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Preliminary Screening of Aqueous Alstonia Scholaris *Linn* Bark Extract for Antivenom Activity in Experimental Animal Model

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Abstract:
Aim: *Snake envenomation is a major cause of death and disability in the developing countries, particularly in South East Asia. Since 1894, commercial snake venom antiserum developed by Calmette is the only specific treatment available for snake bite. The use of plants against the effects of snakes bite has been long recognized; more scientific attention has been given since last 20 years (Santosh et al., 2004). The folklore uses of Indian Medicinal Plant Alstonia scholaris Linn against snakebite treatment has been validated in the present study.*
Methods & Materials: *Viper venom neutralizing activity neutralization with 200mg/kg bw aqueous extract of Alstonia scholaris Linn(AAS) was estimated by in vivo methods in animal models.*
Results: *The aqueous extract of Alstonia scholaris Linn neutralized viper venom induced minimum lethal action(MLD),minimum edema activity(MED), minimum defibrinogenating action(MDD),minimum necrotizing action(MND) in 18-20 gms mice.*
Conclusion: *The present study corroborates the claims of traditional healers and demonstrates the antivenom potential of aqueous Alstonia scholaris Linn(AAS) in treatment of snakebite.*
Keywords*: Aqueous Alstonia scholaris(AAS), Minimum edema dose(MED), minimum defrinogenating dose(MDD), minimum necrotizing dose(MND).*

I. Introduction

Snakebite is an important cause of morbidity and mortality and is one of the major health problems in India[1].The common poisonous snakes found in India are Cobra (N*aja naja*), Krait (*Bangarus Caeruleus*), Russell's viper (*Daboia russelli*) and Saw Scaled Viper (*Echis Carinatus*)[2]. Venoms of Russell's viper of Viperidae family is hemotoxic and mainly affect circulatory system and muscular system causing excessive scarring, hemorrhagic, coagulant defects and hypovolemic shock. The intravenous administration of animal-derived (mostly horse or sheep) antivenoms is the mainstay and the only specific treatment of snake bite envenoming. Anaphylaxis and serum sickness are the major concern with many of the antivenom preparations. These hypersensitivity reactions are mainly caused by the foreign animal proteins present in the antivenom and the probability of a reaction depends partly on the type of antivenom, its manufacturing and concentrating process, and the dose used[3]. Several medicinal plants are used in treatment of snake bite. In this regard, several plants are scientifically studied, *Andrographis paniculata* and *Aristolochia indica* plant extracts inhibits snake venom and could be used for therapeutic purposes in case of snakebite envenomations [4].

Over the years many attempts have been made for the development of snake venom antagonists from plants sources. Several medicinal plants, which appear in old drug recipes or which have been passed on by oral tradition, are believed to be snakebite antidotes and are recommended for the treatment of snakebite [5]. *Alstonia* species are used in traditional medicine. The bark of the *Alstonia constricta* and *Alstonia scholaris* is a source of a remedy against malaria, toothache, rheumatism and snake bites. The latex is used in treating coughs, throat sores and fever. Alstonia is a genus of the family *Apocynaceae* to which many other medicinally important plants belong like *Rauvolfia canescens* , *Alstonia boonei*, *Rauwolfia serpentine* and *Vinca rosea* which have been producing well known remedy for various disorders like schizophrenia and cancer. The traditional method of medications has been long known in the developing countries like India and China[6]. The important plants of genus *Alstonia* includes *Alstonia scholaris, Alstonia boonei, Alstonia congensis* and *Alstonia macrophylla* which have proved to be useful in various diseases.

Alstonia sp are tropical plants growing in various parts of Africa and south Asia. *Alstonia* is named after Professor Dr C. Alston (1685-1760), at Department of Botany in University of Edinburgh. More than 40 known species are there in which most studied species of genus *Alstonia* includes *Alstonia scholaris* and *Alstonia boonei*. The various species of *Alstonia* are highly rich in alkaloids, steroids and triterpenoids, and phenolic compounds which contribute to the toxicity of *Alstonia scholaris* [8].The bark of *Alstonia scholaris* has been extensively used in folklore medicine for treatment of leprosy, dyspepsia, malarial fever, Leishmania

