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Papaya Leaf Having Anti-Snake Venom Property: A Review

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ABSTRACT: ***Traditional systems of medicines are being utilized by a large number of rural people for several ailments. Among the major cause of concern for them are the occurrences of snake bites leading to tissue injury or even death. The recommended mode of treatment for snake bites is the World Health Organization's recommended anti-snake venom serum, commonly known as the anti-dote. The anti-dote or anti-snake venom serum though is freely available at government health care centres yet is marred by lack of specificity, inept handling and storage due to which anti-dotes which are universal for all types of snake venom and can be stored at room temperature are most required. In India, most of the venomous snakes belong to the Viperidae & Elapidae families. In this paper, venom of the Flavanoids has been reported to have anti-snake venom activity due to which the leaf of papaya (Carica papya) was selected for its high flavonoid content. In the present study, the effective concentration 50 (EC_{50}) of the Saw-scaled viper (Echis carinatus) was determined on sheep's plasma at 5.45 µg/mL and the aqueous extract of papaya leaf was found efficient to prevent proteolysis. Next, the minimum coagulant dose (MCD) was calculated at 5.54 µg/ml for sheep's plasma and the aqueous extract of papaya leaf was seen to delay venom induced plasma clotting. An analysis of these results indicate that papaya leaf can be used as a source of anti-snake venom.***

KEYWORDS: ***Antidote Or Anti-Snake Venom, Aqueous Extract Of Carica Papaya, Clotting Time, Effective Concentration, Sheep's Plasma, Minimum Coagulant Dose (MCD), Proteolysis, Saw-Scaled Viper (Echis Carinatus).***

1. INTRODUCTION

Snakebite is one of the most neglected tropical diseases (NTD) plaguing the globe as of now. The World Health Organization (WHO) has estimated that roughly 3 million annual instances of pathological illness following snake bite, with about 5 lakhs of them resulting fatalities or lifelong disability. India is a significant area for snakebites, where approximately 50 thousand deaths and 140 thousand illnesses occur from snakebites annually. In India, the government authorized antidote is a polyvalent antivenom, specific for the 'major four' snake species, namely, the spectacled cobra or Naja naja, saw-scaled viper or Echis carinatus, Russell's viper or Daboia russelii and the common krait or Bungarus caeruleus. Most of the poisonous snakes in India belong to the Elapidae and Viperidae families as listed in Table1. However, the anti-dote created is not unique to any one of these 4 kinds of snakes and nor are they particular for the other poisonous snakes who do not fall under the "big four" thus the need of the hour is to develop low cost anti-dotes which are specific for all the venomous snakes.

Table 1: Venomous snakes commonly found in India. Most of them are from the Elapidae and Viperidae family. The need of the hour is to prepare an antidote against the venom of snakes from both these families[1].

Family	**Common name**	**Snake species**
Elapidae	Indian spectacled cobra	*Naja naja*

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	Krait	*Bungarus caeruleus*
	King cobra	*Ophiophagus hannah*
	Monocellate cobra	*Naja kaouthia*
	Central Asian cobra	*Naja oxiana*
Viperidae	Russell's viper	*Daboia russelii*
	Saw-scaled viper	*Echis carinatus*
	Sochureki's saw-scaled viper	*Echis sochureki*
	Malabar pit viper	*Trimeresurus malabaricus*

The venom of snakes contains a complex brew of enzymes that includes metal ions, peptides, non-enzyme proteins, lipids, amines, carbohydrates, and procoagulants. The venom employs cytotoxic, hemotoxic and neurotoxic properties. Although full of disadvantages, anti-dote treatment for snake bites is the only authorised method.

1.1 Anti-dote and its limitations:

The anti-serpent poison Anti-dote is a brew of antibodies made primarily from horses who are both hyper-immune to the venom of a species (monovalent) and venom of numerous variant species (polyvalent). Administering ASV in people can unfortunately cause adverse reactions such as pyrogenic reactions due to contamination by endotoxins, serum sickness, hives and pruritus, and these events range between 2 and 75% [Table 2][2].

