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In vitro models to estimate the anti-snake venom potential of *Tamarindus indica* and *Cynodon dactylon*

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Abstract
The purpose of this study is to investigate the anti-snake venom characteristics of plants endemic to the western ghats of India. It was observed that traditional healers in Maharashtra, India used these herbs to cure snakebite victims. The freeze-dried *Daboia russelli* snake venom was supplied by the Hindustan Snake Park in Kolkata. Botanists have identified and confirmed the *Tamarindus indica* and *Cynodon dactylon* species. Using three unique study models, including phospholipase A2 activity, procoagulant activity, and fibrinolytic activity, the leaves of both plants were extracted using a hot extraction method and then examined for *in vitro* antivenom activity. Plant extracts at 0.12 to 0.15 mg for *Tamarindus indica* and 0.15 to 0.18 mg for *Cynodon dactylon* reduced PLA2-dependent hemolysis, whereas plant extracts at 1.4 to 1.7 mg for *Tamarindus indica* and 1.7 to 1.95 mg for *Cynodon dactylon* neutralised coagulant activity. *Tamarindus indica* and *Cynodon dactylon* also showed fibrinolytic activity induced by *Daboia russelli* venom.

Keywords: procoagulant activity, fibrinolytic activity, *in-vitro* antivenom activity, *Daboia russelli* venom, phospholipase A2 activity

Introduction
Despite the fact that it is difficult to quantify the total number of cases, snakebite is a widespread medical concern in a number of countries. According to estimates, more than 5 million people may encounter snake envenomation each year. Approximately one million of these are creating the loud scream. There are disparities in health and precision, as well as economic and environmental factors, all around the world, which are represented in epidemiological data. Access to precise statistics is generally limited, making it difficult to do research on topics such as disease transmission or the mortality rate of snake bite patients. Due to the dependence on traditional healers and practitioners of black magic, especially in agricultural regions, clinic numbers are far off from the true number. According to estimates, up to 80% of persons who have been bitten by a snake consult with traditional healers before reaching a treatment facility. This describes the majority of non-industrialized societies. As a result of the delay, between one and three people perish while being transported to the hospital [1-3].

Due to the high cost of treatment at emergency clinics and the restricted availability of venom serums, residents of rural areas frequently find it more cost-effective to consult with local doctors who are known for properly treating patients who have been bitten by snakes. According to these findings, the adoption of plant-based remedies by area specialists is effective, and patients who have advanced to more severe clinical stages of venom harm appear to have a high prevalence of resilience. This study aims to investigate the anti-venom effects of plants indigenous to the western ghats of India. India is the location of these plants. It was discovered that traditional healers in the Indian state of Maharashtra employed these herbs [4-5].

Materials and method
Venom procurement
Powdered lyophilized snake venom was acquired from the Hindustan Snake Park in Kolkata and stored at 4 degrees Celsius.

Medicinal plants and how extracts are produced
Based on a more comprehensive literature assessment, *Tamarindus indica* and *Cynodon dactylon* were selected for use in the current experiment as anti-venom agents. The written survey provided evidence that the selected plants may possess anti-venom effects. Dr. T. Chakraborty, Scientist D and Joint Director of the Botanical Survey of India, identified and confirmed specimens of *Tamarindus indica* Grah. and *Cynodon dactylon* in

India's Koregaon Park. The two specimens were delivered to the respective Institutes for storage. The species voucher specimen sample number for *Tamarindus indica* Grah. is HLT-2, whereas the sample number for *Cynodon dactylon* is HLT-3. The necessary authentication certificates could be purchased. The powdered material (250 g) from the plants *Tamarindus indica* and *Cynodon dactylon* was extracted separately using a hot extraction method including petrol ether, chloroform, 90 percent ethanol, and water. After filtration removed the biomass stores from the system, the pooled waste products were centred around a rotating vacuum evaporator. Except for the water remover, the concentrates were likewise dried at 80 degrees Celsius in an oven. The shower dryer was utilised to evaporate the expelled water. After everything had completely dried, the concentrations were measured [6, 7].

