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RESEARCH ARTICLE

A Pre-clinical Study of Jeerakadi Lepa against Indian Red Scorpion Venom Poisoning

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Abstract

Scorpion stings are very common in India, especially in the Konkan region of Maharashtra state. Scorpion stings show various signs and symptoms. A preclinical study to validate the efficacy of an Ayurvedic preparation called Jeerakadi Lepa was done in Swiss albino mice. A total of 18 Swiss albino mice (n = 18) were divided into 3 groups: the control group (n = 6), the standard group (n = 6) and the experimental group (n = 6). Only Indian red scorpion venom was administered by the SC route in the control group. In contrast, the venom was administered by SC in the standard group, followed by an oral tablet. Prazosin, and in the experimental group, the venom was administered by SC route, followed by local Jeerakadi Lepa. Kruskal-Wallis's test was applied at a 95% confidence interval to assess various toxicity parameters like lacrimation, salivation, etc. The P-value was 0.04433 in toxicity symptoms ($P < 0.05$), but it was 0.3366 ($P > 0.05$) in the number of deaths of Swiss albino mice in the above groups. It was concluded that Jeerakadi Lepa effectively eliminated all the signs and symptoms of toxicity due to scorpion venom in Swiss albino mice. Still, it should not be used independently among humans.

Keywords: Cumin Seeds, Indian Red Scorpion Poisoning, Jeerakadi Lepa, *Mesobuthus tumulus*, Preclinical Study, Vrischikdansha

1. Introduction

Scorpion sting envenomation is an important health issue in certain parts of the world, including India. Out of a total of 1500 different scorpion species found worldwide, 50 species have been identified as being dangerous to humans[1]. The Konkan is a place where a large amount of biodiversity is seen. The Indian red scorpion (*Mesobuthus tumulus*) is a highly poisonous variety of scorpions commonly seen in this area[2]. Envenomation from a scorpion sting can last up to 30 hours, with local to systemic signs and symptoms[3]. A sharp, burning pain at the sting site, followed by pruritus, erythema, local tissue swelling, and ascending hyperesthesia, may be reported[4]. Systemic signs and symptoms include tachycardia, hypertension, pulmonary edema, seizures, salivation, lacrimation, urination, defecation, dysphagia, generalized weakness, priapism, and rigid spastic muscles of the limbs. The scorpion sting envenomation may prove fatal due to cardiac involvement, especially in children[5].

Dr. Himmatrao Bawaskar worked extensively on scorpion bites, specifically the Indian red scorpion *(Mesobuthus tumulus)*. He invented the role of prazosin in the treatment of scorpion bites[6,7].

Despite the advancements in the treatment of scorpion bites, the pain remains a distressing factor and needs to be resolved. Herbal medicines have been used to treat scorpion bites since ancient times. In India, scorpion bites have been treated with 104 different medicinal plants[8]. Jeeraka is a spice used commonly in the Indian kitchen and has been mentioned as a remedy against an Indian red scorpion bite in Ayurveda. The text *Yogratnakar* mentioned Jeerkadi Lepa, which mainly consists of Jeeraka (*Cuminum cyminum)* with other ingredients[9].

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To assess the efficacy of Jeerkadi Lepa, a preclinical study was needed. Therefore, this study was performed. A total of 18 Swiss Albino mice were divided into 3 groups, namely, the control group (n = 6), the standard group (n = 6), and the experimental group (n = 6). In the control group, Indian red scorpion venom was injected by Sub-Cutaneous (SC) route; in the standard group, Indian red scorpion venom was injected by the SC route and tab. Prazosin was administered by the oral route, while in the experimental group, Indian red scorpion venom was injected by SC and *Jeerakadi Lepa* was applied over the injection site. The results were assessed on various toxicity parameters such as lacrimation, salivation, tremors, convulsions, etc.

1.1 Research Question

Is Jeerakadi Lepa efficacious against Indian red scorpion venom poisoning in albino mice?

1.2 Aim

To determine whether Jeerakadi Lepa is efficacious against Indian red scorpion venom poisoning in albino mice.

1.3 Objectives

1.3.1 Primary

To determine the efficacy of Jeerakadi Lepa in the individual case of Indian red scorpion venom poisoning.

1.3.2 Secondary

To study the signs and symptoms produced by '*Mesobuthus tumulus*' and its management.

1.4 Hypothesis

1.4.1 Null Hypothesis (H_0)

H_0 - Jeerakadi Lepa is not efficacious against Indian red scorpion venom poisoning in albino mice.

