

Research Article

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Corresponding Author

Dr. Nilam Vilasrao Malage

2454 'D' ward, Sai Prasad apt.

Shukrawar peth Kolhapur

Email: nilammalage17@gmail.com

Mob. No. - 9890895439



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In vivo Study of the Efficacy of Udumbar (*Ficus - Glomerata*)

Twak Lepa in Honey Bee Sting

Nilam Malage¹, Swapnil Patil², Prashant Shirke³, Smita Lokhande⁴, Adarsh Desai⁵.

1. Dept. of Agadtantra, Hon. Shri. Annasaheb Dange Ayurvedic medical college & Research center, Ashta Tal- Walawa, Dist- Sangli
2. Dept. of Rognidan Hon. Shri. Annasaheb Dange Ayurvedic medical college & Research center, Ashta Tal- Walawa, Dist- Sangli
3. Dept. of Sambhita Sidhant Hon. Shri. Annasaheb Dange Ayurvedic medical college & Research center, Ashta Tal- Walawa, Dist- Sangli
4. Dept. Kayachikitsa Hon. Shri. Annasaheb Dange Ayurvedic medical college & Research center, Ashta Tal- Walawa, Dist- Sangli
5. Dept. Dravyaguna. Hon. Shri. Annasaheb Dange Ayurvedic medical college & Research center, Ashta Tal- Walawa, Dist- Sangli

Abstract:

Today the scope of toxicology continues to grow rapidly and the subject is of profound importance to human and animal life too. Peoples attitude about drug from plant origin are becoming favorable. Human beings came in contact with various poisonous herbs, animals etc. so it is necessary to study branch that is toxicology were it has been mentioned that no substances are harmless only the way of using them are harmless which clearly underlies the concept of toxicology. Being an agricultural country 70% of Indian population is living in villages and remote areas where no medical services available at all incidences of insect bites are very common in such areas.

Now a day's apicultural (culture of honey bees) is developing in rural areas through many schemes conducted by KVIC (Khadi and village industries commission) government of India for rural unemployment. Hence apiculture is establishing vastly survey has proved that death due to bee stings per year in India is 0.007%. Though it is not fatal but for prevention preliminary medicine are important. Apiculturists are often victimized by bee sting, some of them may have anaphylactic shock and have died also hence it is necessity of time to search for such a drug which is easily available and easy to use in such remote areas.

KEY WORDS: Honey bee, Udumber (*ficus glomerata*), Apiculture.

INTRODUCTION:

Agadtantra is an ancient medical science & a special branch of Asthang Ayurveda and is well recognized for the study of toxicity and management of various poisonous bites, toxicity, toxic combinations of food, drugs etc¹... Being an agricultural country, 70% of Indian population lives in villages and forest area, where poor medical service available. In such specific areas animal bites or insects bites are very common. Now a day's Apiculture (Honey bee culture) is a rapidly developing branch of agriculture. Indian Government supports Apiculture through many schemes conducted by KVIC (Khadi and village industries commission). Survey has proved that death due to honey bee sting per year in India is 0.007 %.

Apiculturists are often victimized by honey bee stings. Some of them may have reaction from minor to major. Minor reactions are immediate pain, swelling and warmth at around the skin area that was bitten redness,

itching, sometimes low grade fever etc. though it is not fatal but for prevention preliminary medicine are important. As issue of preliminary medicines comes, the role of natural and holistic treatments has used for centuries to treat insect bites and allergies. The present study attempts to the toxicity of bee venom and anti toxic effect of *Ficus Glomerata* local application. *Ficus Glomerata* is commonly known as Udumber and is distributed throughout the tropical India and easily available.

Various antidotes are mentioned in the texts Udumber is said to be the best among all the anti-inflammatory analgesic having property of healing and coagulation of blood drugs². Hence Udumber should be subjected to experimental studies both animal and phyto chemicals. So this study focused more on antitoxic effect of Udumber and toxicity of honey bee venom.

AIM:

To study the efficacy of Udumber Twak in honey bee sting in albino mice.

