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COMPARATIVE ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) AND PROTHROMBIN TIME (PT) PROFILE OF INDIAN SNAKES *Naja naja, Echis carinatus, Vipera russelli* HELPFUL IN ESTABLISHING THEIR SUPERIOR THERAPEUTIC PROCOAGULANT EFFICACY

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ABSTRACT
Comparative profile studies on the Activated Partial Thromboplastin Time (APTT) and the Prothrombin Time (PT) of Naja naja (Indian cobra), Vipera russelli (Indian Russell's viper) Echis carinatus (Indian saw scaled viper) proving their superior Procoagulant efficacy than the normal platelet poor plasma.

KEYWORDS: Naja naja, Vipera russelli, Echis carinatus, Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT).

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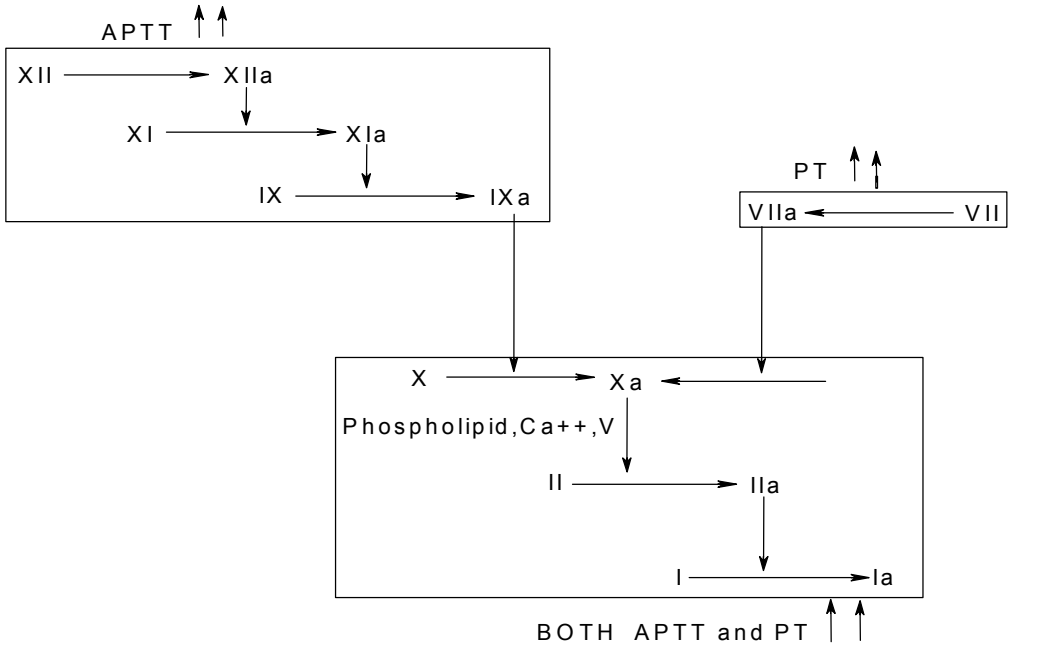
INTRODUCTION

Demonstrative methods for the measurement of blood coagulation time conventionally employed, include those measuring the Activated APTT, PT and fibrinogen levels. Alternatively, a thrombotic event may also be confirmed by measuring the levels of soluble fibrin or fibrin degradation products in circulation[1].The venoms of several species of snakes contain enzymes that convert the Zymogen Prothrombin into the enzyme thrombin and /or its catalytically active precursor meizothrombin. Both the activated products convert fibrinogen into fibrin, thereby resulting in plasma coagulation[2].Activated partial Thromboplastin time's (APTT) normal range is approximately 25-35 seconds, the time taken by citrated platelet poor plasma to clot in the presence of optimum concentration of contact activator, phospholipid (platelet substitute /partial Thromboplastin) and calcium. It screens for all coagulation factors deficiencies; except for factor VII.[3] The Activated Partial Thromboplastin (APTT) tests the integrity of the intrinsic and the final coagulation pathways. Thromboplastin is a tissue extract containing tissue factor and a phospholipid. As the test uses only the phospholipid part as a substitute for the platelet membrane in activating factor XII, it is known as partial Thromboplastin. Deficiency of factors XII, XI, IX, VII, High molecular weight kininogen (HMWK) or kallikerin will prolong the APTT to accelerate the PTT reaction. An activator (Celite-diatomaceous earth, Kaolin) is added and hence the term Activated[4]. An abnormal APTT is associated with quantitative or qualitative deficiencies in factor XII, XI, IX, VIII, and V or X[5]. Abnormal APTT values are obtained in the following conditions like i) Factor deficiencies, which may be corrected by the addition of normal plasma. ii) Presence of inhibitors like heparin, lupus anticoagulant (LA), specific factor inhibitors, fibrinogen degraded products (FDP), which cannot be corrected by normal plasma addition.