Table 2: Major side effects owing to administration of anti-snake venom anti-dote. Though, the anti-dote is the only approved mode of treatment for snake bites yet is marred by many side-effects[3].

Rash, Urticaria, itching, abdominal colic, dry cough, nausea, fever, hypotension, tachycardia.	Type 1 Reactions
Vasodilation, rigor, fever, hypotension.	Type 2 Reactions (pyrogenic reactions)
Monneuritis multiplex, nauseas, vomiting, fever, itching, recurrent urticarial, myalgia, proteinuria with immune complex nephritis and rarely encephalopathy.	Late (serum sickness type) reactions

1.2 Anti-snake venom property of phyto-molecules:

Snake venom includes such components as phosphorus-lipase A2 (PLA2), serpentine venom metalloproteinases (SVMPs) and snake venome hyaluronidases (SVHYs), which include all associated harms. [Tables 3] have indicated the role of phyto-molecules in inhibiting the components of the following venome. [Table 4] shows manmade compounds that have activities against the above-mentioned venom componenets[4].

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Table 3: Plant metabolites useful against snake's phosphor-lipase A_2 (PLA_2). Lysophospholipids and free fatty acids are generated due to the hydrolysis of the sn-2 acyl bond of glycerophospholipids which is catalysed by the Snake PLA_2 in a calcium-dependent manner thereby exhibiting a wide variety of toxic effects[5].

Chemical Form	**Plant race**	**Compound name**	**Genera of Snake**
Protein	*Withania somnifera*	Glycoprotein (WSG)	*Naja naja, Daboia russelii*
	Curcuma longa L	Tumerin	*Naja naja*
Flavonoid	*Pueraria thunbergiana*	Tectoridin	*Naja naja*
	Asperagus, Rheum	Rutin, Curcumin	*Vipera russelii*
Pterocarpus	*Cabeca-de-negro*	Cabenegrins (A-I & A-II)	*Vipers*
	Harpalcye brasiliana	Edunol	
Alkaloid	*Aristolochia radix*	Aristolochic acid	*Vipera russelii*
	Cardiospermum sp	Berberine	*Vipera russelii*
Sterols	*Eclipta prostata*	Stigmasterol, Sitosterol	*C.d.terificus*
Coumestan	*Eclipta prostata*	Wedelolactone	*C.d.terificus*
Polyphenols	*Cordia verbenaceae*	Rosmarinic acid Chlorogenic acid	*Bothrops pirajai Vipera russelii*
Terpenoids/Saponins	*Betula alba, Piper sp*	Clerodane, Betulinic acid	*Naja nigricolis*
Glucosides	*Acalypha*	Acalyphin	*Vipera russelii*

Table 4: Tabulation of artificial agents effective against the main hydrolytic enzymes found in the snake's venom. Artificial agents who have been reported to act as inhibitors of snake venom hyaluronidases (SVHYs), Snake venom metalloproteinases (SVMPs) and against snake's phosphor-lipase A_2 (PLA_2)[6].

Enzyme	**Compound**	**Snake**
Inhibitors of SVMPs	Elaidoylamide	*Vipera sp*
	d2-isoxazoline	*Vipera russelii*
	Indoles, Acenaphthenes	*Viper venom*
	Doxycycline	*Bothrops asper*
	Marimastat	*Bothrops asper*

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Inhibitors of PLA_2	Benzoyl phenyl benzoate derivatives	*Naja melanoleuca*
	Suramin	*Naja melanoleuca*
	Thio-ether amide having phospholipid analogs	*Naja naja naja*
	1,2,3-triazoles	*Lachesis muta*
Inhibitors of SVHY	Benzoyl phenyl benzoate derivates	*Naja melanoleuca*

Moreover, several flavonoids have been found to have anti-snake venom action and since Papaya leaves (Carica papaya) is high in flavonoids thus it has been studied for its anti-snake venom potential.

