

Research

Evaluation of Haemolytic and Morphological Changes in Red Blood Cell on Administration of Lachesis Muta 30, 200 and 1M in - Vitro Study

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ABSTRACT

Introduction

Snake venom has haemolytic action on the blood and reduces the power of its coagulability, with the result that a bloody serum continue to ooze out from wound for many hours.

Objective

The objectives of the study was to evaluate haemolytic and morphological changes in Red Blood Cells on administration of Lachesis muta 30, 200 and 1M.

Methodology

Four milliliters of venous blood was collected under all necessary precautions in amber colour blood sample bottle having 1.5mg of ethylenediaminetetracetic acid and was mixed well and divided into 4 equal parts of 1 ml of blood each. To each blood sample bottle four drops of each of tested medicines (Lachesis muta 30, 200, 1M) and four drops of ethyl alcohol were added and shaken for 2 minutes. Then, tongue shape thin uniform blood smears were made from each sample to be examined under the microscope and packed cell volume of each sample were determined.

Result

The peripheral blood smear showed the presence of echinocytes under the microscope in all the three potency of Lachesis. The packed cell volume showed presence of haemolysis in all the three potency of Lachesis.

Conclusion

Lachesis muta has haemolytic property and tendency for echinocytosis.

Keywords: Echinocytes, Ethylenediaminetetracetic acid, Lachesis muta 30, Lachesis muta 200, Lachesis muta 1M, Packed Cell Volume and Peripheral Blood Smear.

INTRODUCTION

The WHO (World Health Organization) has developed and launched the traditional medicine strategy 2014–2023 in response to the World

Health Assembly resolution on tradition medicine (62.13). The strategy aims to support member states in developing proactive policies and implementing action plans that will strengthen the role traditional medicine plays in keeping population healthy.¹

In Homoeopathy, one of the frequently indicated remedies is *Lachesis muta*. The zoological name of *Lachesis muta* is *Crotalus mutus* belongs to family Crotalidae inhabitant of the hot countries of south America; it attains a length of upwards of seven feet, and its poison fangs are nearly one inch long; the skin is reddish brown, marked along the back with large rhomboidal spots of blackish brown colour, each of which encloses two spots of the colour of the body. The poison resembles saliva, is less viscous, limpid, inodorous, without any marked taste, in colour somewhat greenish at the extremity of the fang; it easily forms into drops, and falls without threading; exposed to air it soon concentrates into a dry yellow mass which for an indefinite time preserves its poisonous qualities. This poison introduced into wound, or injected into vein, produced the most dreadful symptoms, and generally death.²

Snake venom has haemolytic action on the blood and it reduces the power of its coagulability, with the result that a bloody serum continue to ooze out from wound for many hours.³ Haemorrhage from external orifices of the body are common. Functional disturbances are related to the involved organ e.g. convulsions from haemorrhage in the brain.⁴ There may be haemoglobinuria and renal failure from intravascular coagulation.

In Homoeopathy, this venom was used by Dr. Hering (1828) in his experiments was obtained from the living snake which was stunned with a blow; the poison was collected on sugar by pressing the fang upwards against the bag, and the three first attenuation prepared by trituration.²

The drug strength is 1/100. *Lachesis muta* is frequently used anti-haemorrhagic remedy. The ulcer bleeds with black blood, which soon coagulates and looks like charred straw. Small wound bleeds much. A prick of pin will ooze great drop of blood.⁵

Therefore, the present study was design to evaluate haemolytic property and morphological changes in shape of red blood cells on administration of *Lachesis muta* 30, 200 and 1M and this effects were compared with ethyl alcohol. The experiment was approved by the Institutional Ethical Committee of Bakson Homoeopathic Medical College (Ref. No. BHMC/IECHS/781 dated 8th August 2019).

METHODOLOGY

Procurement of Medicine

Lachesis muta 30, 200 and 1M was procured from Dr. Willmar Schwabe India Private Limited and ethyl alcohol was procured from Bakson Drug and Pharmaceutical Private Limited. The Batch number of *Lachesis muta* as:

Lachesis muta 30 CH - 0185127

Lachesis muta 200 CH - 0167482

Lachesis muta 1M - 0185601

Ethyl Alcohol Lot number - D2177

Ethylenediaminetetracetic acid (EDTA) was procured from Central Drug House (P) Ltd., Delhi 110002.

Processing

Four milliliters of the available anonymised blood sample was taken for experiment from Physiology Laboratory of Bakson Homoeopathic Medical College and Hospital, Greater Noida, Uttar Pradesh. The collected venous sample was divided into four equal parts of one milliliter each into amber colour sample bottle having 1.5 mg EDTA as shown in Figure 1. The blood sample bottles were labeled as:

- C-E representing bottle containing ethyl alcohol
- 30 - E representing bottle containing *Lachesis muta* 30
- 200 - E representing bottle containing *Lachesis muta* 200
- 1M-E representing bottle containing *Lachesis muta* 1M

Figure 1. Collected venous blood in blood sample bottle.