Prothrombin time (PT); The Prothrombin time (PT) measures the integrity of the extrinsic pathway and the common coagulation pathway. Deficiencies of factor VII or vitamin K and warfarin therapy cause an elevation in the PT .Inactivation of factor II by large doses of heparin also prolong the PT. PT measures the time taken by citrated platelet poor plasma to clot in presence of optimum concentration of tissue Thromboplastin and calcium. The results are expressed as PR Prothrombin ratio (Patients/control). PR greater than 1.2 is considered as abnormal**.** It is very important that in the event of an injury or accident or a blood disorder, blood has to clot in particular time range (The normal clotting time for a healthy individual weighing 70kgs is (5-15minutes) and therefore, if the clotting time is prolonged, there would be copious loss of blood resulting in the drop of systolic blood pressure, followed by circulatory collapse, ultimately resulting in a state of shock and heart failure .Hence, such severe loss of blood should be prevented and if coagulation cannot be brought about due to reasons like genetic disorders like hemophilic and hemorrhagic syndromes, circulating anticoagulants, thrombocytopenic purpuras, dental extractions, prostractomy, Ophthalmological surgeries, gastroenterology, cosmetic surgeries and post delivery bleedings, one must make use of therapeutic external procoagulant support to hasten the blood coagulation process[6-7].



Implication of raised PT & APTT in the Coagulation system

In the present investigation we have compared the APTT and PT of crude venoms Indian venomous snakes with the normal APTT and PT of human platelet poor plasma.

MATERIALS AND METHODS

Crude venoms of Naja naja (Indian cobra), Vipera russelli (Indian Russell's viper), Echis carinatus (Indian saw scaled viper) were procured from The Irula snake catcher's society, Chennai, India. Human citrated platelet poor plasma was procured from the Karnatak Cancer Research Institute Navnagar Hubli.,Test kits for APTT and PT determination were procured from Tulip Diagnostics (P) LTD. Unit II first floor, Plot nos. 92/96,Phase II C, Verna IND. EST. Verna, Goa -403 722,India

Activated Partial Thromboplastin Time (APTT) Determination

Reagents attained room temperature before prewarming to 37^0C for testing purposes. The kit reagents were mixed well by gentle swirling. To a 12x15mm test tube, add 0.1ml crude venom sample and 0.1ml liquiceline-E, shake the tube gently to mix. Next place the tube in an incubator for 3minutes at 37^0C. Following incubation, add 0.1ml of prewarmed calcium chloride and simultaneously start the stop watch to measure the time of clot formation[8].

Prothrombin Time (PT) Determination

Reagents were brought to room temperature before prewarming to 37^0C for testing purposes. Kit reagents were mixed well. To a 12x15mm tube add0.1ml of crude snake venom which was incubated for 3minutes at 37^0C.Next add 0.2ml of liquiplastin reagent (prewarmed at 37^0C for at least 3minutes) and simultaneously start the stop watch and note the time of appearance of first fibrin strand[9-10].

RESULT

Comparative APTT and PT of the crude venoms of Naja naja, Vipera russelli and Echis carinatus proving their superior procoagulant efficacy is shown in the **table 1**.

DISCUSSION

Indian venomous snakes, Echis carinatus and Naja naja take about 13 times less time for the clot formation, where as Vipera russelli crude venom takes about 2.23 times less time for clot formation, as their APTT and PT times are far less than the normal platelet poor human plasma. Hence, one could

make use of these venoms in the treatment of coagulation disorders, thus proving their superior Procoagulant efficacy over the existing commercial pharmaceutical preparations[11].

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Table 1: The time taken for the APTT and PT by the different snake venoms

Samples	APTT (Time in seconds)	PT (Time in seconds)
Normal platelet poor human plasma	35	5.62
Vipera russelli crude venom	15.66	4.32
Echis carinatus crude venom	2.65	2.57
Naja naja crude venom	2.65	2.53