Research Article

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# AN *IN VITRO* STUDY OF RASAKRIYA AND ETHANOLIC EXTRACT OF CHITRAKA (*PLUMBAGO ZEYLANICA* LINN.) FOR ITS THROMBOLYTIC ACTIVITY WITH SPECIAL REFERENCE TO *"KATUKO RASA SHONITA SANGHATAM BINNATI"*

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**ABSTRACT**

Blood coagulation causes many serious health problems like Myocardial infarction, deep vein thrombosis, renal vein thrombosis etc which are treated with thrombolytic drugs to dissolve the blood clots in modern system of medicine. Thrombolytic agents have certain limitations. Apart from dissolving the clots it also causes serious and occasionally fatal consequences. Herbs and herbal medicines are known for their efficacy in treating diseases effectively without producing any untoward effects. Herbs that are having Katu rasa are known to cause thrombolysis. Hence the research was aimed at understanding and developing rational model to understand action of drug as thrombolytic agent based on classical reference in Ayurvedic science "*Katuko rasa shonitasanghatambhinnati"* by doing an *In vitro* study of Chitraka *Rasakriya* and Ethanolic extract of Chitraka (*Plumbago zeylanica* Linn) for its thrombolytic activity at different dosage using Streptokinase as positive control and distilled water as negative control. Chitraka (*Plumbago zeylanica* Linn) is having *Katurasa, katuvipaka, ushnaveerya, laghurookshateekshnaguna*. Blood samples were treated with different dosage of Chitraka*rasakriya* and Ethanolic extract of Chitraka and compared with Streptokinase as positive control and Distilled water as negative control. Results were assessed based on difference of clot weight before and after treatment for its clot lysis efficacy. Study results showed Chitraka *Rasakriya* and Ethanolic extract of Chitraka possess thrombolytic activity which is statistically highly significant. Chitraka *Rasakriya* had better thrombolytic activity when compared to Ethanolic extract of Chitraka and Streptokinase as positive control.

**Keywords**: *Rasakriya*, thrombolytic, *shonitasanghatambinnati.*

## INTRODUCTION

Blood coagulation causes many serious health problems like Myocardial infarction, deep vein thrombosis renal vein thrombosis etc which are treated with thrombolytic drugs to dissolve the blood clots in modern system of medicine[1]. Dravyaguna shastra /Ayurvedic Pharmacology is a well-developed branch of therapeutics in Ayurveda, similar to modern pharmacology, in aims of drug usage with a difference in understanding the mode of drug action and usage in therapy. The present study aims at understanding basics of Ayurvedic science by reference from Charaka Samhita "*Katuko rasa shonitasanghatam bhinnati*"[2] Ayurvedic Pharmacology considers the drug action is mediated through *Rasa, Guna, Veerya, Vipaka* and *Prabhava* of *Dravya*[3]. Drug action is attributed to *Rasa/Guna/Veerya/Vipaka* or *Prabhava* individually or in combination. According to Ayurveda all the drugs are made up of *Panchamahabhoota*[3] and *Katu Rasa* in the drug is also made up of *panchamahabhoota* with the predominance of *Agni* and *Vayu mahabhoota. Katu rasa* is having *Laghu, Ruksha, Ushna* and *Teekshna* qualities. *Katu Rasa* has the property of *vaktrashodaka* (cleanses the mouth), *Agnideepaka* (appetizer) *Bhuktamshoshayati* (helps in absorption), *Mamsamvilikhati* (scrapes away unwanted growth in the muscle tissue), *Shonitasanghatambinnati* (breaks the blood clots), *bhandanchinnati* (breaks the obstruction), *Margaanvivrunoti* (clears the channel)[4]. Studies are conducted by various researchers to find out the herbs, natural food sources and their supplements having thrombolytic (anticoagulant and anti-platelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke.[5-7] Earlier study published on *Trikatu* reveals that *katu* rasa helps in clot lysis thus indicating thrombolytic activity of *Trikatu*[8]. The present study aims at understanding and proving scientifically, to assess the action of *Katu Rasa*. In the present study Chitraka (*Plumbago zeylanica* Linn) root was used for the Study. Chitraka a perennial herb, having *Katu rasa Katu Vipaka, Ushna Veerya, Laghu, Rooksha, Ushna* and *Teekshna Guna* has been selected for the present study. *Rasakriya* and Ethanolic extract of Chitraka Root was used to assess the thrombolytic activity of the drug. Hence an *in vitro* study of *Rasakriya* and Ethanolic extract of Chitraka (*Plumbago zeylanica* Linn) for its thrombolytic activity with special reference to "*Katuraso Shonitasanghatam Bhinnati"* has been taken up.

