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TO EVALUATE THE ROLE OF *NYAGRODH (FICUS BENGHALENSIS L.) TWAK LEPA* IN HONEY BEE STING

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ABSTARCT

Aim: To evaluate the role of *Nyagrodh (Ficus benghalensis L.) twak lepa* in Honey bee sting

Materials and methods: A observational study was conducted in the Department of ayurveda, after taking the approval of the protocol review committee and institutional ethics committee. Raw sample of Nyagrodh twak was collected from herbal garden and periphery of DBU, Punjab, and was dried for 7 days under shade.

Results: Results show that study drug had worked more efficiently on Erythema and Oedema while Standard drug worked more efficiently on Scaling and Fissure. Histo-pathology showed that wound healed with *Nyagrodh twak lepa* and Standard drug have shown almost similar changes while wound in control group showed extensive areas of necrosis.

Conclusion: *Nyagrodh* is one of the *Panchkshiri Vruksha* and Honey bee comes under the category of *keeta* mentioned in *Ayurveda*. *Nyagrodh twak lepa* on albino mice showed decrease in signs and symptoms due to bee venom. It can be concluded from the findings of present study that *Nyagrodh twak lepa* is effective, helpful in wound healing and can be used in Honey bee sting.

Introduction

Insect bite is very common and usually it is ignored. Cases of honey bee stings are very common in clinical practice. Most of these bites are less harmful and negligible but sometimes poisonous bites could lead to various complications i.e anaphylactic shock. In *Sushruta Samhita* vast description of *Keeta* (insects) is given in *Kalpa Sthana*.(1) There are millions of insects, however Honey bee is among few insects which are very important for human beings and became a part of their culture. Despite the evidence of an enduring reverence for the Honey bee, human beings have an uneasy relationship with it. Honey bees are absolutely essential for the production of agricultural crops as they pollinate1/3rd of what we eat. Apiculturists are easily victimized by bee stings. Most of them die because of anaphylactic shock. Hence it is necessity of time to search for such a drug which is easily available and easy to use even in remote places.

Wasps, bees and hornets secrete a poisonous fluid known as Apitoxin which is a bitter colorless liquid and is hemolytic and neurotropic in toxic amounts. The main component of the venom is a complex amalgamation of proteins, that leads to local inflammation and act as an anticoagulant. As it is impossible to study all the insects in a single study, therefore for thorough and specific study, here only one of the *Makshika* i.e. *Madhumakshika* is selected which comes under *jangama visha*. *Aacharya Charaka* has explained the local application *(Lepa)* of *kshirivruksha twak* to cure all types of *keeta visha*,(2) hence *Nyagrodh twak lepa* is selected as local application on *Apis Cerana Indica* bee sting poisoning. *Nyagrodh* is (one of the *panchkshri vruksha*) considered as anti-inflammatory having properties of healing and coagulation.

Materials and methods

A observational study was conducted in the Department of ayurveda, after taking the approval of the protocol review committee and institutional ethics committee. Raw sample of Nyagrodh twak was collected from herbal garden and periphery of DBU, Punjab, and was dried for 7 days under shade.

Preparation of Nyagrodh twak lepa

Bark of Nyagrodh was grinded in a mixer and powdered, filtered through Sieve no. 120 to obtain fine light brown colored powder. In clean washed Petri dish 8 gm powder of Nyagrodh twak churna was taken and 5 ml water was added to it with the help of 5 ml syringe. This mixture was mixed with

the help of spatula and then slurry lepa was prepared. Each day fresh lepa was prepared for application.

Table 1: Experimental Group Design

Group	Application	Mice
1-Control group	No application	5
3-Study group	Nyagrodh twak lepa	5

Test Drug toxicity test

Prior to the main study, test drug toxicity study was done for which 4 mice were selected (2 male and 2 female).These mice were not included in experimental study. Hair were removed from the back of mice by hair removing cream and Nyagrodh twak lepa was applied on the back of each mice and observed for 48 hours. No toxicity of Nyagrodh twak lepa was seen within stipulated period of 48 hours showing test drug to be safe.

Calculation of dose of Apis Cerena Indica

It was done by giving stings in increasing number to different mice (which were not included in study) and observed for reaction.

Mouse receiving 32 stings died after 4 days.

Sublethal dose is selected to know the anti-toxic effect of Nyagrodh twak lepa.

Hence six stings are selected to be given in each Albino mice.

