IJAYUSH
International Journal of AYUSH
AYURVEDA, YOGA, UNANI, SIDDHA AND HOMEOPATHY
http://internationaljournal.org.in/journal/index.php/ijayush/

International Journal
Panacea
Research library
ISSN: 2349 7025

Original Research Article **Volume 9 Issue 4** **Oct – Dec 2020**

Kaempferia rotunda Linn.:-

EVALUATION OF IN VITRO THROMBOLYTIC ACTIVITY

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ABSTRACT

Chengazhuneerkizhangu, botanically identified as Kaempferia rotunda Linn. is a drug widely used in folklore practice in Kerala, India. The drug even though widely used within the state its medicinal potential is still unknown to a large scientific community. In the present time, with the increase in global demand of herbal medicine and alarming depletion of traditional medicinal plants it is needed to explore the potential of folklore medicines. Kaempferia rotunda Linn is evaluated as part of the study to validate its folklore claim. It was subjected to in vitro analysis to evaluate its thrombolytic activity. The drug was used in its aqueous extract form in three different concentrations 2mg/ml, 4mg/ ml and 6 mg/ml and distilled water was taken as the control. On statistical analysis significant thrombolytic activity was exhibited by the drug in the concentrations 4mg/ml and 6 mg/ml ($p<0.0001$).

INTRODUCTION

Medicinal plants form the base of India's indigenous health care system. Despite of its rich floristic diversity major share of the medicinal plants in our country still remain unknown to the main stream. With the global popularity of Ayurveda there is an increase in the usage of medicinal plants. Due to this over exploitation many of the traditionally used medicinal plants are now facing the threat of extinction. So it is the need of the hour to explore the potential of folklore medicinal plants and bring them into mainstream.

Kaempferia rotunda Linn.is a medicinal plant which is widely popular among the folklore practitioners of Kerala. The plant is known by the name *Chengazhuneerkizhangu* in the State. The Rhizome of the plant is known for its medicinal properties. It is locally used as a part of many medicinal combinations. Folklore practitioners use the drug in the treatment of dropsy, pain and removal of blood. Hortus malabaricus[1], a book describing the Flora of Madras region of British India gives references about the drug. The drug is also described in AyurvediyaOushadha Nighantu[2] a very popular regional lexicon. Ancient medicinal text book Gunapatha[3] also describes about its properties and uses.

Previously done research works ensures that the drug is safe for usage in humanbeings[4]. Anti- inflammatory, analgesic[5]and antioxidant[6]potentials of the drug are proven. It was found to possess antimicrobial,[7]antihyperglycaemic[8] and antinociceptive activities.[8] Moreover it was found to be beneficial against human breast cancer.[9] The phytochemical evaluation of the drug revealed that the drug is rich in flavonoids, alkaloids and phenols[10] which accounts for its medicinal property. No studies are so far conducted for assessing the thrombolytic potential of the drug.

The present study aims at assessing the in vitro thrombolytic activity of aqueous extract of rhizome of *Kaempferia rotunda* Linn.

MATERIALS AND METHODS

Animal procurement

The animals were purchased from College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. (Reg.No. 328/GO/Re/S//01/ CPCSEA).they were acclimatized for standard laboratory conditions for 7 days before use. They were fed

with standard rat pellet diet and purified water for drinking. The pharmacological study had started after they fulfilled the study criteria.

Materials used

Wistar albino rats of both sex weighing 150 to 200 gm, aqueous extract of the drug, feeding bottles, weighing machine, micro centrifuge tubes, syringe filter, micro pipette, soxhlet apparatus, gloves.

Preparation of Extract

The rhizome of the test drug was collected and washed properly to remove the impurities. It was then made into small pieces and was shade dried. It was then coarsely powdered and extracted with aqueous solution using soxhlet apparatus. The extract was concentrated and evaporated to dryness to a constant weight.

Preparation of drug for in vitro analysis

To analyze the thrombolytic activity three different concentrations of the extract of test drug was used. The dried extract was dissolved in aqueous solution to prepare different concentration (2mg/ml, 4mg/ml, and 6mg/ml). The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 microne syringe filter. 100µl of aqueous preparations of the rhizome was added to the micro centrifuge tube containing the clots to check thrombolytic activity.

Procedure for blood sample collection

Whole blood was drawn from 24 healthy Wistar albino rats (12 male & 12 female) by the method of heart puncture. 5 ml of blood was drawn from the heart of each rat as a terminal method of blood collection. 1 ml of blood collected from each rat was transferred into previously weighed sterilized micro centrifuge tubes to form clots.

Thrombolytic assay

5 ml of blood was drawn from 24 Wistar albino rats and was transferred into 24 pre weighed sterile micro centrifuge tubes (1 ml/ tube). The micro centrifuge tubes were then subjected to incubation at 37 0C for 45 minutes. After formation of clot, serum was completely removed from the tubes, without disturbing the clot formed. Each tube containing the clot was the again weighed to determine the weight of the clots.

Clot weight = Weight of clot containing tube – Weight of tube alone

Each micro centrifuge tube containing clot was then properly labelled and grouped into 4 groups containing six samples each. 100µl of the extract of rhizome with various concentration (2,4,6 mg/ml) was added to the tubes. Among the 4 groups first group was added with the extract in the concentration 2 mg/ ml, the second group was added with the extract in the concentration 4 mg/ml, the third group was added with 3 mg/ ml of the extract and the fourth group was the control group to which 100µl ofdistilled water was added. Then all the tubes were again incubated at 370C for 90 minutes and the clot lysis was observed. After incubation the obtained fluid was removed from the tubes and they were again weighed to observe the difference in weight after clot disruption.

