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AN EXPERIMENTAL EVALUATION OF THROMBOLYTIC ACTIVITY OF SHIGRU SEEDS (MORINGA OLEIFERA LAM) - AN IN-VITRO STUDY

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ABSTRACT

Introduction: In the present era, due to unhealthy food and lifestyle people suffer from various life-threatening diseases. Thrombosis is one among such conditions which can suddenly cause blockage in blood vessels leading to Myocardial infarction, cerebrovascular thrombosis and deep vein thrombosis. Treatment of thrombosis involves administration of drugs that dissolve the blood clots. The thrombolytic drugs have limitations due to high risk of hemorrhage, severe anaphylactic reactions and lack of fibrin specificity. Hence, present study has been undertaken to discover the safest, reliable and efficient source for thrombolytic action. **Aims and Objectives:** To evaluate thrombolytic activity of *Shigru* seeds *(Moringa oleifera Lam)*. **Methodology:** Evaluation of thrombolytic activity of Alcohol and Aqueous extract of *Shigru* seeds *(Moringa oleifera Lam)* was carried out on 180 blood samples of 30 healthy volunteers selected randomly, where Streptokinase is taken as positive control and Distilled water as negative control. **Result:** Thrombolytic experiment was conducted in 6 groups among which remarkable clot lysis was shown in aqueous extract group of *Shigru* seeds *(Moringa oleifera Lam)*. It showed similar clot lysis to that of standard Streptokinase group. **Interpretation and Conclusion:** The result suggests that aqueous extract of *Shigru* seeds shows significant thrombolytic activity compared to Streptokinase ≥3500 I.U.

KEYWORDS: *Shigru* seeds *(Moringa oleifera Lam)*, Thrombolytic, Aqueous extract, Alcohol extract.

INTRODUCTION

Ayurveda is one of the most ancient systems of medicine in the world, which is considered as the science of life. Dravyaguna Vijnana is the branch of Ayurveda which deals with medicinal plants, their properties and actions. It always enlightened a path for acceptance of new drug or new pharmacological action of known drugs. In present era, people have adopted number of unhealthy diet and lifestyle, hence develop with many new health disorders. Thrombosis is one such alarming health issue which leads to high mortality rate.

Thrombosis[1] is the process of formation of solid matter in the circulation from the constituents of flowing blood, the mass itself a thrombus. Hemostatic plugs at the cut end of a blood vessel may be considered as the simplest form of thrombosis.

Hemostatic plugs are useful as they stop the escape of blood and plasma, whereas thrombosis developing in the unruptured blood vessels which may be fatal.

Thrombosis is the physiological process that underlies the cardiovascular disorders such as pulmonary emboli, deep vein thrombosis, strokes and myocardial infarction which are the main cause of mortality and morbidity in developing countries.

Thrombolytic[2] agents such as tissue plasminogen activator, urokinase and streptokinase are used worldwide, but their use is associated with high risk of hemorrhages, severe anaphylactic reaction and lacks specificity.

Among the various plants studied, *Moringa oleifera* is medicinally important one. *Shigru* is having *katu rasa*, *ushna virya*, *teekshna* and *pramathi guna (sroto vishodhana)* and also found to be beneficial in *Shonita sanghatam binnati* due to *katu rasa.*[3] In the present study, an attempt to investigate and document the thrombolytic activity of *Shigru (Moringa oleifera)* has been made.

MATERIAL AND METHODS

Source of data:

This being an in-vitro experimental study, human blood is the source and the experiment was conducted in Department of Biochemistry S.D.M Centre for Research in Ayurveda and Allied Science, Udupi.

Method of collection of data:

Dried *Shigru* pods were collected from Bhatkal during the month of April and were dried to obtain *Shigru* seeds (Drumstick seeds).

Preparation of plant extract:

10 grams of dried seeds of *Shigru* were taken separately and coarsely powdered. They were then extracted with 250mL water and ethanol solutions separately. The sediments were filtered and the filtrates were evaporated to dry at 40^{o}C. The extract obtained was the aqueous and alcohol extract of *Shigru* seeds which are used for carrying out experiments.

Fig. 1: Aqueous extract of *Shigru* seeds.

Fig. 2: Alcohol extract of *Shigru* seeds.

Table no 1: Criteria of the study.

1	No. of samples	30 (6 groups of 30 blood samples)
2	Inclusion criteria	Healthy human volunteers of age 20 – 35 year are selected randomly
3	Exclusion criteria	Oral contraceptive, Anti-coagulant therapy.