OBJECTIVES:

1. To study the effect of Udumber Twak Lepa according to signs of honey bee sting in albino mice.
2. To study the toxic effect of honey bee sting in albino mice.
3. To study the antitoxic effect of Udumber Twak Lepa (local application) in honey bee sting in albino mice.

MATERIALS & METHODS:

The materials & technique used in presented work is described as follows. Drugs used for evaluation is as follows.

1. Test group:

Udumbar (ficus glomerata)³

In Ayurveda udumbar is kita nashaka drug.

Rasa: Kashaya

Veerya: Sheeta

Vipaka: Katu

Guna: Laghu, Ruksh

Dosha karma: Kapha pitta shamak

Chemical constituents⁴:

Bark contains around 14% tannin. Tetracyclic triterpene, leucoanthocyanins, lupeol, stigmasterol, B-sitosterol etc.

Parts used- Bark, root, fruits, leaves, separately or collectively.

Authentication & standardization of Drug:

Authentication of Udumber twak was done at Pharmacy College of our institute. To insure the quality of drug standardization is done before using in experiment.

The Phyto- chemical analysis of Udumbar twak :

Table: 1

Sr. No.	Name of the test	Result
1	Total ash content	5.52
2	Alcohol soluble extractives	7%
3	Water soluble extractives	9%

2. Standard drug used as Control group:

The standard drug used Ecziderm the trade name purchased from market. Mild steroids are generally used locally at the site of bee sting. Hence I have chosen Ecziderm ointment as a standard drug for comparison with the Ayurvedic drug ficus glomerata. Knowing the pharmacognosy of the modern allopathic drug mild steroid is not the part my study. Therefore concise description of mild steroid Beclomethasone dipropionate is potent corticosteroid (topical steroids). Generic name is Beclomethasone in the form of cream-ointment, with Beclomethasme Dipropionate 0.025%, having indication as Atopic and discoid eczema,

dermatitis, psoriasis, lichen simplex, interring, discoid, lupus, erythematosus etc. Adult dosage is apply sparingly twice daily and rub in⁵.

ANIMALS:

Sample:

- 1) Albino mice (9 male and 9 female)
- 2) Honey bee (*Apis cerena indica*)

1) Mice:

Mice of 20 to 50 gm were selected from National toxicology centre out of which 50% were males and 50% were females. Mice were also kept under observation before stinging as they should remain infection free. Same diet and water was scheduled to provide.

For calculation of doses of *Apis cerena indica* stings were given in increasing number to different mice and observed for reaction mice receiving 32 stings was died after 4 days. Experiment should have to conduct in sub lethal dose to study the antitoxic effect of Udumber twak lepa on honey bee sting. Hence six stings in each albino mice were decided to give in each albino mice.

Dose calculation of bee venom:

The only source of bee venom in fresh natural bee stings of *Apis cerena indica* were used in the experiment 2.8 mg venom/mg body wt. Number of stings for LD 50/kg body wt. 19 stings/kg⁶ but this data is for *Apis mellifera* species no data is available about *Apis cerena indica*. Hence I had done various trials to determine the sub lethal dose.

Protocol:

Table No.2

Animal species used	Albino mice
Strain	Swiss albino
Source of animal	NTC Pune
Sex of animal	50%males and 50% females
No of animals	18 mice
Average weight	20 – 50 gm
Diet	Pelleted feed supplied by nav Maharashtra Chakan oil mills ltd. Pune
Water	Community tap water <i>ad libitum</i>
Room temperature	20-24 degree Celsius
Relative humidity	40% to50%
Light cycle	12hrs light and 12 hrs dark

2) Honeybee⁷-

Botanical name: *Apis cerena*

Table no. 3-Scientific classification

Kingdom	Animilia	Family	Apidae
Phylum	Arthropoda	Subfamily	Apidae
Class	Insecta	Genus	Apis
Order	Hymenoptera	Subgenus	Apis
Suborder	Apoorita		

