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AN IN-VIVO STUDY OF THE EFFECT OF MUKTA LEPA IN HONEYBEE STING (*Keeta Visha*) IN ALBINO MICE

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ABSTRACT

Background-In *Sushrut Samhita* a total of 67 variety of *Keeta* (insects) under 4 groups are being described. Most of the bites are not very harmful but sometimes poisonous bites can cause complications i.e. anaphylactic shock. Among these, *Makshika* (honeybee) is the subject of concern. In these stings, *Lepa* is useful mode of treatment. **Aim and Objectives-**Experimentally study of the effect of *Mukta Lepa* in Honeybee sting (*Keeta Visha*). To study the anti-inflammatory effect of *Mukta Lepa* due to Honeybee sting (*Keeta Visha*) in Albino Mice.

Material & Methods- This study was carried out in albino mice categorized into three groups- control, standard drug and test drug for comparative evaluation. The control group received nothing, standard drug was beta cyclic ointment and test drug was *MuktaPishti Lepa.* The study was an acute toxicity study. The mice were subjected to honeybee (*Apiscerenaindica*) stings and the sting areas were given an application of test and standard drugs. Results were evaluated after a period of 7 days on the basis of redness, edema. **Result**-As per observation data, it is proved that *Mukta Lepa* is more effective as Standard drug hence it can be useful in Honeybee sting. It helps in reducing edema due to Honeybee sting. **Conclusion-** From this study it is proved that *Mukta Lepa* helps in wound healing. From the detailed pre-clinical study and observational analysis, it is concluded that *Mukta Lepa* is effective and can be used in Honeybee sting.

Keyword: *Mukta Lepa, Keet Visha*, *Apiscerenaindica,*

INTRODUCTION

Honeybees are the subset of bees in the genus Apis, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests out of wax. Honeybees are only extant members of the tribe Apini, all in the genus Apis. Currently, there are only seven recognized species of honeybee with a total of 44 sub species. Honeybees represent only a small fraction of the approximately 20,000 known species of bees[1]. Some other types of related bees produce and store honey, but only members of the genus Apis are true honeybees. Grossly it is divided into 5 types, 1) Apis Cerana 2) Apis Dorsata 3) Apis Mellifera 4) Apis Florae 5) Apis Laboriosa. Most of the data and research papers on bee venom available on internet is about the European bee i.e. Apis Mellifera so the bee venom available in India i.e. Apis Cerana Indica needs to be studied.[2]

Ancient principles should be again and again testified because pure metal regains shining by its polishing. For that purpose, research should be done, and it is impossible to study all the insects in a single study, therefore for thorough and specific study I have selected only one of the *Makshika* i.e., *Madhumakshika.*

In *Ayurveda Visha* is classified into three main types-

1. ***Sthavara Visha*:** It includes plants, minerals and metal poisons etc.
2. ***Jangam Visha***: It includes animal poisons like bites of snakes, insects, spiders, rats, scorpions etc.
3. ***Kritrim Visha*:** It includes poisons prepared chemically.

The incidences of *Jangam vishas* are very common so I developed interest in studying toxic effects of Honeybee sting which comes under *Jangam Visha*[3]. There are many formulations that are mentioned by *Acharya* for the *Agada* practitioners. *Mukta Lepa* is one of such *yoga* mentioned by *Acharya Vagbhata* in *Keeta Luta Visha Pratishedh Adhyay*a. The present study aims towards assessing the effect of *Mukta Lepa* in *Keeta - Visha* through an experimental study. It will not only help in discovering its effect on *Keeta Visha* but also in scientific re-establishing validity of *Agada Yoga*.[4] *Mukta lepa* is considered as anti-inflammatory having properties of healing and coagulation. *Lepa* has its own importance in *Ayurveda* as if whole *Visha* is removed from the body then also some amount may remain in mild form and can show toxic effects later on, which is known as *Dushi Visha*. Therefore, local application *(lepa)* in all types of *Vishas* should be done so considering the importance of *Lepa*, *Mukta Lepa* with water has been selected as a base in Honeybee sting. Preclinical studies have been selected by me for this trial and if this formulation proves effective then further clinical trials can be done with this formulation.[5]

Aim: Experimentally study of the effect of *Mukta Lepa* in Honeybee sting (*Keeta Visha*).

Objectives: To study the anti-inflammatory effect of *Mukta Lepa* due to of Honeybee sting (*Keeta Visha*) in Albino Mice.

