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Original Article

Anti-snakevenom Activity of *Mesua ferrea* Linn. against the Venom of Saw Scaled Viper (*Echis carinatus*)

B. Sivakumar*, K. Koumaraveloue

Department of Pharmacy, Prist University, Trichy-Thanjavur Road, Vallam, Tamil Nadu, INDIA.

ABSTRACT

Background: Traditionally *Mesua ferrea* Linn. used in ethnomedicine for the treatment of various diseases, The leaves and flower are uses as antidote for snake poisoning. The anti-snakevenom activity has not yet reported scientifically. **Objectives:** The designed study is to proof the anti-snakevenom potential of the leaves of aqueous alcoholic extract of *Mesua ferrea* Linn. by *in vitro* enzymatic inhibition followed by neutralization and anti-haemorhagic potential against the venom *Echis carinatus* by *in vivo*. **Materials and Methods: Extraction:** The leaves of *Mesua ferrea* Linn. were collected and subjected to extraction by using different solvent such as hydro alcoholic chloroform and hexane. Further the hydro alcoholic extract was selected to liquid liquid fraction with methanol, chloroform and hexane because of more yield. **Enzyme inhibition activity:** The hydroalcoholic liquid liquid methanol chloroform and hexane fractions were subjected to enzyme inhibition assay such as acetyl cholinesterase, protease, albumin denaturation and anticoagulation activity. **Neutralization potential:** Female mice were divided into four groups (*n*=6, 1 disease control group, 1 normal control, 1 standard control and 1 treatment group). All the mice except Normal control group were injected intraperitoneally with 2.21 mg/kg (LD_{50} dose) of *Echis carinatus* venom in 0.2mL PBS. **Anti-haemorhagic Activity:** Experimental animals were sorted into four groups of 4 animals per group. Animals in Group I and Group II was given intradermal injection of saline and 3µg of *Echis carinatus* Venom (i.e. 3MHD dose*), respectively. In Group III, as a positive control 1:100 w/w Anti-snakevenom (ASV)(mg of ECV/mg of ASV) were administered. For Group IV, the test sample (200mg/kg) was incubated with 3µg of *Echis carinatus* Venom for 30 min and injected intra-dermally. **Results:** The hydroalcoholic liquid-liquid chloroform fraction was shown the good enzymatic inhibition when compared to other fraction and the survival time of animals was increased in the group administered with test extracts was calculated as survival percentage of animals. Similarly the haemorrhagic lesion is reduced in the group administered with test sample. **Conclusion:** The data obtained from this study, it is concluded that the aqueous alcoholic extract of *Mesua ferrea* Linn. has shown the anti-snake venom potential against the venom *Echis carinatus*.

Keywords: *Mesua ferrea* Linn., Anti-snakevenom, Saw scaled viper, *Echis carinatus*, Neutralization potential.

Correspondence:
Dr. Sivakumar B
Research Scholar, Department of Pharmacy, Prist University, Trichy-Thanjavur Road, Vallam-613403, Tamil Nadu, INDIA.
Email: sivapharm003@yahoo.co.in

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INTRODUCTION

Globally snake bite is an public health problem and according to world health organization it is a neglected tropical disease.[1,2] The severe local manifestations are associated with snake bites are blistering, skin damage, inflammation, haemorrhage, coagulopathy and progressive tissue necrosis at the bitten site.[3,4] Even so these local manifestations are nevertheless condition of pathological brought by a mixture of toxin rather than individual toxin present in the venom.[4] Anti-snakevenom is the only treatment against snake bite. On the other hand the anti-snakevenom administration induced progressive tissue necrosis.[5,6] The challenging issue for treating progressive tissue necrosis is still a tough condition for the strategies existing for snakebite management. The direct involvement of metzinicin family matrix degrading snake venom metalloproteases (svmps) revealed clearly by viper bite induced progressive tissue necrosis.[4-8] and hyaluronidase (svhys).[9,10] Saw scaled viper *Echis carrinatus* (EC) is the only echis species found in India.[11] The bite of echis causes severe local manifestation, Due to the high content of zinc (Zn++) dependent smps it is responsible for 10-20% mortality.[12-15] It releases the nuclear and granular content in the extracellular space by activating the neutrophils leading to Neutrophils Extracellular Traps (NETs). It is described as NETosis.[16] The entrapment off venom toxins by the NETs released from neutrophils and as a result tissue necrosis due to the blockage of blood vessel.[16] However the mechanism involved