Table no. 2: Grouping.

Group	Drug	No. of Samples
Group 1	Positive control with Streptokinase (PC).	30
Group 2	Negative control with distilled water (NC).	30
Group 3	Clot lysis by Aqueous extract of *Shigru beeja* (250 mg) (AQ 1)	30
Group 4	Clot lysis by Aqueous extract of *Shigru beeja* (500 mg) (AQ 2)	30
Group 5	Clot lysis by Alcohol extract of *Shigru beeja* (250 mg) (AL 1)	30
Group 6	Clot lysis by Alcohol extract of *Shigru beeja* (500 mg) (AL 2)	30

Method of experimentation

Experiments for clot lysis were carried out earlier by Prasad S. et al., 2007. This study on in-vitro thrombolytic activity had followed the procedure of Prasad S. et al., 2007 with slight modifications.

An in-vitro thrombolytic model was used to evaluate the clot lysis effect of aqueous and alcohol extracts of *Shigru* seeds (*Moringa oleifera* Lam.) along with Streptokinase as a positive control & distilled water as a negative control.

Materials required:

30 Healthy volunteers of age group 20-35 of either sex, Streptokinase, Alcohol and aqueous extract of Test Drugs, Distilled water, Clot tubes, Micropipette, Microtips, Centrifuge apparatus, Incubator, Syringes, Gloves

Selection of volunteers:

Healthy human volunteers of age between 20- 35years of either sex are considered for this study. Volunteers should not be suffering from any bleeding disorders or taking oral contraceptives. Females who are on their menstrual cycle are also excluded.

Specimen:

Whole blood (3mL) was drawn from healthy volunteers (n = 30), using a protocol approved by Institutional Ethical Committee of Muniyal Institute of Ayurvedic Medical Sciences, Manipal. 0.5mL of blood was transferred to each of the six previously weighed micro centrifuge tubes to form clot.

Streptokinase:[4]

Product no: S 3134

Form: lyophilized powder

Storage temp: - 20^0 C

General description:

Streptokinase (STREPTASE) is a 47,000-dalton protein produced by β- hemolytic Streptococcus which is effective in thrombolytic therapy. It has no intrinsic enzymatic activity, but it forms a stable, noncovalent 1:1 complex with plasminogen. This produces a conformational change that exposes the active site on plasminogen that cleaves arginine 560 on free plasminogen to form free plasmin. Streptokinase is rarely used clinically for

fibrinolysis since the advent of newer agents.

Physical form:

Lyophilized powder containing ~ 50% total protein by biuret & sodium glutamate. Total protein composed of enzyme protein & human serum albumin.

Unit definition:

One unit will liquefy a standard clot of fibrinogen, plasminogen & thrombin at pH 7.5 at 37^{o}C in 10 min.

Route of administration:

Intra venous

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 thrombi.

Indications:

Myocardial infarction, ischemic stroke, para pneumonic effusions, emphysema, pulmonary embolism, arterial thromboembolism.

Side effects:

Nausea, bleeding, low blood pressure, allergic reactions.

Complications:

Intra cerebral haemorrhage

Contraindications:

Arteriovenous malformation, Intracranial hemorrhage, Cancer inside skull, suspected aortic dissection, uncontrolled hypertension, Dementia, Active peptic ulcer, recent internal bleeding, Oral anticoagulant therapy.

Preparation of Stock solution:

To the commercially available lyophilized SK vial of >3500 I.U., 5mL distilled water was added & mixed properly. This suspension was used as a stock from which 100µL was used

for in-vitro thrombolysis.

Experimental protocol

Procedure:[5,6]

3mL venous blood drawn from the healthy volunteers was distributed in 6 different pre-weighed micro centrifuge tubes (0.5mL/ tube) & incubated at 37^{o}C for 45mts. After clot formation serum was completely removed without disturbing the clot & each tube having the clot was again weighed to determine the clot weight.

(Clot weight = Weight of the clot containing tube – weight of tube alone)

100µL extract (aqueous and ethanol) of the test drug (*Shigru* seeds) added to the clot containing tube separately. Similarly, 100µL Streptokinase was added to the clot of Standard tube (positive control) and 100µL distilled water added to the clot of blank tube (negative control).

All the tubes were then incubated at 37^{0}C for 90 minutes & observed the clot lysis. After incubation fluid released was removed and tubes were again weighed to observe the difference in the weight after clot disruption. Difference obtained in the weight taken before and after clot lysis was expressed in %. The experiment was repeated 30 times with blood samples of 30 volunteers.

