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Research Article

IN-VITRO EXPERIMENTAL STUDY ON THE ANTICOAGULANT ACTIVITY OF *RAKTA PRAVARTHANA CHURNA*

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ABSTRACT

Coagulation disorders disrupt the delicate balance between clot formation and prevention. Various coagulopathies involve abnormal clotting as a manifestation. *Rakta Pravarthana Churna* (RPC) is an Ayurvedic formulation designed for external application during *Rakta Mokshana* to address *Apravarthana* or clotting tendencies. **Aims & Objectives**: The in-vitro study aims to evaluate its anticoagulant (thrombolytic) activity using blood samples from healthy human volunteers. **Materials & Methods**: The clot lysis activity of aqueous and alcohol extracts of *Rakta Pravarthana churna* at two different concentrations (5% & 20%) along with streptokinase as a positive control and distilled water and alcohol as a negative control was carried out. **Results**: The aqueous extracts showed clot lysis activity whereas alcoholic extracts did not exhibit evidently significant clot lysis. **Discussion**: In comparison with positive control (streptokinase), aqueous extract of RPC at 5% concentration showed 10.76% more clot lysis activity, aqueous extract of RPC at 20% concentration showed 14.31% more activity. **Conclusion**: Aqueous extracts of RPC promote clot dissolution, while alcoholic extracts displayed inhibitory effects. These findings show the complexity of Ayurvedic drug interactions with coagulation processes. The study contributes to the understanding of anticoagulation potential of RPC and its scope for potential therapeutic applications.

INTRODUCTION

Ayurveda system of medicine has elaborate discussions on *Rakta dhatu* (blood), its functions in normalcy, signs and symptoms on vitiation, its disorders, their treatment strategies, and efficient therapeutic formulations. In this context, *Rakta mokshana* (controlled therapeutic blood-letting) is stated to be a prime modality of treatment for *Raktaja vyadhis*[1], and to ensure that *Raktamokshana* itself is not hindered by preexisting clotting tendencies due to *Rakta dushti*, a set of herbo-mineral drugs for *Rakta pravarthana* activity has been mentioned by Acharya Sushrutha.[2] This formulation is prepared in the form of *churna* and intended for external application on the site of *Raktamokshana*.

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Termed as *Rakta Pravarthana churna*, this formulation is tested for its probable coagulation modifying activity in the present in-vitro evaluation.

The ingredients of *Rakta Pravarthana Churna* includes *churnas* of *Ela, Karpura, Kushta, Tagara, Paata, Bhadradaaru, Vidanga, Chitraka, Shunti, Maricha, Pippali, Aagaradhuma, Haridra, Arka, Naktamala* taken in equal quantity and made into a homogenous compound *Churna* preparation which is to be applied on the intended site of *Rakta srava.*

Experiments for clot lysis were carried out earlier by *Prasad S. et. Al., 2006.*[3] Few other studies carried out on anticoagulant activity of Ayurvedic drugs were also referred.[4,5,6] This study on in-vitro anticoagulant (thrombolytic) activity has followed the procedure of *Prasad S. et. Al.*, with slight modifications to evaluate the clot lysis activity of aqueous and alcohol extracts of *Rakta Pravarthana churna* at two different concentrations along with streptokinase as a positive control and distilled water and alcohol as a negative control.

OBJECTIVES

- To evaluate the anti-coagulant (thrombolytic) activity of *Rakta Pravarthana Churna (RPC).*
- To find the % of clot lysis.
- Mean difference of % of clot lysis between positive and negative groups.
- Comparison of clot lysis of test drug with positive control.

Criteria of the Study

- **Number of samples:** 20 samples
- **Inclusion criteria:** Healthy human volunteers of age 20-30 yrs are selected randomly.
- **Exclusion criteria:** Oral contraceptive, anti-coagulant therapy, bleeding disorders.