To each blood sample bottle four drops of respective medicines and ethyl alcohol were added and shaken for two minutes. Then, tongue shape thin uniform smears were made from each sample. The smear was dried in air. The smear was focused under 45X of medical microscope. The packed cell volume of blood from each sample bottle was determined. The wintrobe's tubes were labeled as given above.

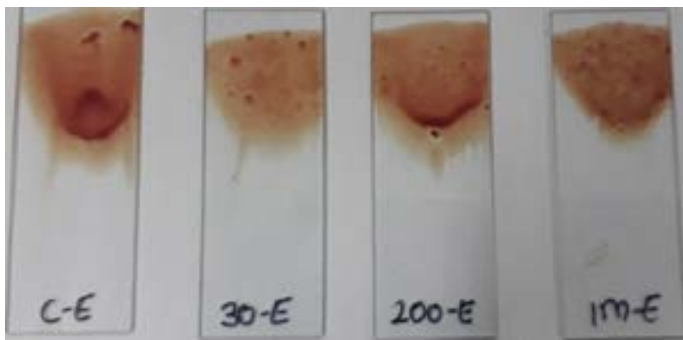
Parameters

- Packed Cell Volume** - The labeled wintrobe's tubes were filled with blood from particular sample bottles upto mark 10 at the top. Then, wintrobe's tubes were centrifuge at 3200 rpm for 30 minutes.⁶
- Peripheral Blood Smear** - A drop of blood from each of blood sample bottle was placed about 1 cm from the end of slide. That slide was supported with the thumb and fingers of left hand. Second slide i.e. spreader, is grasped with thumb and fingers of right hand; its free end extending downwards and to the left (away from the right hand) at an angle of 45° to the horizontal. The edge of the spreader was applied on the first slide just in front of blood drop

and pulled it back so that it just touch the blood drop. Then, wait till the blood touches the corner of the glass slide, the spreader moved forward with a single, smooth, fairly fast gliding motion. The angle of 45° was maintained.⁷

The smear was dried in waving air. The peripheral blood smears were shown in Figure 2. Then, slide was examined under 45X of medical microscope.

Figure 2. Peripheral blood smear of ethyl alcohol, Lachesis muta 30, 200 and 1M.

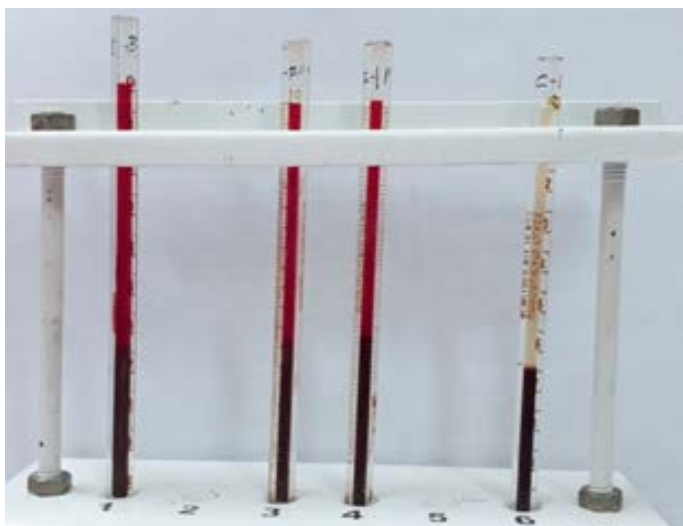


RESULT

Packed Cell Volume

Packed cell volume in Lachesis muta showed haemolysis in 30, 200 and 1M potency as shown in Figure 1,2,3. Packed cell volume in ethyl alcohol was 36%.

Figure 3. Packed cell Volume in Lachesis muta 30, 200,1M and Ethyl alcohol.



Peripheral Blood Smear

Peripheral blood smear under 45X of microscope showed the presence of echinocytes as shown in Figure 4-6, whereas no echinocytes appeared

with ethyl alcohol as shown in Figure 7.

Figure 4. Presence of Echinocytes in peripheral blood smear of Lachesis muta 30.