Activity against venom *in vitro*

The enzyme phospholipase

Using the methods described by Gutierrez *et al*. (1988), the activity of phospholipase A2 was measured using an anomalous hemolytic assay on an agarose-erythrocyte-egg yolk gel plate. Increasing quantities of *Daboia russelli* venom were delivered to 3mm wells in agarose gels containing 1.2% sheep erythrocytes, 1.2% egg yolk as a source of lecithin, and 10mM CaCl2. To measure the venom, micrograms were employed. Following a brief incubation period at 37 degrees Celsius, the diameters of the hemolytic coronas were measured. Each control well contained 15 microliters of a salt solution. MIHD, or the base aberrant hemolytic part, is comparable to a dose of venom that caused an 11 mm hemolytic halo. 6 By combining a set amount of venom (g) with a variable amount of plant extracts (l) and incubating the combination for 30 minutes at 37 degrees Celsius, we were able to determine that the antidote, which is created from plant extracts, is not totally effective at removing phospholipase. The wells of the agarose-egg-yolk-sheep-erythrocyte gels were subsequently filled with 10 l of various combinations. Each well received an equal amount of water. There are no plant extracts in the venom used for control testing. Plates were cooked at 37 degrees Celsius for twenty hours. It was assessed as the ratio of antibodies to venom that, when compared to the action of the venom alone, could halve the hemolytic radiance [6, 7].

Procoagulant Activity

Laing *et al*. adapted significantly Theakston and Reid's technique from 1983 in order to explore the procoagulant action in 1992. At 37 degrees Celsius, human citrated plasma was cooked and various quantities of venom dispersed in 100 l of PBS with a pH of 7.2 were added. The basic coagulant dose10 was not set in situ as a component of the venom, causing the plasma to thicken within sixty seconds of the observed coagulation time. PBS-incubated plasma was the sole component of the control. To maintain equilibrium, a steady amount of venom was coupled with various plant-isolate weakenings. The mixes were incubated for 30 minutes at a temperature of 37 degrees Celsius. When 0.1 millilitre of the combination was added to 0.3 millilitres of citrated plasma, the timing of the plasma's coagulation was then measured. In the control tubes, plasma was either incubated with venom or plant extracts on its own. The effective dosage is the amount of l antibodies (plant extricates) per mg of venom at which the coagulating time is prolonged by a multiple of the thickening season of plasma hatched with two MCD of venom alone (ED) [8].

Activity in the Breakdown of Fibrin

In lieu of this, the modified plaque test was used. The amount of venom that created a 10-millimeter-diameter fibrinolytic glow is known as the basal fibrinolytic focus. Combining a constant amount of venom with varying amounts of plant extracts and incubating the combination for one hour at 37 degrees Celsius allowed for equilibrium testing. After the hatching process was complete, the slurry was poured into each of the plaque's wells. After 18 hours of incubation at 37 °C, fibrinolytic coronas are estimated [9].

Result and Discussion

Phospholipase Activity

Daboia russelli venom is produced to deliver hemolytic haloes in agarose-sheep erythrocyte gels during the phospholipase movement, often known as PLA2. When injected, one unit (1U/10 micrograms) of Russell's snake venom produced an 11-millimeter-wide hemolytic corona. This demonstrates that the molecules (PLA2) required to lyse sheep Rbcs are present in *Daboia russelli*'s venom. Plant extracts successfully reduced the PLA2 subordinate hemolysis of sheep red blood cells, which was partially triggered by the venom of *Daboia russelli*. The PLA2-dependent hemolysis of sheep RBCs induced by *Daboia russelli* venom was totally inhibited by plant extracts of *Tamarindus indica* and *Cynodon dactylon* at concentrations ranging from 0.15 to 0.18 mg and 0.12 to 0.18 mg, respectively.

Table 1: Details the *Daboia russelli* venom's phospholipase activity and how plant extracts combat it

Plant extracts	Dose of *Daboia russelli* venom (µg)	Neutralization of venom by plant extracts (ED_{50} in mg)
PEETI	10 (1 Unit)	0.15
CETI		0.12
EETI		0.12

