

Partial *in vitro* and *in vivo* red scorpion venom neutralization activity of *Andrographis paniculata*

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ABSTRACT

Objective: Red scorpion (*Mesobuthus tamulus*) is the most lethal among all poisonous species of scorpions. Envenoming by *Mesobuthus tamulus* is quite common along the western coast of India, without any established therapy. *Andrographis paniculata* is one of the plants that has 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 the plant *Andrographis paniculata* was obtained using a soxhlet apparatus. Lyophilized venom sample of *Mesobuthus tamulus* was used. Swiss albino mice weighing 20–30 g were used in the study. Calculation of LD₉₉ of *Mesobuthus tamulus* venom was performed using Turner's method. Acute toxicity of *Mesobuthus tamulus* venom and its neutralization by the plant extract at a dose of 1 g/kg and 2 g/kg *in vivo* was seen. Neutralization of the lethal venom effect of *Mesobuthus tamulus* by plant extract at the dose of 1 g/kg and 2 g/kg by Alam and Gome's method (*in vitro*) was also seen. **Results:** The LD₉₉ of *Mesobuthus tamulus* venom from this study was determined to be 25.12 µg/g and the LD₅₀ was 15.85 µg/g. In the acute toxicity and *in vivo* neutralization study, plant extract at the dose of 1 g/kg and 2 g/kg resulted in a mean survival of 62.667 min and 39.333 min, respectively. Neutralization of the lethal venom effect of *Mesobuthus tamulus* by the plant extract at the dose of 1 g/kg and 2 g/kg by Alam and Gome's method (*in vitro*) showed a mean survival of 49.667 min and 42.5 min, respectively. **Conclusion:** The ethanolic extract of *Andrographis paniculata* has some protective effect against the red scorpion venom in mice but does not offer any survival benefit.

KEYWORDS: *Mesobuthus tamulus*, *Adrographis paniculata*, LD₉₉

INTRODUCTION

Red scorpion (*Mesobuthus tamulus*) is the most lethal among all poisonous species of scorpions.^[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 on the 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 edema. About 30–50% fatality due to acute pulmonary edema 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-

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cocktail and insulin-glucose were tried, with no benefits. Even the serotherapy for scorpion envenoming is not established in India.^[8]

Andrographis paniculata (AP) is one of the plants that 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,” belonging to the family Acanthaceae. It is also known as “Bhui-neem” as the plant, although 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* (25 g/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 Tamil Nadu, India. The plant was authenticated by the Department of Botany, of Science College. The plant was then cultivated during the early rainy season (June and July) in the local garden of the college. The plants at the flowering stage, i.e. after 90–120 days of sowing, were cut at the base leaving behind about 10–15 cm of stem for plant regeneration.

Preparation of Extract

Fresh plants were collected, cleaned under running tap water, shade-dried, fine-powdered and stored in an airtight container until further processing. The alcoholic extract was prepared according to the procedure reported by Mahanta and Mukharjee.^[13] Forty grams of dried powder of plant was macerated in 95% of ethanol overnight. It was then packed in the timple of the soxhlet apparatus and was extracted using 95% ethanol refluxing at 60–80°C. The extract thus obtained was dark green to brown in color. The stock extract thus obtained was preserved in an 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 g were used in the study. All the animals were housed in polypropylene cages and maintained at a temperature of $25^{\circ} \pm 2^{\circ}\text{C}$. They were kept in a 12:12 h 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 the Institutional Animal Ethics Committee of the institute where the research was conducted.

Calculation of LD₉₉ of Red Scorpion (*Mesobuthus tamulus*) venom

Lethal dose 99 (LD₉₉) is defined as the least amount of venom (dry weight in grams) injected intraperitoneally to animals resulting in 99% death of the animals within 24 h. The method reported by Turner was adopted for determination of LD₉₉.^[14]

The *Mesobuthus tamulus* venom was dissolved in distilled water and given to mice intraperitoneally (i.p.) in graded doses starting with 1.2 mcg/g and mortality was recorded for 24 h. Five 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 LD₉₉ of *Mesobuthus tamulus* venom i.p. Animals in group 1 received distilled water (DW) and this group was considered as the control. Animals in group 2 and group 3 received plant extract at the dose of 1 g/kg and 2 g/kg i.p., respectively. Plant extract was given 5 min 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 h. All the groups received the same volume of preparations. All the experimental procedures were carried out at the same time of the day, between 09:00 h and 12:00 h.

Neutralization of the Lethal Venom Effect of Red Scorpion (*Mesobuthus tamulus*) by Alam and Gome's Method

The neutralization test described by Alum and Gomes was followed.^[15] Animals were divided into three groups of six animals each: LD₉₉ of *Mesobuthus tamulus* venom was mixed *in vitro* with DW and plant extracts at a dose of 1 g/kg and 2 g/kg, respectively, for groups 1, 2 and 3. Then, the mixture was incubated for 1 h 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 h after admixture injection of venom. Thus, group 1 received DW incubated with LD₉₉

of *Mesobuthus tamulus* venom i.p. and served as the control, group 2 received 1 g/kg plant extract incubated with LD₉₉ of *Mesobuthus tamulus* venom i.p. and group 3 received 2 g/kg of plant extract incubated with LD₉₉ of *Mesobuthus tamulus* venom i.p. All the groups received the same volume of preparations. All the experimental procedures were carried out at the same time of the day, between 09:00 h and 12:00 h.

Blinding

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

Statistical Analysis

The statistical analysis was performed using one-way analysis of variance (ANOVA) using unpaired Student's t-test. A *P*-value ≤0.05 was considered statistically significant and ≤0.005 was considered to be highly significant.