Assessment criteria

Difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the underneath equation.

% of clot lysis = (Wt of released clot / Clot weight) × 100.

RESULTS

In the in vitro analysis of thrombolytic activity water extract of the drug was analyzed for in vitro clot lysing activity. Three different concentrations of the drug (2mg/ml, 4mg/ ml and 6 mg/ml) were used for the study. Distilled water was taken as the control.

Percentage of clot lysis The mean value of percentage of clot lysis at different concentrations of the drug showed that, there was an increase in the percentage of clot lysis with increase in concentration of the drug. The percentage of clot lysis obtained for the group treated with distilled water (Control) was3.488. For the groups treated with 2 mg/ ml, 4 mg/ ml and 6 mg/ml the values were respectively 18.35, 35.20 and 38.22. The highest value was obtained for the group treated with 6mg/ml

The percentage of clot lysis obtained for the group treated with water was compared with the groups treated with the study drug in varying concentrations, the difference in percentage of clot lysis was found to be non-significant for the group treated with the test drug in the concentration of 2 mg /ml and highly significant for other two concentrations (4mg/ ml and 6 mg/ ml). The difference in percentage of clot

lysis for the group treated with 4 mg/ ml and 6 mg/ml when compared with the group treated with 2 mg/ml was found to be highly significant. The difference in percentage of clot lysis for the group treated with 6 mg /ml when compared with the group treated with 4mg/ml was found to be non-significant.

Table No :1 Mean values of percentage of clot lysis at different concentrations of extract

Concentration of extract	Percentage of clot lysis
Control	3.488
2 mg/ml	18.35
4 mg/ml	35.20
6 mg/ml	38.22

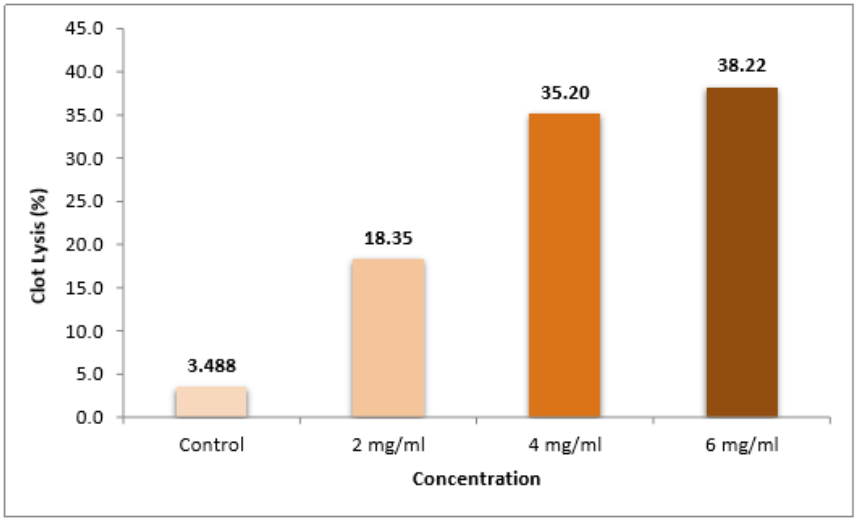

Diagram No: 1 Bar Diagram showing Mean values of percentage of clot lysis at different concentrations of extract

Table No 2 : Comparison of clot lysis at different concentrations

Comparison between groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Water Vs 2mg/ml	-14.86	3.916	No	ns	-29.88 to 0.1591
Water Vs 4mg/ml	-31.71	8.358	Yes	***	-46.73 to -16.70
Water Vs 6mg/ml	-34.73	9.153	Yes	***	-49.75 to -19.71
2 mg/ml Vs 4m/ml	-16.86	4.442	Yes	*	-31.87 to -1.838
2 mg/ml Vs 6 mg/ml	-19.87	5.237	Yes	**	-34.89 to -4.853
4mg/ml Vs 6mg/ml	-3.015	0.7946	No	ns	-18.03 to 12.00

DISCUSSION

In vitro part of the study involved the assessment of thrombolytic potential of the drug in three different concentrations 2mg/ml, 4mg/ml and 6 mg/ml. The data obtained were statistically analyzed using One- Way ANOVA and Tukey's post hoc test. The results obtained showed that the percentage of clot lysis obtained by the concentrations 4mg/ml and 6 mg/ml were statistically highly significant ($p<0.0001$) when compared with the control group which was treated with distilled water and the results obtained for the concentration 2 mg/ml was not significant. Maximum thrombolytic effect was shown by the group treated with the concentration 6 mg/ml.($p<0.0001$).

CONCLUSION

From the results obtained in the study it can be concluded that the rhizome of *Kaempferia rotunda* Linn. possess significant thrombolytic activity.

ACKNOWLEDGEMENT

With deep sense of respect and love, I express my sincere gratitude towards my guide, Dr. P.Y Ansary MD (Ay), PhD, Professor and HOD, Department of Dravyagunavijnanam,

Government Ayurveda College, Tripunithura, for his timely and meticulous guidance, supervisions and suggestions to bring in perfection to every bit of this work. I express utmost gratitude to my beloved teachers Dr. Sara Monsy Oommen, Dr Shincymol V V, Dr.Sariga K S, Dr.Ashima.K.Sasidharan and Dr.Sethu R.

I would like to express my gratitude to the statutory authority of the Kerala University of Health Sciences, Trissur, Kerala, for the valuable support and facilities provided for the completion of the work.

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