INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH
ISSN: 2395-6429, Impact Factor: 4.656
Available Online at www.journalcmpr.com
Volume 8; Issue 04(A); April 2022; Page No.140-143
DOI: http://dx.doi.org/10.24327/23956429.ijcmpr20220032

Research Article

EVALUATION OF THE THROMBOLYTIC ACTIVITY OF KADIKKARA CHENDHOORAM- INVITRO STUDY

Singarajah Janani*[1] and Ajwad M. A. M[2]

[1]Department of Gunapadam, Government Siddha Medical College, Palayamkottai, Tamilnadu, India
[2]Ayurveda Medical Officer, Teaching Hospital of Siddha Medicine, Konesapuri, Trincomalee

ARTICLE INFO

Article History:
Received 10th January, 2022
Received in revised form 2nd February, 2022
Accepted 26th March, 2022
Published online 28th April, 2022

Key words:
Kadikkara Chendhooram, In vitro**,** Streptokinase thrombolytic activity

ABSTRACT

Thrombolysis, also known as thrombolytic therapy, is a treatment to dissolve dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. Several in vitro models have been developed to study clot lytic activity of thrombolytic drugs, but all of these have certain limitations. There is need of an appropriate model to check the clot lytic efficacy of thrombolytic drugs. Thus, the present study, is aimed to investigate **in vitro** thrombolytic activity of *Kadikkara Chendhooram (KC).*

It is a meto - mineral formulation contains three ingredients which prepared as per mentioned in Siddha Literature of *Siddha Vaiththiya Thiraddu* Page Number 157. The Thrombolytic activity of trial drug was evaluated using Healthy Human Blood Samples.

Under this study, Test drug *KC* demonstrated moderate (P <0.001) clot lytic properties in different blood samples. The percent clot lytic activity was compared with water (positive control) and standard enzyme streptokinase (negative control). Then again, the mean percent clot lytic activity of Test drug *KC* was found 60.22%, which is significant compare with the positive and negative control. So, the present research proposes that, the Test drug *KC* has moderate thrombolytic activity. Thus, the formulation is a source of effective herbal drug.

INTRODUCTION

Siddha is the mother medicine of ancient Tamils / Dravidians of peninsular South India. Siddha practitioners believe that five basic elements – earth, water, fire, air, sky – are in food, "humours" of the human body, and herbal, animal or inorganic chemical compounds, such as sulfur and mercury, used as therapies for treating diseases.

According to the theories of humoral pathology, all diseases are caused by the discordant mixture of *vatha*, *piththa*, and *kapha*. The *Siddhar Yugimunivar* in "*Yugivathiya Chinthamani Perunool 800*" says there are eighty type of *vatha* disease in which "*Paarisa Vayu*" is one type.

The symptoms of '*Paarisavayu*' can be correlated with the symptoms of Hemiplegia or CVA or stroke in the modern medicinal system.

In siddha system this is defined as a disease which affects the functions of arms, legs, fingers, tongue, mouth and eyes. The early symptoms that manifest are given as heaviness of body, constipation, irritable mood, rapid pulse and fainting.

A stroke is caused by the interruption of the blood supply to the brain, usually because a blood vessel bursts or is blocked by a clot. This cuts off the supply of oxygen and nutrients, causing damage to the brain tissue. The most common symptom of a stroke is sudden weakness or numbness of the face, arm or leg, most often on one side of the body.

There are various effective Siddha drugs mentioned in Siddha classical literatures and manuscripts by Siddhars or ancient scientists for "*Paarisa vatham*". Therefore Identification and Scientific validation of an effective Siddha drug to prevent and control the *Paarisa vatham* (Stroke / Hemiplegia) is very essential in this current world. Thus, I have selected "*KADIKKARA CHENDHOORAM*" is one of the effective Siddha mineral preparations which is used to treat "*Pakka vatham*" which is mentioned in Siddha Literature of *Siddha Vaiththiya Thiraddu* Pg.No.157. Thus the present study has been aimed to evaluate the Thrombolytic activity of *Kadikkara Chendhooram* - in vitro method.

MATERIALS AND METHODS

Selection of the Drug

Kadikkara Chendooram is a meto - mineral formulation contains three ingredients which mentioned in Siddha Literature of, Dr. Kuppusaamy Muthaliyar, K.N, Dr.Uththamarayan, K.S, 2016, *Siddha Vaiththiya Thiraddu*, Department of Indian Medicine and Homeopathy, Page Number 157. The drug is useful for *Paarisa Vaatham*

Corresponding author:* **Singarajah Janani
Department of Gunapadam, Government Siddha Medical College, Palayamkottai, Tamilnadu, India

(Hemiplegia), hence it has been selected for its Thrombolytic activity.

