AN EXPERIMENTAL STUDY OF EFFICACY OF DARU-SARSHAP-MUSTADI LEPA ON LOCAL SIDE EFFECTS OF BHALLATAKA TAILA

A Thesis

SUBMITTED TO THE

TILAK MAHARASHTRA VIDYAPEETH PUNE

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY (Ph.D.)

In AYURVEDA (AGADTANTRA)

Under the Board of Ayurveda Studies

BY

Vd. NISHANT BHIMRAJ BARAPATRE

(Registration No. **05615008375**)

UNDER THE GUIDANCE OF

Prof. Vd. PRASHANT A. SURU

DEPARTMENT OF AYURVEDA

Year 2020

Tilak Maharashtra Vidyapeeth, Pune

UNDERTAKING

I **Vd. NISHANT BHIMRAJ BARAPATRE** is the Ph.D. Scholar of Tilak Maharashtra Vidyapeeth in **AYURVEDA (AGADTANTRA)** subject. Thesis entitled **"AN EXPERIMENTAL STUDY OF EFFICACY OF DARU-SARSHAP-MUSTADI LEPA ON LOCAL SIDE EFFECTS OF BHALLATAKA TAILA"** under the supervision of **Prof. Vd. PRASHANT A. SURU**, I solemnly affirm that the thesis submitted by me is my own work. I have not copied it from any source. I have gone through extensive review of literature of the related published / unpublished research works and the use of such references made has been acknowledged in my thesis. The title and the content of research is original. I understand that, in case of any complaint especially plagiarism, regarding my Ph.D. research from any party; I have to go through the enquiry procedure as decided by the Vidyapeeth at any point of time. I understand that, if my Ph.D. thesis (or part of it) is found duplicate at any point of time, my research degree will be withdrawn and in such circumstances, I will be solely responsible and liable for any consequences arising thereby. I will not hold TMV, Pune responsible and liable in any case.

I have signed the above undertaking after reading carefully and knowing all the aspects therein.

Signature :

Address : MIG-120, VHB Colony, Nara Road, Bhim Chowk, Jaripatka-Layout, Nagpur (Maharashtra) - 440014

Ph. No. : 8149694530, 8788911978

E-mail : drnishantbarapatre@hotmail.com

Date :

Place : Pune

Tilak Maharashtra Vidyapeeth, Pune

CERTIFICATE OF THE SUPERVISOR

It is certified that the work entitled **"AN EXPERIMENTAL STUDY OF EFFICACY OF DARU-SARSHAP-MUSTADI LEPA ON LOCAL SIDE EFFECTS OF BHALLATAKA TAILA"**

is an original research work done by **Vd. NISHANT BHIMRAJ BARAPATRE** Under my supervision for the degree of Doctor of Philosophy in **AYURVEDA - AGADTANTRA** to be awarded by Tilak Maharashtra Vidyapeeth, Pune.

To best of my knowledge, this thesis –

- embodies the work of candidate himself
- has duly been completed
- fulfills the requirement of the ordinance related to Ph.D. degree of TMV
- up to the standard in respect of both content and language for being referred to the examiner.

Signature of the Supervisor

(Prof. Vd. Prashant A. Suru)

ACKNOWLEDGEMENT

I remain grateful forever to my respected Guide **Prof. Vd. Prashant A. Suru Sir**, MD (Ayu), FIIM, Faculty of Ayurveda, TMV, Pune for his valuable guidance, meticulous supervision, timely given advices, motivational inspiration, consistent moral support, encouragement and co-operation that he extended kindly towards me throughout this research work.

I would like to express my special thanks to my PG Guide **Dr. V.P. Joglekar Sir**, MD (Kaya), MD (Agad), Retd. Prof. & HOD (Agadtantra), Tilak Ayurved College, Pune for his valuable guidance throughout the procedure of Ph. D. research as well as for sharing the knowledge regarding various critical aspects of research.

I want to take this opportunity to express my gratitude towards **Dr. S. P. Sardeshmukh Sir**, Dean, Faculty of Ayurveda, TMV, Pune and **Dr. Abhijit Joshi Sir**, Head, Faculty of Ayurveda, TMV, Pune for their appreciating guidance.

I am also very thankful to **Dr. C. Sunanda Yadav**, HOD, Ph. D. Section as well as all staff of Ph. D. Section and Ayurveda Section, especially **Mrs. Pathak Madam** for their valuable help in administrative procedures of the Ph. D.

I am heartily thankful to my seniors **Dr. Atul Ginode**, Asso. Professor, RT Ayurved College, Akola; **Dr. Sandeep Binorkar**, Assi. Professor, RA Poddar Ayurved College, Mumbai; and **Dr. Pradeep Jadhav**, Asso. Professor, KDMGS Ayurved College, Chalisgaon for their time-to-time directions and support. Also, I am thankful to **Dr. Vinay Pawar**, Asso. Professor, School of Ayurveda, DY Patil University, Mumbai for his guidance and encouragement to perform the statistical analysis.

I wish to convey special thanks to all my colleagues, especially **Dr. Dinesh Naik** and **Dr. Nitin Shende**, to accompany me on this wonderful journey hand-in-hand and for extending their valuable co-operation. I am also thankful to all my juniors, friends and relatives from Pune; particularly **Dr. Dinesh Bahirwad** among the juniors, **Dr. Sandip Daberao** among the friends and my brother-in-law **Mr. Jayesh Sonkusare**, who helped

me with logistics and again extended their helping hands every now and then, to complete all the miscellaneous works and formalities.

I sincerely thank **Dr. Mrs. A. C. Desai**, Director and **Mr. S. U. Deshmukh**, Study Director, Indian Drug Research Association & Laboratory (IDRA&L), Pune, who majorly helped in the Animal Study and **Dr. Chandrashekhar Mote**, Consulting Veterinary Pathologist, Pune, who helped in the Histopathological Examination; leading me to this stage of completion of the research work.

I am very grateful to **Vd. Shailesh Gujar**, New United Pharmacy, Pune to provide Shatadhauta Ghrita for the research study and also **Mr. Kedar Chitale**, Chitale Dairy, Pune for providing the Standardization Certificate of Navaneeta (Butter).

I have no words to express my sincere thanks to **my parents** & **my in-laws** who gave encouragement and cooperation at every step & especially to my wife **Dr. Sujaya** for her emotional and moral support throughout my career.

Finally I thank to **all my well-wishers and helpers**, who helped me knowingly and unknowingly in the completion of my research work. Thanks for all your encouragement.

Vd. Nishant B. Barapatre

ABSTRACT

Title of Thesis - "AN EXPERIMENTAL STUDY OF EFFICACY OF DARU-SARSHAP-MUSTADI LEPA ON LOCAL SIDE EFFECTS OF BHALLATAKA TAILA"

In *Agadatantra* literatures, a scripture named *Anupana Manjari*, authored by *Aacharya Shri Vishraama* in the 18th century, has specified some brief, simplistic and practically useful medicinal combinations for management of many prominent poisons (*Visha*). '*Daru-Sarshapa-Mustadi Lepa*' is one such short and simple drug combination, to cure the poisoning of *Bhallataka* (Semecarpus anacardium).

The *Guna-Karma* (Properties) of all component drugs i.e. *Devdaru, Sarshapa, Mustaka* and *Navaneeta* seem to be beneficial against various skin manifestations. Even the contents are very few and cheap; making it easily available to the more vulnerable lower stratum of society. Considering the lack of any precise allopathic medication and on account of the importance of *Lepa Chikitsa* (Local application) in neutralizing the poison, the present research work was undertaken, to find a solution on this very common, yet neglected problem in our society.

Aim and Objectives - These were determined to firstly assess and then correlate the locally-expressed adverse manifestations of *Taila* (Oil) of *Bhallataka.* The study was mainly intended to investigate the efficacy of the experimental drug i.e. *Daru-Sarshapa-Mustadi Lepa* and every single component of this *Lepa* as well, against these locally-expressed adverse effects of *Bhallataka Taila* in albino mice.

Review of Literature - It was done regarding the toxin "*Bhallataka*", especially for its local side effects, the experimental drug formulation "*Daru-Sarshapa-Mustadi Lepa*" along with its all four ingredient drugs and the *Lepa Kalpana* as mentioned in the *Ayurvedic* literature. Various ancient *Samhita Grantha*, contemporary textbooks, journals and websites related with these topics have been reviewed for this purpose.

Materials - All the herbal raw drugs were procured from a well-known Herbal drugs provider except for the Sarshapa, which was purchased from a local grocery shop. All these were first authenticated in their raw-form, later pulverized and sieved to obtain its powder (*Churna*) and further standardized at IDRAL (Indian Drug Research Association & Laboratory), Pune. The samples of raw-herbal drugs have been deposited to the *Agadatantra* Museum of *MUP's Ayurved College, Risod* and archived as Voucher Specimens.

Navaneeta made up from *Godugdha* (Cow's milk) was purchased from a renowned Dairy "*M/S B. G. Chitale Dairy, Sangli*" and Standardization certificate was obtained from its Quality Control (Q.C.) laboratory. *Shatadhauta Ghrita* was obtained from New United Pharmacy, Pune and its Standardization certificate was also obtained.

The *Taila* (Oil) of *Bhallataka* was extracted by heating it over a flame, after making a few pricks with the help of a large-bore needle. '*Daru-Sarshapa-Mustadi Lepa*' was prepared by mixing all ingredients in equal proportions and utilizing the standard *Lepa* preparation method, using a *Khalva Yantra* (Mortar and Pestle). Both the *Taila* and *Lepa* were standardized in the research laboratory of IDRAL before their use in the experimental study.

Methods - 21 Swiss albino mice, weighing 25-35 gram, were selected for the Experimental study with random sex selection. Thus, 9 males and 12 females got selected for the study and were randomly divided into 7 groups of 3 mice each. The hairs were removed from their back mechanically, in the form of two patches on each side of dorsum. On next day, 0.1 ml *Bhallataka Taila* was applied on each patch of 1 cm diameter and the Swiss albino mice were monitored over a day (24 hours) to study pattern of occurrence of adverse manifestations locally.

The first group (Normal group) was spared from any treatment, while the second group (Control group) was treated with '*Shatadhauta Ghrita Lepa*'. The third group was tested with '*Daru-Sarshapa-Mustadi Lepa*' and next four groups were experimented with the four contents of the *Lepa* separately, though keeping *Navaneeta* as a base. The treatment was given 12 hourly, keeping the *Lepa* applied for 8 hours and keeping the

lesion open for next 4 hours. Daily observations were made for 7 days to assess erythema, edema and formation of blisters and ulceration. Histopathological examination was also performed at the end, to assess different changes occurring at the tissue level.

Observations, Analysis and Interpretation - The erythema of skin, caused due to mechanical irritation of shaving, eventually subsided on its own natural course within the next 24 hours. No other skin reactions were observed during this period.

On the next day of shaving, *Bhallataka Taila* was applied locally on all the shaven patches. During the next 24 hours of observations, the similarity of local side effects in albino mice to those observed in human beings was confirmed.

The mortality in Group 1 (No Treatment Group) was observed to be 100%, where the 1st Mouse died after 1 day only, 2nd Mouse after 3 days and the 3rd one after 7 days. Group 7 (Only *Navaneeta Lepa*) showed 66.66% survival, as only 1 Mouse was found dead after 5 days. All the other Treatment Groups (Groups 2, 3, 4, 5 and 6) showed 100% survival.

Within The Group Comparison - Before & After Treatment (Day 0-Day 7) - It was observed that all the groups showed a significant change (Assessed by applying Wilcoxon Signed Rank Test) in the level of Erythema except Group 1 (Normal Group- No Treatment Group), but the reduction in Erythema was more prominently seen in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and Group 4 (*Devdaru + Navaneeta Lepa*). The reduction in edema was most prominently and significantly seen in Group 3 (*Daru-Sarshapa-Mustadi Lepa*), Group 4 (*Devdaru + Navaneeta Lepa*) and Group 6 (*Mustaka + Navaneeta Lepa*), where the edema subsided completely. Group 4 (*Devdaru + Navaneeta Lepa*) showed a significant decrease in the Blisters and Ulceration as well.

Between The Groups Comparison - After Treatment (On Day 7) - By application of Kruskal Wallis Test, it was significantly evident that the reduction of Erythema was most prominent in Group 4 (*Devdaru + Navaneeta Lepa*) and marginally lesser in Group 3 (*Daru-Sarshapa-Mustadi Lepa*); while it was seen least in Group 1 (Normal Group- No Treatment Group), followed by the Group 7 (Only *Navaneeta Lepa*). The reduction of Edema was significantly seen in three Groups viz. Group 3 (*Daru-*

Sarshapa-Mustadi Lepa), Group 4 (*Devdaru + Navaneeta Lepa*) as well as Group 6 (*Mustaka + Navaneeta Lepa*); while it was least in Group 1 (Normal Group- No Treatment Group). However, the reduction of blisters and ulcerations was not statistically significant; though it was seen most prominently in Group 4 (*Devdaru + Navaneeta Lepa*), and least prominently in Group 7 (Only *Navaneeta Lepa*).

The tissue level changes seen in the Histopathological Examination of the lesions were analyzed according to the pre-formulated semi-quantitative scoring system. The "Formation of Scab in Epidermal Layer" and "Formation of New Epidermal Skin Epithelial Tissue as a Healing" was most prominent in Group 4 (*Devdaru + Navaneeta Lepa*), followed by Group 3 (*Daru-Sarshapa-Mustadi Lepa*); while it was seen least in Group 1 (Normal Group- No Treatment Group), followed by the Group 7 (Only *Navaneeta Lepa*). The "Healing of Skin by Collagen formation and Proliferation of Fibroblast tissue" and "Connective tissue proliferation with granulation tissue formation" was most prominent in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and Group 4 (*Devdaru + Navaneeta Lepa*), while it was least in Group 1 (Normal Group- No Treatment Group). The "Infiltration of acute inflammatory cells (Neutrophils & polymorphonuclear cells-PMN) in the skin section (Dermis & epidermis)" was least in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and Group 4 (*Devdaru + Navaneeta Lepa*), which is a sign of better healing; while it was most prominent in Group 1 (Normal Group- No Treatment Group), which is a sign of lesser healing, though the effects were not statistically significant.

Discussion - It has been put forth from two angles, the *Ayurvedic* aspects and the Experimental aspects. Firstly, the *Visha Samprapti* of *Bhallataka* has been investigated to find the mechanism of its toxicity from an *Ayurvedic* perspective. This investigation ascertains that, the *Laghu-Teekshna Guna, Katu-Tikta-Kashaaya Rasa, Ushna Veerya* and *Katu Vipaaka* of *Bhallataka* contribute to its local toxicity by its *Visha*-alike *Guna;* causing *Pitta Vriddhi, Maansa-Shonita Dushti, Daaha-Paaka-Straava-Chhedana-Bhedana, Vranotpatti and ultimately* generating a hypersensitivity reaction due to *Ojo Visransa*.

Later, the mechanism of pharmacological action *(Samprapti Bhedana)* by the experimental drug i.e. *Daru-Sarshapa-Mustadi Lepa* as well as every single component

of this *Lepa* has been established against the local toxicity of *Bhallataka*. This exercise corroborates that, the *Tikta Rasa* of *Devdaru, Katu-Tikta Rasa* of *Sarshapa* & *Mustaka,* and *Madhura Rasa* of *Navaneeta* specifically act by their *Vishahara/Visha Prasaadana/Visha Prashamana Karma.* Moreover, *Vrana Shodhana, Vrana Straavahara Karma* of *Devdaru; Tvakdoshahara Karma* of *Sarshapa* and *Mustaka; Sheeta Veerya, Pitta-Rakta-Daaha Shamana, Vranaapaha Karma* of *Mustaka* and *Navaneeta;* and all the properties of *Navaneeta,* including its *Madhura Vipaaka* and *Sarva Dhaatu Vardhana-Ojo Vardhana Karma* unambiguously play a role in *Samprapti Bhedana* of local side effects of *Bhallataka* by using *Daru-Sarshapa-Mustadi Lepa.*

In the second half of discussion, some practical difficulties encountered during the Experimental study have been discussed, along with the adopted counter-actions to solve these difficulties; like making a few pricks with the help of a large-bore needle in the *Bhallataka Beeja* to extract its *Taila* (Oil), vigorous activity of *Mardana Sanskaara* to improve the consistency of *Lepa*, keeping the lesions open to dry up for 4 hours in order to avoid the possible occurrence of *Klinna Vrana* (Moist wound), utilizing the mechanical method of shaving with Blade Razor rather than simply using depilatory cream for hair removal, and using ether in a proper dose for anesthesia as overdose can prove fatal for the animals. Also, the rationale behind using *Shatadhauta Ghrita* in Control Group has been discussed, as it has been found more effective than the reference standard drug “Silver nitrate” in a previous research work.

The significant results have been interpreted to draw the key inferences, like attribution of 100% mortality of Group 1 (Normal Group- No Treatment Group) to the systemic toxicity of *Bhallataka* through absorption in this untreated group, and the death of 1 Mouse in Group 7 (Only *Navaneeta Lepa*) due to the solitary use of *Navaneeta* leading to complications of unhealed *Klinna Vrana* (Moist wound).

The difficulties in perceiving the signs of local side effects have been discussed as well; like visibility of erythema, papules, vesicles (Small blisters) and bullae (Large blisters) only at the borders of lesions due to dark discoloration and scab/eschar formation, requiring a careful observation with the help of a magnifying glass; visibility of ulcers only when the scab falling off, and formation of another scab again due to the

exudates, obstructing the visibility of the wound, which caused the waxing & waning of visibility of ulcers & wounds, and thus causing fluctuations in the scoring criteria; measurement of edema possible by visualization and palpation only; and non-availability of practically useful resources to record other symptoms like Pruritus (Itching), Burning sensation and Pain.

The observed differences between the efficacies of all the treatment groups have also been discussed; like attribution of better results of Group 4 (*Devdaru + Navaneeta Lepa*) than the Group 3 (*Daru-Sarshapa-Mustadi Lepa*) to the presence of *Sarshapa* having *Ushna-Teekshna Guna* in the latter group, which also caused a lesser efficacy of Group 5 (*Sarshapa + Navaneeta Lepa*) than the other groups of constituent drugs; the lesser effect of *Shatadhauta Ghrita Lepa* than the experimental drug attributable to the shorter period of the experiment; and the clammy, unhealed appearance of wounds in Group 7 (Only *Navaneeta Lepa*) ascribed to the *Klinnatva* caused by *Navaneeta*, rather than the Groups 3, 4, 5, and 6, where the *Lepa* were not excessively moist due to presence of dry *Churna* (Powder) of herbs, absorbing the *Klinnatva* of *Navaneeta*.

A critical discussion has been propounded to discourse the inadequacies and limitations of the present research work, like the chances of error due to a smaller sample size, imposed by the various rules and regulations regarding animal experiments.

Also, the future directions for further advancement in this area have been discussed, like LD50 study of local toxicity of *Bhallataka Taila* to find out the median lethal dose; clinical trials to investigate the efficacy of *Daru-Sarshapa-Mustadi Lepa* in human participants; further researches to explore the exact mode of action using newer advances of Network Pharmacology and Reverse Pharmacology approach; undertaking animal experimentations of other formulations mentioned in *Anupan Manjari*; research about suitable preservatives for packing and trading of this *Lepa* for the convenience of patients; and exploring the efficacy of this *Lepa* against Urushiol-induced allergic rashes caused due to other plants of the same family like Poison oak, Poison ivy etc.

A very important future prospect is to use "*Daru-Musta-Navaneeta Lepa*" only in future experiments by omitting *Sarshapa*. This is because, "*Devdaru + Navaneeta Lepa*"

has shown better results than the complete formulation, as *Sarshapa* added as an ingredient in '*Daru-Sarshapa-Mustadi Lepa*' was noticed to cause a hindrance in the curative effect of other three constituents. This deterrent action can be specifically attributed to the *Ushna Veerya* as well as the *Teekshna Guna* of *Sarshapa Beeja Churna*, thereby necessitating for its exclusion.

Conclusion - The study validates the claim of *"Anupan Manjari"*, proving that "the experimental drug named *Daru-Sarshapa-Mustadi Lepa* shows significant efficacy against the locally-expressed adverse effects of the *Taila* (Oil) of *Bhallataka* (Semecarpus anacardium) in the experimental animals viz. Swiss albino mice"; and therefore, it can be recommended for further investigations through clinical trials in human population.

Keywords – *Bhallataka*, Local side effects, *Daru-Sarshapa-Mustadi Lepa*.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES / GRAPHS

LIST OF ANNEXURES

INTRODUCTION

INTRODUCTION

Ayurveda is an ancient system of indigenous medicine, which is being practiced since times immemorial. In *Ashtang Ayurveda* i.e. the 8 specialized domains of *Ayurveda*, *Agadatantra* is one special field of science, which draws a parallel with the Modern Toxicology and carries its own importance in the clinical practice.

Agadatantra literally means the *Tantra* (Science) related to the use of *Agada*. Here, the word "*Agada*" has four other synonyms viz. *Bheshaja*, *Aushadha*, *Bhaishajya* and *Jayu*; which have been enumerated in an ancient *Sanskrit* dictionary named "*Amarkosha*".[1] The meaning of all these words is "Medicine" only. Therefore, an "*Agada*" is nothing but a medicine.

गदो रोगः, अगदो रोगप्रतिकारः, तदर्थं तंत्रं अगदतंत्रम् ।

सुश्रुतसंहिता सूत्रस्थान १/१४ (डल्हण टीका)

As the word "*Agada*" suggests from its *Nirukti* (Etymology) that, the substance which acts against "*Gada*" can be called as "*Agada*". In this context, "*Gada*" specifically denotes the "*Visha*" (Poison). However, other synonyms of "*Gada*" are *Vyadhi*, *Aamaya*, *Roga* etc. which mean to be a "Disease" in a broader sense. Therefore, *Agada* is not only an antidote which merely combats the *Visha*, but it can be considered as a medicine which also cures the diseases arising from that poison.

Therefore, *Agadatantra* represents an exclusive branch of *Ayurveda*, which particularly deals with *Agada* (Antidotes) against the toxic effects of *Visha* (Poison) of both *Jangama* (Animate) and *Sthavara* (Inanimate) origin. This *Ayurveda* specialty of *Agadatantra* exemplifies itself in all the major texts of *Ayurveda*. Hence, we find many *Agada Kalpa* mentioned in various *Ayurvedic Samhita* for treatment of poisoning.

However, this branch of *Ayurveda* has faced lots of ignorance by the ancient *Acharyas*. Even though we find citations about *Agadatantra* in all the major textbooks of *Ayurveda*; still *Acharya Sushruta* has been the only one, who dedicated a complete *Sthana* i.e. *Kalpasthana* for its elaboration. All the other *Acharyas* have concluded this subject in just a matter of few *Adhyayas* (Chapters).

In contradiction to this scenario, there are certain texts available in the *Ayurvedic* literatures which are mainly devoted to the subject of *Agadatantra*. *Anupana Manjari* is one such scripture from the 18th century, authored by *Aacharya Shri Vishraama*. The subject matter discussed in this text mainly relates to the treatment of various poisons i.e. *Visha Chikitsaa*. Nevertheless, it comprehensively describes different *Anupana* as well, for the purpose of their use with *Dhatu-Upadhatu* as a part of treatment of diverse diseases. Hence, the name of the text has been given as *Anupana Manjari*.[2]

In the material text of *Anupana Manjari*, some very brief medicinal combinations have been specified for management of many prominent poisons (*Visha*). These formulations are extremely simplistic and practically useful, because they consist of only one or two drugs which are easily available and their preparation doesn't require any special efforts. Even the language used for composition of Sutras of this text appears very simple. Thus, *Acharya Shri Vishrama* has put up his clinical experience in a brief and concise manner.

'*Daru-Sarshapa-Mustadi Lepa*' is such a simplistic, short and snappy medicinal combination described in *Anupana Manjari*, in order to cure the poisoning caused by *Bhallataka* (Semecarpus anacardium Linn).

दारुसर्षपमुस्ताभिः नवनीतेन लेपयेत ।

भल्लातकविकारोऽयम् सद्यो गच्छति देहिनाम् ॥ अनुपान मंजरी ३/६

According to *Acharya Shri Vishrama*, the local manifestations of *Bhallataka* poisoning can be pacified by *Lepa* (External application) of *Devdaru* (Cedrus deodara Roxb.), *Sarshapaa* (Brassica campestris Linn.), *Mustaka* (Cyperus rotundus Linn.) and *Navaneeta* (Butter).

The pharmacological properties for external use of all these herbal raw drugs have already been mentioned in the *Dravyaguna* literature. External application of *Devdaru* is *Shothahara, Vedanasthapana, Kushthaghna, Krimighna, Vrana Shodhana* and *Ropana*. *Sarshapaa* is said to be *Kushthaghna, Jantughna, Vedanahara* and *Varnya*. Also, *Mustaka* is well known for its *Tvagdoshahara, Kandughna, Shothahara* actions.[3]

Navaneeta, which is used as a base for this *Lepa*, has been described as *Varnya* and is used in *Vata-Pitta-Rakta Vikara*.[4] Therefore, on the first look, the general *Guna-Karma* (Properties) of every single component of '*Daru-Sarshapa-Mustadi Lepa*' seems to be favorable in its role against various skin manifestations.

Being a *Lepa Kalpana* i.e. Local application, it is very clear that it has been intended for use in local manifestations of *Bhallataka*. Even *Charaka* has included *Lepa Chikitsa* into *Chaturvinshati Upakrama* for treatment of *Visha* (24 Modalities for treatment of poisoning). Therefore, *Lepa Chikitsa* (External application) is an important measure in the local treatment of any dermatological lesion caused by contact of *Visha*.

According to the modern medical science, an important principle in the treatment of poisoning is the removal of unabsorbed poison. The poison should always be removed from its local site of application. In case of contact poisoning, the local area should be firstly washed out with water or soap and water.[5] Therefore, the modern medical science also emphasizes on the local treatment of the contact poisoning.

Ayurveda appears to be one-step ahead of the modern science, as it has suggested for the neutralization of the remaining poison as well as the absorbed poison at the local site of poisoning only, by application of *Lepa* (External application) locally at that site. If the poison is not removed completely, then its remains may cause the toxic effects again or it may get converted into *Dooshivisha* (Latent poison). Therefore, *Lepa Chikitsa* (Local application) is necessary in all types of local manifestations of poisoning.

Considering the importance of *Lepa Chikitsa* (Local application) in all types of poisoning, '*Daru-Sarshapa-Mustadi Lepa*' has been selected for the present research work as a local application for local side-effects caused due to *Bhallataka Taila*.

RATIONALE OF THE STUDY

The inadvertent appearance of contact dermatitis as an adverse manifestation of *Bhallataka* toxicity has always been a frequently suffered problem. Even in the *Ayurveda* community, lots of *Vaidya* are now-a-days using several self-manufactured preparations which include *Bhallataka* as an ingredient. In the course of production of these formulations, some inappropriately carried out procedures, like direct contact with the utensils or media utilized in the process of *Bhallataka Shodhana*, lead to the appearance of contact dermatitis.[6] As a result, these *Vaidya* suffer from local manifestations like irritation, erythema, blister formation etc.

The inadvertently manifested contact dermatitis can also appear, when the patient applies juice of *Bhallataka* on the external surface of skin, either by himself as an anecdotal home remedy or used by the quack doctors. Occasionally the juice of Marking Nut is smeared over the genital area in order to penalize the person for adultery, which is a usual practice observed in the tribal areas. At times, the skin is soiled with Marking Nut juice, in order to create bruise-like injuries over the skin; so that allegations of (physical) violence can be deceitfully levied on the enemies. The use of Marking Nut juice is also brought about for the purpose of Vitriolage (Acid attack) by flinging it on the body of others with an intention of harm or torture. Sometimes it is applied locally to fabricate the deliberate wounds with the aim of malingering as well, especially by the army-men, navy people, and air-force personnel to give up their duties and also by the prisoners to escape from the rigorous activities during imprisonment.[7]

Washermen use the Semecarpus fruits for marking the clothes with certain signs before their washing, so that they can be identified afterwards. That's why, the term "Marking Nut" came into existence for the *Bhallataka* fruits. The paste of marking nut is sometimes used for removal of tattoo. In the Southern parts of India, especially in the state of Aandhra Pradesha, the Hindu people celebrate the festival of Dashehara with a traditional custom of tying the Semecarpus fruits to their automobiles as well as to the entry gates of their homes.[8]

In the *Samhita* literature of *Ayurveda*, we find the references for medicinal use of *Bhallataka* in many ailments like *Kushthha* (Skin disorders), *Gulma* (Benign abdominal tumor), *Udara Roga* (Ascitis), *Arsha* (Hemorrhoids or Piles), *Aamavaata* (Rheumatoid arthritis), *Kapha Roga* (Diseases due to vitiated Kapha Dosha) etc.[9] Also, some ethnic groups in the Central part of India reveal a conventional tradition of setting the Semecarpus fruits on fire, with a belief that the emitted fumes safeguard the patient from Black Magic and Supernatural powers. However, these fumes often cause allergic reactions leading to dermatitis.[10] Even some people in the villages use it as a hair dye and also for the treatment of Alopecia areata.

