ejpmr, 2021,8(9), 438-442

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THE ANTICOAGULANT ACTIVITY OF *VIBHITAKA PHALA TWAK* (*TERMINALIA BELLIRICA* (GAERTN.) ROXB.) – INVITRO STUDY

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Article Received on 26/06/2021 **Article Revised on 16/07/2021** **Article Accepted on 06/08/2021**

ABSTRACT

The *Kashaya kalpana* is one of the liquid dosage forms which can provide maximum therapeutic efficacy prepared by using drug and water in specific proportion and reducing it to particular quantity. The concept of *Saptavidha kashaya* explained by *Acharya Harita* is a reductive dosage form. The *Saptavidha Kashayas* are *Pachana kashaya, Deepana kashaya, Shodhana kashaya, Shamana kashaya, Tarpana kashaya, Kledana kashaya*, and *Vishoshi kashaya.* The *vishoshi kashaya* which has highest reduction that is *Shodhashamsha* (1/16th) and has *shoshana karma* and this concept is considered in study to evaluate the anticoagulant activity. The drug, *Vibhitaka phala twak*, selected for the present study based on its *kashaya rasa, laghu* and *ruskha guna* is assessed to have *shoshana karma* in turn *lekhana karma*. The *vishoshi kashaya* of *vibhitaka phala twak* was subjected to analytical evaluation and an in-vitro study for screening Anti-coalgulant activity and was carried out by Lee- white method and Haem-hydrolysis method. Invitro study carried out, revealed the Anti-coalgulant activity. Presence of Ellagic acid, Triterpenoids, and Aglycone Saponins are responsible for the anti-coagulant activity of *Vishoshi kashaya* of *Vibhitaka phala twak.*

KEYWORDS: *Ayurveda, Vibhitaka phala twak, Vishoshi Kashaya, Shoshana* and *Lekhana karma*, Anticoagulant activity.

INTRODUCTION

Aushadha/ Dravya is one among *Chatuspada*.[1] In *bahukalpam*, different dosage forms can be prepared of *aushadhi*. *Acharya Harita*, has given more importance to various dosage form. Single drug can show many actions in different dosage form and the *bahukalpam*, that is drug can be used in different dosage form plays a main role in the treatment. *Kashaya*, one of the liquid dosage forms, is easily absorbed in the gut. *Acharya Harita* has mentioned *Saptavidha Kashaya* those are *Pachana, Deepana, Shodhana, Shamana, Tarpana, Kledana*, and *Vishoshi kashaya*.[2] In these *kashayas'* where, pharmacological activities changes depending on boiling and reduction which is unique concept adopted and practically utilized.

Vishoshi kashaya is prepared by reducing to one-sixtheenth. It exhibits the action of *shoshana* in turn *lekhana*, Anticoagulant activity, anti-hyperlipidaemic activity and also in thyroid disorders. Anticoagulants reduces the coagulability of blood. This can inhibit the deposition of fibrin, keep the thrombi loosely anchored and can inhibit the formation of the thrombus which consists of fibrin clots. Anti-coagulants can reduce the size of the thrombus.[3] *Vibhitaka phala twak*, which is *Kashaya rasa, laghu* and *ruksha guna*[4] can be attributed for anti-coagulant activity with the *lekhana karma*. By considering all these the present research topic entitled "The Anticoagulant Activity of *Vibhitaka Phala Twak* (*Terminalia bellirica* (Gaertn.) Roxb.) – Invitro study" was carried out by Lee -white and Haem hydrolysis method.

METHODOLOGY

- The *Vishoshi Kashaya* was prepared from 1part (25gms) of *Vibhitaka phala twak churna* and 16 parts (400ml) of water and reduced to 1/16th (25ml).[5]
- The sensory evaluation and phytochemical evaluation of the *Vishoshi kashaya* was carried out.
- Determination of pH and specific gravity was carried out.[6]
- The invitro study for Anti- coagulant activity of *Vishoshi Kashaya* of *Vibhitaka Phala twak* was carried out by following two methods.
- Lee - white method[7]
- Haem hydrolysis method[8]

1. Lee - white method

Principle and Rationale

Anticoagulants achieve their effect by suppressing the synthesis or functions of various clotting factors that are normally present in the blood. The venous blood was collected in the tubes and kept on water bath at 37^0c and time was noted from the time of vein puncture till the blood clots.

Requirements: Eppendorf tubes – 2**,** Blood sample**,** Syringes**,** Micropipette**,** Pipette tips**,** Stopwatch**,** Water bath.

