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Research Article

ANTI RED SCORPION VENOM ACTIVITY OF ANDROGRAPHIS PANICULATA

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ABSTRACT

Objective: Red scorpion (*Mesobuthus tamulus*) is the most lethal among all poisonous species of scorpion. Envenoming by *Mesobuthus tamulus* is quite common along the western coast of India, without any established therapy. *Andrographis paniculata* is one of the plants which have long been used in traditional herbal medicine for the treatment of poisoning by animal bites. Hence the study was planned to evaluate the ethanolic extract of *Andrographis paniculata* for the treatment of *Mesobuthus tamulus* envenoming.

Materials and Methods: Ethanolic extract of plant *Andrographis paniculata* was obtained using soxhelet apparatus. Lyophilized venom sample of *Mesobuthus tamulus* was used. Swiss albino mice weighing 20-30 gm were used in the study. Calculation of LD_{99} of *Mesobuthus tamulus* venom was done using Turner's method. Acute toxicity of *Mesobuthus tamulus* venom and its neutralization by plant extract at the dose of 1gm/kg and 2gm/kg in vivo was seen. Neutralization of the lethal venom effect of *Mesobuthus tamulus* by plant extract at the dose of 1gm/kg and 2gm/kg by Alam and Gome's method (in vitro) was also seen.

Results: The LD_{99} of *Mesobuthus tamulus* venom from this study was determined to be 25.12 µg/gm and LD_{50} was 15.85 µg/gm. In the acute toxicity and in vivo neutralization study plant extract at the dose of 1gm/kg and 2gm/Kg resulted in mean survival of 62.667 mins. and 39.333 mins. respectively. Neutralization of the lethal venom effect of *Mesobuthus tamulus* by plant extract at the dose of 1gm/kg and 2gm/kg by Alam and Gome's method (in vitro) showed mean survival of 49.667 mins and 42.5 mins respectively.

Conclusion: Ethanolic extract of *Adrographis Paniculata* has some protective effect against the Red Scorpion Venom in mice but doesn't offer any survival benefit.

Keywords: Mesobuthus tamulus, Adrographis Paniculata, LD_{99}

INTRODUCTION

Red scorpion (*Mesobuthus tamulus*) is the most lethal among all poisonous species of scorpion.[1] Scorpion venom is a potent sodium channel activator[2] and envenoming by *Mesobuthus tamulus* results in sudden pouring of endogenous catecholamines into circulation due to the autonomic storm evoked by delayed inactivation of neuronal sodium channels.[1] Vomiting, profuse sweating, priapism in males and cold extremities precede the development of severe cardiovascular manifestations.[3] Clinical manifestations depend upon dose of venom, season of sting and time elapsed between sting and hospitalization.[4] Alpha-receptor stimulations play a major role in the pathogenesis of acute pulmonary oedema. About 30–50% fatality due to acute pulmonary oedema with scorpion sting has been reported from India.[5] Early reporting of a case and immediate hospitalization to facilitate the administration of prazosin arrest the development of severe life-threatening cardiovascular manifestations.[6]

Scorpion antivenin did not reverse and prevent the cardiovascular morbidity and mortality due to envenoming by red scorpion sting. [7]

There is no standard protocol for the treatment of scorpion venom poisoning. Various regimens including decongestive treatment, beta blocker, nifedipine, excessive diuretics, lytic-cocktail and insulin-glucose were tried, with no benefits even the serotherapy for the scorpion envenoming is not established in India.[8]

Andrographis paniculata (AP) is one of the plants which have long been used in traditional herbal medicine. It is widely found and cultivated in tropical and subtropical Asia, south-east Asia and India. [9, 10] It is a herbaceous plant commonly known as "King of bitters" due to its extreme bitter taste or "Kalmegh" belongs to family Acanthaceae. It is also known as 'Bhui-neem', since the plant, though much smaller in size, shows similar appearance and has bitter taste as that of Neem (*Azadirachta indica*). In Tamil it is called as 'Sirunangai' or 'Siriyanangai'.