Table no.4⁸

Toxicological effect of various component of bee venom

Component	% venom	Chemical nature	Activity/pharmacology
Melittin	30-50%	Small, highly basic, 26 amino acid Polypeptide of 2840mole.wt	Pain,cardiotoxin/haemolysin, Membrane activity release histamine, depress B.P.
Phosphotipane	10-20	Basic stable protein of 15800roote.wt	Membrane and phospholipid Disruptant, Toxic, pain, Cellulysis, Lunges are target
Apamin	3	Highly basic 18 amino acid polypeptide	Neurotoxin causes tremors
Hyalnoonidae	2	Protein of 35000 mole wt.	Promotes, spreading of other components, no other activities, hydrolysis connectivity tissue.
Must cell degranulating peptide	2	Highly basic 22 amino acid polypeptide.	Release histamine etc. from must cells, pain anti-inflammatory
Histamine	<1	Small unstable biogenic amino of 3 mole wt	Burning, itching, redness immediate local skin effect pain.

METHODS:

Preparation of Udumber twak lepa -

Sting Experiment was conducted under the guidance of expert at NTC Pune. Raw sample of Udumber bark was collected from herbal garden of college.For preparation of Udumber twak lepa two main ingredients Udumber twak churna and water were required.After grinding the bark of Udumber in mixer powder form was obtained. Then it was allowed to filter in cotton cloth so that fine light pink coloured powder of Udumber bark is obtained. Then in clean washed Petri dish 8gm powder of Udumber twak churna was taken. Then 5 ml water was added to it with the help of 5ml syringe. This mixture mixed with the help of spatula and thin slurry lepa was prepared for each day fresh lepa was prepared.

Procedure for handling mice during experiment:

First of all preliminary drug toxicity study was done, and then removed fur of all albino mice with the help of scissors. Honey bees were collected from honey bee research centre Pune. Given anesthesia for honey bees putting them in deep forever about 5 min. After giving anesthesia honey bees are collected in plastic tin. For provision of oxygen to honey bees some holes made to plastic tin. By using plain forceps bees collected one by one from plastic tin and 6 stings were given on each fur removed area of albino mice. In this way 18 mice were sting by honey bee in that 9 were male and 9 were female. Then in each mouse out of six stings, 3 stings were removed by using forceps and 3 stings to study there.

EXPERIMENTAL STUDY:

These mice were divided in three groups

- 1) Test/trail group: 3 male and 3 female
- 2) Standard: 3 male and 3 female
- 3) Control: 3 male and 3 female

After dosing animals were observed for 1 hr up to 7 days. Sensitivity and duration of action of sting i.e.Apis cerena Indica in mice were observed.

Test group:

For test group Udumber Twak Churna lepa was locally applied on the whole fur removed area of 6 albino mice (3 male and 3 female)

Standard group:

For standard group beclomethasone and dipropionate 0.025% ^sis used as local application on fur removed area of albino mice 6 mice were applied locally with this medicine 3 male and 3 female.

Control group:

6 albino mice were in control group 3 male and 3 female. They had not applied any medicine.

OBSERVATIONS:

Udumber twak churna lepa and ecziderm ointment were applied up to 7 days in trail groups and standard groups, control group albino mice respectively signs and symptoms were observed. Normal allergic reactions at the time of sting life are Pain, Burning sensation, Erythema around the sting site; Swelling, Tenderness on touch etc. are observed. Then Normal allergic reaction hours/days after the sting like Itching, Residual redness, a small brown/ red damage spot at the purchase site, swelling at the sting site are observed. Duration of lowering or vanishing the local effect of bee sting in Udumber twak lepa group (test group), Standard drug & control group are observed & Comparative observations were tabulated.