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in cellular level is unclear in formation of NETs because of venom toxins. The degradation of Extracellular Matrix (ECM) proteins resulting in haemorrhage at the site of injection is a well-known pathophysiology due to the SVMPs of ECV which is already demonstrated.[4,8,17] Scientifically many reports explained the SVMPs direct involvement in damaging the tissue architecture by ECM protein degradation.[7,18,19] These haemorrhagic SVMPs acting on the membrane basement and damage capillary walls leads to extravasation[7,18-20] Further scientific evidences revealed that the disrupt in micro vessel is intervened by type IV collagen degradation because of the action of SVMPs [7,20] The MMPs and ADAMs are the metzinicin family proteases which have the similar structural domains and catalytic site architecture like SVMPs.[21] The activation of MAPKs by MMPs through activated Protease Receptor (PAR)-1 has shown in many reports.[22]

Since MMPs are catalytically related to SVMPs and it is assumed that the NETosis and intracellular signalling cascade via PAR-1 is induced by SVMPs of Echis carrinatus. So the severe tissue necrosis due to the NETosis which is mediated by PAR-1-ERK signalling axis due to the SVMPs present in the venom of *Echis carinatus.*[23] Many alternative therapies used for neutralizing the snake venom induce tissue damage. The Neutralising abilities of ZN++ specific chelators are successfully demonstrated against the tissue damage induce by snake venom.[4] 2,3 dimrcapto-1-propane sulfonic acid used for the therapeutic intervention of remodel drug used against haemotoxic snake bite.[24] For inhibition of SVMPs many chelating agent have been studied extensively and are pharmacologically approved.[25,26] Because of their non-specific chelation property many molecules are fail to reach the clinical trial[27] So the tissue damage and necrosis caused by the venom of *Echis carrinatus* is highly challenging to treat. All the alternative therapies are done based on the hypothesis, even so ASV also. Because of highest complexities and immunogenic reaction the envenomed person continue to suffer throughout the life time. Plant based medicines are used as antidote from many years for the treatment of snake bite which do not produce any immunogenic and complex reaction and many of the plants has been proofed scientifically towards different types of snake bite. Among that the snake belongs to viperidae family *is Echis carinatus* generally called as saw scaled viper which contains the highly toxic venom when compared to all other snakes. The haemotoxicity, cell damage, and necrosis even after the treatment of anisnake venom is common due to the high content of SVMPs. In this study the plant *Mesua ferrea* Linn. is demonstrated for the neutralization of the venom of *Echis carinatus* by enzyme inhibition assays and neutralization potential and antihaemorhagic activity against the venom of *Echis carinatus.*

MATERIALS AND METHODS

Venom Collection

The lyophilized venom of *Echis carinatus* where collected from sigma Aldrich, India, and it was stored in 20°C.

Plant Collection and Extraction

The plant was collected from the foot hills of Western Ghats of Palakkad district from Kerala. The plant specimen was identified and authenticated by the Government Arts and Science College Ooty, The Nilgiris, Tamil Nadu. The leaves were washed, shade dried, powdered and stored in airtight container for future use. 50 g of powdered leaves were extracted with aqueous alcohol in a soxhelet extractor by continuous hot percolation method. Extracts were concentrated by rotary vacuum evaporator and the residue obtained was dried, weighed

Animals and Ethical Committie Approval

Adult Swiss albino mice (8 to 9-week-old female) weighing 20–25 g were obtained from the Central Animal House Facility, Skanda life sciences, Bengaluru, India. The animal experiments were approved by the Institutional Animal Ethical Committee, Skanda life sciences (Organization), Bengaluru, India (Approval number: IAEC-SLS-2021-039), for the purpose of venom neutralization potential in test sample in envenomed mice (LD_{50}). During all experiments, animal care and handling were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Extraction

The plants were subjected to soxhelet extraction by using three different solvents such as hydroalcoholic, chloroform and hexane. The final extract were collected and it is further subjected to different evaluation.

