



Research Article

EXPERIMENTAL STUDY OF THE ROLE OF *PIPPALYAADI LEPA* AGAINST INDIAN RED SCORPION VENOM IN ALBINO MICE

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ABSTRACT


Indian red scorpion (*Hottentotta tamulus*) venom produces severe local and systemic manifestations due to autonomic nervous system imbalance. Ayurveda describes various *Viśaghna* formulations for envenomation, among which topical applications (*Lepa*) are advocated for immediate management. **Aim:** To evaluate the efficacy of *Pippalyadi Lepa* against Indian red scorpion venom in Swiss albino mice. **Materials and Methods:** Indian red scorpion venom obtained from an authorized institute was administered subcutaneously to Swiss albino mice at a standardized lethal dose. Animals were divided into two groups of six each. Group I received venom alone, while Group II received venom followed by local application of *Pippalyadi Lepa* at the site of injection. The formulation was prepared using equal proportions of classical ingredients with *Kuṭāla Kṣarodaka* as the base. Clinical signs such as irritation, convulsions, tremors, increased heart rate, excessive salivation and lacrimation, locomotor dysfunction, loss of labyrinthine righting reflex, dyspnoea, and mortality were recorded. Statistical analysis was performed to assess significance. **Results:** The *Pippalyadi Lepa* treated group showed a significant delay in the onset and progression of local and systemic manifestations when compared to the control group. Survival time was markedly prolonged, indicating a protective effect of the formulation. **Conclusion:** Local application of *Pippalyadi Lepa* effectively delays systemic toxicity and improves survival in Indian red scorpion envenomation. The formulation may be considered a simple, economical, and supportive therapeutic option, especially in rural settings.

INTRODUCTION

Ayurveda is one of the most ancient medical sciences in the world. It conceives and describes the basic and practical aspects of life processes, health, illness, and its management in terms of its own conceptions and methodologies. Even though modern science has been creating cutting-edge tools and treatments for the detection and treatment of various illnesses, the great majority of people still receive their medical care via antiquated medical systems.

There are 1000 numerous types of scorpions in the world, including 86 species found in India. Two species, *Palmanus gravimanus* (big black scorpion) and *Hottentotta tamulus* (Indian red scorpion), are mostly found in India.^[1]

The annual number of scorpion stings exceeds 1.2 million leading to more than 3250 deaths (0.27%); India is the most affected, with a reported incidence of 0.6 %.^[2] The majority of patients in rural regions undergo treatment to traditional healers, who neither record the case nor notify the authorities. As a result, there is a lack of trustworthy epidemiological data, and many envenomation instances go unreported, making it challenging to determine the actual occurrence. It is found that case fatality rates of 3-22% were reported among the children hospitalized for scorpion stings in India.^[3,4]

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There are two possible forms of scorpion stings: local manifestation, which causes excruciating local pain, and systemic manifestation. The pain at the site is very severe in nature, and in systemic manifestation there is an autonomic storm (autonomic system crisis).

An autonomic storm with short-term parasympathetic and long-term sympathetic stimulation is brought on by scorpion venom's activation of neuronal sodium channels and inhibition of calcium-dependent potassium channels, which results in an abrupt release of endogenous catecholamines.^[5]

Scorpion's venom is a cocktail of several low molecular weight basic proteins, neurotoxins, nucleotides, aminoacids, oligopeptides, cardipotoxins, nephrotoxin, hemolytic toxins, phosphodiesterase, phospholipase A, hyaluroinidase. Acetylcholineesterase, glycosaminoglycans, histamine, serotonin. 5-hydroxyptamine and proteins that inhibit protease, angiotnsinase and succinate - dehydrogenese, ribonuclease, 5- nucleotidase. The venom of a single species of scorpion may contain several chemicals that have powerful synergistic effects on the victim.^[6]

Although the antivenin is available, its efficacy is unclear in relation to the current situation, when cases manifest late and long after the toxin's maximal tissue concentration period. An alternative strategy needs to be created because there is no clarity on how to handle this issue because antivenin is not a common drug in hospitals. This research is being done to find a remedy because it is vital to develop some antivenin alternatives.