1.4.2 Alternative Hypothesis (H_A)

H_A - Jeerakadi Lepa is efficacious against Indian red scorpion venom poisoning in albino mice.

1.5 Study Type

Experimental study; preclinical study; animal study.

1.6 Place of Study

The study was carried out at APT Testing and Research Pvt. Ltd. (approved by Maharashtra state LIC no. 37/PD/TL7) ISO -2001 -2015 certified laboratory, Sr. No. 36/1/1, M.N. 199, Vadgaon Khurd, Pune – 411041, Maharashtra, India.

To determine the efficacy of Jeerakadi Lepa in the preclinical study, a total of 18 (n = 18) albino mice were divided into 3 groups, the control group (n = 6), the experimental group (n = 6), and the standard group (n = 6).

2. Materials and Methods

2.1 Materials

Jeerakadi Lepa is an Ayurvedic preparation mentioned in the text Sharangdhara[6] (Table 1). This preparation comprises *Cuminum cyminum* medicinal paste, honey, cow ghee, and rock salt. It is advised to apply locally at the bite site in a lukewarm state. The ingredients were collected from the market, and authentication and standardization were made at Vasantidevi College of Pharmacy, Kodoli, Dist. – Kolhapur and the prepared Lepa were standardized as per the parameters.

2.2 Animal Ethical Committee Approval

The preclinical study was conducted in compliance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines -2010. The approval of the Institutional Animal Ethical Committee was obtained. (20/1617 dated 27/8/2016) (Research Project No. 2APTRF/IBSC/19).

2.3 Procurement of Venom

Indian red scorpion venom was obtained from Haffkin Biopharmaceutical, Parel, Mumbai, (Batch No: 841) with prior application of the venom purchase by the researcher, forwarded by the Head of the Department and the Dean of the Yashwant Ayurvedic College, PGTRC, Kodoli. A dried and lyophilized form of 300 mg of Indian red scorpion was obtained from the Haffkin Institute.

Table 1. Constituents of Jeerakadi Lepa

Sr No.	Constituent	Latin name	Quantity	form
1	Jeeraka	*Cuminum cyminum* Linn.	2 gm	Powder
2	Cow ghee	----	1 gm	--
3	Honey	---	1 gm	--
4	Rock salt	-----	1 gm	---

The other materials, such as the food for Swiss albino mice, etc., were obtained from the APT Research Institute, Pune.

2.4 Methods

2.4.1 Study Design

A total of 18 (n = 18) Swiss albino mice were obtained from the APT Research Institute, Pune (male 9 and female 9) and were randomly selected. Their average age was 7 ± 1 week. The Average weight was 30 gm ± 8 gm. The female mice were non-pregnant. They were kept on pelleted feed and community tap water was added with Libitum. They were kept at an average room temperature of 22°C ± 2°C. The relative humidity was 50% ± 10%. 12 hours of light and 12 hours of the dark cycle were used.

A total of 18 Swiss albino mice was divided into 3 groups (Table 2)

- Group 1 (control group) – 6 Swiss albino mice (3 males and 3 females).
- Group 2 (standard group) – 6 Swiss albino mice (3 males and 3 females).
- Group 3 (experimental group) – 6 Swiss albino mice (3 males and 3 females).

2.4.2 Dose of Indian Red Scorpion Venom

In an earlier study by Tiwari Anil Kumar and Deshpande SB, the LD_{50} of scorpion venom was estimated as 7.2 ± 1.35 mg/kg[10]. A lyophilized form was diluted to inject mice with the appropriate fatal dose. In a vial of 0.29 gm of Indian Red, Scorpion Venom added PBS (Phosphate Buffer Solution) 1ml. A total of 0.01 ml was taken from this solution and added to 5 ml of PBS in a glass container. Then it was sealed with rubber cork. From this dilution, 1.5 ml was taken out and injected into mice. The scorpion venom obtained was 110 mg. Therefore, we administered 0.4 ml of scorpion venom into a 40 gm bodyweight mouse. The Indian red scorpion venom was injected into all the Swiss albino mice involved in this study (n = 18) (Figures 1, 2 and 3).

Table 2. Study design

Group I (control group)	Only Indian red scorpion venom was injected in the SC route.
Group II (Standard group)	Indian red scorpion venom was injected in the SC route and then Prazosin was given by oral route.
Group III (Experimental group)	Indian red scorpion venom was injected in the SC route then applied Jeerakadi Lepa on injected part and then was applied honey over it.