Liquid Liquid Extraction

The Hydroalcoholic crude extract were dissolved in 10mL of Methanol (Initially-500 mg/10mL) was used for liquid-liquid partition chromatography. Chloroform, Methanol and Hexane soluble fractions were separated using separating funnel.

In vitro Enzyme Inhibition Assays

Acetylcholinnesterase inhibition assays

The AChE enzyme was incubated with various concentrations of test (40, 80, 160, 320, 640, 1280 µg/mL) compounds and incubated for 5 min. Post incubation, 100 µM Acetythiocholine iodide was added to each Microtiter well. The contents were further incubated for 5 min. After incubation, 180µL of DTNB reagent from the stock of 10mg/mL was added. The kinetic absorbance was measured at 2nd and 10th min at 412nm.[28]

Protease inhibitory Activity

Protease inhibitor activity was assayed according to the method of Universal Protease Activity Assay: Casein as a substrate with slight modifications and soya extract used as standard. 100 µg of venom was pre-incubated with 50 µL of a suitable dilution of the sample (40, 80, 160, 320, 640, 1280µg/mL) at 37°C for 10 min. To the above mixture 500 µL of 0.65% Hammerstein casein (SRL, India) (prepared in 50 mM phosphate buffer) was added and incubated at 37°C for 10 min. The reaction was terminated by the addition of 500 µL of 110 mM Trichloroacetic Acid (TCA) solution and incubated at 37°C for 30 min. The precipitated protein was removed by centrifugation at 10,000 rpm for 15 min. To 200 µL of supernatant 500 µL of 500 mM sodium carbonate was added. Immediately 100 µL of Folins reagent is added. The absorbance was measured at 510 nm in Spectromax i3X with appropriate blanks. The protease inhibitory activity was expressed in terms of percent inhibition.[29,30]

Albumin Denaturation Inhibitory Activity

The test was performed following the method described by Gambhire *et al.* (2009) with slight modifications. 24 µL of fresh egg albumin was pre-incubated with 20 µL of a suitable dilution of the sample at 37°C for 15 min. To the above mixture 556 µL of 1X phosphate buffer (pH-6.3) was added, mix well. Denaturation was induced by adding venom (100µg). The turbidity was measured using a spectrophotometer at 660 nm against appropriate blanks. The albumin denaturation inhibitory activity was expressed in terms of percentage inhibition.[31]

Anticoagulation Assay

To study the plasma clotting activity, goat blood obtained from a slaughter house was collected in 3.8% tri-sodium citrate. The Platelet Poor Plasma (PPP) was prepared by centrifuging the blood twice at 5000 rpm for 20 min at 4°C. A different concentration of test sample was pre-incubated with 300µL of PPP for 3 min at 37°C, and adding 100µL of snake venom (100ug/mL). For control, PPP incubated with PBS before treatment with test sample and incubation for 3 min and add 50ul of $Cacl_2$. One unit of anti-coagulant activity has been defined as test sample induced 1 s increase in clotting time of the control PPP.[32]

In vivo efficacy for anti-snakevenom activity

Neutralization Potential of Test Sample against the Percentage increased In Survival Rate of Envenomed Mice (LD_{50}).

Mice housing and procurement

Twenty four female mice (Swiss albino, 8–10 weeks old, *n*=6), weighing 22–25 g, was procured for the study. Animals were group-caged housed by dose. The temperature of the experimental animal room were maintained at 22°C (+ 3°C) with relative humidity 50-60%. Artificial lighting was in sequence of 12 hr light, 12 hr dark. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water. The animals were kept for quarantine for 7 days, followed by acclimatization for 5 days in study condition.

Venom Administration

24 mice sorted into 4 groups (4 groups- 1 disease control group, 1 normal control, 1 standard control and 1 treatment group) of 6 animals per group. All the mice except Normal control group were injected intraperitoneally with 2.21 mg/kg (LD_{50} dose) of *Echis carinatus* venom in 0.2mL PBS.

Treatment

30 min post venom administration, mice in group 3 and group 4 was given intraperitoneal injection of Anti-snake venom (ED_{50}) (0.0086 mL/g)) and Test sample (200 mg/kg), respectively. Mice in group 1 and group 2 were given Normal saline.

