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Anti-snake venom activities of ethanol and aqueous extract of *Asparagus racemosus* against Indian cobra (*Naja naja*) venom induced toxicity

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ABSTRACT

Background: Snake bite is an occupational hazard that leads to significant number of fatalities and medical emergencies in developing countries. The focus of research in the field of snake bite management is now on the plant kingdom as across countries from time immemorial traditional healers have been using herbal drugs for management of snake bite.

Objective: To evaluate anti-snake venom activities of ethanol and aqueous extracts of *Asparagus racemosus* (AR) against Indian cobra (*Naja naja*) venom induced toxicity.

Materials and Methods: Anti-snake venom activities of *Asparagus racemosus* against Indian cobra (*Naja naja*) venom induced toxicity was studied via *in vitro* phospholipase A_2 induced hemolysis on sheep RBC and *Naja naja* venom induced anticoagulation, *in- vivo* neutralization activity against lethality induced by *Naja naja* venom in rats, *Naja naja* venom induced edema and effect on bleeding time of venomized rats.

Results: The minimum lethal dose (MLD) was found as 2mg/kg and both ethanol and aqueous extracts of *Asparagus racemosus* possess significant neutralization activity against *Naja naja* venom induced lethality. Both AR extracts inhibited phospholipase A_2 dependent hemolysis of sheep RBC and exhibited significant reduction ($p<0.001$) in the anti-coagulant activity of venom with 71.87% and 78.87% inhibition by ethanol and aqueous extract of *Asparagus racemosus* (EAR) at 300 µg respectively. EAR and AAR at doses of 300, 600 and 900 mg/kg exhibited significant ($p<0.001$) decrease in bleeding time and both extracts also displayed anti-inflammatory activity by inhibiting edema significantly ($p<0.001$).

Conclusion: These findings suggest a potential antivenom role of *Asparagus racemosus* against *Naja naja* envenomation.

Key words: *Naja naja*, Hemolysis, lethality, edema, bleeding, anticoagulation.

Introduction

Snake bite is an occupational hazard in tropical and sub-tropical countries like India [(1)]. Snake bite mortalities occur primarily in rural areas (about 97%) and it is estimated that about 50,000 deaths annually occur due to snake bites [(2)]. An accurate estimate of incidences of snake bite in India is difficult as rural population is the one that is mostly affected by snake bites and this population usually approach traditional healers for treatment and management. Snake envenomation leads to many pathophysiological conditions like, pain, necrosis, inflammation, hemorrhage, nephrotoxicity, cardio toxicity, respiratory paralysis and finally death [(3)]. *Naja naja* (Indian Cobra) is commonly found snake specie in most of the Indian states and its bite leads to several deaths per year. It is clear from studies of large series of snake-bite deaths that many hours usually elapse between bite and death in the case of elapid envenoming, and several days in the case of viper envenoming. Elapidae venom possesses several proteins, including cardiotoxins, neurotoxins and phospholipase A_2, that are responsible for their toxicity. Phospholipase A_2 (lecithinase): is one of the most extensively studied venom enzymes [(4)]. Antisera therapy is currently the only recognized treatment approach for venomous

snake bites by the medical fraternity. The development of anti-sera for snake bite management is associated with high cost as it is derived from animal source and the production is time consuming. There is serious shortage/non availability of antiserum in remote rural areas due to special storage requirements and cost associated with it. To overcome these drawbacks, there is a need to search and develop new universal, affordable and safe antidote against snake bites. *Asparagus racemosus* plant belongs to the family *liliaceae* and is commonly known as satawar, saatamuli, satavari and is found at low altitudes throughout India. The roots are said to possess diuretic and ulcer healing effects. Ayurvedic practitioners have used it for nervous disorders and inflammation [5]. It is reportedly used as potent antioxidant, immunostimulant, and for its anti-dyspepsia and antitussive effects [6]. The roots are also used for treatment of epilepsy, leprosy and body pains. *Asparagus* is also used by traditional healers for snake bite management [7]. It is evident that *Asparagus racemosus* has been traditionally used for treatment and management of variety of medical conditions and emergencies including snake bite, thus the aim of our study was to provide scientific basis for the use of *Asparagus racemosus* in treatment and management of *Naja naja* envenomation.