Collection of Raw Drugs

1. *Kadikkaram* (Nitrate of Silver) - 01 Palam (35 grams)
2. *Lingam* (Red Sulphide of Mercury - Natural) - 01 Palam (35 grams)
3. *Rasa Chendhooram* (Red Sulphide of mercury) - 01 Palam (35 grams)

All three ingredients were bought from *Gopalan aasan* shop, Nagercoil at Kanyakumari District, Tamil Nadu.

Identification and Authentication

All raw drugs were identified and Authenticated by the experts of *Gunapadam* (pharmacology) Department, Government Siddha Medical College Palayamkottai, Tirunelveli.

The specimen samples of the identified raw drugs were presented in the laboratory of PG *Gunapadam* for future references.

METHOD OF PREPARATION

Take above three purified ingredients in mentioned amount and make as powder separately. Then take a glass – corked air tight glass container *(Kal kaarkkup puddi)* and put half amount of powder of Purified *Rasa Chendhooram*, then place half amount of powder of Purified *Lingam* above it. Then put whole part of powdered *Kadikkaram*.

After that again put rest half part of the *Rasa Chendhooram* powder above the powder of *Kadikkaram*. Finally lay the rest half part of the *Lingam* on top of whole layer of powders.

After that close the bottle by a glass cork very tightly and cover the bottle by a piece of leather. Then after place the bottle in the middle part of the heap of boiled rice (*Nel*) and keep it until become cool.

Finally take the medicine out, powdered well by using stone mortar (*Kalvam*) and store in a clean container.

Shelf life: 75 years.
Dosage: ½ to 1 *Arisi Pramanam* (32.5 to 65mg)
Adjuvent: Honey, Ginger juice, Basil leave juice.
Indication: *PaarisaVaatham* (Hemiplegia)
Vatha rogankal, Oozhi, Pethy

Reference: Dr. Kuppusaamy Muthaliyar, K.N, Dr. Uththamarayan, K.S, 2016, *Siddha Vaiththiya Thiraddu*, Department of Indian Medicine and Homeopathy, Chennai 600 106, Pg No: 157

Pharmacological Analysis

Thrombolytic Activity of *Kadikkara Chendhooram*

Reagents and chemicals

Streptokinase (SK) vials of 15, 00, 000 I.U.10 blood (5ml) sample drawn from healthy human volunteers, *KC*, Distilled Water

Apparatus

Micro centrifuge tube (0.5ml/tube), Micropipette, Vortex mixer, 0.22-micron syringe filter, Beaker, Electric Balance, Incubator

Experimental procedure

Streptokinase (SK)

To the commercially available lyophilized SK vial (Polamin Werk GmbH Herdecke, Germany) 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 100 µl (30,000 I.U.) was used for in vitro thrombolysis.

Specimen

Whole blood (5 ml) was drawn from healthy human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy. 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots.

Sample preparation

The *KC* 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.

Thrombolytic assay

Experiments for clot lysis were carried as reported earlier. Venous blood drawn from healthy volunteers was transferred in different pre- weighed sterile eppendorf tube (500µl/tube) 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 clot containing tube-weight of tube alone). Each eppendorf tube containing clot was properly labelled and 100 µl of *KC* 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. 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

RESULTS

Addition of 100 µl SK, a positive control (15,00,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 72% ± 1.95 % clot lysis. Clots when treated with 100µl sterile distilled water (negative control) showed only negligible clot lysis (3.80%). The in vitro thrombolytic activity study revealed that *KC* showed 60.22%. Statistical representation of the effective clot lysis percentage by our Siddha preparation, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated below.

Table Thrombolytic Activity of *KC*

	Weight of the Empty Tube [A]Gm	Weight of Tube With Clot[B]Gm	Weight Clot[C] C=BA	Weight of the Tube With Clot After Lysis [D]Gm	Weight of Lysis [E][B-D]	% Of Clot Lysis	Average % Of Clot Lysis
1	0.82	1.54	0.72	1.07	0.47	65.27	
2	0.83	1.48	0.65	1.16	0.32	49.23	
3	0.83	1.64	0.81	1.24	0.40	49.38	
4	0.82	1.40	0.58	1.10	0.30	51.72	
5	0.84	1.56	0.72	1.02	0.54	75.00	
6	0.82	1.48	0.66	1.04	0.44	66.66	
7	0.80	1.59	0.79	1.03	0.56	70.58	
8	0.81	1.41	0.60	1.04	0.37	61.66	**60.22%**
9	0.82	1.40	0.58	1.06	0.34	58.62	
10	0.82	1.43	0.61	1.10	0.33	54.09	

