Phytochemical screening and anti-venom effect of leaves extracts of *Andrographis paniculata and Morus alba*

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ABSTRACT:

It is estimated that the true incidence of snake envenomation could exceed 5 million per year. About 1,00,000 of these develop severe squeal. The present study aims to study the anti-snake venom activities of the local plants, which are native to the western ghats of India. These plants were found to be used by traditional healers in Maharashtra, India to treat patients bitten by snakes. *Andrographis paniculata* plant extracts and incubated at 37°C for 30 minutes prior to injection. We found that 0.15- 0.17 mg of *Andrographis paniculata* plant extracts were able to completely neutralize the lethal activity of $2LD_{50}$ of *V. russelli* venom and 0.16-0.19 mg of *Andrographis paniculata* plant extracts were able to completely neutralize the lethal activity of $2LD_{50}$ of *Najanaja*venom and 0.14-0.18 mg of *Morus alba* plant extracts were able to completely neutralize the lethal activity of $2LD_{50}$ of *V. russelli* and *Najanaja* venom.. The minimum edematicdose of the venom was found to be 4µg in rat. Significant inflammation was seen after 1hr of venom injection and maximum inflammation was seen at 180 min. Plant extractsat dose level 100mg/kg, 200mg/kg and 400mg/kg showed significant activity when compared with control and minimum % inhibition 29.13 27.1 at 100mg/kg, at 60 min 58.18 and 57.21 at 240 min for 400mg/kg, and std polyvalent antivenom 60.89 for *Andrographis paniculata and* 31.03 ,31.7 at 100mg/kg, at 60 min 59.02 and 58.34 at 240 min for 400mg/kg for *Morus alba*for*V. Russelli*and *Najanaja*venom respectively.

Key words: phospholipase *Andrographis paniculata*, antivenom activity, *Morus alba Najanaja, D. russelli* venom,

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INTRODUCTION:

Snakebites are an occupational and environmental disease. It is a common occurrence in tropical and subtropical countries. It is common in agricultural countries affecting farmers and plantation workers

leading to significant increase in mortality and morbidity[1]. In 2009, World Health organization (WHO) has declared snakebites as a neglected tropical disease[1]. The precise number of deaths due to snakebite is not properly established and it continues to be a public health problem in most of the countries. Joseph Fayrer quantified the snakebite deaths for the first time in 1869 for half of the British India (Including Pakistan, Bangladesh and Burma) and reported about 11,416 deaths. Many surveys have been carried out to assess global envenoming but estimate remains elusive[2-3]. The analysis of snakebite mortality by Swaroop and Grab provided two interesting facts "primarily a considerable number of variations exist from one region to another and secondly the topographicallysimilar regions were found to have higher rates of snakebite". A total number of 30,000-40,000 deaths[4-5] due to snakebite were witnessed worldwide excepting in China, USSR and Central European countries[6-7]. The highest numbers were observed in Asia (25,000-30,000) followed by South America. North America, Europe and Oceania have reported low figures. In Africa, an exact number of estimates could not be provided, but the annual snakebite deaths were found to be 400-1000.

Many plants have been used to treat various effects induced by snake bite[8]. Therefore, much of the research efforts have been published since years on the potential use of medicinal plant extracts to reduce or to evaluate compounds that could efficiently minimize the activity of phospholipase A_2 (PLA_2) enzymes which constituted a rich source in snake venoms. Several PLA_2 inhibitors have been isolated from plants. A compound AIPLAI purified from the methanol leaf extract of *Azadirachta indica* (Neem) inhibits the Cobra and Russell's viper venoms (RVVs) PLA_2 enzymes[9] in a dose-dependent manner. Chemical constituents like alkaloids, flavonoids, sitosterol or glucoside, lupeol, gymnemagenin, phenolics, triterpenes like oleanoic acid, tannins, α and β amyrin have been reported for anti-snake venom activity. All these classes of chemical compounds are capable of interacting with macromolecular targets with enzymes or receptors and it can effectively inhibit the toxic effect of snake venoms *in vitro* than *in vivo*.[10-11]

MATERIALS AND METHOD:

The *Andrographis paniculata, Morus alba* and*Vitex negundo* were identified and authenticated by Bhima Ram choudhary Deputy conservator of forest, Botanical Survey of India, Sikar, Rajasthan India. The voucher specimens were deposited at the respective Institutes.