Weight loss after application of extract solution was taken as functional indication of thrombolytic activity.

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

Experimental study

Thrombolytic activity:

- Mature seeds of *Shigru* were collected. It was then dried under sunlight.
- Dried *Shigru* seeds were then coarsely powdered & extracted with 250mL of distilled water & ethanol separately. It was then filtered, evaporated & the sediments obtained were the alcohol and aqueous extracts of *Shigru* seeds.
- Empty clot tubes were collected, weighed & marked.
- A 3mL venous blood was drawn from each healthy human volunteer & was transferred to 6 clot tubes using micro pipette.
- These clot tubes were incubated at 37^{0}C for 45 minutes.

- After clot formation, serum was aspirated, centrifuged & again aspirated & weighed.
- 100µL extract (aqueous and ethanol) of the test drug (*Shigru* seed) was added to the clot containing tube separately. Similarly, 100µL Streptokinase was added to the clot of Standard tube (positive control) &100µL distilled water added to the clot of blank tube (negative control).
- Clot tubes were then incubated at 37^{o} C for 90 minutes.
- Fluid collected was removed, centrifuged & again aspirated & weighed. % of clot lysis was calculated.

RESULT

Result of thrombolytic activity

Table no. 3: Determination of clot lysis by streptokinase.

Sl. No	Clot Weight (g)	Weight of the released clot (g)	% of Clot lysis
1	0.24	0.05	20.8
2	0.194	0.06	30.93
3	0.178	0.08	44.943
4	0.244	0.04	16.393
5	0.29	0.08	27.59
6	0.24	0.04	16.67
7	0.237	0.08	33.755
8	0.198	0.02	10.101
9	0.246	0.04	16.2601
10	0.239	0.04	16.74
11	0.294	0.06	20.41
12	0.271	0.09	33.210
13	0.255	0.04	15.69
14	0.249	0.04	16.064
15	0.254	0.04	15.75
16	0.205	0.05	24.390
17	0.232	0.05	21.551
18	0.213	0.05	23.474
19	0.211	0.04	18.96
20	0.265	0.06	22.64
21	0.269	0.06	22.304
22	0.286	0.07	24.48
23	0.232	0.05	21.551
24	0.211	0.06	26.431
25	0.25	0.08	32
26	0.231	0.04	17.32
27	0.24	0.07	29.17
28	0.228	0.07	30.701
29	0.237	0.02	8.44

30	0.251	0.03	11

Table no. 4: Determination of clot lysis by distilled water.

Sl. No	Clot Weight (g)	Weight of the released clot (g)	% of Clot lysis
1	0.635	0.016	2.51
2	0.464	0.035	7.5
3	0.339	0.003	0.88
4	0.471	0.023	4.88
5	0.442	0.007	1.5
6	0.144	0.011	7.6
7	0.09	0.005	5.5
8	0.085	0.001	1.1
9	0.062	0.001	1.6
10	0.156	0.003	1.9
11	0.124	0.001	0.8
12	0.117	0.004	3.4
13	0.159	0.001	0.6
14	0.158	0.008	5.06
15	0.22	0.01	4.54
16	0.13	0.01	7.6
17	0.206	0.006	2.9
18	0.096	0.006	6.25
19	0.16	0.001	0.6
20	0.16	0.02	12.5
21	0.14	0.001	0.7
22	0.25	0.001	0.4
23	0.21	0.002	0.9
24	0.149	0.001	0.6
25	0.155	0.005	3.2
26	0.1	0.002	2
27	0.202	0.002	0.99
28	0.13	0.01	7.69
29	0.151	0.012	7.9
30	. 0.152	0.013	8.5

Table no. 5: Determination of Clot lysis by AQ1 (250 mg Aqueous extract of seeds of *Shigru*).

Sl. No	Clot Weight (g)	Weight of the released clot (g)	% of Clot lysis
1	0.23	0.1	43.48
2	0.189	0.05	26.46
3	0.226	0.12	53.097
4	0.233	0.05	21.46
5	0.208	0.04	19.230
6	0.247	0.06	24.291
7	0.202	0.04	19.801

8	0.222	0.06	27.027
9	0.18	0	0
10	0.286	0.1	34.97
11	0.257	0.03	11.673
12	0.247	0.05	20.242
13	0.241	0.05	20.75
14	0.201	0.02	9.950
15	0.246	0.03	12.195
16	0.235	0.08	34.042
17	0.245	0.06	24.49
18	0.212	0.04	18.87
19	0.184	0.03	16.304
20	0.245	0.07	28.571
21	0.261	0.08	30.651
22	0.278	0.04	14.39
23	0.251	0.05	19.920
24	0.184	0.03	11.583
25	0.228	0.02	8.771
26	0.229	0.02	8.733
27	0.21	0.03	14.29
28	0.211	0.01	4.74
29	0.224	0.02	8.93
30	0.204	0.03	14.705

Table no. 6: Determination of Clot lysis by AQ2 (500 mg Aqueous extract of seeds of *Shigru*).

