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RESEARCH ARTICLE

Evaluation of Anti-Scorpion-Venom Property of Ethanolic Extract of *Mimosa pudica* in Albino Mice

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ABSTRACT

Mesobuthus tamulus (red scorpion) is common in many parts of India, where morbidity and mortality due to stinging have been reported. Prazosin is widely used for the management of the scorpion sting. However, before the patient is taken to the hospital, certain herbal therapy can be tried in order to minimize the morbidity and complications. *Mimosa pudica* is one such plant used for this purpose. Therefore, we did a prospective randomized trial of scorpion venom versus *Mimosa pudica* plant extract alone and with prazosin in the treatment of severe *Mesobuthus tamulus* sting. Swiss albino mice were used in the study. Calculation of LD99 (Lethal dose) of *Mesobuthus tamulus* venom was done using Turner's method. The mean survival time for the mice treated with LD99 dose was 3 minutes. Acute toxicity of the scorpion venom and its neutralization by the plant extract in-vivo was done. In the acute toxicity and in-vivo neutralization by plant extract in the dose of 1gm/Kg and 2gm/Kg resulted in the mean survival time of 7 minutes and 9.83 minutes respectively. In combination of prazosin + plant extract the mean survival time was increased to 20.83 minutes.

KEYWORDS

Mesobuthus tamulus, Mimosa pudica, LD99 (Lethal dose)

INTRODUCTION

Scorpion sting can pose a life threatening acute medical emergency and is a neglected public health problem in tropical and sub-tropical countries, especially in North Africa, the Middle East, Latin America, and India. Mesobuthus tamulus, an Indian red scorpion is the most lethal species of Buthidae family in India[1].

The scorpion venom delays the closing of neuronal sodium channels[2], resulting in "autonomic storm" owing to sudden pouring of endogenous catecholamines into the circulation[3].

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In the past various regimens, including a lytic-cocktail, insulin, atropine, beta blocker, nifedipine, and captopril have failed to reduce morbidity and mortality[4]. However, since the advent of the alpha1 blocker prazosin the fatality rate has been reduced to 1%.

Scorpion anti-venom is a specific antidote capable of neutralizing the circulating venom toxins if administered soon after sting. Whether the antivenom can reverse the cardiac pathophysiological effects of scorpion venom is uncertain[5].

De Rezende and colleagues[6] found that although venom antigen in plasma from people who had been stung by a scorpion was not detected one

hour after antivenom therapy, and pain and agitation disappeared within a few hours, patients with pulmonary oedema recovered only 48 hours after serotherapy.

Mesobuthus tumulus is common in the western Maharashtra, Saurashtra, Kerala, Andhra Pradesh, Tamil Nadu, and Karnataka states in India where more morbidity and mortality due to stinging have been reported. Prazosin is widely used for the management of Mesobuthus tumulus sting[7]. Being a competitive post-synaptic α1 adrenoceptor antagonist, is used as the first line of management[8]. We did a prospective, randomized trial of scorpion antivenom plus prazosin versus *Mimosa pudica* plant extract alone in the treatment of severe Mesobuthus tumulus sting.

Mimosa pudica is one of the plants that have long been used in traditional herbal medicine[8]. It is widely found and cultivated in South America and Central America but is now a pan tropical weed. *Mimosa pudica is* in the family of Leguminosae[9] and is one of the plants long been used in traditional herbal medicine against scorpion sting.

Hence, we decided to try the ethanolic extract of *Mimosa pudica* for the treatment of Mesobuthus tumulus sting in mice.

Aim and Objective

The aim was to evaluate anti-scorpion-venom property of the ethanolic extract of *Mimosa pudica* in mice.

The objective was to compare the efficacy of *Mimosa pudica* with that of prazosin.

Mimosa pudica

The plant *Mimosa pudica* invites attention of researchers worldwide for its pharmacological activities such as anti-diabetic, antitoxin, anti-hepatotoxin, antioxidant, and wound healing activities[10].

Mimosa pudica (Leguminosae) is a small plant about 3 feet in height cultivated throughout India. It is a multipurpose plant as vegetable, spice, and a source of cooking and cosmetic oil and as a medicinal drug. It is known as Lajjabate in Bengali, Hadergitte in Kannada, Kasirottam in Tamil, and Manugumaramu in Telugu. The leaves, roots, or the whole plant is used for the above mentioned purposes.