AIM AND OBJECTIVES

AIM

To study the role of *Pippalyaadi lepa* against Indian red scorpion poison in albino mice.

OBJECTIVES

1. To study the signs produced by Indian red Scorpion poison in albino mice.
2. To observe changes in signs of Indian red scorpion poisoning in albino mice after local application of *Pippalyadi lepa*.

MATERIAL AND METHODS

Material

Pippalyaadi lepa^[7]

Ingredients of *Pippalyaadi lepa*

1. *Pippali*- *Pipper longum* (*Phala*)
2. *Marich*- *Pipper nigrum* (*Phala*)
3. *Yashti*- *Glycyrrhiza glabra* (*Mula*)
4. *Gambharee*- *Gmelina arborea* (*Twak* (bark))
5. *Surasmanjiri*- *Ocimum sanctum* (*Pushpmanjiri*)
6. *Sahadeva*- *Sida rhombifolia* (*Mula*)

7. *Apamarga*- *Achyranthus aspera* (*Mula*)

8. *Tanduliya*- *Amaranthus spinosus* (*Mula*)

9. *Kutalkshar*- *Sesamum indicum* (*Kshar*)

Methods

Collection of Indian red Scorpion venom

Dried form of Indian red scorpion venoms attained from Haffkine Institute for Training, Research and testing (HITRT); Mumbai. Total 500mg of venom purchased from this institute for animal experimentation. Its details are as follows.

Detail of Venom

| Vial No. | Quantity of IRSV | Sealing Date |
|----------|------------------|--------------|
| 697E-280 | 280 mg | AUG-1997 |

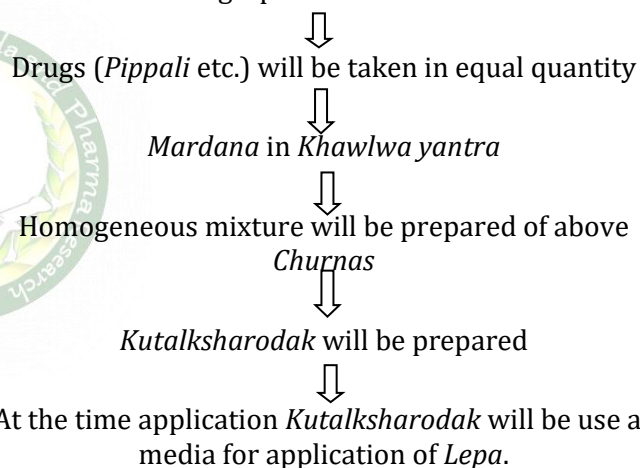
Collection of raw drugs (*Pippalyadi lepa*)

Raw drugs (*Churna*) were collected from botanical garden of Ayurvedic Mahavidyalaya Pharmacy.

Preparation of *Pippalyaadi lepa*

All above drugs (*Churnas*) should be taken in equal quantity and *Kutalksharodak* used as base for *Lepa*.

Preparation of *Pippalyaadi lepa* can be present in graphic as below



Thickness of *lepa*- *Vishaghna lepa* (*Sharangdhara*)^[8] - 1/3rd *Anguli praman*.

Authentication standardization of the drug was done from authorized and standard laboratory of pharmacy college.

Pilot study for fatal dose calculation

In order to assess the lethal dose of venom for mice, a pilot study was conducted. The conversion factor between humans and mice is 0.0026. This was taken into account while calculating the venom dosage.

The Indian red scorpion's lethal dose for humans is 1.5mg since the conversion factor for dose conversion from humans to mice is 0.0026/70kg. Therefore, 0.3mg of Indian red scorpion venom is the lethal dose for mice using the conversion factor.