Grouping

Table 1: Grouping for Anticoagulant study of RPC

Group	Drug	No. of samples
Group 1	Positive control with streptokinase	20
Group 2	Negative control with distilled water	20
Group 3	Negative control with alcohol	20
Group 4	Clot lysis by 5% conc. of aqueous extract of RPC (AQ1)	20
Group 5	Clot lysis by 20% conc. of aqueous extract of RPC (AQ2)	20
Group 6	Clot lysis by 5% conc. of alcoholic extract of RPC (AL1)	20
Group 7	Clot lysis by 5% conc. of alcoholic extract of RPC (AL2)	20

MATERIALS AND METHODS

Equipment: 20 healthy volunteers of age group 20-30 years of either sex, streptokinase, alcohol and aqueous extracts of RPC and its testing solution at 5% and 20% concentration, distilled water, test tubes, Eppendorf tube, micropipette, microtips, dropper, spatula, incubator, cyclo mixer, orbital shaker, hot air oven, syringes, gloves, digital balance.

Preparation of drug extract

Rakta Pravarthana churna is taken and separate aqueous and alcoholic solutions are prepared with 20gm of *Churna* dissolved in 200ml of distilled water and ethanol respectively.

The solutions are kept mixed in orbital shakers for 10-12 hours. Then the solutions are filtered and evaporated to dryness in a hot air oven at 57°C such that the solvent is completely removed.

The aqueous and alcoholic extracts thus obtained are weighed and stored in Eppendorf tubes for further use.

Selection of volunteers: Healthy human volunteers of age between 20-30 years of age of either sex are considered for this study. Volunteers not suffering from any bleeding disorders or taking oral contraceptives, females who are menstruating are also excluded.

Specimen: Whole blood (4ml) was drawn from healthy volunteers. 0.5ml blood was transferred to each of the 7 previously weighed test tubes to form clot.

Positive control – Streptokinase

General description: Streptokinase is a 47,000-dalton protein produced by β-hemolytic streptococcus which is effective in anticoagulant (thrombolytic) therapy. Currently streptokinase is rarely used clinically since the advent of newer agents. Physical form - lyophilized powder. Mechanism of action: Streptokinase belongs to a family of medications known as fibrinolytics. Complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin. Plasmin produced in the blood breaks down fibrin, thereby dissolving clots.

Preparation of solutions

- Positive control: Add 5ml sterile distilled water to streptokinase vial (15,00,000 IU) and mix properly. This suspension will be used as stock solution from which 100µl will be used for each test tube containing clot.
- Negative control: 100µl distilled water and alcohol will be used as 2 separate negative control solutions for each blood sample.
- Test drug: The aqueous extract of RPC is dissolved in distilled water in 5% and 20% concentrations. Similarly, the alcoholic extract of RPC is dissolved in alcohol in 5% and 20% concentrations. These serve as test drug solutions and 100µl of each is used against each blood sample.

PROCEDURE

4ml of venous blood was drawn from the healthy volunteers and distributed in 7 different pre-weighted sterile test tubes, and incubated at 37°C for 45mins. After clot formation the serum was aspirated and completely removed without disturbing the clot.

Each tube having the clot was again weighed to determine the clot weight.

(Weight of clot = weight of clot containing tube – weight of empty tube)

100µl extract (aqueous and alcohol) of *Rakta Pravarthana churna* at 5% and 20% concentrations, 100µl streptokinase, 100µl distilled water and 100µl alcohol were added to the tubes containing clots. All the tubes after adding the drug samples, positive and negative controls were incubated at 37°C for 90mins and observed for clot lysis. After incubation, the fluid released from the clot was removed and the tubes were again weighed to get the clot weight after clot lysis. The difference in weight of clot before and after the clot lysis was expressed in %. The experiment was repeated 20 times with blood samples of 20 volunteers.