## MATERIAL AND METHODS

To evaluate thrombolytic activity of *rasakriya* and Ethanolic extract of Chitraka (*Plumbago zeylanica* Linn) with special reference to action of *Katu rasa* on *Shonitasanghata* or thrombosis.

Study was initiated after receiving Institutional Ethics Committee Clearance (IECNOSKACH/IEC/005/2019) and informed consent from the volunteers. Volunteers were screened for their health status by checking Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), Clotting time (CT) and Bleeding time (BT). Among 16 volunteers screened, 10 were selected for the study based on inclusion criteria.

### Inclusion Criteria

- Age of 18-25 years.
- Both the gender
- Normal CBC, ESR, BT-CT

### Exclusion criteria

- A history of oral contraceptive
- On anticoagulant therapy
- Any other systemic disease.

### Preparation of Study material

Chitraka roots were collected and identified by experts in Department of Dravyaguna, Sri Kalabhyraveshwaraswamy Ayurvedic Medical College Hospital and Research Centre Bangalore, Karnataka, India.

1. **Chitraka *Rasakriya***

Dried Chitraka Root (*Plumbago zeylanica* Linn) was taken cleaned for its physical impurities. It was weighed and made into coarse powder. 500 grams of Chitraka *Kashaya choorna* was added with 8 liters of water on *mandagni* and reduced to 1/4$^{th}$ as per the *Kashaya* preparation[9]. It was filtered, and the filtrate was kept on *mandagni* till it was reduced to solid form. *Rasakriya* was collected, weighed and preserved in airtight container and designated as RC

Chitraka *Rasakriya* yield: 48 grams

2. **Ethanolic extract**

The Ethanolic extract of the Chitraka (*Plumbago zeylanica* Linn) was done by using cold maceration technique[8]. 500 grams of the Chitraka *choorna* was soaked in 2500 ml of Ethanol for about 10 days at room temperature with occasional stirring. The solution was filtered through filter cloth followed by Whatman's filter paper and the filtrate thus obtained was designated as EC. This was done by evaporation method under the fan. The extract was collected, weighed and preserved in air tight container.

Ethanol extract of Chitraka Yield: 43 grams

### Standard control

To the commercially available lyophilized Streptokinase vial (15, 00, 000 IU), 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 micro litres was used for *in vitro* thrombolytic study.[8]

### Negative Control

100 micro litres of distilled water was used.

### Procedure

Sterile micro centrifuge tube was taken and weighed (W1). Following all aseptic condition 10 ml of venous blood was drawn from healthy volunteers and 1 ml of blood was transferred into 10 pre-weighed sterile micro centrifuge tubes. Weight of the tube recorded (W2). These tubes were incubated at 37$^0$c for 45 minutes. After clot formation serum was completely aspirated through Pasteur pipette without disturbing the clot formed and weight of the tube recorded once again (W3). Chitraka *Rasakriya* (RC) at the dose of 25 mg (RC1), 50 mg (RC2), 75 mg (RC3) and 100 mg (RC4) and Ethanol extract of Chitraka (EC) at the dose of 25 mg (EC1), 50 mg (EC2),75 mg (EC3) and 100 mg (EC4) were added respectively in the first eight tube and one was kept as standard control (SC) where 100 micro litres of Streptokinase suspension was used and one tube was kept as negative control (NC) where 100 micro litre distilled water was used. Tubes were incubated at 37$^0$c for 90 minutes and were observed for clot lysis. After incubation, serum was separated and aspirated without disturbing the clot. The tubes were weighted by electronic weighting device and the weight of the clot was taken giving due consideration to the weight of the interventional drug (W4).