OBSERVATION TABLES:

**TABLE NO.5
REDNESS**

Group	Test	Standard	Control
Sample size	6	6	6
Mean difference	1.35	0.89	0.78
S.D.	0.37	0.56	1.41
S.E.	0.15	0.22	0.57
Degree of freedom	5	5	5
Calculated 't' value	9	4.04	1.36
't' table value	2.57	2.57	2.57
P or level of significance	5%	5%	5%.
Inference	Highly significant	Significant	Insignificant

At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 9 times the standard error. So the result is highly significant in Test group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 4.04 times the standard error. So the result is significant in standard group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 1.36 times the standard error. So the result is insignificant in control group

**Table No.6
Swelling**

Group	Test	Standard	Control
Sample size	6	6	6
Mean difference	1.38	0.97	0.85
S.D.	0.37	0.87	1.36
S.E.	0.15	0.35	0.55
Degree of freedom	5	5	5
Calculated 't' value	9.2	2.77	1.54
't' table value	2.57	2.57	2.57
P or level of significance	5%	5%	5%
Inference	Highly significant	Significant	Insignificant

At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 9.2 times the standard error. So the result is significant in test group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 2.77 times the standard error. So the result is significant in standard group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 1.54 times the standard error. So the result is insignificant in control group.

**Table No.7
Tenderness**

Group	Test	Standard	Control
Sample size	6	6	6
Mean difference	1.41	0.98	0.87
S.D.	1.28	0.68	1.51
S.E.	0.52	0.27	0.61
Degree of freedom	5	5	5
Calculated 't' value	2.71	3.62	1.42
't' table value	2.57	2.57	2.57
P or level of significance	5%	5	5%
Inference	Significant	Significant	Insignificant

At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 2.71 times the standard error. So the result is significant in test group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 3.62 times the standard error. So the result is significant in standard group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 1.42 times the standard error. So the result is significant in control group.

**Table No.8
Blue discoloration**

Group	Test	Standard	Control
Sample size	6	6	6
Mean difference	1.16	1.17	20.3
S.D.	0.73	2.95	5.31
S.E.	0.29	1.20	2.16
Degree of freedom	5	5	5
Calculated 't' value	4	0.97	0.93
't' table value	2.57	2.57	2.57
P or level of significance	5%	5%	5%
Inference	Significant	Insignificant	Insignificant

At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 4 times the standard error. So the result is significant in test group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 0.97 times the standard error. So the result is insignificant in standard group. At 5 degree of freedom 5% significant limit is 2.57 the observed 't' value is 0.93 times the standard error. So the result is insignificant control group.

Histopathological Observation

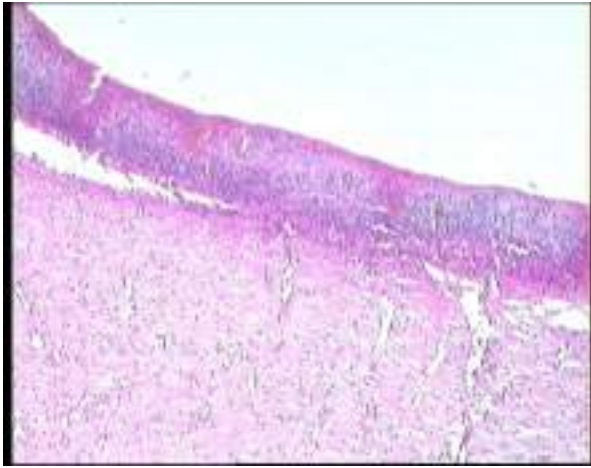


Photo No. 1 (with sting)

C1=control male albino mice

Focal epidermal ulceration with infiltration by polymorphs and lymphocytes dermis underneath show fibrosis.

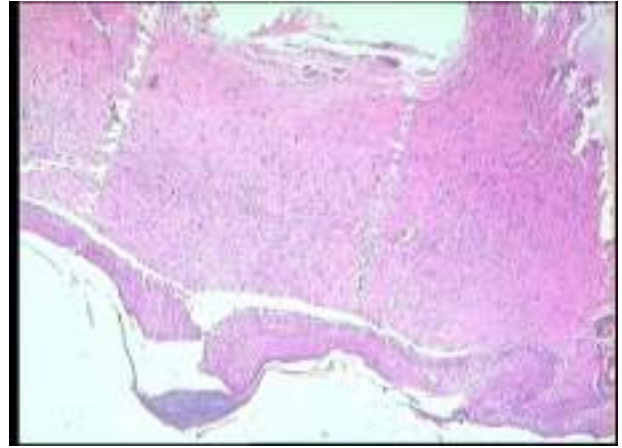


Photo No. 4 (without sting)

S2= Standard female albino mice

Epidermal ulcer with infiltration by polymorphs superficial dermis also shows few inflammatory cells.