Constituents of Mimosa Pudica

The phytochemicals present in Mimosa pudica are tyrosin 3,4-dihydroxypiridine, mimosinamine, mimosinic acid 7,8,3',4'-tetrahydroxyl-6-C-[alpha-L-rhamnopyranosyl-(-->2)]-beta-D-glucopyranosyl flavones; 5,7,3',4'tetrahydroxyl-6-C-[alpha-L-rhamnopyranosyloxy-(1-->2)]-beta-D-glucopyranosyl; ascorbic acid; beta-carotene; crocetin; crocetin-diethyl-ether; catcher; mimosine; norepinephrine; thiamin[9,11].

The whole plant of *Mimosa pudica* is very useful for various pharmacological and biological activities. Mostly roots and leaves are shown to have maximum pharmacological activity.

MATERIALS AND METHODS

Laboratory bred albino mice of either sex, weighing 18 – 22g were used for the study. The animals were maintained under standard laboratory conditions at 25°C environment, commercial pellet diet with water at lebitum and normal photo period (12hr dark/12hr light).

The experimental protocol was approved by the Institutional Animal Ethics Committee, Mamata Medical College, Khammam. [proposal number – IAEC/2012 – 7].

Drugs and Chemicals

Prazosin, 5mg tablets from Cipla, India; alcoholic extract of *Mimosa pudica.*

Ryle's infant feeding tube (IFT), wooden mouth gag, syringes, mortar and pestle, and stop-watch.

The *Mimosa pudica* ethanolic extract was obtained using Soxhlet apparatus by following Turner's method[12].

Acute Toxicity Study and Establishment of Dose of the Extract

Acute toxicity tests in rats and mice have proven the LD50 of *Mimosa pudica* extract to be more than 5g/Kg. Based on the results obtained this

study, the dose of ethanolic extract of *Mimosa pudica* for Anti-scorpion venom activity was fixed to be 1g/Kg and 2g/Kg body weight.

Inclusion criteria:

1. Albino mice of either sex weighing between 18 and 22g.
2. Healthy mice with normal behaviour and activity.

Exclusion criteria:

1. Albino mice weighing less than 18g or more than 22g.
2. Pregnant and lactating animals.

Methodology and Experimental Design

After calculating the LD99 of scorpion venom, the venom neutralizing ability of the plant extract in the dose of 1g/Kg and 2g/Kg was determined using in-vitro and in-vivo methods.

The alleviation of the mean survival time of the animals was used to infer the anti-venom property of the drug/extract after challenging with LD99 of scorpion venom.

The animals were divided into six groups, each containing six animals.

- All groups were administered LD99 dose of scorpion venom intraperitoneally.
- Group I was taken as control and distilled water was administered.
- Group II & III were administered the *Mimosa pudica* extract in the dose of 1g/Kg and 2g/Kg respectively.
- Group IV was administered prazosin in the dose of 0.1 mcg/Kg intraperitoneally which was taken as standard.
- Group V & VI were administered *Mimosa pudica* extract in the dose of 1g/Kg and 2g/Kg and prazosin (dose – as in group IV) respectively administered intraperitoneally.

Mimosa pudica extract and prazosin were administered intraperitoneally after 5 minutes of administration of scorpion venom. The duration of survival and the animals survived were recorded for 24 hours.

RESULTS AND DISCUSSION

Statistical Analysis

The statistical analysis of data was done using one way analysis of variance (ANOVA), followed by Dunnett's test using the software 'Primer of biostatistics'. P value of less than 0.05 was considered to be significant.

LD99 (Lethal dose) of Mesobuthus tamulus venom by probity analysis was found to be 20 mcg/g (Table 1 and Figure 1). 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. Treatment protocol is followed for the envenoming of Mesobuthus tamulus and prazosin was taken as standard.

When LD99 is injected in the mice, it produced 100% deaths in 3minutes. The ethanolic extract of the plant *Mimosa pudica* significantly increased the mean survival time to 7 minutes and the protection fold but could not protect animals from death when used alone. The best results were obtained at the dose of 2g/Kg as compared to the dose of 1g/Kg, by increasing the survival time to 9.8 minutes, which may be due to some pharmacokinetic and dynamic reasons that further be evaluated in a separate study (Table 2 and Figure 2). Similar findings were also reported by Mariana AOB, et al, 2014.[13]

Prazosin administration also increases the mean survival time to 11.67, but the more increase in mean survival time was seen when the plant extract was administered along with prazosin 20.83 minutes. None of the groups showed complete protection from the lethal effect of the poison.

It was observed that the plant extract of *Mimosa pudica* provides some protection against the lethal dose of venom. Further studies are required to potentiate this claim.

This protective property of *Mimosa pudica* can be exploited 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.