Extraction procedure:

The leaves powder of *Andrographis paniculata*and*Morus alba* of plants were pounded and the powdered material (250 g) was extricated independently with petroleum ether, chloroform, ethanol (90%) and water utilizing hot extraction strategy. In the wake of expelling the biomass deposits by filtration, pooled removes were focused on rotating vacuum evaporator[12-13]. The concentrates were additionally dried utilizing stove at 80°C withthe exception of water remove. The water extricate was dried utilizing shower dryer (at delta temperature: 168 ± 2°C, outlet temperature: 107 ± 3°C, blow Speed: 12 units and air Pressure: 0.6 kg/cm^2). At last totally dried concentrates were gauged and yields were determined.[4]These extracts were labeled as PEEAP, CEAP, EEAP and AEAP for *Andrographis paniculata*and PEEMA, CEMP, EEMP and AEMP for *Morus alba* (Moraceae).

Evaluation of antivenom activity:

Evaluation of antivenom activity by using *In vitro* anti-venom study was carried out by using five different types of research models. *Viz*. Phospholipase activity, Procoagulant activity, Fibrinolytic activity, Proteolytic activity and Hyaluronidase activity and *in vivo* techniques for different extracts of *Andrographis paniculata*and*Morus alba*)[14-17]. *In vivo* antivenom study was carried out for PEEAP, CEAP, EEAP and AEAP of *Andrographis paniculata*and PEEMA, CEMA, EEMA and AEMA of *Morus alba*.

***In vivo* anti-venom activity:**

In vivo anti-venom study was carried out by using five different types of research models. *Viz.* Determination of Lethal Toxicity, Haemorrhagic activity, Defibrinogenating activity, Edema forming activity and Myonecrotic activity.

Defibrinogenating Activity determination:

The minimum defibrinogenating dose (MDD) of *V.russelli*and *Najanaja* venom is defined as the minimum amount of venom which when injected i.v. into mice causes incoagulable blood 1 h later (Theakston & Reid, 1983). Neutralization of this activity was estimated by mixing different amount of venom with fixed amounts of plant extract[18], incubating at 37°C for 1 h, and centrifuging. The supernatant was injected i.v. into mice and the nature of the blood observed after 1 h.

Hemorrhagic activity:

The minimum haemorrhagic dose (MHD) of *V.russelli*and *Najanaja* venom was determined by the method described by Theakston and Reid, 1983. The minimum haemorrhagic dose was defined as the least amount of venom which when injected intradermaly (i.d.) into mice results in a haemorrhagic lesion of 10mm diameter in 24 hours. Neutralization of the haemorrhagic activity was estimated by mixing a fixed amount of venom with different amounts plant extracts. The plant extract venom mixture was incubated at 37°C for 1 h and 0.1 ml of the mixture was injected intradermaly into mice[19]. The haemorrhagic lesion was estimated after 24 h.

RESULT AND DISCUSSION:

Leaves powder was characterized by morphological features like light yellowish green colour, presence of specific characteristic and characteristic taste.

TABEL 1: Pharmacognostic Study of *Andrographis paniculata*

Sr. No.	Plant	Extracts	Abbreviation	Appearance	Consistency	Yield % (w/w)
1.	***Andrographis paniculata***	Pet Ether	PEEAP	Greenish Yellow	Semisolid	2.0 %
2.		Chloroform	CEAP	Pale Yellow	Semisolid	2.6 %
3.		Ethanol	EEAP	Dark Green	Semisolid	3.2 %
4.		Aqueous	AEAP	Dark Brown	Solid (Fine Powder)	4.9 %

TABEL 2: TLC Studies of PEAP, CEAP, EEAP, AEAP extract of *Andrographis paniculata*

Fraction/ Extract	Solvent system	No of spots	TLC profile	
			R_f value	Color
PEEAP extract	Toluene:Ethylacetate:Metenol: Water(7:6:5:2)	3	0.94; 0.90; 0.74	Dark green, green, light yellow, light green
CEAP extract	Ethyl acetate: methanol: toluene: water (5:4:6:5)	6	0.82; 0.57; 0.60; 0.68; 0.71; 0.76	Dark green, light yellow light green, yellow, yellow, Cream

EEAP extract	Ethyl acetate: methanol: toluene: water (5:4:6:5)	4	0.50; 0.59; 0.62; 0.80	light yellow light green, yellow, yellow
EEAP extract	Ethyl acetate: methanol: toluene: water (5:4:6:5)	4	0.48; 0.51 0.75; 0.84	Yellow, Cream light green, yellow