This experiment provides the percentage of clot lysis which will be taken as the measure of anti-coagulant activity.

$$\text{\% of clot lysis} = \frac{\text{weight of the released clot x 100}}{\text{Clot weight}}$$

RESULTS

In the in-vitro experimental study done on human blood samples, the aqueous extracts of *Rakta Pravarthana Churna* taken in two different concentrations showed clot lysis activity whereas alcoholic extracts did not exhibit evidently significant clot lysis.

The experimental results of clot weight, weight of the released clot and % of clot lysis for 20 samples each treated with streptokinase, distilled water, alcohol, aqueous and alcoholic extracts of RPC in 5% and 20% concentrations, and other results are tabulated as follows.

Table 2: Determination of clot lysis by Streptokinase

S.No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	0.27	0.14	51.85
2.	0.25	0.15	60
3.	0.25	0.09	36
4.	0.30	0.12	40
5.	0.24	0.10	41.67
6.	0.19	0.09	47.36
7.	0.28	0.11	39.28
8.	0.13	0.07	53.85
9.	0.13	0.07	53.85
10.	0.14	0.06	42.86
11.	0.22	0.11	50
12.	0.17	0.07	41.17
13.	0.13	0.07	53.85
14.	0.18	0.08	44.44
15.	0.20	0.11	55
16.	0.17	0.05	29.41
17.	0.19	0.05	26.31
18.	0.16	0.05	31.25
19.	0.13	0.04	30.76
20.	0.18	0.05	27.77

Table 3: Determination of clot lysis by distilled water

S.No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	0.26	0.09	34.61
2.	0.30	0.15	50
3.	0.29	0.11	37.93
4.	0.31	0.12	38.71

5.	0.14	0.04	28.57
6.	0.30	0.11	36.67
7.	0.31	0.12	38.71
8.	0.22	0.07	31.82
9.	0.21	0.09	42.85
10.	0.16	0.05	31.25
11.	0.26	0.12	46.15
12.	0.15	0.06	40
13.	0.22	0.09	40.90
14.	0.22	0.08	36.36
15.	0.16	0.07	43.75
16.	0.24	0.07	29.16
17.	0.21	0.04	19.04
18.	0.20	0.06	30
19.	0.12	0.02	16.66
20.	0.25	0.05	20

Table 4: Determination of clot lysis by alcohol

S.No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	-	-	-
2.	-	-	-
3.	0.25	0.05	20
4.	0.29	0.10	34.38
5.	0.25	0.04	16
6.	0.23	0.05	21.73
7.	0.30	0.09	30
8.	0.11	-0.03	0
9.	0.15	-0.02	0
10.	0.18	0	0
11.	0.31	0.13	41.93
12.	0.21	0.06	28.57
13.	0.21	0.02	9.52
14.	0.27	0.08	29.62
15.	0.23	0.02	8.69
16.	0.20	0.01	5
17.	0.25	0.03	12
18.	0.09	-0.02	0
19.	0.13	0.01	7.69
20.	0.25	0.02	8

Table 5: Determination of clot lysis by AQ1 (5% concentration of aqueous extract of RPC)

S.No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	0.21	0.12	57.14
2.	0.16	0.10	62.5
3.	0.15	0.05	33.34
4.	0.17	0.07	41.18
5.	0.20	0.11	55
6.	0.08	0.03	37.5
7.	0.20	0.09	45
8.	0.12	0.07	58.33
9.	0.08	0.03	37.5
10.	0.10	0.05	50
11.	0.10	0.05	50
12.	0.12	0.05	41.66
13.	0.12	0.08	66
14.	0.17	0.10	58.82
15.	0.18	0.11	61.11
16.	0.12	0.05	41.66
17.	0.13	0.06	46.15
18.	0.10	0.03	30
19.	0.22	0.10	45.45
20.	0.23	0.07	30.43

Table 6: Determination of clot lysis by AQ2 (20% concentration of aqueous extract of RPC)