MATERIALS AND METHD

Collection of plant and preparation of extracts

The *Asparagus racemosus* plants were collected from herbal garden of BVV's Ayurvedic Medical College and was identified by botanist SA Kappali, Department of Botany, Basaveshwar Science College, Bagalkot. The roots were cleaned, air dried and then subjected to coarse powdering followed by passage through sieve # 44 to get powder of uniform size. The collected powder was successively extracted with petroleum ether to defat and then by ethanol for 24 hr by using soxhlet apparatus and water extraction by hot maceration. After the extraction, solvents were distilled off to get concentrated residue and completely dried by lyophilization and stored in air tight container under refrigeration [8].

Animals

Wistar albino rats of both sexes (200-250 gm) were used. The animals were housed at room temperature (22-28 °C) with 65±10% relative humidity for 12 hr dark and12hr light cycle and were given standard laboratory feed (Amruth, Sangli, Maharashtra) and water *ad libitum.* The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee.

Chemicals

Naja naja venom was procured from M/s Haffkines Institute, Mumbai after obtaining permission from Chief Wildlife Warden, Bangaluru, Karnataka. Tween-80 (Rankem, New Delhi, India) and phosphate buffer solution (PBS) pH 7.2 (Sigma-Aldrich, USA. CO). All other solvents and chemicals used for extraction were of analytical grade.

Preparation of Venom Sample

The lyophilized *Naja naja* venom was preserved in a desiccator at 4℃. It was weighed and dissolved in PBS pH 7.2, centrifuged and clear supernatant solution was used for Phospholipase A_2 activity. For animal studies the lyophilized *Naja naja* venom was dissolved in normal saline, centrifuged and clear supernatant solution was used.

LD_{50} and Minimum lethal dose (MLD) of Naja naja venom

The median lethal dose (LD_{50}) and minimum lethal dose (MLD) of *Naja naja* venom was assayed by injecting different concentrations of venom prepared in 0.2 ml of saline into the peritoneum of Wistar albino rats.

Neutralization activity of Asparagus racemosus extracts against lethality induced by Naja naja venom in rats

The ability of the ethanol and aqueous extract of *Asparagus racemosus* to neutralize lethal toxicity of venom was assessed by *in-vivo* neutralization test. The rats were administered with ethanol and aqueous extracts (50,100, 200, 300, 400 and 500 mg/kg) of *Asparagus racemosus* through oral route one hour prior to administration of 2 mg/kg of MLD of venom by intraperitoneal route and all the animals were observed for mortality till 24 hr [9].

Phospholipase A_2 Activity [10, 11]

Phospholipase A_2 activity was assessed by indirect hemolytic activity on agarose-erythrocyte-egg yolk gel plate as described by Gutierrez *et al.*, (1988). 300 µl packed sheep erythrocytes were washed four times with saline solution, 300 µl of 1:3 egg yolk solution (prepared in saline solution) was added to it followed by addition of 250 µl of 0.01 M $CaCl_2$

solution to 25 ml of 1% (w/v) of agar at 50 °C dissolved in PBS pH 7.2. The mixture was poured into petri dishes, allowed to gel and 3 mm diameter wells were made. To determine the minimum hemolytic dose (MHD) of *Naja naja* venom, different concentration (1 to 18 µg) of venom dissolved in 15 µl of Phosphate buffer pH 7.2 were dispensed into the wells. Control wells contained 15 µl of Phosphate buffer pH 7.2. After 20 h of incubation at 37°C, the diameters of hemolytic haloes were measured by digital vernier calipers [Mitutoyo, Model No. CD-6"CSX, Resolution 0.01 mm]. The minimum hemolytic dose (MHD) was defined as the amount of venom that induced a hemolytic halo of 11-mm diameter. 15µg venom in 15 µl of Phosphate buffer pH 7.2 was found as MHD.

Neutralization of phospholipase A_2 activity (indirect hemolytic activity) by ethanol and aqueous extracts of *Asparagus racemosus* was performed by incubating constant amount of venom [15 µg venom per 15 µl of Phosphate buffer pH 7.2 (1 MHD)] with different amounts of EAR and AAR (6.25 to 800 µg) for 30 min at 37°C. The 15 µl aliquots of the mixtures (venom and extracts) were added to wells in agarose-egg yolk-sheep-erythrocytes gels, plates were incubated at 37°C for 20 h. Control samples (15 µg venom per 15 µl of Phosphate buffer pH 7.2) contained venom without extract. Plates were incubated at 37°C for 20 hr. Neutralization was expressed as % inhibition that reduced 50% diameter of the hemolytic halo, when compared to the effect induced by venom alone.