Following treatment animals was observed for mortality for a period of 24 hr. The survival time and mortality was recoded for each animal.

Mice housing and procurement

Sixteen female mice (Swiss albino, 8–10 weeks old, *n*=4), weighing 22–25 g, was procured for the study. Animals were group-caged housed by dose. The temperature of the experimental animal room was maintained at 22°C (+ 3°C) with relative humidity 50-60%. Artificial lighting was in sequence of 12 hr light, 12 hr dark. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water. The animals were kept for quarantine for 7 days, followed by acclimatization for 5 days in study condition.The back of the animals were shaved one day prior to the study.

Haemorrhagic Activity

Experimental animals were sorted into four groups of 4 animals per group. Animals in Group I and Group II was given intradermal injection of saline and 3µg of *Echis carinatus* Venom (i.e. 3MHD dose*), respectively. In Group III, as a positive control 1:100 w/w ASV (mg of ECV/mg of ASV) were administered. For Group IV, the test sample (200 mg/kg) was incubated with 3µg of *Echis carinatus* Venom for 30 min and injected intra-dermally.

Terminal Procedure

After a time interval of 3 hr, animals was sacrificed, their skin removed (Injection site), and the size of the haemorrhagic lesion on the inner side of the skin was measured by vernier caliper.

Neutralization was expressed as ED_{50}, i.e., the volume of the venom/anti-venom or test sample ratio, in which the size of the haemorrhagic spot is reduced by 50%.

(*Minimum Haemorrhagic Dose (MHD), defined as the dose of venom that induces a haemorrhagic area of 10 mm diameter. The MHD for *Echis carinatus* Venom is 1 µg).[33]

RESULTS

The neutralization potential of test samples in envenomed mice evaluated and the results are represented in terms of % survival rate. It was observed that the mean survival time in group administered with venom alone is 13.05 hr and 21.40 hr in group tested with polyvalent anti-snake venom. Whereas, in the group tested with test sample has shown mean survival time of 19.03 hr. Survival % of animals was calculated and animals treated with test sample has shown 66.66% survival, animals tested with polyvalent anti-venom and venom alone has shown the % survival of 83.33% and 33.33% respectively.

The neutralization potential of test samples in hemorrhagic activity in mice evaluated and the results are represented in terms of hemorrhagic activity. It was observed that the minimum hemorrhagic dose in group administered with ECV Control is 10.15±0.77 in mm. whereas in the group tested with test sample has shown MHD is 6.17±0.45 in mm. Animals tested with polyvalent anti-venom of MHD is 4.25±0.31 in mm respectively.

DISCUSSION

Antivenom is considered the standard treatment for snakebites. However, a detailed mechanism of action of snake antivenom is essential to understanding its potential and limitations. Polyclonal immunoglobulins administered intravenously bind to snake venom in the circulation. Antibody binding blocks active site interactions between toxins and target tissues. Second, antivenom-venom complexes remain in circulation due to their massive molecular mass and block the distribution of toxins to peripheral tissues. Third, the production of anti-venom-venom complexes promotes the removal of toxins from the circulation by the reticuloendothelial system[27] Therefore, early administration of antivenom is important to stop venom distribution to peripheral sites.[34]

More than a hundred different antivenoms are used for coagulopathic poisoning of snakes throughout the world. Despite its therapeutic potential, anti-venom can cause life-threatening anaphylaxis.[35] Many deaths due to antivenin still occur, but are underreported for a variety of reasons. Therefore, it is crucial to explore the available evidence to establish the effect of the antivenom.[34]

The common cause of viper bites is the severe necrosis at the local tissue of the bitten site[36] followed by it continues their complication such as coagulopathy and systemic haemorrhage which tends to lead forward hypoxia followed by multiorgan failure and death[37,38] The major toxin present in ECV and most viper venoms

Table 1: Yield summary after crude extraction.

Sample	Sample Taken for extraction	Solubility	Yield	Percentage yield
Leaves of *Mesua ferrea* Linn. (In powder form)	50g	Hydro alcoholic	1200mg	2.4%
	50g	Chloroform	500mg	0.1%
	50g	Hexane	360 mg	0.072%

Table 2: Yield summary liquid-liquid fraction.