Animal experiment ^[9]

Route for injection of venom: Subcutaneous

Site: Neck

Details of Animal use for the experiment:

| | |
|----------------|-------------------------------------|
| Animal Species | Swiss Albino mice |
| No. of Animals | 6 in each group |
| No. of Groups | 2 |
| Weight of Mice | Between 20-40gm |
| Sex of Mice | 3 males and 3 females in each group |

Details of groups for animal experiment

| | |
|---------|--|
| Group 1 | Only Indian red scorpion venom injected by subcutaneous route in albino mice. |
| Group 2 | Indian red scorpion venom (subcutaneously) + <i>Pippalyaadi lepa</i> at the site of injection. |

Observations

Overall signs observed in animal experiment

- Irritation
- Irritation at site
- Tremors
- Increased heart rate
- Convulsion
- Locomotor dysfunction
- Excessive salivation
- Excessive lacrimation
- Distention of abdomen
- Paralysis of hind limbs
- Loss of Labyrinthine Righting reflex (LRR)
- Dyspnea
- Death

Some observations, such as hind limb paralysis, nasal hemorrhage, and stomach distention, are not observed in every animal experiment. For statistical analysis and to access the changes, common observations are made.

Observations of Group I (In seconds)

| Signs | Male T | Male HT | Male RH | Female T | Female HT | Female RH |
|-----------------------|--------|---------|---------|----------|-----------|-----------|
| Irritation | 1 | 1 | 1 | 1 | 1 | 1 |
| Irritation at site | 15 | 26 | 13 | 17 | 22 | 15 |
| Convulsion | 30 | 58 | 42 | 35 | 60 | 60 |
| Distension of abdomen | 40 | - | - | 48 | 80 | 65 |
| Tremors | 60 | 85 | 83 | 55 | 90 | 58 |
| Increased heart rate | 100 | 90 | 88 | 88 | 110 | 90 |
| Hind limb paralysis | 110 | - | 105 | - | - | 80 |
| Excessive salivation | 550 | 900 | 840 | 800 | 960 | 900 |
| Excessive lacrimation | 550 | 950 | 900 | 800 | 1125 | 900 |
| Locomotor dysfunction | 663 | 1100 | 988 | 980 | 1160 | 950 |
| Nasal bleeding | - | 1600 | 1105 | 1000 | - | - |
| Loss of L.R.R. | 700 | 1825 | 1207 | 1130 | 1861 | 1090 |
| Dyspnoea | 802 | 2380 | 1410 | 1401 | 2295 | 1206 |
| Death | 900 | 2400 | 1500 | 1500 | 2400 | 1320 |

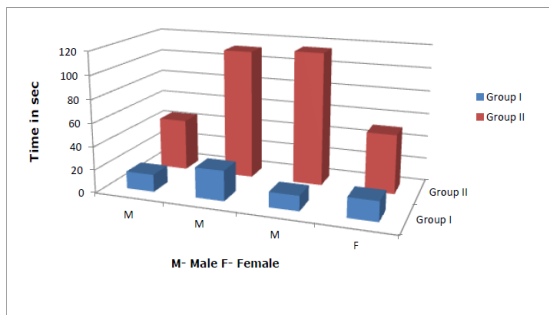
Observations of Group II (In seconds)

| Signs | Male T | Male HT | Male RH | Female T | Female HT | Female RH |
|------------|--------|---------|---------|----------|-----------|-----------|
| Irritation | 1 | 1 | 1 | 1 | 1 | 1 |

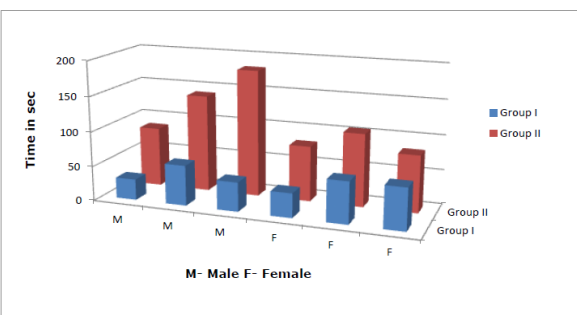
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|-----------------------|------|------|------|------|------|------|
| Irritation at site | 45 | 112 | 115 | 51 | 75 | 50 |
| Convulsion | 86 | 140 | 182 | 80 | 105 | 82 |
| Distension of abdomen | 110 | - | 212 | 85 | - | - |
| Tremors | 180 | 370 | 379 | 207 | 250 | 146 |
| Increased heart rate | 292 | 739 | 752 | 305 | 380 | 265 |
| Hind limb paralysis | 315 | - | 840 | - | - | 290 |
| Excessive salivation | 800 | 1120 | 1105 | 950 | 980 | 740 |
| Excessive lacrimation | 850 | 1150 | 1130 | 1005 | 1050 | 900 |
| Locomotor dysfunction | 1870 | 5030 | 5070 | 1895 | 2070 | 1600 |
| Nasal bleeding | 1690 | - | - | 1744 | 2005 | - |
| Loss of L.R.R. | 2007 | 5912 | 5970 | 2014 | 3139 | 1970 |
| Dyspnoea | 2311 | 6505 | 6520 | 2430 | 3777 | 2190 |
| Death | 2520 | 6720 | 6780 | 2640 | 3900 | 2400 |