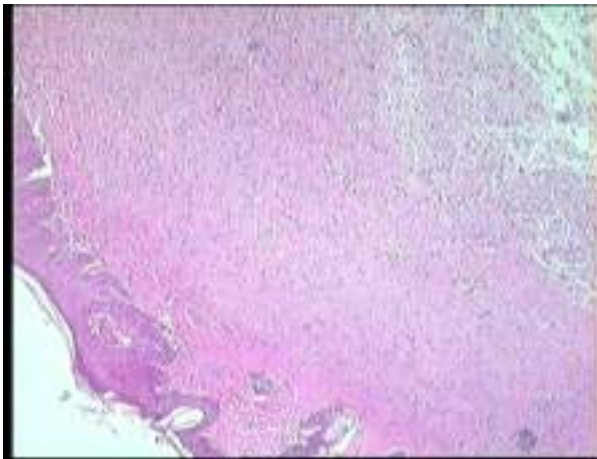


Photo No.2 (without sting)

C2= control male albino mice

No abnormality detected

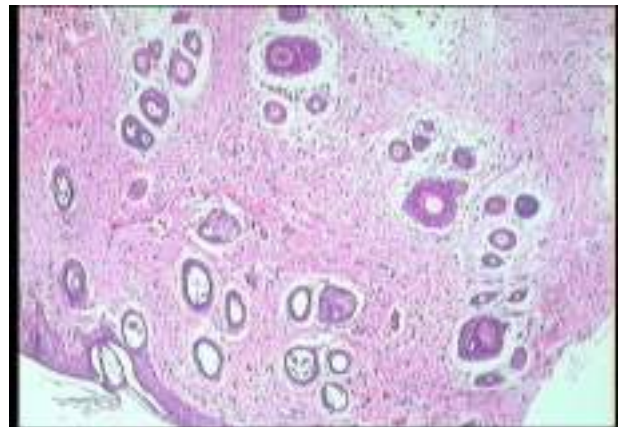


Photo No.5 (with sting)

T1= RH female

No abnormality detected

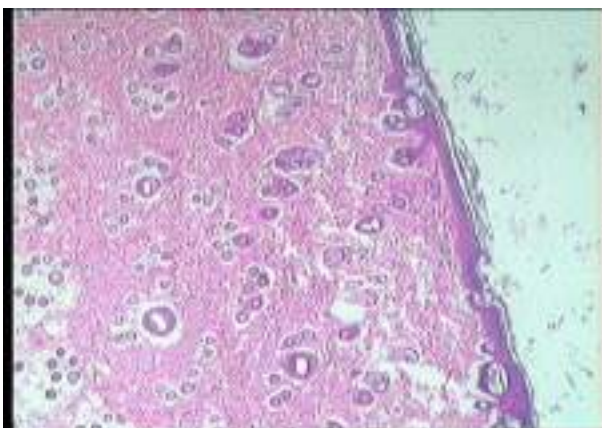


Photo No.3 (with sting)

S1= Standard female albino mice

Large ulcer with dense infiltration by polymorphs. Infiltrate is seen in subcutaneous fat also few bacterial colonies are also seen.

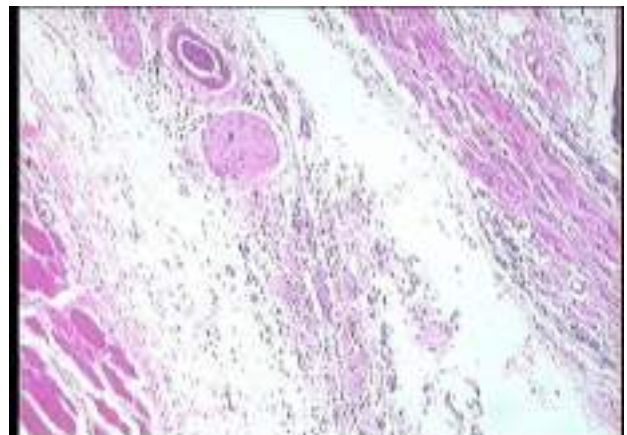


Photo No.5 (without sting)