AETI		0.15
PEECD		0.15
CECD		0.12
EECD		0.12
AECD		0.18

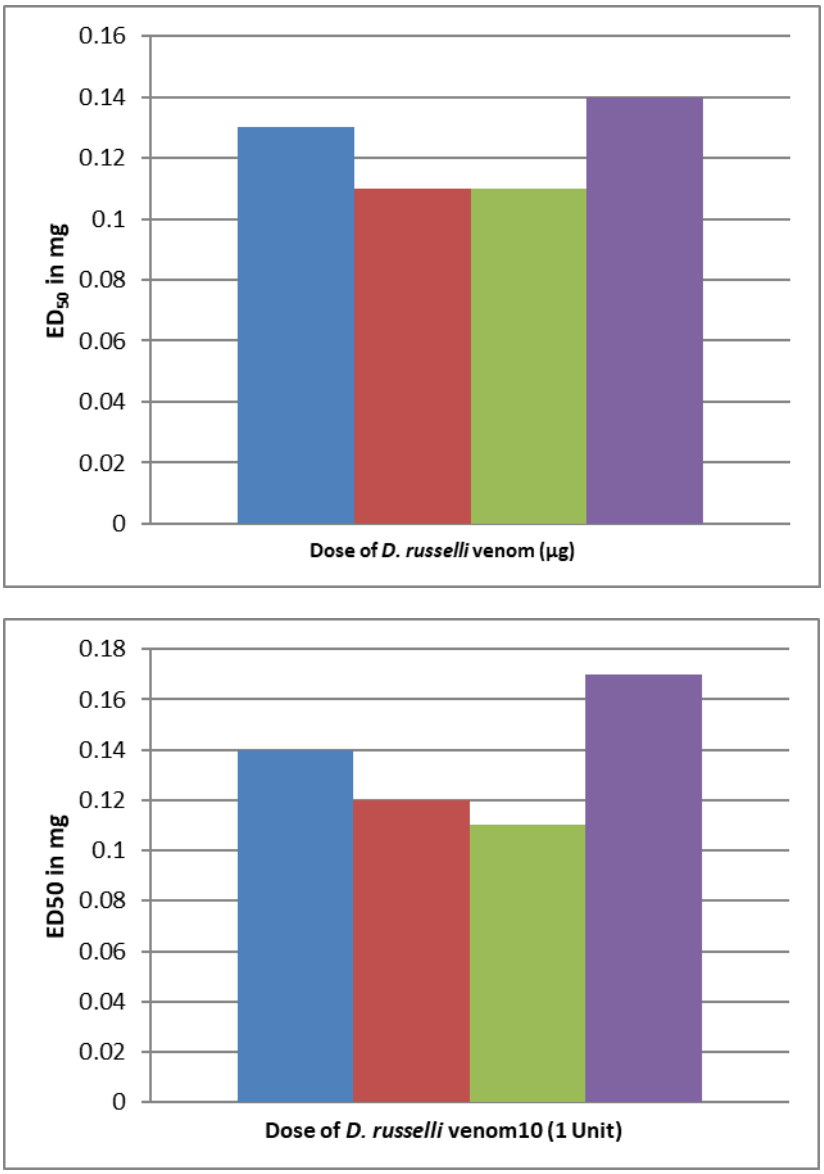

Fig 1: demonstrates that plant extracts inhibit the phospholipase activity of *Daboia russelli* venom

Procoagulant Activity

Despite the fact that the venom component is responsible for the plasma thickening in sixty seconds, the nature of the coagulant substance is yet unknown. In 60 seconds, 120 grammes of Russell's snake venom thickened human citrated plasma. As part of the equilibrium evaluation, the absence of clumping represents the respective killing abilities of the two plant isolates. According to our findings, 1.4 to 1.95 mg of *Cynodon dactylon* plant extract and 1.7 to 1.95 mg of *Tamarindus indica* plant extract were able to totally halt coagulant movement. Rapid thickening induced by the enormous quantity of venom indicated that a very high dose of antidote was required to be fatal.

Table 2: Details the procoagulant action of *Daboia russelli* venom and the manner in which plant extracts inhibit it

Plant extracts	**Dose of *Daboia russelli* venom (µg)**	**Neutralization of venom by plant extracts (ED_{50} in mg)**
PEETI	16 ($2LD_{50}$)	1.7
CETI		1.7
EETI		1.7
AETI		1.4