Sample	Sample Taken for extraction	Yield
LLF MeoH	1800 mg	450mg
LLF Chloroform		899mg
LLF Hexane		50 mg

Table 3: Acetylcholinesterase inhibition activity of standard.

Donepezil concentration(µg/mL)	Venom ACHE (units/mL)	%	IC_{50}(µg/mL)
0	4929.98	0.00	13.62
3.12	4009.70	18.67	
6.25	3437.41	30.28	
12.5	2610.49	47.05	
25	2130.34	56.79	
50	1676.87	65.99	
100	1178.54	76.09	

Table 3a: Albumin denaturation inhibitory activity of standard.

Sample	Conc	OD @ 510nm	% Inhibition	IC_{50} (µg/mL)
C	0	1.0910	0.0	0
Indomethacin	0.3125	0.9460	13.3	1.99
	0.625	0.7207	33.9	
	1.25	0.6500	40.4	
	2.5	0.5350	51.0	
	5	0.3480	68.1	
	10	0.2200	79.8	

Table 3b: Albumin denaturation inhibitory activity of Hydro alcoholic liquid liquid methanol, hexane and chloroform fraction.

C	0	0.654	0.0	0.37
HALLF Chloroform	0.02	0.56061	14.3	
	0.04	0.502029	23.2	
	0.08	0.384867	41.2	
	0.16	0.351756	46.2	
	0.32	0.272799	58.3	
	0.64	0.086868	86.7	
HALLF MeoH	0.02	0.612908	6.7	0.29
	0.04	0.550167	16.9	
	0.08	0.479531	28.5	
	0.16	0.395904	42.1	
	0.32	0.270931	62.5	
	0.64	0.078887	93.8	
HALLF Hexane	0.02	0.5900	10.9	-
	0.04	0.5591	16.1	
	0.08	0.5288	21.2	
	0.16	0.4974	26.5	
	0.32	0.4842	28.8	
	0.64	0.4753	30.3	
	1.2	0.4284	38.2	

Table 3c: Anti-coagulation activity of standard EDTA.

EDTA µg/mL	Time (s)	Unite Increase in time of anticoagulation
0	20	0
3.125	26	6
6.25	32	12
12.5	41	21
25	54	34
50	65	45
100	78	58

Table 3d: Anti-coagulation activity of HALLCF.

HALLCF extract (mg/mL)	Time (s)	Unit Increase in time of anticoagulation
0	20	0
0.04	31	11
0.08	39	19
0.16	55	35
0.32	64	44
0.64	70	50
1.2	82	62

Table 3e: Anti-coagulation activity by HALLMF.

HALLMF extract (mg/mL)	Time (s)	Unite Increase in time of anticoagulation
0	20	0
0.04	20	0
0.08	20	0
0.16	21	1
0.32	23	3
0.64	25	5
1.2	28	8

Table 3f: Anti-coagulation activity of HALLHF.

HALLHF extract (mg/mL)	Time (s)	Unite Increase in time of anticoagulation
0	20	0
0.04	20	0
0.08	20	0
0.16	20	0
0.32	20	0
0.64	21	1
1.2	23	3
2.4	24	4

Table 3g: Treatment groups for the study of neutralization potential of the plant extract against the venom *Echis carrinatus*.

Group	Treatment	Dose (µg/mice)	No. of animals
G1	Normal Control	Normal saline	6
G2	Disease Control	*Echis carinatus* Venom (2.21mg/kg)	6
G3	Standard control	ECV+ Anti-snake venom (ED_{50})	6
G4	Test compound	TS-1 (200mg/kg)	6
No. of Animals per study			24

Table 3h: Neutralization potential of test sample on the % increase in survival rate in envenomed mice.

Groups	Treatment	N	Survival time (24 hr)	% Survival	No of mortality
G-I	Normal control	6	-	100	0
G-II	Venom control	6	13.05	33.33	4
G-III	Standard control	6	21.40	83.33	1
G-IV	Treatment group	6	19.03	66.66	2

Table 3i: Treatment groups for antihaemorhagic activity of the test extract against the venom *Echis carrinatus*.