2. LITERATURE REVIEW

Timothy Omara in his study discuss about that some plants employ the activities of different snake venom to oppose. This review aims at identifying antiviral herbs and certain pharmaceutical data to support their usage in Uganda. A interdisciplinary literature study found that 77 plant species from 65 genera and 42 families are utilised for snakebite therapy in Uganda. The bulk of these species are Fabaceae (31%), Euphorbiaceae (14%), Asteraceae (12%), Amaryllidaceae (10%) and Solanaceae (10 percent). Shrubs (41%), trees (33%) and plants constitute the major growth habit of the species (18 percent)[7].

O. Kadiri et al. in a study discloses fact that snakebite envenoming coexists with bacterial, viral, and parasitic NTDs in many rural contexts indicates patterns of comorbidity that are susceptible to integrated management. There are several features of snakebite control that are similar to the principal aspects used to combat the infectious NTDs, indicating that incorporation of snakebite prevention, treatment, and rehabilitation resources into the strategies to fight NTDs would offer a great health benefit to vulnerable communities. For example, encouraging the wearing of appropriate shoes, the use of a torch after dark, sleeping under an insecticide-impregnated bed net, and speeding patient transport to medical care in remote areas using trained volunteer motorcyclists are all effective in reducing the incidence of snakebite. These methods would also decrease the burden of soil-transmitted helminthic diseases, tropical and Buruli ulcer, podoconiosis, malaria, and kala-azar and other vector-borne illnesses. Most of the main components of the WHO Global Plan to Combat Neglected Tropical Diseases 2008–2015 also apply to decrease the incidence of snakebite envenoming[8].

J P Chippaux in yet another study discloses that the real overall incidence and severity of envenomations are still mostly unknown, with the exception of a few countries that are rare or documented correctly. This information is nevertheless vital if recommendations are to be established on snake bites, drug supply plans, especially antivenin, and medical professionals are to be trained in the treatment of snake-bites. No global study on snake-bite epidemiology has been conducted since Swaroop & Grab's extensive 1954 review. This article is aimed at

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bringing snake envenomation to attention by health authorities and encourages them to develop therapy procedures tailored to their needs[9].

3. DISCUSSION

3.1 Experimental Design:

Firstly, aqueous extract of papaya leaves was prepared and its anti-snake venom activity was determined on the following parameters: anti-proteolysis activity, anti-hemolytic activity and anti-clotting activity.

3.2 Reagents:

All reagents were of the best analytical grade.

3.3 Venom:

Snake venom of Saw-scaled viper (*Echis carinatus*) in a lyophilized state was sanctioned from the Alipur Zoo, Kolkata, India and was kept at 4˚C for further use. It was dissolved in 1 % Phosphate Buffer Saline (PBS) and centrifuged for 10 min at 1000 rpm and stored at 4˚C until further use. Venom concentration was expressed in terms of dry weight as reported earlier.

3.4 Snake Venom anti-dote:

Monovalent snake venom anti-dote in lyophilized form (for reference) was purchased from the Bengal chemicals in Kolkata was dissolved in 1 % PBS.

3.5 Botanical material:

The leaves of Papaya (*Carica papaya)* obtained from the local market were washed, dried (50ºC), grained and suspended in water so as to obtain an aqueous suspension. Following which, the suspensions were exposed to ultra-sonication for 120 minutes, at 25ºC, in order to isolate its secondary metabolites. The aqueous extracts were filtered with 0.22 µm sterile membrane and lyophilized[3].

3.6 Determination of proteolytic Activity of Saw-scaled viper venom:

As per earlier publications, Saw-scaled viper (*Echis carinatus*) venom's proteolytic activity was determined using sheep's plasma. Briefly, aliquots of *Echis carinatus* venom (0–10 μg/mL) were incubated along with sheep's plasma at 37∘C for 60 min in a total volume of 2 mL. An effective concentration 50 (EC_{50}) was denoted as the concentration of *Echis carinatus* venom (5.45 μg/mL) that is able to induce 50 % proteolysis.

