

Thrombolytic Property of Homoeopathic Medicine Vipera Berus 6c and Lachesis 6c on Human Blood Clot

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ABSTRACT

Thrombosis or intravascular blood clotting refers to coagulation of blood inside blood vessels. Normally blood does not clot in blood vessels, because of some factors like injury to blood vessels, toughened endothelial lining (e.g.; arteriosclerosis), sluggishness of blood flow (e.g.; reduced cardiac action, hypotension, low metabolic, and immobility), agglutination of RBC, and congenital absence of protein c. Major complications of thrombosis are Deep vein thrombosis, embolism (pulmonary embolism, cerebral embolism, and coronary embolism), ischemia, necrosis, and infarction. This invitro study aimed to evaluate the thrombolytic property of vipera 6c and Lachesis 6c. thrombolytic assay method used to analyse. In this study Human blood samples will be used to assess the thrombolytic property of homoeopathic medicines Lachesis 6c and vipera 6c. The peak incidence of Lachesis 6c is obtained at 250 micrograms/ml whereas the peak incidence of vipera 6c is obtained at 500 micrograms/ml. It is concluded from the study that both Lachesis 6c and vipera 6c have anti thrombolytic activity. The peak thrombolytic activity of Lachesis 6c is obtained at 250 microgram the peak thrombolytic activity of vipera 6c is obtained at 500 micrograms

Keywords: Thrombolytic property, Vipera6c, Lachesis 6c, Human Blood clots, Thrombolytic assay

I. INTRODUCTION

Thrombosis or intravascular blood clotting refers to coagulation of blood inside blood vessels. Normally blood does not clot in blood vessels, because of some factors like injury to blood vessels, toughened

endothelial lining (e.g.; arteriosclerosis), sluggishness of blood flow (e.g.; reduced cardiac action, hypotension, low metabolic, and immobility), agglutination of RBC, and congenital absence of protein c. Major complications of thrombosis are Deep vein thrombosis, embolism (pulmonary embolism,

cerebral embolism, and coronary embolism), ischemia, necrosis, and infarction. Modern medicine uses anticoagulants like heparin, warfarin, EDTA, oxalate compounds, citrates which gives relief with side effects. Patients treated with anticoagulants due to venous thromboembolism have a risk of major bleeding of 7.2 events per 100 person years, the risk of fatal bleeding of 1.31 per 100 person-years, and a case fatality rate of 13.4% due to major bleed.

WHO states that 70% fatal of acute myocardial infarction are attributed by occlusion cause by atherosclerosis plaque. The world's biggest killer is ischemic heart disease, responsible for 10% of world total deaths since 2000, largest increase in death has been for this disease rising by 2million to 8.9 million deaths in 2019. Stroke is 2nd and 3rd leading cause of death. Heart attack and stroke are usually acute and events are mainly caused by a blockage that prevents blood from flowing to heart and brain Second purpose need for study is to change the statement, homoeopathy is pseudoscientific system of alternative system. Homoeopathic medicine Lachesis is indicated in blood coagulable disorder, decreases rate of coagulation of blood, whereas in viper coagulability is lost. Both Lachesis and viper are Ophidia group remedies. Generally, snake poison constitutes proteolysis, haemolysis, and thromboplastic property. Here we use potentized homeopathic medicine of Lachesis 6c and vipera 6c. Potentization believed that substance which are medicinally inert in their crude state are thus rendered active and effective for healing the sick by process of trituration and succession. It is believed that quantity of drug on homoeopathic dose is very small and order of 100th 30,100th-100,100th-200,100 th- 1000000 and cannot measured by present scientific instruments and techniques but when given on basis of principal similia similibus curentur gives cure or recovery to patient.

