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Pharmacological Study

In vitro thrombolytic activity of *Dhamasa* (*Fagonia arabica* Linn.), *Kushta* (*Saussurea lappa* Decne.), and *Guduchi* (*Tinospora cordifolia* Thunb.)

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Abstract

Introduction: Thrombotic disorders are among the major fatal conditions affecting the society. Treatment modalities used for such disorders are either surgical interventions or use of drugs such as urokinase, streptokinase (SK), or tissue plasminogen activators to dissolve the blood clots. These modalities have their own limitations and side effects apart from being expensive. There is a need for safer and cost effective antithrombolytic agents. **Aim:** To evaluate *in vitro* thrombolytic property of *Dhamasa (Fagonia arabica* Linn.), *Kushta* (*Saussurea lappa* Decne.), and *Guduchi* (*Tinospora cordifolia* Thunb.) plant extract. **Materials and Methods:** Venous blood drawn from 20 healthy volunteers was allowed to form clots which was weighed and treated with the extract of test plant materials to disrupt the clots. Weight of clot after and before treatment provided a percentage of clot lysis. SK was used as a positive and water as a negative control. **Statistical Analysis Used:** The significance between % clot lysis of five groups by means of weight difference was tested by the one-way ANOVA. **Results:** Clot lysis observed were 68.06%, 14.85%, 25.01%, 92.54%, and 3.00% for *Dhamasa*, *Kushta*, *Guduchi*, SK, and distilled water, respectively. **Conclusion:** Herbal extracts possess thrombolytic properties and lyse blood clots *in vitro*.

Key words: *Dhamasa*, *Fagonia arabica*, *Guduchi*, *Kushta*, *Saussurea lappa*, streptokinase, thrombolytic activity, *Tinospora cordifolia*

Introduction

Thrombosis is defined as "hemostasis in the wrong place," and is a major cause of morbidity and mortality. Arterial thrombosis is a common cause of myocardial infarction, ischemic stroke, and limb gangrene whereas venous thrombosis leads to deep vein thrombosis which can be complicated by the post thrombotic syndrome, and pulmonary embolism, chronic thrombo-embolism, pulmonary hypertension.[1] Number of other condition that can arise according to the location of the thrombus and the organs affected. The major risk factors for such thrombotic disorders are acquired disorders of hyper-coagulation, others being the exogenous factors such as surgery, hospitalization, immobility, trauma, pregnancy and the puerperium, and endogenous factors such as cancer, obesity.[2]

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Current treatment modalities of thrombotic disorders include surgical interventions or use of drugs such as alteplase, anistreplase, streptokinase (SK), urokinase, and tissue plasminogen activators.[3] These modalities are costly as well as have serious side-effects which may be life threatening such as intracranial haemorrhage,[4] spontaneous pulmonary haemorrhage,[5] and angioedema.[6] Moreover, these drugs are not used in patients who have undergone surgery or those with a history of nervous lesions, gastrointestinal bleeding, or hypertension.[7] There is a need for safer and cost effective anti-thrombolytic agents. In order to find the blood thinning agents this work was conceived as it is known that herbal

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products are often perceived as safe because they are "natural."[8] This study aims to investigate the multiple solvent extracts of the three medicinal plants viz., *Dhamasa* (*Fagonia arabica* Linn.), *Kushta* (*Saussurea lappa* Decne.), and *Guduchi* (*Tinospora cordifolia* Thunb.) for their clot lysis (thrombolytic activity) by using thrombolytic *in vitro* model.

Materials and Methods

The whole dry plant of *Dhamasa*, dry bark of *Kushta*, and dry stem of *Guduchi* were collected from the local market and were identified and authenticated by experts of the Department of Dravyaguna, National Institute of Ayurveda, Jaipur. The plants were cleaned, powdered and dried up in the hot air oven to remove the moisture content. The multiple solvent (methanol: Isopropyl alcohol: Acetone 100 ml each) extraction procedure was used to prepare extract by using Soxhlet apparatus. The extract was dried in the hot air oven and weighed to find out the percentage of extract formed from the dried powder.

About 100 mg of the extract thus formed was suspended in 10 ml distilled water (DW) and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant. Supernatant was filtered through a 0.22 µ syringe filter and was used to check clot lysis. An *in vitro* thrombolytic model was used to check the clot lysing effect of aforesaid three plants. SK was used as a positive control and DW as a negative control.

Clot lysis

Experiment for clot lysis was carried as reported earlier.[9] In brief, 2.5 ml venous blood drawn from healthy volunteers was distributed in five different pre-weighed sterile micro-centrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube − weight of tube alone).

As a standard control, 100 µl of SK and as a non-thrombolytic control, 100 µl of DW along with 100 µl of each samples were separately added to the micro-centrifuge tubes. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated 20 times with the blood samples of 20 volunteers.