Intraperitoneal injection of an ethanol extract of the aerial parts of the plant *Andrographis paniculata* (25gm/kg body weight) to mice poisoned with cobra venom had markedly delayed the occurrence of respiratory failure and death.[11, 12]

Hence we decided to try the ethanolic extract of Andrographis paniculata for the treatment of Mesobuthus tamulus.

MATERIALS AND METHODS

Collection of the Plant Materials

The Plant material was brought from the Tamil Nadu, India. The plant was authenticated by Department of Botany, of Science College. The plant was then cultivated during early rainy season (June and July) in the local garden of the college. The plants at flowering stage that is after 90–120 days of sowing, were cut at the base leaving behind about 10–15cms of stem for plant regeneration.

Preparation of extract

Fresh plants were collected, cleaned under running tap water, shade dried, fine powdered and stored in airtight container until further processing. The alcoholic extract was prepared according to the procedure reported by Mahanta & Mukharjee.[13] Forty grams of dried powder of plant was macerated in 95% of ethanol overnight. It was then packed in the timble of soxhelet apparatus and was extracted using 95% ethanol refluxing at 60-80° C. The extract thus obtained was dark green to brown in colour. The stock extract thus obtained was preserved in airtight glass container and kept inside the refrigerator at 4° C.

Venom Sample

Lyophilized venom sample of *Mesobuthus tamulus* was purchased from Haffkine Institute, Parel, Mumbai, India and was stored at 2-8° C for future use, taking all the precautionary measures of handling and storage.

Experimental animals

Swiss albino mice weighing 20-30 gm were used in the study. All the animals were housed in polypropylene cages and maintained at a

temperature of 25º ± 2ºC. They were kept in a 12:12 hour light : dark cycle and fed on standard laboratory chow and water ad libitum. Animals were acclimatized to laboratory conditions before the test for 10 days.

Ethical clearance

The protocol was submitted and due clearance was taken from Institutional Animal Ethics Committee of the institute where the research was conducted.

Calculation of LD_{99} of Red Scorpion (Mesobuthus tamulus) venom

Lethal dose 99 (LD_{99}) is defined as the least amount of venom (dry weight in grams) injected intraperitoneally to animals resulting in the 99% death of animals within 24 hours. The method reported by Turner was adopted for determination of LD_{99}. [14]

The *Mesobuthus tamulus* venom was dissolved in distilled water and given to mice intraperitoneally (i.p.) in graded doses starting with 1.2mcg/gm and mortality was recorded for 24 hours. 5 animals were taken in each group.

Acute toxicity of Mesobuthus tamulus venom and its neutralization by plant extract

Animals were divided into three groups of six animals each. Each animal in the groups 1-3 was administered LD_{99} of *Mesobuthus tamulus* venom i.p. Animals in Group 1 received distilled water (DW) and this group was considered as control. Animals in Group 2 and Group 3 received plant extract at the dose of 1gm/kg and 2gm/kg i.p. respectively. Plant extract was given 5 minutes after the dose of *Mesobuthus tamulus* venom. In all the groups the duration of survival and the number of animals survived was recorded for 24 hours. All the groups received same volume of preparations. All the experimental procedures were carried out at the same time of the day, between 0900hrs and 1200hrs.

Neutralization of the lethal venom effect of Red Scorpion (Mesobuthus tamulus) by Alam and Gome's method

Neutralization test described by Alum and Gomes was followed.[15] Animals were divided into three groups of six animals each. LD_{99} of *Mesobuthus tamulus* venom was mixed in vitro with distilled water (DW) and plant extracts at the dose of 1gm/kg and 2gm/kg respectively for group 1, 2 and 3; then the mixture was incubated for 1 hour at 37° C and centrifuged at 2000 rpm for 10 min. The supernatant was injected i.p. into mice. The duration of survival and the number of animals survived was recorded for 24 hours after admixture injection of venom. Thus Group 1 received distilled water incubated with LD_{99} of *Mesobuthus tamulus* venom i.p. and served as control Group 2 received 1gm/kg plant extract incubated with LD_{99} of *Mesobuthus tamulus* venom i.p. and Group 3 received 2gm/kg of plant extract incubated with LD_{99} of *Mesobuthus tamulus* venom i.p. All the groups received same volume of preparations. All the experimental procedures were carried out at the same time of the day, between 0900hrs and 1200hrs.