Group	Treatment	Dose (µg/mice)	No. of animals
G1	Normal Control	Normal saline	4
G2	Disease Control	*Echis carinatus* Venom (3µg)	4
G3	Positive control	Anti-snake venom (1:100 w/w)	4
G4	Test compound-1	Test sample (200mg/kg)	4
No. of Animals per study			16

Table 3j: Neutralization potential of test sample of *Echis carinatus* venom induced haemorrhagic activity in mice.

Group	Treatment	MHD (in mm) mean±SD
I	Normal saline	0
II	*Echis carinatus* Venom (3 µg)	10.15±0.77
III	ECV + Anti-snake venom (1:100 w/w)	4.25±0.31
IV	ECV + Test sample (200 mg/kg)	6.17±0.45

are SVMPs, in which they preferentially acting on extracellular matrix components that are responsible for haemorrhagic activity.[7-8,19,20,4] It is the attribution of viper bites toward SVMPs leads to induce progressive tissue necrosis because of particular action on PIII class metalloproteases. Naturally SVMPs are Haemotoxic and interfere with Haemostatic system of the victim of the snake bites[39] Disintegrin and Metalloproteinases (ADAMS) Are in close relationship with SVMPs. So they are called as snake venom ADAMs[40,41] The components of SVMPs are disintegrin –like (D), Cysteine rich ©, metalloproteinase (M) domain and ADAMDEC that harbor's putative zinc (Zn++)binding sequence which have the similar morphological structure of the metzinicin family of MMPs[21,41-43] The restricted catalytic activity of SVMPs towards M.domain and Zn++ on the ADAMDEC-1 plays a major role in their enzymatic activities.[21,41]

Because of this many anti-isnake venom failure to neutralize the enzymatic activities of the venom of *Echis carrinatus*. Due to the lack of placebo controlled randomised evidence, no studies were available to conduct meta analysis. Cochrane review s concluded that there is a lack of placebo controlled randomised evidence to give antivenom for VICC.[44] In order to debilitate the action of SVMPs, A specific Zn++ chelators to chelate the Zn++ metal ions from SVMPs, was introduced as an new theraphy towards the management of local tissue necrosis effectively induced by viper venoms.[33] TTD and an derivative of Dimercaprol antagonize the activity of Zn++ dependent SVMPs. which neutralize the action of Saw scaled viper venom.[33] These are the repurposed drug to treat toxicity induced by *Echis carinatus* venom that are mediated by SVMPs. On the other hand the side effects of chelating therapy is unstoppable such as fever and chills headaches nausea and vomiting diarrhoea and more serious side effects may include seizures, drop in pressure, respiratory failure, low, irregular heartbeat, severe allergic reactions, severe hypersensitivity, anemia, kidney damage, liver damage, loss of vitamins and nutrients.[45] Hence approaching the plant based medicine is good choice for the progressive tissue necrosis and also for haemorrhagic disorders followed by coagulopathy. In this study the *Mesua ferrea* Linn. leaf was initially subjected for the extraction with different solvent like hydroalcoholic, chloroform and hexane and its yield percentage was reported in Table 1.