Sl. No.	Clot Weight (g)	Weight of the released clot (g)	% of Clot lysis
1	0.286	0.15	52.45
2	0.19	0.06	31.58
3	0.215	0.07	32.56
4	0.237	0.06	25.32
5	0.26	0.07	26.923
6	0.218	0.05	22.935
7	0.221	0.06	27.149
8	0.224	0.05	1.12
9	0.216	0.03	13.89
10	0.255	0.05	19.68
11	0.296	0.06	20.270
12	0.295	0.09	30.51
13	0.262	0.07	26.72
14	0.25	0.06	24
15	0.24	0.05	20.833
16	0.261	0.09	34.482
17	0.26	0.1	38.461
18	0.218	0.08	3.697
19	0.237	0.11	46.413

20	0.264	0.08	30.303
21	0.288	0.1	34.722
22	0.275	0.07	25.454
23	2.540	0.09	34.75
24	0.237	0.09	35.294
25	0.234	0.08	34.188
26	0.258	0.07	27.131
27	0.193	0.08	41.450
28	0.239	0.08	33.47
29	0.243	0.07	28.806
30	0.221	0.05	22.624

Table no. 7: Determination of Clot lysis by AL1 (250 mg Alcohol extract of seeds of *Shigru)*.

Sl. No.	Clot Weight (g)	Weight of the released clot (g)	% of Clot lysis
1	0.263	0.05	19.01
2	0.241	0.03	12.45
3	0.224	0.04	17.86
4	0.244	0.02	8.196
5	0.282	0.02	7.092
6	0.222	0	0
7	0.215	0.01	4. 651
8	0.237	0.05	21.097
9	2.690	- 0.01	0
10	0.254	-0.02	0
11	0.278	0	0
12	0.26	0.02	7.692
13	0.249	0.01	4.02
14	0.233	0.01	4.291
15	0.254	0	0
16	0.245	0	0
17	0.269	0.03	11.152
18	0.224	-0.02	0
19	0.229	-0.03	0
20	0.252	-0.01	0
21	0.272	0.02	7.352
22	0.286	0	0
23	0.239	-0.02	0
24	0.229	0.02	7.905
25	0.233	-0.01	0
26	0.25	-0.01	0
27	0.214	-0.02	0
28	0.213	-0.04	0
29	0.233	-0.02	0
30	0.231	-0.02	0

Table no. 8: Determination of Clot lysis by AL2 (500 mg Alcohol extract of seeds of *Shigru*).

Sl. No	Clot Weight (g)	Weight of the released clot (g)	% of Clot lysis
1	0.282	0.05	17.73
2	0.213	0.01	0
3	0.179	-0.02	0
4	0.275	0.01	3.64
5	0.281	0.01	3.56
6	0.245	-0.01	0
7	0.216	-0.02	0
8	0.226	0	0
9	0.245	0	0
10	0.239	-0.01	0
11	0.29	-0.01	0
12	0.233	-0.01	0
13	0.252	-0.01	0
14	0.234	0	0
15	0.25	0	0
16	0.255	-0.02	0
17	0.24	0	0
18	0.21	-0.02	0
19	0.241	-0.01	0
20	0.27	-0.01	0
21	0.276	-0.03	0
22	0.283	0	0
23	0.243	-0.02	0
24	0.241	-0.02	0
25	0.206	-0.06	0
26	0.234	-0.04	0
27	0.236	-0.01	0
28	0.186	-0.02	0
29	0.243	-0.02	0
30	0.252	0.02	7.936

Table no. 9: % of clot lysis when compared with positive control.

Group	Clot lysis %	% change
Positive control	22.32±1.468	-
Negative control	3.73±0.58	83.28↓
Test drug (aq1)	20.12±2.091	9.856↓
Test drug (aq2)	28.23±1.949	26.47↑
Test drug (al1)	4.425±1.158	80.17↓
Test drug (al2)	1.09±0.649	95.11↓

P VALUE < 0.0001 can be considered extremely significant.