W1: Weight of the tube
W2: Weight of the tube with the blood sample
W3: Weight of the tube with the blood clot
W4: Weight of the tube after blood clot lysis

Blood clot weight i.e. B1 = W3-W1
Blood clot weight i.e. B2 = W4-W1

### Assessment criteria

Results were assessed based on difference of clot weight before and after the lysis i.e. B2 and B1 and is expressed as percentage of clot lysis.

### Statistical Analysis

For the statistical analysis, the data obtained in the groups were recorded and tested by paired t test. The significance between percentage clot lysis by Streptokinase and herbal extract by means of weight difference is tested by the ANOVA test analysis. Data are expressed as mean ± standard deviation. The corresponding p value was noted and the obtained results were interpreted as p value < 0.005 as significant, p value < 0.0001 as highly significant.

## RESULTS

Results were tabulated and analyzed. Analysis of Clot weight before and after treatment of Chitraka *Rasakriya*, Ethanolic extract of Chitraka, Streptokinase (Standard control) and distilled water (Negative control) was evaluated using paired t test.

### Correlation between the Groups

There was strong positive correlation between before treatment and after treatment of RC1 dose, RC2 dose and RC3 dose of Chitraka *Rasakriya* group (r = .896, .961, .907 p = ≤.001), RC4 dose of Chitraka *Rasakriya* group (r = .805, p = ≤.005).There was a strong positive correlation between before treatment and after treatment of EC1, EC 3 and EC4 dose of Ethanolic extract of Chitraka (r = .966, .961, .862 p = ≤.001). There was positive

correlation between before treatment and after treatment of EC2 dose of Ethanolic extract of Chitraka (r = .756, p = ≤.045). There was positive correlation between before treatment and after treatment of Standard Control i.e. SC (r = .763, p = ≤.010) and positive correlation between before treatment and after treatment of negative control i.e. NC (r = .626, p = ≤.05).