Material & Methods

Review of concerned literature from traditional and modern texts. The Collection of *Mukta* from Authentic sources. Preparation and Authentication of *Mukta Lepa* in our *Rasa Shashtra* Department of Uttaranchal Ayurvedic College Dehradun. Standardization of *Mukta churna* in Authorized Laboratory of Pharmacy College (Adarsh Vijendra institute of pharmaceutical sciences (Gangoh).

Experimental Study –

Animal species to be used - Albino Mice

Place of experiment - National Toxicology Centre, Pune.

Source of animals to be used - National Toxicology Centre, Pune.

Sex of animals to be used - (50% males) and (50% females), in each Group will be taken.

Average weight of animals - 27-32 grams

Strain - Swiss albino

Diet - Pelleted feed

Water - Normal community supplied clean water

Room temperature - 20-24 °C
Relative humidity - 40-50%
Light cycle - 12 hours light and 12 hours dark

No. of groups -
1) Control group-6 (Albino mice), No application.
2) Trial group – 6 (Albino mice)
3) Standards drug group – 6 (Albino mice)

Table - 1

	Control group	**Trial group**	**Standard drug group**
No. of animals	6	6	6
Sex of animals	3 male, 3 females	3 male, 3 females	3 male, 3 females
Feeding	Animal food	Animal food	Animal food
Local application	No application	*Mukta Lepa*	Standard drug
Period of acclimatization	7 days	7 days	7 days
Dosing	Fresh natural Honeybee sting produced on bare skin of Albino Mice will remain as it is.	**1)** Fresh natural Honeybee sting produced on bare skin of Albino Mice. **2)** *Mukta Lepa* will be used as local application externally in the form of *Lepa* with water.	**1)** Fresh natural Honeybee sting produced on bare skin of Albino Mice. **2)** Local application of mild steroid ointment (BETASALIC) Content – Beclomethasone Dipropionate (0.1%), Salicylic acid (3.0%)

Image of Procedure

Fig 1. Hair Removal of Mice

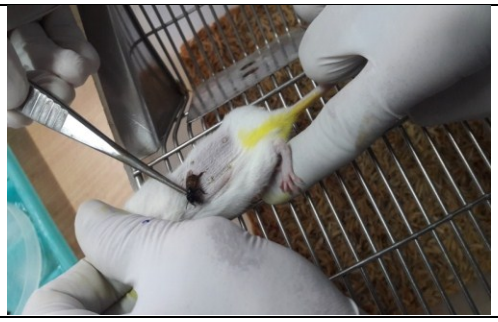

Fig 2. Honeybee Sting to Mice

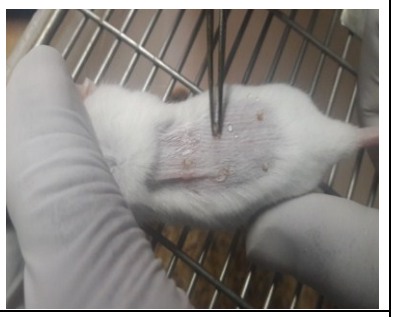

Fig 3. Removal of Sting

Fig 4. Apply *Mukta Lepa*

Fig 5. Apply Betasalic Drug

Fig 6. *MuktaLepa*

Fig 7. Betasalic Ointment

For experimental rating of skin reactions following observations were noted.

Table - 2

	Skin Reaction	**Rating**
a)	**Edema**	
1)	No edema	0 (-)
2)	Very slight edema	1 (+)
3)	slight edema (edges of area well defined by definite raising)	2 (++)
4)	Moderate edema (raised app 1 mm)	3 (+++)
5)	Severe edema (raised more than 1 mm& extending beyond area of exposure) total possible edema score	4 (++++)