RESULTS

Calculation LD₉₉ of *Mesobuthus tamulus* Venom

Lethality data of *Mesobuthus tamulus* venom is shown in Table 1. LD₉₉ was calculated by probit analysis. The LD₉₉ of *Mesobuthus tamulus* venom from this study was determined to be 25.12 µg/g. LD₅₀ was also calculated from the same data and was found to be 15.85 µg/g [Table 1, Graph 1].

Acute Toxicity of *Mesobuthus tamulus* Venom and its Neutralization by the Plant Extract

The *Mesobuthus tamulus* venom at a dose of 25.12 µg/g (LD₉₉) produced 100% death in mice. The ethanolic extract of the plant *Andrographis* significantly increased the 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 1 g/kg was found to be more effective against *Mesobuthus tamulus* venom, showing a mean survival of 62.67 min as compared to 39.33 min seen with the plant extract at the dose of 2 g/kg [Table 2].

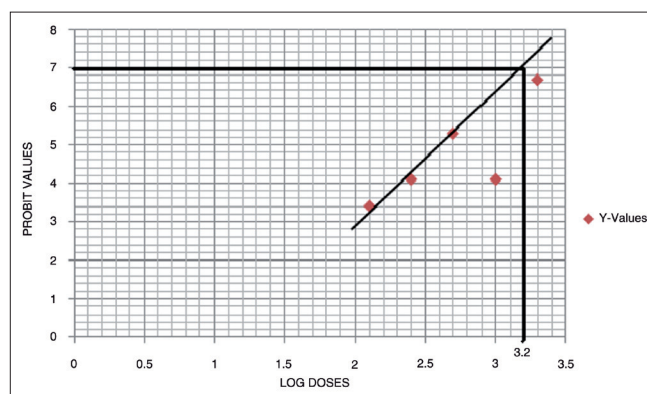
Neutralization of the Lethal Venom Effect of Red Scorpion (*Mesobuthus tamulus*) by Alam and Gome's Method

The LD₉₉ of *Mesobuthus tamulus* venom that was mixed with DW_i as control, resulted in 100% mortality of mice. However, the LD₉₉ of *Mesobuthus tamulus* venom when mixed with the ethanolic extract of plant *Andrographis* resulted in a significant increase in the mean survival time and the protection fold but had no effect on animal mortality.

The plant extract when used at the dose of 1 g/kg was found to be more effective against *Mesobuthus tamulus* venom, showing a mean survival of 49.67 min as compared to 42.5 min shown by the plant extract at a dose of 2 g/kg [Table 3].

DISCUSSION

LD₉₉ of *Mesobuthus tamulus* venom by probit analysis was found to be 15.85 µg/g. This LD₉₉ was taken to analyze the anti-scorpion venom effect of the plant under study. LD₉₉ value was preferred as the chances of the mortality of mice with LD₉₉ dose is more than LD₅₀. Because no standard treatment protocol is followed for the venom of *Mesobuthus tamulus*, no standard was taken.^[8]



Graph 1: Calculation LD₉₉ of *Mesobuthus tamulus* venom in mice receiving various doses of *Mesobuthus tamulus* venom by Turner's method (*n* = 5)

Table 1: Calculation of LD₉₉ of *Mesobuthus tamulus* venom in mice receiving various doses of *Mesobuthus tamulus* venom by Turner's method (*n* = 5)

Dose (mcg/g)	Adjusted (dose × 100)	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	250	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)	5/5	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

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

Groups (n = 6)	Mean survival time (min)	Protection fold	Total animal survival/total no. of animals in the 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 are expressed in mean ± SD; Unpaired Student's "t"-test; *P < 0.05; **P < 0.005; LD₉₉SV: LD₉₉ of scorpion venom; PE1: Plant extract of *Andrographis paniculata* at a dose of 1 g/kg; PE2: Plant extract of *Andrographis paniculata* at a dose of 2 g/kg; DW: Distilled water; Protection fold: Time duration as compared with group 1

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

Groups (n = 6)	Mean survival time (min)	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 are expressed in mean ± SD; Unpaired Student's "t"-test; *P < 0.05; **P < 0.005; LD₉₉SV: LD₉₉ of red scorpion venom; PE1: Plant extract of *Andrographis paniculata* at a dose of 1 g/kg; PE2: Plant extract of *Andrographis paniculata* at a dose of 2 g/kg; DW: Distilled water; ASV; Protection fold: Time duration as compared with group 1

When LD₉₉ is injected in the mice, it produced 100% deaths. The ethanolic extract of plant *Andrographis* significantly increased the 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 1 g/kg (62.67 min) as compared to the dose of 2 g/kg (39.33 min), which may be due to some pharmacokinetic and dynamic reasons that can further be evaluated in a separate study.

Neutralization of the lethal venom effect of red scorpion (*Mesobuthus tamulus*) when studied by Alam and Gome's method showed that when the plant extract was used at a dose of 1 g/kg, it was found to be more effective against *Mesobuthus tamulus* venom, showing a mean survival 49.67 min as compared to 42.5 min shown by the plant extract at a dose of 2 g/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 terpenes, 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 the active constituent is a diterpene and is responsible for the 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 a significant amount of time is lost while shifting the patient from the Primary Health Care Centre to the Tertiary Health Care Centre.

CONCLUSION

The ethanolic extract of *Andrographis pinniculata* has some protective effect against the red scorpion venom in mice. Further studies are required in humans to potentiate this claim.

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