[A] [B] [C] [D]

Fig 1: TLC studies of extract PEEAP [A], CEAP [B], EEAP [C], and AEAP [D] for *Andrographis paniculata*

TABLE 3: Percentage of phytoconstituents present in *Andrographis paniculata*

Constituents presents	**Quantity of phytoconstituents in (%)**			
	PEEAP	CEAP	EEAP	AEAP
Alkaloids	7.12	6.23	5.14	4.43
Phenols	6.25	7.21	7.87	8.2
Tannins	4.51	4.78	5.01	5.31
Flavonoid	6.71	6.03	-	-

Neutralization of *V. russelli* venom induced lethality by plant extracts

Plant extracts		**Dose of *V. russelli* venom (µg)**	**Neutralization of venom by plant extracts (ED_{50} in mg)**
Andrographis paniculata	PEEAP	16 ($2LD_{50}$)	0.16 mg
	CEAP	16 ($2LD_{50}$)	0.15 mg
	EEAP	16 ($2LD_{50}$)	0.15 mg
	AEAP	16 ($2LD_{50}$)	0.17 mg

TABLE 4: Neutralization of *Najanaja*venom induced lethality by plant extracts

Plant extracts		**Dose of *Najanaja* venom (µg)**	**Neutralization of venom by plant extracts (ED_{50} in mg)**
Andrographis paniculata	PEEAP	16 ($2LD_{50}$)	0.18 mg
	CEAP	16 ($2LD_{50}$)	0.17 mg
	EEAP	16 ($2LD_{50}$)	0.16 mg
	AEAP	16 ($2LD_{50}$)	0.19 mg

Edema forming activity:

The minimum edematic dose (MOD) of venom/ carrageenan is defined as the least amount of venom/ carrageenan which, when injected in to male albino rats**,** produced inflammation (edema) in the paw. The minimum edematic dose of the venom was found to be 4µg in rat. Significant inflammation was seen after 1hr of venom injection and maximum inflammation was seen at 180 min. Plant extracts at dose level 100mg/kg, 200mg/kg and 400mg/kg showed significant activity when compared withcontrol.

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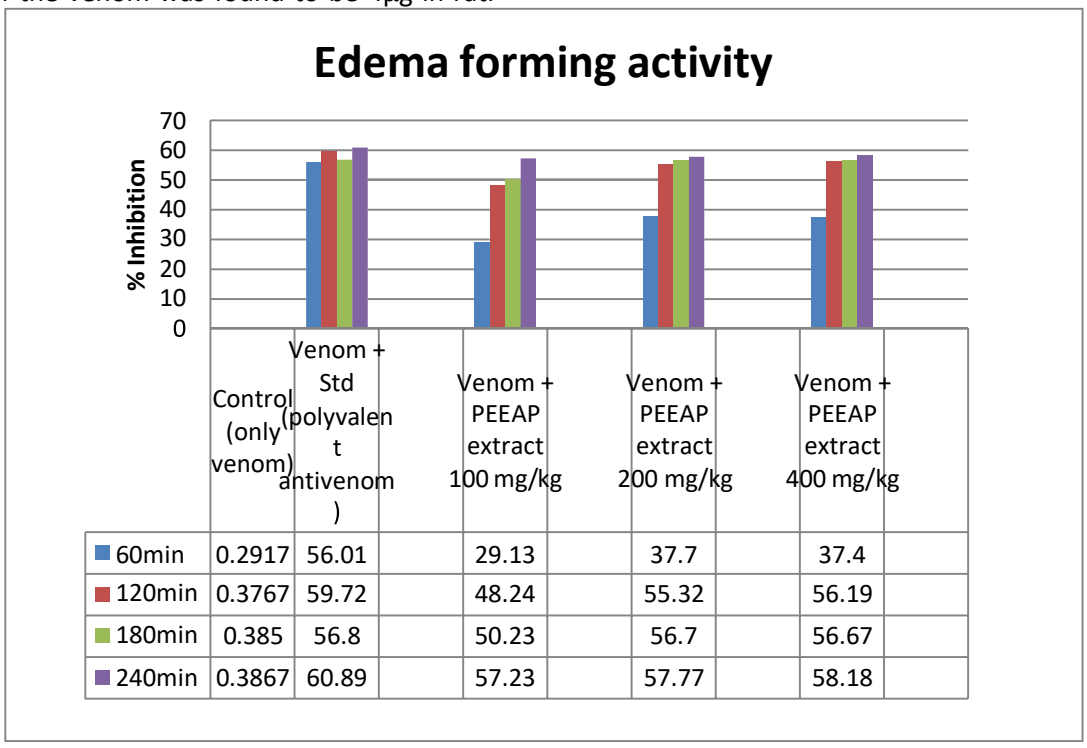