S. No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	0.28	0.17	60.71
2.	0.14	0.10	71
3.	0.24	0.10	41.67
4.	0.25	0.12	48
5.	0.23	0.11	47.83
6.	0.11	0.04	36.4
7.	0.22	0.11	50
8.	0.11	0.06	54.55
9.	0.06	0.03	50
10.	0.12	0.07	58.33
11.	0.05	0.01	20
12.	0.18	0.10	55.55
13.	0.10	0.05	50
14.	0.25	0.17	68
15.	0.17	0.11	64.71
16.	0.09	0.04	44.44
17.	0.17	0.07	41.17
18.	0.11	0.04	36.36
19.	0.23	0.10	43.47
20.	0.27	0.10	37.03

Table 7: Determination of clot lysis by AL1 (5% concentration of Alcohol extract of RPC)

S.No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	0.25	0.02	8
2.	0.18	-0.02	0
3.	0.23	-0.02	0
4.	0.29	0.07	24.14
5.	0.21	0	0
6.	0.17	-0.04	0
7.	0.26	0.04	15.38
8.	0.16	-0.04	0
9.	0.08	-0.04	0
10.	0.14	0	0
11.	0.08	-0.01	0
12.	0.19	-0.04	0
13.	0.14	-0.05	0
14.	0.21	-0.05	0
15.	0.19	-0.03	0
16.	0.12	-0.02	0
17.	0.21	-0.01	0
18.	0.15	-0.03	0
19.	0.19	-0.02	0
20.	0.22	-0.02	0

Table 8: Determination of clot lysis by AL2 (20% concentration of Alcohol extract of RPC)

S. No	Clot Weight (g)	Weight of the released clot (g)	% of clot lysis
1.	0.26	0.03	11.54
2.	0.20	-0.02	0
3.	0.24	-0.05	0
4.	0.30	0.06	20
5.	0.26	0.06	23.08
6.	0.13	-0.04	0
7.	0.26	0.05	19.23
8.	0.18	-0.08	0
9.	0.09	-0.06	0
10.	0.13	-0.05	0
11.	0.15	-0.04	0
12.	0.19	-0.04	0
13.	0.20	-0.03	0
14.	0.21	0.01	4.76
15.	0.19	-0.04	0
16.	0.11	-0.06	0
17.	0.20	-0.05	0
18.	0.14	-0.05	0
19.	0.19	-0.05	0
20.	0.20	-0.03	0

Table 9: % of clot lysis when compared with Negative control (Distilled water)

	Group	Clot lysis %	% change
A	-ve control (Distilled water)	34.65 ± 2.00	-
B	-ve control (alcohol)	15.17 ± 3.12***	56.2↓
C	+ve control (Streptokinase)	42.83 ± 2.45	23.61↑
D	AQ1	47.44 ± 2.45**	36.91↑
E	AQ2	48.96 ± 2.75***	41.3↑
F	AL1	2.38 ± 1.42***	93.1↓
G	AL2	3.93 ± 1.74***	88.6↓

A vs B *** P<0.001, A vs C ns P>0.05, A vs D ** P<0.01, A vs E *** P<0.001, A vs F *** P<0.001, A vs G *** P<0.001

- P value: Mean ± SEM
- P value < 0.001 can be considered highly significant
- P value > 0.05 can be considered as not significant
- P value < 0.01 can be considered moderately significant

Table 10: % of clot lysis when compared with Negative control (Alcohol)

	Group	Clot lysis %	% change
A	-ve control (Distilled water)	34.65 ± 2.00	128.4↑
B	-ve control (alcohol)	15.17 ± 3.12	-
C	+ve control (Streptokinase)	42.83 ± 2.45***	182.3↑
D	AQ1	47.44 ± 2.45***	212.7↑
E	AQ2	48.96 ± 2.75***	222.7↑
F	AL1	2.38 ± 1.42**	84.3↓
G	AL2	3.93 ± 1.74*	74.1↓

B vs C *** P<0.001, B vs D *** P<0.001, B vs E *** P<0.001, B vs F ** P<0.01, B vs G * P<0.05