Thus, accidental appearance of contact dermatitis due to the local toxicity of *Bhallataka* has been a usual problem in our civilization all the time. However, the treatment in allopathic medicine for local side effects of *Bhallataka* is simply based on the presenting symptoms without any precise medication. It usually includes the cleaning of affected body parts using fresh water only, followed by the use of either mild emollients or the medicinal creams having drug ingredients like Silver Nitrate, Silver Sulfadiazine etc.[11] As compared to this, '*Daru-Sarshapa-Mustadi Lepa*' can prove to be a specific treatment for the local side effects of *Bhallataka*.

The contents of this *Lepa* are very few, cheap and easily available in the market; therefore, it can be easily prepared and administered even to the lower stratum of the society, in which the contact dermatitis due to *Bhallataka* is more common.

Till date, only a few researches have been completed regarding the local manifestations of *Bhallataka*. This area of research has been generally untouched by most of the researchers and hence, there is a need of research in this direction. So, it has prompted me to select this topic for my Ph.D. research work.

For this purpose, an experimental study has been proposed and the experimental drug viz. '*Daru-Sarshapa-Mustadi Lepa*' has been tried and tested in the Swiss albino mice, to assess its efficacy on the local side effects of *Bhallataka*. The rationale behind the animal experiments is also as ancient as *Ayurveda* science is, because such experiments have already been described and advocated in the age-old *Samhita*

Granthas like *Charaka* and *Sushruta Samhita*. *Acharya Charaka* has suggested mixing the blood with food and serving it to the crows or dogs, in order to check whether the Rakta (Blood) is *Shuddha* or not (*Charaka Samhita Siddhisthana 6/79*).[12] *Acharya Sushruta* has also advised to test the food of king, whether it is poisoned or safe, by feeding it to the animals (*Sushruta Samhita Kalpasthana 1/28-33*).[13] This shows the long-standing tradition of animal experiments in *Ayurveda*, which is still being practiced to study the safety and efficacy of any new drugs, because human life is of utmost importance among all the living beings.

If this formulation is proven to be safe and effective during the in vivo study, then it can be further developed for clinical trials as well. Therefore, the present research has been proposed to study the effect of local application of '*Daru-Sarshapa-Mustadi Lepa*' to alleviate the locally-observed side-effects of *Bhallataka* (Semecarpus anacardium Linn) *Taila* (Oil). In the pursuit of this endeavor, the thesis work presented here is an attempt to find a solution on this very common, yet neglected problem in our society, which can prove to be beneficial to the community at large.

PREVIOUS WORK DONE

PREVIOUS WORK DONE

1) Study of role of Bhallataka in the treatment of Rheumatoid Arthritis.
   By- Dr. Geetha C. R., Trivendrum, 1987.

2) Effect of Shodhana on Bhallataka. w.s.r. to phytochemistry and acute toxicity.
   By- Dr. Pramod K., Haridwar, 1993.

3) A clinical study on the role of Bhallataka on Kaphaj Shwitra Dushti.
   By- Dr. Prasad L., Haridwar, 1999.

4) Kushtha me Bhallataka ka bahya prayog.
   By- Dr. Shweta Sawarkar, Nagpur, 2003.

5) Comparative study of Sthavara Visha Bhallataka in relation to its Shodhana Sanskara.
   By- Dr. Uday Pawade, Nagpur, 2004.

6) Urushiol-induced Contact dermatitis caused during Shodhana (Purificatory measures) of Bhallataka (Semecarpus anacardium Linn.) fruit.
   By- R. Ilanchezhian, Roshy Joseph C. and Acharya Rabinarayan, Jamnagar, 2012.

7) An experimental study to evaluate the efficacy of Shatadhauta Ghrita application in contact poisoning caused by Bhallataka Beeja (Semecarpus anacardium Linn.)
   By- Dr. Sruthy Das, Karnataka, 2013.

8) Experimental study of Tila-Navnitadi Lepa as an antidote against local action of Bhallataka (Semecarpus anacardium Linn.) in albino mice.
   By- Dr. Abhijeet Gawai, Pune, 2015

AIM AND OBJECTIVES

AIM

"An Experimental Study of Efficacy of *Daru-Sarshapa-Mustadi Lepa* on Local Side Effects of *Bhallataka Taila*"

OBJECTIVES

1. To evaluate and compare the local side effects of *Bhallataka Taila*.
2. To study the effect of *Daru-Sarshapa-Mustadi Lepa* on the local side effects produced by *Bhallataka Taila*.
3. To study the anti-toxic effect of all the individual ingredients of *Daru-Sarshapa-Mustadi Lepa*.

RESEARCH QUESTION

Whether *Daru-Sarshapa-Mustadi Lepa* shows any efficacy on local side effects of *Bhallataka Taila* in albino mice?

NULL HYPOTHESIS (Ho) -

There is no significant action of *Daru-Sarshapa-Mustadi Lepa* on local side effects of *Bhallataka Taila* in albino mice.

ALTERNATE HYPOTHESIS (H_1) -

Daru-Sarshapa-Mustadi Lepa is significantly effective on local side effects of *Bhallataka Taila* in albino mice.

REVIEW OF LITERATURE

- ***REVIEW OF THE TOXIN - BHALLATAKA***
- ***REVIEW OF LOCAL SIDE-EFFECTS OF BHALLATAKA***
- ***REVIEW OF THE EXPERIMENTAL DRUG FORMULATION***
- ***REVIEW OF LEPA KALPANA***

REVIEW OF THE TOXIN - BHALLATAKA

Bhallataka finds its mention since the very ancient period, even in the literatures of *Vaidika Kaala* (2500-600 BC), *Pauranika Kaala* (1500-300 BC) and *Samhita Kaala* (1000 BC-7th Century AD). It has been described under the *Upavisha Varga*[14] in *Dhanvantari Nighantu* (10th-13th Century) and *Rasa Tarangini* (20th Century). More recently, it has also been included under poisonous medicinal plants in Drug and Cosmetics Act, 1940[15] which is currently in practice in India. This shows that *Bhallataka* is well-known to the mankind as a *Visha Dravya* (Toxic substance) and our ancestors have taken its cognizance from time to time.

However, if a poison is used in a proper dose and manner, then it acts as an excellent medicine, rather than showing its harmful effects in the body. Also, if a medicine is used in an improper way and wrong amount, then it can act as a poison. The ancient sages of *Ayurveda* knew this wisdom very well; and therefore, *Acharya Charaka* has explained this principle in the first and foremost chapter of his writing itself.

योगाद् अपि विषं तीक्ष्णं उत्तमं भेषजं भवेत् ।
भेषजं च अपि दुर्युक्तं तीक्ष्णं सम्पद्यते विषम् ॥

चरकसंहिता सूत्रस्थान १/१२७

Therefore, *Bhallataka* has been in use for medicinal purpose since very ancient times. In *Ayurveda*, *Bhallataka* has been mentioned in therapeutics for various diseases like *Kushtha, Gulma, Udara, Arsha, Aamvaata, Kaphaja Rogas* etc.[16] It has been widely used for internal administration, like in the form of *Rasayana Kalpa* (Rejuvenating medicine) or curative medicines like *Sanjivani Vati* etc.; and also as an external application to cure a variety of diseases like piles, skin diseases etc. Thus, a poison has also been converted into useful medicines by making use of the principle established by sage *Charaka*.

As *Bhallataka* exhibits such long-standing historical background, it's important to take a look at the timeline of its references. These glimpses from the history

will let us know about the earlier situations confronted by the humans due to contact of *Bhallataka*. It also reveals the sequential development of knowledge and beliefs about *Bhallataka* right from its oldest references till today.

HISTORICAL REFERENCES

1) ***Vaidika Kaala* (2500-600 BC) –** In *Veda Vaangmaya* (Vedic Literature), we find the description about various herbs, but there is no mention of the plant *Bhallataka* in any *Veda*. The most primitive reference of *Bhallataka* can be found in *Panini Sutra* (4th Century BC). However, as it is a book of *Sanskrit* Grammar, *Bhallataka* has been mentioned here just as an example for grammatical elaboration. Still it confirms that *Bhallataka* was known to the mankind in those early days as well.[17]

पश्य भल्लातकान् फुल्लान् नरैः अनुपसेवितान् ।
फल पत्रैः अवनतान् नूनम् शक्ष्यामि जीवितुम् ॥

रामायण अयोध्याकांड २/५६/७

In *Valmiki Ramayana* also, we find mention about *Bhallataka* in its *Ayodhya Kaand*, where Lord *Rama* asks *Seeta* to look at the trees of *Bhallataka*, rich with plentiful flowers and bent down with the weight of fruits and leaves, unplucked by men.[18] This indicates that the use of flowers, fruits and leaves of *Bhallataka* was in practice in the period of *Ramayana* also.

2) ***Pauranika Kaala* (1500-300 BC) –** *Agni Purana* has included *Bhallataka* in the category of "*Ratna*". Therefore, the marking nut was supposed to be a precious stone. Also, we find references of *Bhallataka Taila* being used as a *Kushthaghna* and *Rasayana Dravya*. Therefore, it seems that the medicinal use of *Bhallataka* was common at that time. However, the leaves of *Bhallataka* have been advised to be discarded from the rituals. So, it indicates that the side-effects of *Bhallataka* were probably known at the time of *Agni Purana*.[19]

Neelamata Purana has mentioned the usage of *Bhallataka Taila* in the cosmetics used in ancient *Kashmir*. It was utilized in the preparation of various scents, perfumes, garlands etc. It was used for the worship of horses as well.

Devi-Bhagavata-Purana has mentioned *Bhallataka* as a tree found in *Mani Dveepa* (A fictional island of Gems and Pearls, *Devi Shakti's* residence). According to the beliefs of this *Purana*, *Bhallataka* trees always used to bear new leaves, fruits and flowers, which spread its sweet fragrance all over this place. This attracted the birds and bees. Even the rivers flowing through the forests of *Bhallataka* were carrying the juicy liquids.[20]

Apart from these, we find some references of *Bhallataka* in *Garud Purana* and *Brahma Purana* as well, regarding its qualities and medicinal uses.

In *Kautilya Arthashastra*, the references of *Bhallataka* are found in the 14th part of the book i.e. *Aupanishadikam* (Secret means). The chapter named *Paraghaata Prayoga* (Means to injure an enemy) contains different *Yoga* (Formulations) for causing death, disease or injury to other person. There are a number of formulations described, which contain *Bhallataka* as an ingredient. This indicates that it was a well-known poison at the time of *Kautilya* as well and its criminal use was a common practice in that time.[21]

Brihat Samhita by *Varah Mihir* (509-577 AD) has described the Adamantine glues and binders, used in the construction of temples, mansions etc., which were expected to last for a million years. *Bhallataka* was also used as one of the ingredient of these Adamantine glues and binders. The use of *Bhallataka* in treatment of horses has also been mentioned by *Varah Mihir*.[22]

3) ***Samhita Kaala* (1000 BC-7th Century AD) –** It was the most important period in the history of *Ayurveda*, as the most commendable works in the form of *Samhita Grantha* (Texts) have been compiled by the *Brihattrayi* (Great Trios of *Ayurveda*) in this period. As *Ayurveda* was very well-established during this period, *Bhallataka* finds its mention in almost all the texts written during this time.

A) ***Charaka Samhita* (1000 BC-4th Century AD) –** *Acharya Charaka* has mentioned *Bhallataka* in the *Phala Varga* (Class of Fruits). He has also included it under various *Mahakashaya* viz. *Deepaniya Gana, Bhedaniya Gana, Kushthaghna Gana* and *Mutra Sangrahaniya Gana*.

The qualities and the medicinal uses of *Bhallataka* were well-known in the period of *Charaka Samhita* and therefore, it has been advised in a variety of ailments for both internal administration as well as for external application. However, *Bhallataka* was most extensively used for *Rasayana Karma* (Rejuvenation therapy) & in *Kaphaja Roga* by *Acharya Charaka*. As the side-effects of *Bhallataka* were well-known, they were administered in the form of 10 types of preparations for *Rasayana Karma* and certain precautions were taken to avoid its direct contact, by coating the oral cavity by *Ghrita* (*Charaka Samhita* Chikitsa Sthana 1-2/13-19).[23]

भल्लातकानि तीक्ष्णानि पाकीनि अग्निसमानि च ।
भवन्ति अमृतकल्पानि प्रयुक्तानि यथाविधि ॥

चरकसंहिता चिकित्सास्थान १/२/१७

कफजो न स रोगो अस्ति न विबन्धो अस्ति कश्चन ।
यं न भल्लातकं हन्यात् शीघ्रं मेधाग्निवर्धनम् ॥

चरकसंहिता चिकित्सास्थान १/२/१९

B) ***Sushruta Samhita* (1000 BC-5th Century AD) –** *Acharya Sushruta* has mentioned leaves of *Bhallataka* in the *Shaaka Varga* (Class of Vegetables). He has also included it under *Nyagrodhadi Gana* and *Mustadi Gana*. *Sushruta* has described the properties of *Bhallataka Taila* like *Rasa*, *Veerya*, *Doshaghnata* for the very first time. Therefore, it seems that he had a detailed knowledge about *Bhallataka* (*Sushruta Samhita Sutra Sthana 45/122*).[24]

Acharya Sushruta had good knowledge about the side-effects of *Bhallataka* as well. Therefore, he added *Bhallataka* in the class of *Phala Visha* (Poisonous fruits) by using its synonym "*Khadyotaka*". He was the first *Acharya* to label *Bhallataka* as a *Visha* (Poison). He also advised to take *Sheeta Jala* (Cold water) as *Anupana* (Adjuvant) after consumption of *Bhallataka Taila*. As *Sushruta* might have had a better knowledge about *Bhallataka*, he has advised it for various diseases internally as well as externally. Even he has used it in the formulation of *Anjana* (Collyrium) for eye diseases.

C) ***Ashtang Sangraha* (6th Century AD) and *Ashtang Hridaya* (7th Century AD) –** Both these texts, written by *Acharya Vriddha Vagbhata* and *Laghu Vagbhata* respectively, generally reiterate the facts mentioned by both *Acharya Charaka* and *Sushruta*. However, as they are more contemporary to these two, they add certain new dimensions of knowledge.

The *Shodhana* (Process of Purification) of *Bhallataka* has been described by *Ashtang Sangraha* for the first time, by way of cutting the fruits of *Bhallataka* into pieces and keeping them in *Ishtika Churna* (Brick powder) until the oil is completely soaked in brick powder (*Ashtang Sangraha Uttarasthana 49/99*).[25]

Ashtang Hridaya Uttarasthana 39/75 has mentioned the method for extraction of *Bhallataka Taila*. It has also mentioned *Bhallataka* as the *Agra Aushadha* (Drug of choice) for treatment of *Shushka Arsha* (Non-bleeding type of Piles). It has also been advised in many other diseases internally and externally.[26]

4) *Nighantu Kaala* (8th -19th Century AD) – Due to the development of *Dravyaguna Vaangmaya* (Literature about herbal drugs and their properties) in the form of compilation of various *Nighantu Grantha* in the later period, we find the information of all herbal drugs like their synonyms, properties, pharmacological actions etc. described in much details than the *Samhita Grantha*. *Nighantu* have classified all the herbal drugs into different groups, termed as *Varga*, according to their similarities. The following list describes the *Varga* in various *Nighantu*, in which *Bhallataka* has been included, which demonstrates the trend of its classification.

Table No. 1 – Trend of Classification of *Bhallataka* in *Nighantu Grantha*

Sr. No.	*Nighantu Grantha*	*Kaala* (Time Period)	*Varga* of *Bhallataka*
1	*Dhanvantari Nighantu*	10th Century AD	*Chandanadi, Upavisha Varga*
2	*Sodhala Nighantu*	12th Century AD	*Chandanadi Varga*
3	*Madanapaala Nighantu*	14th Century AD	*Abhayadi Varga*
4	*Kaiyadeva Nighantu*	15th Century AD	*Aushadhi Varga, Taila Varga*
5	*Bhavprakasha Nighantu*	16th Century AD	*Haritakyadi Varga*
6	*Raaj Nighantu*	17th Century AD	*Aamradi Varga*
7	*Priya Nighantu*	20th Century AD	*Haritakyadi Varga*
8	*Nighantu Aadarsha*	20th Century AD	*Bhallatakadi Varga*

5) ***Rasa Grantha Kaala* (5th-20th Century AD) –** Usually the description of herbal drugs is found in the *Nighantu Grantha* only. However, *Bhallataka*, being a *Visha Dravya* (Poisonous drug), has been described in some of the *Rasa Grantha* as well, as it is an ingredient in the preparation of various medicines. Therefore, we find the *Shodhana* (Purification) procedure of *Bhallataka* and its *Kalpa* (Formulations) described in several *Rasa Granthas*.

However, only *Rasa Tarangini* (20th Century AD) has enumerated *Bhallataka* under the category of *Upavisha*. No prior *Rasa Grantha* has given it much importance, even though an expert in *Agadatantra* (Toxicology) like *Acharya Sushruta* had mentioned it as *Phala Visha*. As it is a sub-lethal type of poison usually manifesting the skin and superficial tissues only, with lesser chances of systemic toxicity or loss of life; all the *Rasa Acharyas* have refrained from its inclusion in the classification of *Visha*. But *Rasa Tarangini*, a very contemporary *Rasa Grantha*, decided to increase the total number of *Upavisha* from being seven to total eleven, in order to accommodate some less toxic yet important poisons like *Bhallataka*. Therefore, *Rasa Tarangini* seems to have brought *Bhallataka* under the category of *Upavisha*, in order to highlight its importance as a poison.

NIRUKTI (ETYMOLOGY)

भल्ल इवातति वृणोति वा भल्लातकः । (निघन्टु आदर्श)

As the touch of *Bhallataka* may cause a pricking pain and *Vrana* (Wound), as of with the prick of a lancet (*Bhalla*); therefore, it is called as *Bhallataka*.

OR

As *Bhallataka* scrapes away the vitiated *Kapha Dosha* with its penetrating sharpness as a javelin (*Bhalla*), it is called as *Bhallataka*.

PARYAYA (SYNONYMS)

Bhallataka has many synonyms, which are mostly based on its irritating nature. However, some of the synonyms are also based on the kind of manifestations caused by it in the body, while some others indicate the external appearance of the plant or plant parts as well. Therefore, some important synonyms have been quoted here according to its etymology.

1) ***Agnika, Anala, Vanhi, Dahana, Tapana*** – अग्निवत् तीक्ष्णकारी । (निघन्टु आदर्श)

   As it has the corrosive property, it is comparable with the nature of fire.

2) ***Agnimukhi*** - अग्निवत् मुखं अस्या दाह करत्वात् अग्निमुखी । (निघन्टु आदर्श)

   As it produces ulcerations and burns due to contact with the skin.

   Also because, the nuts are seated in fleshy orange cup, looking like Agni (Fire).

3) ***Arushkara*** - अरुवर्णं करोति । अरुषिं स्फोटैः व्रणान् करोति अरुष्करः । (निघन्टु आदर्श)

   As it produces *Arunshika* (Rashes) on the body.

4) ***Shophahetu, Shophakrita*** - शोफस्य हेतु इति । (निघन्टु आदर्श)

As it produces edematous rashes all over the body.

5) ***Veeravriksha, Veerataru*** - विशेषेणेरयेति वीरा वीराणां वा वृक्षो दुःस्पर्शत्वात् । (निघन्टु आदर्श)

As its touch is intolerable, just as a worrier can't be touched by an ordinary man.

6) ***Vatari*** - विकृतरूपं वातं शमयति इति । (निघन्टु आदर्श)

As it is *Vaataghna*.

7) ***SphotaBeejaka, Vranakrita, Kshatakrita*** - स्फोटजनकं बीजं यस्या सा । (निघन्टु आदर्श)

As it produces *Sphota/Vrana/Kshata* (Rashes/Wounds) all over the body.

8) ***Krimighna*** - कृमिन् हन्ति इति । (निघन्टु आदर्श)

As it kills the *Krimi* (Worms).

9) ***Bhedana*** - भिनत्ति अर्बुदादीन् इति । (निघन्टु आदर्श)

As it can destroy the tumours.

10) ***Ranjaka*** - रंजयति त्वगादीन् इति । (निघन्टु आदर्श)

As it produces blackish discoloration of the skin.

11) ***Beeja Paadapa, Prithaka Beeja*** - बीजयुक्तः पादपः । (निघन्टु आदर्श)

As the tree is full of externally visible seeds (nuts).

12) ***DhanurBeeja, Dhanurvriksha*** – As the nuts are obliquely ovoid.

13) ***TailaBeeja, SnehaBeeja*** – As the nuts contain oil.

14) ***Arshohita*** – As it is the drug of choice in Piles.

15) ***Antah Satva*** – As its main content i.e. its oil lies inside the fruit.

VERNACULAR NAMES

- English - Marking nut tree, Oriental cashew tree, Dhobi nut tree.
- Hindi - *Bhilawa, Bhela.*
- Marathi - *Bibba, Bhilawa.*
- Gujarati - *Bhilamu.*
- Punjabi - *Bhilawa, Bhela, Bhiladar.*
- Bengali - *Bhela, Bhelatuki.*
- Assami - *Bhelaguti, Bhala, Bholaguti.*
- Oriya - *Bhollataki, Bholai, Balia.*
- Kannada - *Bilawa, Bhallataka, Goddugeru, Karigeri, Bhallika.*
- Malayalam - *Chera, Cheru, Alakkucheru.*
- Tamil - *Senkottui, Tatamkottai, Sherankottai, Scramkotati.*
- Telugu - *Phidivittalu, Nallajidi, Nallajidiginga.*
- Arabic - *Habbul-fahm, Habbul-Kalb.* (*Kalb*= Heart)
- Persian - *Baladur*. (*Bala*= Pain, *Dur*= Remove)
- Urdu - *Baladur, Bhilavan.*
- Nepali - *Bhilai*
- Spanish - Anacardo
- French - Anacardier d'orient

BOTANICAL NAME

- **Latin name** - Semecarpus anacardium Linn.
- **Family** - Anacardiaceae (Aamra Kula)
- **Etymology** - In Greek Language,

  Semecarpus means -

  Simeion = Marking or Tracing

  Carpos = Nut

  Anacardium means - Cardium like i.e. Heart-shaped.

As the black juice of the heart-shaped nut was commonly used in the past by the washermen (*Dhobi*) for marking or tracing some designs on the cloth for their identification, it acquired the botanical name "Semecarpus anacardium" lexicologically.

AYURVEDIC CLASSIFICATION

Table No. 2 – Classification of *Bhallataka* in *Ayurveda (Brihattrayi)*

Constitution	*Chetana Dravya – Antashchetana Dravya*
Origin	*Audbhida Dravya – Vaanaspatya/Vriksha*
Morphology	*Kaarya Dravya*
Mahabhuta	*Teja Mahabhuta Pradhan Dravya*
Use	*Aushadhi Dravya*
Gana (According to *Charaka Samhita*)	*Deepaniya Gana* *Bhedaniya Gana* *Kushthaghna Gana* *Mutra Sangrahaniya Gana* *Katuka Skandha*
Gana (According to *Sushruta* & *Vagbhata*)	*Nyagrodhadi Gana* *Mustadi Gana* *StanyaShodhana Gana* *Kashaya Varga*

भल्लातकः कषायोष्णः शुक्रलो मधुरो लघुः ।
वातश्लेष्मोदरानाहकुष्ठार्शोग्रहणीगदान् ।
हन्ति गुल्मज्वरश्वित्रवन्हिमान्द्यकृमिव्रणान् ॥

भावप्रकाश निघंटु

BOTANICAL CLASSIFICATION

Table No. 3 – Classification of *Bhallataka* in Botanical Science

Kingdom	Plantae - All plants
Sub-Kingdom	Tracheobionata - Vascular plants
Super Division	Spermatophyta - Plants having seeds
Division	Magnoliophyta - Plants having flowers
Class	Magnoliopsida - Dicotyledons
Sub-Class	Rosidae
Order	Sapindales
Family	Anacardiaceae - Sumac Family
Genus	Semicarpus
Species	Anacardium

BOTANICAL DESCRIPTION

Bhallataka is a medium-sized tree, having height of about 25-40 feet. The leaves are simple, large, obviate-oblong, rounded at the apex, 9-30 inches long, 5-12 inches in breadth and are crowded towards the extremities of the branches. The surface of leaves is glabrous above and ashy grey or buff and pubescent beneath. However, the tree becomes leafless between February and April. The leaves reappear in the month of May.

Its flowers are small, greenish white or dull greenish yellow in color, dioeciously and can be seen in terminal panicles. The fruits (Marking nuts) are 2-5 cm long, heart-shaped, smooth and shiny, black when ripe, with rough projection at base containing an edible kernel. They are located on fleshy orange-colored receptacle. They ripen from December to March. The bark is grey in color and exudes an irritant secretion on taking an incision.[27]

The fruit of *Bhallataka* is known as Marking nut (*Bhilawa*). It weighs about 1.6 to 3.6 grams and has a hard black outer layer within which is a thick pericarp. The pericarp (Fleshy pulp) of the fruit or seed contains a brownish oily acrid juice, which turns black when mixed with lime and exposed to air. Therefore, it is used by *Dhobis* (Washermen) as "Marking ink" for making some marks on the clothes.[28]

GEOGRAPHICAL DISTRIBUTION

Bhallataka tree is fairly common all over the India, especially in the tropical regions having hot climatic conditions. It is found in the sub-Himalayan tract towards the east of Sutlej. It can be found ascending the outer hills of Himalaya up to 3500 feet height and grows till the Far-East regions of Assam, including Bihar, West Bengal and Orissa.

PRAYOJYA ANGA (USEFUL PARTS)

Mainly, the Phala (True fruit) of *Bhallataka* i.e. the Black-colored Marking nut is used for the medicinal purposes. Sometimes only *Taila* (Oil) of *Bhallataka* can be used, as its therapeutic properties are mainly due to the oil present inside the nut. Apart from this, the seed kernel (*Godambi*) is widely used as a dry fruit.

ACTIVE PRINCIPLES

The pericarp of *Bhallataka* contains a blackish juice, which is corrosive in its action. In the initial phase of researches to investigate the chemical constituents of *Bhallataka*, it was found that the Tarry oil present in this juice consists of –

1) 90% **Anacardic acid**, which is an oxy-acid &
2) 10 % **Cardol**, which is a non-volatile alcohol.

In 1925, Naidu carried out further investigations and isolated the following chemical constituents from the bitter, astringent and highly vesicant juice of *Bhallataka*.

1) **Catechol**
2) **Anacardol** ($C_{18}H_{13}O_{3,}COOH$), which is a mono-hydroxy phenol.
3) **Two phenolic acids** - $C_{16}H_{15}O_{3,}COOH$ and $C_{14}H_{13}O_{3,}COOH$.
4) A sweet-tasted **fixed oil** from the kernel of the nut, which contains linoleic, myristic, oleic, palmitic and stearic acids.

In 1931, Pillay and Siddiqui carried out a systematic work in order to study the exact composition of the juice of pericarp of *Bhallataka*. However, they couldn't find any of these four chemical constituents which were testified by the earlier researchers. In lieu, they detected below mentioned active principles in *Bhallataka* juice.

1) **Semecarpol**, which is a mono-hydroxy phenol.
   Boiling Point - 185-190^{o} C at 2.5 mm of pressure
   Congealing Point - Below 25^{o} C [Congeals to a fatty mass]
   Quantity - 0.1 % of the juice.

2) **Bhilawanol**, which is an O-di-hydroxy compound, including the cis and trans isomers of **Urushiol** (3-pentadecenyl-8-catechol).[29]
   Boiling Point - 225-226^{o} C at 3 mm of pressure
   Congealing Point - Below 5^{o} C
   Quantity - 46 % of the juice (15-17 % of the nut).

3) **Tarry residue**, which is non-volatile and corrosive in its action.
   Quantity - About 18 % of the nut.[30]

FATAL DOSE & PERIOD

FATAL DOSE: 10 grams.

FATAL PERIOD: 12 to 24 hours.[31]

PREVALENT USAGES

Bhallataka has been used for medicinal and non-medicinal purposes since very ancient times. There have been various kinds of practices prevalent in the Indian society where *Bhallataka* fruits or its *Taila* (Oil) is used, which make the people vulnerable to endure the local side-effects of *Bhallataka* due to its contact. The following usages of *Bhallataka* can mainly be found prevalent at the present times.

In *Ayurveda*, *Bhallataka* has been mentioned in therapeutics for various diseases like *Kushtha, Gulma, Udara, Arsha, Aamvaata, Kaphaja Rogas* etc.[32] It is widely used therapeutically as local application to cure diseases like piles, warts, joint pain and a variety of skin diseases like Leukoderma (Vitiligo), Psoriasis, Leprosy etc. Hence, the accidental local toxicity of *Bhallataka* causing contact dermatitis is a very common problem.

Many *Ayurvedic Vaidyas* use various self-prepared *Ayurvedic* formulations containing *Bhallataka* or these formulations are manufactured in the *Ayurvedic* pharmaceutical units. During preparation of medicines, contact dermatitis is caused due to improper handling of utensils and disposal of media used in the *Shodhana* (Purification) procedure.[33] Therefore, many *Vaidyas* and workers from *Ayurvedic* Pharma industry suffer due to local manifestations of *Bhallataka* toxicity.