Table 1: Percentage of death of mice receiving various doses of scorpion venom (n=4)

Dose mcg/Kg	Log dose	Dead/ total	Death %	Corrected *%	Probit
125	2.1	0/4	0	6.25	3.5
250	2.4	2/4	50	50	5.0
500	2.7	1/4	25	25	4.3
1000	3.0	3/4	75	75	5.7
2000	3.3	4/4	100	93.75	6.5

Corrected formula*:

For the 0% dead: 100(0.25/n) = 6.25

For the 100% dead: 100[(n-0.25)/n] = 100[(4-0.25)/4]=93.75

n - Number of animals in the group.

Table 2: Mean survival time, protective fold, survived animals and % survival against the LD99 of scorpion venom when channelized by plant extract and anti-venom immediately after venom administration

Group n=6	Mean survival time in min (Mean ± SEM)	Protection Fold	Total animals survived/total animals in each group	% of survival
Group – I LD99 + DW	3 ± 1.095	-----	0/6	0%
Group – II LD99 + PE1	7 ± 1.673	2.33	0/6	0%
Group – III LD99 + PE2	9.83 ± 1.47	3.276	0/6	0%
Group – IV LD99 + P	11.67 ± 2.16	3.89	0/6	0%
Group – V LD99 + PE1 + P	15 ± 2.19	5	0/6	0%
Group – VI LD99 + PE2 + P	20.83 ± 2.317	6.943	0/6	0%

Result were expressed in Mean ± SEM; Unpaired Student t test; *P<0.001;

LD99SV: LD99 Scorpion venom

PE1= Plant Extract of mimosa pudica at the dose of 1Gm/Kg,

PE2= Plant Extract of mimosa pudica at the dose of 2Gm/Kg.

P= prazosin

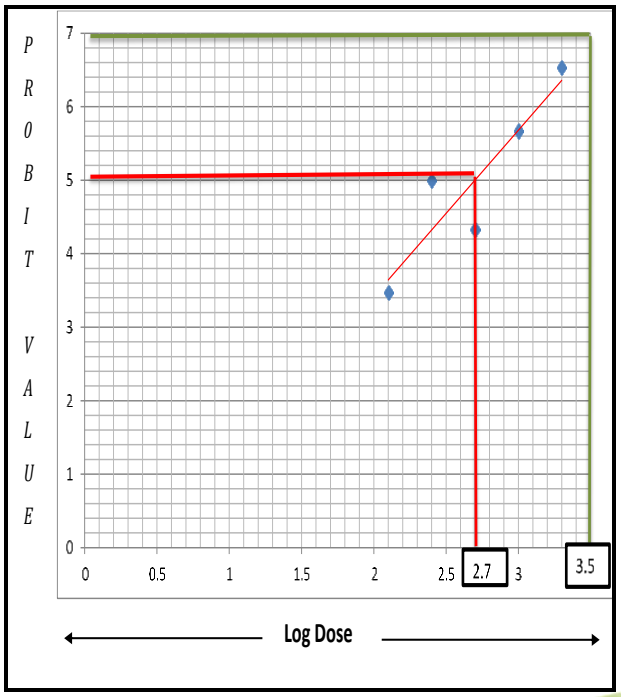

Figure 1: Graph showing the probit value against the log dose

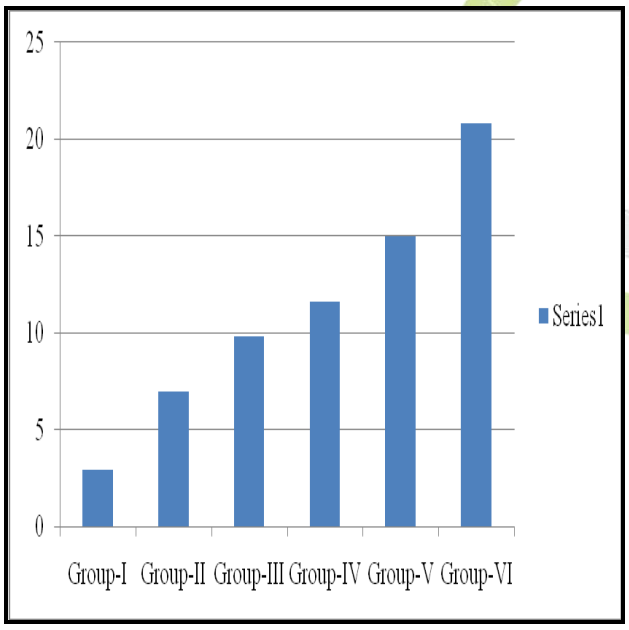

Figure: 2 Bar- diagram showing the survival time of the animals against LD99 (Lethal dose) of scorpion venom when challenged by the plant extract and anti-venom

CONCLUSION

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

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