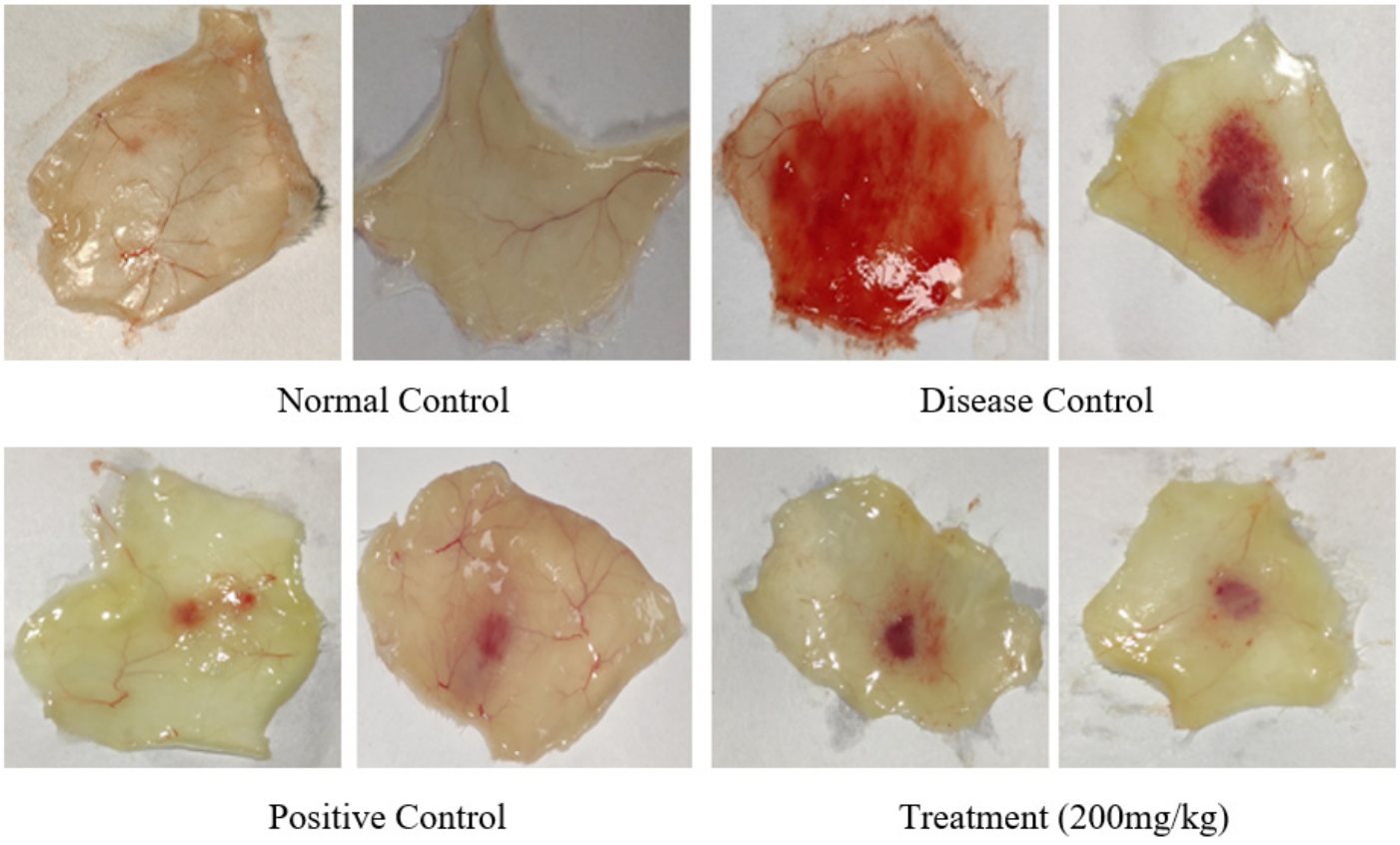

Figure 1: Haemorrhagic activity of ECV venom and its neutralization of test sample. Dorsal surface of the skin showing haemorrhagic spot.

Table 4: Acetylcholinesterase inhibitory activity of hydroalcoholic liquid liquid methanol, hexane and chloroform fraction.

Sample	Conc	OD @ 510nm	% Inhibition	IC_{50} (µg/mL)
C	0	4321.46	0.0	743
HALLCF	0.4	3730.53	13.7	
	0.8	3184.41	26.3	
	0.16	2715.75	37.2	
	0.32	2372.19	45.1	
	0.64	2149.77	50.3	
	1.2	1489.41	65.5	
HALLMF	0	4321.46	0.0	IC_{50} is not calculated due to lesser inhibition.
	0.4	4245.63	1.8	
	0.8	3915.74	9.4	
	0.16	3824.41	11.5	
	0.32	3533.63	18.2	
	0.64	3265.26	24.4	
	1.2	2915.38	32.5	
C	0	4321.46	0.0	IC_{50} is not calculated due to lesser inhibition.
HALLHF	0.4	3993.263	7.6	
	0.8	3854.936	10.8	
	0.16	3631.717	16.0	
	0.32	3558.274	17.7	
	0.64	3472.839	19.6	
	1.2	3289.508	23.9	
	2.4	3199.746	26.0	

Further the hydroalcoholic extract was subjected to liquid liquid fraction Iike hydroalcoholic liquid liquid chloroform and methanol and hexane fraction along with the yield was shown in the Table 2.

