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Study on the Anti-snake venom property of Cabbage

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ABSTRACT- Snake poison causes death and tissue disfiguration among the rural people. Though anti-dote or anti-snake venom serum is freely accessible at government health care facilities but is hampered by poor handling, storage and lack of specificity. The necessity of the hour is to find new anti-dotes which are not only inexpensive but can work against a broad variety of snake venoms. In India, most of the poisonous snakes belong to the Elapidae & Viperidae families. In this study, venom of the Flavonoids has been found to have anti-snake venom action owing to which the cabbage was chosen for its high flavonoid content. For this, initially the effective concentration of the Russell's viper was found on goat's plasma at 4.54 μg/mL and the aqueous extract of cabbage was observed to inhibit proteolysis. Next, the minimal coagulant dosage (MCD) was estimated as 3.69 µg/ml for goat's plasma and the aqueous extract of cabbage was found to delay venom induced plasma clotting. An examination of these findings suggest that cabbage may be utilized a source of anti-snake venom.

KEYWORDS- Antidote, Anti-Snake Venom, Clotting, Concentration, Minimum Coagulant Dosage

I. INTRODUCTION

In most rural areas of the globe, death or disfiguration of body parts owing to the untreated instances of snake bites is a frequent work danger. Major causes for this include inadequate rural healthcare facilities, delay in the administration of the anti-snake venom, non-specificity of the anti-snake venom (AVS) against that particular kind of snake venom. In India, more than 2 lakhs of snake bites out of which more than 35,000 instances are reported to deadly yearly. However, majority of these instances are estimated arbitrarily since not only are these cases under-reported but most of the patients are sent to traditional healers[1].

Table 1: Major side effects owing to administration of anti-snake venom (AVS)

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

Out of 3000 known species of snakes, 300 are known to be venomous. There are about 53 species of poisonous snakes in India, out of 216 species of snakes documented. Venomous snakes in India are mostly from the Elapidae or Viperidae families, including individuals including the saw scaled viper Echis carinatus, the Russell's vipers Daboia russelii, the ubiquitous krait Bungarus caeruleus, or the Indian spectacled cobra Naja naja. From the southern parts of India, Hypnale hypnale and H. nepa both as Hump-nosed pit vipers has been reported[2–4]. Snake venoms have enzymes like metalloproteases, serine proteases, phospholipases A2, etc. & the snake envenomation's pathophysiology involves a multistep series of occurrences depending upon the synergistic actions of the components of the venom.

A. Antisnake venom and its limitations

ASV is a cocktail of antibodies generated mostly by horses that have been hyper-immunized against a particular species' venom (monovalent) or a variety of species' venoms at the very same time (polyvalents) (polyvalent). Unfortunately, administering ASV to people can cause pyrogenic process [5–9] this is due to endotoxin contamination, serum sickness, urticaria, or pruritus, as well as potentially fatal anaphylaxis, with the frequency of these events ranging from 4 to 79 percent (Table 1).

Though, the AVS is the only approved mode of treatment for snake bites yet is marred by many side-effects.

B. Availability and issues in stockpiling

There are economic difficulties marketing, logistical and storage issues associated with supply and production of AVS. Storage at 0-4°C is needed to prevent the rapid degradation of liquids form of the ASV whose half-life is 2 year. However, its lyophilized form retains its efficiency till 5 years provided it's stored in a cool and dark place[10].

C. Issue of specificity

One of the main problems with ASV is the lack of Absolute specificity. Due to the vast taxonomic and geographic variety among the snake species, a substantial difference in the antigenicity and the composition of the AVS has been found which thus limits it to a geographical area exclusively [11],[12]. Interestingly, traditional healers have traditionally relied upon herbs to offer a treatment during instances of snake bites. Various new phytochemicals with anti-snake venom effects have been

identified and some of them have been summarized in Table 2 and Table 3.