Table 2: Animal Housing and Feeding Conditions

Animal species used	Albino mice
Strain	Swiss albino
Source of animal	National Toxicology Centre ,Pune
Sex of animal	50% male and 50% female
No. of animals	10 mice
Average weight	20-50 gm
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
Period of Acclimatization	7 days

Methodology

All 10 mice (50 % male and 50% female) were prepared for experiment. For removing hair a strong depilatory (hair removal) cream was used at the back of each albino mice. Only desired area on the back was depilated on each mice.Foraged bees colony was selected for the experiment. For identification of groups of male and female mice, they were stained (yellowish) with picric acid which remains for three to four weeks. Each mice was given stings one by one at their back maintaining a safe distance between two stings. After sting operation, out of six stings in each albino mice three stings were removed and another three stings were kept as it is.After giving stings all mice were observed for 1 hour for toxic signs and symptoms and mortality, up to a period of 7 days.

Table 3: Rating of skin reactions following observations were noted:

	Skin Reaction	Rating
(A)	Erythema	
	Slight , spotty \ diffuse	1
	moderate uniform redness	2

	Intense	3
	Fiery red with edema or epidermal	4
	dryness shiny	1
	fine scale	2
	Moderate	3
	severe with large flakes	4
(C)	Fissures	
	fine cracks	1
	single / multiple broader fissure	2
	made cracks with hemorrhage or	3
(D)	Oedema	
	No oedema	0
	Very slight oedema	1
	Slight oedema(edges of area well	2
	Moderate oedema (raised app 1 mm)	3
	Severe oedema (raised more than 1 mm & extending beyond area of	4

Results

Signs and symptoms were observed for 7 days in the two groups.

Table 4: Erythema in Study group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	3	2	2	1	1	1	0
2	T	F	3	3	2	1	2	1	1
3	W	F	2	2	2	1	2	1	1
4	H	M	3	2	Dead	-	-	-	-
5	T	M	2	2	2	1	1	1	0

Table 5: Erythema in Control group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	2	2	2	2	1	1	1
2	RH	F	3	3	Dead	-	-	-	-
3	W	F	2	2	2	2	2	2	2
4	H	M	2	2	2	2	1	1	1
5	RH	M	3	3	Dead	-	-	-	-

Table 6: Scaling in Control group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	1	1	1	1	1	1	1

2	RH	F	2	2	Dead	-	-	-	-
3	W	F	3	3	2	2	2	2	2
4	H	M	2	2	2	2	2	2	2
5	RH	M	3	3	Dead	-	-	-	-

Table 7: Scaling in Study group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	2	2	2	1	1	1	1
2	T	F	2	2	2	2	1	1	0
3	W	F	3	3	2	2	2	2	2
4	H	M	2	2	Dead	-	-	-	-
5	T	M	3	2	2	2	1	1	0

Table 8: Fissure in Study group
Table 9: Fissure in Control group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	1	1	1	1	0	0	0
2	T	F	2	2	2	2	2	2	2
3	W	F	3	3	2	2	1	1	1
4	H	M	2	2	Dead	-	-	-	-
5	T	M	1	1	1	1	0	0	0

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	1	1	1	1	1	1	1
2	RH	F	2	2	Dead	-	-	-	-
3	W	F	1	1	1	1	1	0	0
4	H	M	2	2	2	2	2	2	2
5	RH	M	2	1	Dead	-	-	-	-

Table 10: Edema in Study group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	2	1	1	1	1	1	1
2	T	F	2	2	2	2	2	2	2
3	W	F	3	2	2	2	2	2	2
4	H	M	2	1	Dead	-	-	-	-
5	T	M	4	3	3	3	3	3	3

Table 11: Oedema in Control group

Sr. No.	Animal Marking	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	H	F	1	1	1	1	1	0	0
2	RH	F	2	2	Dead	-	-	-	-
3	W	F	2	2	2	2	2	2	2
4	H	M	4	4	3	3	3	3	3
5	RH	M	3	3	Dead	-	-	-	-

Table 12: Inter group Comparison

Groups	Symptoms	Reduction	Cured mice
Study Control	Erythema	Day 4 Day 5	2 0
Study Control	Scaling	Day 5 Day 4	2 0
Study Control	Fissure	Day 5 Day 6	2 1
Study Control	Edema	Day 2 Day 4	0 1

Table 13: Paired ‘t’ test for Erythema in Inter groups

Analytical Parameters	Study Group	Control Group
Sample Size	5	5
Mean Difference	1.69	0.38
Standard Deviation	0.85	0.55
Standard Error	0.37	0.26
Calculated t value	5.1	1.55
Table t value	2.67	2.67
Degree of Freedom	5.5	5.5
Two tailed p Value	0.019	0.184
Inference	Very Significant	Not Significant

Table 14: Paired ‘t’ test for Scaling in Inter groups

Analytical Parameters	Study Group	Control Group
Sample Size	5	5
Mean Difference	1.7	0.35
Standard Deviation	1.14	0.55
Standard Error	0.44	0.23