**Table 1: Correlation between the Groups before treatment to after treatment**

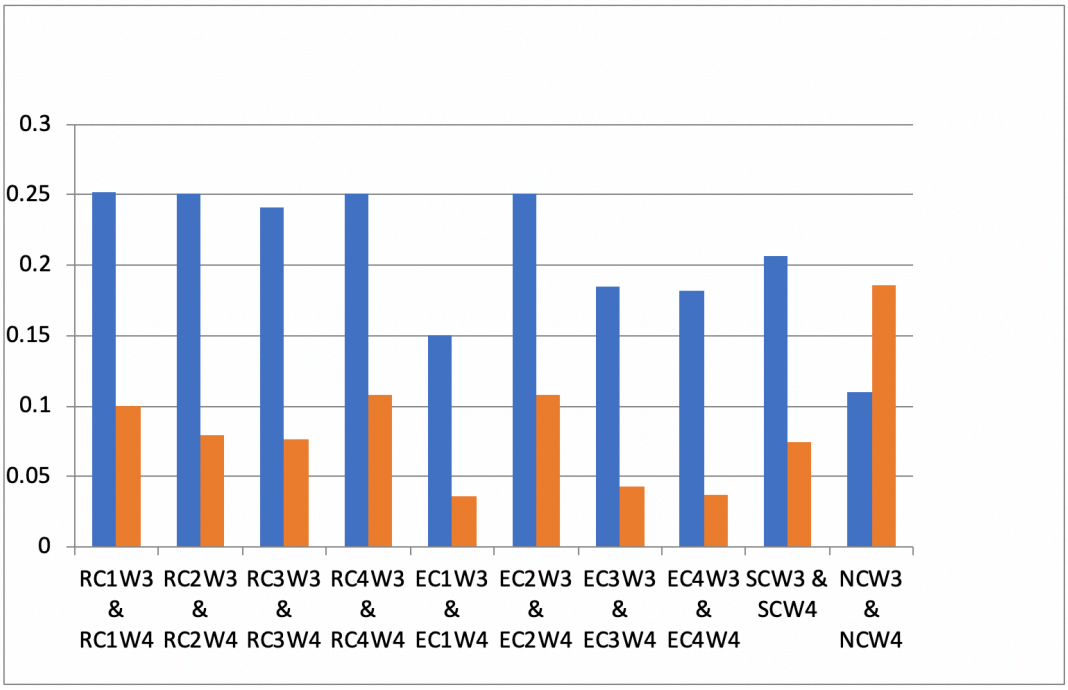
| Groups | N | Correlation | Sig. | Remarks |
|---|---|---|---|---|
| RC1W3 & RC1W4 | 10 | .898 | .0001 | HS |
| RC2W3 & RC2W4 | 10 | .961 | .0001 | HS |
| RC3W3 & RC3W4 | 10 | .907 | .0001 | HS |
| RC4W3 & RC4W4 | 10 | .806 | .005 | HS |
| EC1W3 & EC1W4 | 10 | .966 | .001 | HS |
| EC2W3 & EC2W4 | 10 | .756 | .045 | S |
| EC3W3 & EC3W4 | 10 | .961 | .001 | HS |
| EC4W3 & EC4W4 | 10 | .862 | .001 | HS |
| SCW3 & SCW4 | 10 | .763 | .010 | S |
| NCW3 & NCW4 | 10 | .626 | .05 | S |

HS: Highly significant, S: Significant.

**Table 2: Analysis of Clot lysis before treatment to after treatment within the group**

| Groups | Mean | Std. Deviation | Std. Error Mean | T | df | Sig. (2-tailed) | Remarks |
|---|---|---|---|---|---|---|---|
| RC1W3 & RC1W4 | .25200 | .10031 | .03172 | 7.944 | 9 | .0001 | HS |
| RC2W3 & RC2W4 | .25100 | .07909 | .02501 | 10.036 | 9 | .0001 | HS |
| RC3W3 & RC3W4 | .24100 | .07680 | .02429 | 9.923 | 9 | .0001 | HS |
| RC4W3 & RC4W4 | .25100 | .10785 | .03411 | 7.359 | 9 | .0001 | HS |
| EC1W3 & EC1W4 | .15000 | .03559 | .01125 | 13.328 | 9 | .0001 | HS |
| EC2W3 & EC2W4 | .25100 | .10785 | .03411 | 7.359 | 9 | .0001 | HS |
| EC3W3 & EC3W4 | .18500 | .04301 | .01360 | 13.601 | 9 | .0001 | HS |
| EC4W3 & EC4W4 | .18200 | .03736 | .01181 | 15.406 | 9 | .0001 | HS |
| SCW3 & SCW4 | .20700 | .07484 | .02367 | 8.746 | 9 | .0001 | HS |
| NCW3 & NCW4 | .11000 | .186 | .05851 | 1.865 | 9 | .095 | NS |