However, it is acknowledged that such therapy may cause problems due to anti-venom features such as a rapid hypersensitivity reaction to foreign immunoglobulins, which can include anaphylactic or pyrogenic symptoms including chills, stiffness, headache, and tachycardia. During 8–12 days of therapy, delayed allergic venom responses or serum sickness is detected, which is characterised by inflamed skin, fever, as well as allergens, among other things; • Ineffectiveness of the anti-venom due to significant geographical distribution inside the composition of both the venom; • Antigenic reactivity due to all the taxonomic diversity of something like snakes; • Improper the use of such a anti-venom leads to errors in medical management, which either contributes significantly to a higher prevalence of allergic events. All of these plants include phytochemicals that can be utilised as anti-venom antidotes for snakes [13–16]

Table 2: Phytochemicals originated from plants which are used against snake-bites

Active principle	Plant
Amenth of lavone, Quercetin, Myricetin	Davilla eliptica, Mouriri pusa,
Alkylamides, Ketoalkenes, Polysaccahrides	Echinacea sp
Polyphenols	Pentaceburminica, Areca catechu
Ellagic acids	Casearia sylvestris
Edunol	Harpalyce brasiliana
Lupeolactate	Hemidesmus indicus
Cucurbitane glycosides, trichotetral	Trichosanthes tricuspidata
ß-sitosterol, stigma sterol	Pluchea indica
Pthalates	Vitis negundo
Tannins, steroids, Alkaloids	Delonix elata
Tannins	Guiera senegalensis
Quercetin	Alium cepa
Resverotrol	Cissus assamica
Stigmasterol, D-mannitol	Ecliptica prostrate
Aristolochic acid	Aristolchia sp
Rosmarinic acid	Cordia verbenacea
Turmerin	Curcuma longa
Glycoproteins	Withania somnifera
Amides	Strychnos nux vomica

Table 3: Following plants are being used by traditional healers as a treatment towards snake bites

Parts of plants used	Family	Plant
Whole plant	Avanthaceae	Andrographis paniculata
	Asteraceae	Eclipta alba
	Lamiaceae	Leucus aspera
	Leguminaceae	Mimosa pudica
	Punicaceae	Punica granatum
Aerial parts	Loranthaceae	Viscum articulatum
	Bombaceae	Bombax ceba
Stem	Musaceae	Ensete edule
	Moringaceae	Moreinga olifera
Stem bark	Apocyanaceae	Alstonia scholaris
	Fabaceae	Deris scandens
Bark	Rubiaceae	Butea monosperma
Leaf	Iridaceae	Allium cepa
	Liliaceae	Asparagus racemosus
	Bombaceae	Bombax ceba
	Moringaceae	Moreinga olifera
Root	Leguminaceae	Cassia tora
	Loganaceae	Strychnos nux vomica
	Solanaceae	Withania somnifera
	Apocyanaceae	Rauwolfia serpentina
Rhizome	Acoraceae	Acorus calamus
Tuber	Iridaceae	Allium cepa
Flower	Meliaceae	Azadirachta indica
Seeds	Apocyanaceae	Nerium oleander

Moreover, many flavonoids have been reported to have anti-snake venom activity and as Cabbage (Brassica oleracea) is rich in flavonoids thereby it has been investigated for its anti-snake venom activity [17].
Brassica oleracea includes several cultivars such as Cauliflower, Cabbages etc. It is especially rich in flavonoids and glucosinolates. All these including phenolic compounds are to play a protective role against ultraviolet radiation and also protects against the deleterious activities of Reactant Oxygen Species (ROS) as tabulated under Table 4 and Table 5 [18–20].

Table 4: List of Flavanoids and their relevance in medical research. Wide range of medical applications can be found in this phyto-chemicals

Flavonoid (Subclass)	Relevances in the Medical Research
Luteolins (Flavone) & Apigenins	Phytoestrogens with the anti-inflammatory & anti-bacterial function, inducer of apoptosis
Cyanidins (Anthocyanidins)	Anti-cancer, Anti-oxidant, beneficial to eyes and nerves
Glycitein, Daidzein	Cardio-protective
Kaempferol (Flavonol)	Cardio-protective, anti-gastric cancer
Quercetins (Flavonol)	Cardio-protective, anti-gastric cancer

Table 5: Anthocyanin and flavonoid contents in Brassica oleracea. All these phyto-molecules have medicinal activities

B. oleracea Variety Verification	Anthocyanins (C-Cyanidin)
var.capitata.f.rubra	C-3 (caffeoyl)-(p-coumaroyl)-diglycosides 5-glycoside
var.capitata.f.rubra	C-3 (caffeoyl) diglycoside, 5-glycosides
var.capitata.f.rubra	C-3-(caffeoyl) diglycoside, 5-glycosides
B. oleracea Variety Verification	Flavonol (Q-Quercetin; K-Kaempferol)
var.acephala, var. botrytis italica	Q-3-O sophorotriosides 7-O sophoroside
var.acephala, var. botrytis italica	Q-3-O sophorotrioside 7-O-glycoside
var. botrytis italica	Q-3-O glycosides