Graphical presentation of observations are done and after this statistical analysis done according to parameters.

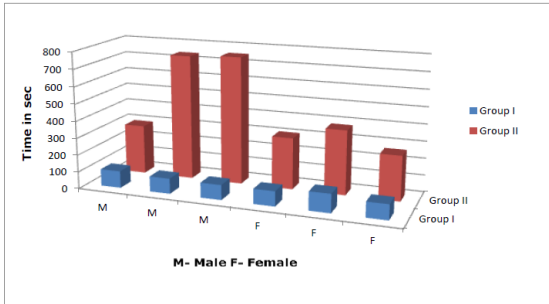
Graphical presentation of observations



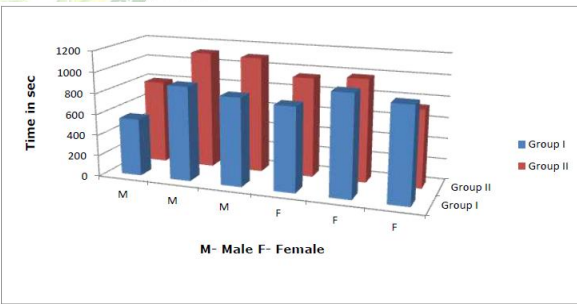
Graphical presentation of observations of Irritation of site



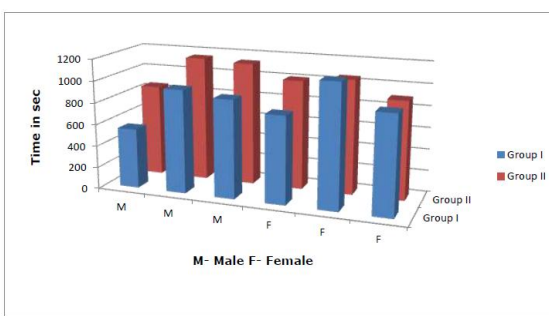
Graphical presentation of observations of convulsion



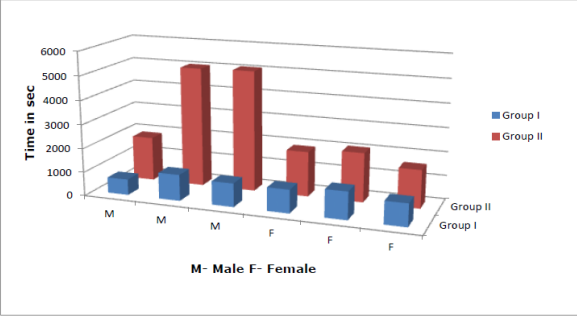
Graphical presentation of observations of increased heart rate



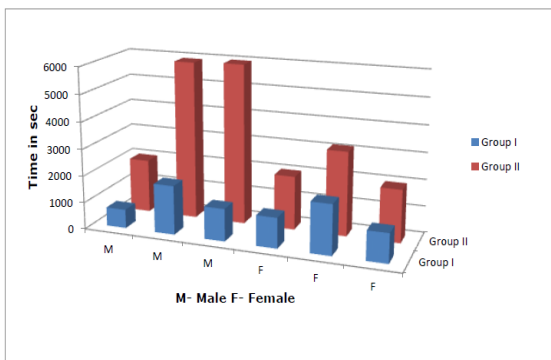
Graphical presentation of observations of Excessive salivation