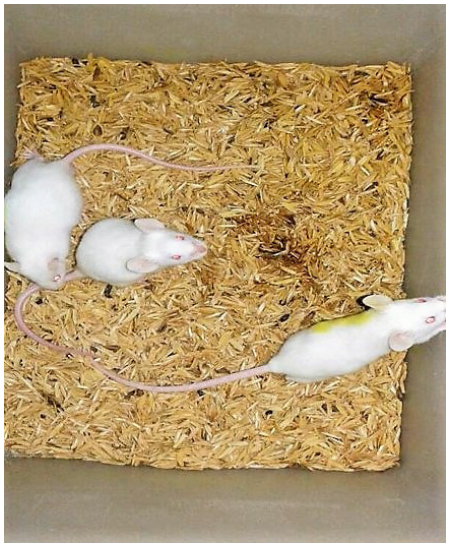

Figure 1. Swiss albino mice before injecting the venom.

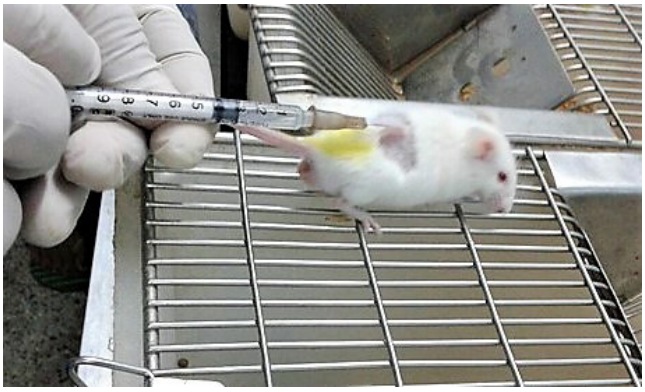

Figure 2. Injecting Indian red scorpion venom.

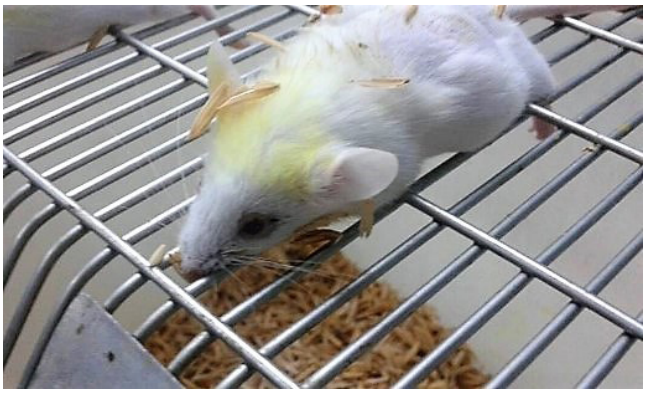

Figure 3. Swiss albino mice after Indan red scorpion venom injection.

2.4.3 Dose of Prazosin

It is a selective α_1 blocker having a selectivity ratio of 1000:1. It has been used as an established drug for severe or moderate envenomation due to the Indian red scorpion (*Mesobuthus tumulus*)[11]. Therefore, prazosin was used as the control. The brand Minipress 5 mg by Pfizer Pharma was used. It is used by humans. So, the dose of prazosin was calculated in mice by the conversion formula, and it was 0.0065 mg/kg. 1 mg of prazosin tablet was dissolved in 10 ml of distilled water, and 6.5 ml out of 10 ml were administered by oral route to the albino mice. The prazosin was administered only to the Swiss albino mice assigned to the standard group (Group II) (n = 6).

2.4.4 Application of Jeerakakdi Lepa

Lepa means the local application of medicinal paste over the affected part. Sushruta[12] and Sharangdhara[13] mentioned 3 types of Lepa. Sharangdhara mentioned that Vishaghna Lepa is one of the three types applicable in this study. All the raw material required for the Jeerakadi Lepa was authenticated and standardized in the pharmacy. It was within the expiry date.

The Jeerakadi Lepa was prepared as per Lepa Vidhi. The sequence for the preparation was followed, as described in the text Yogratnakar.

The first medicinal paste of fine powder of the cumin seeds (*Cuminum cyminum*) was taken on a plate, cow ghee, and rock salt in the dose of 1 gm each was added. The mixture was homogenized with a spoon. Lastly, honey 1 gm was added and mixed. The preparation was made lukewarm in the water bath and applied over the injected site in the Swiss albino mice assigned to the experimental group. (n = 6).

The thickness of the applied material was 4-5 mm, and it was kept until it dried. The time was approximately 4-5 minutes[14].

The Swiss albino mice were very restless after the Indian red scorpion venom injection, but after the application of Jeerakadi Lepa, they slowly became calm.