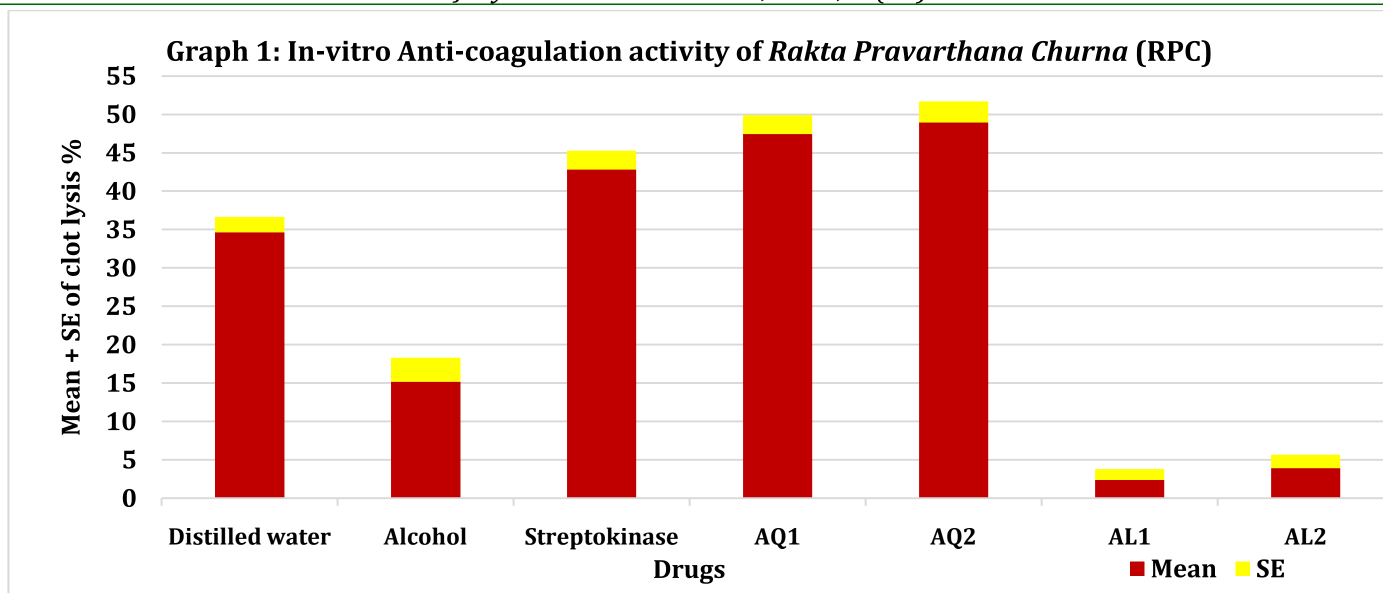
- P value: Mean ± SEM
- P value < 0.001 can be considered highly significant
- P value < 0.01 can be considered moderately significant
- P value < 0.05 can be considered as mildly significant

Table 11: % of clot lysis when compared with Positive control (Streptokinase)

	Group	Clot lysis %	% change
A	-ve control (Distilled water)	34.65 ± 2.00	19.1↓
B	-ve control (alcohol)	15.17 ± 3.12	64.6↓
C	+ve control (Streptokinase)	42.83 ± 2.45	-
D	AQ1	47.44 ± 2.45	10.76↑
E	AQ2	48.96 ± 2.75	14.31↑
F	AL1	2.38 ±1.42***	94.4↓
G	AL2	3.93 ± 1.74***	90.8↓

C vs D ns P>0.05, C vs E ns P>0.05 , C vs F *** P<0.001 , C vs G *** P<0.001

- P value: Mean ± SEM
- P value > 0.05 can be considered as not significant
- P value < 0.001 can be considered highly significant