infection[9]. Various alkaloids that have been reported in stem bark of *A.scholaris* includes alstonidine, *O*-methylmacralstonine, macralstonine *O*-acetylmacralstonine, alstonine, ditamine, echicaoutchin, corialstonidine, corialstonine chlorogenine, villalstonine, pleiocarpamine, villalstonine, macrocarpamine, and triterpenoids which have been reported are alpha-amyrin linoleate, lupeol palmitate and lupeol linoleate[10,11]. The pentacyclic triterpenes (free or as glycosides) are found widely in several antisnake venom plants (*Aegle marmelos*, *Centipeda minima*, *Aloe vera*, *Phyllanthus niruri*, *Alstonia scholaris*, *Phyllanthus emblica*, *Elephentopus scaber*, etc.) and provide nearly 20% protection against snake venom [12]**.** In the present study an attempt has been made to study the antivenom potential of the aqueous bark extract of *Alstonia scholaris Linn* in mice models.

II. Materials

Snake venom and snake venom antiserum

Lyophilized snake venom **VRV(*Vipera russellii)*** venom was be purchased commercially from Irula Snake Catcher's Cooperative Society(ISCICS), Kancheepuram, Chennai, India & dissolved in 0.9% NaCl, centrifuged at 2,000 rpm (revolution per minute) × 10 mins. The venom concentration was expressed in terms of dry weight(mg/ml). Lyophilized polyvalent snake venom antiserum I.P (batch no. 4066016) was commercially purchased from Bengal Chemical Pharmaceutical Pvt. Ltd., Calcutta, India.

Animals

Male Swiss albino mice (20±2 g) were obtained from enlisted supplier of Vidyasagar University and maintained in standard laboratory conditions. They were given standard laboratory diet and water *ad libitum*. All animal experiments were approved by the University Animal Ethics Committee, Department of Physiology with Community Health, Vidyasagar University, *Paschim Medinipur*, India and in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animal (CPCSEA),Government of India.

Aqueous extract

Plant parts of *Alstonia scholaris* Linn identified by traditional medicinemen and taxonomists in the study for snakebite treatment was be collected, washed thoroughly and dried in shade(Voucher specimen No: SS105). They were then crushed and taken in a round-bottomed flask separately with distilled water and refluxed in a water bath for 1 h at 90 - 95ºC. The extract obtained was filtered through a Whatman No. 1 filter paper. The filtrate was stored in refrigerator for future use.

Acute Toxicity

The acute toxicity study was carried out as per OECD 423 guidelines. The limit test for acute toxicity was carried out at 2 g/kg oral dose were observed continuously for 24 h for behavioral, neurological and autonomic profiles and, after a period of 24 and 72 h, for any lethality, morbidity state or death[13]

Snake venom: Neutralization studies with crude plant extracts

The plant materials of *Alstonia scholaris* Linn collected from the ethnobotanical survey of *Paschim Medinipur* district from the traditional healers was be investigated in the laboratory for antivenom potential. The following methods will be used to screen the plants for antivenom activities.

1.Minimum Lethal action

The minimum lethal dose (MLD) of the venoms (*Vipera russellii venom*-VRV) will be assessed by injecting 0.1 ml of different concentrations of venom into the tail vein of male albino mice (18±2 n=6) and the number of deaths within 24 h will be recorded **(Theakston and Reid, 1983).** To study the lethal action neutralization of crude plant extracts (aqueous and methanolic) various doses of venoms (VRV) will be mixed with fixed dose (Effective Dose 50-ED50 value) of the crude plant extract (incubated at 37°C×1 h, centrifuged at 2,000 rpm×10 min). Supernatant will be injected intravenously (*i.v*) and the fold of lethality neutralization will be determined.

2. Necrotizing activity

The minimum necrotizing dose is the least amount of venom which when injected intradermally into mice's results in a necrotizing lesion of 5 mm diameter in 3 d later[14]. Neutralization of the necrotizing activity was estimated by mixing a fixed amount of venom with different amounts of AAS. The AAS–venom mixture was incubated at 37 °C for 1 h and 0.1 ml of the mixture was injected intradermally into mice. The necrotizing lesion was estimated after 3 d. The standard reference group was administered snake venom antiserum (2mg/kg bw) *ip* after administration of MND dose of the venom.