II. REVIEW OF LITERATURE:

Thrombosis or intravascular blood clotting refers to coagulation of blood inside blood vessels. Normally blood does not clot in blood vessels, because of some factors like injury to blood vessels, toughened endothelial lining (e.g.; arteriosclerosis), sluggishness of blood flow (e.g.; reduced cardiac action, hypotension, low metabolic, and immobility), agglutination of RBC, and congenital absence of protein c. Major complications of thrombosis are Deep vein thrombosis, embolism (pulmonary embolism, cerebral embolism, and coronary embolism), ischemia, necrosis, and infarction. Modern medicine uses anticoagulants like heparin, warfarin, EDTA, oxalate compounds, citrates which gives relief with side effects

THROMBOSIS: Thrombosis or intravascular blood clotting refers to coagulation of blood inside the blood vessels. Normally, blood does not clot in the blood vessel because of some factors which are already explained. But some abnormal conditions cause thrombosis.

Causes of Thrombosis

1. Injury to blood vessels During infection or mechanical obstruction, the endothelial lining of the blood vessel is damaged and it initiates thrombosis.
2. Roughened endothelial lining In infection, damage or arteriosclerosis, the endothelium becomes rough and this initiates clotting.
3. Sluggishness of blood flow Decreased rate of blood flow causes aggregation of platelets and formation of thrombus. Slowness of blood flow occurs in reduced cardiac action, hypotension, low metabolic rate, prolonged confinement to bed and immobility of limbs.
4. Agglutination of RBCs Agglutination of the RBCs leads to thrombosis. Agglutination of RBCs occurs by the foreign antigens or toxic substances.
5. Toxic thrombosis Thrombosis is common due to the action of chemical poisons like arsenic compounds, mercury, poisonous mushrooms and snake venom.

6. Congenital absence of protein C Protein C is a circulating anticoagulant, which inactivates factors V and VIII. Thrombosis occurs in the absence of this protein. Congenital absence of protein C causes thrombosis and death in infancy.

Factors predisposing to venous thrombosis: Patient factors:

- increasing age
- Obesity
- Varicose veins
- Previous deep vein thrombosis
- Family history, especially of unprovoked venous thromboembolism when young
- Transient additional risk factors: Pregnancy/puerperium Oestrogen-containing oral contraceptives and hormone replacement therapy Immobility, e.g. long-distance travel (>4 hrs) Intravenous drug use involving the femoral vein Surgery (see below) Medical illnesses (see below)

Surgical conditions:

- Major surgery, especially if >30 mins' duration Abdominal or pelvic surgery, especially for cancer
- Major lower limb orthopaedic surgery, e.g. joint replacement and hip fracture surgery

Medical conditions:

- Myocardial infarction/heart failure Inflammatory conditions:
- inflammatory bowel disease, connective tissue disorders and vasculitis
- Malignancy (anti-cancer chemotherapy increases the risk of venous thromboembolism compared with cancer alone)
- Nephrotic syndrome
- Chronic obstructive pulmonary disease
- Severe infection, bacterial or viral
- Neurological conditions associated with immobility, e.g. stroke, paraplegia, Guillain-Barré syndrome
- Any high-dependency admission Haematological disorders

- Polycythaemia rubra vera
- Essential thrombocythemia
- Deficiency of natural anticoagulants: antithrombin, protein C, protein S
- Paroxysmal nocturnal haemoglobinuria • Gain-of-function prothrombotic mutations: factor V Leiden, prothrombin gene G20210A
- Myelofibrosis Antiphospholipid syndrome

III.COMPLICATIONS OF THROMBOSIS:

1. Thrombus: During thrombosis, lumen of blood vessels is occluded. The solid mass of platelets, red cells and/or clot, which obstructs the blood vessel, is called thrombus. The thrombus formed due to agglutination of RBC is called agglutinative thrombus.
2. Embolism and embolus: Embolism is the process in which the thrombus or a part of it is detached and carried in bloodstream and occludes the small blood vessels, resulting in arrests of blood flow to any organ or region of the body. Embolus is the thrombus or part of it, which arrests the blood flow. The obstruction of blood flow by embolism is common in lungs (pulmonary embolism), brain (cerebral embolism) or heart (coronary embolism)
3. Ischemia: Insufficient blood supply to an organ or area of the body by the obstruction of blood vessels is called ischemia. Ischemia results in tissue damage because of hypoxia (lack of oxygen). Ischemia also causes discomfort, pain and tissue death. Death of body tissue is called necrosis.
4. Necrosis and infarction: Necrosis is a general term that refers to tissue death caused by loss of blood supply, injury, infection, inflammation, physical agents or chemical substances. Infarction means the tissue death due to loss of blood supply. Loss of blood supply is usually caused by occlusion of an artery by thrombus or embolus and sometimes by atherosclerosis. Area of tissue that undergoes

