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In Vitro Study on Thrombolytic Effect of the Poly Herbal Combination "Trikatu"

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ABSTRACT

Thrombolytic drugs such as streptokinase, urokinase and tissue plasminogen activators have been conventionally used to effectively dissolve the formed thrombus inside blood vessels. However, these drugs are associated with serious clinical shortcomings[1] and they are expensive. Hence there is scope in finding out a safe and affordable alternative. *Trikatu* is a Polyherbal combination of drugs *Pippali (Piper longum* Linn.), *Maricam* (*Piper nigrum* Linn) and *Nagaram* (*Zingiber officinale* Roscoe) in the ratio 1:1:1. The drug combination is mentioned in all the great treatises of Ayurveda and considered to possess the ability to break the blood clot. Though this drug combination is accepted and used in various ailments, no scientific data are available to substantiate its thrombolytic potential. The present study was taken up to determine the thrombolytic effect of these drugs and its drug combination Trikatu using *in vitro* clot lysing model. One Way ANOVA and POST HOC Tukey's HSD test were done for the statistical analysis in the experiment. In vitro experiment revealed that all the study drugs possess significant thrombolytic effect with P value= 0.000. Drug *Piper nigrum L* showed a greater thrombolytic effect compared to the other drugs. Their combination *Trikatu* had more effectiveness compared to drug *Piper longum L*.

1. INTRODUCTION

Thrombosis is the formation of clot or thrombus inside blood vessels. Atherosclerotic diseases such as myocardial infarction, cerebral venous sinus thrombosis and deep vein thrombosis are the consequences of thrombus formed inside blood vessels[2]. These diseases are leading causes of morbidity and mortality throughout the world[3]. Thrombolytic drugs are used to dissolve the formed clot inside blood vessels. All available thrombolytic agents still have significant shortcomings[3]. Continuous investigations are going on in this area to develop an ideal drug with minimal side effects. The study is thereby trying to find out an alternative for the present day thrombolytic drugs.

Ayurveda preparations are used since ancient times to uphold a healthy physical and mental state. The significance of these preparations is increasing with the advancement of phytochemistry and compound isolation. Thrombolytic effect of some Ayurvedic drugs like *Ocimum sanctum* L., *Curcuma longa* L., *Azadirachta indica* etc has already been assessed[4]**.** In classical treatises, several drugs and combinations are mentioned in the context of thrombolysis. *Trikatu* is one such drug combination likely to possess thrombolytic activity as it is mentioned remedial in diseases like "*Makkalla*" (Post partum condition) where clot formation (thrombosis) is the pathology. *Trikatu*[5,6,7,8], is a combination of the drugs *Pippali* (*Piper longum* Linn), *Maricam* (*Piper nigrum* Linn) and *Nagaram* (*Zingiber officinale* Roscoe) in the ratio 1:1:1[9]. The present study was attempted to determine the thrombolytic effect of *Trikatu* by *in vitro* clot lysis model[10,11].

2. MATERIALS AND METHODS

Collection of plant material

Dried fruits of *Piper longum* L was obtained from "Aromatic and medicinal plant research station", Odakkali, Ernakulam, Kerala. Fruits of Piper nigrum L and tuber of *Zingiber officinale* Roscoe were collected from Anchalummodu, Kollam, Kerala.

Preparation of extract

Aqueous extract of coarsely powdered drugs were prepared using soxhlet apparatus. The extracts was concentrated and evaporated to dryness to a constant weight. The dried extracts of the drugs individually and in combination (*Trikatu)* were dissolved in the aqueous solution

in the concentrations 10mg/ml. The suspension was kept overnight and decanted to remove the soluble supernatant. The prepared extracts were kept in separately labeled conical flasks.

Preparation of Standard drug: Streptokinase (SK)

To the commercially available lyophilized SK vial of 15, 00,000 IU, 5 ml 0.9% normal saline was added and mixed properly. This suspension was used as working solution, from which 100 µ l (30,000IU) was taken for the experiment each time.

Specimen for Thrombolytic test

5 ml blood was drawn from 10 healthy human volunteers of either sex between age group 20 to 40 without a history of oral contraceptive or anticoagulant therapy.

Preparation of blood clot

5.0 ml of venous blood was drawn from 10 healthy volunteers. Blood samples from each individual were transferred to six separate eppendorf tubes, each tube dedicated to one study group. Hence each group contains 10 samples. The Eppendorf tubes were subjected to incubation at 37°C for 45 minutes for clot formation, then the serum was completely removed from the tubes without disturbing the clot and each tube having clot was again weighed to determine the initial clot weight.

(Initial clot weight = weight of clot containing tube – weight of tube alone).