PEECD		1.7
CECD		1.7
EECD		1.4
AECD		1.95

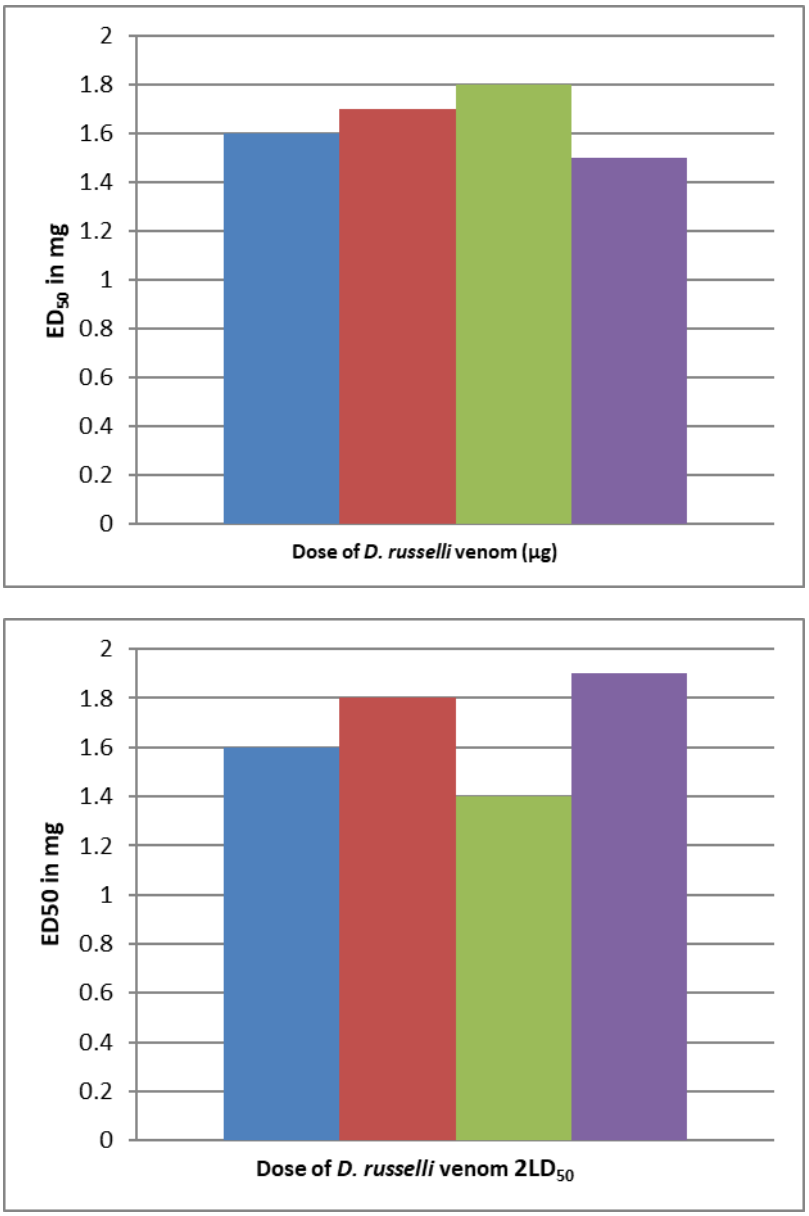

Fig 2: demonstrates that plant extracts neutralise the procoagulant effect of *Daboia russelli* venom

Fibrinolytic Activity

Plant extracts have the potential to effectively inhibit the fibrinolytic effect of *Daboia russelli* venom. *Tamarindus indica* and *Cynodon dactylon* plant extracts suppressed the fibrinolytic activity (as determined by a modified plaque assay) caused by *Daboia russelli* venom.

Table 3: includes the fibrinolytic activity of *Daboia russelli* venom as well as the plant extracts that inhibit it

Plant extracts	Dose of *Daboia russelli* venom (µg)	Neutralization of venom by plant extracts (ED_{50} in mg)
PEETI	16 ($2LD_{50}$)	0.16
CETI		0.12
EETI		0.18
AETI		0.16
PEECD		0.18
CECD		0.18
EECD		0.15
AECD		0.15