Table - 3

Report on Histopathological observation of skin (Table Q)										
		1	2	3	4	5	6	7	8	
Sr No	Code No	Skin surface (epi-dermis)	Break in length of epitheli-um, Ul-ceration etc.	Colla-gen for-mation	Granulation tissue & Neovascu-lar-ization and Fibrosis	Hemor-rhages	Infiltration of Neutro-phils, In-flammatory cells	Infiltration of mononu-clear cells (MNC) /PMN.Giant cells etc.	Hyper- ke-ratinization, Re-epitheliza-tion	Overall Patho-Logical Grade (Lesion Score)
1	Discon-trol Sec-tion 1	+	+	+	++	+++	++	++	+	Moderate (+3)
2	Discon-trol Sec-tion 2	+	++	++	++	+++	++++	++++	+++	Severe (+4)
3	STD A Section 1	NAD	+	+++	++	+	++	+	+(focal)	Mild (++)
4	STD A Section 2	NAD	NAD	+	++	+	+	+	NAD	Minimal (+)
5	STD B Section 1	NAD	NAD	+	++	+	+(focal)	+(focal)	NAD	Minimal (+)
6	STD B Section 2	NAD	NAD	+	++	+	+(focal)	+	NAD	Minimal (+)
		Scab for-mation	Break in length of epitheli-um, Ul-ceration etc.	Colla-gen for-mation	Granulation tissue & Neovascu-lar-ization and Fibrosis	Hemor-rhages	Infiltration of Neutro-phils, In-flammatory cells	Infiltration of mononu-clear cells (MNC) /PMN.Giant cells etc.	Hyper- ke-ratinization, Re-epitheliza-tion	Overall Patho-Logical Grade (Lesion Score)
7	TEST A	+	+	+	++	+++	++	++	+	Moderate (+3)
8	TEST A	+	+(focal)	+++	++	+	++	+	+	Mild (++)
9	TEST B	+	NAD	+++	++	+	+	+	+	Mild (++)
10	TEST B	NAD	+(focal)	+	+	+(focal)	+	+	+(focal)	Minimal (+)
Note: Overall Grade score as- NAD =No Abnormality Detected, Minimal changes (+), Mild changes (++), Moderate changes (+++), Severe changes (++++).										
Point no 3, 4 and 8 suggest healing reaction while others suggest inflammatory reaction.										

Observation

Male Albino mice were found to be more sensitive than female mice to Honeybee sting. Death of mice occurred usually after 24 hours. Sting reactions are more on male mice. The signs and symptoms were reduced usually after 4 to 5 days. Immediate signs observed after stinging were Erythema, Inflammation, Tenderness, and Edema. Maximum stings were removed by mice themselves within 24 hours as mice always keep themselves clean. Study drug (*Mukta lepa*) worked more efficiently on Edema (*Shopha*) as observed from statistical analysis. The effect of *Mukta lepa* is moderately effective as compare to standard drug. One symptom i.e., edema, was observed in the mice so as it is significant hence included in the study for experimental rating of skin reactions in my thesis.

From observation tables: Two male mice died on day 2 of trial group. Three mice died on day 2 of control group: one male, two females.

Observation table for Edema in trial group

Table - 4.1

Sr. no.	Animal marking	Sex	Day1	Day2	Day3	Day4	Day5	Day6	Day 7
1	TLF	M	_	Dead	_	_	_	_	_
2	T-LLS	M	_	1	2	2	1	1	_
3	T	M	_	Dead	_	_	_	_	_
4	W	F	_	1	2	2	1	1	1
5	LH	F	_	1	1	2	1	1	_
6	LLS	F	_	1	1	1	1	1	_

TLF= tail leg front, T-LLS= tail left leg side, T= tail marking, W= white (no marking) LH= head marking, LLS= left leg side. M= male, F= female

Observation table for Edema in Standard group

Table - 4.2

Sr. no.	Animal marking	Sex	Day1	Day2	Day3	Day4	Day5	Day6	Day 7
1	H	M	_	1	2	2	3	2	2
2	T	M	_	1	2	2	2	1	1
3	RF	M	_	1	2	2	2	2	2
4	W	F	_	1	2	2	2	2	2
5	T	F	_	1	2	3	2	2	2
6	RLS	F		1	2	3	2	2	1

H= head marking, T= tail marking, RF= right front, W= white (no marking), RLS= right leg side, M= male, F= female

Observation table for Edema in control group

Table - 4.3

Sr. no.	Animal marking	Sex	Day1	Day2	Day3	Day4	Day5	Day6	Day 7
1	HT	M	_	Dead	_	_	_	_	_
2	T	M	_	1	2	2	2	2	1
3	W	M	_	1	2	2	2	2	1
4	H	F	_	Dead	_	_	_	_	_
5	T	F	_	1	2	2	2	2	1
6	W	F	_	Dead	_	_	_	_	_

HT= head tail, T= tail, W= white (no marking) H= head marking, M= male, F= female

From Observation table no.4.1, 4.2, 4.3:

1. Observation of edema in trial group: Maximum reduction of edema was observed from day 5 and then remained constant. There mice have shown good result on trial group 2 female and 1 male

2. Observation of edema in standard group: Maximum reduction of edema was observed from day 6 and on day 7, two mice have shown good results on standard drug (one male, one female).