Accidental contact dermatitis may result from the application of juice externally by quacks or the patient self-medicating himself using it as a quack remedy for rheumatic pain. Also, the juice of *Bhallataka* is applied locally in the rural areas by patients of injury, probably with a misconception that its disinfectant property will aid in the healing of wound. This oily acrid juice of *Bhallataka* is used as abortifacient as well, by applying it locally to the cervical os, by means of an abortion stick. Some people in villages use the juice alone as a hair dye or *Bhallataka* oil is mixed as a component into the indigenous hair dye preparation. It is also used for the treatment of Alopecia areata as a folk medicine.[34]

Sometimes the juice is applied at genitals as punishment for infidelity, especially in the rural areas. To support the false charge of assault, it is sometimes applied to arms, thighs, breasts etc. which produces lesions simulating bruises. Sometimes the juice is used for Vitriolage i.e. it may be thrown on others body, in fit of anger or jealousy, to cause a disfigurement or injury. Malingerers may also use the juice to produce local injuries like conjunctivitis or bruises to leave a job in army, navy, air force etc. or to avoid work in the prison.[35]

Dhobis (Washermen) often mix the oil of *Bhallataka* with alum or lime-water to prepare the "Marking ink" in order to put some indelible identification marks on the clothes, hence the name "Marking nut". This marking ink is sometimes used for the purpose of tattooing as well. However, the paste of marking nut is more commonly used for tattoo removal. Though it's a very painful option, but very much cheaper than other expensive alternatives for removal of tattoo.

In central India, predominantly in the tribal population where witchcraft is a common practice, the marking nuts are worn around the neck by children as a protective measure against evil's eye (*Buri Nazar*). In addition to this, burning the *Bhallataka Beeja* for protection of children from evil's eye is a very common practice. The seeds are firstly waived in front of the children and then they are burnt to produce a smoke which can cause contact dermatitis in susceptible individuals.[36] In Andhra Pradesh, the marking nuts are also tied to the vehicles and entrance doors, on the occasion of *Vijaya Dashami (Dasara)* festival, for protection from the evil.

In rural areas, the leafy branches of *Bhallataka* tree are burnt in the brick kilns as a fuel. As a result, the workers usually suffer from contact dermatitis due to smoke. Sometimes, even a direct contact with plant parts of *Bhallataka* or its smoke isn't required to precipitate the local symptoms. The people, who are allergic to *Bhallataka*, may get suffered by just passing through the shadow of its tree, as the allergen may be carried through the wind.

There are some industries which convert the Bhilawa Shell Liquid (BSL) into semisolid or solid resin. The Bhilawa Shell Liquid loses its irritant and vesicant action on conversion into the resin and then it's used as a resinous base in various industries for manufacturing varnishes, paints, enamels, water proofing materials as well as electrical insulating materials.

In this way, the use of *Bhallataka* can be observed in different parts of India, in the form of diverse practices in different social groups and therefore, the contact dermatitis caused by *Bhallataka* is seen as a very common problem in Indian society.

LOCAL SIDE-EFFECTS

भल्लातस्य तु रसः स्वल्पोऽपि पतितः त्वचि ।
करोति दारूणं दाहं व्रणं चैव अतिदारूणम् ॥
शोथं सञ्जनयति आशु तीव्रदाहसमन्वितम् ।
मुखे निपतितो घोरं वीसर्पं प्रकरोति वै ॥

रसतरंगिणी चतुर्विंशः तरङ्ग

According to *Rasa Tarangini*, even a very little quantity of juice of *Bhallataka* dropped on the skin causes severe *Daaha* (Burning sensation), severe *Vrana Utpatti* (Ulceration) and quickly generates *Shotha* (Swelling). If it is dropped on the mouth, then it causes severe symptoms like *Visarpa* (Erysipelas).

Even *Acharya Charaka* has described that contact with fruit or flower of *Bhallataka* causes *Aagantuja Shotha* (Exogenous swelling) in the body.[37] That's why; the synonyms of *Bhallataka* like *Shophahetu, Shophakrita, Vranakrita* have been mentioned, which mean it causes inflammation and ulceration. If juice of *Bhallataka* comes in contact with body even in traces, it produces severe *Daaha* (Burning sensation), *Vrana* (Ulcer) and *Sphota* (Blisters). When it comes in contact with face, it produces *Daaha* with *Shotha* (Edema) and *Visarpa* (Erysipelas).[38]

According to Modern Toxicology, *Bhallataka* is an irritant poison. When its juice is applied to the skin, it causes irritation, erythema, edema, burning sensation and painful blisters containing acrid serum. This serum causes an eczematous eruption on any part of the body, which comes into contact with. The lesion is painful and resembles a bruise with marginal papules; followed by vesicles (Small blisters) and bullae (Large blisters). This lesion shows marked pruritus and may later ulcerate after sloughing. Normally, the blisters are filled with clear fluid; however, sometimes the vesicles filled with whitish fluid may indicate a secondary infection by staphylococci or streptococci, resulting due to excessive scratching.[39]

The rashes commonly occur at the site of contact or sometimes at distant sites like face and genitals as well, due to transmission of toxin through hand contact. The lesions usually begin within a few hours or at the most within 3 days of application. It may take about 1–2 weeks for healing and normally it doesn't leave any scar. However, without treatment, it may last up to 3 weeks. A post-inflammatory pigmentation may also appear in dark-skinned persons, which remains permanent throughout the life.[40]

Although these are the most common patterns of clinical reaction, sometimes the clinical presentation may be variable and misleading to reach final diagnosis. The lesions may rarely manifest with Erythema multiforme, Morbilliform rash, Scarlatina, Urticaria or Cellulitis.

Notably, the local manifestations of *Bhallataka* are more common than the systemic manifestations, as the juice is much less irritant when administered internally.[41] However, it is important to note that even nephrotoxicity may occur in rare cases, as a result of systemic absorption of toxin even through the local application.[42]

CASE_REPORTS OF LOCAL SIDE-EFFECTS

It has been observed in a number of cases that, the use of marking nut causes dermal toxicity and can gravely affect the sensitive individuals.[43] Therefore, some reported cases of local toxicity of *Bhallataka* can be noted, which reveal the various patterns of clinical presentations of local toxicity of *Bhallataka*.

1) In the 1940s, the "Marking ink" prepared from the "Marking nuts" was recognized to be a cause of "*Dhobi* mark dermatitis" or "Washerman's dermatitis" in the British soldiers stationed in pre-independent India during the period of Second World War.[44] The garments of these servicemen were labeled by the *Dhobis* (Washermen) with this ink, which resulted in the occurrence of dermatitis among 15-20% of soldiers. The markings thus made, were indelible and persisted even after boiling the clothes, causing dermatitis throughout the life of that clothing.[45]

2) A 36 years male patient was admitted to the hospital with signs-symptoms of cellulitis, with excessive swelling of right lower limb, difficulty in walking and excessive itching. On examination, right lower limb had extensive erythema, pitting edema, raised local temperature and tenderness, while the left lower limb showed mild erythema only. After 48 hours of treatment on the line of cellulitis, the edema of right lower limb increased progressively and a maculo-papular blanchable rash was also developed on the trunk.

   After taking the case-history carefully, patient revealed that he had applied the marking nut for fissure feet a day before. On examination of the feet, hyperkeratosis and fissures were revealed on both soles, with blackish staining of *Bhallataka* on right sole. His total IgE level was also raised up to 1200 IU/L, in comparison to the reference range of <100 IU/L. A patch test was also conducted, showing a strong positive reaction within 12 hours. The line of treatment was now changed and it was successfully managed with a diagnosis of Allergic Contact Dermatitis. Thus, it is a very rare clinical presentation of local toxicity of *Bhallataka* representing as a cellulitis.[46]

3) A 42 years male patient presented with florid erythematous vesico-bullous lesions, with a thick dark-brown to black crust over the entire scalp region, accompanied by a severe burning sensation. The symptoms occurred after an hour of topical use of marking nut sap as quack remedy for alopecia areata. Blisters & erythema were also observed over his arms, neck, and trunk. It was diagnosed and treated as severe marking nut dermatitis.[47]

4) Certain plant species like Oak, Red maple etc. are well-known to cause Nephrotoxicity when consumed; however, there are no clear reports of any plant latex being Nephrotoxic on external contact. A reported case of Nephrotic syndrome, following local contact with *Bhilawa* latex can possibly give a clue about this unusual cause of Nephrotoxicity.

A 27 years male patient, with a history of local contact with latex of *Bhallataka*, presented with signs-symptoms of Nephrotic syndrome. Initially a usual picture of local toxicity developed in the form of generalized urticarial pruritic rash, which resolved spontaneously within 24 hours. However, from 3rd day onwards, generalized body edema was observed with a reduced urine-output.

The lab investigations confirmed a heavy proteinuria (more than 3 grams per day) resulting in hypo-albuminemia and an elevated serum Creatinine level (156.2 μml/l), which were very suggestive of the Nephrotic syndrome. The Renal histology and Light microscopy revealed an acute interstitial nephritis.

This patient was completely healthy previously and showed no history of any drugs, insect bites or infections. On systemic examination, no abnormalities were found clinically within the cardiac, respiratory and neurological systems, which could have led to a generalized edema. Therefore, this renal injury can be attributed to systemic absorption of the locally applied latex of *Bhallataka* only.

A hypothesis can be generated that, the plants having chemical constituents exhibiting some COX-inhibitory activities might be responsible for the development of Nephrotic syndrome and Interstitial nephritis, because a very similar presentation is often seen in the cases of NSAID-induced acute renal injury. Therefore, such acute kidney damage similar to NSAIDs exposure can

possibly occur due to systemic absorption of locally applied latex of *Bhallataka*, as it popularly shows the COX-1 and COX-2 inhibitory activities.[48] There has also been another similar case reported, where Renal Cortical Nephrosis was observed following the exposure to sap of *Bhallataka*.[49]

5) A case of *Bhallataka*-induced contact dermatitis was reported for the novelty of its mode of transmission. A sealed bottle of "Bhilawanol Oil" was shipped from India by Airmail, which partially opened and contaminated many other pieces of mail with the thick black oil. This affected 16 employees of government departments in Washington out of 50 in total, who unpacked and wiped off the oil, as well as carried and distributed the mails.

   The workers acquired itching and burning of hands, arms and faces, followed by eruption of erythematous patches and vesicular eruption of various sizes on exposed body parts within 24 hours. The lesions were mostly seen on the flexor surfaces of the forearms and their appearance was very similar to a typical dermatitis venenata caused by Rhus toxicodendron.[50]

6) A middle-aged couple was admitted to the hospital with a severe irritant contact dermatitis, following the application of fruit extract of marking nut for treating the fissure feet. Within an hour, the male patient witnessed severe itching in right hip with erythematous vesicles and painful rashes. Later, they both developed multiple vesicles and maculo-papular rashes over inner thighs as well as on the forearm and chest. Further, they presented with blisters, scaly itching, erythematous rashes, edema and burning sensation over both the thighs. The edema was more prominent over the face and feet.[51]

7) A 27 years male patient complained of pruritus, burning sensation, erythema and edema on the front part of left thigh, immediately after wearing a recently laundered pant. It was revealed that the inside portion of pant was marked with a black smudge by the *Dhobi* (Washerman). Within 24 hours, the symptoms spread to the opposite limb as well as to the upper limbs and trunk, even after discarding the garment.

On clinical examination, closely grouped wheals of 0.5-1 cm diameter were seen on the anterior part of left thigh, which were spread in a total area of 8 cm. A few wheals were observed scattered on the opposite thigh, upper limbs and trunk as well. Such a contact urticarial presentation induced by the skin contact of *Bhallataka* is an uncommon pattern and therefore is more likely to be missed.[52]

8) A 5 years girl was admitted for temporary loss of vision, as she couldn't open her eyes due to the marked surrounding facial edema which involved the forehead and periorbital tissues. The girl's mother had smeared the juice of *Bibba* nut to her forehead as an *Ayurvedic* cure for Vitiligo. It also resulted in non-painful skin ulceration, which ultimately left a 1 cm^2 area of scar at the site of contact.

   Interestingly, the mother had also applied the same juice to her skin to darken her own patches of Vitiligo, and was needed admission to another hospital with gross ulceration and edema of both legs.[53]

9) A 50 years female patient reported with severe burning sensation & erythematous rashes on legs, 2 days after self-application of *Bhallataka* Oil on the right ankle joint, in order to reduce her joint pain. There was diffuse erythema and edema on his right ankle, along with brownish-black spotty discoloration and vesication on the sites of contact. The subject presented the same lesions on the other leg, face and neck as well, which were caused by transfer of toxin due to hand contact.[54]

10) A 14 years old girl presented with itching and multiple blisters on feet, 6 weeks after applying the juice of *Bhallataka* by a *Baiga* (Local Traditional Healer), to ease the pain in her twisted right ankle joint. The blisters were tense, rounded or oblong, large in size, measuring about 40–60 mm x 20–40 mm in size, along with many small papulo-vesicular edematous lesions on the right ankle and over both the feet. An important thing to note that, the symptoms appeared almost after a month and half in this case.[55]

11) A 30 years female beautician suffered from severe burning sensation and itchy erythematous lesions on left forearm and behind right pinna, following application of a herbal hair dye which was accidentally contaminated with a few drops of *Bhallataka* Oil. The lesion on left forearm revealed many circular-shaped discrete erythematous patches with central necrotic blackish area or vesicles, which resembled a lesion of erythema multiforme. Also, multiple small papules were observed behind the right pinna, with diffuse edema and erythema.[56]

12) A 16 years girl, who used juice of *Bhallataka* as a hair dye for her grey hairs, presented with a slight itching and pain, but severe burning sensation on her face, neck, right forearm and right hand. There was diffuse erythema with small vesicles on the margin of hairs, which was found extended to her back, side areas of neck and forehead. The scalp exhibited some oozing and eyelids were somewhat edematous. The distal part of forearm and hand showed reddish brown eschars and slight erythema with few vesicles, caused due to splashing of juice while grinding *Bhallataka* with a mortar and pestle.[57]

13) A 42 years male patient from the tribal area of Bhilai (Chhattisgarh) presented with severe itching and marked erythema on back, originated after applying diluted *Bhallataka* juice over the knife-etched human figures on his back. This strange action was performed by a local traditional healer, who promised a cure to the patient's repeated episodes of severely itchy facial skin lesions and peri-orbital edema, caused by exposure to fumes of *Bhallataka* boiled by his two neighbors. The patient was annoyed with the act of his neighbors, which had subjected him to hospitalization on previous two occasions. Therefore, he consulted the healer, who etched the two figures of his enemies and supposedly tried to expose both of them to same allergen.[58]

14) A 10 years old girl reported with severe pruritus & erythema, oozing of secretions from the pre-existing chronic skin lesions over occipital scalp and right foot, which were further worsened by *Bhallataka* sap application by her grandmother, in order to heal the lesions and get rid of recurrent allergic reaction to the fumes. On clinical examination, lesions were circular-shaped, erythematous, edematous, eroded and crusted plaques, measuring about 30-40 mm in diameter.[59]

15) A 31 years male patient was diagnosed for “Spreading Cellulitis” of the left forearm. He presented with an erythematous, eczematous rash, itching and blistering, oozing with secretions over the inner forearm, where necrosed area was about 60 mm x 60 mm in diameter. The acute eczema was further seen extending towards the upper arm as well. He later revealed a history of application of a crushed nut extracts paste before 2 weeks obtained from a traditional healer, in order to remove a previously inscribed tattoo on the left forearm, which showcased the initials of his ex-girlfriend. Though he succeeded in removing the tattoo, he ended up with a severe reaction to application of *Bhilawa* juice.[60]

16) A 31 years female patient presented with the "Beak" sign since 2 days i.e. a diffuse eczematous reaction on both cheeks and eyelids; but the tip of nose was spared. She had developed an itchy, ill-defined, erythematous rash on her face with edematous eyelids. The cause was revealed to be the handling of marking nuts, extracting the sap by puncturing with needle, and applying it directly with her finger over her mother’s scalp, as a home remedy for treatment of patchy hair loss (alopecia areata). However, it was interesting to note that she didn’t get any reaction on her fingers or hands.[61]

17) A 59 years male priest, who consumed a homemade sweet offered by a devotee, suffered with an anaphylactic reaction, within 30 minutes of consumption. He developed peri-orbital swelling, swelling on the lips, a sensation of throat swelling and wheezing. All these symptoms resulted due to the oral ingestion of that sweet, which was found to be containing *Bhallataka*.[62]

18) While conducting the chemical examination of juice of *Bhallataka*, some quantity of it was rubbed to the dorsum of left hand of the person carrying out the analysis, which produced very severe irritation and blistering after 2 days. The blister spread along the margin and ultimately the whole dorsum of hand was blistered and swollen, with very intense itching and oozing of the serum. He further developed a severe complication in the form of suppurative lymphadenitis of the axilla and required surgery for its cure.

19) A 12 years old child died due to application of an oily substance to the paralyzed limbs by a *Hakim*. The corrosive action of the substance led to the demise of the child, which was later analyzed to be a preparation of *Bhallataka*. Therefore, it is important to note that, even death can occur due to local toxicity of *Bhallataka*, though in very rare cases.

20) Some twigs were thrown into the bed of a person with a criminal intention. The person suffered with severe vesication, when his feet touched the twigs. On chemical analysis, the juice of *Bhallataka* was detected on those twigs.

21) Also, there have been some criminal offences of vitriolage, causing grievous hurt to the victim. In one case, a woman poured *Bhallataka* juice mixed with oil on the private parts of another woman when she was asleep; with an intention to drive her husband apart who was previously involved in adultery with her.[63]

22) Five research workers and lab assistants from an *Ayurveda* Institute reported with a clinical presentation of contact dermatitis, subsequent to the exposure to fruits of *Bhallataka* during their storage, processing (*Shodhana*), cleaning the equipments, and disposing off the media used for *Shodhana*.

A 33 years male, who collected and dried the marking nuts, presented with itching and blisters on the leg. As the oil from a ruptured nut spilled on his leg accidentally, he suffered with itching & slight burning sensation in the beginning, which turned into blisters after 10 hours of contact.

A 40 years male research worker, who assisted in cleaning the equipments used for *Bhallataka Shodhana*; presented with itching, erythema and burning sensation of the skin and also suffered with severe blisters after 11 hours.

Two female research workers of 55 years and 43 years age, who disposed off the media used for *Bhallataka Shodhana,* also suffered with itching, erythema and burning sensation on both the upper extremities. However, there was no occurrence of blisters in these 2 cases.

The 33 years male, experienced only pruritus all over the body and black patches of discoloration on the face, as he was exposed to the fumes of *Bhallataka* during its *Swedana* (Boiling).[64]

TREATMENT OF LOCAL SIDE-EFFECTS

Though local toxicity of *Bhallataka* is a common occurrence in the society, there's no drug of choice to manage this condition through allopathic medicine. The treatment in allopathy is only symptomatic i.e. washing the parts with water and application of bland liniments or other ointments containing Silver Nitrate, Silver Sulfadiazine etc. at the local area.[65]

In the above described case reports, the local treatments included Calosoft lotion [Containing Calamine, Aloe vera, Liquid paraffin], Fusidic-BNF cream [Containing Fusidic acid-2% w/w, Beclomethasone-0.02% w/w], Mupirocin-2% ointment, topical steroids like Betamethasone cream and Potassium permanganate soaks, wet dressings of Boric acid or Burrow's 1:20 solution, topical Hydrocortisone acetate ointment, Fluticasone and Mupirocin ointment. As evident from the lines of treatment administered locally in these case reports, it is quite clear that there's great amount of diversity of local applications in practice.

It is a striking fact that there's no specific local treatment for the localized toxicity of *Bhallataka* other than using such symptomatic liniments. Therefore, the drugs described in *Ayurvedic* Literature for treatment of local manifestations caused by contact of *Bhallataka* (Semecarpus anacardium Linn) can be tried and tested. *Ayurveda* provides a wide range of local applications i.e. *Lepa Kalpa*na, which can be used at the household level or can be developed into a formulation. These treatments are -

i) *Tila Kalka* (Paste of Sesamum indicum) should be triturated with *Mahisha Dugdha* (Milk of buffalo) and mixed with *Navaneeta* (Butter) **or**

ii) *Yashtimadhu Kalka* (Paste of Glycerrhiza glabra) and *Tila* (Sesamum indicum) should be triturated with *Dugdha* (Milk) **or**

iii) Paste of *Shaala Patra* (Desmodiun gangeticum) to be applied locally.[66]

iv) *Meghanad Swarasa* (Juice of Amaranthus spinosa) with *Navaneeta* (Butter) **or**

v) *Devdaru* (Cedrus deodara), *Sarshapa* (Brasica campestris), *Mustaka* (Cyperus rotundus) and *Navaneeta* (Butter) mixed together to form a *Lepa* **or**

vi) *Tila* (Sesamum indicum) mixed with *Navaneeta* (Butter), *Mishri* (Sugar) and *Dugdha* (Milk) **or** *Sharkara* (Sugar) mixed with *Ghee* **or**

vii) *Nimba Patra* (Leaves of Azadirachta indica), seeds of *Tila* (Sesamum indicum) and *Tila Taila* (Sesamum oil) should be boiled together to form a concentrated solution, which can be applied locally.[67]

viii) *Tila Kalka* (Paste of Sesamum indicum) mixed with *Aja Dugdha* (Goat milk) and *Navaneeta* (Butter) **or** Singular use of *Krishna Mrittika* (Black clay) pacifies the *Shotha* (Edema) produced by *Bhallataka*.[68]

ix) For external manifestations of *Bhallataka* toxicity, Coconut oil, *Ghrita*, *Raala* (Resin) ointment or Lead lotion is applied.[69]

x) *Dhanyak Patra Kalka* (Coriander leaves pulp) **or** *Navaneeta* (Butter) mixed with *Musta* (Cyperus rotundus) are also used as antidotes.[70]

xi) Local application of paste made of leaves of *Kasamarda* (Cassia occidentalis) or leaves of *Arjuna* (Terminalia arjuna) or *Haridra* (Curcuma longa) pacifies the toxic effects of *Bhallataka*.

xii) When *Aamra Haridra* (Curcuma amada), *Sathi Chawal* (Red rice, which gets ready in 60 days) and milk triturated with stale water are locally applied with pressure, it relieves the edema caused due to the smoke of *Bhallataka*.

xiii) *Krishna Tila* (Sesamum indicum) triturated with milk or curd should be applied as *Lepa*, when there are symptoms like wounds, blisters and edema, caused due to contact with oil or smoke of *Bhallataka*.

xiv) The corrugated-meaty seeds of Walnuts, Coconut meat, *Chironji* seeds (Buchanania lanzan) and *Krishna Tila* (Sesamum indicum) should be finely powdered and the local lesion of *Bhallataka* toxicity should be coated with it. After 4-5 hours, it should be washed off using buttermilk and it should be kept uncoated for some time. After 30 minutes or 1 hour, it should again be coated with fresh preparation of the same *Lepa*. All the local toxic effects of *Bhallataka* can be pacified by using *Lepa Kalpa*na in this way.[71]

xv) *Nimba Patra Kalka* (Neem leaves paste) can externally be used due to its *Pitta Shamaka* property, as it's indicated for both of its expounding factors viz. *Vrana* (Wound) and *Visha* (Poison).[72]

xvi) All types of *Pitta Shamaka* treatments like Milk, *Ghee, Navaneeta, Shatadhauta Ghrita* etc. and all the drugs with cold potency can be used in this condition, both externally as well as internally.[73]

REVIEW OF THE EXPERIMENTAL DRUG FORMULATION

Anupan Manjari is a scripture written by *Acharya Shri Vishram* in the 18th century. The main content of this script is *Visha Chikitsa* (Treatment of Poisoning), along with the description of various *Anupana* for the use of *Dhatu-Upadhatu* for the treatment of various ailments.

In *Anupan Manjari*, only one or two drug formulations have been described for the treatment of every *Visha* (Poison). Thus, *Acharya Shri Vishram* has tried to put up his clinical experience very briefly and has used very simple language for composition of the *Sutras* of this text.

One of such simple and brief drug formulation mentioned in *Anupan Manjari* is '*Daru-Sarshapa-Mustadi Lepa*' which has been indicated for the treatment of *Bhallataka* (Semecarpus anacardium Linn.) poisoning.

दारुसर्षपमुस्ताभिः नवनीतेन लेपयेत ।

भल्लातकविकारोऽयम् सद्यो गच्छति देहिनाम् ॥

अनुपान मंजरी ३/६

The contents of '*Daru-Sarshapa-Mustadi Lepa*' are -

i. *Devdaru* (Cedrus deodara Roxb.) *Kaand* (Stem) *Churna* (Powder)
ii. *Sarshapa* (Brassica campestris Linn.) *Beeja* (Seeds) *Churna* (Powder)
iii. *Mustaka* (Cyperus rotundus Linn.) *Kanda* (Tuber) *Churna* (Powder)
iv. *Navaneeta* (Butter)

DEVDARU

- **Latin name** - Cedrus deodara Roxb.
- **Family** - Pinaceae (Sarala Kula)
- **Other names** -
  - **Sanskrit** – *Bhadradaru, Surbhuruh, Suradaru, Devakashtha, Daru, Amaradaru, Amarataru*
  - **Marathi** – *Devdar*
  - **Hindi** – *Devdar*
  - **English** – Deodar, Himalayan Cedar

- **Classical categorization** –
  According to *Charaka – Anuvasanopaga & Stanya Shodhana Gana, Katuka Skandha*
  According to *Sushruta – Vaata Sanshamana Varga*

- ***Swarupa*** - It is a large coniferous *Vruksha* (Tree) approx. 75-80 meters (Nearly 250 feet) in height and the girth about 15 meters, as the branches are well spread. The tree looks attractive and evergreen with dark green-colored leaves (*Patra*). The shape of leaves is slender like needles. They are 2.5 to 5 cm in length (up to 7 cm) and are clustered at the end of its arcuate-shaped branchlets. The wood (*Kaand*) is oily, aromatic and very strong. Even it was being used for construction of religious temples historically. The heartwood (*Kaandsaara*) is light yellowish-brown to brown in color, while the sapwood in the periphery of heartwood appears white.[74]

  As *Devdaru* is a monoecious species, it has both male and female flowers (cones) on the same tree. The male flowers (Pollen cones) are 4 to 7.5 cm in length, numerous, solitary, erect, cylindrical or elongated-oval shaped, which grow at the tip of the branchlets. The female flowers (Seed cones) are barrel-shaped or ellipsoid, borne singly or in pairs at the tip of peripheral branches of the crown. The seeds are oily, irregular, triangular-shaped and rounded. The winged seeds are 6 mm long, light-brown and the size of the wing is 2.5 cm across. The flowering and fruiting period is usually August to November. The life span of tree is 600 years.[75]

- **Distribution** - *Devdaru* is distributed in North-West Himalaya at height of 3500-12000 feet from Kashmir to eastwards in Jammu, Himachal Pradesh, Uttaranchal and Uttar Pradesh. It is also distributed in Pakistan, Afghanistan & Nepal.

- **Pharmacognosy** -
  a) Macroscopic – The wood is moderately hard and light yellowish-brown to brown in color. It splits readily in longitudinal direction; the annual rings are well-marked and the medullary rays can be seen as whitish lines. The resin canals may or may not be present. If present, they are seen arranged in long tangential rows as dark, narrow lines on the radial surface of the pieces of the wood. The odor of the wood is distinct as it is aromatic, but the taste is not distinct.
  b) Microscopic – Under the microscope, the mature wood appears almost entirely of narrow, quadrangular or rarely five or six sided tracheids. They have a very thick wall, along with pits and a narrow lumen. The xylem rays are numerous, very fine and are seen running straight throughout the region. They are uniseriate and 2 to 16 cells high as seen in the tangential section; but the vessels are absent.
  c) Powder – *Devdaru* in powder form appears oily and brownish-yellow in color. It shows tracheids and xylem ray cells entirely or in the form of fragments.[76]

- **Parts used** - *Kaandsaara* (Heartwood) and *Taila* (Oil)
- **Chemical constituent** - Sesquiterpenes (A and B – Himochalene, Himachalol etc.), P- methyl-acetophenon, Atlantone

- **Dosage** - *Kaandsaara Churna* (Heartwood powder) – 3 to 6 gram
  Kwatha (Decoction) – 50 to 100 ml
  Taila (Oil) – 20 to 40 drops

- **Therapeutic Uses** -

देवदारु लघु स्निग्धं तिक्तोष्णं कटुपाकि च ।
विबंधाध्मान शोथामतंद्राहिक्का ज्वरास्रजित् ।
प्रमेहपीनसश्लेष्मकासकण्डूसमीरनुत् ॥ (भावप्रकाश निघंटु)

According to *Bhavprakash Nighantu, Devdaru* can be therapeutically used in *Vibandha, Aadhmana, Shotha, Aama, Tandra, Hikka, Jvara, Raktavikara, Prameha, Pinasa, Kapha Vikara, Kaasa, Kandu, Vaata Vyadhi*.

It is also useful in *Krumi, Kushtha, Amavata, Sutika Roga* and very effective as *Dushta Vrana Shodhaka* according to *Kaiyadev Nighantu*.

- **Pharmacological activities with reference to side-effects of *Bhallataka* -**
    1) Anti-inflammatory (*Shothaghna*) & Analgesic (*Vedanahara*) activity - The volatile oil extracted from *Devdaru* has been found to be inhibiting the Carrageenan-induced edema significantly in the albino rats. It has showed an analgesic activity as well in the same animal model. The anti-inflammatory activity observed here can be attributed to its membrane stabilizing action.