T2= RH female

No abnormality detected

In Histopathological observation wound healed with Udumber Lepa showed no abnormality. Wound healed by Ecziderm shows large ulcer with dense infiltration by polymorphs. Infiltrate is seen in subcutaneous fat & also

few bacterial colonies are seen. Superficial dermis showed few inflammatory cells. Wound healed by without any application of medicine shows focal epidermal ulceration with infiltration by polymorphs and lymphocytes. Dermis underneath show fibrosis.

RESULTS:

After experimental study the data collected was statistically analyzed. To see the effect of udumber twak lepa on signs and symptoms observed after stinging paired test was applied. To see the effect of udumber twak churna lepa in comparison with ecziderm ointment and control group analysis variance that is ANOVA or F-test was applied.

Comparison of redness, in test, standard and control group:

On looking at the means for test, standard and control group we find that the mean redness in test group, standard group and control group are comparable but mean redness was apparently lowest in test group.

Comparison of swelling in test, standard and control group

On looking at the means for test, standard and control group we find that the mean swelling in test group, standard group and control group are comparable but mean swelling was apparently lowest in test group.

Comparison of tenderness in test, standard and control group:

On looking at the means for test, standard and control group we find that the mean tenderness in test group, standard group and control group are comparable but mean tenderness was apparently lowest in test group.

Comparison of blue discolorations in test, standard and control group

On looking at the means for test, standard and control group we find that the mean blue discolorations in test group, standard group and control group are comparable but mean blue discoloration was apparently lowest in test group.

Discussion:

This study was focused on antitoxic effect of ficus glomerata and toxicity of honey bee venom in comparison with Ecziderm as standard group and control group on the bee sting. The grades of parameters used in the experiment were approved by 1st standards of Indian government for skin reaction. After applying Udumber Twak Lepa for 7 days, it was found that, in all three groups pain, itching, redness, swelling, tenderness, blue discoloration this symptoms were observed. In trail and standard group wound was healed. In standard group one albino mice (LH) was sacrificed after 4th day. In control group two albino mice (H and H-RF) was sacrificed after 2nd day and on 3rd day respectively. In test

group all albino mice were survived. Hence we can say that honey bee stings can be fatal. Application of udumber twak lepa can be recommended as first line of treatment on honey bee sting. Hypersensitive response show individual variation. But responsiveness is common in redness, pain etc. On observation after study Females were found to be more sensitive to honey bee sting. They show severe reaction on third and fourth day of stinging. Hair growth was fast in male than females. Also the wounds which were remained with sting were healed in good manner but with large scar. Maximum stings were removed by themselves within 24 hrs.

OBSERVED PROBABLE MODE OF ACTION OF UDUMBER TWAK LEPA:

Bark of Udumber is acts as antibacterial and astringent. Topical use of Udumber bark is reduces burning sensation. It has healing property, reduces edema. It acts on Rakta Dhatu⁹, and cures hemorrhagic conditions. Bark is potent antioxidant, also having anti-inflammatory property.

In Ayurveda Udumber is classified under following categories according to its mode of actions as Sothahara, vedanasthapan, varnya, raktapittashamak, varanarapaka, daahprashmana¹⁰ etc. so these properties can be correlated with above observed results in honey bee sting.

Conclusion:

Honey bee sting can be fatal. So this study can be concluded, that Udumber bark should be recommended on honey bee sting poisoning as local application with water. In current experiment, Mild steroid (Beclomethasme and dipropionate) is used in honey bee sting as control group. Study was limited on experimental basis. Exact chemical changes occurred by Udumber on stinged albino mice not investigated, as they were beyond the scope of this study. Further experimentation is required to explain the mechanism of action to identity the systemic changes occurred in Albino mice when Udumber Lepa application on it. Finally Udumber twak lepa is more effective as it is easily available, applicable & cost effective.

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