Fig 2: Effect of PEEAP extract on the *V. russelli*venom induced paw edema in rats

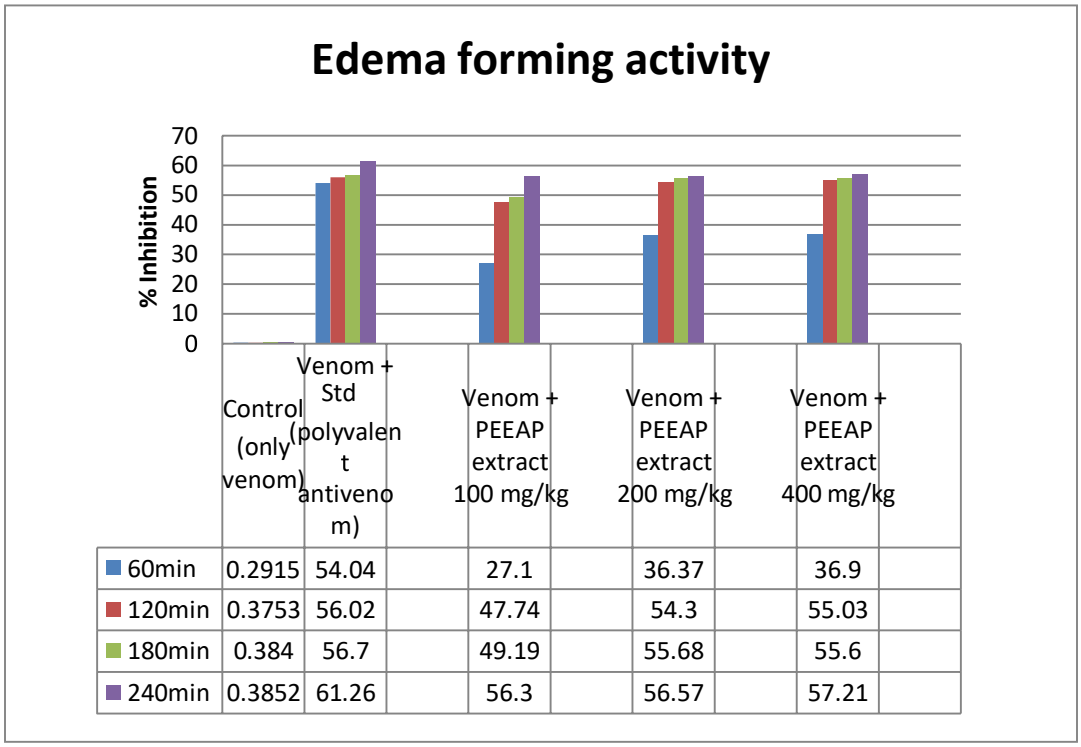


Fig 3: Effect of PEEAP extract on the *Najanaja*venom induced paw edema in rats

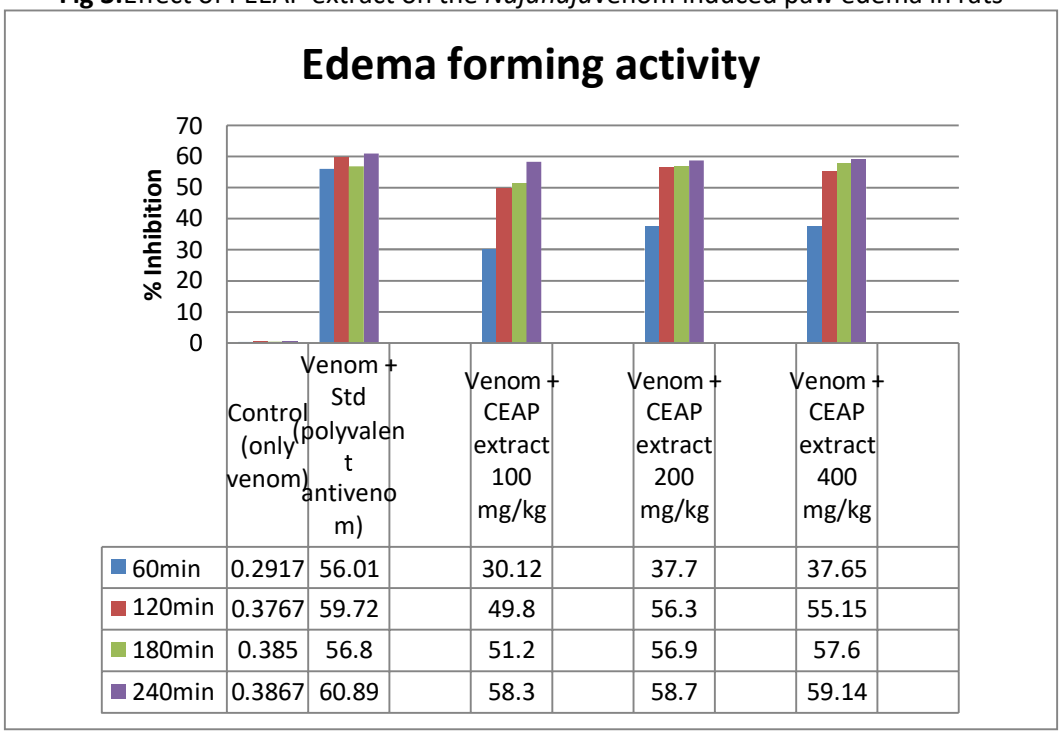


Fig 4: Effect of CEAP extract on the *V. russelli*venom induced paw edema in rats

