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In Vivo Study To Evaluate The Efficacy Of Shirisha Pushpa And Punarnava Mula Lepa In Honey Bee Sting In Swiss Albino Mice.

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ABSTRACT

Ayurveda is a timeless science based on principles of prevention and healing. Although diseases are described in various forms in modern medicine, their fundamental imbalance often corresponds with Ayurvedic concepts. Ayurveda states that diseases and remedies are infinite, and treatment depends on proper understanding of principles rather than fixed formulations.Honey bee sting (Makshika Dansha) is a common toxicological emergency in both rural and urban populations. In Ayurveda, Agadtantra describes various herbal antidotes for insect bites. The present in vivo study was conducted to evaluate the efficacy of Shirisha (Albizia lebbeck) pushpa lepa and Punarnava (Boerhavia diffusa) mula lepa in honey bee sting in albino mice. The study focuses on anti-inflammatory and anti-toxic properties of these herbal formulations in comparison with standard treatment. Parameters such as Swelling,Redness,Tenderness ,Discoloration, and histopathological changes were assessed.

Keywords- Shirisha pushpa, Punarnava mula, lepa,Honey bee,Agadtantra,Ayurveda.

INTRODUCTION

Agadtantra is a specialized branch of Ayurveda dealing with toxicology and management of poisons, including insect bites and animal venoms. Ushna, which explain its rapid and harmful effects on the body.Charaka Samhita describes fifty Mahakashayas, each containing ten herbs selected based on therapeutic action. Vishaghna Mahakashaya includes herbs used for detoxification and treatment of various poisons.Some herbs in this group have ambiguous identification, requiring interpretative understanding based on classical texts and clinical application.

Danshtra chikitsa, Agad tantra and Vishagarvairodhik prashaman are synonym of each other. Danshtra chikitsa is the sixth branch of Ayurveda which mainly deals with agada i.e. The medicine with anti-poisonous effects. Visha means the dravya which causes vishaad (sorrow or depression) is known as visha. Laghu, *Ruksha, Aashu, Vishad, Vyavayi, Tikshna, Vikaashi, Sookshma, Ushna, Anirdeshyarasa* are the ten properties of poisonous drugs. Drugs which act against toxic effect of substances are called as vishghna. Honey bee (Apis cerana indica) sting is known to cause local inflammatory reactions such as pain, swelling, erythema, and burning sensation.

Bee venom contains biologically active compounds such as melittin, phospholipase A, hyaluronidase, histamine, and other peptides responsible for inflammation and allergic reactions.

In Ayurveda, lepa (external application of herbal paste) is considered an effective local therapeutic approach for managing visha (toxicity). Shirisha and Punarnava are well-known Vishaghna (anti-toxic) herbs mentioned in classical texts.

Aim

To evaluate the efficacy of Shirisha pushpa and Punarnava mula lepa in honey bee sting in Swiss albino mice.

OBJECTIVES:-

1. Primary Objective:

To study the efficacy of Shirisha pushpa and Punarnava mula lepa (as local application) as an Antidote in Honey Bee sting in Swiss albino mice.

2. Secondary objectives:

* To study the Toxic Effect of Honey Bee (Apis cerana indica) Sting in Swiss Albino Mice.

* To see the effect of Shirisha pushpa and Punarnava mula lepa and Hydrocortisone Cream (local application) against local toxicity of Honey bee sting in Swiss Albino mice.

* To study the effects of Shirisha pushpa and Punarnava mula lepa on sign and symptoms against local toxicity of Honey bee sting in Swiss Albino mice.

* To study the Shirish pushpa and punarnava mula Lepa as per Ayurvedic Samhita.

RESEARCH QUESTION :-

Does Shirisha pushpa and Punarnava mula lepa as a local application1-2mm in thickness for 7 days once in a day on the back of Swiss albino mice having age of 8-10weeks and average weight of 20-25 gm have antitoxic effect in honey bee[apis cerana indica] sting?

HYPOTHESIS

(H0): Null Hypothesis :

Shirisha pushpa and Punarnava mula lepa is not significantly effective in honey bee sting poisoning in Swiss albino mice

(H1) Alternative Hypothesis :

Shirisha pushpa and Punarnava mula lepa is significantly effective in honey bee sting poisoning in Swiss albino mice

Materials and Methods

Materials

- Shirisha pushpa (Albizia lebbeck)
- Punarnava mula (Boerhavia diffusa)
- Hydrocortisone cream (standard control)
- Distilled water

Animals

- Swiss albino mice (20–25 g, 8–10 weeks age)
- Honey bees (Apis cerana indica)

Instruments

- Electronic balance
- Forceps
- Rectal thermometer
- Cooling pads

Experimental Animals

- Species: Swiss Albino Mice
- Total animals: 18
- Age: 6–10 weeks
- Weight: 20–30 g
- Sex: Equal distribution of males and females
- Study duration: 7 days

Group Distribution

Group I – Control

Honey bee sting only.