DISCUSSION

The term *Rakta Pravarthana* essentially translates to "initiating the flow of blood." *Rakta Pravarthana churna*, as elucidated within the context of *Raktamokshana*, it can be understood as a promoter of the dissolution of blood clots or stagnant blood that may cause blockages during bloodletting. Thus, in the context of *Raktamokshana*, the resolution of pre-existing blood clots- referred to as clot lysis- becomes synonymous with ensuring unobstructed blood flow or anticoagulation. Therefore, in this context, clot lysis serves as a measure of anticoagulation activity. The current study delves into the thrombolytic potential of *Rakta Pravarthana churna* extracts, with the primary objective of evaluating their clot lysis activity. Through this assessment, the study indirectly aims to infer the anti-coagulation potential of *Rakta Pravarthana churna.*

The methodology employed for this study was based on the work of Prasad et al. (2007), with minor adaptations to assess the clot lysis activity of *Rakta Pravarthana churna* extracts.

Table 12: Consolidated comparison with positive control (Streptokinase) in RPC study

Groups	Clot lysis by negative control (Distilled water)	Clot lysis by negative control (alcohol)	Clot lysis by positive control (Streptokinase)	Clot lysis by Aqueous extract of RPC at 5% conc. AQ1	Clot lysis by Aqueous extract of RPC at 20% conc. AQ2	Clot lysis by Alcoholic extract of RPC at 5% conc. AL1	Clot lysis by Alcoholic extract of RPC at 20% conc. AL2
Mean clot lysis %	34.65±2.00	15.17±3.12 ***	42.83±2.45	47.44±2.45	48.96±2.75	2.38±1.42 ***	3.93±1.74 ***
% change	19.1↓	64.6↓	-	10.76↑	14.31↑	94.4↓	90.8↓

The results showed that in comparison with positive control (streptokinase), distilled water showed 19.1% less clot lysis activity, alcohol showed 64.6% less clot lysis activity, aqueous extract of RPC at 5% concentration showed 10.76% more clot lysis activity, aqueous extract of RPC at 20% concentration showed 14.31% more activity, alcoholic extract of RPC at 5% concentration showed 94.4% less clot lysis activity, alcoholic extract of RPC at 20% concentration showed 90.8 % less clot lysis activity.

Statistically speaking, when compared to streptokinase, water showed almost same activity (P>0.005), alcohol showed high significance negatively (P<0.001), aqueous extract of RPC at both 5% and 20% concentration showed not much significant activity (p>0.05) while alcoholic extract of RPC at both concentrations showed high significance negatively (p<0.001)

Table 13: Consolidated comparison with negative control (distilled water) in RPC study

Groups	Clot lysis by negative control (Distilled water)	Clot lysis by negative control (alcohol)	Clot lysis by positive control (Streptokinase)	Clot lysis by Aqueous extract of RPC at 5% conc. AQ1	Clot lysis by Aqueous extract of RPC at 20% conc. AQ2	Clot lysis by Alcoholic extract of RPC at 5% conc. AL1	Clot lysis by Alcoholic extract of RPC at 20% conc. AL2
Mean clot lysis %	34.65±2.00	15.17±3.12 ***	42.83±2.45	47.44±2.45 **	48.96±2.75 ***	2.38±1.42 ***	3.93±1.74 ***
% change	-	56.2↓	23.61↑	36.91↑	41.3↑	93.1↓	88.6↓

The results showed that in comparison with distilled water, alcohol showed 56.2% less clot lysis activity, streptokinase showed 23.61% more clot lysis activity, aqueous extract of RPC at 5% concentration showed 36.91% more clot lysis activity, aqueous extract of RPC at 20% concentration showed 41.3% more activity, alcoholic extract of RPC at 5% concentration showed 93.1% less clot lysis activity, alcoholic extract of RPC at 20% concentration showed 86.6% less clot lysis activity.