    2) Mast cell stabilizing (Anti-allergic) activity – The volatile oil of *Devdaru* has been found to be inhibiting the degranulation of mast cells isolated from the peritoneum of rats.

    3) Wound healing (*Vrana Ropana*) activity – The application of 5% and 15% Cedar wood oil (*Devdaru Taila*) along with the Castor oil (*Eranda Taila*) on the surgical wounds produced artificially in cow calves has shown promising results by enhancing the wound healing.[77]

    4) Anti-bacterial (*Krimighna*) activity – An in-vitro study was conducted to evaluate the effect of water-soluble extract of *Devdaru* on five types of bacteria. The activity was directly observed under the electron microscope. It confirmed that the *Devdaru* extract possesses remarkable anti-bacterial activity against Staphylococcus aureus, E. coli, Proteus vulgaris, Bacillus subtilis and Bacillus cereus.[78]

SARSHAPA

- **Latin name** - Brassica campestris Linn.
- **Family** - Cruciferae (*Rajika Kula*)
- **Other names** -
  - **Sanskrit** – *Katusneha, Tantubha, Kadambak, Siddharth, Teekshnaka*
  - **Marathi** – *Shirasi, Mohari*
  - **Hindi** – *Saraso, Rai*
  - **English** – Field Mustard, Turnip Rape.

- **Classical categorization –**
  According to *Charaka – Asthapanopaga Gana, Kandughna Gana*
  According to *Sushruta – Shimbi Varga, Pipalyadi Varga, Sthavara Sneha Varga*

- ***Swarupa*** - It is an erect, stout, simple or branched, glaucous *Varshayu Kshupa* (Annual Herb) of 1-2 meter height. The surface of its stem is bristly, because of the clearly visible coarse hairs. The amplexicaul leaves are generally 30-45 cm in length, alternate and stalked. There are coarse hairs on the blade of leaves as well. However, the leaves in the upper part are 12.5 cm long and their distal end has a sharp tip.

  The flowers are yellow in color, about 7-10 mm in length and 1.5 cm in diameter. They have four petals and equal number of sepals. *Sarshapa* is a hermaphrodite plant i.e. it has both male and female organs. The stamens are six in number, out of which 4 stamens are long while the other 2 are short. The gynoecium is fused and appears as a single carpel. Therefore, it is self fertile. However, the pollination occurs with the help of bees. The plant is in flower from May to August. The fruits are small in size, and in the form of Siliqua (Bean pods), which are approximately 5 cm in length and 1 cm in breadth, 3 veined and densely covered by stiff hairs. They contain multiple tiny seeds, hard, round-shaped, about 1 to 1.5 mm in diameter, and the color appears as beige or yellow or light brown or even red.[79]

- **Distribution** - *Sarshapa* is extensively distributed all over India and exclusively cultivated as an oil-yielding crop. It seems to have introduced first in Punjab from the north-west, from where it propagated eastwards to Bihar, West Bengal & Uttar Pradesh. Sometimes, it is also found grown in the river banks, arable and waste lands.

- **Pharmacognosy** –
  a) Macroscopic – The seeds of *Sarshapa* are small in size, slightly oblong in their shape, pale or reddish-brown in color. They look bright and smooth in their appearance. The diameter of each seed is 1.2 to 1.5 mm. If seen under a magnifying glass, it appears to be minutely reticulated. The taste of *Sarshapa* seeds is bitter and sharp.
  b) Microscopic – The *Sarshapa* seed shows single-layered colorless testa. It is followed by 3 to 5 layered, non-lignified, hexagonal, thick-walled cells, which are filled up with yellowish-brown contents. The embryo and endosperm consists of hexagonal, thin-walled parenchymatous cells, which contain the oil globules.
  c) Powder – The powdered *Sarshapa* appears yellow-colored with brown particles and oily. The powder is slightly bitter and sharp in taste. It frequently shows thick-walled, fragments of reddish-brown cells of hypodermis and yellowish hyaline masses.

- **Parts used** - *Beeja* (Dried seeds), *Taila*.
- **Chemical constituent** - Sinalbin, Sinapin, Sulfocyanide, Lecithine, Sinigrin. Fixed oil content is at least 23-25 % in the seeds.

- **Dosage** - *Churna* (Powder) – 1 to 3 grams
  Kalka (Paste) – 0.5 to 1 gram

- **Therapeutic Uses –**

सर्षपस्तु रसे पाके कटुः स्निग्धः सतिक्तकः ।
तीक्ष्णोष्णः कफवातघ्नो रक्तपित्ताभिवर्धनः ।
रक्षोहरो जयोकण्डूकुष्ठकोष्ठकृमिग्रहान् ॥ (भावप्रकाश निघंटु)

According to *Bhavprakash Nighantu*, *Sarshapa* can be therapeutically used in the disease conditions like *Kandu, Kushtha, Koshtha Krimi* and *Graha Badha.*

It is *Jantughna* and therefore it proves beneficial for the skin (*Tvachya*). It also acts locally as *Vedana Sthaapana, Lekhana* and *Varnya*.

- **Pharmacological activities with reference to side-effects of *Bhallataka* -**

1) Anti-inflammatory (*Shothaghna*) & Nociceptive (*Vedanahara*) activity - *Sarshapa* has shown in-vitro as well as in-vivo anti-inflammatory activity, which can be attributed to the presence of Flavonoids as chemical constituent in *Sarshapa*. The anti-inflammatory effect of flavonoids on blood vessels, inflammatory cells and inflammatory mediators is well recognized universally.

   Sarshapa also shows a topical nociceptive effect and therefore can be used as a rubefacient. Various analgesia experiments carried out in the animal models have revealed the mechanism of action of different nociceptive chemicals including *Sarshapa Taila* (Mustard oil) to be based upon the transient receptor potential ankyrin-1 and related ion flow within specific neurons.[80]

2) Wound healing (*Vrana Ropana*) activity – Sinigrin, a phyto-chemical constituent of *Sarshapa*, has shown the potential to cure the wounds (*Vrana*). The wound healing (*Vrana Ropana*) activity has been tested by in-vitro assay in the normal human keratinocytes.

3) Anti-bacterial (*Jantughna*) activity - Allyl iso-thyocynate, another phyto-chemical constituent of *Sarshapa*, carries the anti-microbial and anti-fungal activity. The *Sarshapa Churna* (Powder) and *Sarshapa Taila* (Oil) have been proven to demonstrate their inhibitory effects on Escherichia coli and Salmonella.[81]

4) Even in *Ayurveda*, it has been advised for its *Vishaghna* and *Vedanahara Karma.* According to *Yoga Ratnakara (Keeta Jalaukadi Visha Chikitsa Adhyaya)*, burnt oil of seeds (*Beeja*) of *Sarshapa* should be applied externally on the bite of Centipede (*Shatapadi*), in order to alleviate severe pain caused by its venom.[82]

MUSTAKA

- **Latin name** - Cyperus rotundus Linn.
- **Family** - Cyperaceae (*Mustaka Kula*)
- **Other names** -
    - **Sanskrit** – *Varid, Jalad, Neerad, Ambud, Ghan, Shishira, Kachchharuha, Krodakaseruka*
    - **Marathi** – *Moth, Nagarmoth*
    - **Hindi** – *Motha, Nagarmotha*
    - **English** – Nut grass

- **Classical categorization –**

  According to *Charaka* – *Truptighna Gana, Trushna Nigrahaniya Gana, Lekhaniya Gana, Kandughna Gana, Stanya Shodhana Gana*

  According to *Sushruta* – *Mustadi Varga, Vachadi Varga*

- ***Swarupa*** - It is a *Bahuvarshayu Kshupa* (Perennial Herb) in the form of slender, erect sedge of 0.33 to 1 meter in height. It has underground rhizomes, which are fleshy and white in color initially, but they become woody and brown-colored later. When the rhizomes reach the surface of land, they become rounded in shape and are known as Basal Bulb. This Basal Bulb then gives rise to roots as well as other rhizomes. The rhizome also gives birth to the Tubers, which are food reserves in the form of starch and have the capacity to generate new rhizomes or new plants. Tubers are 1-3 cm long and their shape is round, hence the name "rotundus".

  The stem of *Mustaka* sedge is 30-40 cm long, smooth and erect. Leaves are long but narrow, 20-30 cm long and 0.2-1 cm in breadth, dark-green colored, smooth and shiny. Their upper surface is grooved and the tip is sharp, and they always arise in a group of 3 leaves. The flowers grow in clusters at the end of stem. There are 3 to 9 stalks of different lengths, having reddish-brown or purple spikelets at their ends, which are 3.5 cm long and bear 10-40 flowers without petals. The fruits are 2 mm long, brown or black, dry, having single seed and network of grey lines.[83]

- **Distribution** - *Mustaka* is a weed, which occurs in tropical, subtropical and temperate regions. It is found throughout the country (India) commonly in the marshy areas, waste lands, roadsides and gardens up to an elevation of about 1800-2000 meters. It is specifically seen in the South India, West Bengal, Rajasthan and Chhota Nagpur region of Bihar.

- **Pharmacognosy** –

  a) Macroscopic – *Mustaka* comprises of rhizomes and stolons, which have many wire-like roots. The stolons are 10 to 20 cm in length and bear many rhizomes crowded together on their surface. The rhizomes are roughly conical in shape, but their size and thickness varies a lot. They are capped up with remnants of stem and the leaves, which form a scaly covering on the top of the rhizomes. Their color is dark-brown or black on the external side, while they appear creamish-yellow internally. The odor smells pleasant.

  b) Microscopic – The rhizome shows a single-layered epidermis, which is followed by two to six layers of suberized sclerenchymatous cells. A dark-brown content is filled within the epidermis and the outer sclerenchymatous layers. The thin-walled parenchymatous cells, which are circular to oval, are seen in the ground tissue of cortex, along with the small intercellular spaces. This region comprises of few fibro-vascular bundles as well. The endoderm is seen distinct and it surrounds the stele. There is a wide central zone under the endodermis, which is composed of thin-walled parenchymatous cells, which are circular to oval, along with the intercellular spaces. This region also comprises of richly scattered collateral and closed vascular bundles, which are surrounded by the bundle sheath. The vessels are narrow, with simple reticulate and scalariform thickening and oblique pores. The starch grains appear simple, round or oval in shape and they measure 6-28 microns in diameter. Many pigmented cells, which are filled up with reddish-brown content, can be seen throughout the cortex and the stele.

c) Powder – The powdered *Mustaka* is creamish-brown in color. Under the microscope, it shows reddish-brown cells as well as reticulate and simple pitted vessels. The closely packed sclerified cells can also be seen, which are fibre-like in their appearance. The vessels are narrow, with scalariform thickness and oblique pores due to the remnants of leaves. The starch grains appear simple, round or oval in shape and measure 6-28 microns in diameter.[84]

- **Parts used** - *Kanda* (Dried rhizome)
- **Chemical constituent** - Volatile aromatic essential oils, flavonoids, terpenoids, monoterpenes and sesquiterpenes.

- **Dosage -** *Churna* (Powder) – 3 to 6 grams
  Kwath (Decoction) – 20 to 30 ml

- **Therapeutic Uses –**

मुस्तं हिमं कटु ग्राही तिक्तं दीपनपाचनम् ।
कषायं कफपित्तास्रतृड्ज्वरारुचिजन्तुजित् ॥

(भावप्रकाश निघंटु)

According to *Bhavprakash Nighantu*, *Mustaka* can be therapeutically used for *Kapha-Pitta* and *Rakta Vikara, Trushna, Jvara, Aruchi* and it also demonstrates *Jantughna Karma.*

It is also *Tvagdoshahara* and *Shothahara*. Therefore, it's beneficial in *Kandu* and various types of *Tvak Roga.*

- **Pharmacological activities with reference to side-effects of *Bhallataka* -**

1) Anti-inflammatory (*Shothaghna*) activity – The essential oils in *Mustaka Kanda* have shown the anti-inflammatory activity in the Swiss albino rats, in which edema was induced using carrageenan. The aqueous, ethanol and ether extracts of *Mustaka* showed good anti-inflammatory activity in comparison to Indomethacin.

2) Anti-bacterial (*Jantughna*) activity – The essential oils present in *Mustaka Kanda* have shown a significant anti-bacterial activity, specifically against the Gram +ve bacteria like Staphylococus aureus. However, it doesn't possess much potential against the Gram –ve bacteria.[85]

3) Anti-allergic activity – The ethanol extract of *Mustaka Kanda* and other isolated chemical constituents were found effective in the inhibition of immediate-type as well as delayed-type hypersensitivity in the in-vitro and in-vivo studies. The sesquiterpenes showed good activity, while the monoterpenes didn't show any significant effect. Even in-vivo studies proved the sesquiterpenes to be most effective in anti-allergic activity.[86]

4) Analgesic (*Vedanahara*) activity - The ethanolic extract of *Mustaka* has been proven significantly effective, when its analgesic effect was evaluated for 1.2 % acetic acid solution induced pain in albino mice. The experiment confirmed that there was a considerable reduction in the number of writhes and stretches in the experimental mice. Even when it was used in the mice in addition to other established analgesics like morphine and pethidine, it commendably potentiated their analgesic effect.

5) Wound Healing (*Vrana Ropana*) activity - The effect of alcoholic extract of *Mustaka Kanda* was studied in rats for its wound healing activity, in comparison to the standard drug Nitrofurazone ointment (0.2 % w/w). Three types of wound models were used for this purpose - excision wound, incision wound and dead-space wound model. The ointment of alcoholic extract of *Mustaka Kanda* exhibited significant difference in the wound closure time as well as the tensile strength in all these three wound models.[87]

NAVANEETA

It is a milk product, which is commonly manufactured in the Indian households and also as a Dairy product. It is a *Jangama Dravya* according to *Ayurveda*, which is derived by churning out the curd.

- **Sanskrit Synonyms** – *Mrakshana, Saraja, Haiyangveena, Navaneetaka, Ksheerittha, Manthaja, Ksheer Nirmathodbhava,*
- **Marathi Name** – *Loni*
- **Hindi Name** – *Makhkhana*
- **English Name** – Indian Butter

- **Classical categorization** –

  According to *Charaka* – *Gorasa Varga (Dugdha Varga)*

  According to *Sushruta* – *Takra Varga*

  According to *Bhavprakash Nighantu* – *Navaneeta Varga*

- **Composition of *Navaneeta* (Indian butter)**[88] **-**

  Navaneeta (Indian butter) predominantly consist the milk fat (which is approximately 80 %) and other solids except the fat (Vitamins, minerals, protein and lactose) in a little quantity. It contains moisture, salt (except in unsalted butter) and curd as well. Apart from this, phospholipids, acids, enzymes, microorganisms and air are also contents of the butter.

Table No. 4 – Composition of Indian butter *(Navaneeta)*

Constituents	Quantity (% w/w)
Fat	80-83
Moisture	15.5-16.0
Salt	0-3 (Absent in unsalted butter)
Curd	1-1.5

Nutritional composition of butter –

Butter provides a high amount of calories. It is also rich in minerals and Vitamins. The nutritional constituents of butter are –

Table No. 5 – Nutritional Composition of Indian butter *(Navaneeta)*

Constitutent	**Per 100 Gram**	
Energy	744 Kcal	
Fat	82 Gram	
	Saturates	52.1 Gram
	Monounsaturates	20.9 Gram
	Polyunsaturates	2.8 Gram
	Trans fatty acids	2.9 Gram
Protein & Carbohydrate	0.6 Gram each	
Riboflavin	0.07 mg	
Vitamin B12	0.3 µg	
Pantothenate	0.05 mg	
Biotin	0.2 µg	
Thaimin, Niacin, Vitamin B6, Vitamin C and Folate	Traces	
Retinol	958 µg	
Carotene	608 µg	
Vitamin D	0.9 µg	
Vitamin E	1.85 mg	
Sodium	606 mg (Absent in unsalted butter)	
Potassium	27 mg	
Calcium	18 mg	
Magnesium	2 mg	
Phosphorus	23 mg	
Iodine	38 µg	
Copper	0.01 mg	
Zinc	0.1 mg	
Chloride	994 mg	
Iron, Selenium & Manganese	Traces	

Fatty acid composition of butter –

As *Navaneeta* (Indian butter) is a mixture of triglycerides, the variety of fatty acids can be observed in its overall composition as mentioned below.

Table No. 6 – Fatty acid composition of Indian butter *(Navaneeta)*

Fatty Acids	Average (Gram/100 Gram)
Unsaturated	
Monounsaturated Fatty Acids	21.021
Palmitoleic Acid	0.961
Oleic Acid	19.961
Polyunsaturated Fatty Acids	3.043
Linoleic Acid	2.728
Linolenic Acid	0.315
Saturated Fatty Acids	
Butyric Acid	3.226
Caproic Acid	2.007
Caprylic Acid	1.19
Capric Acid	2.529
Lauric Acid	2.587
Myristic Acid	7.436
Palmitic Acid	21.697
Stearic Acid	9.999

- **Therapeutic Uses –**

संग्राहि दीपनं हृद्यं **नवनीतं नवोद्धृतम्** ।

ग्रहण्यर्शोविकारघ्नं अर्दितारुचिनाशनम् ॥

चरक संहिता सूत्रस्थान २७/२२९

Freshly derived *Navaneeta* is *Sangraahi* (Constipating), *Dipana* (Apetizer), *Hridya* (Good for cardiac health) and it cures the diseases like *Grahani* (Inflammatory bowel syndrome), *Arsha* (Piles), *Ardita* (Facial paralysis) and *Aruchi* (Loss of taste).

नवनीतं पुनः सद्यस्कं लघु सुकुमारं मधुरं कषायमीषदम्लं शीतलं मेध्यं दीपनं
हृद्यं संग्राहि पित्तानिलहरं वृष्यमविदाहि क्षयकासश्वासव्रणशोषार्शोऽर्दितापहं,
चिरोत्थितं गुरु कफमेदोविवर्धनं बलकरं बृहणं शोषघ्नं विशेषेण बालानां प्रशस्यते ॥
क्षीरोत्थं पुनर्नवनीतं उत्कृष्टस्नेहमाधुर्यमतिशीतं सौकुमार्यकरं
चक्षुष्यं संग्राहि रक्तपित्तनेत्ररोगहरं प्रसादनं च ॥

सुश्रुत संहिता सूत्रस्थान ४५/९२–९३

Sadyaska Navaneeta (Freshly prepared butter) is *Laghu* (Light and easy to digest), *Sukumar* (Makes the body soft and tender), *Madhur* (Sweet), *Kashay* (Astringent) and slightly *Amla* (Sour/Acidic), *Sheetal* (Cooling), *Medhya* (Improves memory and intellect), *Dipana* (Appetizer), *Hridya* (Good for cardiac health), *Sangraahi* (Constipating), *Pitta Anilahara* (Mitigates *Pitta* and *Vaata*), *Vrishya* (Aphrodisiac), *Avidahi* (Doesn't cause heart burn) and cures the diseases like *Kshaya* (Consumption), *Kaasa* (Cough), *Shwas* (Dyspnoea), *Vrana* (Wounds or ulcers), *Shosha* (Emaciation), *Arsha* (Piles) and *Ardita* (Facial paralysis).

Chirotthita Navaneeta (Butter after few days of standing) becomes *Guru* (Heavy), increases *Kapha* (A body humor) and *Meda* (Fat), *Balakara* (Bestows strength), *Brihana* (Stoutening), *Shoshaghna* (Cures phthisis) and especially beneficial for children.

Ksheerottha Navaneeta (Butter made of thickened milk) is the best of all oily substances (or milk products), *Madhur* (Sweet in taste), *Atisheeta* (Extremely cooling), *Saukumaaryakara* (Makes the body soft and tender), *Chakshushya* (Improves eye-sight), *Sangraahi* (Constipating), *Raktapittahara* (Cures hemoptysis), *Netrarogahara* (Cures eye diseases) and *Prasaadana* (Pleasant).

शीतं स्वादुकषायाम्लं **नवनीतं नवोद्धृतम्** ।
यक्ष्मार्शोऽर्दित पित्तासृग्वातजिद् ग्राहि दीपनम् ।
क्षीरोद्भवं तु संग्राहि रक्तपित्ताक्षिरोगजित् ॥

अष्टांग संग्रह सूत्रस्थान ६/६७

Navoddhruta Navaneeta (Freshly prepared butter) is *Sheeta* (Cooling), *Swaadu* (Sweet), *Kashaay* (Astringent) and *Amla* (Sour/Acidic). It cures *Yakshma* (Tuberculosis), *Arsha* (Hemorrhoids), *Ardita* (Facial paralysis) and the disorders caused by *Pitta*, *Asruk (Rakta)* and *Vaata*, as it is *Graahi* (Constipating) but *Dipana* (Appetizer).

Ksheerodbhava Navaneeta (Butter made of thickened milk) is also *Sangraahi* (Constipating) and it cures *Raktapitta* (Hemoptysis) and *Akshiroga* (Diseases of eye).

नवनीतं नवं वृष्यं शीतं वर्णबलाग्निकृत् ।
संग्राहि वातपित्तासृक क्षयार्शोऽर्दितकासजित् ।
क्षीरोद्भवं तु संग्राहि रक्तपित्ताक्षिरोगजित् ॥

अष्टांग हृदय सूत्रस्थान ५/३६–३७

Even *Ashtang Hridaya* has reiterated the properties of *Navaneeta* explained by the earlier *Acharyas*. He states that, *Nava Navaneeta* (Fresh butter) is *Vrishya* (Aphodisiac) and *Sheeta* (Cooling), increases *Varna* (Skin complextion), *Bala* (Strength) and *Agni* (Digestive fire), *Sangraahi* (Constipating). It cures the diseases caused by *Vaata, Pitta* and *Asruk (Rakta)*; and also cures *Kshaya* (Consumption), *Arsha* (Hemorrhoids), *Ardita* (Facial paralysis) and *Kaasa* (Cough).

Ksheerodbhava Navaneeta (Butter made of thickened milk) is also *Sangraahi* (Constipating) and it cures *Raktapitta* (Hemoptysis) and *Akshiroga* (Diseases of eye).

After *Samhita Grantha*, *Navaneeta* and its therapeutic uses have also been described in details in the *Nighantu Grantha* as well. Though the description is almost a repetition of all the facts which have been already stated by the *Brihattrayi* (Three great trios of *Ayurveda*); however, we find the details of *Navaneeta* in a comprehensive manner in these *Nighantu Grantha*, especially in *Kaiyadev Nighantu* and *Bhavprakash Nighantu.* The description is mainly on the lines of *Acharya Sushruta*, reiterating the therapeutic uses of *Sadyaska*, *Chirasthita* and *Dugdhottha Navaneeta* separately.

नवनीतं हितं **गव्यं** वृष्यं वर्णबलाग्निकृत् ।
संग्राहि वातपित्तासृक्क्षयार्शोऽर्दितकासहृत् ।
तद्धितं बालके वृद्धे विशेषादमृतं शिशोः ॥

दुग्धोत्थं नवनीतं तु चक्षुष्यं रक्तपित्तनुत् ।
वृष्यं बल्यमतिस्निग्धं मधुरं ग्राहि शीतलम् ॥

नवनीतं तु सद्यस्कं स्वादु ग्राहि हिमं लघु ।
मेध्यं किञ्चीत्कषायाम्लमीषत्तक्रांशसंक्रमात् ॥

सक्षारकटुकाम्लत्वात् छर्द्यर्शःकुष्ठकारकम् ।
श्लेष्मलं गुरु मेदस्यं **नवनीतं चिरन्तनम्** ॥

भावप्रकाश निघंटु नवनीतवर्ग १–५

- **Pharmacological activities with reference to side-effects of *Bhallataka* -**
  1) Anti-bacterial activity – As very less research studies have been conducted to explore the pharmacological effects of *Navaneeta* (Butter) till today, therefore, only one research study could be found about the activity of butter in relation to its dermatological action. Though the experimental drug i.e. butter was administered orally in this research experiment, still the results can be extended to assume its anti-bacterial activity in dermal lesions as well.

     The sphingolipids present in butter have shown their protective activity against some varieties of bacteria, viruses and some toxins as well. This protective activity has been found due to the competitive inhibitory mechanisms. Even addition of sphingolipids to the diet has shown to prevent the adhesion of bacteria to the colon and thus, and reducing the microbial load. It has also shown the effect of excretion of pathogenic bacteria in the feces of the infants.[89] Therefore, a similar activity of butter may be possible even for protecting the local lesions due to contact of *Bhallataka* from bacteria, viruses and the toxins.

LEPA KALPANA

Lepa is an *Ayurvedic* medicinal formulation, which is prepared in the form of a paste of the herbal drugs and is used as an external application.

- **Synonyms** – *Lepana, Lipta, Aalepa.*
- **Types** –

  <u>According to *Sushruta Samhita*</u> –

  1) ***Pralepa*** – It is a *Sheeta* (Cold) *Lepa*, which is prepared from the drugs of *Sheeta Guna* and without heating. It is applied as a thin-layer (*Tanu*) and is specifically useful in the skin disorders with prominency of *Pitta Dosha* and *Rakta Dhaatu Dushti*.

  2) ***Pradeha*** – It is usually an *Ushna* (Lukewarm) *Lepa*, which is mostly prepared from the drugs of *Ushna Guna*. It is commonly applied as a thick (*Bahala*) *Lepa* and is specifically useful in the skin disorders with prominency of *Vaata* and *Kapha Dosha*.

  3) ***Aalepa*** – It is prepared by using the drugs of both *Ushna Guna* and *Sheeta Guna.* The thickness of this type of *Lepa* is moderate and it is useful in the skin disorders with prominency of *Rakta* and *Pitta Dosha*.

  <u>According to *Sharangdhara Samhita*</u> –

  1) ***Doshaghna Lepa*** – It is prepared by mixing the herbal drugs with *Aarnal* (Fermented gruel). It's specifically indicated in *Shotha* (Edema), *Kushtha* (Skin diseaases) etc. and thickness of this *Lepa* is one-forth *Anguli* (Finger).

  2) ***Vishaghna Lepa*** – It is prepared by mixing the herbal drugs with *Ghee* and hot water. It's specifically indicated in *Jwara* (Fever), *Shotha* (Edema), *Visarpa* (Erycipelas) and *Kushtha* (Skin diseases). The thickness of this *Lepa* is one-third *Anguli* (Finger).

3) ***Varnya Lepa*** – It is prepared by mixing the herbal drugs with *Nimbu Swaras* (Lemon juice), *Dugdha* (Milk) or egg albumin. It's specifically indicated in *Vyanga* (Facial melanosis) and for improving the *Varna* (Color and complexion of skin).[90] The thickness of this *Lepa* is half *Anguli* (Finger).

- **General actions of *Lepa*** – *Vrana Shodhana, Vrana Ropana, Daaha Shamana, Shothaghna, Todahara, Vedanaapaha, Kandughna, Twak Prasaadana.*

- **General method of *Lepa* preparation** – Usually the drugs are pulverized to form a fine powder of the drugs. Then this fine powder is mixed with some liquid media like *Jala* (Water), *Swarasa*, *Kwath* (Decoction), *Gomutra* (Cow urine), *Sneha* (Oil, *Ghee*), Milk or milk products etc. and is well triturated to obtain a uniform paste. [Note- Addition of *Sneha* should be quantified according to the vitiated *Dosha*. In case of *Vaata* disorder- $1/4^{th}$ *Sneha*, *Pitta* disorder- $1/6^{th}$ *Sneha*, *Kapha* disorder- $1/8^{th}$ *Sneha*)

- **Rules of *Lepa* application** –
  1) *Lepa* should always be prepared freshly. Stale *Lepa* should never be applied.
  2) New *Lepa* should never be applied over the previous *Lepa*. It should always be applied after cleaning the previous one; otherwise it causes *Vedanaa* and *Daaha*.
  3) The thickness of *Lepa* should be like that of *Aardra Maahish Charma* (Wet skin of the buffalo).
  4) *Lepa* should be applied in *Pratiloma* direction (Against the direction of hair follicles), so that it penetrates the pores of the hairs and gets absorbed through the ducts of sweat glands and the capillaries.
  5) The dried *Lepa* should not be left as such on the skin. It must be removed at the earliest after drying, because it becomes ineffective after drying and causes irritation of the skin and the skin loses its lustre.
  6) *Lepa* should never be applied during night hours, because the internal heat cannot be released due to cold weather at the night, causing exaggeration of the disease.
  7) *Lepa* once used should not be reused, because it loses its potency and becomes ineffective.