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

P value: Mean ± SEM; **: P < 0.01

Table no. 10: % of clot lysis when compared with negative control.

Group	Clot lysis %	% change
Positive control	22.32±1.468	498↑
Negative control	3.73±0.58	-
Test drug (aq1)	20.12±2.091	486↑
Test drug (aq2)	28.23±1.949	656↑
Test drug (al1)	4.425±1.158	18.6↑
Test drug (al2)	1.09±0.649	70↓

P VALUE < 0.0001 can be considered extremely significant.

B	vs	A	**	P	<	0.01
B	vs	C	**	P	<	0.01
B	vs	D	**	P	<	0.01
B	vs	E	ns	P	<	0.05

B vs F ns P < 0.05

% Change for positive control

B= B-A/A = 3.73 - 22.32/ 22.32 = -18.59/22.32 = -0.83* 100 = - 83.28

C= C-A/A = 20.12- 22.32/ 22.32 = -2.2 /22.32 = - 0.098 * 100 = - 9.856

D= D-A/A = 28.23- 22.32/ 22.32 = 5.91/ 22.32 = 0.264 * 100 = 26.47

E= E-A/A = 4.425- 22.32/ 22.32 = - 17.895/22.32 = - 0.801 * 100 = - 80.17

F= F-A/A = 1.09 - 22.32/ 22.32 = - 21.23/22.32 = - 0.95 * 100 = - 95.11

% Change for negative control

A= A-B/B = 22.32 – 3.73/3.73= 18.59/3.73 = 4.98 * 100 = 498

C= C-B/B = 20.12- 3.73/3.73 = 16.39/3.73 = 4.86 * 100 = 486

D= D-B/B = 28.23- 3.73/3.73= 24.5/3.73 = 6.568 * 100 = 656

E= E-B/B = 4.425- 3.73/3.73= 0.695/3.73 = 0.186 * 100 = 18.6

F= F-B/B = 1.09 - 3.73/3.73= -2.64/3.73 = 0.70 * 100 = - 70

DISCUSSION

Seeds of *Moringa oliefera* are selected for evaluation of its thrombolytic activity. The unwholesome food and regimen cause *doshadusti* leading *sroto dusti* and hence *sroto avarodha.* Sharngdhara and Bhavaprakasha has explained that *Pramati* drugs have ability to clear such *sroto dusti vaishyamyas* especially *avarodha*. Adamalla comments *srotas* as the channels of *Karna*, *Mukha*, *Nasa*, and other channels where there is accumulation of dosha. Kasirama comments that *srotas* here refers to *Rasavahasira* and *Pramati Dravya* removes the accumulated *Dosas* in *Rasadisrotas*. Thrombosis is also an obstruction in the normal circulatory pathway. Drugs possessing these properties dissolve the thrombus and clear the pathway. *Shigru* is having *Pramathi guna (sroto vishodhana)* according to Manasi Deshpande[7] and also found to be beneficial in *Shonita sanghatam binnati* (thrombolytic activity) due to *katu rasa.*[8] Seeds of *shigru* is possessing *Katu rasa, Laghu, teekshna guna, Ushna veerya* and *Katu vipaka*, because of which it acts on the *avarodha* or *sangha* of *srotas* and removes the obstruction. The *Moringa oleifera* contains many potent phytoconstituents such as Nillin, Moringine, Moringinine, Bayrenol, Cartotene, Flavonoids, Polysaccharide, Protein Components, Essential Amino Acids, Minerals, Vitamins etc. which possess wide range of biological activities.

There is a complex physiology system associated with maintaining haemostasis of circulation. During blood vessel injury or certain pathological conditions there is a formation of haemostatic plug of platelets at the site of injury and which reinforces the fibrin deposition to form thrombus. Once the repair process is over, the fibrinolytic system is activated to remove fibrin. However, in certain disorders such as acute myocardial infarction, deep vein thrombosis, pulmonary embolism and stroke there is a great requirement of fibrinolytic medication to overcome the clinical conditions. There are many clinically used drugs such as Streptokinase, Alteplase and Reteplase which are potent but associated with more side effects and costlier. Thus, in the current scenario there is a search for potent, cost effective and no adverse effect drugs. Hence, the new drug discovery has been focused more towards the natural source especially plant products. The present study was undertaken to screen the in vitro thrombolytic activity of aqueous and alcoholic extract of *Shigru* seeds.