Coagulation Activity [12]

The coagulation activity of the EAR and AAR against the anticoagulant response caused by *Naja naja* venom was estimated as described by Theakston et.al. (1983) [24]. Activity was initiated by estimating minimum coagulation dose of plasma (MCD-P) of venom and assayed by taking various concentration of venom (1 to150 µg), dissolved in 100 µl of PBS pH 7.2 and to this 300 µl of human citrated plasma was added followed by addition of 100µl of 0.25M $CaCl_2$ and the clotting time was observed every 15 sec by gentle tilting until coagulation occurred. Plasma incubated with PBS alone served as control. MCD-P was defined as the least amount of venom that clotted plasma in 60 sec at 37 °C.

The neutralization of anticoagulant activity of venom by EAR and AAR was done by taking constant amount of venom (2 MCD-P/100 µl) which was mixed with 50-300 µg of EAR and AAR. The AR extracts (50-300 µg) in 2 MCD-P/100µl mixture of different aliquots were incubated for 30 min at 37 °C and then 100 µl of each aliquots were added to 300 µl of citrated plasma, the plasma was recalcified by addition of 100 µl of 0.25 M $CaCl_2$ to each of the aliquots and the clotting times were recorded by gentle tilting at every 15 sec. till coagulation occurred.

Percentage Inhibition of anti-coagulation= {Formation of clot by venom[sec] - formation of clot by extract [sec] / Formation of clot by venom[sec]} X 100

Effect of EAR and AAR on Naja naja induced bleeding [13]

Bleeding time test was performed using male Wistar albino rats weighing 200-250 gm. The rats were grouped in 8 groups, each group contained 6 animals and group 1 received vehicle orally, group 2 received vehicle orally and *Naja naja* venom LD_{50} dose by i.p route and group 3 to 8 received required doses of 300, 600 and 900 mg/kg EAR and AAR by oral route one hour prior to administration of *Naja naja* venom respectively. After 1, 2, 3 and 4 hr of venom administration the tail of each rat was gently pierced with the lancet. The blood was absorbed on Whatman no.1 filter paper at every 15 sec interval till no blood stain appeared on the filter paper; the end point was recorded as total bleeding time.

Percentage Inhibition of bleeding time= {[Bleeding time of Venom control- Bleeding time of Treated group] / Bleeding time of Venom control} X 100

Edema-forming activity

The Minimum edema-forming dose (MED) of *Naja naja* venom was determined by the method given in Shenoy et al. (2013) [14]. The Minimum edema-forming dose was defined as the least amount of venom which when injected subcutaneously into rat footpad results in 30% edema within 6 hours of venom injection. The volume of each footpad was measured at 0, 1, 2, 3, 4 and 5 hr after venom injection with a digital plethysmometer-7140 (UGO Basile, Italy). The *Naja naja* venom MED was found to be 6 µg/100 µl. The rats were grouped in 8 groups and each group contained 6 animals. Group 1 received vehicle orally, group 2 received vehicle

orally and 100 µl *Naja naja* venom by intraplantar route and group 3 to 8 received required doses of 100, 200 and 300 mg/kg EAR and AAR by oral route one hour prior to the administration of *Naja naja* venom (6 µg/100 µl) by intraplantar route. One hour after venom injection change in the paw volume was recorded every hour till completion of 5 hours.

Statistical analysis

All the data is expressed as mean±SEM. The significance of differences in means between control and treated animals for different parameters was determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The minimum level of significance was at $p<0.05$.

RESULTS

LD_{50} and Minimum lethal dose (MLD) of Naja naja venom

Lethality tests using *Naja naja* venom were performed for determination of median lethal dose (LD_{50}) and the minimum lethal dose (MLD) after intraperitoneal injection in rats and the median lethal dose (LD_{50}) of the venom was determined as 1.5 mg/kg and minimum lethal dose (MLD) as 2 mg/kg. Results are summarized in table 1.

