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Preliminary Screening of Herbal Plant Extracts for Anti-venom activity against Common Sea Snake *(Enhydrina schistosa)* Poisoning.

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ABSTRACT - Various Indian herbal plants were screened for antivenin activity against common sea snake venom Enhydrina schistosa. As the venom of Enhydrina schistosa is the most toxic among the common sea snakes, an attempt was made to screen 12 plants from different families for antivenin potential. Alcoholic extracts of authenticated plant materials were examined for their venom neutralization ability using in-vitro and in-vivo methods. The alleviation in the mean survival time of experimentally protected laboratory animals were used to infer the antivenom property of the drug, after challenging with median lethal venom dose. Mean survival time study indicated appreciable protective action of three herbal extracts namely Mucuna pruriens, Mimosa pudica and Andrographis paniculata among the 12 screened plants. Mean survival time was higher in in-vitro experiments as compared to the in-vivo experiments.

KEY WORDS - *Enhydrina schistosa,* Herbal extract, Mucuna pruriens, Mimosa pudica, Andrographis paniculata Venom.

INTRODUCTION

Snake bite is a major health hazard leading to high mortality rate especially in Indian fisherman community. *Enhydrina schistosa* (common sea snake) is most widely distributed sea snake in the world and its venom is among the most toxic snake venoms (1). The intravenous median lethal dose of *Enhydrina schistosa* was more than 10 times for most terrestrial venomous snakes (2). *Enhydrina schistosa* is a common sea snake found in the tropical and subtropical regions of Indian Ocean and Arabian Sea. The venom of *Enhydrina schistosa* is extremely potent, and a complete envenomation by an adult sea snake may contain enough venom to kill 3 adult people. Antivenom against snakes bites are lacking in the rural areas of coastal region. Antiserum; being the only therapeutic agent, its development from animal source is time consuming and expensive. Although, use of plants against the effects of snakes bite has been long recognized, more scientific attention has been given since last 20 years. Many Indian medicinal plants are recommended for the treatment of snake bite (3). In almost any part of the world, where venomous snakes occur, numerous plant species are used as folk medicine to treat snake bite (4). Topical application of the plant or its sap onto the bite area, chewing leaves or bark or drinking plant extracts or decoctions are some procedures intended to counteract snake venom activity. In most cases the efficacy of this treatment regimen is unproven. Many studies dealing with anti snake bite testing of folk medicines, plant extracts are either mixed with snake venom and injected into mice or are given to the experimental animals before the venom is administered neglecting actual circumstances of snake bite accidents (5). Therefore most plant extracts failed to show any curative effect when applied after envenoming. This necessitates the search for plant constituents which have supportive effect in antivenom and most rational approach would be to treat some the some symptoms or responses.

However, literature review has failed to reveal any scientific studies taken up to screen locally used herbs for neutralizing capacity of sea snake envenomation Hence, our work aims to screen some herbs freely available in India for antivenom activity against sea snake poisoning.

MATERIALS AND METHODS

Collection of plant material

12 plant materials reported in Table no 1 were collected from Gandhi Krishi Vidyan Kendra (GKVK), Hebbal Bangalore, India. The plants were authenticated by Dr. M Vasundhara, Asst. Professor,

Table 1: Plants screened for antivenom activity

Sl No	Plant name	Family
1	*Mucuna pruriens*	Fabaceae
2	*Vitex nigundo*	Verbenaceae
3	*Coleus aromatica*	Lamiaceae
4	*Wrightia tomentosa*	Apocynaceae
5	*Sapindus laurifolius*	Sapindaceae
6	*Tecoma stans*	Bignoniaceae
7	*Mimosa pudica*	Mimosaceae
8	*Tephrosia purpurea*	Fabaceae
9	*Aristolochia indica*	Aristolochiaceae
10	*Andrographis paniculata*	Acanthaceae
11	*Alangium salviifolium*	Alangiaceae
12	*Acorus calamus*	Araceae

University of Agricultural Sciences, GKVK campus, Hebbal, Bangalore, India. A voucher specimen (AAC/3/6/2005) has been deposited. The whole plant material was dried in shade, powdered and stored in airtight containers until it was used for further studies.

Preparation of alcoholic extracts

Alcoholic extracts were prepared according to the procedure reported by Mahanta and Mukherjee (6). The dried powder was packed in the thimble of soxhlet apparatus and was extracted with 95 % ethanol by refluxing at 60 to 80^0 C for 12 hours. The filtrate was concentrated at 40^0 C following drying at vacuum and kept in a dessicator at room temperature. The plants extracts were dissolved in saline and centrifuged at 2000 rpm for 10 min at room temperature. The supernatant was used for further investigation and kept at 4 ^{o}C. The plants were expressed in terms of dry weight.