infarction is called infarct. Infarction commonly occurs in heart, brain, lungs, kidneys and spleen.

LACHESIS:

bluish or purplish discoloration of the affected area, suggesting venous stasis. The condition tends to worsen with slight pressure or touch. Lachesis is often used when symptoms predominantly affect the left side of the body. Symptoms are aggravated in the morning, upon waking. Accompanying the clot, there may be throbbing or pulsating pain. Patients needing Lachesis often experience heat, hot flushes, and an inability to tolerate warmth. Lachesis is also indicated in conditions where there is a tendency for spontaneous bleeding, often following the formation of clots

VIPERA:

The affected veins appear extremely swollen and sensitive to the slightest touch. Intense pain in the affected limb, especially when the limb is lowered; the patient feels as if the veins would burst due to congestion. Symptoms often worsen with any movement or when exposed to warmth. There is a characteristic sensation of the veins bursting, especially when the limb is hanging down. The affected area may appear bluish or purplish due to venous stasis. The pain and swelling often improve when the limb is elevated.

IV. MATERIALS AND METHODS:

STUDY SETUP: Tri bio lab, Trichy.

STUDY DESIGN: Invitro study

STUDY PARAMETER: weight of the Blood clot

STUDY INTERVENTION: Lachesis 6c, Vipera 6c

Thrombolytic Assay

In vitro clot lysis activity of different extract will be carried out according to following method. 5ml of venous blood will be drawn from healthy volunteers (n = 2) and it will be transferred to different pre weighed sterilized micro-centrifuge tube (1 ml/tube). The micro centrifuged tubes will be incubated at 37°C for 45minutes to form clot. Serum will be completely

removed from the tubes without disturbing the clot formed and discarded. Each of the tube having clot will be weighed again to determine the weight of the clot.

The clot weight = weight of clot containing tube — weight of tube alone

Each micro-centrifuge tube containing clot will be appropriately labelled and 100 µl of the samples Lachesis 6c, Vipera 6c will be added to the centrifuged tube in different concentrations such as 500µg, 250µg, 100µg, 50µg, 10µg will be added. The tubes containing clot and vipera 6c, Lachesis 6c extract will be incubated at 37°C for 90 minutes for observation of clot lysis. The experimental tubes will be then centrifuged and the supernatant fluid will be completely removed and discarded. The experimental tubes will be again weighed to observe the difference in weight after clot disruption. Finally, difference obtained in weight of the experimental tubes will be calculated and the result was expressed as percentage of clot lysis by the following equation,

Percentage of clot lysis = (Clot weight - Weight of released clot / Clot weight)×100

V. OBSERVATION AND STUDY:

THROMBOLYTIC PROPERTY OF VIPERA 6C:

In vitro clot lysis activity of different extract was carried out according to following method.

5ml of venous blood was drawn from healthy volunteers (n = 2) and transferred to different pre weighed sterilized micro-centrifuge tube (1 ml/tube). The micro centrifuged tubes were incubated at 37°C for 45minutes to form clot. Serum was completely removed from the tubes without disturbing the clot formed and discarded. Each of the tube having clot was again weighed to determine the weight of the clot.

The clot weight = weight of clot containing tube — weight of tube alone.