Regarding the inhibitory experiments, an EC of *Echis carinatus* venom (5.45μg/mL) was kept at room temperature separately with various amounts of the aqueous extracts of papaya leaves and commercial anti-dote so as to determine its proteolytic activity accordingly in sheep's plasma. Positive control experiments were undertaken by incubating venom with PBS (1% v/v)[2].

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3.7 Clotting Activity:

Citrated normal sheep plasma (diluted with equal volume of PBS) was prepared accordingly and was mixed with *Echis carinatus* snake venom (0–10 μg/mL) and the clotting time was monitored using an Amelung coagulometer, model KC4A (Labcon). The concentration of venom (5.54 μg/mL) that clotted plasma in 60 seconds was considered as the minimum coagulant dose (MCD). To evaluate the inhibitory effect, the aqueous extracts of papaya leaves and the commercial anti-dote separately were kept at room temperature for 45 minutes with 1 MCD of venom (5.54 μg/mL) and then the mixture was added to plasma to record the clotting time as reported earlier[6].

3.8 Statistical Analysis:

P values of ≤ 0.05 were considered to be statistically significant as reported earlier.

The main objective of this paper is to identify whether aqueous extract of papaya leaves can act as an anti-dote against snake venom. In this regard, the anti-proteolysis and prevention of venom induced clotting both in sheep's plasma was identified[2].

3.9 Anti-Proteolysis role of the aqueous extract of papaya leaves:

Sheep's blood incubated with snake's venom yielded an effective concentration 50 (EC_{50}) at 5.45 μg/mL after 60 mins. Keeping the EC_{50} value constant for the snake venom, the percentage inhibition of proteolysis of sheep's blood in the presence of the aqueous extract of papaya leaves and the commercial anti-dote was observed with the papaya leaves extract preventing hemolysis more efficiently than the commercially available anti-dote (Figure 2A and Figure 2B)[4].

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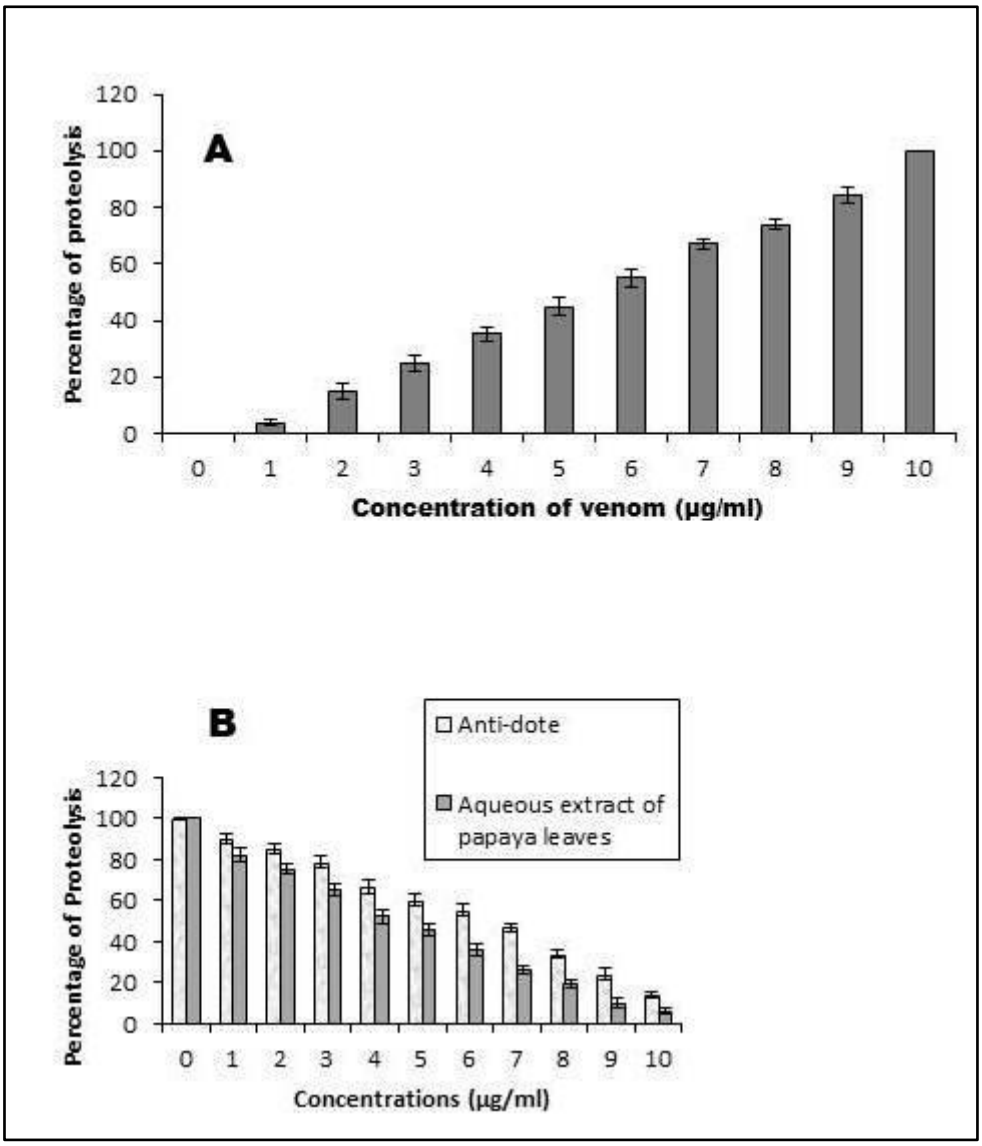