Statistical analysis

Data are expressed as mean ± standard error of the mean. The significance between % clot lysis by SK, DW, test plants by means of weight difference was tested by the one-way ANOVA by using Instat GraphPad version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Addition of 100 µl SK, the standard control to the clots along with 90 min of incubation at 37°C, showed 92.54% ±1.25% clot lysis. Clots when treated with 100 µl sterile DW (negative control) showed only negligible clot lysis (3.00% ±0.597%). The mean difference in clot lysis percentage between positive and negative control was very significant ($P < 0.001$). After treatment of clots with 100 µl of *Dhamasa* (*F. arabica*), *Guduchi* (*T. cordifolia*), *Kushta* (*S. lappa*) clot lysis, i.e. 68.06% ±3.53%, 25.01% ±2.11%, 14.85% ±1.37%, respectively, was obtained. When compared with the negative control (water) the mean clot lysis % difference of *Dhamasa* and *Guduchi* was significant ($P < 0.001$ in both) but mean clot lysis % difference of *Kushta* was not found to be significant ($P < 0.01$). When compared with the positive control (SK) the mean clot lysis % difference of all the three test plants was significant ($P < 0.001$ in all). Statistical representation of the effective clot lysis percentage by test plants, positive thrombolytic control (SK), and negative control (sterile DW) is tabulated in Table 1. Between group comparisons of percentage clot lysis is tabulated in Table 2.

Discussion

In earlier study, thrombolytic effect of *Dhamasa* has been reported so it was selected to confirm the reported findings.[10] *Guduchi* has been mentioned as best in "*Shonitavibandhprashmana*" (one that removes obstruction in blood) in *Agrya Prakarana* by *Acharya Charaka*.[11] *Kushta* has been mentioned by *Sushruta* during description of *Rakta mokshana* (blood-letting). *Sushruta* says that in the process of *Rakta mokshana* if the bleeding does not occur due to clot formation then area should be rubbed by the powdered form of some drugs. *Kushta* is one among the drug mentioned to facilitate bloodletting.[12] Textual references suggest that *Guduchi* and *Kushta* have thrombolytic property and so they were screened for their thrombolytic activity in the present study.

Herbal preparations are used since ancient times to maintain health and to prevent and treat various ailments. Advancement in field of phytochemistry have paved path for identification

Table 1: Thrombolytic activity of test drugs

Drug name	Mean±SEM			
	Weight of clot (g)	Weight of clot after lysis (g)	Clot different (g)	Percentage of clot lysis
Dhamasa	0.29495	0.095505	0.19945±0.006995	68.06±2.110
Guduchi	0.28	0.21	0.07±0.01236	25.01±3.532
Kushta	0.301815	0.25568	0.04614±0.004876	14.85±1.377
Streptokinase	0.2512325	0.019695	0.23154±0.007454	92.54±1.257
Distilled water	0.268435	0.26051	0.00793±0.001571	3.00±0.5973

Degrees of freedom (between columns)=4; Degrees of freedom (within columns)=95, F=170.97. SEM: Standard error of mean

Table 2: Between group comparisons of percentage clot lysis

Comparison between the five groups in terms of mean difference of percentage of clot lysis (*n*=20)			
Comparison	**Difference of percentage of clot lysis**	***Q***	***P***
Guduchi versus *Dhamasa*	43.051	20.553	<0.001
Guduchi versus *Kushta*	10.153	4.847	<0.01
Guduchi versus SK	67.534	32.241	<0.001
Guduchi versus DW	22.011	10.508	<0.001
Dhamasa versus *Kushta*	53.205	25.400	<0.001
Dhamasa versus SK	24.483	11.688	<0.001
Dhamasa versus DW	65.062	31.061	<0.001
Kushta versus SK	77.688	37.089	<0.001
Kushta versus DW	11.857	5.661	<0.01
SK versus DW	89.545	42.750	<0.001

SK: Streptokinase, DW: Distilled water, Degrees of freedom (between columns)=4, Degrees of freedom (within columns)=95, *F*=347.52

and isolation of plant compounds for curing diseases. Presently, about 30% of the pharmaceuticals are prepared from plants worldwide.[13] Researches are going on extensively to find new alternative herbal drugs in various areas. Treatment of hyper coagulable state still remain a great challenge and to combat vascular diseases number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having antithrombotic effect.[7,9,13] SK is a widely used thrombolytic drug by the modern science but it can cause serious and life threatening side effects.[14] SK has its own complications like, bleeding which may be fatal with intracranial haemorrhage, it is also ineffective in individuals with anti-streptococcal and anti-prothrombin antibodies, and in patients who had multiple SK injections.[15,16] All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency.