RESEARCH METHODOLOGY

PLAN OF WORK

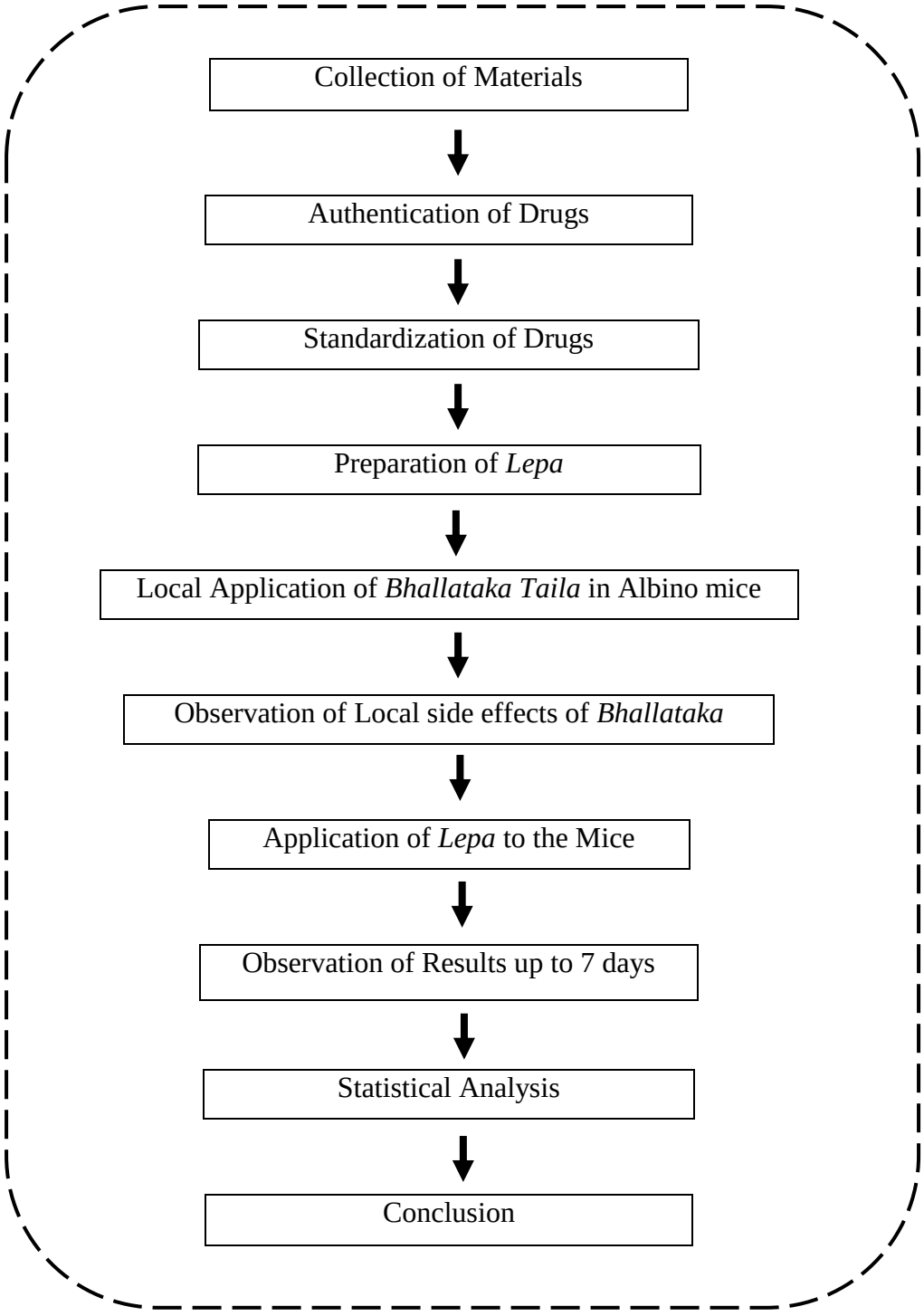


Figure 1 – Flow Chart about Plan of research work

MATERIALS

INGREDIENTS OF *'DARU-SARSHAPA-MUSTADI LEPA'*

i. *Devdaru* (Cedrus deodara Roxb.) *Kaand* (Stem) *Churna* (Powder)
ii. *Sarshapa* (Brassica campestris Linn.) *Beeja* (Seeds) *Churna* (Powder)
iii. *Mustaka* (Cyperus rotundus Linn.) *Kanda* (Tuber) *Churna* (Powder)
iv. *Navaneeta* (Butter)

Out of these four contents, two herbal drugs viz. *Devdaru* and *Mustaka* were procured in the raw-form from a well-known trader of *Ayurvedic* herbal drugs and an Authentication certificate was obtained from the trader stating that – "The supplied raw herbs are authentic and genuine products, which are purchased from an authentic supplier and are original in nature (with their established botanical names)." (Please see Annexure No. 1)

Sarshapa, being a routinely used food ingredient and therefore being readily available at the grocery shops, is not supplied by the above mentioned *Ayurvedic* herbal drugs trader. Hence, it was purchased from a local grocery shop, where it was available by the name "*Mohari*" (Mustard). It was later authenticated at the research laboratory of IDRAL (Indian Drug Research Association and Laboratory), Pune and was found to be genuine "Brassica campestris". (Please see Annexure No. 2)

Navaneeta (Butter) was purchased from a renowned milk products manufacturing company "*M/S B. G. Chitale Dairy, Sangli*" and the Standardization certificate was obtained from the Quality Control (Q.C.) laboratory of the same manufacturing unit for ensuring its quality standards. (Please see Annexure No. 3 and 4)

COLLECTION OF BHALLATAKA BEEJA/NUT

The *Bhallataka Beeja* or marking nuts were also procured in the raw-form from the same trading firm of *Ayurvedic* herbal drugs. The raw drug '*Bhallataka*' was properly identified according to its morphological characteristics and an Authentication certificate was also obtained from the trader himself stating that – "The supplied raw herb is authentic and genuine products, which is purchased from an authentic supplier and is original in nature with the botanical name as Semecarpus anacardium." (Please see Annexure No. 1)

The *Bhallataka Beeja* or marking nuts were again authenticated at the research laboratory of IDRAL (Indian Drug Research Association and Laboratory), Pune; which reconfirmed its identity after studying its macroscopic and microscopic characters. (Please see Annexure No. 5)

The samples of all the above-mentioned raw-herbal drugs as well as *Bhallataka Beeja* have been deposited to the *Agadatantra* Museum maintained by *MUP's Ayurved College, Degaon Tq. Risod Dist. Washim* and have been archived in properly preserved condition as Voucher Specimens. (Please see Annexure No. 6)

PROCUREMENT OF *SHATADHAUTA GHRITA*

Shatadhauta Ghrita, a very famous classical *Ayurvedic* formulation, was obtained from New United Pharmacy, Pune. This *Ayurvedic* manufacturing unit specializes in the production of *Shatadhauta Ghrita*, as it was the pioneering product of this pharmacy. It is a GMP (Good Manufacturing Practices) certified pharmacy by the Government of Maharashtra.

A Standardization certificate was also obtained from the Pharmaceutical Company stating that – "The supplied product is an authentic and genuine product, which is manufactured according to the standard manufacturing processes in this GMP-certified pharmacy and is original in nature." (Please see Annexure No. 7)

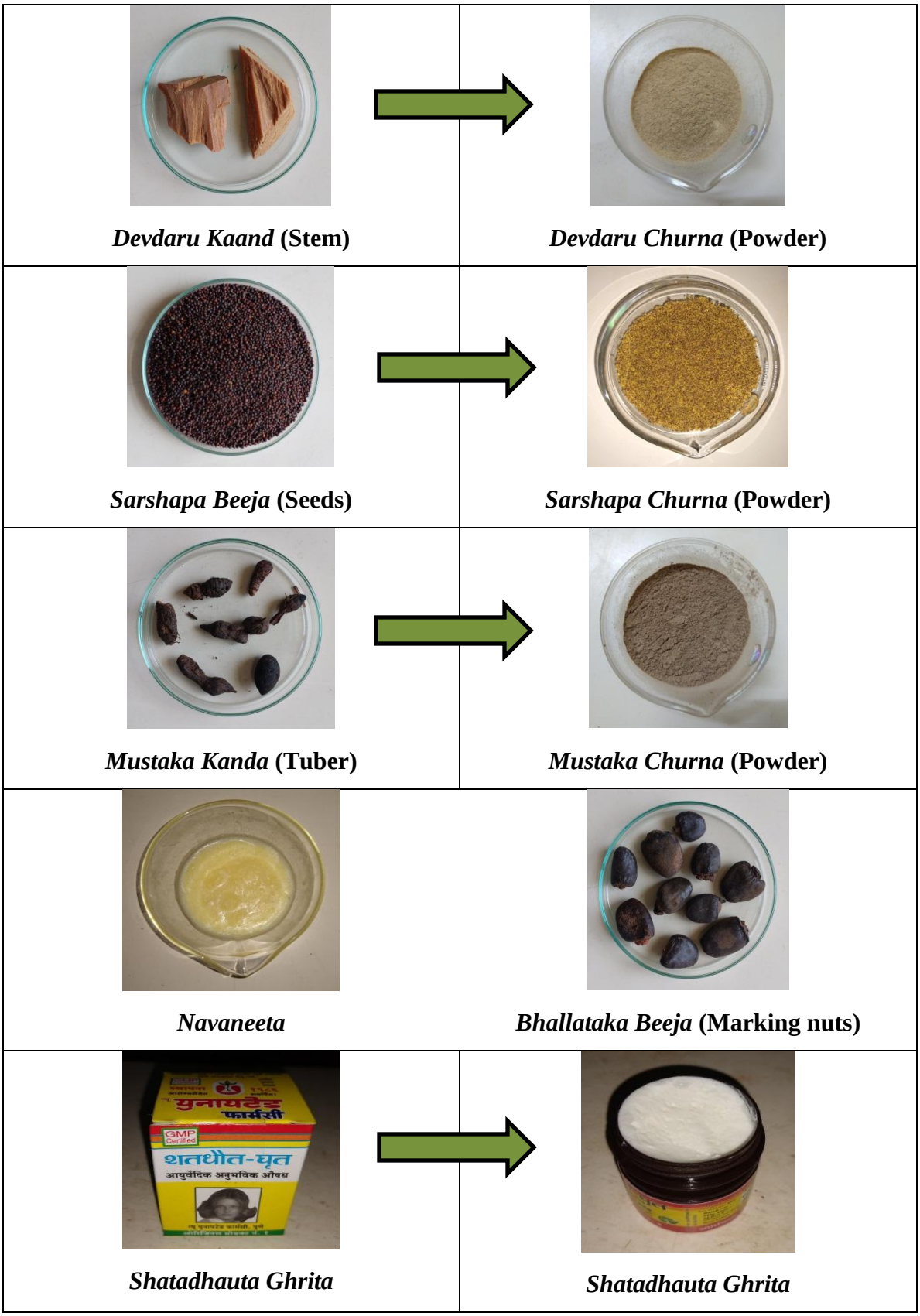


Figure 2 – Collection of all the materials for the research work

METHODS

STANDARDIZATION OF RAW DRUGS

In order to test the efficacy of drugs, it is very necessary to standardize these drugs before we use them in the experimental study. Therefore, authentication and standardization of these drugs has been done in the research laboratory of IDRAL (Indian Drug Research Association and Laboratory), Pune.

The authentication of all the herbal raw drugs i.e. *Devdaru*, *Sarshapa* and *Mustaka* was carried out by critically studying their macroscopic, microscopic and organoleptic characters; which reconfirmed the identity of these raw drugs with specific botanical name and family of each herb. (Please see Annexure No. 2, 8 and 9)

After confirming that the raw drugs are original and authentic in nature, they were first pulverized in a pulverizer and then sieved with the help of a mesh, in order to obtain a fine and homogenous powder (*Churna*). This procedure was performed separately for *Devdaru*, *Sarshapa* and *Mustaka*; and all the drugs were converted into their powdered-form.

Later, the standardization of *Devdaru* (Cedrus deodara), *Sarshapa* (Brassica campestris) and *Mustaka* (Cyperus rotundus) *Churna* (Powder) was done with the help of tests and determinations given in the *Ayurvedic* Pharmacopoeia of India (API) in order to ensure that the herbal raw drugs meet the quality standards prescribed by API. The results of tests for standardization were as follows, which confirmed the purity and strength of the herbal drugs.

1) ***Devdaru* –** The light yellowish-white powder, which was having characteristic odor and insoluble in water, was identified as *Devdaru Churna* of standard quality, purity and strength, as it complied with the standards prescribed by API. (Please see Annexure No. 10)

Table No. 7 – Standardization Criteria for *Devdaru Churna*

Sr. No.	Name of Test	Test Result	Permissible Limits given in API
1	Total Ash	0.42 % w/w	Not more than 2 %
2	Acid-insoluble Ash	0.12 % w/w	Not more than 1 %
3	Alcohol-soluble Extractive	7.55 % w/w	Not less than 7 %
4	Water-soluble Extractive	1.62 % w/w	Not less than 1.5 %

2) ***Sarshapa* –** The yellowish-brown powder, which was having characteristic odor and insoluble in water, was identified as *Sarshapa Churna* of standard quality, purity & strength, as it complied with the following standards prescribed by API. (Please see Annexure No. 11)

Table No. 8 – Standardization Criteria for *Sarshapa Churna*

Sr. No.	Name of Test	Test Result	Permissible Limits given in API
1	Total Ash	4.86 % w/w	Not more than 5 %
2	Acid-insoluble Ash	0.39 % w/w	Not more than 0.5 %
3	Alcohol-soluble Extractive	21.71 % w/w	Not less than 8 %
4	Water-soluble Extractive	18.55 % w/w	Not less than 16 %
5	Fixed Oil Content	36.70 % w/w	Not less than 35 %

3) ***Mustaka* –** The light brownish colored powder, which was having characteristic odor and insoluble in water, was identified as *Mustaka Churna* of standard quality, purity & strength, as it complied with the following standards prescribed by API. (Please see Annexure No. 12)

Table No. 9 – Standardization Criteria for *Mustaka Churna*

Sr. No.	Name of Test	Test Result	Permissible Limits given in API
1	Total Ash	6.72 % w/w	Not more than 8 %
2	Acid-insoluble Ash	3.70 % w/w	Not more than 4 %
3	Alcohol-soluble Extractive	6.22 % w/w	Not less than 5 %
4	Water-soluble Extractive	12.22 % w/w	Not less than 11 %

STANDARDIZATION OF *NAVANEETA* (BUTTER)

Navaneeta made up from *Godugdha* (Cow's milk) was taken for the purpose of present study, because *Acharya Sharangdhar* has mentioned that animate substances like milk, butter etc. should be considered from cow only if not indicated otherwise (*Sharangdhar Samhita Purva Khanda 1/51*).[91]

दुग्धे सर्पिषि मूत्रे च ग्राह्यं गोसम्भवं बुधैः ।

शारंगधर संहिता पूर्वखंड १/५१

As *Navaneeta* (Butter) was purchased from a renowned milk products manufacturing company "*M/S B. G. Chitale Dairy, Sangli*", the certificate of Standardization was also obtained from the Quality Control (Q.C.) laboratory of the same manufacturing unit, which ensured the quality standards of the *Navaneeta* (Butter) used. (Please see Annexure No. 3 and 4)

Table No. 10 - Standardization Criteria for Indian butter *(Navaneeta)*

Sr. No.	Name of Test	Test Result	Permissible Limits by FSSAI
1	Moisture %	14.84 % m/m	Not more than 16 %
2	Milk Fat %	83.55 % m/m	Not less than 80 %
3	Curd %	1.61 % m/m	Usually lesser than 1.5 %
4	RM (Reichert Meissl) Value	28.68	The lesser is better.
5	PV (Polenske) Value	1.58	The lesser is better.
6	Acidity % (As Lactic Acid)	0.023 % m/m	Not more than 0.15 %
7	FFA (Free Fatty Acid)	0.29	The lesser is better.
8	Baudouin Test	Negative	Negative = No adulteration
9	Color-added Test	Negative	Only permissible colors.
10	Total plate count	540/g	Not more than 10,000/g
11	Coliform Count	Absent	Not more than 10/g
12	Yeast and Mold Count	Absent	Not more than 20/g
13	E. Coli Count	Absent	It should be Absent/g
14	Salmonella Count	Absent	It should be Absent/25 g
15	Shigella Count	Absent	It should be Absent/25 g
16	S. Aureus Count	Absent	Not more than 10/g
17	Anaerobic Spore Count	Absent	The lesser is better.
18	Listeria monocytogens Count	Absent	It should be Absent/g

BHALLATAKA TAILA (OIL) EXTRACTION & STANDARDIZATION

After proper authentication of *Bhallataka Beeja* or marking nuts, the *Bhallataka Taila* (Oil) was extracted by heating the *Beeja* over a flame. However, the yield of oil seemed lesser by this method, when undamaged nuts were exposed to the flame directly. Therefore, a few pricks were made in the marking nuts with the help of a large-bore needle and then they were held over the flame. After using this technique, there was a remarkable improvement in the yield of the oil. Each nut yielded approximately 4-5 ml of oil. The smell of oil was characteristically unpleasant. The oil, thus extracted, was finally collected in a glass bottle with an air-tight lid.

This *Bhallataka Taila* was then standardized in the research laboratory of IDRAL (Indian Drug Research Association and Laboratory), Pune. The following parameters were recorded which ensured the standard quality of *Bhallataka Beeja* as well as the extracted *Taila* (Oil). (Please see Annexure No. 13)

Table No. 11 – Standardization Criteria for *Bhallataka Taila*

Sr. No.	Parameters	Findings
1	Color	Black
2	Appearance	Thick viscous liquid (Oil)
3	Specific Gravity (Weight per ml)	0.9993 grams/ml
4	Refractive Index	1.583
5	Viscosity	431.78 centistokes/sq.cm
6	Iodine Value	115.45
7	Saponification Value	222.77
8	Peroxide Value (Rancidity)	17.89
9	Acid Value	15.20
10	TLC (Thin Layer Chromatography)	Confirmed the chemical constituents of *Bhallataka* and established the identity.

METHOD OF PREPARATION OF '*DARU-SARSHAPA-MUSTADI LEPA*'

'*Daru-Sarshapa-Mustadi Lepa*' was prepared according to the reference given in *Anupan Manjari*. However, in this yoga, no *Pramaana* (proportion) of contents is mentioned. But the classical textbook for *Bhaishajya Kalpana* i.e. *Sharangdhar Samhita* has mentioned that, "All the contents in a formulation should be taken in same proportions, when no proportion of the contents is mentioned".

" ..... भागे अनुक्ते तु साम्यम् ।"

शारंगधर संहिता पूर्वखंड १/५०

Therefore, all the four contents of the *Lepa* viz. *Devdaru* (Cedrus deodara), *Sarshapa* (Brassica campestris), *Mustaka* (Cyperus rotundus) *Churna* (Fine powder) and *Navaneeta* (Butter) as a base were intermixed in equal proportions.

The preparation of *Lepa* was done by standard *Lepa* preparation method described in the *Ayurvedic* Pharmacopoeia of India (API). Accordingly, the mixture was triturated in a *Khalva Yantra* (Mortar and Pestle) till a homogenous, soft paste is obtained. Finally, the consistency of this prepared *Lepa* was not so thick and not so thin.

STANDARDIZATION OF '*DARU-SARSHAPA-MUSTADI LEPA*'

After fresh preparation of the *Lepa*, it was further standardized in the research laboratory of IDRAL (Indian Drug Research Association and Laboratory), Pune before its use in the experimental study. The following parameters were recorded which ensured the standard quality of this prepared *Lepa*. (Please see Annexure No. 14)

Table No. 12 – Standardization Criteria for *Daru-Sarshapa-Mustadi Lepa*

Sr. No.	Parameters	Findings
1	Color	Yellowish brown
2	Appearance	Oily *Lepa* having characteristic odor, insoluble in water.
3	pH	6.5
4	Rancidity Test	Complied (No color development).
5	Total Fatty Matter	58.35 % w/w
6	Loss on Drying	7.55 % w/w
7	Total Ash	2.31 % w/w
8	Acid Insoluble Ash	0.86 % w/w
9	Microscopy	Brownish-yellow color fragments, oil globules. Fragments of reddish-brown cells. Yellowish oil globules. Pigmented cells filled with reddish-brown content. Thin-walled pieces of parenchymatous cells.

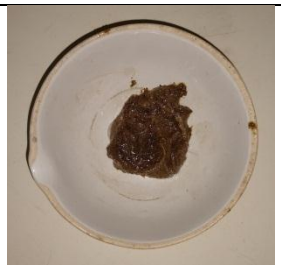
	
***Bhallataka Taila* (Oil) Extraction**	***Bhallataka Taila* (Oil)**
	
***Daru-Sarshapa-Mustadi Lepa* (Before)**	***Lepa* (After *Mardana Sanskaara*)**
	
Devdaru + Navaneeta	***Devdaru + Navaneeta Lepa***
	
Sarshapa + Navaneeta	***Sarshapa + Navaneeta Lepa***
	
Mustaka + Navaneeta	***Mustaka + Navaneeta Lepa***

Figure 3 – Extraction of *Bhallataka Taila* and Preparation of Lepa

THE EXPERIMENTAL STUDY

SELECTION OF MICE -

Swiss albino mice weighing 25-35 gram were selected, so as to get sufficient body surface for the experimental study. Animals were obtained from the animal house of IDRAL, Pune itself. No sex selection was done. The animals of either sex were randomly selected. Therefore, 9 males and 12 females got selected for the study.

Only healthy mice, having no skin infection or any other disease were carefully chosen. Mice were kept under observation before the local application of *Bhallataka Taila*, so that they remained infection free and healthy at the time of experimentation. Same diet and water were provided to all the selected mice.

Table No. 13 – Distribution of Sex and Body weight of experimental animals

Code	Sex	Weight	Code	Sex	Weight	Code	Sex	Weight
1-A	Female	32	4-A	Female	32	7-A	Female	30
1-B	Male	34	4-B	Male	30	7-B	Male	27
1-C	Female	29	4-C	Female	35	7-C	Female	28
2-A	Female	32	5-A	Male	28	**Total Mice = 21**		
2-B	Male	32	5-B	Female	31	Male - 9		
2-C	Male	31	5-C	Male	27	Female -12		
3-A	Female	28	6-A	Female	30			
3-B	Male	32	6-B	Female	25	**Average Weight =**		
3-C	Female	27	6-C	Male	27	25-35 gram		

DOSE OF *BHALLATAKA TAILA* -

The *Bhallataka Taila* was applied in such a dose, that it formed a circular patch, measuring 1 cm in diameter on the skin of mice. For this purpose, 0.1 ml of *Bhallataka Taila* was required.

DOSE CALCULATION OF *DARU-SARSHAPA-MUSTADI LEPA* [92] -

According to *Sharangdhar Samhita Uttara Khanda 11/3*, the thickness of *Vishaghna Lepa* (Anti-poisonous topical application) should be one-third of the *Anguli*.

But considering the age and weight factor of the Albino mice, *Lepa* should be applied in such a dose that it should cover the whole wound and inflammation around it. For this purpose, a *Lepa* of 1 mm thickness over the area of wound and inflammation was applied uniformly.

PLACE OF ANIMAL EXPERIMENT -

The animal study was performed in the Swiss albino mice and it was conducted at the animal house facility of IDRAL (Indian Drug Research Association and Laboratory), Pune. The animals for the study were provided by this animal house itself.

CONSENT OF INSTITUTIONAL ANIMAL ETHICS COMMITTEE -

Permission of Institutional Animal Ethics Committee (IAEC) of above-said institution was obtained prior to this animal study and a **Research Project No. 294 T-17** was allotted to the present study during this process. (Please see Annexure No. 15)

PROTOCOL FOR ANIMAL EXPERIMENT –

Table No. 14 – Selection Criteria for the experimental animals

Animal species	Albino mice
Strain	Swiss Albino
Source of Animal	IDRAL, Pune
Average Weight of Mouse	25 – 35 gram
Total Number of Mice	21 (Males-9, Females-12)
Age of Mice	6 – 8 weeks
Sex of Mice	No sex selection, random selection
Period of Acclimatization	7 days
Route of Drug Administration	Local application of *Lepa*

GROUPS OF ANIMAL EXPERIMENT –

Table No. 15 – Allocation of the experimental animals in different groups

Group	Number of Mice	Drug	Purpose
Group 1 (Normal Group)	Three	No drug application, only washed with warm water	To observe the natural changes occurring in the skin reaction and also to compare with the wound healing process of other groups.
Group 2 (Control Group)	Three	*Shatadhauta Ghrita Lepa*	To assess the healing process and to compare with the wound healing process of other groups, as it's a proven effective measure.[93]
Group 3	Three	*Daru-Sarshapa-Mustadi Lepa*	To assess the healing process.
Group 4	Three	*Devdaru + Navaneeta Lepa*	To assess the healing process.
Group 5	Three	*Sarshapa + Navaneeta Lepa*	To assess the healing process.
Group 6	Three	*Mustaka + Navaneeta Lepa*	To assess the healing process.
Group 7	Three	Only *Navaneeta Lepa*	To assess the healing process.

Total no of Animals - 21

Route of administration - External application (Dorsal surface).

METHOD OF EXPERIMENT –

1. The animals were brought to the Animal house 7 days prior to the experiment, for their physiological, psychological and nutritional stabilization following the transportation and the change in their environment.

2. After this 7 days lag period of acclimatization, all the mice were prepared before the experiment. Only desired surface area was prepared i.e. hairs from the back of each mouse were removed in the form of two patches on each side of dorsum. The hairs were shaved mechanically using Blade Razor, a day before the experiment.

3. Every mouse was kept in separate cage, so that animals would not inflict injuries to each other. For simple identification of groups, their cages were marked with the Code numbers specified for each mouse. Also, the sex of each animal was written on the cage itself, in order to identify the male and female mice separately.

4. On the next day of shaving, local application of *Bhallataka Taila* was done to each mouse one by one; at the back side, one patch on each side of the dorsum, by maintaining a safe distance between the two patches. Thus, every mouse was having two patches and all the 21 mice were having a total number of 42 patches. (The desired area of *Bhallatak Taila* application was marked earlier on each spot.)

5. After local application of *Bhallataka Taila* – Out of the total 21 mice having 42 patches, three mice having six patches were kept as it is and no treatment was provided. Then another three mice having six patches were treated with '*Shatadhauta Ghrita Lepa*' and next three mice (six patches) were treated with '*Daru-Sarshapa-Mustadi Lepa*'. In the same way, all four contents of the *Lepa* were applied separately to every group of three mice (six patches), so as to test their individual efficacy against the local side effects of *Bhallataka Taila*.

6. After local application of *Bhallataka Taila*, all mice were observed for 24 hours for the development of any local side effects like erythema, eruption of skin, blister formation, edema, irritability, mortality etc.

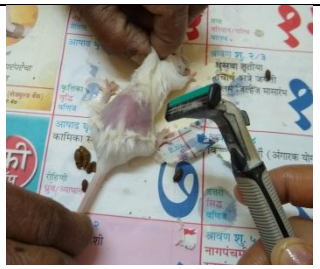
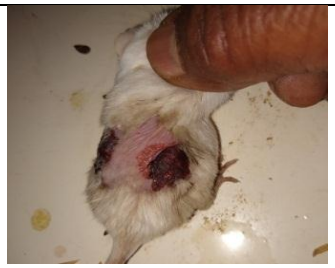
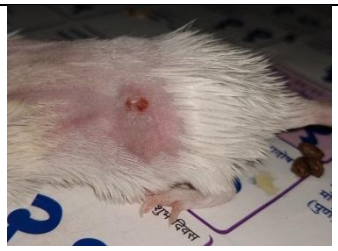
 **Selection of Mice according Body weight**	 **Separate labeled cage for each mouse**
 **Administration of Anesthesia by Ether**	 **Mechanically shaving off the hairs**
 **Application of *Bhallataka Taila* (0.1 ml)**	 **Observation of side-effects after 24 hour**
 **Application of different *Lepa* for 8 hour**	 **Assessment when wound open for 4 hour**
 **Punch Biopsy at the end of Experiment**	 **Sample for Histopathology Examination**

Figure 4 – Methodology of the Animal Experiment

SCORING SYSTEM FOR LOCAL SIDE EFFECTS OF *BHALLATAKA TAILA* -

Table No. 16 – Pre-formulated Scoring System for assessment of Erythema

Skin Reaction	Score
(A) Erythema [94]	
No reaction	0
Very slight erythema (Barely perceptible)	1
Slight erythema (Well defined, spotty or diffuse)	2
Moderate erythema (Moderately uniform redness)	3
Severe erythema (Fiery red, Beet like redness)	4

Table No. 17 – Pre-formulated Scoring System for assessment of Edema

(B) Edema [95]	
No Edema	0
Very slight Edema (Barely perceptible)	1
Slight Edema (Edges of area well defined by definite raising)	2
Moderate Edema (Area raised approximately 1 mm)	3
Severe Edema (Area raised more than 1 mm & extending beyond area of exposure)	4

Table No. 18 – Scoring System for assessment of blisters and ulceration

(C) Formation of blisters and ulceration [96]	
No elevated skin lesions	0
Formation of papules	1
Formation of vesicles (Small blisters)	2
Formation of bullae (Large blisters)	3
Formation of ulcers	4

PROCEDURE –

1. Local application of *Bhallataka Taila* was done to each mouse in 2 patches, one on each side of the vertebral column.

2. After local application, mice were observed for sensitivity reaction and duration of onset of local side effects.

3. Skin reactions and other signs were observed for 24 hours.

4. Rating of skin reaction was done as per the pre-formulated scoring system.

5. During observations, Erythema, Edema, Formation of blisters and ulceration, Diameter of wound, Mortality if any etc. were assessed.

6. '*Daru-Sarshapa-Mustadi Lepa*' was applied over the inflicted area twice daily (12 hourly). It was kept as it is for 8 hours and was washed away with plain water after 8 hours. It was kept open for the next 4 hours and assessment of the wound was done during this period. After 4 hours, the *Lepa* was applied again.