Table 1: LD_{50} and Minimum lethal dose (MLD) of *Naja naja* venom

Groups	Venom Dose(mg/kg)	No. of deaths/No. of rats	% Death
1	-	0/6	0
2	0.5	0/6	0
3	1	1/6	16.67
4	1.5	3/6	50
5	2	6/6	100
6	2.5	6/6	100

Neutralization activity of Asparagus racemosus against lethality induced by Naja naja venom in rats

Both EAR and AAR significantly inhibited the lethality induced by *Naja naja* venom at 2 mg/kg. EAR at 500 mg/kg dose showed 100% survival and AAR showed 83.33% survival rate. Effective dose 50% (ED_{50}) of EAR and AAR after intraperitoneal injection in venom pretreated rats was found to be 300mg/kg body weight for the both extracts and results were summarized in table 2.

Table 2: *In- vivo* Neutralization activity of *Asparagus racemosus* against lethality induced by *Naja naja* venom (2 mg/kg) in rats.

Groups	Extract Dose (mg/kg)	No. of death/No. of rats	% Survival (24h)
EAR	0	6/6	0
	50	6/6	0
	100	6/6	0
	200	4/6	33.33
	300	3/6	50
	400	2/6	66.67
	500	0/6	100
AAR	0	6/6	0
	50	6/6	0
	100	6/6	0
	200	5/6	16.67
	300	3/6	50
	400	2/6	66.67
	500	1/6	83.33

Phospholipase A_2 Activity

Effect of EAR and AAR on Phospholipase A_2 activity was performed by measuring the *Naja naja* venom induced hemolytic haloes in agarose-sheep erythrocytes gels, these haloes were produced as *Naja naja* venom contains the enzyme Phospholipase A_2 that has the ability to lyse sheep RBC. About 15 µg of *Naja naja* venom in 15 µl of Phosphate buffer pH 7.2 resulted in haemolytic haloe of 11.26±0.2205 mm diameter (Table 3). *Naja naja* venom 15 µg/15 µl of phosphate buffer pH 7.2 was taken as minimum hemolytic dose (MHD). Both EAR and AAR inhibited phospholipase A_2 dependent haemolysis of sheep RBC induced by *Naja naja* venom in a dose dependent manner. From fig 1 and 2 (Results are expressed as mean ± SEM of 3 observations), it was evident that 71.4% inhibition of haemolysis with 3.22±0.6221 mm haloes was due to 800 µg of EAR and 62.52% inhibition with 4.22±0.7149 mm haloes can be attributed to AAR.

Table 3: Determination of MHD of *Naja naja* venom by Phospholipase A_2 induced hemolysis on sheep RBC.

Venom (µg/15 µl) in PBS pH 7.2	Haloes (mm)
1	02.55
3	04.60
6	06.50
9	08.57
12	10.09
15	11.26
18	13.08

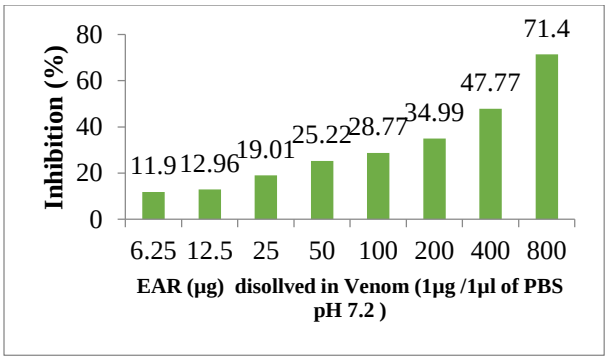

Fig 1. Effect of EAR on Phospholipase A_2 induced hemolysis by *Naja naja* venom on sheep RBC

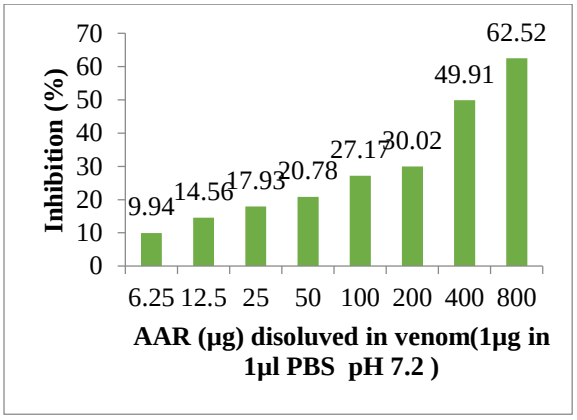

Fig 2. Effect of AAR on Phospholipase A_2 induced hemolysis by *Naja naja* venom on sheep RBC