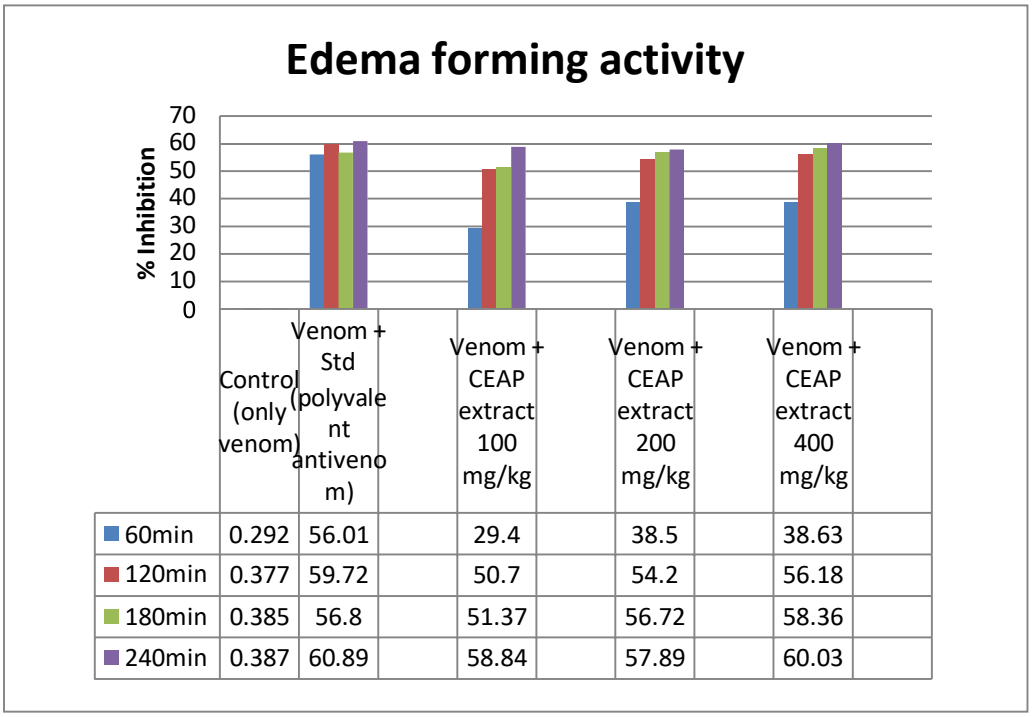


Fig 5: Effect of CEAP extract on the *Najanaja*venom induced paw edema in rats

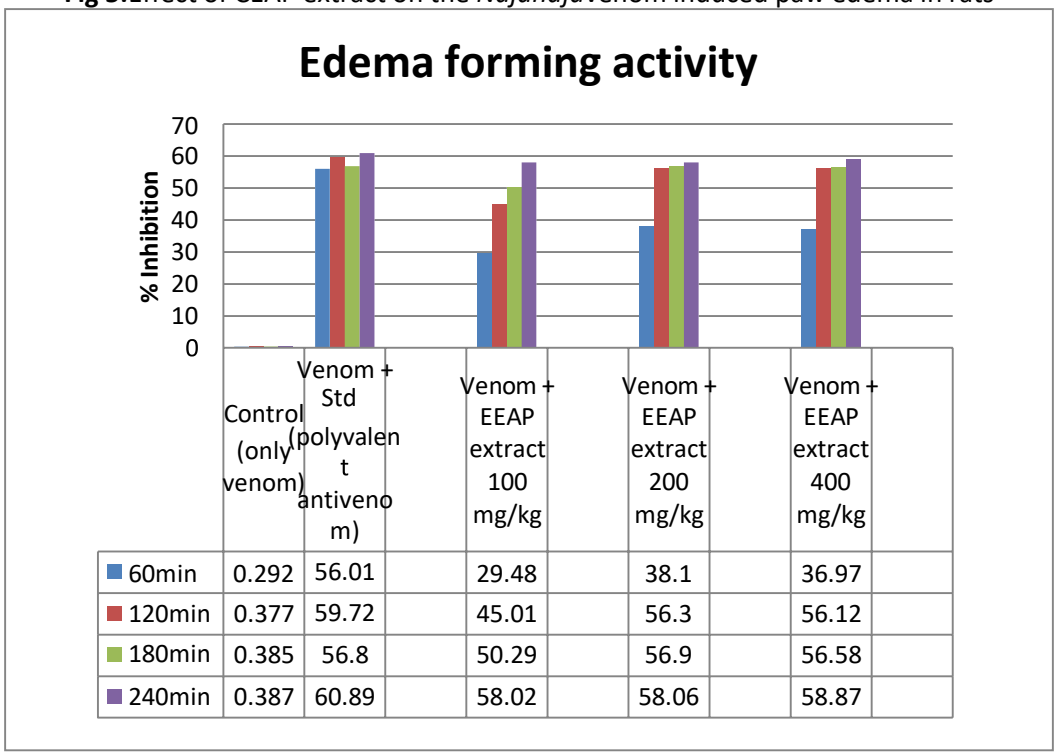