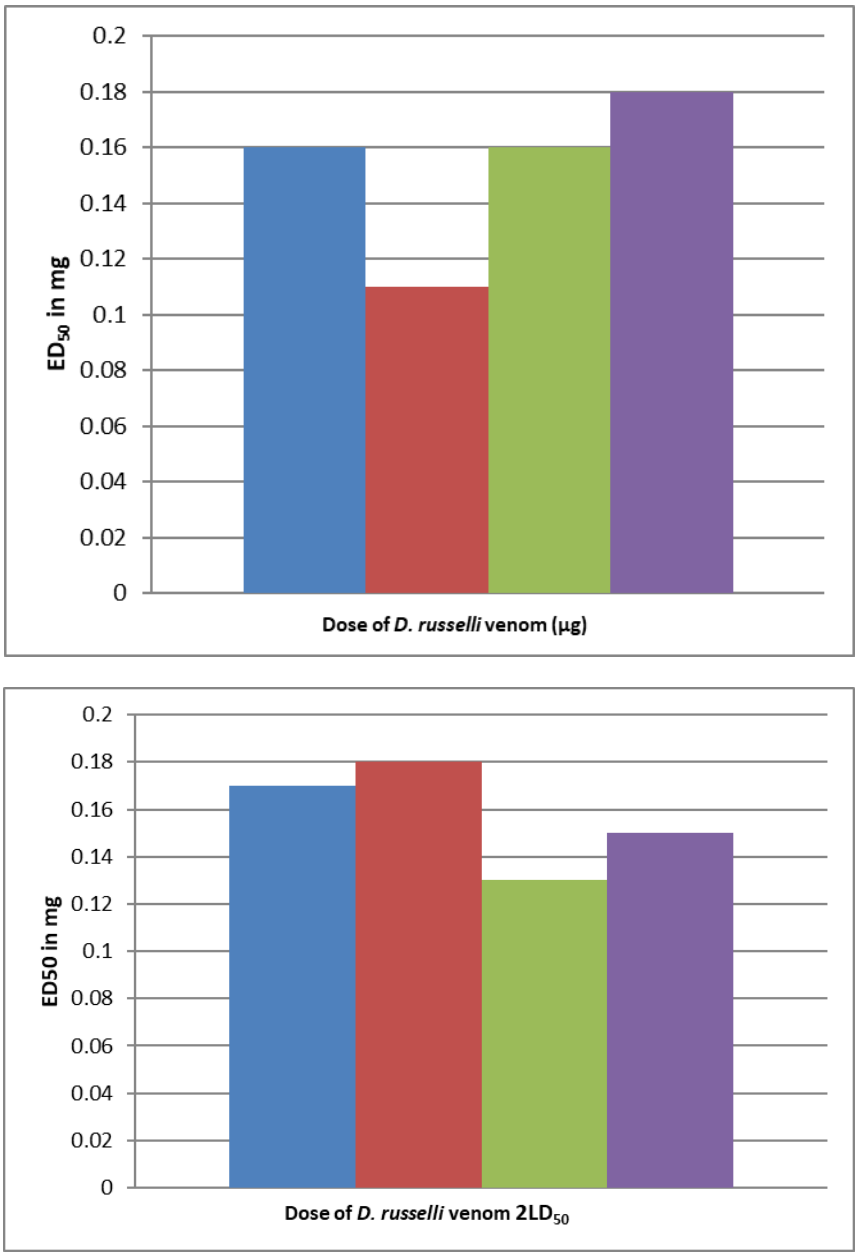

Fig 3: Illustrates the fibrinolytic action of *Daboia russelli* venom and the manner in which plant extracts inhibit it

Synopsis and Discussion

Sheep red blood cells (RBCs) exposed to the venom of *Daboia russelli* were subjected to subservient PLA2 hemolysis, which was somewhat suppressed by plant extracts. It was believed that *Tamarindus indica* plant extract and *Cynodon dactylon* plant extract doses of 0.12 to 0.15 mg and 0.18 to 0.24 mg, respectively, could totally prevent PLA2 subordinate hemolysis of sheep RBCs induced by *Daboia russelli* venom[10]. In the 1960s, it was not entirely proven that the component of the venom with the lowest coagulant activity was the one that caused plasma coagulation. At doses of plant extracts between 1.4 and 1.7 mg for *Tamarindus indica* and 1.7 and 1.95 mg for *Cynodon dactylon*, the coagulant action can be completely removed, according to our findings. To be fatal, an exceptionally large amount of the antidote was required, as a significant amount of the venom generated a rapid thickening. The plant extracts successfully prevented the fibrinolytic reaction caused by the venom of *Daboia russelli*. *Tamarindus indica* and *Cynodon dactylon* extracts at doses of 0.12 to 0.18 mg and 0.15 to 0.18 mg, respectively, were able to completely inhibit the fibrinolytic effect, also known as an altered plaque measure, generated by *Daboia russelli* venom [11].

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