Procedure

- Ten healthy volunteers were selected for getting the sample of blood.
- Two Eppendrof tubes were taken with the internal diameter 8mm and placed in 37^0C water bath.
- A clean dry syringe was used to draw the venous blood.
- Stop watch was used to note the time immediately after drawing the blood.
- 1ml of venous blood was put into Eppendorf tube.
- The test tube was observed at 30 seconds of interval to note the clotting time by gentle tilting the tube.
- Time was noted when no blood flows even after the inverting the tube. This was noted as the clotting time.
- Then to other tube 100microliteres of *kashaya* and 1ml of blood was added.
- The tube was observed at 30 seconds of intervals for noting the clotting by gently tilting the tube.
- The same procedure was repeated with the blood of ten hyperlipidaemic volunteers.
- Procedure was done by triplicate method, standardized and SOP was established.

2. Haem Hydrolysis Method

Principle and Rationale

The clotting factors are made up of proteins and the anti-coagulant activity is achieved by the proteolysis of these clotting factors. Agar is dissolved in water and autoclaved. Haem is also dissolved in distilled water and pasteurized. Once cooled both the media are mixed in aseptic condition inside Laminar Air Flow chamber. Immediately after mixing, the media will be poured on a sterile petri plates and allowed to solidify. After the media solidifies, two wells are made using gel borer and samples are loaded in respective plates wells and incubated at 37°C for 17-24 hours. After 24hrs the plates were observed for clear transparent zone which indicates proteolytic activity.

Requirements: 2% Agar**,** 1% Haem**,** Petri plates**,** Gel borer.

Procedure

- 2% Agar (1g) dissolved in 30ml water and autoclaved at 121°C for 15 mins
- 1% Haem (0.5g) dissolved in 20mL distilled water and pasteurized.
- Once cooled both the media were mixed in aseptic condition inside Laminar Air Flow chamber.
- Immediately after mixing, the media was poured on a sterile petri plates and allowed to solidify.
- After the media solidifies, two wells measuring 0.5cm was made using gel borer and 100µL of the *Vishoshi kashaya* and supernatant of *Vishoshi kashaya* samples were loaded in respective petri plates wells and incubated at 37°C for 17-24 hours.
- After 24hrs the plates were checked for clear transparent zone.

RESULTS

Results of *Vibhitaka phala twak vishoshi kashaya* for anticoagulant activity carried out by Lee - White and Haem hydrolysis method revealed as following,

- Time taken for preparation of *Vishoshi Kashaya* was 4 hours 20mins.

Figure. 1 *vishoshi kashaya*

- Sensory evaluation of *vishoshi kashaya*

Table no. 1: Showing the Sensory evaluation of *vishoshi kashaya.*

Sample	Colour	Odour	Taste	Form
Vishoshi Kashaya	Dark brown	Strong	Astringent	Liquid

- Phyto-chemical screening of *vishoshi kashaya* reveals presences of Secondary metabolites like Triterpenoids, aglycone Saponins, and Phenolic Compounds.
- pH value- 4.5
- Specific gravity value- 1.12
- **Invitro Study-** Anticoagulant Activity of *Vishoshi Kashaya* of *Vibhitaki Phala twak*

1. Screening of *Vishoshi kashaya* for anticoagulant activity by Lee and white method

Table No 2: Showing Anticoagulant Activity evaluated on the blood of healthy volunteers.

Sample No.	Clotting time	Observations at intervals of				
		Blood samples treated with *Vibhitaka phala twak Vishoshi Kashaya*				
		15mins	30mins	1hr	2hrs	24hrs
1.	4mins 10 sec	No clotting	No clotting	No clotting	No clotting	No clotting
2.	4mins 12sec	No clotting	No clotting	No clotting	No clotting	No clotting
3.	4mins 15 sec	No clotting	No clotting	No clotting	No clotting	No clotting
4.	4mins 24sec	No clotting	No clotting	No clotting	No clotting	No clotting
5.	4mins 27sec	No clotting	No clotting	No clotting	No clotting	No clotting
6.	4mins 36sec	No clotting	No clotting	No clotting	No clotting	No clotting
7.	4 mins 40 sec	No clotting	No clotting	No clotting	No clotting	No clotting
8.	5 mins	No clotting	No clotting	No clotting	No clotting	No clotting
9.	5mins 11sec	No clotting	No clotting	No clotting	No clotting	No clotting
10.	5 mins 20 sec	No clotting	No clotting	No clotting	No clotting	No clotting

Table No 3. Showing Anticoagulant Activity evaluated on the blood of Hyperlipidaemic volunteers.