Grouping

Total samples were divided into six groups. Four study drug groups corresponding each for the extracts of *Piper longum* L, *Piper nigrum* L, *Zingiber officinale* Roscoe and their drug combination (Trikatu). Fifth and sixth group respectively for positive control (streprokinase) and negative control (distilled water). Groups were named as NCD (Negative control Group), PCD (Positive control Group), SDT (study drug Trikatu), SDP (study drug Pippali or *Piper longum* L), SDM (study drug Maricam or *Piper nigrum* L), SDN (study drug Nagaram (*Zingiber officinale* Roscoe).

Method of Thrombolytic assay

To the Eppendorf tubes containing pre weighed clot, respective drugs in five groups and sterile water in the sixth group were added at the quantity of 100µl. All the tubes were

incubated again at 37°C for 90 minutes and observed for clot lysis. After incubation, the fluid formed was removed from the tubes and tube containing the clot was again weighed (Final clot weight) 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.

$$\% \text{ of clot lysis} = (\text{Final clot Wt} / \text{initial clot wt.}) \times 100$$

3. RESULT AND DISCUSSION

Percentage reduction in the clot weight is taken as the outcome variable for the experiment. Aqueous extracts of drug combination 'Trikatu' exerted 16.83 % reduction in clot weight. Drugs *Piper nigrum* L, *Piper longum* L and *Zingiber officinale* Roscoe exerted respectively 22.10 %,14.76%,13.29% reduction in clot weight against negative control group which exerted only 6.84 % reduction. One way ANOVA followed by POST HOC Tukey's HSD test was done for the statistical analysis.

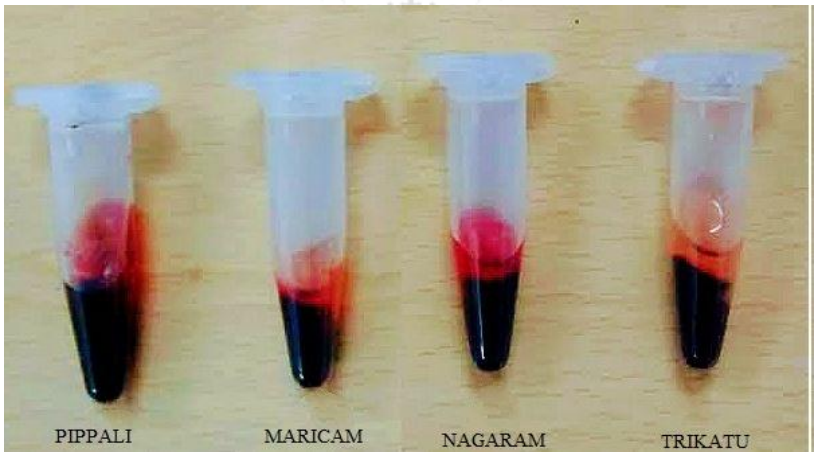
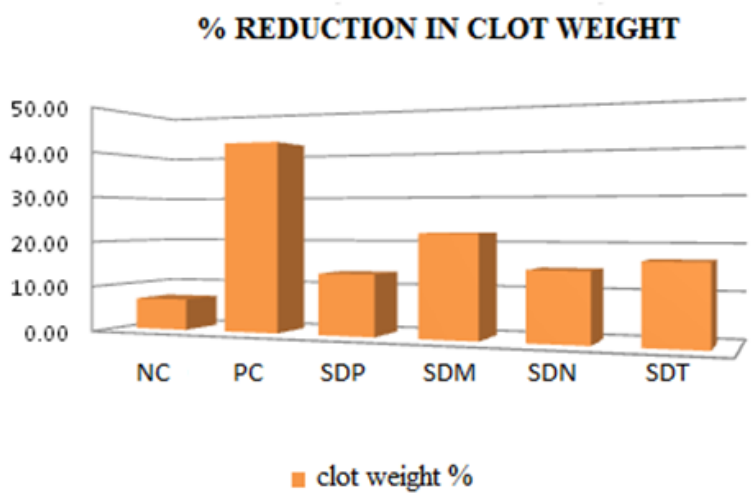


Figure No. 01: Clot lysis after extract addition

Graph 01: Bar diagram representing mean value of % Reduction in clot weight for each group



NC = (Negative control Group)

PC = (Positive control Group)

SDT = (study drug Trikatu)

SDP = (study drug Pippali or *Piper longum* L)

SDM = (study drug Maricam or *Piper nigrum* L)

SDN = (study drug Nagaram or *Zingiber officinale* Roscoe).