Table no. 11: Consolidated form when compared with positive control.

Groups	Clot lysis by Streptokinase	Clot lysis by Distilled water	Clot lysis by AQ1 (250 mg Aqueous extract of *Shigru* seeds)	Clot lysis by AQ2 (500 mg Aqueous extract of *Shigru* seeds)	Clot lysis by AL1 (250 mg Alcohol extract of *Shigru* seeds)	Clot lysis by AL2 (500 mg Alcohol extract of *Shigru* seeds)
	A	B	C	D	E	F
MEAN ± SEM	22.32 ± 1.468	3.73 ± 0.58	20.12 ± 2.091	28.23 ± 1.949	4.425 ± 1.158	1.09 ± 0.649

Streptokinase is the standard drug used for thrombolytic activity, shows 22.32 mean clot lysis percentage. The mean clot lysis percentage analysis of Test drug in Aqueous extract in two different concentrations 250 mg (AQ1) and 500 mg (AQ2) is 20.12 and 28.23 respectively. The statistical analysis showed there is no significance difference between percentage of clot lysis of streptokinase and Aqueous extract of *Shigru* seeds in two different concentrations (250 mg & 500 mg). This indicates that the percentage of clot lysis of Aqueous extract in 250 mg (AQ1) and 500 mg (AQ2) concentrations having almost same activity of streptokinase of 3500I.U. While the percentage of clot lysis of AQ2 showed increased percentage of clot lysis (28.23) when compared to the percentage of clot lysis by streptokinase (22.32). Alcohol extract of *Shigru* seeds in both concentrations 250 mg (AL1) and 500 mg (AL2) showed no thrombolytic activity when compared to streptokinase of 3500I.U.

Table no. 12: Consolidated form when compared with negative control.

Groups	Clot lysis by Streptokinase	Clot lysis by Distilled water	Clot lysis by AQ1 (250 mg Aqueous extract of *Shigru* seeds)	Clot lysis by AQ2 (500 mg Aqueous extract of *Shigru seeds*)	Clot lysis by AL1 (250 mg Alcohol extract of *Shigru* seeds)	Clot lysis by AL2 (500 mg Alcohol extract of *Shigru* seeds)
	A	B	C	D	E	F
MEAN ± SEM	22.32 ± 1.468	3.73 ± 0.58	20.12 ± 2.091	28.23 ± 1.949	4.425 ± 1.158	1.09 ± 0.649

The results showed that Streptokinase has remarkable clot lysis ($P < 0.01$) when compared with negative control. Alcohol extract of *Shigru* seeds both 250 mg (AL1) and 500 mg concentration (AL2) showed similar clot lysis activity as compared to negative control. Remarkable level of clot lysis was shown by Aqueous extract of *Shigru* seeds (AQ1, AQ2) &

was found highly significant with negative control. Hence, test drug AQ1 and AQ2 can be at par to Streptokinase.

Thus, the present study provides a preliminary insight that the aqueous extract of *Shigru* seeds having almost same thrombolytic activity of streptokinase.

Further research is required to support the present data.

CONCLUSION

Experiment was conducted to evaluate the clot lysis of aqueous and alcoholic extracts of *Shigru* seeds. Thrombolytic activity study conducted on 250 mg and 500 mg concentration of both aqueous and alcohol extract of *Shigru* seeds taking Streptokinase as positive control and distilled water as negative control. Study revealed that aqueous extract of *Shigru* seeds both in 250 mg (AQ1) and 500 mg (AQ2) concentration have clot lysis activity which is equal to Streptokinase of 3500 IU. Thrombolytic activity of aqueous extract of test drug in high concentration (AQ2) showed increased mean clot lysis percentage when compared to that of standard drug Streptokinase. This concord the interpretations of author Manasi Deshpande about *Shigru* seeds possessing *pramati guna* that does *Srotho vishodhana.*[7] *Rasa- Guna* of *Shigru* seeds ie., *katu, tikta rasa, laghu, usna veerya* and *kaphavaatanashaka* also holds this opinion.

Limitations

- It is not possible to explain effect of drug in living body**.**

Scope for further study

- Experimental study was conducted on a small sample size, so larger sample size can be taken.
- Further phytochemical analysis of Aqueous extract of *Shigru* seeds can be done to know which specific phytoconstituent is responsible for thrombolytic activity.
- This study being in vitro is very much restricted; so clinical trials can be taken for the same and can be used for further experimentation proving the efficacy of drug.

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