5 micro-centrifuge tubes containing clot were appropriately labelled as 10VR6, 50 VR6, 100VR6, 250 VR6, 500VR6 and one tube was labelled as control. The homoeopathic remedy Vipera 6c was added to the clot at various concentration such as 10,50,100, 250, 500 microlitres using micro pipette as mentioned in the label. All the 5 tubes containing clot and Vipera 6c extract were incubated along with the control tube at 37°C for 90 minutes for observation of clot lysis. The experimental tubes were then centrifuged and the supernatant fluid was completely removed and discarded. The experimental tubes were again weighed to observe the difference in weight after clot disruption. Finally, difference obtained in weight of the experimental tubes was calculated and the result was expressed as percentage of clot lysis by the following equation,

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



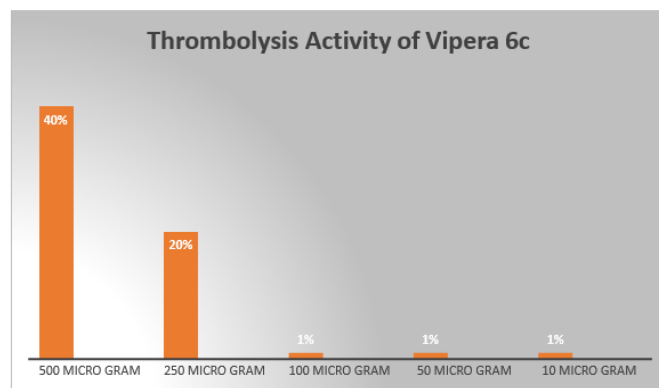
| S. No | Sample concentration (µg/ml) | Weight of clot (g) |
|-------|------------------------------|--------------------|
| 1. | Control | 0.05 |
| 2. | 500 µg/ml | 0.03 |
| 3. | 250 µg/ml | 0.04 |
| 4. | 100 µg/ml | 0.05 |
| 5. | 50 µg/ml | 0.05 |
| 6. | 10 µg/ml | 0.05 |

Percentage of clot lysis

| S. No | Tested sample concentration (µg/ml) | Percentage of clot lysis (%) |
|-------|-------------------------------------|------------------------------|
| 1. | Control | 100 |

| S. No | Tested sample concentration (µg/ml) | Percentage of clot lysis (%) |
|-------|-------------------------------------|------------------------------|
| 2. | 500 µg/ml | 40 |
| 3. | 250 µg/ml | 20 |
| 4. | 100 µg/ml | 0 |
| 5. | 50 µg/ml | 0 |
| 6. | 10 µg/ml | 0 |

Thrombolysis Activity of Vipera 6c



THROMBOLYTIC PROPERTY OF LACHESIS 6C:

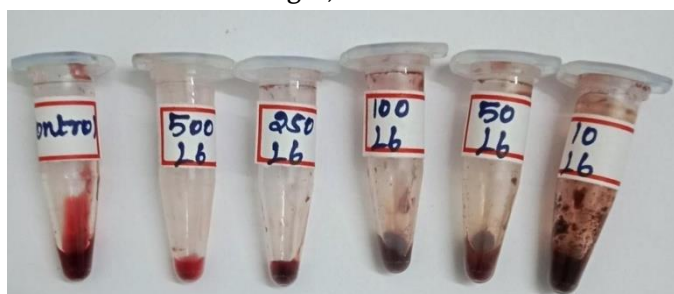
In vitro clot lysis activity of different extract was carried out according to following method. 5ml of venous blood was drawn from healthy volunteers (n = 3) and transferred to different pre weighed sterilized micro-centrifuge tube (1 ml/tube). The microcentrifuge tubes were incubated at 37°C for 45minutes to form clot. Serum was completely removed from the tubes without disturbing the clot formed and discarded. Each of the tube having clot was again weighed to determine the weight of the clot.

The clot weight = weight of clot containing tube — weight of tube alone.