D. Determination of Proteolytic Activity of Daboia russelli venom

As per earlier publications, Daboia russelli venom's proteolytic activity was determined using goat's plasma [21]. In a total amount of 1.5 mL, aliquots of Daboia russelli venom (0–11 g/mL) seem to have been fermented with goat's plasma at 37°C for 100 minutes. The concentration of Daboia russelli venom (g/mL) required to produce 50% proteolysis was designated as an increasing concentration 50 (EC50).
In the inhibitory studies, an EC of Daboia russelli venom (4.54 g/mL) was incubated overnight with various quantities of cabbage aqueous extracts as well as commercial AVS to assess its protein expression in goat plasma. Gestating venom with PBS (1 percent v/v) served as a positive control sample.

E. Clotting Activity

The clotting time was measured using an Amelung coagulometer, model KC4A, after citrated normal goat plasma (diluted with equal amount of PBS) was combined with Daboia russelli snake venom (0–10 g/mL) and the clotting time was observed (Labcon). The minimal coagulant dosage was determined to be the concentration of venom (3.69 g/mL) that clotted plasma in 60 seconds (MCD). To test the inhibitory action, the aqueous extracts of cabbage and the commercial antidote were stored separately at room temperature for 30 minutes with 1 MCD of venom (3.69 g/mL), and then the combination was added to plasma to measure the clotting time, as previously described[22–25].

F. Anti-Proteolysis role of the aqueous extract of cabbage

Goat's blood incubated with snake's venom yielded an effective concentration 50 (EC50) at 4.54 μg/mL after 100 mins. Keeping the EC50 value constant for the snake venom, the percentage inhibition of proteolysis of goat's blood in the presence of the aqueous extract of cabbage and the commercial anti-dote was observed with the cabbage extract preventing hemolysis more efficiently than the commercially available anti-dote (Figure 1).