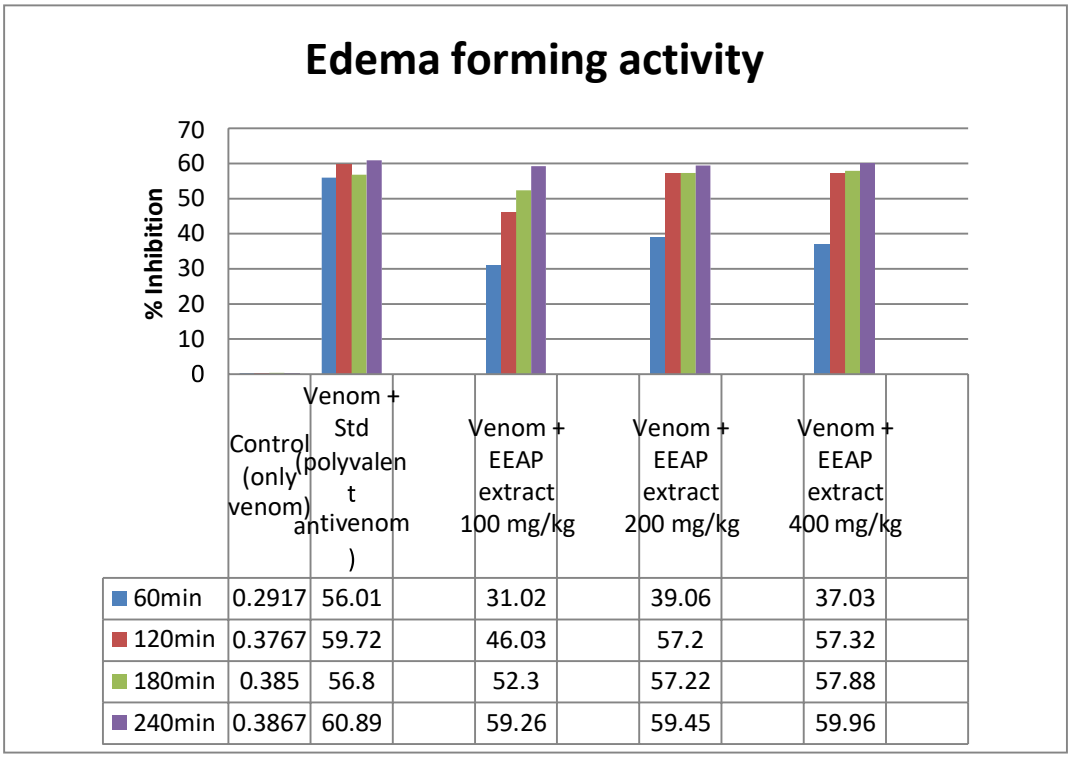
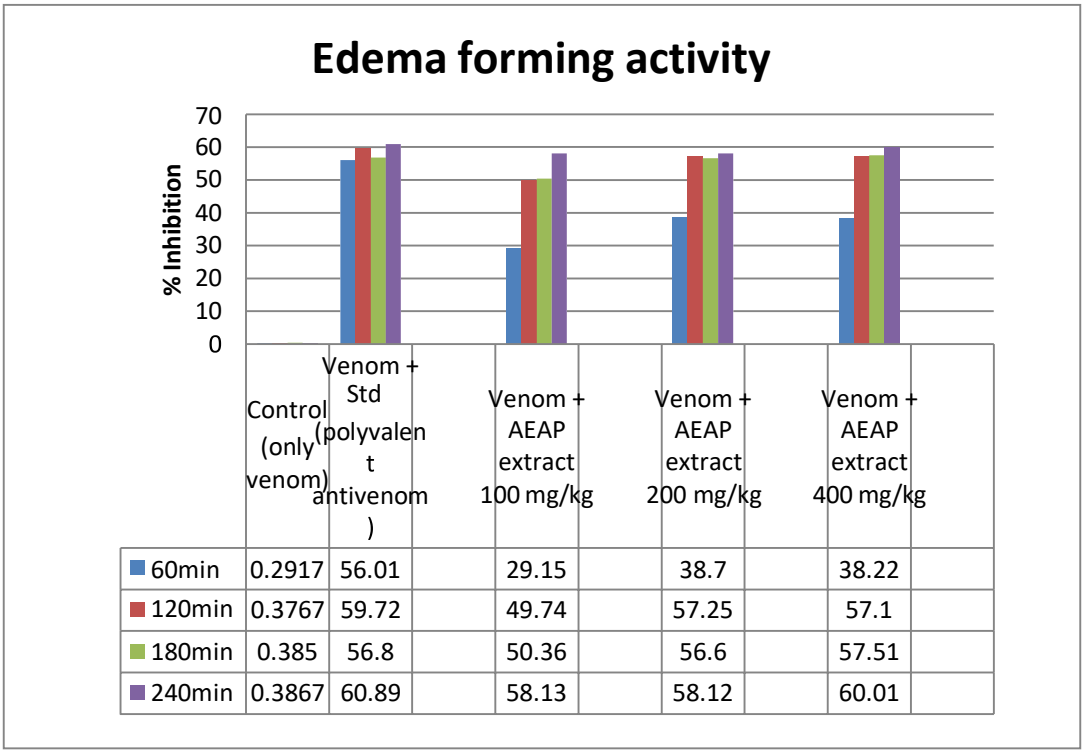