5 micro-centrifuge tube containing clot was appropriately labelled as 10L6, 50L6, 100L6, 250L6, 500L6 and one tube was labelled as control. The homoeopathic remedy Lachesis 6c was added to the clot at various concentration such 10, 50, 100, 250, 500 micro litres using micro pipette as mentioned in the label. All 5 tubes containing clot and Lachesis 6c

extract were incubated along with control tube at 37°C for 90 minutes for observation of clot lysis. The experimental tubes were then centrifuged and the supernatant fluid was completely removed and discarded. The experimental tubes were again weighed to observe the difference in weight after clot disruption. Finally, difference obtained in weight of the experimental tubes was calculated and the result was expressed as percentage of clot lysis by the following equation,

Percentage of clot lysis = $\left(\frac{\text{Clot weight} - \text{Weight of released clot}}{\text{Clot weight}} \right) \times 100$

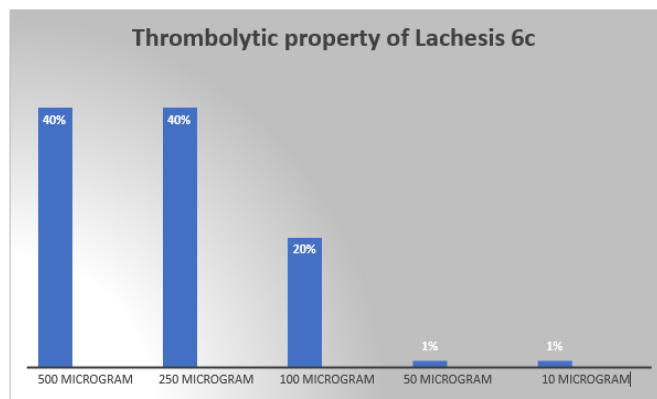


| S. No | sample concentration (µg/ml) | Weight of clot (g) |
|-------|------------------------------|--------------------|
| 1. | Control | 0.05 |
| 2. | 500 µg/ml | 0.03 |
| 3. | 250 µg/ml | 0.03 |
| 4. | 100 µg/ml | 0.06 |
| 5. | 50 µg/ml | 0.08 |
| 6. | 10 µg/ml | 0.12 |

Percentage of lysis

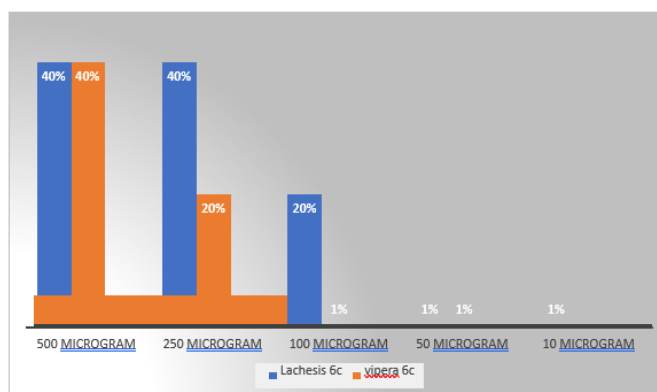
| S. No | Tested sample concentration (µg/ml) | Percentage of clot lysis (%) |
|-------|-------------------------------------|------------------------------|
| 7. | Control | 0 |
| 8. | 500 µg/ml | 40 |
| 9. | 250 µg/ml | 40 |
| 10. | 100 µg/ml | 20 |
| 11. | 50 µg/ml | 1 |
| 12. | 10 µg/ml | 1 |

Thrombolytic property of Lachesis 6c



VI. RESULTS AND DISCUSSION:

This study was conducted at Tribio lab, Trichy. Human blood samples were used to assess the thrombolytic property of homoeopathic medicines Lachesis 6c and vipera 6c. Below are the results of study



The peak incidence of Lachesis 6c is obtained at 250 micrograms whereas the peak incidence of vipera 6c is obtained at 500 micrograms.

VII. CONCLUSION:

It is concluded from the study that both Lachesis 6c and vipera 6c anti thrombolytic activity. The peak thrombolytic activity of Lachesis 6c is obtained at 250 microgram the peak thrombolytic activity of vipera 6c is obtained at 500 microgram further study are required to access the anti-thrombolytic property in higher potencies

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