7. Changes in the local side effects of *Bhallataka Taila* were observed daily at 48 hrs, 72 hrs, 96 hrs etc. after removal of previous *Lepa* for 7 days in the following groups -

    a) Normal group
    b) Control group
    c) *Daru-Sarshapa-Mustadi Lepa* group (Topical application)
    d) Each separate content drug *Lepa* group

8. The comparative observations were tabulated. A Photographic Record was also maintained to visually compare the observations.

9. Histopathological tests were also performed to compare the efficacy of all the seven groups against the local side effects of *Bhallataka Taila*, as per the following method.

HISTOPATHOLOGICAL EXAMINATION -

Histopathology is the preparation (by preservation, thin slicing/sectioning, and staining with various dyes) and microscopic examination of samples of tissue. With this type of laboratory examination, changes occurring in the tissues can be determined very accurately. For this purpose, one needs to obtain the tissues by procedure of biopsy.

A biopsy is the surgical removal and microscopic examination of a sample of tissue. One of the most common technique of biopsy is a punch biopsy, where a small, circular amount of tissue is removed using a biopsy punch.[97]

During this experiment, punch biopsy was done under anesthesia (Ether Vapors induced), with the help of circular blade of 5 mm diameter, attached to a pencil-like handle. The skin was stretched and then blade was rotated down through epidermis and dermis into the subcutaneous fat, producing a cylindrical core of tissue.[98]

The samples for Histopathology were placed in a fixative (10% formalin solution in Normal Saline), to prevent from autolysis and decomposition. The volume ratio of tissue to formalin was kept 1:10. Then the samples were submitted to the Histopathologist in wide-mouthed, properly sealed, leak and shatter-proof containers.[99]

HISTOPATHOLOGICAL ASSESSMENT CRITERIA -

During the Histopathological examination, the changes occurring in the skin tissues at the histological level were observed. The following parameters were assessed and a semi quantitative scoring system was used for grading these criteria as per the Standard Guidelines for Histopathology [100] -

Table No. 19 – Scoring System for assessment of Scab tissue formation

(A) Scab tissue formation in Epidermal layer	**Score**
No Scab tissue formation or very focal formation (Below 5%)	0
5 to 25% Scab tissue formation	+1
25 to 50% Scab tissue formation	+2
50 to 75% Scab tissue formation	+3
More than 75% Scab tissue formation	+4

Table No. 20 – Scoring System for assessment of new Epidermal skin formation

(B) Formation of new Epidermal skin Epithelial tissue as a Healing process	**Score**
No formation or very focal formation	0
Minimal formation, covering the wound incompletely with less than 25% of tissue section	+1
Mild formation, covering the wound incompletely with less than 50% of tissue section	+2
Moderate formation, covering the wound incompletely with more than 50 % of tissue section	+3
Severe formation, covering the wound completely	+4

Table No. 21 – Scoring System for assessment of Collagen and Fibroblast tissue

(C) Healing of skin by Collagen formation and proliferation of Fibroblast tissue in Dermis layer and Subcutaneous tissue	Score
No healing or very focal healing (Below 5%)	0
5 to 25% healing with collagen deposition & fibrous tissue proliferation	+1
25 to 50% healing with collagen deposition & fibrous tissue proliferation	+2
50 to 75% healing with collagen deposition & fibrous tissue proliferation	+3
More than 75% healing with collagen deposition & fibrous tissue proliferation	+4

Table No. 22 – Scoring System for assessment of Connective & Granulation tissue

(D) Connective tissue proliferation with Granulation tissue formation	Score
No changes or very few changes (Below 5%)	0
Minimal and INCOMPLETE healing of tissue with formation of granulation tissue and connective tissue (5 to 25%)	+1
Mild and INCOMPLETE healing of tissue with formation of granulation tissue and connective tissue (25 to 50%)	+2
Moderate and INCOMPLETE healing of tissue with formation of granulation tissue and connective tissue (50 to 75%)	+3
COMPLETE healing of tissue with formation of granulation tissue and connective tissue (More than 75%)	+4

Table No. 23 – Scoring System for assessment of Acute Inflammatory Cells

(E) Infiltration of Acute Inflammatory Cells (Neutrophils and Polymorphonuclear Cells - PMN) in the skin section (Dermis and Epidermis)	**Score**
No infiltration or very focal infiltration	0
Minimal and focal infiltration of cells in the skin section : Average 1-5 cell count in various fields of tissue section per high power field	+1
Mild infiltration of cells in the skin section : Average 6-10 cell count in various fields of tissue section per high power field	+2
Moderate infiltration of cells in the skin section : Average 11-30 cell count in various fields of tissue section per high power field	+3
Diffuse infiltration of cells in the skin section : More than 30 cell count in various fields of tissue section per high power field	+4

The overall Histopathological Grades were given on the basis of all the above mentioned observations in the tissue sections and not from any one single parameter. For example, in a tissue where Healing may be seen Moderate (+3) or Severe (+4) grade with very good formation of Granulation tissue and Collagen, may also contain higher number of inflammatory cells of Mild (+2) or Moderate (+3) grade; and therefore, the overall Histopathological Grade might regress to Mild (+2) or Moderate (+3) grade, because presence of inflammatory cells indicates incomplete healing.

Thus, the histopathological criteria were assessed with the help of such semi-quantitative scoring system and later subjected to the statistical analysis.

OBSERVATIONS

ANALYSIS

INTERPRETATION

OBSERVATIONS AFTER REMOVAL OF HAIRS

After the 7 days period of acclimatization, hairs of all the mice were mechanically shaved using Blade Razor, one day prior to the experiment. However, only desired surface area was shaved i.e. hairs from the back of each mouse were removed in the form of two patches on each side of dorsum.

After removal of the hairs, the following changes were observed in the skin of the patches on the back side of the mice on each side of dorsum.

Table No. 24 – Observation of skin reactions after removal of hairs

Sr. No.	Skin Reaction	Observation	Score after Hair removal	Score after 24 hours
1	Erythema	Slight erythema (Well defined and diffuse)	2	0
2	Edema	No Edema	0	0
3	Blisters & Ulceration	No elevated skin lesions	0	0

As observed from the above table, there was appearance of slight erythema on the skin of mice after removal of the hairs; which was well-defined and diffuse. The erythema appeared as a normal response to the mechanical irritation of the skin caused due to shaving by Blade Razor. However, within the next 24 hours, the erythema eventually subsided on its own natural course.

There was no development of edema on the skin and no elevated skin lesions like papules, blisters or ulcers as well. All these skin changes were uniform in the entire population of 21 albino mice.

OBSERVATIONS AFTER *BHALLATAKA TAILA* APPLICATION

On the next day of shaving (after 24 hours), *Bhallataka Taila* was applied locally on both the patches at the back side of each mouse. The following method was employed for *Bhallataka Taila* application and making the observations, in order to evaluate and compare the local side effects of *Bhallataka Taila*.

1. A desired area of 1 cm diameter on each patch was marked with the help of a marker pen, prior to the application of *Bhallataka Taila*.

2. *Bhallataka Taila* was applied over the marked area, in a dose of 0.1 ml, by measuring with the help of a syringe.

3. The signs produced by local application of *Bhallataka Taila* in albino mice were observed and these observations were carefully noted after 24 hours.

4. The same procedure was carried out in all the animals.

5. The observations were made on the basis of direct visualization and palpation.

Table No. 25 – Observation of skin reactions after application of *Bhallataka Taila*

Group – Mouse - Patch			Erythema	Median	Edema	Median	Ulcers	Median
Group 1	**A**	**Lt.**	4	4	2	3	3	3
		Rt.	4		3		3	
	B	**Lt.**	4		2		2	
		Rt.	4		3		3	
	C	**Lt.**	4		3		2	
		Rt.	4		3		3	
Group 2	**A**	**Lt.**	4	4	3	3	2	2
		Rt.	4		3		3	
	B	**Lt.**	4		2		2	
		Rt.	4		2		2	
	C	**Lt.**	4		3		3	
		Rt.	4		3		2	

Group – Mouse - Patch			Erythema	Median	Edema	Median	Ulcers	Median
Group 3	**A**	**Lt.**	3	4	2	2.5	2	2.5
		Rt.	3		2		2	
	B	**Lt.**	4		2		2	
		Rt.	4		3		3	
	C	**Lt.**	4		3		3	
		Rt.	4		3		3	
Group 4	**A**	**Lt.**	4	4	3	2.5	3	2.5
		Rt.	4		2		2	
	B	**Lt.**	4		2		3	
		Rt.	4		2		3	
	C	**Lt.**	4		3		2	
		Rt.	4		3		2	
Group 5	**A**	**Lt.**	4	4	2	2	2	2
		Rt.	4		2		2	
	B	**Lt.**	4		2		2	
		Rt.	4		2		2	
	C	**Lt.**	4		3		3	
		Rt.	4		3		3	
Group 6	**A**	**Lt.**	4	4	2	2	3	3
		Rt.	4		2		2	
	B	**Lt.**	3		2		3	
		Rt.	4		2		3	
	C	**Lt.**	4		2		2	
		Rt.	4		2		3	
Group 7	**A**	**Lt.**	4	4	2	2.5	2	2
		Rt.	4		2		2	
	B	**Lt.**	4		3		2	
		Rt.	4		3		2	
	C	**Lt.**	4		3		3	
		Rt.	4		2		3	

Based on the observations from the above table, the following conclusions can be drawn in order to evaluate and compare the local side effects of *Bhallataka Taila*.

1) The median of all the groups of animals was 4 for the score of erythema. This confirms that almost all the mice presented with Severe Erythema, which was fiery red-colored like the Beet Root.

2) The median for the score of edema was in between 2 and 3. This confirms that all the mice exhibited with edema ranging from Slight Edema (Where the edges of area were well defined by definite raising) to Moderate Edema (Where area was raised approximately 1 mm).

3) The median score for formation of blisters and ulceration was in between 2 and 3. This confirms that the mice presented with formation of vesicles (Small blisters) or formation of bullae (Large blisters).

4) There was also an increase in the irritability of the animals, as evidenced from a rise in their scratching and writhing tendency.

5) In this way, the inflammatory property of local application of *Bhallataka Taila* was observed in the albino mice as well. Thus, the local side effects of *Bhallataka Taila* were very similar as compared to those observed in the human beings.

6) This also confirms that the *Bhallataka Taila* used for the present research work was potent and effective to cause its local side-effects.

(**Note** – For all the observed Experimental data, please see Annexure No. 16)

RATIO OF SURVIVAL OF THE EXPERIMENTAL ANIMALS

Table No. 26 – Data of survival in different groups of Albino mice

Group	Code of Mice	Days of Survival	Final Outcome
Group 1 (No Treatment)	1-A	1	Dead
	1-B	7	Dead
	1-C	3	Dead
Group 2 (*Shatadhauta Ghrita Lepa*)	2-A	7	Live
	2-B	7	Live
	2-C	7	Live
Group 3 (*Daru-Sarshapa-Mustadi Lepa*)	3-A	7	Live
	3-B	7	Live
	3-C	7	Live
Group 4 (*Devdaru + Navaneeta Lepa*)	4-A	7	Live
	4-B	7	Live
	4-C	7	Live
Group 5 (*Sarshapa + Navaneeta Lepa*)	5-A	7	Live
	5-B	7	Live
	5-C	7	Live
Group 6 (*Mustaka + Navaneeta Lepa*)	6-A	7	Live
	6-B	7	Live
	6-C	7	Live
Group 7 (Only *Navaneeta Lepa*)	7-A	7	Live
	7-B	7	Live
	7-C	5	Dead

The above "Survival Table" shows the data of survival of all the albino mice involved in the present experimental research in terms of days of survival. This data can be presented in the form of "Kaplan-Meier Curve" as given below.

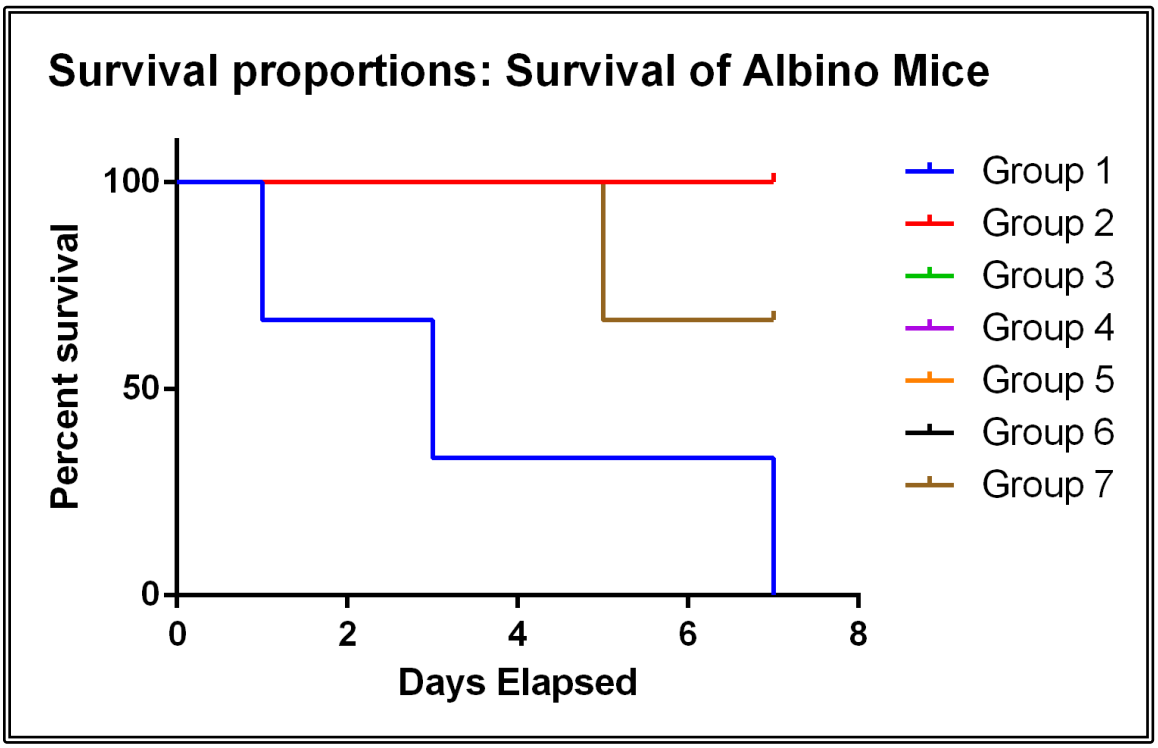


Figure 5 – Kaplan-Meier Curve showing Survival Ratio

The Kaplan-Meier Curve shows 100% mortality in Group 1 (No Treatment Group), where all the 3 Albino Mice were dead, 1st Mouse died after 1 day only, 2nd Mouse after 3 days and the 3rd one after 7 days.

Group 7 (Only *Navaneeta Lepa*) shows 66.66% survival, as only 1 Mouse out of 3 in total was found dead after 5 days.

All the other Treatment Groups (Groups 2, 3, 4, 5 and 6) showed 100% survival, as there was no mortality within these Groups.

BEFORE & AFTER TREATMENT (DAY 0-DAY 7) - WITHIN THE GROUP

ERYTHEMA

1) NORMAL GROUP (NO TREATMENT) –

Table No. 27 – The experimental data of Group 1 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1	A	Lt.	4	4	*	*	*	*	*	*
		Rt.	4	4	*	*	*	*	*	*
	B	Lt.	4	4	4	4	4	4	4	4
		Rt.	4	4	4	4	4	4	4	4
	C	Lt.	4	4	4	4	*	*	*	*
		Rt.	4	4	4	4	*	*	*	*

2) CONTROL GROUP (*SHATADHAUTA GHRITA LEPA*) –

Table No. 28 – The experimental data of Group 2 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 2	A	Lt.	4	4	3	3	3	3	3	2
		Rt.	4	4	4	4	3	3	3	2
	B	Lt.	4	4	3	3	3	3	2	2
		Rt.	4	4	3	3	3	3	3	2
	C	Lt.	4	4	3	3	3	3	3	2
		Rt.	4	4	4	3	3	3	3	2

3) EXPERIMENTAL GROUP (*DARU-SARSHAPA-MUSTADI LEPA*) –

Table No. 29 – The experimental data of Group 3 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 3	**A**	**Lt.**	3	3	2	2	2	2	1	1
		Rt.	3	3	2	2	2	2	2	1
	B	**Lt.**	4	4	3	3	3	2	2	1
		Rt.	4	4	4	4	3	3	3	1
	C	**Lt.**	4	4	3	2	2	2	2	2
		Rt.	4	4	4	2	2	2	2	2

4) EXPERIMENTAL GROUP (*DEVDARU + NAVANEETA LEPA*) –

Table No. 30 – The experimental data of Group 4 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 4	**A**	**Lt.**	4	4	4	3	2	2	2	2
		Rt.	4	4	3	3	3	2	2	2
	B	**Lt.**	4	4	3	3	2	2	2	1
		Rt.	4	3	3	3	3	3	2	1
	C	**Lt.**	4	4	3	3	2	1	1	1
		Rt.	4	3	3	3	2	1	1	0

5) EXPERIMENTAL GROUP (*SARSHAPA + NAVANEETA LEPA*) –

Table No. 31 – The experimental data of Group 5 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 5	**A**	**Lt.**	4	4	3	2	2	2	2	2
		Rt.	4	4	4	3	3	2	2	2
	B	**Lt.**	4	4	3	3	3	3	2	2
		Rt.	4	4	3	3	3	3	2	2
	C	**Lt.**	4	4	3	3	3	3	2	2
		Rt.	4	4	4	3	3	3	2	2

6) EXPERIMENTAL GROUP (*MUSTAKA + NAVANEETA LEPA*) –

Table No. 32 – The experimental data of Group 6 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 6	**A**	**Lt.**	4	4	4	3	3	3	3	2
		Rt.	4	4	4	4	3	3	3	2
	B	**Lt.**	3	3	3	3	3	3	2	2
		Rt.	4	4	4	3	3	2	2	2
	C	**Lt.**	4	4	3	3	3	3	3	2
		Rt.	4	4	4	3	3	2	2	2

7) EXPERIMENTAL GROUP (ONLY *NAVANEETA LEPA*) –

Table No. 33 – The experimental data of Group 7 - Day wise variation in Erythema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 7	**A**	**Lt.**	4	4	4	3	3	3	3	3
		Rt.	4	4	4	3	3	3	3	3
	B	**Lt.**	4	4	4	4	4	3	3	3
		Rt.	4	4	4	4	3	3	3	3
	C	**Lt.**	4	4	4	4	4	4	*	*
		Rt.	4	4	4	4	4	4	*	*

<u>MEDIAN CHANGE</u> –

Table No. 34 – Day wise variation in Erythema - The median change in all groups

Follow-up	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1	4	4	4	4	4	4	4	4
Group 2	4	4	3	3	3	3	3	2
Group 3	4	4	3	2	2	2	2	1
Group 4	4	4	3	3	2	2	2	1
Group 5	4	4	3	3	3	3	2	2
Group 6	4	4	4	3	3	3	2.5	2
Group 7	4	4	4	4	3.5	3	3	3

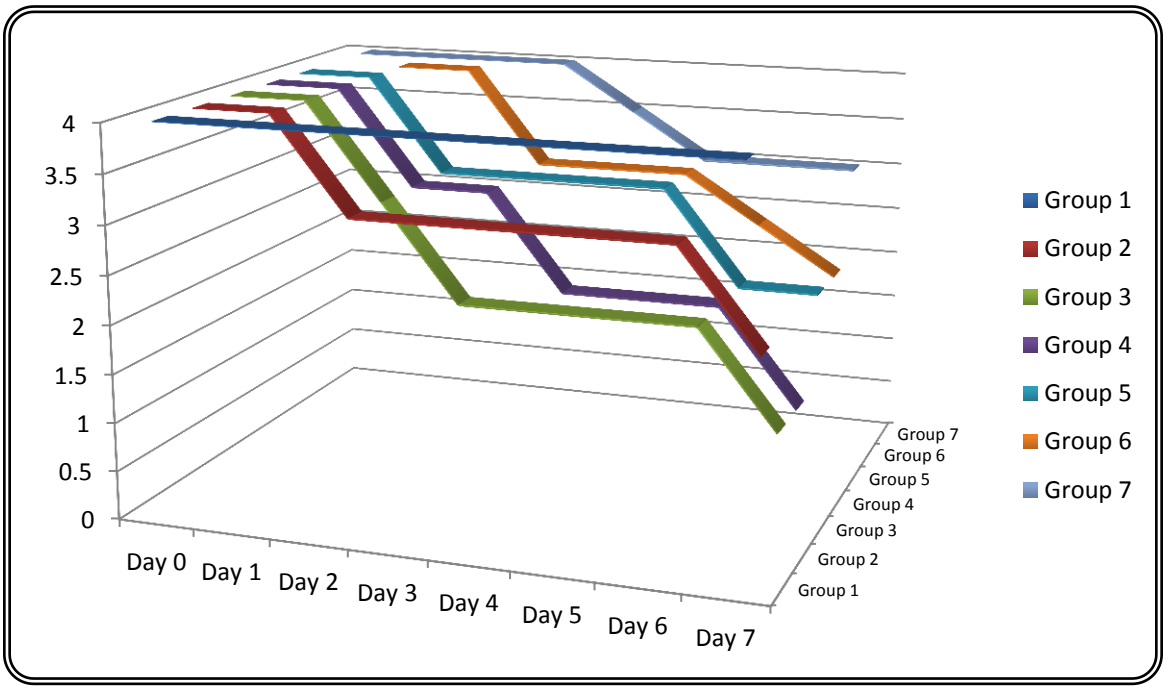


Figure 6 – Day wise variation in Erythema - Within Group Comparison

From the above Line Diagram, it can be observed that all the groups show changes in the level of Erythema except Group 1 (Normal Group- No Treatment Group). But the reduction in Erythema is more prominently seen in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and Group 4 (*Devdaru + Navaneeta Lepa*).

However, we need to assess whether this change in medians across Day 0 to Day 7 is statistically significant or it may appear by chance as well. Therefore, Wilcoxon Signed Rank Test has been used for this Ordinal Data.

Table No. 35 - Statistical Analysis for significance of Day wise variation in Erythema

Erythema	Median		Z-Value	P-Value (Asymptotic Significance – 2 Tailed)	Result
	Day 0	Day 7			
Group 1	4	4	0.000	1.000	Not Significant
Group 2	4	2	-2.449	0.014	Significant
Group 3	4	1	-2.271	0.023	Significant
Group 4	4	1	-2.232	0,026	Significant
Group 5	4	2	-2.449	0.014	Significant
Group 6	4	2	-2.333	0.020	Significant
Group 7	4	3	-2.000	0.046	Significant

As observed from the P-values of Wilcoxon Signed Rank Test for all 7 Groups, it is evident that all the other 6 groups show significant decrease in Erythema except Group 1 (Normal Group - No Treatment Group).

EDEMA

1) **NORMAL GROUP (NO TREATMENT) –**

Table No. 36 – The experimental data of Group 1 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1	**A**	**Lt.**	2	2	*	*	*	*	*	*
		Rt.	3	3	*	*	*	*	*	*
	B	**Lt.**	2	2	3	3	3	3	3	3
		Rt.	3	3	3	3	3	3	3	3
	C	**Lt.**	3	3	3	3	*	*	*	*
		Rt.	3	3	3	3	*	*	*	*

2) **CONTROL GROUP (*SHATADHAUTA GHRITA LEPA*) –**

Table No. 37 – The experimental data of Group 2 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 2	**A**	**Lt.**	3	3	2	2	2	2	2	2
		Rt.	3	2	2	2	2	2	2	2
	B	**Lt.**	2	2	2	2	2	2	2	2
		Rt.	2	3	2	2	2	2	2	2
	C	**Lt.**	3	3	2	2	2	2	1	1
		Rt.	3	3	2	2	2	2	2	2

3) EXPERIMENTAL GROUP (*DARU-SARSHAPA-MUSTADI LEPA*) –

Table No. 38 – The experimental data of Group 3 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 3	**A**	**Lt.**	2	2	2	2	2	2	1	0
		Rt.	2	3	3	2	2	2	2	0
	B	**Lt.**	2	2	2	1	1	1	1	0
		Rt.	3	2	2	2	2	2	1	0
	C	**Lt.**	3	2	2	1	1	1	1	1
		Rt.	3	2	2	1	1	1	1	1

4) EXPERIMENTAL GROUP (*DEVDARU + NAVANEETA LEPA*) –

Table No. 39 – The experimental data of Group 4 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 4	**A**	**Lt.**	3	3	3	2	2	2	1	1
		Rt.	2	3	2	2	2	2	2	1
	B	**Lt.**	2	2	2	2	1	1	1	0
		Rt.	2	2	2	2	2	2	2	0
	C	**Lt.**	3	2	2	2	2	1	1	0
		Rt.	3	2	2	2	2	1	1	0

5) EXPERIMENTAL GROUP (*SARSHAPA + NAVANEETA LEPA*) –

Table No. 40 – The experimental data of Group 5 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 5	**A**	**Lt.**	2	3	1	1	1	1	1	1
		Rt.	2	2	2	2	1	1	1	1
	B	**Lt.**	2	2	2	2	2	2	1	1
		Rt.	2	3	2	2	2	2	1	1
	C	**Lt.**	3	3	2	2	2	2	2	2
		Rt.	3	3	2	2	2	2	2	1

6) EXPERIMENTAL GROUP (*MUSTAKA + NAVANEETA LEPA*) –

Table No. 41 – The experimental data of Group 6 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 6	**A**	**Lt.**	2	2	2	2	1	1	1	1
		Rt.	2	2	2	2	1	1	1	1
	B	**Lt.**	2	2	2	2	2	2	1	0
		Rt.	2	3	2	2	2	1	1	0
	C	**Lt.**	2	2	2	2	2	2	1	0
		Rt.	2	2	2	2	2	1	1	0

7) EXPERIMENTAL GROUP (ONLY *NAVANEETA LEPA*) –

Table No. 42 – The experimental data of Group 7 - Day wise variation in Edema

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 7	**A**	**Lt.**	2	2	2	1	1	1	1	1
		Rt.	2	2	2	1	1	1	1	1
	B	**Lt.**	3	3	3	2	2	2	2	2
		Rt.	3	3	3	3	2	2	2	2
	C	**Lt.**	3	3	3	2	2	2	*	*
		Rt.	2	3	3	2	2	2	*	*

<u>MEDIAN CHANGE</u> –

Table No. 43 – Day wise variation in Edema - The median change in all groups

Follow-up	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1	3	3	3	3	3	3	3	3
Group 2	3	3	2	2	2	2	2	2
Group 3	2.5	2	2	1.5	1.5	1.5	1	0
Group 4	2.5	2	2	2	2	1.5	1	0
Group 5	2	3	2	2	2	2	1	1
Group 6	2	2	2	2	2	1	1	0
Group 7	2.5	3	3	2	2	2	1.5	1.5

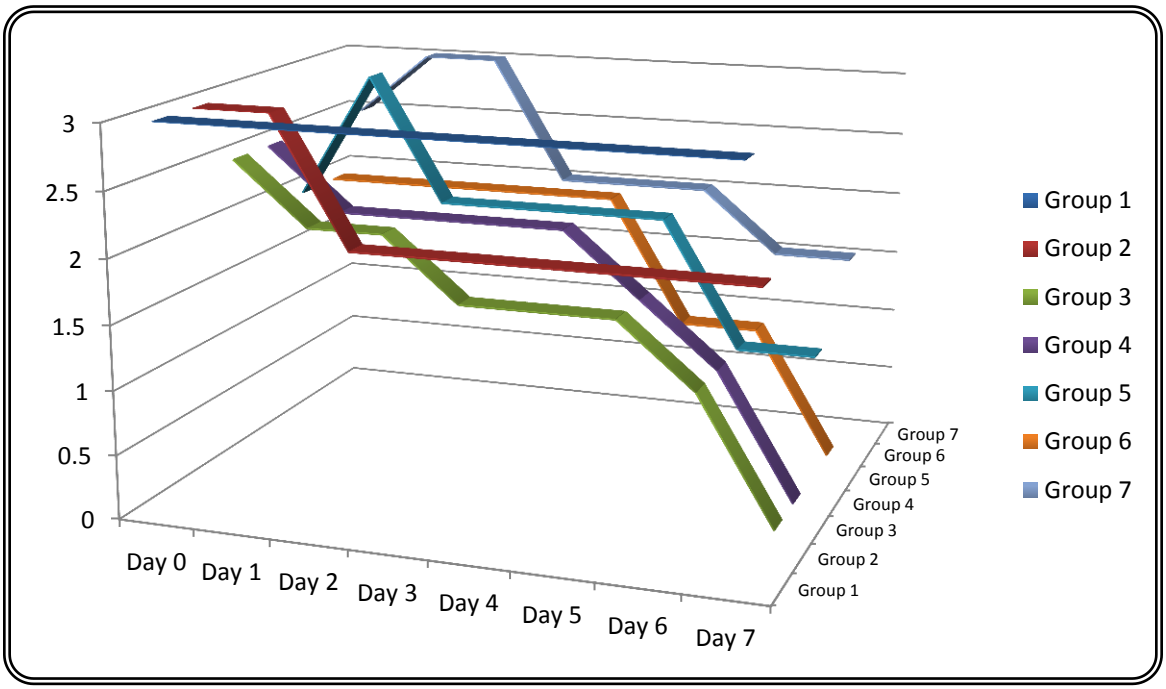


Figure 7 – Day wise variation in Edema - Within Group Comparison

From the above Line Diagram, it can be observed that all the groups show changes in the level of Edema except Group 1 (Normal Group- No Treatment Group). But the reduction in edema is most prominently seen in Group 3 (*Daru-Sarshapa-Mustadi Lepa*), Group 4 (*Devdaru + Navaneeta Lepa*) and Group 6 (*Mustaka + Navaneeta Lepa*), where the edema subsided completely.