3. Observations

The albino mice were observed for the signs and symptoms of toxicity due to Indian red scorpion venom. The following parameters were assessed:

- Lacrimation
- Nasal secretions
- Salivation
- Tremors and convulsions
- Urination
- Swelling
- Bloody diarrhoea
- Increased heart rate

These parameters were assessed as mild, moderate, or severe. During the data analysis, 3 points were assigned in the case of severe symptoms, 2 points for moderate symptoms, and 1 point for the calm presence of the symptoms. Another parameter was the deaths of Swiss albino mice among the groups. The observations have been tabulated below (Tables 3 and 4).

Table 3. Lacrimation, nasal secretion, salivation, tremors and convulsions, urination, swelling, bloody diarrhoea and heart rate in various groups

Sr. No.	Parameter	Control Group (n = 6)	Standard Group (n = 6)	Experimental Group (n = 6)	H Value	P Value
1	Lacrimation	7 (46.67)	7 (46.67)	1 (7)	6.2413	0.04413 (P < 0.05)
2	Nasal Secretion	7 (31.82)	6 (27.27)	3 (13.64)		
3	Salivation	6 (46.15)	6 (46.15)	1 (7.69)		
4	Tremors and Convulsions	5 (38.46)	4 (30.77)	4 (30.77)		
5	Urination	4 (40)	4 (40)	2 (20)		

(*Continued*)

Table 3. (Continued)

Sr. No.	Parameter	Control Group (n = 6)	Standard Group (n = 6)	Experimental Group (n = 6)	H Value	P Value
6	Swelling	6 (35.29)	6 (35.29)	5 (29.41)		
7	Bloody Diarrhea	0	0	0		
8	Heart Rate	6 (35.29)	6 (29.41)	5 (29.41)		

Table 4. Body weight and survived/died details in different groups

Sr. No.	Group (n = 18), (6 in each group)	Bodyweight (n = 18) (6 in each group)		Survived/died (n = 18) (6 in each group)		H - value	P - value
		Mean	SD	SU	DI		
1	Control Group (Male)	23	3	1	2	2.2105	0.3366 (P > 0.05)
2	Control group (female)	20.7	0.6	0	3		
3	Standard group (Male)	23	2.6	2	1		
4	Standard group (female)	23.7	3.5	0	3		
5	Experimental group (male)	21.7	1.5	2	1		
6	Experimental group (female)	22	2.6	2	1		

4. Statistical Analysis

Since three groups were present in the study and the data were ordinal, the non-parametric Kruskal Wallis test was applied at the 95% confidence interval level. It was carried out using the IBM SPSS software version 20. For another parameter, the number of deaths of Swiss albino mice, which is a nominal variable, the same statistical test was applied.

5. Discussion

Lepa is a formulation in Ayurveda that can be applied for various purposes like palliation, improving the complexion, or treating toxicity due to the bite of poisonous animals or insects. In the latter case, as there is wound formation, the absorption rate can be higher than in the earlier, with intact skin. A paste was made with the seeds of the cumin plant (*Cuminum cyminum*) and was applied to treat cutting wounds. It was shown that the alcohol extract of the seeds of cumin promoted wound healing on excision, incision, and granuloma wound models[15]. Cumin seeds are a rich source of essential oils and affect insulin metabolism; they are also reported to have antioxidant, antiallergic, and antiplatelet effects. The cumin seed oil has also been shown to produce significant analgesia in a chemical model of nociception (the formalin test)[15].

This study found that the total score of all the signs and symptoms of toxicity in the experimental group was lower in lacrimation, nasal secretions, and salivation. But in the case of tremors and convulsions, the score is equal to that of the standard group. Urination was also seen in equal proportions to that of the standard group. Bloody diarrhoea was absent in all the animals in the study. Significant changes in heart rate were seen among all the groups, with marginal differences only.

The Kruskal Wallis test was applied to assess the difference among the 3 groups, and the P value was 0.04413 (P < 0.05). Thus, the null hypothesis was rejected, and the alternative hypothesis was accepted.

However, in the case of the number of deaths, the value of P was 0.3311 (P > 0.3311), showing no significant difference.

6. Conclusion

It is concluded that Jeerakadi Lepa is efficacious in relieving all the signs and symptoms of toxicity due to Indian red scorpion venom envenomation in Swiss albino mice. Still, it is doubtful to treat moderate or severe cases independently.

7. Limitations

First, it was an animal study with a less (n = 6 in each group) sample size. While treating humans without prazosin will be unethical and life-threatening.

It is believed that Jeerakadi Lepa can act as an add-on therapy to treat scorpion sting cases.

8. Acknowledgements

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