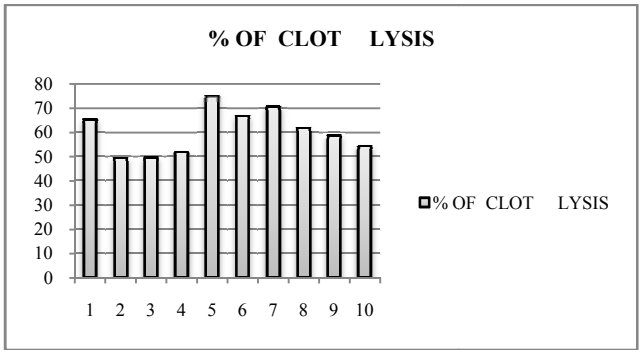

Fig Percentage of clotlysis

Table Effect of *KC* on clot lysis formulation

Blood sample	Control (water)	Streptokinase	% of Clot lysis Formulation
1.	3.18	70.32	65.27
2.	3.37	70.55	49.23
3.	3.58	71.32	49.38
4.	3.62	71.86	51.72
5.	3.77	72.13	75.00
6.	3.91	72.18	66.66
7.	4.05	72.55	70.58
8.	4.13	73.56	61.66
9.	4.20	73.89	58.62
10.	4.22	74.28	54.09
Mean	**3.80 %**	**72.264**	**60.22%**

Table Effect of clot lysis

Sample	Result%
Streptokinase (SK)	72.26
Distilled Water (Control)	3.80
Trial drug (*KC*)	60.22

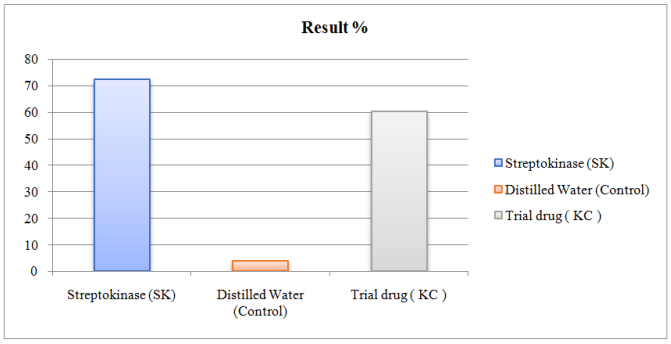

Fig Effect of clot lysis

DISCUSSION

The present study was undertaken to evaluate the thrombolytic activity of *KC*. In the thrombolytic bioassay result suggested that the *KC* showed very significant activity. The *KC* can be evaluated to further research for thrombolytic activity to a specific disease.

Atherosclerosis-induced heart attacks and strokes are leading reasons of morbidity and mortality. Current essential and auxiliary prevention strategies emphasize control of different atherosclerotic danger components, including smoking, hypertension, hypercholesterolemia, diabetes mellitus, weight, irritation, and homocysteine. Current pharmacological studies recommend remedial estimations of these natural preparations, including lowering of blood pressure and lipids, antioxidation, thrombolytic activity and the promotion of microcirculation. There is a requirement for more goal and scientific approaches to authenticate individual herbs to identify chemical constituents, detect adulteration or contamination of herbs, and screen the quality of herbs and herbal medicines.

There is also a need to check the consistency of different batches of herbs utilized as a part of this study and to distinguish bioactive parts in herbs reported to have physiological effects.

CONCLUSION

Under this study, Test drug *KC* demonstrated moderate (P <0.001) clot lytic properties in different blood samples. The percent clot lytic activity was compared with water (positive control) and standard enzyme streptokinase (negative control). The mean % of clot lysis for water and streptokinase was found 3.8% and 72% separately. Then again, the mean percent clot lytic activity of Test drug *KC* was found 60.22%, which is significant compare with the positive and negative control. So, the present research proposes that, the Test drug *KC* has moderate thrombolytic activity. Thus, the formulation is a source of effective herbal drug.

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How to cite this article:

Singarajah Janani and Ajwad M. A. M (2022) ' Evaluation of the Thrombolytic Activity of Kadikkara Chendhooram- Invitro Study', *International Journal of Current Medical and Pharmaceutical Research,* 08(04), pp 140-143.

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