Coagulation activity

Anti-coagulant activity was exhibited by *Naja naja* venom; hence the coagulation activity of EAR and AAR was performed using the human plasma. Minimum coagulation dose of plasma (MCD-P) of *Naja naja* venom was observed to be 60 µg/100 µl. To evaluate the coagulant activity of the EAR and AAR the 2 X MCD-P was used (120 µg venom/100 µl of PBS pH 7.2). 120 µg/100 µl venom dose was kept constant and varying doses 50-300 µg of EAR and AAR were used. In control (venom treated) the coagulation time was found to be 121.5±2.172sec, which was significantly (p<0.001) higher as compared to coagulation time observed with blank (Plasma + PBS+ $CaCl_2$). The coagulation time for blank was 23.83±0.307 sec. In contrast, the 100, 150, 200, 250 and 300 µg dissolved in 120 µg venom/100 µl of PBS pH 7.2 of EAR and AAR displayed significant reduction (p<0.001) in the anti-coagulant activity of venom. Anticoagulation was inhibited by EAR and AAR at 300 µg by 71.87% and 78.87% respectively. The results are summarized in table 4.

Table 4: Effect of EAR and AAR on *Naja naja* induced anticoagulation [Coagulation activity]

Group	Dose (µg)*	Formation of clot Mean ± SEM (Seconds)	% Inhibition of anti-coagulation
Normal	PBS+$CaCl_2$	23.83±0.307	-
Venom	120	121.5±2.172[a]	-
EAR	50	116.3± 4.462	4.28
	100	100.3± 1.687***	17.44
	150	77.17± 2.535***	36.48
	200	60.00± 2.033***	50.61
	250	34.83± 4.078***	71.33
	300	34.17± 4.316***	71.87
AAR	50	120.2± 1.682	1.07
	100	98.67± 5.024***	18.79
	150	65.17± 1.493***	46.36
	200	42.33± 1.764***	65.16
	250	27.50±2.540***	77.36
	300	25.67±1.726***	78.87

All the values are expressed as mean ±SEM, n=6, [a]p<0.001 as compared to normal and *p<0.05, **p<0.01, ***p<0.001 (One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test) as compared to venom. [EAR= Ethanol extract of *Asparagus racemosus*, EAR= Ethanol extract of *Asparagus racemosus*].

*Extracts (µg) added to 120 µg venom in 100 µl PBS pH 7.2 and calcium chloride solution.

Effect of EAR and AAR on Naja naja induced bleeding

Bleeding time significantly (p<0.001) increased in venom treated group at first hour and was found to be 207.5±4.61 sec as compared to normal group (102.5±6.021). EAR 900 mg/kg displayed significant (p<0.001) decrease in bleeding time with percentage of inhibition of bleeding time being 34.78%, 32.09%, 25.67% and 31.08% at 1, 2, 3, and 4 hr respectively. Similarly, AAR 900 mg/kg also displayed significant (p<0.001) decrease in bleeding time with percentage of inhibition of bleeding time being 30.36%, 33.33%, 27.02% and 28.37% at 1, 2, 3, and 4 hr respectively as compared to venom treated group.

Edema-forming activity

In edema forming activity, the rats injected with *Naja naja* venom showed increase in paw volume. About 6 µg/100 µl of venom induced more than 30% edema formation within 3 hrs which is considered as 100% activity and it was considered as MED. The venom treated control animals displayed significant (p<0.001) increase in paw edema at 2 hr and onwards as compared with normal group. In contrast, animals pre-treated with EAR and AAR (100, 200 and 300 mg/kg) one hour before

challenging with MED of venom displayed significant (p<0.001) decrease in paw edema at 3, 4 and 5 hr as compared with control treated animals. The 50% inhibition was shown by both extracts at 2 hr and results are summarized in fig 3.

Table 5: Effect of EAR on *Naja naja* induced bleeding

Bleeding time in hours	Groups							
	Normal	Venom control	**EAR 300 mg/kg**		**EAR 600 mg/kg**		**EAR 900 mg/kg**	
	Bleeding time (Sec)	Bleeding time (Sec)	**Bleeding time (Sec)**	% Inhibition	**Bleeding time (Sec)**	**Bleeding time (Sec)**	% Inhibition	**Bleeding time (Sec)**
1	102.5±6.021	207.5±4.610a	170.8±5.833***	17.68	151.7±4.410***	170.8±5.833***	**17.68**	151.7±4.410***
2	100.0±5.000	202.5±3.354a	150.0±5.477***	25.92	137.5±4.610***	150.0±5.477***	25.92	137.5±4.610***
3	97.50±5.123	185.0±6.325a	162.5±4.610*	12.16	150.8±3.962***	162.5±4.610*	12.16	150.8±3.962***
4	95.00±3.162	185.0±6.325a	150.0±3.873***	18.91	142.5±5.123***	150.0±3.873***	18.91	142.5±5.123***