Statistical Analysis

Table No. 01: One Way Anova Test Result

	% change in clot weight	
	Mean	**Sd**
NCD	6.84	1.48
PCD	41.93	4.94
SDP	13.29	3.51
SDM	22.10	4.02
SDN	14.76	3.57
SDT	16.83	4.27

F= 102.86 **p<0.001**

Table 02: Post Hoc (Tukey's Hsd) Test Result

Comparison	Mean Difference	Std. Error	P
NCD vs PCD	-35.08400	1.694	0.000
NCD vs SDP	-6.44400	1.694	0.000
NCD vs SDM	-15.25700	1.694	0.000
NCD vs SDN	-7.91700	1.694	0.000
NCD vs SDT	-9.98400	1.694	0.000
PCD vs SDP	28.64000	1.694	0.000
PCD vs SDM	19.82700	1.694	0.000
PCD vs SDN	27.16700	1.694	0.000
PCD vs SDT	25.10000	1.694	0.000
SDP vs SDM	-8.81300	1.694	0.000
SDP vs SDN	-1.473	1.694	0.388
SDP vs SDT	-3.54000	1.694	0.041
SDM vs SDN	7.34000	1.694	0.000
SDM vs SDT	5.27300	1.694	0.003
SDN vs SDT	-2.067	1.694	0.228

Study drugs were compared with the negative control group. The result indicates that the study drugs had significant effect (P value 0.000). That affirms the thrombolytic effect of study drugs. Further, study drugs were compared with each other. Study drug *Piper nigrum L* had shown significant difference against *Trikatu*, *Zingiber officinale* and *Piper Longum L* with P value 0.003, 0.000, 0.000 respectively. The drug *Trikatu* had shown significant difference against *Piper longum L* with P value=0.041. There was no significant difference observed between *Zingiber officinale & Piper Longum L* or between *Zingiber officinale Roscoe & Trikatu*. This result denotes *Piper nigrum L* is more efficacious compared to all other study drugs and *Trikatu* is efficacious than *Piper Longum L.* However the effect of study drug compared to positive control drug was not significant.

4. CONCLUSION

It is revealed from the *in vitro* experiment that: all the study drugs possess significant thrombolytic effect with P value= 0.000. Drug *Piper nigrum L* showed a greater thrombolytic effect compared to all other drugs. Drug *Piper longum L* alone is less potent than other drugs.

Ethical concern

Ethical clearance was obtained from the Institutional Ethical Committee. Informed consent was obtained from the volunteers in writing.

Hereby declare that there is no conflict of interest in this study.

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6. REFERENCES

1. Aslanabadi, Naser et al. "The Streptokinase Therapy Complications and its Associated Risk Factors in Patients with Acute ST Elevation Myocardial Infarction." *Iranian Journal of pharmaceutical research: IJPR* vol. 17,Suppl (2018): 53-63.
2. Palta, Sanjeev et al. "Overview of the coagulation system." *Indian journal of anaesthesia* vol. 58,5 (2014): 515-23. doi:10.4103/0019-5049.144643
3. Ali, Md Ramjan et al. "Aspect of thrombolytic therapy: a review." *The Scientific World Journal* vol. 2014 (2014): 586510. doi:10.1155/2014/586510

4. Khan, Irfan Newaz et al. "Thrombolytic potential of Ocimum sanctum L., Curcuma longa L., Azadirachta indica L. and Anacardium occidentale L." *Journal of basic and clinical pharmacy* vol. 2,3 (2011): 125-7.
5. Vaidya Jadavji Trikamji acharya, Nibandhasangraha commentary of susrutha samhitha by Dalhana acharya, chaukhamba sanskrit sansthan, varanasi, 2010. p 64
6. Bhishagaacharya Harishaastri Paraadakara Vaidya, Astanga hridayam by Vagbhata, Chaukhambha orientalia,Varanasi, 2005. P 10
7. Vaidya Jadavji Trikamji acharya, Nibandhasangraha commentary of susrutha samhitha by Dalhana acharya, chaukhamba sanskrit sansthan, varanasi, 2010. P 65
8. Vaidya Jadavji Trikamji acharya, Nibandhasangraha commentary of susrutha samhitha by Dalhana acharya, chaukhamba sanskrit sansthan, varanasi, 2010. p 390
9. Prof. K. R. Srikanthamurthy, Bhavapraksha of Bhavamisra Vol I, Chowkhamba Krishnadas Academy, Varanasi,2008, p 167 &168
10. ER -Kishore, Kamal. (2013). In-vitro and In-vivo Screening Methods for Antithrombotic Agents. American Journal of Phytomedicine and Clinical Therapeutics.1.497-506. https://www.researchgate.net/publication/306208338_Invitro_and_Invivo_Screening_Methods_for_Antithrombotic_Agents
11. 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;4:14. Published 2006 Sep 12. doi:10.1186/1477-9560-4-14