Blinding

All the experiments were singly blinded, to prevent observational bias, [16] in which one of the post graduate student recorded the survival time and animals survived in each experiment.

Statistical Analysis

The statistical analysis was done using one way analysis of variance (ANOVA) using unpaired student's t test. P value ≤0.05 was considered as statistically significant and ≤0.005 was considered to be highly significant.

RESULTS

Calculation LD_{99} of Mesobuthus tamulus venom

Lethality data of *Mesobuthus tamulus* venom is shown in Table 1 LD_{99} was calculated by probit analysis. The LD_{99} of *Mesobuthus tamulus* venom from this study was determined to be 25.12 µg/gm. LD_{50} was also calculated from the same data and was found to be 15.85 µg/gm. (Table 1, Graph 1)

Acute toxicity of Mesobuthus tamulus venom and its neutralization by plant extract

The *Mesobuthus tamulus* venom at the dose of 25.12 µg/gm (LD_{99}) produced 100% death in mice. The ethanolic extract of plant *Andrographis* significantly increased mean survival time and the protection fold but could not protect animals from death when used alone.

The plant extract when used alone at the dose of 1gm/kg was found more effective against *Mesobuthus tamulus* venom showing mean survival of 62.67 mins as compared to 39.33 mins seen with plant extract at the dose of 2 gm/kg. (Table 2)

Table 2: Acute toxicity of Mesobuthus tamulus venom and its neutralization by plant extract

Groups (n=6)	Mean Survival time(mins)	Protection fold	Total animal Survival/total no. of animals in group	% Survival
Group 1 LD_{99}SV+DW	17.833± 13.166	-	0/6	0
Group 2 LD_{99}SV+PE 1	62.667± 22.214**	3.51	0/6	0
Group 3 LD_{99}SV+PE 2	39.333± 13.049*	2.21	0/6	0

Results were expressed in Mean ± SD; unpaired student "t" test; *P < 0.05; **P < 0.005 , LD_{99}SV: LD_{99} of scorpion venom. PE1: Plant Extract of Andrographis paniculata at the dose of 1gm/kg. PE2: Plant Extract of Andrographis paniculata at the dose of 2 gm/kg. DW: Distilled water. Protection fold: Time duration as compared to Group 1

Neutralization of the lethal venom effect of Red Scorpion (Mesobuthus tamulus) by Alam and Gome's method

The LD_{99} of *Mesobuthus tamulus* venom which was mixed with distilled water (DW), as control, resulted in 100% mortality of mice. Whereas, LD_{99} of *Mesobuthus tamulus* venom when mixed with ethanolic extract of plant Andrographis, resulted in significant increase in mean survival time and the protection fold but had no effect on animal mortality.

The plant extract when used at the dose of 1gm/kg was found more effective against *Mesobuthus tamulus* venom showing mean survival of 49.67 mins as compared to 42.5 mins shown by plant extract at the dose of 2 gm/kg. (Table 3)

Table 3: Neutralization of the lethal venom effect of Mesobuthus tumulus by Alam and Gome's method

Groups (n=6)	Mean Survival time (mins)	Protection fold	Total animal Survival /total no. of animals in group	% Survival
Group 1 LD_{99}SV+ DW	18.833 ± 13.527	-	0/6	0
Group 2 LD_{99}SV+ PE 1	49.667 ± 15.908**	2.64	0/6	0
Group 3 LD_{99}SV+ PE 2	42.5 ± 13.838*	2.26	0/6	0

Results were expressed in Mean ± SD; unpaired student "t" test; *P < 0.05; **P < 0.005; LD_{99}SV: LD_{99} of Red scorpion venom. PE1: Plant Extract of Andrographis paniculata at the dose of 1gm/kg. PE2: Plant Extract of Andrographis paniculata at the dose of 2 gm/kg. DW: Distilled water; ASV. Protection fold: Time duration as compared to Group 1

Table1: Calculation LD_{99} of Mesobuthus tamulus venom in mice receiving various doses of Mesobuthus tamulus venomby Turner's method (n=5).