Group II – Standard

Honey bee sting + Hydrocortisone cream.

Group III – Test

Honey bee sting + Shirisha Pushpa and Punarnava Mula Lepa.

Experimental Procedure

The dorsal skin of mice was shaved. Six honey bee stings were administered to each animal. Three stings were removed and three were retained. Observations were recorded daily for seven days. The standard drug and test formulation were applied once daily.

Assessment Parameters

Assessment Parameters

- Swelling
- Redness
- Tenderness
- Discoloration
- Histopathological Changes

- Mortality Rate

Methodology

1. Drug Preparation

- Shirisha pushpa and Punarnava mula were collected, authenticated, and standardized.
- Lepa formulations were prepared as per classical method.

2. Experimental Design

- Honey bee sting was induced on the dorsal surface of albino mice.
- Treatment groups received:
- Shirisha pushpa & Punarnavamula lepa

Standard drug (Hydrocortisone cream)

- Control group (no treatment / base)

3. Application Protocol

- Lepa applied locally (1–2 mm thickness)
- Once daily for 7 days

TEST PROCEDURE

I. Weight of each Swiss Albino Mice will be taken.

II. Animals will be observed for food & water intake.

III. Random selection will be done.

IV. Five days prior animal will be kept in cage

V. Each animal will be marked for individual identity. With help of some colour, identification of groups of male and female Swiss Albino Mice is done

VI. Hair will be removed from the desired part, will be used for experimentation that is back region of animal body.

VII. Each spot will be marked earlier and then one by one sting will be given to the back by maintaining safe interval between the two stings.

VIII. Out of six stings in each mouse three stings will be removed and three stings will be kept as it is.

IX. After procedure, all Swiss Albino Mice will be observed for any sign and symptoms. Rating of skin reaction will be evaluated as per BIS

(Bureau of India Standard) 1992

During observation – Swelling,Redness,Tenderness,Discoloration observed

After the occurrence of any signs on site where honey bee sting toxicity is seen as per group mentioned that

1]Control group (no medicine will be applied)

2]Standard group (apply hydrocortisone cream)

3] Test group (apply shirisha pushpa and punarnava mula lepa)

Assessment Parameters

- Swelling
- Redness
- Tenderness
- Discoloration

Observations

General Clinical Observations

Following honey bee sting administration, animals exhibited local inflammatory signs characterized primarily by erythema and edema. No bluish discoloration or tenderness was observed throughout the study period.

Erythema

The control group exhibited persistent erythema throughout the observation period. In contrast, both standard and test groups demonstrated gradual reduction in erythema scores, reaching minimal or absent erythema by Day 7. The findings suggest effective suppression of venom-induced inflammatory responses by the test formulation.

Body Temperature

Animals in the control group showed fluctuations associated with inflammatory reactions. The standard and test groups maintained near-normal body temperature throughout the study period. Statistical comparison revealed significant differences between treated groups and controls.

Body Weight

Initially, all animals experienced reduction in food intake following envenomation, resulting in weight loss. However, after treatment, animals in the standard and test groups resumed normal feeding behavior and gained weight progressively. The increase in body weight reflected recovery from venom toxicity and improvement in general health status.

Mortality

A notable observation was mortality in the untreated control group. Three animals died by Day 4, likely due to progressive venom toxicity. No deaths occurred in either the standard or test groups, indicating a protective effect of treatment.

Statistical Analysis

Paired t test –

* In experimental study, the data obtained was quantitative data .

* Paired t test were used to compare day 1 and day 7 data of control , Standard and test group of observations Erythema , body temperature and body weight

* After application of drugs in Standard group and Test group , there was significant decrease in Erythema and body temperature was maintained as well as there was no significant weight loss in animals but was difference in weight gain in standard and test groups. So there is significant difference in observations.

Unpaired t test –

* Unpaired t test were used to compare Standard and Test groups to study the significance

* After the Experimental study , It was observed that in both standard and Test group Erythema was decreased , in body temperature there was slight difference , but body weight was slightly decreased in test group compare to standard group .

Discussion on efficacy of Shirish Pushpa and Punarnava Mula Lepa –

* The data obtained from observations and statistical analysis, showed that there was significant difference in observations and showed positive results .

* Thus the null hypothesis is rejected and the hypothesis is that shirisha pushpa and punarnava mula Lepa is as effective in honey bee sting in Swiss albino mice.

Histopathological Findings

Microscopic examination provided objective evidence regarding tissue recovery.