However, we need to assess whether this change in medians across Day 0 to Day 7 is statistically significant or it may appear by chance as well. Therefore, Wilcoxon Signed Rank Test has been used for this Ordinal Data.

Table No. 44 - Statistical Analysis for significance of Day wise variation in Edema

Edema	Median		Z-Value	P-Value (Asymptotic Significance – 2 Tailed)	Result
	Day 0	Day 7			
Group 1	3	3	-1.000	0.317	Not Significant
Group 2	3	2	-1.890	0.059	Not Significant
Group 3	2.5	0	-2.333	0.020	Significant
Group 4	2.5	0	-2.232	0.026	Significant
Group 5	2	1	-2.333	0.020	Significant
Group 6	2	0	-2.271	0.023	Significant
Group 7	2.5	1.5	-2.000	0.046	Significant

As observed from the P-values of Wilcoxon Signed Rank Test for all 7 Groups, it is evident that all the other 5 groups show significant decrease in Edema except Group 1 (Normal Group - No Treatment Group) and Group 2 (Control Group - *Shatadhauta Ghrita Lepa*).

FORMATION OF BLISTERS & ULCERATION

1) NORMAL GROUP (NO TREATMENT) –

Table No. 45 – The experimental data of Group 1 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1	**A**	**Lt.**	3	3	*	*	*	*	*	*
		Rt.	3	3	*	*	*	*	*	*
	B	**Lt.**	2	3	3	3	3	3	3	3
		Rt.	3	3	3	3	3	3	3	3
	C	**Lt.**	2	3	3	3	*	*	*	*
		Rt.	3	3	3	3	*	*	*	*

2) CONTROL GROUP (*SHATADHAUTA GHRITA LEPA*) –

Table No. 46 – The experimental data of Group 2 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 2	**A**	**Lt.**	2	2	2	2	2	2	4	4
		Rt.	3	3	2	2	2	2	2	4
	B	**Lt.**	2	2	2	2	2	2	1	1
		Rt.	2	3	3	3	3	2	2	1
	C	**Lt.**	3	3	3	2	2	2	2	1
		Rt.	2	3	3	3	3	2	2	1

3) EXPERIMENTAL GROUP (*DARU-SARSHAPA-MUSTADI LEPA*) –

Table No. 47 – The experimental data of Group 3 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 3	**A**	**Lt.**	2	2	2	2	2	2	1	1
		Rt.	2	3	3	2	2	2	2	1
	B	**Lt.**	2	2	2	2	2	2	4	1
		Rt.	3	3	2	2	2	2	4	1
	C	**Lt.**	3	3	4	4	4	4	4	4
		Rt.	3	2	2	2	2	2	4	4

4) EXPERIMENTAL GROUP (*DEVDARU + NAVANEETA LEPA*) –

Table No. 48 – The experimental data of Group 4 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 4	**A**	**Lt.**	3	3	3	2	2	2	4	1
		Rt.	2	3	3	2	2	2	2	1
	B	**Lt.**	3	2	2	2	1	1	4	1
		Rt.	3	2	2	2	2	2	2	1
	C	**Lt.**	2	2	2	2	2	1	1	1
		Rt.	2	2	2	2	2	1	1	0

5) EXPERIMENTAL GROUP (*SARSHAPA + NAVANEETA LEPA*) –

Table No. 49 – The experimental data of Group 5 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 5	**A**	**Lt.**	2	2	4	4	4	4	4	4
		Rt.	2	2	2	2	4	4	4	4
	B	**Lt.**	2	2	2	2	2	2	1	1
		Rt.	2	3	2	2	2	2	1	1
	C	**Lt.**	3	3	3	3	2	2	4	4
		Rt.	3	3	2	2	2	2	4	4

6) EXPERIMENTAL GROUP (*MUSTAKA + NAVANEETA LEPA*) –

Table No. 50 – The experimental data of Group 6 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 6	**A**	**Lt.**	3	3	4	4	2	1	1	1
		Rt.	2	2	2	4	2	1	1	1
	B	**Lt.**	3	2	2	2	2	2	4	1
		Rt.	3	3	2	2	2	1	4	4
	C	**Lt.**	2	2	2	2	2	2	4	1
		Rt.	3	3	2	2	2	1	4	2

7) EXPERIMENTAL GROUP (ONLY *NAVANEETA LEPA*) –

Table No. 51 – The experimental data of Group 7 - Day wise variation in Blisters

Mouse		Patch	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 7	**A**	**Lt.**	2	2	4	2	2	4	2	4
		Rt.	2	2	4	2	2	2	2	4
	B	**Lt.**	2	3	3	3	3	2	2	2
		Rt.	2	3	3	3	3	2	2	2
	C	**Lt.**	3	3	3	3	3	3	*	*
		Rt.	3	3	3	3	3	3	*	*

<u>MEDIAN CHANGE</u> –

Table No. 52 – Day wise variation in Blisters - The median change in all groups

Follow-up	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1	3	3	3	3	3	3	3	3
Group 2	2	3	2.5	2	2	2	2	1
Group 3	2.5	2.5	2	2	2	2	4	1
Group 4	2.5	2	2	2	2	1.5	2	1
Group 5	2	2.5	2	2	2	2	4	4
Group 6	3	2.5	2	2	2	1	4	1
Group 7	2	3	3	3	3	2.5	2	3

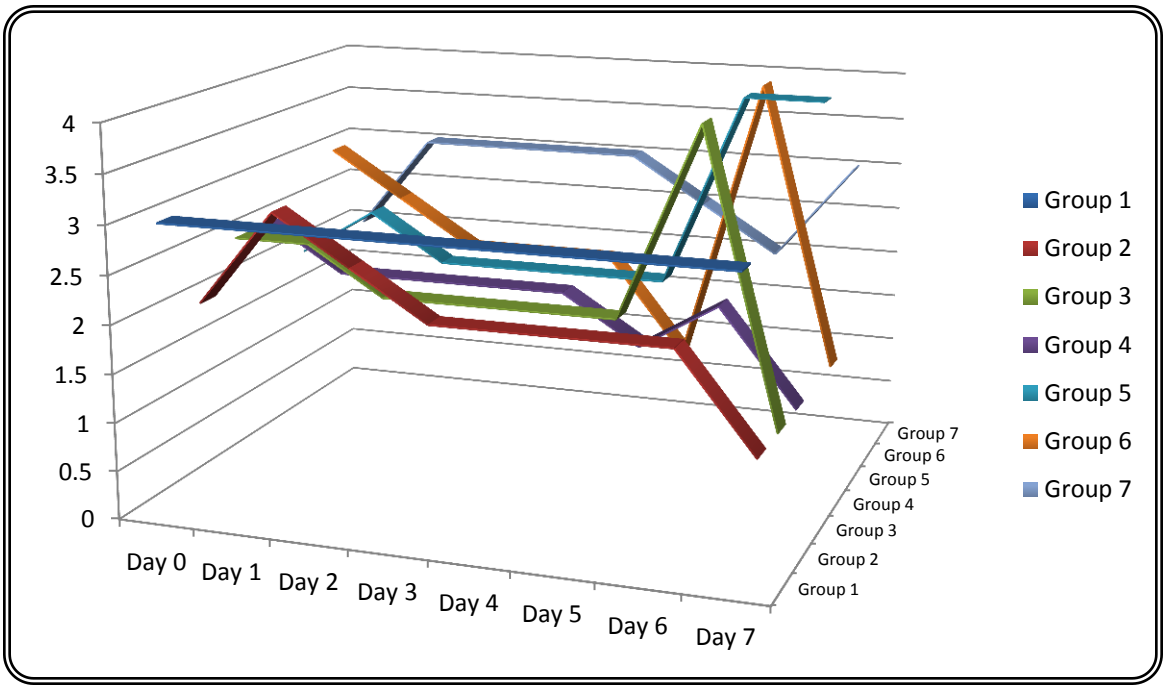


Figure 8 – Day wise variation in Blisters - Within Group Comparison

From the above Line Diagram, it can be observed that Group 2 (*Shatadhauta Ghrita Lepa*), Group 3 (*Daru-Sarshapa-Mustadi Lepa*), Group 4 (*Devdaru + Navaneeta Lepa*) and Group 6 (*Mustaka + Navaneeta Lepa*) show good decrease in the Blisters and Ulceration. On the other hand, Group 5 (*Sarshapa + Navaneeta Lepa*) and Group 7 (Only *Navaneeta Lepa*) have shown an increase in the Blisters and Ulceration.

However, we need to assess whether this change in medians across Day 0 to Day 7 is statistically significant or it may appear by chance as well. Therefore, Wilcoxon Signed Rank Test has been used for this Ordinal Data.

Table No. 53 - Statistical Analysis for significance of Day wise variation in Blisters

Formation of Blisters and Ulceration	Median		Z-Value	P-Value (Asymptotic Significance – 2 Tailed)	Result
	Day 0	Day 7			
Group 1	3	3	-1.000	0.317	Not Significant
Group 2	2	1	-0.541	0.589	Not Significant
Group 3	2.5	1	-1.000	0.317	Not Significant
Group 4	2.5	1	-2.271	0.023	Significant
Group 5	2	4	-1.190	0.234	Not Significant
Group 6	3	1	-1.730	0.084	Not Significant
Group 7	2	3	-1.414	0.157	Not Significant

As observed from the P-values of Wilcoxon Signed Rank Test for all 7 Groups, it is evident that all the other 6 groups do not show significant decrease in Blisters and healing of ulcerations except the Group 4 (*Devdaru* + *Navaneeta Lepa*).

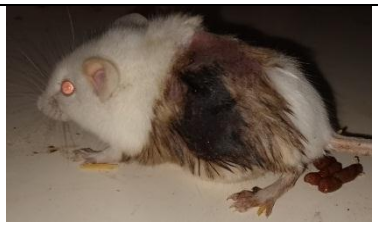
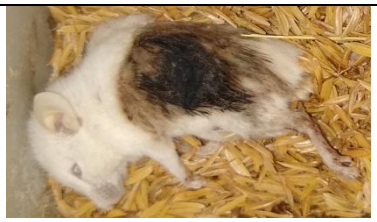
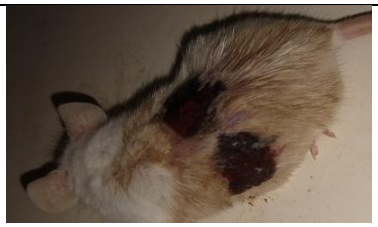
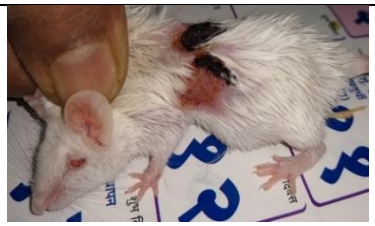
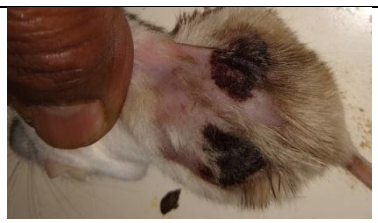
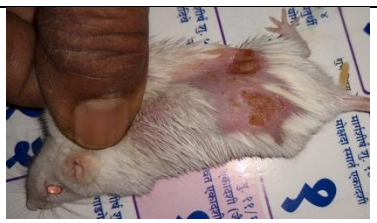
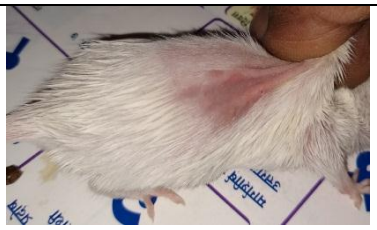
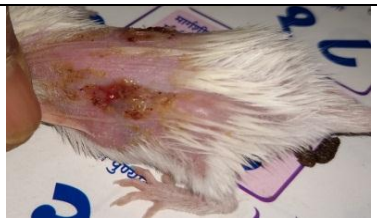
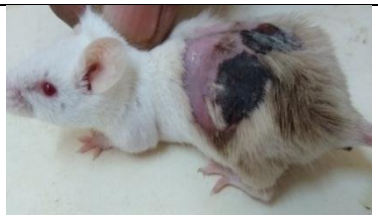
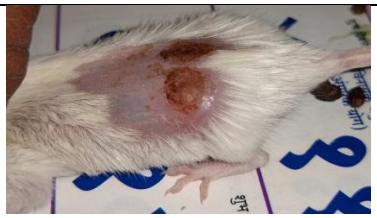
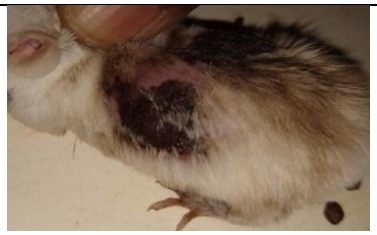
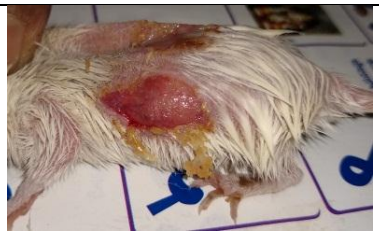
	Day 0	Day 7
Group 1		
Group 2		
Group 3		
Group 4		
Group 5		
Group 6		
Group 7		

Figure 9 – Photographic demonstration of results (Before & After Treatment)

AFTER TREATMENT (ON DAY 7) - BETWEEN THE GROUPS COMPARISON

ERYTHEMA

Table No. 54 - Experimental data of Group wise difference in Erythema on Day 7

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	1	2	2	2	3
	Rt.	*	2	1	2	2	2	3
B	**Lt.**	4	2	1	1	2	2	3
	Rt.	4	2	1	1	2	2	3
C	**Lt.**	*	2	2	1	2	2	*
	Rt.	*	2	2	0	2	2	*

Table No. 55 - Statistical Analysis of Group wise difference in Erythema

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	35.50	27.77	6	0.0001
Group 2	6	19.50			
Group 3	6	9.83			
Group 4	6	9.17			
Group 5	6	19.50			
Group 6	6	19.50			
Group 7	4	32.50			
Total	36				

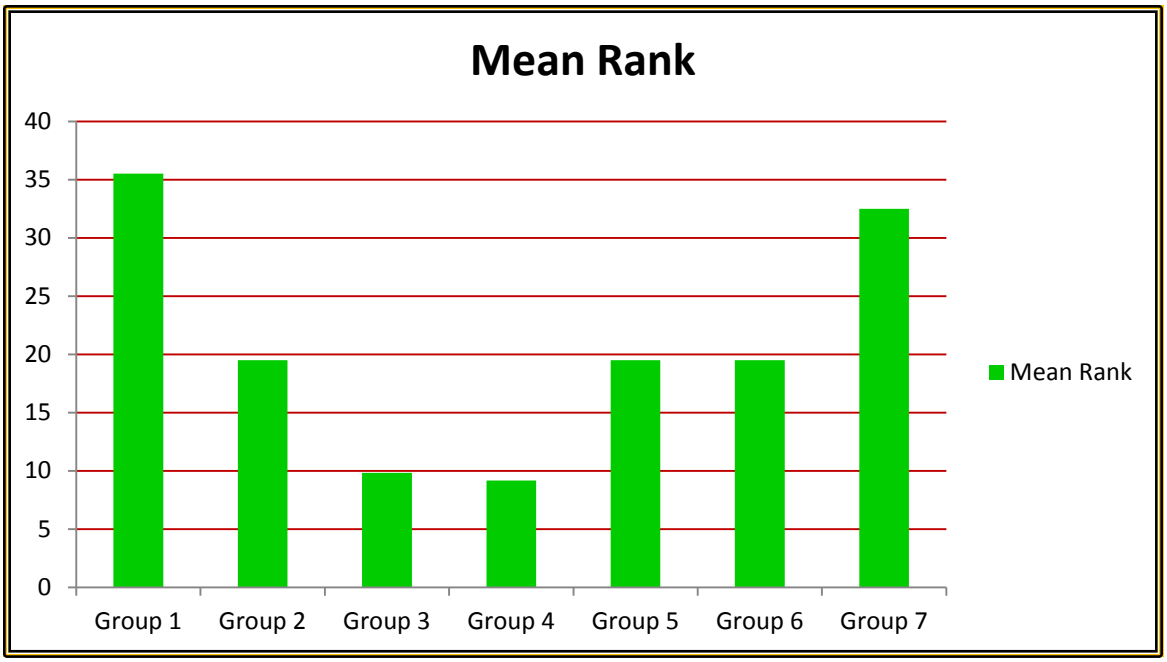


Figure 10 – Group wise difference in Erythema (After Treatment - On Day 7)

For comparison between effects of all the 7 Groups, a non-parametric test was more suitable, as the data is on ordinal scale and we cannot assume a Gaussian distribution for this data. Also, the number of groups is more than three. Therefore, Kruskal Wallis Test has been used to assess whether the difference in the results of 7 Groups are statistically significant or not.

From the above findings, we can observe that the P-Value is less than 0.05 and therefore, we can conclude that there is significant difference in all the 7 Groups for Erythema.

From the above Bar Diagram, it is evident that the effect of Group 4 (*Devdaru + Navaneeta Lepa*) is most prominent for reduction of Erythema, while the second most prominent results are found in Group 3 (*Daru-Sarshapa-Mustadi Lepa*), which is marginally lesser than the Group 4.

On the other hand, the reduction of Erythema is least seen in Group 1 (Normal Group- No Treatment Group), followed by the Group 7 (Only *Navaneeta Lepa*).

EDEMA

Table No. 56 - Experimental data of Group wise difference in Edema on Day 7

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	0	1	1	1	1
	Rt.	*	2	0	1	1	1	1
B	**Lt.**	3	2	0	0	1	0	2
	Rt.	3	2	0	0	1	0	2
C	**Lt.**	*	1	1	0	2	0	*
	Rt.	*	2	1	0	1	0	*

Table No. 57 - Statistical Analysis of Group wise difference in Edema

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	35.50	24.93	6	0.0004
Group 2	6	28.67			
Group 3	6	10.83			
Group 4	6	10.83			
Group 5	6	21.33			
Group 6	6	10.83			
Group 7	4	25.00			
Total	36				

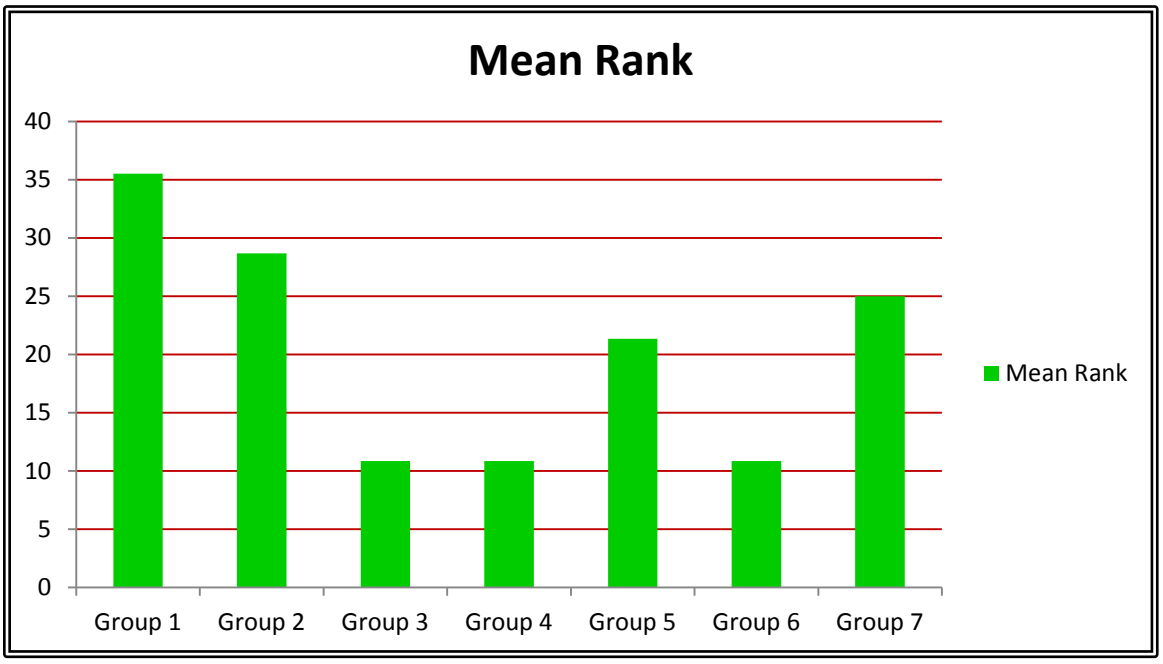


Figure 11 – Group wise difference in Edema (After Treatment - On Day 7)

From the above findings, we can observe that the P-Value is less than 0.05 and therefore, we can conclude that there is significant difference in all the 7 Groups for Edema.

From the above Bar Diagram, it is evident that the effects of three Groups viz. Group 3 (*Daru-Sarshapa-Mustadi Lepa*), Group 4 (*Devdaru + Navaneeta Lepa*) as well as Group 6 (*Mustaka + Navaneeta Lepa*) are most prominent for reduction of Edema, while the reduction in Edema is least in Group 1 (Normal Group- No Treatment Group).

FORMATION OF BLISTERS & ULCERATION

Table No. 58 - Experimental data of Group wise difference in Blisters on Day 7

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	4	1	1	4	1	4
	Rt.	*	4	1	1	4	1	4
B	**Lt.**	3	1	1	1	1	1	2
	Rt.	3	1	1	1	1	4	2
C	**Lt.**	*	1	4	1	4	1	*
	Rt.	*	1	4	0	4	2	*

Table No. 59 - Statistical Analysis of Group wise difference in Blisters

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	24.50	11.77	6	0.0672
Group 2	6	17.67			
Group 3	6	17.67			
Group 4	6	9.33			
Group 5	6	24.33			
Group 6	6	16.17			
Group 7	4	26.50			
Total	36				

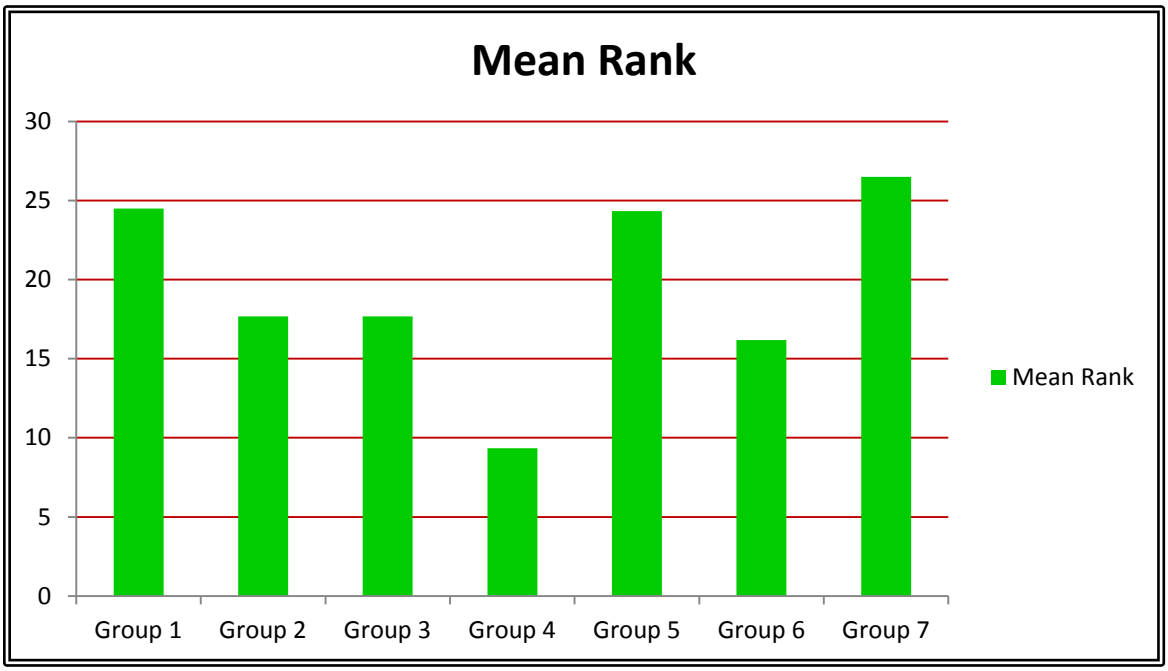


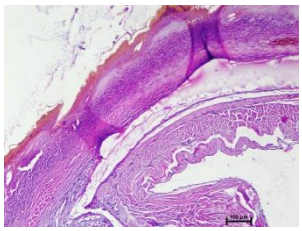
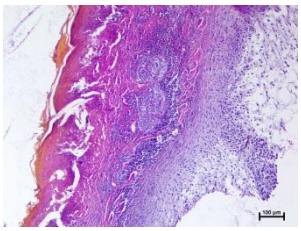
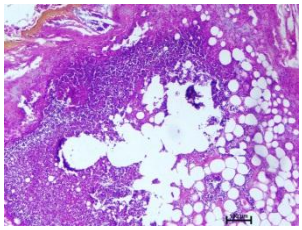
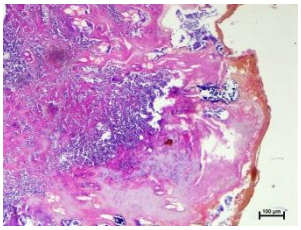
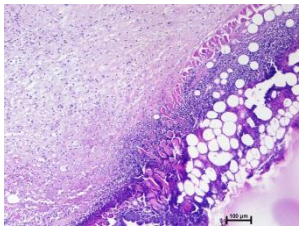
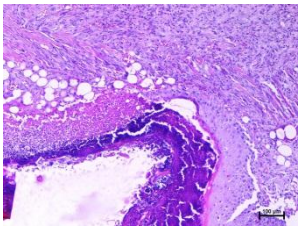
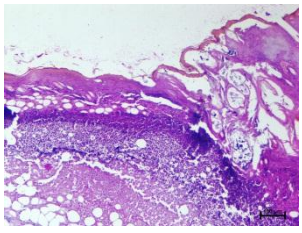
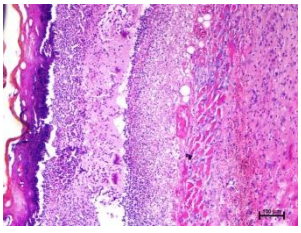
Figure 12 – Group wise difference in Blisters (After Treatment - On Day 7)

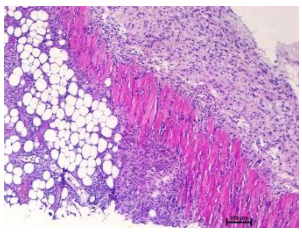
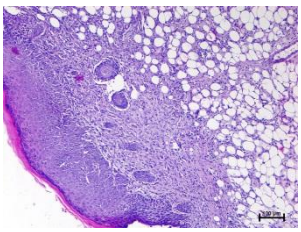
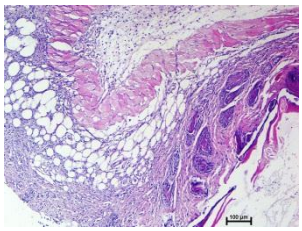
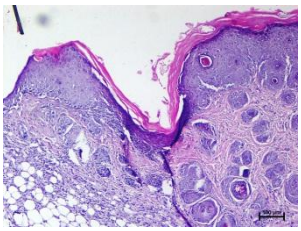
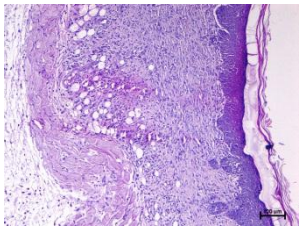
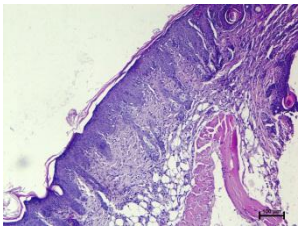
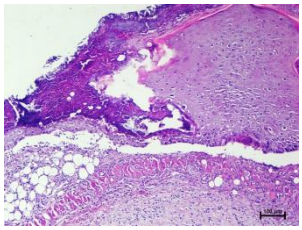
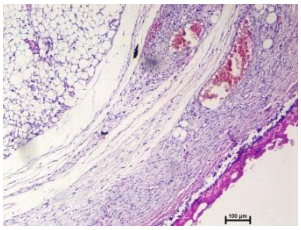
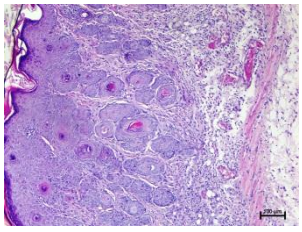
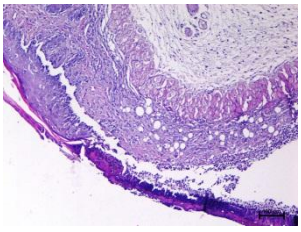
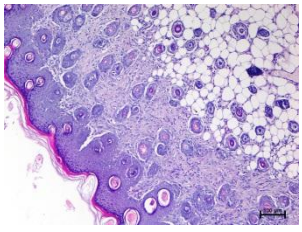
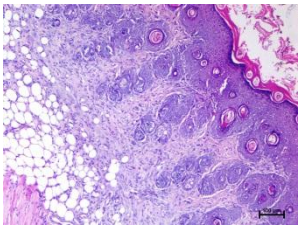
From the above findings, we can observe that the P-Value is greater than 0.05 and therefore, we can conclude that though there seems a difference in all the 7 Groups for Healing of Blisters and Ulcers, this difference is not statistically significant and it can occur by chance also.