Venom sample

Live common sea snakes *Enhydrina schistosa* were collected locally, at Arabian seacoast, Malpe beach, Udupi district, Karnataka, India. Permission to milk the venom was obtained from Ministry of Conservation of Wild life, Malleshawarm, Bangalore. The milking procedure reported by Gawade and co workers was followed with slight modification (7). The venom was pooled and lyophilized (subsequently referred to as the venom). The venom was preserved in a dessicator at 4 ^{0}C in an amber colored glass vial until further use. The venom was dissolved in distilled water and kept at 8 ^{0}C until further use. The venom concentration was expressed in terms of dry weight (mg/ml stock venom solution).

Test animals

Male inbreed Swiss albino mice 18-20 gm were used for the studies of acute extract or venom toxicity and in the experiments of venom neutralization. Institutional Animal Ethics Committee clearance at Al-Ameen College of Pharmacy, Bangalore, was obtained to conduct the experiment. All the animals were conditioned in standard cages. They were kept in a 12/12 hr light-dark cycle. Food pellets (Amrut Biotec, Maharashtra) and water was available *ad libitum*. Each experimental group was matched with parallel control group treated only with saline solution (0.9%). Experiments were carried out at laboratory temperature (30-35 ^{0}C).

Acute toxicity of the extracts and venom

The method reported by Turner was adopted for the determination of LD_{99} (Median lethal dose MLD) of the venom and extracts (8). Mice (n=4) were injected i.p. with the graded doses of the venom. The plants extracts were given by per oral route at dose of 1gm/kg on body weight basis. Mortality was recorded within 24 hours.

Neutralization of the lethal effect of venom

In- vitro experiments

In-vitro neutralization test as described by Alam and Gomes was followed (9). One MLD of venom (200µg/kg) was mixed with a fixed amount (1gm/kg) of plant extracts; the mixture was incubated at 37 ^{0}C for 1 hr and centrifuged at 2000 rpm for 10min. The supernatant was injected i.p. into male albino mice (n=6). The deaths were recorded for 24 hr after admixture injection of venom and extracts.

In- vivo experiments

In-vivo neutralization test as described by Martz was followed with modifications (4). Mice were injected with extract preparation (1gm/kg) i.p. 30 min prior to the administration of one MLD of the (200µg/kg). The deaths were recorded for 24 hr after admixture injection of venom.

Statistical analysis

All values were expressed as mean (± SEM). Comparison between groups of data was conducted using Student's unpaired *t*-test. *P* values of <0.05 were considered significant.

RESULTS

Total 385 sea snakes were collected locally, at Arabian seacoast, Malpe beach, Udupi district, Karnataka and milked on alternative days which resulted in the 1.785 gm, at an average of 4.63mg/snake. The venom of

Enhydrina schistosa was highly lethal to mice with a median lethal dose 200µg/kg body weight of mouse by i.p. route. The mean survival time of *Enhydrina schistosa* venom injected by i.p. route was 1.25 ± 1.33 hr (Mean ± SEM). All the plant extracts given at a dose of 1gm/kg by p.o. route in three mice were found to be safe as observed for 7 days. Appreciable protective action against the venom was observed with the three herbal extracts (*Mucuna pruriens, Mimosa pudica and Andrographis paniculata*) out of 12 plants extract screened. The % mean survival time was increased by 12.22, 9.99 and 9.90 protection fold by *in-vivo* method and 16.02, 24.92 and 15.32 fold by *in-vivo* method respectively as compared to the one median lethal dose of the venom. The results were summarized in the Table 2 and 3 for *in-vivo* and *in-vitro* test respectively.

DISCUSSION

Snake bite is a major health hazard that leads to high mortality and great suffering in victims. Most common conventional remedy is administration of antiserum with a major draw back being its prohibitive cost and chance that victims are often some distance away from medical care when bitten. Although it is well known that antiserum is invariably unavailable, the role of medicinal plants remains largely ignored. 578 species of higher plants from 94 families have been cited in literature in treatment of snake bite (10). Many plant extracts have been reported to posses a detoxifying effect on snake venoms (11, 12, and 13). Some of these plant extracts has shown antivenom property

Table 2: Mean survival time (hrs) and protection fold against the LD_{99} of extracts administrated (i.p.) 30 min before challenging with one median lethal dose of the venom (i.p)

Group	Treatment	Mean survival time (hr)	Protection fold (LD_{99})
1	LD_{99}	1.25 ± 1.33	
2	LD_{99} + *Mucuna pruriens*	15.27 ± 1.25 ***	12.22
3	LD_{99} +*Vitex nigundo*	3.48 ± 0.25 ***	2.78
4	LD_{99} +*Coleus aromatica*	6.45 ± 0.68 ***	5.16
5	LD_{99} +*Wrightia tomentosa*	5 ± 0.75***	4
6	LD_{99} +*Sapindus laurifolius*	5.45 ± 0.23***	4.36
7	LD_{99} +*Tecoma stans*	3.17 ± 1.56 ***	2.54
8	LD_{99} +*Mimosa pudica*	12.49 ± 0.25***	9.99
9	LD_{99} +*Tephrosia purpurea*	2.13 ± 0.25**	1.70
10	LD_{99} +*Aristolochia indica*	2 ± 0.38**	1.6
11	LD_{99} +*Andrographis paniculata*	12.37 ± 0.98***	9.90
12	LD_{99} +*Alangium salviifolium*	2 ± 1.45 **	1.6
13	LD_{99} +*Acorus calamus*	2.1 ± 2.3 **	1.68

*Results were expressed as Mean ± SEM (n=6) ; Unpaired students "t" test ; * * P < 0.001 ; * * * P < 0.0001*

Table 3: Mean survival time (hrs) and protection fold against the LD_{99} of incubated mixture of extracts (i.p.) and one median lethal dose of the venom (i.p).

