



## RESEARCH ARTICLE

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# A factor of anti-atherosclerotic activity-fibrinolytic activity of venom of *Buthus tumulus* species (Indian red scorpion)

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### ARTICLE INFO

### ABSTRACT



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**Introduction:** Atherosclerosis (AS) is an inflammatory disease of the arterial blood vessels (arteries), in which the walls of the blood vessels become thickened and hardened specifically due to atheromatous plaques. The plaques are composed of cholesterol and other lipids, inflammatory cells, and calcium deposits. There are five different types of atherosclerosis such as in the aorta, coronary, cerebral, renal, and pulmonary arteries. The scorpion species that present medical importance belonging to the family Buthidae Scorpion toxins consist of several peptides isolated from the venom of different species of scorpion and are based on their pharmacological action and also agree well with the structural properties of this peptide family.

**Method:** The study was conducted in the Department of Pharmacology, Modern College of Pharmacy, Nigadi, Pune in the year June 2012- June-2013 by In-vitro blood clot dissolving activity of SV and artificial blood clot degradation method

**Results:** It was observed that SV was evaluated for its fibrinolytic properties in the In-vitro blood clot dissolving method and shown to decrease the stained area of the blood-stained cloth as compared to the control.

**Conclusion:** In the artificial blood clot degradation method, the color intensity is higher in the test tube containing SV compared to the control and hence, it can be stated that SV has fibrinolytic activity.

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### Introduction

High fibrinogen levels in patients with the atherosclerotic disease have generated a large volume of clinical pieces of evidence in the past few decades and have been considered a risk marker for cardiovascular events. High fibrinogen levels are also known to promote atherosclerotic disease by increasing blood viscosity,

stimulating fibrin formation, and increased platelet aggregation. In the setting of an inflammatory status such as that seen in individuals with excess weight, the higher hepatic production of fibrinogen regulated by inflammatory cytokines may be an important link in the progression of atherosclerotic disease in its different subclinical and clinical stages (Figure 1).



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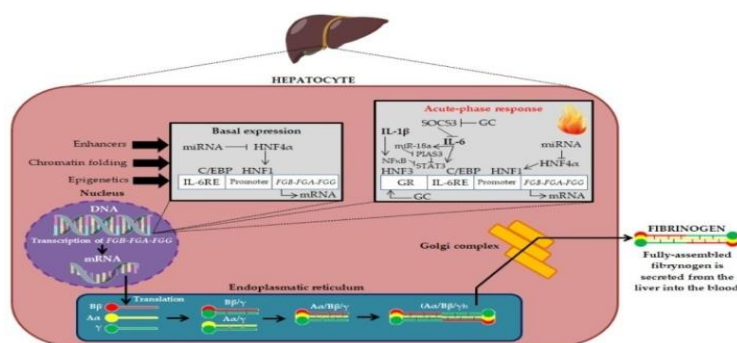


Figure 1: Hepatic production of fibrinogen regulated by inflammatory cytokines

However, several investigators have suggested that fibrinogen may play a more active role in the development and progression of atherosclerotic plaque. The simultaneous presence of fibrinogen, its degradation products, and low-density lipoproteins cholesterol (LDL-C) has been observed to influence atherogenesis in the arterial wall. Furthermore, smooth muscle cell proliferation and migration stimulated by fibrinogen and fibrin degradation products suggest that fibrinogen is involved in the earliest stages of plaque formation.

Fibrinogen may play an active role in the development and progression of atherosclerotic plaques. The association between fibrinogen levels and atherosclerotic plaques over three different arterial sites in an asymptomatic never-treated male population with increased cardiovascular risk.

The photocopy of the scorpion is presented in Figure 2.



Figure 2: Image of Buthus tumulus

#### Classification

|              |                                                                                                     |
|--------------|-----------------------------------------------------------------------------------------------------|
| Kingdom      | Animalia                                                                                            |
| Phylum       | Arthropoda                                                                                          |
| Subphylum    | Chelicerata                                                                                         |
| Class        | Arachnida                                                                                           |
| Order        | Scorpiones                                                                                          |
| Family       | Buthidae                                                                                            |
| Genus        | Buthus or Mesobuthus                                                                                |
| Species      | B. tumulus                                                                                          |
| Common names | Indian red scorpion,<br>eastern Indian scorpion                                                     |
| Synonyms     | B. tamulus concanensis,<br>B. tamulus sindicus, B.<br>tamulus gujaratensis, B.<br>tamulusgangeticus |

#### Scorpion venom (SV).

Scorpions belong to the most ancient group of animals on earth as they have been roaming this planet for more than 400 million years. More than 1500 different species have been described and they are classified in the order Scorpiones within the Arachnida.