All the values are expressed as mean±SEM, n=6, [a]p<0.001 as compared to normal group and *p<0.05, **p<0.01, ***p<0.001 (One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test) as compared to venom control group. [EAR= Ethanol extract of *Asparagus racemosus*, EAR= Ethanol extract of *Asparagus racemosus*].

Table 6 : Effect of AAR on *Naja naja* induced bleeding.

Bleeding time in hours	**Groups**							
	Normal	**Venom control**	**AAR 300 mg/kg**		**AAR 600 mg/kg**		**AAR 900 mg/kg**	
	Bleeding time (Sec)	**Bleeding time (Sec)**	**Bleeding time (Sec)**	% Inhibition	**Bleeding time (Sec)**	% Inhibition	**Bleeding time (Sec)**	% Inhibition
1	102.5±6.021	207.5±4.610[a]	176.0±3.077***	15.18	153.2±3.371***	26.16	144.5±3.294***	30.36
2	100.0±5.000	202.5±3.354[a]	175.0±5.000***	13.58	150.0±3.873***	25.92	135.0±5.477***	33.33
3	97.50±5.123	185.0±6.325[a]	177.5±4.610	4.05	148.5±4.161***	19.72	135.0±3.873***	27.02
4	95.00±3.162	185.0±6.325[a]	175.0±5.000	5.40	147.5±4.610***	20.27	132.5±4.610***	28.37

All the values are expressed as mean±SEM, n=6, [a]p<0.001 as compared to normal group and *p<0.05, **p<0.01, ***p<0.001 (One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test) as compared to venom control group. [EAR= Ethanol extract of *Asparagus racemosus*, AAR= Aqueous extract of *Asparagus racemosus*].

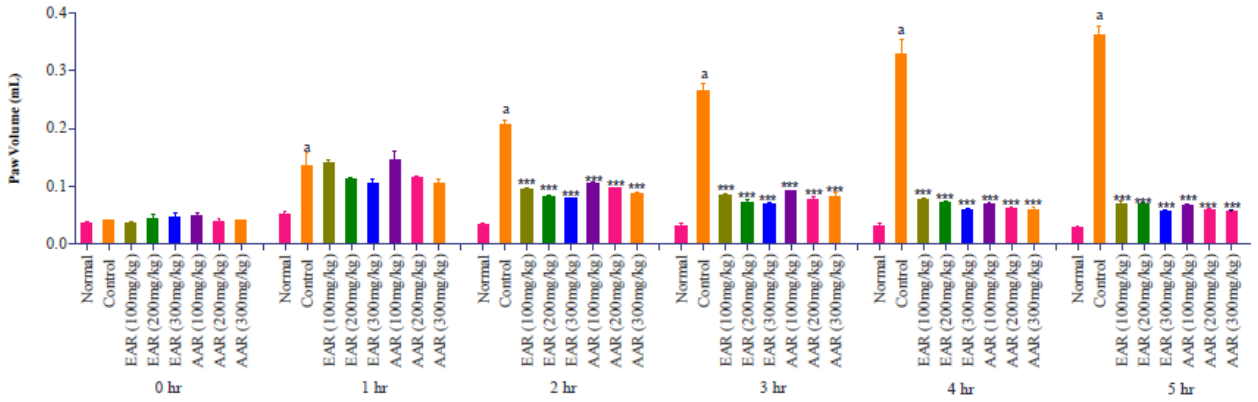

Fig 3. *Naja naja* venom induced edema and its inhibition by ethanol and aqueous extracts of *Asparagus racemosus*. All the values are expressed as mean±SEM, n=6, [a]p<0.001 as compared to normal group and *p<0.05, **p<0.01, ***p<0.001 (One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test) as compared to venom control group. [EAR= Ethanol extract of *Asparagus racemosus*, AAR= Aqueous extract of *Asparagus racemosus*].