Group	Treatment	Mean survival time (hr)	Protection fold (LD_{99})
1	LD_{99}	1.25 ± 1.33	
2	LD_{99} + *Mucuna pruriens*	20.02 ± 0.45 ***	16.02
3	LD_{99} +*Vitex nigundo*	6.68 ± 0.57 ***	5.34
4	LD_{99} +*Coleus aromatica*	4.57 ± 0.78***	3.65
5	LD_{99} +*Wrightia tomentosa*	5.68 ± 0.25***	4.54
6	LD_{99} +*Sapindus laurifolius*	5.58 ±1.25***	4.54
7	LD_{99} +*Tecoma stans*	5.15 ± 2.02***	4.12
8	LD_{99} +*Mimosa pudica*	31.15 ± 0.85***	24.92
9	LD_{99} +*Tephrosia purpurea*	3.19 ± 1.02***	2.55
10	LD_{99} +*Aristolochia indica*	2.3 ± 0.25**	1.84
11	LD_{99} +*Andrographis paniculata*	19.16 ± 1.23***	15.32
12	LD_{99} +*Alangium salviifolium*	2.35 ± 1.45**	1.88
13	LD_{99} +*Acorus calamus*	2.45 ± 2.35**	1.96

*Results were expressed as Mean ± SEM (n=6) ; Unpaired students "t" test ; * * P < 0.001 ; * * * P < 0.0001*

on land snakes and their possible mechanism of action have been suggested (14, 9, 7). So far, none of these plants have been screened for the antivenom or neutralization property against sea snake poisoning. Therefore, the present investigation aimed at screening 12 plants extracts for antivenom property against the sea snake poisoning which have a folkloric use in treatment of land snake bites.

To infer the antivenom property of the drug alleviation in the mean survival time of experimentally protected laboratory animals, after challenging with median lethal venom dose was used. The results indicated a promising alleviation in the survival time by three alcoholic extracts, *Mucuna pruriens, Mimosa pudica and Andrographis paniculata* out of 12 species of different plant extracts screened. It was also observed in the present investigation mean survival time was higher in *in-vitro* experiments as compared to the *in-vivo* experiments. This could be possibly due to inactivation or precipitation of active venom components by the plant extracts.

The pharmacological properties of snake venom are mainly associated with proteins, particularly with enzymes (15). Compared with terrestrial snake venom, sea snake venoms contain fewer different proteins and enzymes. *Enhydrina schistosa* venom contains long and short chain post – synaptic (alpha) neurotoxic polypeptides and toxic phospolipase A_2 with (beta) presynaptic neuromuscular blocking activity and myotoxic effects (2). PLA_2 is almost invariably the most toxic component of the venom and responsible for wide range of pharmacological effects including neurotoxicity, cardiotoxicity, hemolytic and damage to biological membranes (15, 16). Severe *Enhydrina schistosa* envenomation develops pronounced myoglobinuria within three to six hours after the bite[17]. In most animals, *Enhydrina schistosa* venoms kill by producing respiratory paralysis attributable to their content alpha neurotoxins. This activity depends on the neurotoxins binding to the alpha subunit of the acetylcholine receptor at the neuromuscular junction (18). Apart from neurotoxins *Enhydrina schistosa* also reported for hyaluronidase, alkaline phospgatase, phosphodiesterase and cholinesterase activities (19). Besides AchE of venom is also toxic in nature (15).

The extracts were observed to provide some protection against the lethal dose of the venom. Certain naturally occurring substances are known to modify the actions of proteins and enzymes, especially the plant polyphenols (20). Several plant constituents like flavonoids, quinonoid, xanthene, polyphenols and terpenoids possessed protein binding and enzyme inhibiting properties (21, 22). Which also inhibit snake venom Phospholipase A_2 (PLA_2) activities (23). Therefore, significant neutralization of toxic enzymes of *Enhydrina schistosa* venom by alcoholic extracts of *Mucuna pruriens, Mimosa pudica and Andrographis paniculata* might lead to inhibition of lethality of venom.

CONCLUSION

Results of the present investigation has indicated the presence of antivenom activity of the *Enhydrina schistosa* venom in the alcoholic extracts of the three plants namely *Mucuna pruriens, Mimosa pudica and Andrographis paniculata* which have not been reported previously. *In-vitro,* alcoholic extracts could have neutralized the toxic enzymes of the venom and *in-vivo,* it could have antagonized the lethality of the venom. Further purification work is necessary for the better understanding of the mechanism of venom inhibition.

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