Coagulation factor or natural anticoagulant factor levels influence the risk of venous thrombosis along with other risk factors such as ageing, obesity, protein C deficiency, etc. Oxidative stress, which is defined as an imbalance between pro-oxidant and antioxidant systems, can be both a cause and consequence of many vascular complications and serve as one of the biomarkers for these conditions.[17] *F. arabica* has been found to be beneficial in reducing oxidative stress by virtue of its antioxidant potential.[18] Different parts of *F. arabica* have also been used to cure various ailments, namely hematological, neurological, endocrinological, and inflammatory disorders.[19] *T. cordifolia*[20] and *Saussurea lappa* are also found to have good anti-oxidant activity.[21] These plants have properties which can help in preventing vascular diseases. The results of this study shows mild to moderate thrombolytic activity of test plants and so it gives an opportunity to explore their use in field of hypercoagulable state. These plants have *Tikta/Katu* (bitter/pungent) predominant *Rasa* (taste) and *Ushna Virya* (hot potency). *Katu Rasa* has property of "*Shonitasanghata Bhinnati*" (dissociates blood clots).[22] *Tikta Rasa* has *Lekhana* and *Kleda*, *Meda*, *Shleshma Upshoshana* properties which may help in lysis of formed thrombus in blood vessels.[23] It may be hypothesized that thrombolytic activity of these drugs might be due to above mentioned properties. In earlier studies also herbal medicines such as *Hemidesmus indicus* (L.) R.Br.,[24] *Allium sativum* L.,[25] *Zingiber officinale* Roscoe.,[26] *Ocimum sanctum* L., *Curcuma longa* L., and *Azadirachta indica* A. Juss[27] have been shown to exert thrombolytic or fibrinolytic effects. Positive results give hope to develop drugs in future but there is need of extensive research to find out active constituents so that development of alternative novel thrombolytic drugs can be done.

Conclusion

Dhamasa showed a significant percentage of clot lysis which is comparable with SK, a well-known thrombolytic drug. *Guduchi* and *Kushta* also showed mild thrombolytic activity. This study indicates possibility of finding novel thrombolytic drugs. However, there is need of thorough phytochemical and pharmacological research to discover their therapeutic potential. Once proved on scientific grounds these herbal preparations may be incorporated as thrombolytic agent for the improvement of the patients suffering from atherothrombotic diseases.

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Conflicts of interest
There are no conflicts of interest.

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हिन्दी सारांश

धमासा, कुष्ठ और गुडूची के कृत्रिम परिवेशीय में रक्त स्कंदन की क्रियाविधि

श्वेता चौधरी, पवनकुमार गोदडवार, रीतु शर्मा

रक्त स्कंदन जन्य विकार समाज में व्याप्त अत्यंत घातक विकारों में से एक है। इन विकारों की चिकित्सा या तो शल्य कर्म द्वारा अथवा यूरोकाइनेज, स्ट्रेप्टोकाईनेज या टिश्यू प्लाज्मिनोजेन एक्टिवेटर्स के माध्यम से किया जाता है। मंहगी होने के अलावा इन चिकित्सा विधियों की अपनी परिसीमाएं तथा साइड इफेक्ट हैं। अतएव अपेक्षाकृत निरापद एवं सस्ते रक्त स्कंदन प्रतिबंधक औषधियों की आवश्यकता है। कृत्रिम परिवेशीय परिस्थिति में धमासा (फेगोनिया एराबिग), कुष्ठ (सासुरिया लेप्पा) तथा गुडूची (टिनोस्पोरा कोर्डिफोलिया) निर्यास से स्कंदित रक्त की विलयन शक्ति का आंकलन करना इस अध्ययन का उद्देश्य है। २० स्वस्थ व्यक्तियों की सिरा से रक्त को एकत्रित कर स्कंदित किया गया। परिक्षणीय औषधियों के निर्यास द्वारा स्कंदित रक्त का विलयन करने का प्रयास किया गया। परीक्षण के पूर्व एवं पश्चात् स्कंदित रक्त का भार लिया गया एवं विलयन का प्रतिशत निकाला गया। स्ट्रेप्टोकाइनेज का सकारात्मक एवं विशुद्ध जल का नकारात्मक नियामक के रूप में प्रयोग किया गया। स्कंदित रक्त विलयन प्रतिशत के सांख्यकीय अध्ययन हेतु पांचों औषध समूह के भार के अंतर का परीक्षण वन–वे एनोवा द्वारा किया गया। स्कंदित रक्त विलयन प्रतिशत ६८.०६%, ९४.८५%, २५.०१%, ९२.५४% एवं ३.००% क्रमशः धमासा, कुष्ठ, गुडूची, स्ट्रेप्टोकाइनेज एवं विशुद्ध जल में प्राप्त किया गया। कृत्रिम परिवेशीय परिस्थितियों में परिक्षणीय औषधियों में स्कंदित रक्त की विलयन शक्ति पाई गई।