatore for the support and facility rendered to carry out the research work .

9. CONFLICT OF INTEREST

Conflict of interest declared none

10. REFERENCES

1. Mya Mu Aye, HninThanda Aung, Myint Myint Sein Chabaco Armijos. A Review on the Phytochemistry, Medicinal Properties and Pharmacological Activities of 15 Selected Myanmar Medicinal Plants, Mol 2019, Jan: 24, 293 doi:10.3390/molecules24020293.
2. Zaheer Z, Robinson T, Mistri AK. Thrombolysis in Acute Ischaemic Stroke: An Update Ther Adv in Chr Dis .2011 Mar. 2(2):p.119-131. doi: 10.1177/2040622310394032.
3. Wardlaw JM, Recombinant Tissue Plasminogen Activator for Acute Ischaemic Stroke: An Updated Systematic Review and Meta-Analysis. Cochrane Database of Systematic Reviews, 2003 Jun 3(1): p. 1-12. Doi: 10.1016/S0140-6736(12)60738-7.4.Wu D.H., Shi GY, Chuang WJ Hsu J.M., Young K.C., Chang C.W., Coiled coil region of streptokinase gamma domain is essential for plasminogen activation, *Jl of Bioll Chem, 2001* ;May,(276):15025-33. doi: .10.1074/ibc.M005935200
4. Choudhari Sand Kareppa BM. Identification of bioactive compounds of *Zingiber officinale* Roscoe rhizomes through gas chromatography and mass spectrometry. Int J of Pharma Res and Dev. 2013 Oct 5(08): 016-020.
5. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thromb J*.2006 Sep; 4:14. doi: 10.1186/1477-9560-4-14
6. Oladimeji HO., Nia R. and Essien EE. *In vitro* anti-microbial and brine shrimp lethality potential of the leaves and stem of *Calotropisprocera,* (Ait), *Af J of Biomed, Res*, 2006, Dec, 9: 205-211. Doi: 10.4314/ajbr.v9i3.48906.
7. Rolf B and Per O. Studies on the Thrombolytic Activity of a Protease from *AspergillusOryzae*, Article first published online: 8 DEC 2008 DOI: 10.1111/j.1748- 1716.1964.tb02899.
8. Sikder AA, Abu B, AKM. Nawshad Hossain, Md. Kowser Miah, Mohammad A. Kaisar and Mohammad A. Rashid, Evaluation of Thrombolytic Activity of Four Bangladeshi Medicinal Plants, as a Possible Renewable Source for Thrombolytic Compounds, *J of P and Nut Sci*, 2011 Sep, 1, 4-8. Doi: 2223-3806/11.
9. Middleton P, Stewart F, Al-Qahtani S, Egan P, O'Rourke C, Abdulrahman A, Antioxidant, antibacterial activities and general toxicity of *Alnusglutinosa*, Fraxinus excelsior and Papaverrhoeas," *Iran J of Pharma Res*, 2005;2, pp. 81-86, 10.22037/ijpr.2010.620.
10. ShahanaSharmin, Md. TanvirKabir, Md. Nasiful Islam, Mohd. RaeedJamiruddin, Imon Rahman, Arifur Rahman, Mahboob Hossain. Evaluation of Antioxidant, Thrombolytic and Cytotoxic Potentials of Methanolic Extract of *Aporosawallichii* Hook.f. Leaves: An Unexplored Phytomedicine, J of Appl Pharma Sci (2018), 8(07): 051-056. Doi;10.7324/JAPS.2018.8709
11. Hasanuzzaman M, Ali MR, Hossain M, Kuri S, Islam MS. Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of Averrhoabilimbi (fruits), Int curr pharma j, 2013,May,2:92-96, doi.org/10.3329/icpj.v2i4.14058.
12. Brown MS and Goldstein JL. Regulation of the activity of the low density lipoprotein receptor in human fibroblasts. PMID, Cell, 1975 Nov; 6(3):307-16. Doi: 10.1016/0092-8674(75)90182-8
13. Shivasharanappa K and Londonkar R. Clot lysis and antimitotic study of *Ficus glomerata* Roxb fruit extracts. ISRN Pharma, 2014, Doi, 2014:975303.
14. Mushiur Rahman SM, Sohel Rana, Ariful Islam AFM, Alok Kumer, Abu Kayes Hashem, Habibur Rahman, Mohaimenul, Tonmoy Kumar Biswas, Sayeed Sabbir AA, Antithrombotic and anti-inflammatory activities of leaf methanolic extract of *Euphorbia hirta* Lin. Int J of Com & Alt Med 2019 Aug, 2(4). 154–162. Doi: 10.15406/ijcam.2019.12.00466.
15. Prawej Ansari, Md. Josim Uddin, Md. Masudur Rahman, Md. Abdullah-Al-Mamun, Md. Rabiul Islam, Md. Hazrat Ali and A.S.M. Ali Reza, Anti-inflammatory, anti-diarrheal, thrombolytic and cytotoxic activities of an ornamental medicinal plant: *Persicaria orientalis*, J of Basi and Clin Physio and Pharmaco, 2016, Aug, 28(1), https://doi.org/10.1515/jbcpp-2016-0023