Sample No.	Clotting time	Observations at intervals of				
		Blood samples treated with *Vibhitaka phala twak Vishoshi Kashaya*				
		15mins	30mins	1hr	2hrs	24hrs
1.	2mins 30 sec	No clotting	No clotting	No clotting	No clotting	No clotting
2.	2mins 35sec	No clotting	No clotting	No clotting	No clotting	No clotting
3.	2mins 42sec	No clotting	No clotting	No clotting	No clotting	No clotting
4.	2mins 50sec	No clotting	No clotting	No clotting	No clotting	No clotting
5.	3mins	No clotting	No clotting	No clotting	No clotting	No clotting
6.	3 mins 10 sec	No clotting	No clotting	No clotting	No clotting	No clotting
7.	3mins 25sec	No clotting	No clotting	No clotting	No clotting	No clotting
8.	3 mins 30sec	No clotting	No clotting	No clotting	No clotting	No clotting
9.	3mins 43sec	No clotting	No clotting	No clotting	No clotting	No clotting
10.	3 mins 50 sec	No clotting	No clotting	No clotting	No clotting	No clotting

Figure 2a: With *Vishoshi Kashaya* B. Without *Vishoshi Kashaya*

2. Screening of *Vishoshi kashaya* for anticoagulant activity by Haem Hydrolysis method.

- After 24hrs the petri plates were checked for clear transparent zone in which Haem was hydrolysed and clear zone was observed.
- This shows that, both the *Vishoshi kashaya* and supernatant of *Vishoshi kashaya* of *Vibhitaka phala twak*, has anti-coagulant activity.

Figure. 3: Haem Hydrolysis.

DISCUSSION

Vibhitaka phala, one of the ingredients in *Triphala*, easily and abundantly available drug. *Vibhitaka phala* is also used in many formulations. The *Kashaya rasa*, *Laghu* and *ruksha guna* contributes in the *shoshana karma* of *Vishoshi kashaya*. *Vishoshi kashaya* of *Vibhitaka phala twak* was prepared by reducing to 1/16th at 25^{0}C. The time taken for the preparation of *kashaya* was 4hours 20mins because water soluble principle is concentrated to give the desirable pharmacological activity. The *kashaya* was dark brown in colour and strong odour which suggest that it was concentrated. The pH of *Vishoshi kashaya* was 4.5 suggests the presences of more phenols which are basically hydroxyl (OH) compounds.

Lee-white method and Haem hydrolysis method was followed because it is an ideal and suitable to screen the anti-coagulant activity. In Lee-white method, healthy volunteers with clotting time from 4mins 10 sec to 5 mins 20 sec and Hyperlipidemia volunteers with clotting time from 2mins 30 sec to 3 mins 50 sec, samples of blood revealed that *vishoshi kashaya* has anti-coagulant effect by not clotting the blood even after 24hrs. In Hyperlipidemia volunteers' lesser the time of clot time indicates the viscosity of blood is increased. The coumarin derivatives resembles Vitamin K (which is an element for synthesis of number of clotting factors), gives rise to clotting factors that are incapable of binding Calcium ions which is an important element in the activation of coagulation factors. Therefore, it acts as anti-coagulant. In Haem-hydrolysis method, the *Vishoshi kashaya* as it showed the clear zone, which revealed proteolytic nature thereby confirming anti-coagulant activity.

The coagulation of blood can be considered as different aspects of *Shonita dusti* like *Shonita sanghata* and *Shonita kleda*.[9,10] The *kaphahara* property plays pivotal role. *Kashaya rasa* has *lekhana karma* which is *medohara*.[11] *Laghu* and *ruksha guna* is *lekhana* and *kaphaghna*.[12,13,14] As **Ellagic acid** (coumarin) is proved to have Anti-coagulant activity[15], **Triterpenoid**s have Anti-hyperlipidaemic activity and Anti-coagulant[16], **aglycone saponins** have Haemolytic activity.[17] By considering the above-mentioned *rasa*, *guna*, *karma* and phytoconstituents and its activity, *vishoshi kashaya* of *Vibhitaka phala twak* exihibited the anti-coagulant activity.

CONCLUSION

There is a need of anti-coagulant in coronary artery diseases (CAD), Myocardial infarction (MI), Ischemic heart diseases (IHD) etc., as a preventive and prophylactic measures. Anti-coagulant study carried out for *Vibhitaka phala twak* has given significant pharmacological activity by Lee- white and Haem hydrolysis method which can be clinically efficacious further.

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