Statistically speaking, when compared to distilled water, alcohol was highly significant negatively (P<0.001), streptokinase showed similar activity (p >0.05), aqueous extract of RPC at both 5% showed moderate significance positively (p<0.01) and at 20% concentration showed high significance positively (p<0.001) while alcoholic extract of RPC at both concentrations showed high significance negatively (p<0.001)

Table 14: Consolidated comparison with negative control (alcohol) in RPC study

Groups	Clot lysis by negative control (Distilled water)	Clot lysis by negative control (alcohol)	Clot lysis by positive control (Streptokinase)	Clot lysis by Aqueous extract of RPC at 5% conc. AQ1	Clot lysis by Aqueous extract of RPC at 20% conc. AQ2	Clot lysis by Alcoholic extract of RPC at 5% conc. AL1	Clot lysis by Alcoholic extract of RPC at 20% conc. AL2
Mean clot lysis %	34.65±2.00 ***	15.17±3.12	42.83±2.45 ***	47.44±2.45 ***	48.96±2.75 ***	2.38±1.42 **	3.93±1.74 *
% change	128.4↑	-	182.3↑	212.7↑	222.7↑	84.3↓	74.1↓

The results showed that in comparison with alcohol, distilled water showed 128.4% more clot lysis activity, streptokinase showed 182.3% more clot lysis activity, aqueous extract of RPC at 5% concentration showed 212.7% more clot lysis activity, aqueous extract of RPC at 20% concentration showed 222.7% more activity, alcoholic extract of RPC at 5% concentration showed 84.3% less clot lysis activity, alcoholic extract of RPC at 20% concentration showed 74.1% less clot lysis activity.

Statistically speaking, when compared to alcohol, water, streptokinase, and aqueous extract of RPC at both 5% and 20% concentrations showed high significance positively (P<0.001), while alcoholic extract of RPC at 5% concentration showed moderate significance negatively (P<0.01), and alcoholic extract of RPC 20% concentration showed mild significance negatively (p<0.05).

When evaluated in comparison with the negative control of distilled water, the extracts displayed varying degrees of clot lysis activity. The negative control of alcohol exhibited significantly reduced clot lysis percentages, indicating a hindrance to clot dissolution. This could be attributed to the interaction between alcohol and the coagulation cascade, which might interfere with the natural clot lysis process. The positive control, streptokinase, demonstrated an increase in clot lysis percentages, validating its established thrombolytic activity.

The aqueous extract in both concentrations (AQ1 and AQ2) exhibited almost similar clot lysis percentages compared to positive control, and exhibited enhanced clot lysis percentages compared to both negative controls, suggesting their potential to promote clot dissolution. This observation indicates that these extracts could influence the fibrinolytic pathways, leading to increased clot breakdown. Importantly, the significant change in clot lysis percentages for AQ1 and AQ2 highlights their dose-dependent effect, with higher concentrations leading to greater clot dissolution.

Conversely, the alcoholic extract in both concentrations (AL1 and AL2) displayed remarkably lower clot lysis percentages across all comparisons. This unexpected outcome underscores the complexity of interactions between alcoholic extracts and coagulation factors. Further investigation is required to elucidate the mechanisms behind this inhibitory effect.

The inter-group comparisons reveal interesting insights. While there were no significant differences observed between the positive control and the aqueous extract (AQ1 and AQ2), alcoholic extract (AL1 and AL2) demonstrated significantly reduced clot lysis percentages compared to the positive control. The alcoholic extracts also show significantly reduced clot lysis in comparison with alcohol itself. This suggests that the alcoholic extracts may in fact promote coagulation or perhaps favour platelet aggregation, warranting a closer examination of their impact on thrombolytic and coagulation pathways.

CONCLUSION

In conclusion, the study highlights the differential effects of *Rakta Pravarthana churna* extracts on clot lysis, with aqueous extracts promoting clot dissolution and alcoholic extracts displaying inhibitory effects. These findings emphasize the complexity of Ayurvedic drug interactions with coagulation processes and highlight the need for further research to elucidate the mechanisms underlying these observations. The study contributes to the understanding of the thrombolytic and anticoagulation potential of *Rakta Pravarthana churna*

and its implications for potential therapeutic applications.