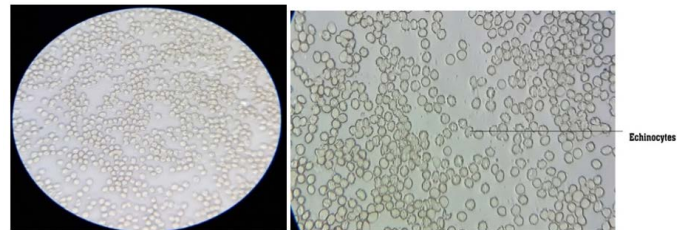


Figure 5. Presence of Echinocytes in peripheral blood smear of Lachesis muta 200.

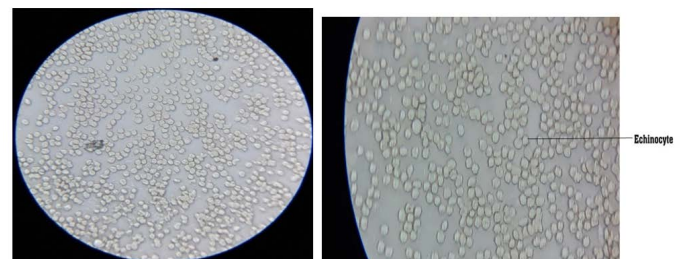


Figure 6. Presence of Echinocytes in peripheral blood smear of Lachesis muta 1M.

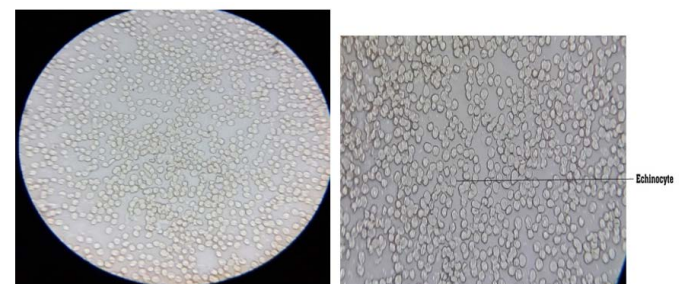
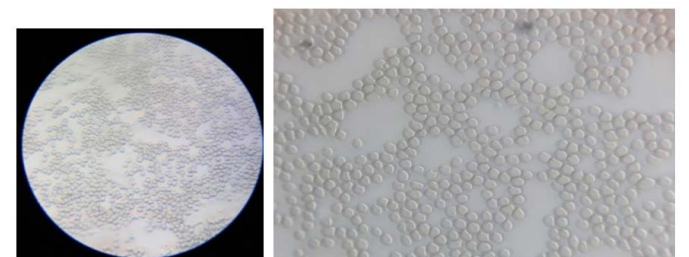


Figure 7. Peripheral blood smear of ethyl alcohol.



DISCUSSION

Lachesis muta has haemolytic property. Blood was mixed with EDTA to prevent the coagulation of blood by removing calcium ions by chelation, thereby decreases the ionic calcium level so much that blood coagulation is blocked.

Packed cell volume with *Lachesis muta* showed haemolysis of blood in 30, 200 and 1M potency. Packed cell volume in ethyl alcohol was 36%.

Normally RBCs are seen as biconcave disc with thickened periphery and thin center in unstained peripheral blood smear. The lipid bilayer of normal Red blood cell is asymmetric in lipid composition. The outer half of the lipid bilayer is relatively enriched in sphingomyelin and phosphatidylcholine, whereas the inner half is preferentially enriched in negatively charged phosphatidylserine and phosphatidylethanolamine. Any causative agent that preferentially accumulate in the outer half of the red blood cell lipid bilayer, resulting in expansion of outer lipid bilayer to produce echinocytes. Echinocytes are also called burr cells

Echinocytic transformation occurs in the presence of fatty acids, lysophospholipids. Echinocytes also form when RBCs are dehydrated, pH is increased, intracellular calcium is increased, and RBC ATP is depleted. Echinocytes are the predominant RBC shape abnormality in human burn patients. Several haemolytic anaemias have been reported in association with echinocytes on peripheral blood films. Echinocytes occurs in dog following coral snake and rattlesnake envenomation, presumably secondary to the action of phospholipase A₂, a calcium dependent enzyme present in venom may be responsible for echinocytic transformation through production of lysolecithin a known echinocytic agent. Higher venom doses produced spherocytic and spherocytic transformation.

Echinocytosis are more in number in slide of *Lachesis* 30 than in *Lachesis* 200 and 1M.

This study enhance the pathophysiological understanding of *Lachesis muta* and will help the clinician to identify the sphere of action of *Lachesis muta*.

The study shows that *Lachesis muta* has haemolytic property and tendency to produce echinocytosis.

This study does not give mechanism through which echinocytosis occur on administration of *Lachesis*. Also, study would be perform in in-vivo in future.

CONCLUSION

Lachesis muta has haemolytic property and tendency to produce echinocytosis.

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CONFLICTS OF INTEREST

None.

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