3. Observation of edema in control group: Maximum reduction in edema was observed from day 6 and one male mouse cured.

Histopathological observation:

Disease Control: A minimum change in Scab Formation was observed. There was mild break in length of epithelium and ulceration. There was mild collagen formation in the skin. Granulation tissue, neovascularization and fibrosis were observed in the diseased animals. Moderate hemorrhage was also observed. There was severe infiltration of Neutrophils, mononuclear cells/PMN cells which indicate inflammation. Hyper keratinization and Reepithelialization was also observed.

Standard: No Scab Formation was observed. There was minimal to no break in length of epithelium and ulceration. There was moderate collagen formation which shows healing. Mild Changes in Granulation tissue, neovascularization and fibrosis was observed in the standard material treated animals. Mild hemorrhage was also observed. There was mild infiltration of Neutrophils, mononuclear cells/PMN cells. No hyper keratinization and Reepithelialization was also observed.

Test: No Scab Formation was observed. There was no break in length of epithelium and ulceration. There was moderate collagen formation which shows healing. Mild Changes in Granulation tissue, neovascularization and fibrosis was observed in the test material treated animals. Mild hemorrhage was also observed. There was mild infiltration of mononuclear cells/PMN cells. No Hyper keratinization and Reepithelialization was also observed.

Result

As per observation data it is proved that *Mukta lepa* is more effective as Standard drug hence it can be useful in Honeybee sting. It helps in reducing edema due to Honeybee sting. It is economical and easily available all over India, hence can be used as preliminary treatment in Honeybee sting before reaching hospital.

DISCUSSION

Insects form nearly 3/5th of Anthropoids. The branch of Zoology that deals with the study of insects is known as Medical Entomology. Honeybees are the most familiar Anthropoids. Honeybees are the useful insects for human beings. They help in pollination of crops on the other hand their bite in human beings can cause life threatening conditions.

Discussion regarding Honeybee: Honeybees are the social bees' nest in the colonies headed by a single fertile female; ***Queen***, the only egg layer in the colony. Foraging for nectar and other tasks such as feeding the queen and the larvae, cleaning brood cells and removing debris are carried out by a caste of females known as ***workers.*** Honey and pollen are stored, and larvae are reared in cells made from wax secreted by the worker bees.[6] ***Drones*** are the caste of males in the hive whose function is only mating. They develop from eggs that have not been fertilized and they don't sting.

The manifestation of *Makshika damsha* varies widely from pain to physiological disturbances and even upto death. At the site of bite patient feels itching *(kandu*), swelling *(shopha*), burning sensation (*daha*) and pain *(ruja*). Apart from these symptoms *Aacharya Sushruta* has specifically described symptoms of *Kashayee* and *Sthalika* as grayish black pustules (*shyaav pidika*) and fever (*jwara*) like *upadrvas*.[7]

Charak Aacharya in *Vishachikitsa Adhayaye of Chikitsa Sthaan* has explained in detail about *Visha Samanya Chikitsaupakram* in which twenty-four treatment modalities are explained:

From which 24th *upkrama* is *lepa* which means local application. There is a good reference from *Pratishedh Adhyaya Mukta lepa* works effect on *keeta Vishabaadha*.[8]

Mukta Lepa is one of such *yoga* mentioned by *Acharya Vagbhatta* in *Keet Luta Visha Pratishedh Adhyaya*. *Mukta* is commonly available and easily identified by a common man. *Mukta* is having various chemical constituents which work on Honeybee venom. An attempt was made to observe the effect of *Mukta lepa* on Honeybee sting whatever may be the result, but the work will surely be proved its contribution in the field of service to humanity.[9]

Drug procurement: *Mukta* is easily available all over India. *Mukta* was collected from Ayurvedic medicine store. Authentication and standardization of *Mukta* was done in *Rasashastra* department of UAC.

(Table - 5)

	Test	Standard values	Results
1	Total ash content	Not more than 8 percent	7.2 percent
2	Water soluble extractive	Not less than 8 percent	11.2 percent
3	Alcohol soluble extractive	Not less than 6 percent	8 percent
4	Acid insoluble ash	Not more than 3 percent	2.1 percent
5	Foreign matter	Not more than 2 percent	1 percent

From the above values it is proved that the study drug is standard.