Fig 6: Effect of EEAP extract on the *V. russelli*venom induced paw edema in rats



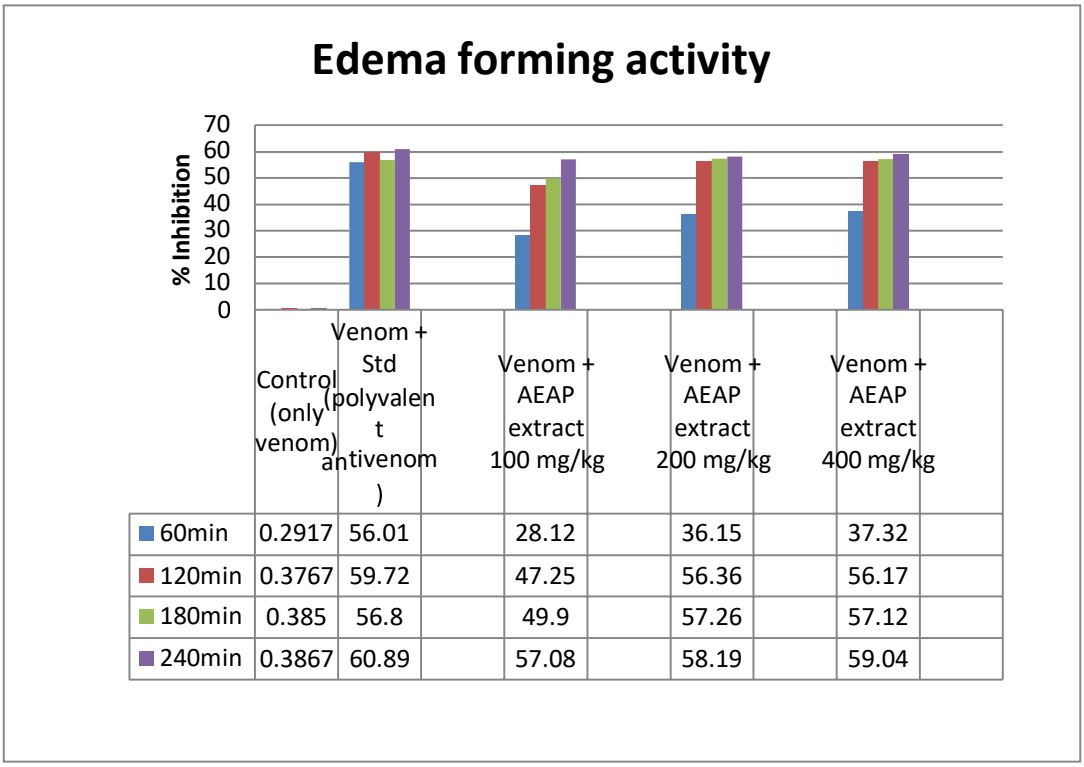
	Control (only venom)	Venom + Std (polyvalent antivenom)	Venom + EEAP extract 100 mg/kg	Venom + EEAP extract 200 mg/kg	Venom + EEAP extract 400 mg/kg
60min	0.2917	56.01	31.02	39.06	37.03
120min	0.3767	59.72	46.03	57.2	57.32
180min	0.385	56.8	52.3	57.22	57.88
240min	0.3867	60.89	59.26	59.45	59.96

Fig 7: Effect of EEAP extract on the *Najanaja*venom induced paw edema in rats



	Control (only venom)	Venom + Std (polyvalent antivenom)	Venom + AEAP extract 100 mg/kg	Venom + AEAP extract 200 mg/kg	Venom + AEAP extract 400 mg/kg
60min	0.2917	56.01	29.15	38.7	38.22
120min	0.3767	59.72	49.74	57.25	57.1
180min	0.385	56.8	50.36	56.6	57.51
240min	0.3867	60.89	58.13	58.12	60.01

Fig 8: Effect of AEAP extract on the *V. russelli*venom induced paw edema in rats



	Control (only venom)	Venom + Std (polyvalent antivenom)	Venom + AEAP extract 100 mg/kg	Venom + AEAP extract 200 mg/kg	Venom + AEAP extract 400 mg/kg
60min	0.2917	56.01	28.12	36.15	37.32
120min	0.3767	59.72	47.25	56.36	56.17
180min	0.385	56.8	49.9	57.26	57.12
240min	0.3867	60.89	57.08	58.19	59.04

Fig 9: Effect of AEAP extract on the *Najanaja*venom induced paw edema in rats

Summary and conclusion:

Based on escalated writing overview; *Andrographis paniculata*, *Morus alba* and *Vitex negundo* were chose for utilized as anti-venom activity in present examination. The writing survey demonstrated that the chose plants have potential as anti-venom activity. The *Andrographis paniculata*, *Morus alba* and *Vitex negundo* were identified and authenticated by Bhima Ram choudhary Deputy conservator of forest, Botanical Survey of India, Sikar, Rajasthan India. The Leaves of *Andrographis paniculata* and *Morus alba* plants wereconceal dried, decreased to coarse powder with the assistance of processor and put away in water/air proof compartment till further use.The leaves powder of *Andrographis paniculata* and *Morus alba* of plant was extracted by Hot extraction method with petroleum ether, chloroform, ethanol (90%) and water utilizinghot extraction strategy and These extracts were labeled as PEEAP, CEAP, EEAP and AEAP for *Andrographis paniculata* and PEEMA, CEMA, EEMA and AEMA for *Morus alba*

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