Dose (mcg/gm)	Adjusted(Dosex100)	Log dose	Dead/total	Dead %	Corrected* formula %	Probit
1.25	125	2.096 ≈ 2.1	0/5	0%	5	3.36≈ 3.4
2.5	25	2.3979≈ 2.4	1/5	20%	20	4.16≈ 4.2
5	500	2.6990≈ 2.7	3/5	60%	60	5.25≈ 5.3
10	1000	3.000≈ 3.0	1/5	20%	20	4.16≈ 4.2
20	2000	3.3010≈3.3	575	100%	95	6.64≈ 6.6

Corrected formula*: For the 0% dead: 100(0.25/n) = 100(0.25/5) = 5, For the 100% dead: 100[(n-0.25)/n] = 100; [(5-0.25)/5] = 95, n is the number of animals in the group

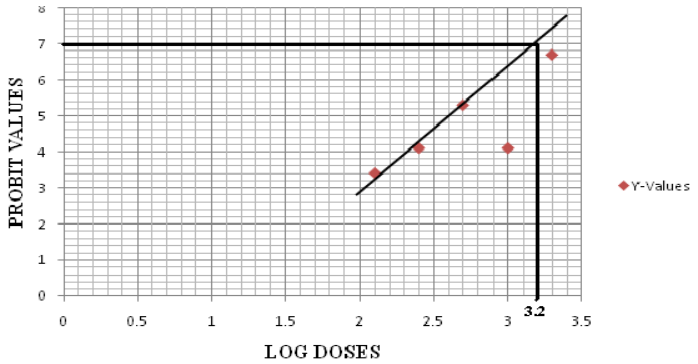

Fig. 1: Calculation LD_{99} of Mesobuthus tamulus venom in mice receiving various doses of Mesobuthus tamulus venom by Turner's method (n=5).

DISCUSSION

LD_{99} of *Mesobuthus tamulus* venom by probit analysis was found to be 15.85µg/gm. This LD_{99} was taken to analyze the anti-scorpion venom effect of the plant under study. LD_{99} value was preferred as the chances of the mortality of mice with LD_{99} dose is more than LD_{50}. Since no standard treatment protocol is followed for the evnoming of *Mesobuthus tumulus*, hence no standard was taken.[8]

When LD_{99} is injected in the mice it produced 100% deaths. The ethanolic extract of plant Andrographis significantly increased mean survival time and the protection fold but could not protect animals from death when used alone. The best results are obtained at the dose of 1gm/kg (62.67 mins) as compared to the dose of 2 gm/kg (39.33 mins) this may be due to some pharmacokinetic and dynamic reasons which can further be evaluated in separate study.

Neutralization of the lethal venom effect of Red Scorpion (*Mesobuthus tamulus*) when studied by Alam and Gome's method showed that when plant extract was used at the dose of 1gm/kg, it was found more effective against *Mesobuthus tamulus* venom showing mean survival 49.67 mins as compared to 42.5 mins shown by plant extract at the dose of 2 gm/kg. None of the groups showed complete protection from the lethal effects of the poison.

It was observed that the plant extract of *Andrographis paniculata* provides some protection against the lethal dose of venom. Certain naturally occurring substances in *Andrographis paniculata* such as sitosterol, pentacyclic terpines, nitro compounds (aristolchic acid), cinnamic acid derivatives, curcumimoids, polyphenolic compounds and flavonoids are known compounds possessing protein binding and enzyme inhibiting properties. The leaves of Andrographis paniculata contain andrographolide, and it is claimed that active constituent of which is diterpene and is responsible for anti-scorpion-venom property by modifying the actions of proteins and enzymes. Further studies are required to potentiate this claim.

This protective property of *Andrographis paniculata* can be explored in practice where significant amount of time is lost while shifting the patient from the Primary Health Care Centre to the Tertiary Health Care Centre.

CONCLUSION

Ehanolic extract of *Androgrphics pinniculata* have some protective effect against the red scorpion venom in mice.Further studies are required in humans to potentiate this claim.

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