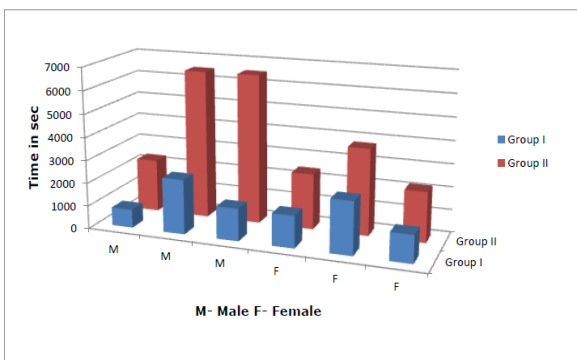
Graphical presentation of observations of Excessive Lacrimation



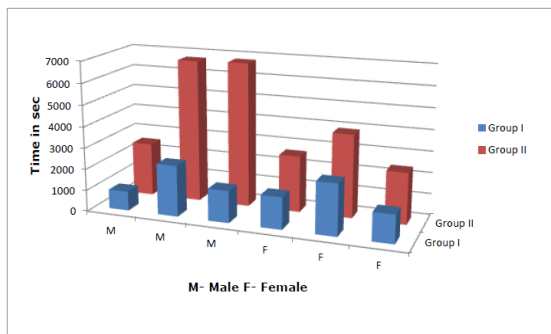
Graphical presentation of observations of Locomotor Dysfunction



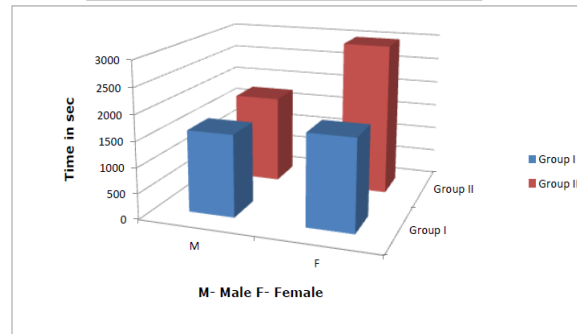
Graphical presentation of observations of Loss of L.R.R.



Graphical presentation of observations of Dyspnoea



Graphical presentation of observations of Death



Graphical presentation showing average time of survival



Figure 1: Indian Red scorpion venom



Figure 2: Injection of Indian Red scorpion venom subcutaneously



Figure 3: Prepared Pippalyaadi Lepa



Figure 4: Dissection of Albino mice

DISCUSSION

According to Dr. H. S. Bawaskar, a scorpion injects 1.5mg maximum.^[10] The conversion formula from humans to mice, which is 0.3mg, should be used for conversion. But when no mice died at this dose, we injected 0.5mg of venom, which killed 50% of the

animals; we next injected 0.7mg. Since 50% of the mice perished at this dose as well, we directly injected 0.9mg of venom. At this dose all the animals died. But to evaluate, we injected 0.8mg of venom here; 50% of the animals died, so the final higher dose finalized after

was 0.9mg. At this higher concentration also our experimental drug shows experimental and statistically significant activity.

The LD50 of scorpion venom differs from batch to batch because its protein and its potency change according to various criteria.

In all of group II, observation times of irritation at the site, convulsion, distension of abdomen, tremors, increased heart rate, excessive salivation, excessive lacrimation, locomotor dysfunction, nasal bleeding, loss of L.R.R., dyspnoea, and death are increased by 56.66 seconds, 65 seconds, 183.50 seconds, 347.95 seconds, 124.16 seconds, 143.33 seconds, 1949 seconds, 2199.84 seconds, 2373.17 seconds, and 2490 seconds, respectively.

CONCLUSION

- Weight may play a significant role in how envenomation symptoms manifest.
- Every individual can show different signs according to its sensitivity to Indian red scorpion venom.
- The manufacture of *Pippalyaadi lepa* needs no expert people; hence, it can be made in-house only and can be used as a household remedy.
- The application also does not need expert people.
- After application of *Pippalyaadi lepa*, signs were delayed in albino mice of Indian red scorpion poisoning.
- *Pippalyaadi lepa* increased the survival rate in albino mice of Indian red scorpion envenomation.
- The present study proves that local application can be effective in systemic manifestation of scorpion envenoming.
- Hence, the efficacy of *Pippalyaadi lepa* against Indian red scorpion poison in albino mice has been proved.

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