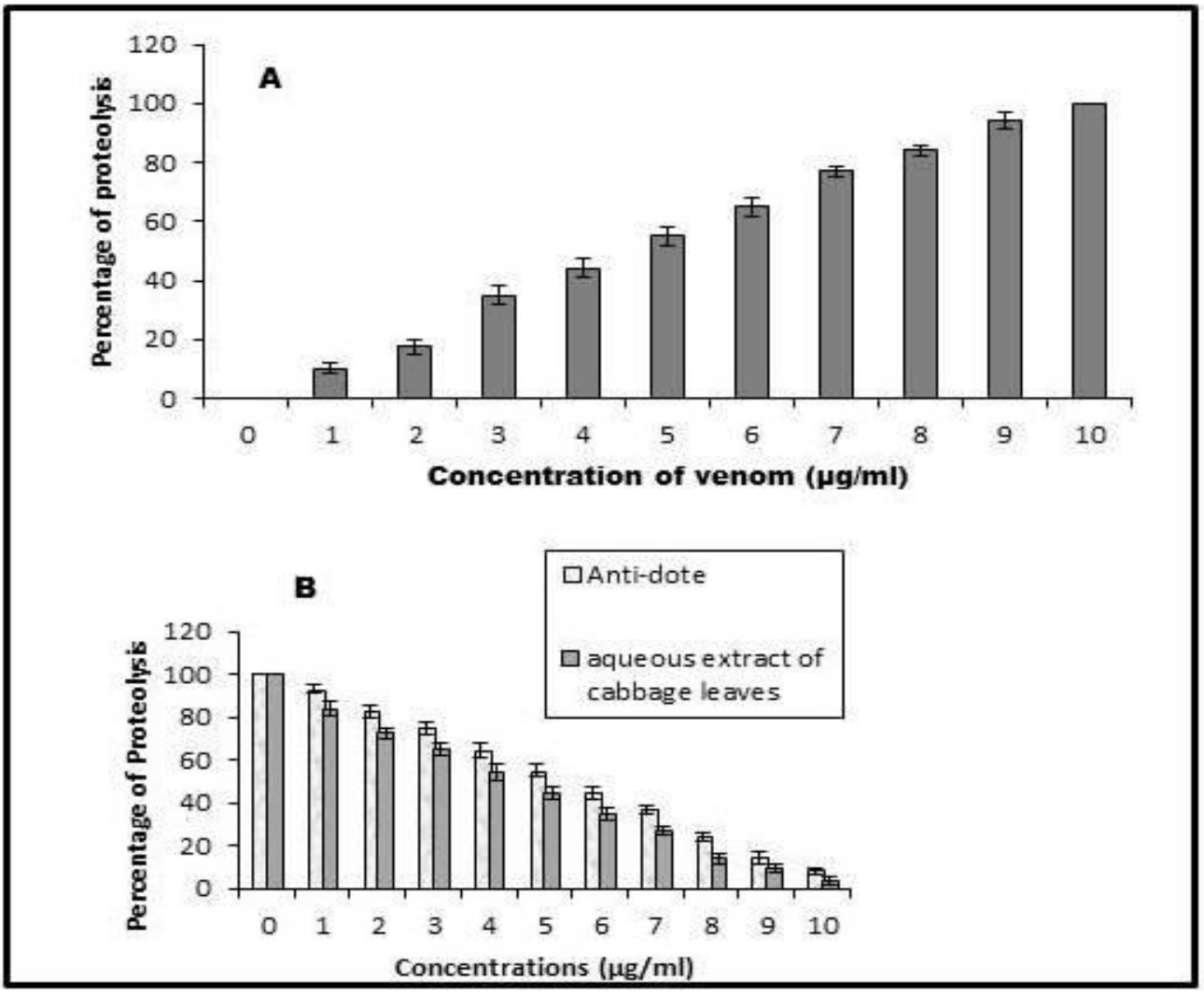

Figure 1: Effect of cabbage aqueous extracts and commercial Anti-dotes on proteolysis induced by Daboia russelli venom

Effective concentration 50 (EC50) of Daboia russelli on goat's blood was determined at 4.54 μg/mL after 100 mins (B) Keeping the EC50 value constant for the snake venom, the percentage inhibition of proteolysis of goat's blood in the presence of the aqueous extract of cabbage and the commercial anti-dote was observed with the cabbage extract preventing hemolysis more efficiently than the commercially available anti-dote.

Firstly, minimum coagulant dose (MCD) was calculated at 3.69 µg/ml for goat's plasma i.e. this amount coagulated the goat's plasma at 60 s. The crude extract of cabbage or the commercialized antidote were stored separately at room temperature for 30 minutes with 1 MCD of venom (3.69 g/mL) and then the combination was introduced to plasma to measure the sedimentation rate, keeping this quantity constant. When compared to the control group, the aqueous extract of cabbage had a better preventative effect than the commercial antidote through extending the clotting time (Figure 1).