3. Defibrinogenating activity(MDD)

The minimum defibrinogenating dose (MDD) of the venoms (VRV) was assessed by injecting 0.1 ml of different concentrations of venom, intravenously (*i.v*) in male albino mice (20±2 g, n=6) and the formation of incoagulable blood was recorded after 2 h **[14].** To study the defibrinogenating action neutralization, various doses of venoms (VRV) was mixed with fixed dose of the crude plant extracts (incubated at 37°C×1 h, centrifuged at 2,000 rpm×10 min). Supernatant was injected intravenously (*i.v*) and the fold of defibrinogenating action neutralization was determined.

III. Statistics

Results are expressed as mean±SEM. The data were analyzed by ANOVA complemented by the Differences were considered significant when $P < 0.05$.

IV. Results

The MLD of the venom was recorded at **1µg/ mice *i.p*.** The minimum edema dose(MED) was found to **be 1 µg/ mice** *i.p*. Oral administration of aqueous *Alstonia scholaris* did not produce any toxicity upto 1gm/kg *bw* after 72 hours. Significant protection was observed at 200mg/kg of AAS. The MND of venom injected intradermally into mice's shaved dorsal skin was 10µg/mice. The AAS at 200 mg/kg reduced the necrotizing lesions significantly **(Table 1).**

Table 1: Neutralization of Viper venom(VRV) necrotizing lesion

Groups(n=6)	Necrotic lesion in mm
VRV(10µg)	6.2±0.03
VRV(10µg)+ Antivenin(2mg/kg) *bw*	0.0
VRV(10µg)+AAS(200mg/kg) *bw*	4.5±0.02 [a]
VRV(10µg)+AAS(400mg/kg)*bw*	3.9±0.01 [a]
VRV(10µg)+AAS(800mg/kg)*bw*	2.9±0.02 [a]

[a]P<0.05 vs control

Table 2: Neutralization of Viper russelli venom activity by aqueous bark extract of Alstonia scholaris

Neutralization of Venom activity	Venom +AAS extract	Venom dose (p/n)	Fold of Protection	Effective dose of neutralization(mg)
Lethal action	**VRV(1µg)+AAS(200mg/kg bw)**	**1(0/6)** **2(0/6)** **4(0/6)** **5(6/6)**	**1** **2** **4** **NP**	98±0.5
Defibrinogenation	**VRV(1µg)+AAS(200mg/kg bw)**	1(6/6) 2(0/6)	**1** **NP**	90±0.5
Edema activity	**VRV(0.5µg)+AAS(200mg/kg bw)**	0.5(0/6) 1(0/6) 2(6/6)	**1** **2** **NP**	95±0.5

[1]p/n= No of animals killed /total number of animals

[2]p/n= No of clotted blood samples/ Total no of blood samples

[3]p/n=No of animals with inflamed paws/ total number of animals

NP: No Protection

The Minimum Defibrinogenating dose of the venom was **1µg.** 200 mg /kg *bw* AAS gave two fold protection against viper venom induced defibrinogenation and could clot VRV**(1µg)** in **5±0.2mins**. Viper venom induced significant edema in rat paw. Maximum inflammation was seen at 2 h which gradually decreased over a period of time. Intraplantar *Viper* venom injection followed by AAS(*p.o*) produced significant reduction in inflammation at 200 mg/kg dose **(Table 2).**

V. Discussion

According to the WHO, the anti snake venom compounds should be tested regarding its capacity to neutralize venom effects such as lethality, hemorrhagic-necrotizing effects, neutralization of the coagulant and defibrinogenating activity[14].The use of plants against the effects of snakes bite has been long recognized; more scientific attention has been given since last 20 years[15]. Most venom possesses the ability to cause local necrosis and hemorrhage when introduced intradermally. Hence, the minimum necrotizing dose and minimum hemorrhagic dose estimation proves a reasonable test for assessing the antivenom activity[16]. Venom releases an enormous amount of histamine into circulation by mast cell degranulation. The released substances could also add to the various toxic signs and in fact may be responsible for some of the toxicity such as anaphylaxis. Mast cells are a rich source of mediators like histamine and platelet activating factors. As a consequence of *Viper* bite local necrosis occur, which is often due to clot formation[17]. In patients who suffer from snake bites marked

progress of defibrination has been observed. Further studies are required to establish the efficacy of AAS as potent anti-snake venom drug. Further experimental work is being carried out to isolate and identify the active principles present in the AAS that are responsible for anti-snake venom activity.

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