Control Group

- Distorted epidermal architecture.
- Marked inflammatory cell infiltration.
- Congested blood vessels.
- Moderate to severe pathological changes.

Standard Group

- Normal epidermal and dermal architecture.
- Minimal or absent inflammatory cells.
- No significant pathological abnormalities.

Test Group

- Mild epidermal distortion.
- Very few inflammatory cells.
- Reduced vascular congestion.
- Histological grading ranged from mild to moderate.
- Significant improvement compared with disease control.

These findings indicate that the test formulation effectively controlled tissue inflammation and accelerated healing.

The study evaluated reduction in:

- Inflammation at sting site
- Skin redness and swelling
- Systemic temperature changes
- Tissue-level inflammatory damage

Results

* According to Histopathological Findings:

The Standard Group (treated with 1% hydrocortisone cream) showed 70–80% improvement, whereas the Trial Group (treated with Shirisha Pushpa and Punarnava Mula) demonstrated 65–75% improvement.

* According to Statistical Analysis:

Both the Standard Group and the Trial Group showed a significant reduction in erythema, swelling, and Stable body weight. Overall, both groups exhibited statistically significant improvement.

* From above given observations we can say that application of Shirish Pushpa and Punarnava Mula Lepa in honey bee sting in Swiss albino mice has shown positive results.

* Hence its efficacy is proved from the observations, statistical analysis and histopathology reports

Discussion

The study was-

"IN VIVO STUDY TO EVALUATE THE EFFICACY OF SHIRISHA PUSHPA AND PUNARNAVA MULA LEPA IN HONEY BEE STING IN SWISS ALBINO MICE".

Discussion on Experimental study

1. Probable made of action

2. chemical constituents

3. Discussion on Statistical Analysis

4. Discussion on efficacy of test drugs

Animal ethical committee permission was taken prior to animal experiment which was

* of bite shows its properties by its Rasa & karma

* Madura – Vishagna (□. □□ 26/3 & □. □□ 10/7-8)

Tikta carried out in approved animal testing center

No swelling and bluish discoloration were observed throughout the study.

Probable Mode of action

* Shirish Pushpa and Punarnava Mula Lepa when applied externally at the site – Vishagna (□ . □□ 26/42)

* Kashay – Vranaropan (□ .□□□. □□ 10/20)

* Katu – Vranashodhan (□□ . □□ 42/15 & □. □□ 10 / 17-18)

* Vishagna (□□ . □□ 42/15)

By chemical constituents:

Shirish Pushpa and Punarnava Mula Lepa contains tannin which has Anti inflammatory , Antitoxic & Astringent properties , promotes wound healing.

* Besides , it also contains Gallic acid and β – sitosterol which shows Antioxidant , Anti -inflammatory properties . Which also significantly reduces levels of histamines , IgE & mast cells

* Initially , after the sting there was decrease in the body weight but after the application of lepa in both standard & test groups the signs were reduced and animals started consuming there pelleted feeds properly, hence the weight increased

* In Control Group at Day 4 total 3 animals were dead as no treatment was given and toxicity was increased (probable reason of death may be due to bradycardia, hematuria, bloody diarrhea. No animals were dead in both the Standard and Test Groups.

The efficacy observed in this study can be attributed to the pharmacological properties of both ingredients.

Shirisha

Shirisha contains flavonoids, tannins, saponins, gallic acid, and β-sitosterol. These compounds possess anti-inflammatory, antioxidant, antihistaminic, and wound-healing activities. Shirisha is also described in Ayurvedic texts as a potent Vishaghna drug.

Punarnava

Punarnava possesses anti-inflammatory, anti-edematous, and detoxifying actions. Traditionally it is indicated in Shotha and Visha conditions.

Ayurvedic Interpretation

The formulation acts through:

- Vishaghna Karma
- Shothahara Karma
- Vranashodhana
- Vranaropana

These actions collectively reduce venom-induced inflammation and facilitate tissue healing.

Honey bee venom induces inflammatory mediators such as histamine and phospholipase A2 leading to local tissue damage. Shirisha possesses anti-inflammatory, anti-allergic, and detoxifying properties. Punarnava is known for its anti-inflammatory and diuretic activity.

The observed reduction in erythema and edema suggests inhibition of inflammatory cascade. Lepa formulation likely acts through local absorption and neutralization of venom-induced inflammation.

Conclusion

Shirisha pushpa and Punarnava mula lepa demonstrated significant anti-inflammatory and anti-toxic effects in honey bee sting model in swiss albino mice.

These formulations may be further explored for clinical application in insect bite management.

Further Scope

- Clinical trials in human subjects
- Standardization of dosage and formulation
- Molecular mechanism studies
- Comparative study with modern antihistamines

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