These fraction were subjected to different enzyme inhibition study such as acetycholinesterase, protease, albumin denaturation and anticoagulant activity was summarised in Tables 3, 4, 5 and supplementary Table 3a, 3b, 3c, 3d, 3e, 3f.

By compiling all the above results the plant *Mesua ferrea* Linn. was shown the good enzyme inhibition activity particularly the hydroalcoholic liquid liquid chloroform fraction. On the other side the crude hydroalcoholic was subjected to *in vivo* evaluation for the neutralization potential, and anti-haemorhagic activities against the venom *Echis carinatus* in envenomed mice was evaluated and the results are represented in terms of % survival rate. The animal groupings and its neutralization potential is shown in supplementary Table 3g, 3h. It was observed that the mean survival time in group administered with venom alone is 13.05 hr and 21.40 hr in group tested with polyvalent anti-snake venom. Whereas, in the group tested with test sample has shown mean survival time of 19.03 hr. Survival % of animals was calculated and animals treated with test sample has shown 66.66% survival, animals tested with polyvalent anti-venom and venom alone has shown the % survival of 83.33% and 33.33% respectively.

The neutralization potential of test samples in haemorrhagic activity in mice evaluated and the results are represented in terms of haemorrhagic activity. The grouping of animals and its neutralization potential is shown in supplementary Table 3i, 3j and Figure 1 It was observed that the minimum haemorrhagic dose in group administered with ECV Control is 10.15±0.77 in mm. whereas in the group tested with test sample has shown

Table 5: Protease inhibitor activity for standard and test sample.

Sample	Conc	OD @ 510nm	% Inhibition	IC_{50} (µg/mL)
C	0	1.2860	0.0	20
Soya extract	0.3125	1.1410	11.3	1.46
	0.625	0.9157	28.8	
	1.25	0.8450	34.3	
	2.5	0.7300	43.2	
	5	0.5430	57.8	
	10	0.4150	67.7	
C	0	0.849	0.0	IC_{50} was not calculated due to lesser inhibition.
HALLMF	0.4	0.756	11.0	
	0.8	0.703	17.2	
	0.16	0.670	21.1	
	0.32	0.656	22.7	
	0.64	0.578	31.9	
	1.2	0.528	37.8	
HALLCF	0.4	0.8079084	4.84	717
	0.8	0.7451673	12.23	
	0.16	0.6745305	20.55	
	0.32	0.590904	30.4	
	0.64	0.4659312	45.12	
	1.2	0.2738874	65.74	
HALLHF	0.4	0.840	1.09	IC_{50} was not calculated due to lesser inhibition.
	0.8	0.810	4.59	
	0.16	0.783	7.81	
	0.32	0.776	8.60	
	0.64	0.742	12.66	
	1.2	0.726	14.47	
	2.4	0.678	20.10	

MHD is 6.17±0.45 in mm. Animals tested with polyvalent anti-venom of MHD is 4.25±0.31 in mm respectively.

CONCLUSION

Based on the results of different evaluation hence it is concluded that the plant *Mesua ferrea* Linn. contains the effective antivenom phytochemical against the venom of *Echis carinatus*. It shows protection against haemorrhagic bleedings and histotoxic poisons towards tissues by neutralizing the haemotoxic and neurotoxic enzymes. Hence the plant *Mesua ferrea* Linn. is highly recommended for the treatment for the envenomation by the snake *Echis carinatus*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The specific treatment against snake bite is the administration of the anti-snakevenom. Due to the action of nonimmunoglobulin proteins at high concentration at commercially available hyper immune anti-venom which lead to anaphylactic shock, pyrogen

reaction and serum sickness. Most of the anti-snakevenom failed to neutralize the enzymes present in the venom *Echis carinatus*, because it has the restricted catalytic activity of SVMPs towards M Domain and Zn++ on the ADAMDEC-1 plays a major role in enzymatic activities. Since pharmaceutical industries are producing the antibody against the venom of *Echis carinatus* and is not enough to neutalize the enzymes present in the venom of *Echis carinatus*. Since the plant showing enough potential against the neutralization towards the venom of *Echis carinatus* and will be the good choice and safest medicine inorder to avoid the hyper immune reaction and other serious complication from the marketed antibodies.

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