Calculated t value	3.7	1.59
Table t value	2.55	2.55
Degree of Freedom	5.5	5.5
Two tailed p Value	0.019	0.19
Inference	Significant	Not Significant

Table 15: Paired 't' test for Fissure in Inter groups

Analytical Parameters	Study Group	Control Group
Sample Size	5	5
Mean Difference	1.10	0.43
Standard Deviation	0.99	0.62
Standard Error	0.35	0.26
Calculated t value	2.83	1.69
Table t value	2.67	2.67
Degree of Freedom	5.5	5.5
Two tailed p Value	0.041	0.18
Inference	Significant	Not Significant

Table 16: Paired 't' test for Edema in Inter groups

Analytical Parameters	Study Group	Control Group
Sample Size	5	5
Mean Difference	0.81	0.55
Standard Deviation	0.44	0.57
Standard Error	0.156	0.28
Calculated t value	5.2	2.43
Table t value	2.67	2.67
Degree of Freedom	5.5	5.5
Two tailed p Value	0.003	0.071
Inference	Significant	Not Quite Significant

Discussion

Allergic reactions observed immediately after the sting includes Erythema (redness around the sting site), Inflammation, Tenderness, loss of locomotor activity and Dizziness. Allergic reactions observed hours/ days after the sting includes Itching, Residual redness, A small brown / red damage spot at the sting site, Oedema, Swelling, Bluish discoloration and pus formation at or around the sting site. Female Albino mice were found to be more sensitive than male mice to Honey bee sting. Death of mice usually occurred after 48 hours. Sting reactions are more on female mice. The signs and symptoms were reduced usually after 3 days. Maximum stings were removed by mice themselves within 24 hours as mice always keep themselves clean. One female mouse died on day 3 of study group. Two mice died on day 3 of control group; one male and one female due to toxicity of Bee-venom.

Statistical Analysis revealed that the Study drug had worked more efficiently on Erythema and Oedema while standard drug worked more efficiently on Scaling and Fissure. There is natural healing mechanism of wound which was observed in control group. Study drug helps in speedy healing of wound. Leucoanthocyanin, a chemical constituent present in the bark of Nyagrodh (Ficus benghalensis L.) have anti-inflammatory properties which help in healing of wound. Leucoanthocyanin has anti enzymatic effect against various enzymes out of which hyaluronidase and

glucoronidase are two of them present in Honey bee venom. These enzymes are responsible for the spread of venom by increasing the cell wall and capillary permeability. Application of Nyagrodh twak lepa reduces the further spread of venom. Ethanolic and Petroleum ether extracts present in the bark of Ficus benghalensis L. (Nyagrodh) have anti-inflammatory action which helps in wound healing. Nyagrodh has kashaya 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 bark of Ficus benghalensis L. has haemostatic properties which helps in coagulation of blood.

Lepa is one of the treatment modality out of 24 mentioned in *Charak Samhita. Vishaghan lepa* is adviced 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 *Nyagrodh twak 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.

Probable mode of action of *Nyagrodh twak lepa Nyagrodh* has *sheeta virya* which helps in reducing the symptoms like itching *(kandu),* burning sensation *(daha),* skin rashes *(pidika). Nyagrodh* has *kashaya rasa* and *kashaya rasa* is considered as *Sthambhak*, it helps in reducing symptoms like bleeding *(rakta straav),* erythema on the sting site.(4)

According to biomedical science: The chemical constituent . **leucoanthocyanin** present in the bark of *Ficus benghalensis L. (Nyagrodh)* possess anti-inflammatory property. Leucoanthocyanin is haemostatic, antiseptic, astringent and reported to improve biological properties of blood vessels. Leucoanthocyanin has anti-enzymatic effect on Xanthine oxidase, Elastase, Collagenase, Hyaluronidase and Glucuronidase. Out of these enzymes Hyaluronidase and Glucosidase are present in bee venom. Glucosidase are the group of members of Glucoronidase enzyme. (5) *Nyagrodh twak* works against the two enzymes present in the venom of worker bee. The bark of *Ficus benghalensis L.* helps in healing of wound due to the presence of Ethanolic and petroleum ether extracts. These extracts have anti-inflammatory action.(6,7)

Conclusion

Nyagrodh is one of the *Panchkshiri Vruksha* and Honey bee comes under the category of *keeta* mentioned in *Ayurveda*. *Nyagrodh twak lepa* on albino mice showed decrease in signs and symptoms due to bee venom. It can be concluded from the findings of present study that *Nyagrodh twak lepa* is effective, helpful in wound healing and can be used in Honey bee sting. The effect of *Nyagrodh twak* is similar to the standard drug so both the drugs can be used in Honey bee sting. It is economical or free of cost and easily available all over India, hence can be used as an emergency drug for the same.

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