The scorpion species that present medical importance belonging to the family Buthidae are represented by the genera Androctonus, Buthus, Mesobuthus, Buthotus, Parabuthus, and Leirus located in North Africa, Asia, the Middle East, and India (Petricevich, 2010). The venom is constituted of mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, enzyme inhibitors, and proteins namely neurotoxic peptides (Muller, 1993; Gwee et al., 2002). Scorpion toxins are classified according to their structure, mode of action, and binding site on different channels or channel subtypes (Possani et al., 2000; Tytgat et al., 1999; Possani et al., 1999). Each class consists of several peptides isolated from the venom of different species of scorpion and are based on their pharmacological action and also agree well with the structural properties of this peptide family. The long-chain toxins affecting sodium channels have been subdivided primarily into two major subtypes,  $\alpha$ - and  $\beta$ -toxins (Jover et al., 1980; Wheeler et al., 1982).

#### Objective

To investigate and evaluate for fibrinolytic, the activity of Indian red scorpion venom using In vitro model.

#### Material and method

##### Plan of work

Scorpion venom (SV) was a gift sample from Prime Serum, Narayangaon, Pune, Maharashtra, India in the year 2012 and stored at 4 °C until use. Scorpion venom was weighed and dissolved in phosphate buffer.

##### Study protocol

Fibrinolytic activity of *Buthustamulus venom* by-

- In-vitro blood clot dissolving activity
- Artificial blood clot degradation method

## Methods

### In-vitro blood clot dissolving activity of SV

In-vitro blood clot dissolving activity of SV was studied on 3 white cotton cloth pieces (5 × 5) stained with blood (sheep blood). The following sets were prepared & studied:

1. Control set - Petri plate with D.W. (10 mL) + stain cloth
2. Standard set – Petri plate with D.W. (10 mL) + Streptokinase 5000 IU/mL + stain cloth
3. Test -Petri plate with D.W. (10 mL) + stain cloth + SV (3µg/mL)

The above Petri plates were incubated at 37 °C for 1h 20 minutes. After incubation, cloth pieces were taken out, rinsed with water, and dried. Visual examination of cloth pieces using SV and streptokinase was done. (Badhe et al., 2013; Adinarayana et al., 2003)

### Artificial blood clot degradation method

The fibrinolytic effect of SV was observed by the artificial blood clot degradation method. An artificial blood clot was made by spontaneous coagulation in a glass test tube using 500 µL of fresh sheep blood. After 60 mins the

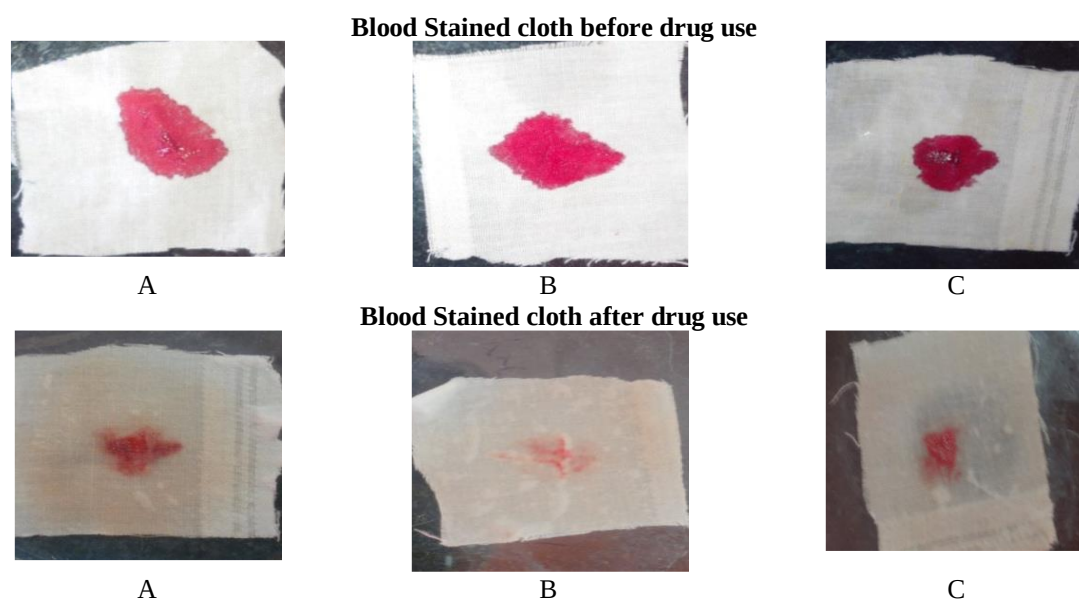
artificial blood clot was rinsed out and cut uniformly and dipped in (test drug) SV (3µg/mL) & streptokinase (5000IU/mL) as a standard and in control, normal saline was used at room temperature. The degradation of the clot was visually estimated by a change in color intensity after 1h at room temperature (Omura et al., 2005; Jonatas et al., 2011).