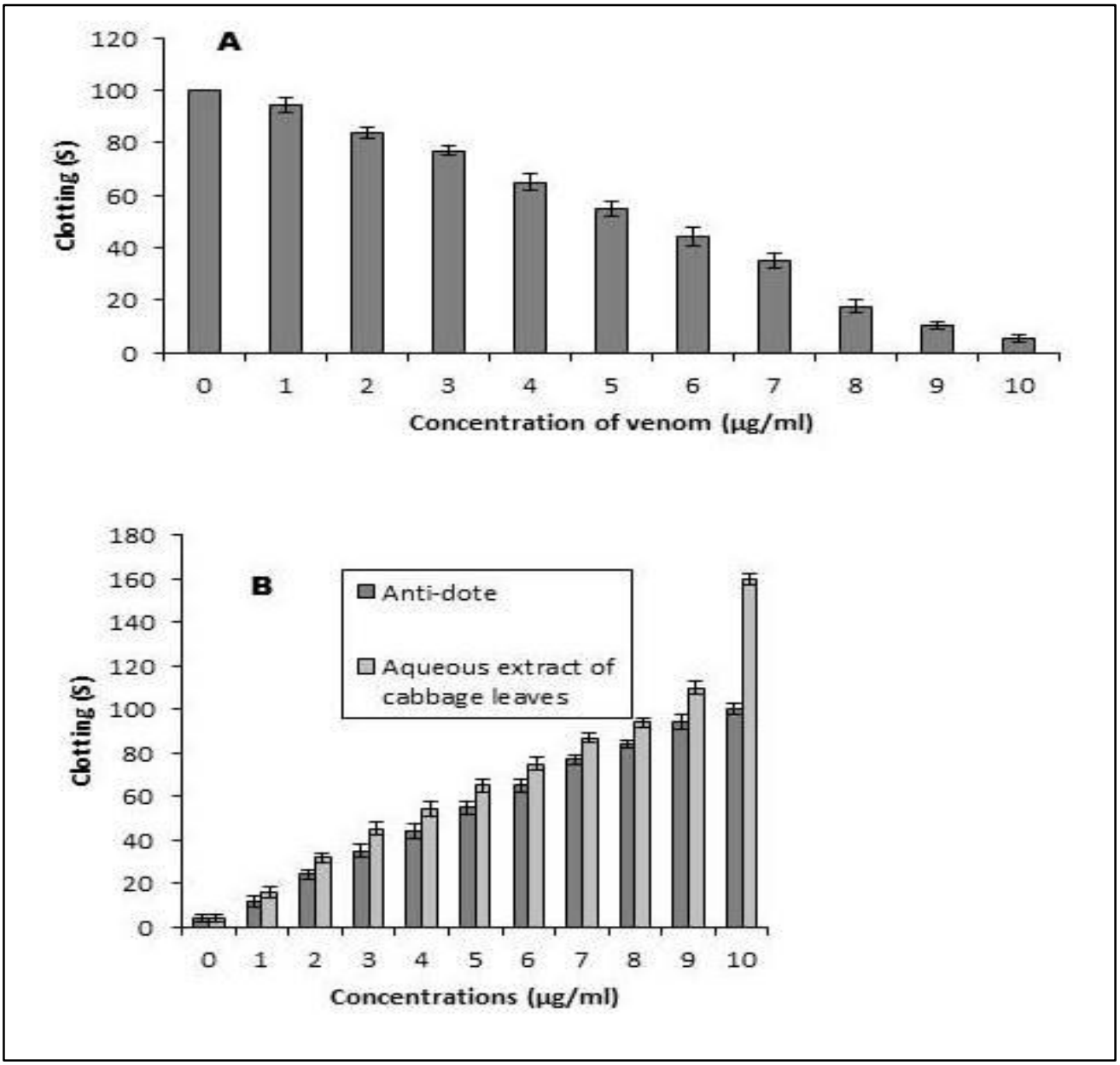

Figure 2: Effect of aqueous extract of cabbage on clotting induced by snake venom

Minimum coagulant dosage (MCD) was estimated at 3.69 µg/ml for goat's plasma i.e. this quantity coagulated the goat's plasma at 60 s. The aqueous extracts of cabbage as well as the professional antidote were individually kept at room temperature for 30 minutes with 1 MCD of venom (3.69 g/mL) and then the mixture was administered to plasma to measure the clotting time, maintaining this quantity constant. When compared with the control group, the extract showed of cabbage showed stronger preventive action than the conventional antidote by increasing the clotting time. Snakebite envenomation is a diseases in the world sickness that kills over 100,000 people each year and injures over 400,000 others. According to latest WHO estimates, snakebite envenomation causes around.80–140,000 fatalities per year, 400,000 amputations, as well as other long-term incapacities and disabilities. The venom of the Russell Viper (Daboia russelli) was chosen for this study because it has hemotoxic properties. Furthermore, flavonoids have been shown to have anti-snake venom activity, hence the cabbage (Brassica) was chosen to test for anti-snakes venom action.

II. DISCUSSION

The primary aim of this study is to determine if aqueous extract of cabbage may function as an anti-dote against snake venom. Throughout this context, the anti-proteolysis and prevention of venom induced clotting including both goat's plasma was found. 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 are poor owing to lack of explanation, inappropriate handling and storage conditions etc. Most of the venomous snakes in India are from the India are from the Elapidae and Viperidae families. Since most of the rural people prefer to flock to traditional healers who tend to patients of snake-bites with therapeutic herbs, which tend to be high in flavonoids thus

cabbage was selected as for its common vegetable status as well as being rich in flavonoids.

III. CONCLUSION

Elapidae (African or Asian cobras, Asian kraits, African mambas, American coral snakes, Australian or New Guinean poisonous snakes, or sea snakes) and Viperidae (Old World vipers, American rattlesnakes and pit vipers, as well as Asian pit vipers) are the snake family with the most clinical values (Old World vipers, American rattlesnakes or pit vipers, or Asian pit vipers). To begin, commercially available snake venom and a polyvalent anti-venom serum or anti-dote were procured. Next goat's plasma was likewise collected. Daboia russelli venom's proteolytic activity was evaluated using goat's plasma. Secondly, after determining the minimal coagulant dosage (MCD) the clotting time was measured in presence of the aqueous extract of cabbage leaves. Effective concentration 50 (EC50) of the goat's blood was at 4.54 μg/mL after 100 minutes. The cabbage extract prevented haemolysis more effectively than the commonly produced anti-dote. Secondly, the aqueous extract of cabbage exhibited greater preventative activity than the commercial anti-dote by increasing the clotting time as compared to the control set. Thereby the humble cabbage maybe utilized for big scale synthesis of anti-snake venom there in upcoming years.

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