Figure 2. Effect of papaya leaves aqueous extracts and commercial Anti-dotes on proteolysis induced by *Echis carinatus* venom. (A) Effective concentration 50 (EC_{50}) of *Echis carinatus* on sheep's blood was determined at *µ*g/mL after 60 mins (B) Keeping the EC_{50} value constant for the snake venom, the percentage inhibition of proteolysis of sheep's blood in the presence of the aqueous extract of papaya leaves and the commercial anti-dote was observed with the papaya leaves extract preventing hemolysis more efficiently than the commercially available anti-dote[10].

4. CONCLUSION

Among the rural population, snake bites are a frequent cause of mortality and tissue disfiguration. Though anti-venom serum or anti-dote is accessible at the government basic health care facilities but the effectiveness of such medications is poor owing to lack of specificity, inappropriate handling and storage conditions etc. Most of the venomous snakes in India are from the India are from the Elapidae and Viperidae families. In this study, the Saw-

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Scaled Viper's (Echis carinatus) venom was selected for since this snake's venom possesses hemotoxic action. Moreover, there are reports of flavonoids having anti-snake venom action; thus, the papaya leaves (Carica papaya) were selected to examine for its anti-snake venom activity.

Sheep's blood incubated with snake's venom produced an effective concentration 50 (EC50) at μg/mL after 60 minutes. Keeping the EC50 value constant for the snake venom, the percentage inhibition of proteolysis of sheep's blood in the presence of the aqueous extract of papaya leaves and the commercial anti-dote was observed, with the papaya leaves extract preventing hemolysis more efficiently than the commercially available anti-dote. Secondly, minimum coagulant dosage (MCD) was estimated as 5.54 µg/ml for sheep's plasma. Keeping this quantity constant, the aqueous extracts of papaya leaves and the commercial anti-dote separately were maintained at room temperature for 45 minutes with 1 MCD of venom (5.54 μg/mL) and then the combination was introduced to plasma to record the clotting time. The aqueous extract of papaya leaves exhibited greater preventative activity than the commercial anti-dote by increasing the clotting time as compared to the control set.

As most of the rural people prefer to flock to traditional healers who tend to patients of snake-bites with medicinal herbs, which tend to be high in flavonoids thus papaya leaves was selected as for its common vegetable status and also being rich in flavonoids. Thereby the modest papaya leaves may possibly be utilized for big scale synthesis of anti-snake venom in the future.

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