DISSCUSSION

Asparagus racemosus showed significant antivenom activity against *Naja naja* venom. Envenomation by *Naja naja* causes pathological changes in the victims and necrosis is the chief manifestation, occasionally it is also associated with neurological dysfunction. In absence of antivenom therapy, humans envenomed with *Naja naja* venom die a distressful death. *Naja naja* venom contains neurotoxins that are relevant clinically and they bind to nicotinic Ach receptors exhibiting depolarizing neuromuscular blockade. Irreversible cell depolarization is caused by cardio toxins and leads to clinical manifestations like dysrhythmia, hypotension and death. Some of

the toxins activate complement via C3-C9 sequence [15].

Many Indian medicinal plants are recommended for management of snake envenomation [16]. *Asparagus racemosus* contains alkaloids, flavonoids, glycosides and tannins[17, 18, 19]. *Asparagus racemosus* roots are known to possess several active constituents that have been established by scientific research to possess inherent antivenom activity by researchers involved in venom research.

Ethanol and aqueous extracts of *Asparagus racemosus* were found to inhibit the lethality induced by the *Naja naja* venom. The ethanol extract (500 mg/kg) was more effective in inhibiting the venom lethality, when compared to the aqueous extract (500 mg/kg). Superior performance of ethanol extract may be attributed to presence of tannins, alkaloid and flavonoids.

Naja naja venom contains toxic enzymes like Phospholipase A_2 that has variable toxicity, hyaluronidase that is responsible for facilitation of dispersion of other toxins, L-aminoacid oxidase that imparts characteristic yellow color to the venom. It also has acetyl choline, acetyl hydrolase [20]. The concentrations and potencies of various site specific toxins and hydrolytic enzymes of venom are known to vary depending on various factors [21] thus complicating the management of snake bite.

Phospholipase A_2 activity was estimated by using sheep RBC. The lyses of sheep RBC were inhibited by both EAR and AAR. Ethanol extract resulted in greater protective activity as compared to aqueous extract. Higher degree of protection was observed with ethanol extract at a dose of 800 µg. The inhibition of PLA_2 activity evidenced by inhibition in size of haloes with ethanol and aqueous extracts of *Asparagus racemosus* is a marker towards the protective action of these extracts against *Naja naja* venom induced toxicity.

Since *Naja naja* venom exhibited increase in coagulation time, ethanol and aqueous extracts of *Asparagus racemosus* were subjected to coagulant activity. The results obtained suggest that both ethanol and aqueous extracts have significant coagulant activity. The ethanol extract was capable of inhibiting the anticoagulant activity of venom by 71.87% at a dose of 300 µg/kg and the aqueous extract inhibited anticoagulant activity of venom by 78.87% at a dose of 300 µg/kg. Since *Asparagus racemosus* is a rich source of vitamin K and calcium and both being key factors in coagulation mechanism, the ability of ethanol and aqueous extracts to inhibit anticoagulant activity of *Naja naja* venom supports the observations of this research. Additionally, the inhibitory effect on the anticoagulant activity of *Naja naja* venom may also be due neutralization of venom components.

Ethanol and aqueous extracts of *Asparagus racemosus* were subjected to bleeding time activity, the results obtained indicate that both the extracts managed to reduce the bleeding time significantly when compared with bleeding time observed with venom treated animal. Bleeding time was inhibited by about 36.44% for ethanol extract at 2 hr with dose of 900 mg/kg. Similarly, reduction in bleeding time was observed by about 33.72% for aqueous extract.

Protective action of ethanol and aqueous extracts of *Asparagus racemosus* were evaluated against *Naja naja* induced edema. At 5 hr time point significant decrease in edema was observed with both the extracts at dose level of 300 mg/kg. Ethanol extract inhibited edema by 84.88% and aqueous extract inhibited by 84,53%.

Antivenom activity by glycoside, tannins, alkaloids and flavonoids showed potential against snake venom [20]. This indicates that that various tannins, alkaloids and flavonoids along with vitamin K and calcium ions that are the active constituents of *Asparagus racemosus* may be responsible for the antivenom activity of the extracts of *Asparagus racemosus.*

CONCLUSION

The present research indicates that *Asparagus racemosus* is potential candidate for use in the management of *Naja naja* envenomation. Further studies are required to elucidate the molecular mechanism of action and to evaluate its usefulness in management of envenomation by *Naja naja.*

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CONFLICTS OF INTEREST

There is no conflict of interest.

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