## Result

Determination of Fibrinolytic activity by In-vitro blood clot dissolving activity of SV - The blood clot was taken on a white cloth as shown in Figure 3. The visual results were observed for the blood clot-dissolving activity of both SV and streptokinase. Both possessed fibrinolytic properties. The clot-dissolving effect of SV and Streptokinase is comparative.

### Determination of Fibrinolytic activity

The in-vitro blood clot was taken on a white cloth as shown in Figure 4. The visual results were observed for the blood clot-dissolving activity of both SV and streptokinase. Both possessed fibrinolytic properties. The clot-dissolving effect of SV and Streptokinase is comparative.

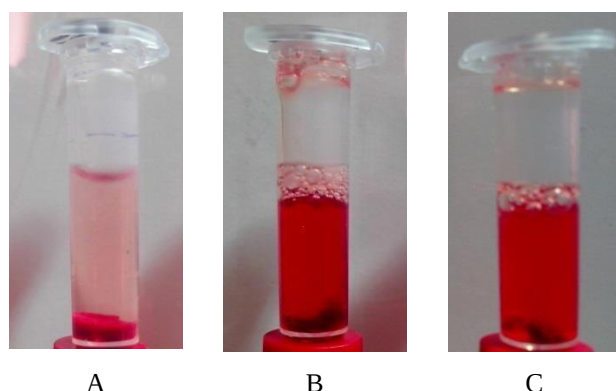


**Figure 3:** In-vitro blood clot dissolving activity of SV: Cloth stained with sheep blood -A, Blood stained cloth washed with water -B, Blood stained cloth washed with Streptokinase and C with test drug SV.

### Fibrinolytic effect by artificial blood clot degradation method

The blood clot degradation was observed in the test tube containing test drugs (SV) with spreads of red blood cells trapped by multiple fibrins net. In the test tube of normal saline, the blood clot degradation was not observed until

60 min. The color density is higher in the streptokinase tube than in the test tube containing the test drug presented in Figure 2.



**Figure 4.** Fibrinolytic activity of SV by degrading the blood clot within 60 min. A– Control, B– Standard (streptokinase 5000 IU/mL), C– SV (3 µg/mL).

### Discussion

It has been reported that SV contains a complex mixture of toxins, such as  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Cl^-$  channel toxins (Petricevich, 2010), Peptides such as Hypotensin peptide (2008), lipolytic peptide, bradykinin potentiating peptides (Meki et al., 1995),  $PGI_2$  and NO-releasing peptide (Song et al., 2013), enzymes like phospholipase A<sub>2</sub> (Hariprasad et al., 2011) and many unrevealed mixtures. These constituents of SV shows several physiological and pharmacological activity such as anti-inflammatory (Ahmadi et al., 2009), antiosteoporotic, (Gomes et al., 2009), antiplatelet aggregation (Song et al., 2005), antiproliferative and apoptogenic (Gupta et al., 2007), antithrombotic and fibrinolytic activities (Song et al., 2013). In the present study, Indian red scorpion venom was explored for fibrinolytic, activities that give scientific authentication to its use. In our study, SV was evaluated for its fibrinolytic properties in the In-vitro blood clot dissolving method and shown to decrease the stained area of the blood-stained cloth as compared to the control. In the artificial blood clot degradation method, the color intensity is higher in a test tube containing SV compared to the control and hence, it can be stated that SV has fibrinolytic activity.

### Conclusion

In this study, the fibrinolytic activity was confirmed by both the In-vitro blood clot dissolving activity of SV and the artificial blood clot degradation method showing a competent effect as Streptokinase.

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