Experimental study: Honeybee collection was done from Authorized research center. Drug (*Mukta lepa*) toxicity test was done prior to the start of experimental study on 4 mice: 2 male and 2 females, other than 18 mice included in the experiment. To see the effect of *Mukta lepa* Preclinical trials were carried out at an Authorized laboratory to have an authenticated and unbiased results. For this study: 18 mice were taken.

(Table - 6)

	Trial group	Standard group	Control group
No. of mice	06	06	06
Sex of mice	3 male, 3 females	3 male, 3 females	3 male, 3 females
Local application	*Mukta lepa*	Betasalic (Beclomethasone Dipropionate 0.1%, Salicylic acid 3.0%)	No application

Probable mode of action of *Mukta lepa* - According to *Acharya Sushrut*, the signs and symptoms of *Makshika Damsh* are itching (*Kandu*), edema (*Shopha*), burning sensation on the site of sting (*Daha*), pain *(Ruja),* skin rashes *(Pidika*), fever (*Jwara*) etc. *Muk*ta has *Sheeta Virya* which helps in reducing the symptoms like itching *(Kandu*), burning sensation (*Daha*), skin rashes (*Pidika*). *Mukta* has *Madhura rasa* and it helps in reducing symptoms like bleeding (*Rakta Straav*), Erythema on the sting site.

Discussion regarding *Keeta*: In *Charka Samhita*: Description of *Agadtantra* is in chapter no. 23 of *Chikitsa Sthaan*. There is brief description of *Keeta Visha*, *Makshika* and its *Chikitsa.*

In *Sushrut Samhita*: There is wide description of *Agadtantra* as well as *Keeta Visha. Acharya Sushrut* has described *Agadtantra* in 8 chapters of *Kalpa Sthaan. Keeta Kalpa* is chapter 8 of *Kalpa Sthaan* in which wide description of *Keeta* as well as *Makshika Damsh*, its symptoms and treatment are mentioned.

In *Ashtang Sangraha*: *Keeta are* classified into four types: *Vayavya*, *Agneya*, *Soum*ya and *Sankirna*. In the treatment context of *Makshika Damsh Acharya Sushrut and Acharya Vagbhata* has given comprehensive treatment with local and internal medications.

Discussion regarding *Lepa:* *Lepa* is one of the treatment modalities out of 24 mentioned in *Charak Samhita*. *Vishaghan Lepa* is advised to use in 1/3 *Angula Pramaan* in thickness which is approximately 0.625

cm. Thickness of human skin is approximately 3-4mm. Human skin is similar to those mammals in which skin is not covered with fur; hence fur was removed before application of *Mukta lepa* on Albino mice. *Shodhan Chikitsa*, though the main line of treatment in *Visha Badha* but still small amounts of *visha* remains in the body which can be removed locally by *Lepa* application.

CONCLUSION

Conclusion is that part of the research work where facts observed during study and are being put forward from the discussion based on concepts, observations and results. These conclusive points may support the hypothesis of research study or may give new concepts. So, on the basis of discussion of the present study, following conclusions have been brought out.

Conclusion from Review of Literature: It is mentioned in *Aacharya Vagbhatta* that *Mukta Lepa* reduces *Keeta Visha* poisoning, *Mukta Lepa* on albino mice showed decrease in signs and symptoms due to bee venom. *Mukta* have anti-inflammatory properties which help in healing of wound. *Mukta* has *Madhura rasa,* which is considered *Sthambhak* in Ayurvedic classics, this property can also help in prevention of spread of venom, as compared to modern texts the pearl has hemostatic properties which helps in coagulation of blood.

Conclusion from Authentication and Standardization of *Mukta:* As per standards of *Mukta* in API (Ayurvedic pharmacopeia of India), collected things were good standard and were of pearl.

Conclusion from Experimental study: Male Albino mice are more sensitive to Honeybee sting. During study it was found that hypersensitive response had shown individual variations. Death of mice occurred usually after 24 hours. In pilot study, drug toxicity was done which showed no reaction after 24 hours and there is no mortality of albino mice seen. It revealed that *Mukta Lepa* is not toxic for Albino mice. Study drug *(Mukta Lepa)* had given better results in Edema than standard drug. From Observational data it is proved that *Mukta Lepa* has shown highly significant result. Hence can be used as preliminary treatment in Honeybee sting. From the study it is proved that *Mukta lepa* helps in wound healing. From the detailed pre-clinical study and observational analysis, it is concluded that *Mukta lepa* is effective and can be used in Honeybee sting. *Mukta* has showed significant results on the signs and symptoms of Honeybee venom.

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