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FIGURES

Fig 1: Mixing drug solutions in orbital shaker

Fig 2: Filtering the drug solutions

Fig 3: Alcoholic & aqueous solutions of RPC

Fig 4: Drying the solutions in hot air oven at 57^{0}C

Experimental evaluation of anti-coagulant activity of RPC

Fig 5. Concentrating the extracts in heating mantle

Fig. Prepared extracts of RPC stored in eppendorf tubes

Fig 7: Pre-weighing of empty test tubes

Fig 8: Collection of blood samples from volunteers

Fig 9: Blood sample divided among test tubes

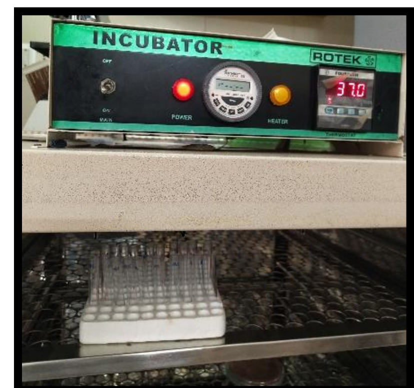

Fig 10: Incubating the tubes at 37°C

Fig 11: Aspirating the serum after clot formation

Fig 12: Determining clot weight

Fig 13: Adding test solutions to tubes containing clot

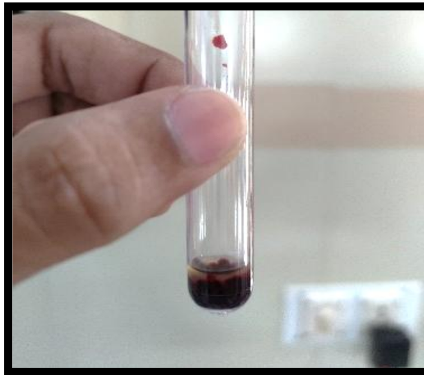

Fig 14: Tubes along with test drugs after incubation

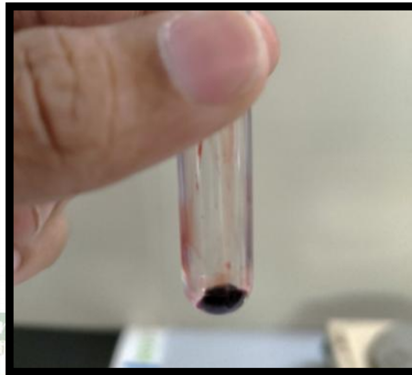

Fig 15: Showing clot after aspirating the fluid

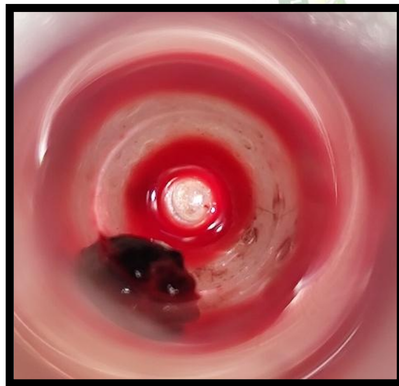

Fig 16: Clot in a tube added with Aquoeous solution of RPC

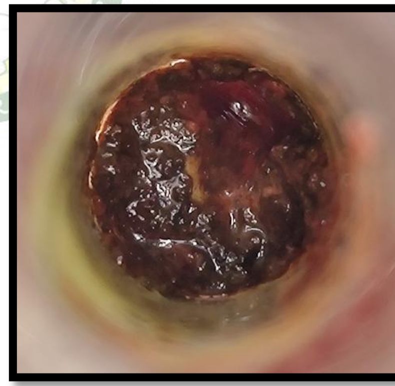

Fig 17: Clot in a tube added with alcoholic solution of RPC