HS: Highly significant, NS: Not significant



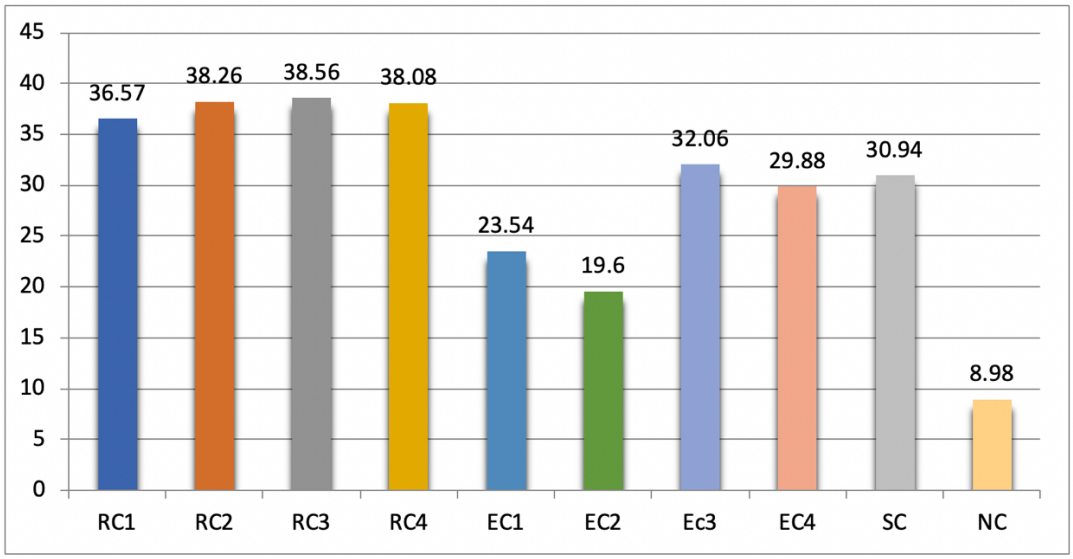

**Graph 1: Analysis of Clot lysis within the group before treatment to after treatment**

Analysis of Clot lysis within Chitraka *Rasakriya* group was statistically highly significant in RC1, RC2, RC3 and RC4 dose from before treatment to after treatment (t – 7.944, 10.036, 9.923, 7.359 respectively, P value ≤ 0.0001). Clot lysis in Ethanolic extract of Chitraka group was statistically highly significant in EC1, EC2, EC3 and EC4 dose from before treatment to after treatment (t – 13.32, 7, 7.359, 13, 601, 15, 406 respectively P value ≤.0001). Clot lysis in standard control was statistically highly significant from before treatment to after treatment (t – 8.746 P value ≤ .0001) and Clot lysis in Negative Control group was not statistically significant from before to after treatment (t – 1.865 P value 0.095).

**Table 3: Analysis of Clot lysis between the groups**

| Clot Lysis | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 66533.817 | 1 | 66533.817 | 8.02 | .001 |

There is statistically significant difference in lysis of clots among groups treated with Chitraka *Rasakriya*, Ethanolic Extract of Chitraka; Standard Control in comparison to Negative Control Group (F = 8.02, p 0.001).




**Graph 2: Analysis of Clot lysis between the groups**

Chitraka *Rasakriya* had better thrombolytic activity when compared to Ethanolic extract of Chitraka and Strptokinase (Standard control)

## DISCUSSION

Results indicate that Chitraka Rasakriya and Ethanolic extract of Chitraka has thrombolytic activity which is highly significant and comparable to standard control, Chitraka by virtue of its *Katu rasa* probably helping in the lysis of blood clot which can be substantiated by quotation given by Acharya Charaka "*Katurasoshonitasanghatam binnati*[2]. *Katu rasa* penetrates into minute vessels of the body by its *Laghu, rooksha, teekshna, vishadaguna* and due to its *Vayu* and *Agni mahabhoota* predominance helps in *vighatana* (breakdown) of *Shonitasanghata* (clots) thus helping in the lysis of thrombus in the blood vessel and facilitate in the circulation of blood.

## CONCLUSION

Present study results reveal that Chitraka *Rasakriya,* Ethanolic extract of Chitraka are having thrombolytic activity comparable to Positive control (Streptokinase). Chitraka *Rasakriya* has better thrombolytic activity when compared to Ethanolic extract of Chitraka and Streptokinase as positive control. Chitraka can be used as thrombolytic agent as the present study is also supported by Acharya Charaka. However extensive *in vivo* study (experimental and Clinical study) will help in giving acost effective safer drug for the treatment of disease caused due to thrombosis. There is a scope for doing research on all the drugs and food items having *katu rasa* for its thrombolytic activity, so that diet modification may help in managing thrombosis.

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