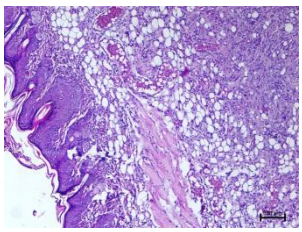
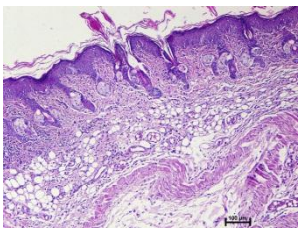
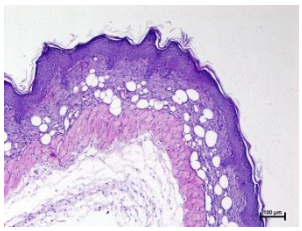
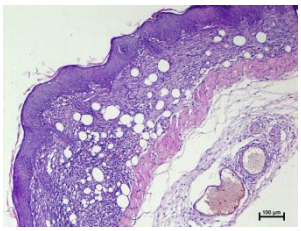
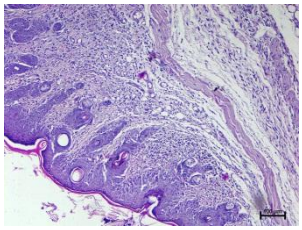
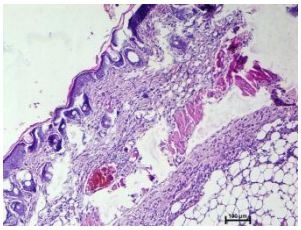
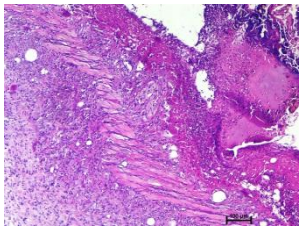
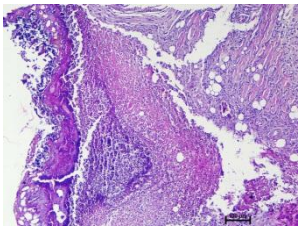
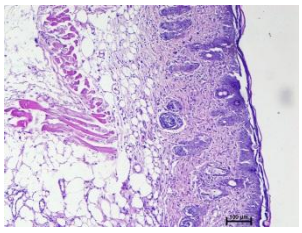
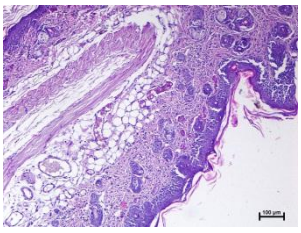
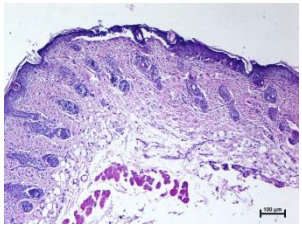
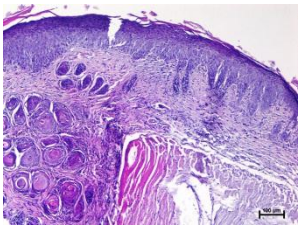
From the above Bar Diagram, it is evident that the effect of Group 4 (*Devdaru + Navaneeta Lepa*) is most prominent for reduction of blisters and ulcerations, while it's least prominent for Group 7 (Only *Navaneeta Lepa*), though the effects are not statistically significant.

OBSERVATIONS OF HISTOPATHOLOGICAL EXAMINATION

Figure 13 – Photographical record of Histopathological Examination

Group	Code	Left-side Lesion	Right-side Lesion
Group 1 (No Treatment)	1-A	Not Available	Not Available
	1-B		
	1-C	Not Available	Not Available
Group 2 (*Shatadhauta Ghrita Lepa*)	2-A		
	2-B		
	2-C		

Group 3 (*Daru-Sarshapa-Mustadi Lepa*)	3-A		
	3-B		
	3-C		
Group 4 (*Devdaru + Navaneeta Lepa*)	4-A		
	4-B		
	4-C		

Group 5 (*Sarshapa + Navaneeta Lepa*)	5-A		
	5-B		
	5-C		
Group 6 (*Mustaka + Navaneeta Lepa*)	6-A		
	6-B		
	6-C		

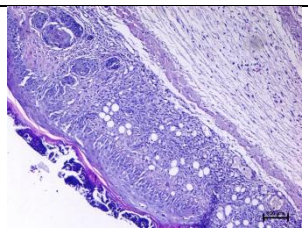
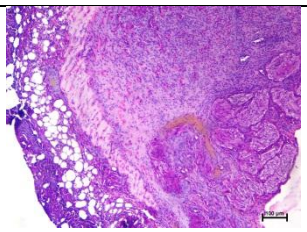
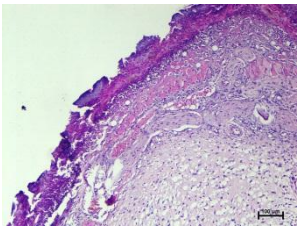
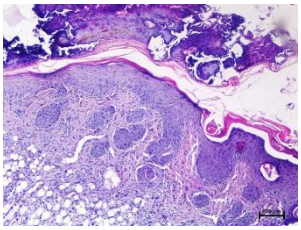
Group 7 (Only *Navaneeta Lepa*)	7-A		
	7-B		
	7-C	Not Available	Not Available

Figure 13 – Photographical record of Histopathological Examination

The above images of Histopathology Examination show different changes at the tissue level in each section obtained from the lesions. The following changes are mainly examined and the score is given for each criterion separately according to the pre-formulated semi-quantitative scoring system. (Please see Annexure No. 17)

1. Absence or presence of healing according to the formation of scab in Epidermal layer (In percentage).

2. The formation of new Epidermal skin Epithelial tissue covering the wound as a measure of Healing process (In percentage) (As Minimal, Mild, Moderate & Severe).

3. Assessment of healing of skin by Collagen formation and proliferation of Fibroblast tissue in Dermis layer and Subcutaneous tissue (In percentage).

4. The proliferation of Connective tissue with Granulation tissue formation as a process of healing (In percentage) (As Minimal, Mild, Moderate & Complete).

5. The infiltration of Acute Inflammatory Cells (Neutrophils and Polymorphonuclear Cells - PMN) in the skin section (Dermis and Epidermis) (As average cell count per high power field of tissue section) (As Minimal, Mild, Moderate & Diffuse).

(A) SCAB FORMATION IN EPIDERMAL LAYER

Table No. 60 - Experimental data of Group wise difference in Scab tissue formation

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	3	3	3	3	2
	Rt.	*	3	3	3	3	3	2
B	**Lt.**	1	2	3	4	3	3	3
	Rt.	1	1	4	4	3	4	2
C	**Lt.**	*	3	3	4	3	3	*
	Rt.	*	3	4	3	3	2	*

Table No. 61 - Statistical Analysis of Group wise difference in Scab tissue formation

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	2.00	19.44	6	0.0035
Group 2	6	12.50			
Group 3	6	24.50			
Group 4	6	26.75			
Group 5	6	20.00			
Group 6	6	20.00			
Group 7	4	9.88			
Total	36				

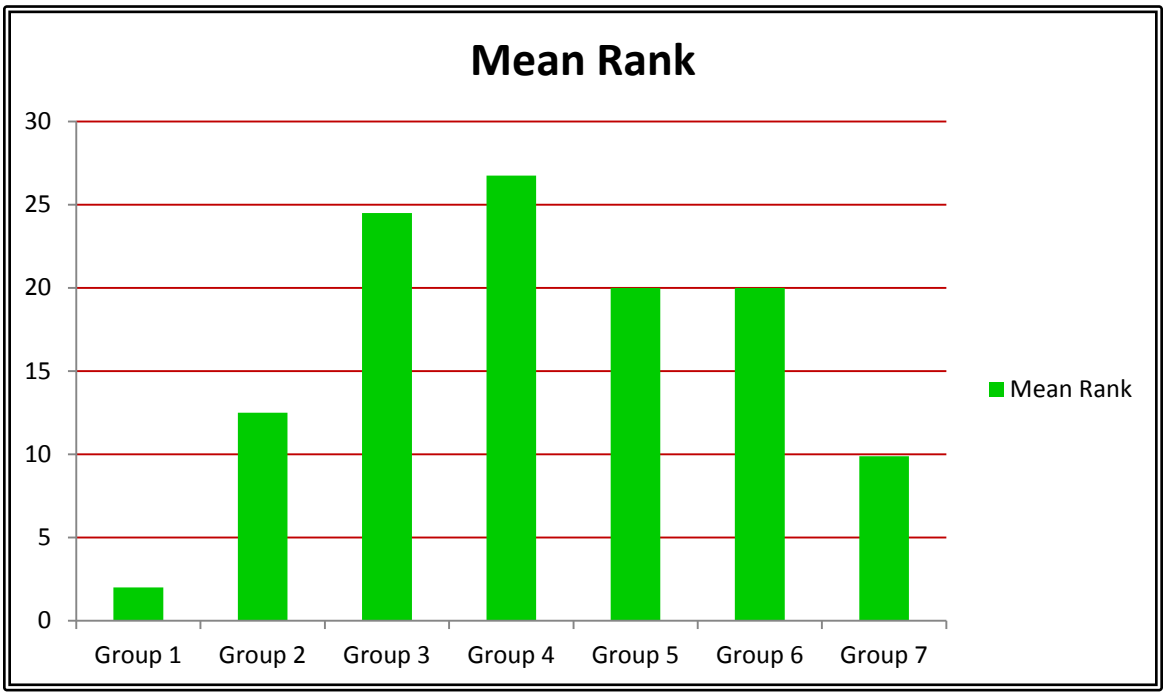


Figure 14 – Group wise difference in Scab tissue formation

From the above findings, we can observe that the P-Value is less than 0.05 and therefore, we can conclude that there is significant difference in all the 7 Groups for Scab Formation in Epidermal Layer.

From the above Bar Diagram, it is evident that the Formation of Scab in Epidermal Layer is most prominent in Group 4 (*Devdaru + Navaneeta Lepa*), while the second most prominent results are found in Group 3 (*Daru-Sarshapa-Mustadi Lepa*), which shows somewhat lesser formation of Scab than the Group 4.

On the other hand, the Scab Formation in Epidermal Layer is least seen in Group 1 (Normal Group- No Treatment Group), followed by the Group 7 (Only *Navaneeta Lepa*).

(B) FORMATION OF NEW EPIDERMAL SKIN EPITHELIAL TISSUE AS A HEALING PROCESS

Table No. 62 - Experimental data of difference in new Epidermal skin formation

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	3	3	3	3	3
	Rt.	*	3	2	3	2	2	2
B	**Lt.**	1	2	4	3	2	3	2
	Rt.	1	2	4	4	2	2	2
C	**Lt.**	*	3	4	4	3	3	*
	Rt.	*	3	3	4	2	4	*

Table No. 63 - Statistical Analysis of difference in new Epidermal skin formation

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	1.50	17.67	6	0.0071
Group 2	6	15.75			
Group 3	6	25.50			
Group 4	6	27.75			
Group 5	6	13.50			
Group 6	6	19.75			
Group 7	4	12.38			
Total	36				

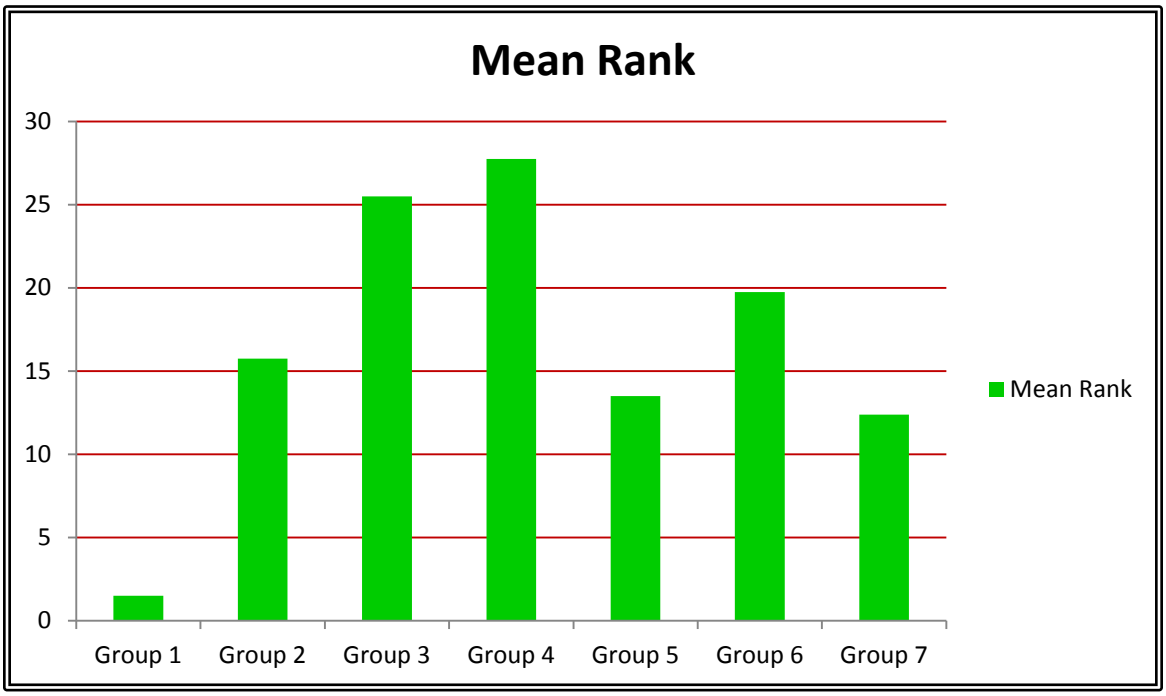


Figure 15 – Group wise difference in new Epidermal skin formation

From the above findings, we can observe that the P-Value is less than 0.05 and therefore, we can conclude that there is a difference in all the 7 Groups for Formation of New Epidermal Skin Epithelial Tissue as a Healing.

From the above Bar Diagram, it is evident that the Formation of New Epidermal Skin Epithelial Tissue as a Healing is most prominent in Group 4 (*Devdaru + Navaneeta Lepa*), while the second most prominent results are found in Group 3 (*Daru-Sarshapa-Mustadi Lepa*), which shows somewhat lesser formation of New Epidermal Skin Epithelial Tissue as a Healing than the Group 4.

On the other hand, the Formation of New Epidermal Skin Epithelial Tissue as a Healing is least seen in Group 1 (Normal Group- No Treatment Group), followed by the Group 7 (Only *Navaneeta Lepa*).

(C) HEALING OF SKIN BY COLLAGEN FORMATION & PROLIFERATION OF FIBROBLAST TISSUE IN DERMIS LAYER & SUBCUTANEOUS TISSUE

Table No. 64 - Experimental data of difference in Collagen and Fibroblast tissue

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	3	3	3	3	2
	Rt.	*	3	4	4	3	4	3
B	**Lt.**	1	2	4	3	3	2	3
	Rt.	1	3	4	4	3	4	2
C	**Lt.**	*	2	4	4	4	4	*
	Rt.	*	2	3	4	2	3	*

Table No. 65 - Statistical Analysis of difference in Collagen and Fibroblast tissue

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	1.50	19.68	6	0.0032
Group 2	6	10.17			
Group 3	6	26.17			
Group 4	6	26.17			
Group 5	6	17.83			
Group 6	6	22.17			
Group 7	4	12.00			
Total	36				

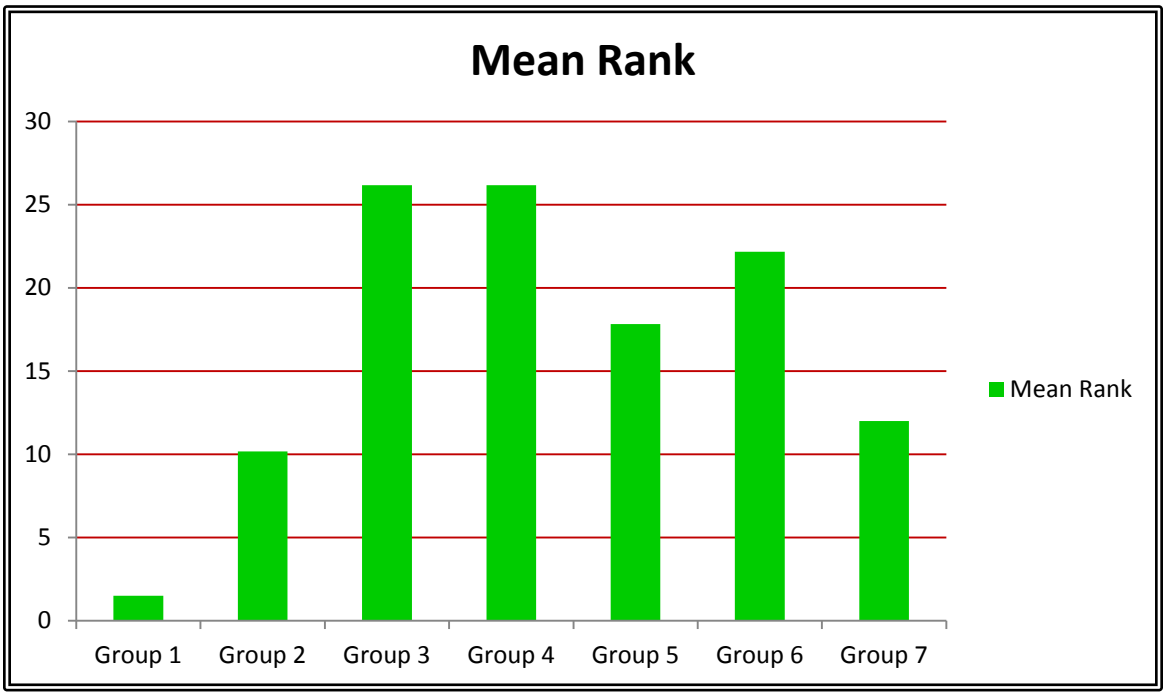


Figure 16 – Group wise difference in new Collagen and Fibroblast tissue

From the above findings, we can observe that the P-Value is less than 0.05 and therefore, we can conclude that there is significant difference in all the 7 Groups for Healing of Skin by Collagen formation and Proliferation of Fibroblast tissue in Dermis layer and Subcutaneous tissue.

From the above Bar Diagram, it is evident that the Healing of Skin by Collagen formation and Proliferation of Fibroblast tissue is most prominent in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and Group 4 (*Devdaru + Navaneeta Lepa*), while it is least in Group 1 (Normal Group- No Treatment Group).

(D) CONNECTIVE TISSUE PROLIFERATION WITH GRANULATION TISSUE FORMATION

Table No. 66 - Experimental data of difference in Connective & Granulation tissue

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	3	4	3	3	3
	Rt.	*	3	4	3	2	3	2
B	**Lt.**	1	2	3	3	3	3	2
	Rt.	1	3	4	4	3	2	2
C	**Lt.**	*	2	3	2	2	3	*
	Rt.	*	3	3	4	2	3	*

Table No. 67 - Statistical Analysis of difference in Connective & Granulation tissue

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	1.50	17.07	6	0.0090
Group 2	6	15.25			
Group 3	6	26.33			
Group 4	6	25.83			
Group 5	6	15.25			
Group 6	6	20.08			
Group 7	4	11.63			
Total	36				

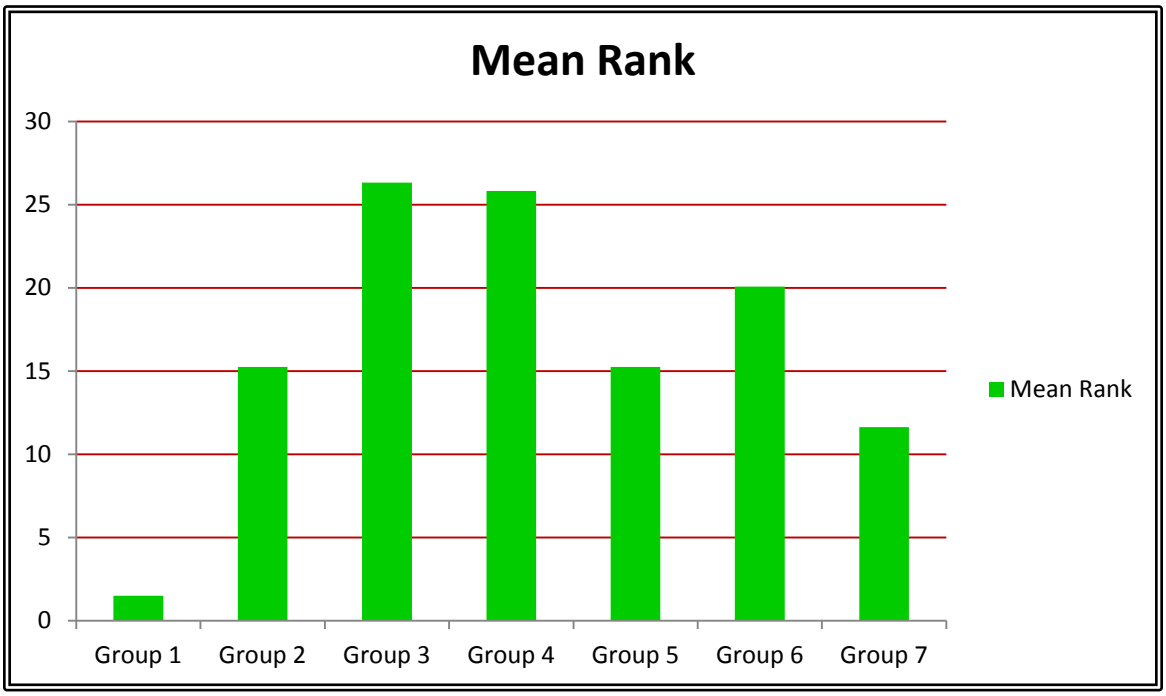


Figure 17 – Group wise difference in Connective & Granulation tissue

From the above findings, we can observe that the P-Value is less than 0.05 and therefore, we can conclude that there is significant difference in all the 7 Groups for Connective tissue proliferation with granulation tissue formation.

From the above Bar Diagram, it is evident that Connective tissue proliferation with granulation tissue formation is most prominent in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and slightly lesser in Group 4 (*Devdaru + Navaneeta Lepa*), while it is least in Group 1 (Normal Group- No Treatment Group).

(E) INFILTRATION OF ACUTE INFLAMMATORY CELLS (NEUTROPHILS & POLYMORPHONUCLEAR CELLS-PMN) IN THE SKIN SECTION

Table No. 68 - Experimental data of difference in Acute Inflammatory Cells

Mouse	Patch	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	**Lt.**	*	2	1	2	1	1	2
	Rt.	*	1	1	1	2	2	1
B	**Lt.**	4	3	2	1	1	3	2
	Rt.	2	2	1	2	3	2	2
C	**Lt.**	*	2	2	1	2	1	*
	Rt.	*	1	1	1	1	1	*

Table No. 69 - Statistical Analysis of difference in Acute Inflammatory Cells

Group	N	Mean Rank	Kruskal Wallis H	df	P-Value
Group 1	2	30.50	6.18	6	0.4035
Group 2	6	21.17			
Group 3	6	14.33			
Group 4	6	14.33			
Group 5	6	18.50			
Group 6	6	18.50			
Group 7	4	21.00			
Total	36				

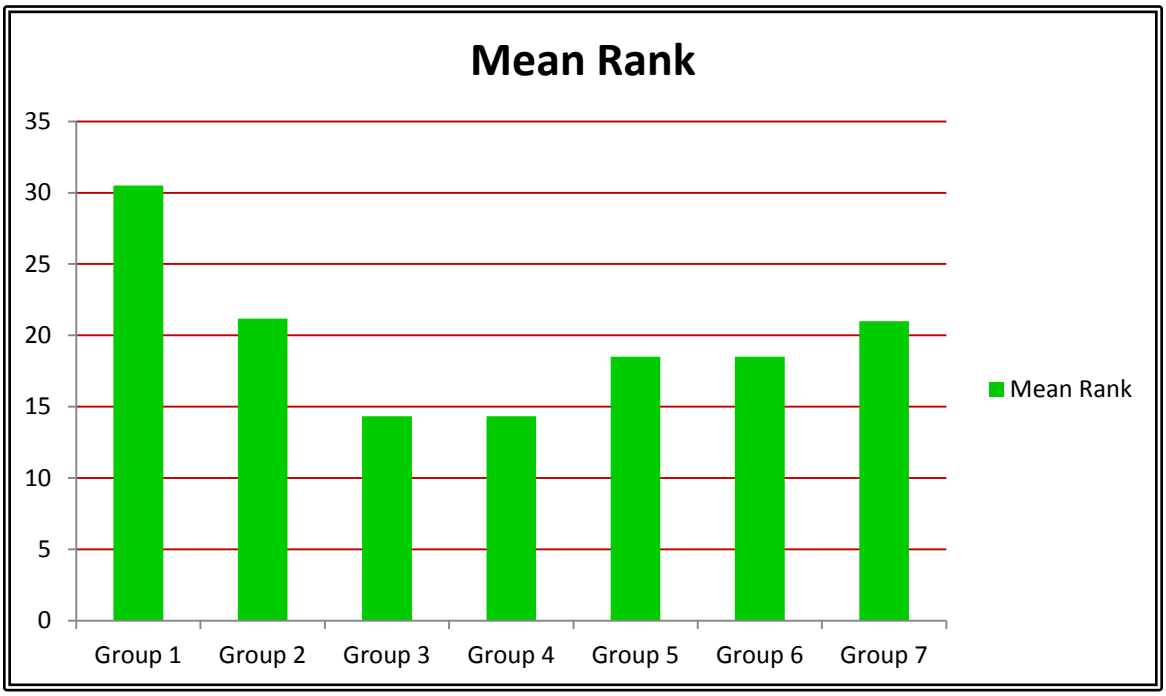


Figure 18 – Group wise difference in Acute Inflammatory Cells

From the above findings, we can observe that the P-Value is greater than 0.05 and therefore, we can conclude that though there seems a difference in all the 7 Groups for Infiltration of acute inflammatory cells (Neutrophils & polymorphonuclear cells-PMN) in the skin section (Dermis & epidermis), this difference is not statistically significant and it can occur by chance also.

From the above Bar Diagram, it is evident that the Infiltration of acute inflammatory cells (Neutrophils & polymorphonuclear cells-PMN) in the skin section (Dermis & epidermis) is least in Group 3 (*Daru-Sarshapa-Mustadi Lepa*) and Group 4 (*Devdaru + Navaneeta Lepa*), which is a sign of better healing; while it's most prominent in Group 1 (Normal Group- No Treatment Group), which is a sign of lesser healing, though the effects are not statistically significant.

RESULTS

RESULTS

The present in-vivo study has resulted to prove that, the experimental drug "*Daru-Sarshapa-Mustadi Lepa*" is effective against the local adverse effects of *Bhallataka* (Semecarpus anacardium) *Taila* (Oil) in the experimental conditions.

The present experimental study was also aimed to investigate the efficacy of every single component of *Daru-Sarshapa-Mustadi Lepa*, which has been found to be variable as follows.

1) The topical application of "*Devdaru Kaand Churna* + *Navaneeta Lepa*" has better results even than the complete formulation of "*Daru-Sarshapa-Mustadi Lepa*".

2) The topical application of "*Sarshapa Beeja Churna* + *Navaneeta Lepa*" has demonstrated a decreased effectiveness as compared to the rest of the ingredients of "*Daru-Sarshapa-Mustadi Lepa*".

3) The topical application of "*Mustaka Kanda Churna* + *Navaneeta Lepa*" has exhibited a superior effect in reduction of edema and has also been effective in reducing the blisters and ulcers.

4) The topical application of "Only *Navaneeta Lepa*" has failed to show the desired results and it delayed the healing of the wound.

Therefore, the results of the present experimental research point out that, "*Daru-Sarshapa-Mustadi Lepa*" and more specifically the "*Devdaru* + *Navaneeta Lepa*" are useful on local side-effects produced by *Bhallataka Taila* in albino mice, because –

1) They prominently reduced the Erythema caused by *Bhallataka Taila*.

2) They also reduced the Edema caused by *Bhallataka Taila* very prominently.

3) They also decreased the Blisters and healed the Ulcerations caused by *Bhallataka Taila*. However, only results of "*Devdaru* + *Navaneeta Lepa*" are statistically significant.

4) They enhanced the formation of Scab in the Epidermal Layer of skin, where the ulcer was caused by local contact of *Bhallataka Taila*.

5) They also helped to grow the epidermal layer of skin by regeneration of the epithelial tissue, which is considered as a sign of good healing of the ulcers inflicted by *Bhallataka Taila*.

6) They also showed a good healing effect at the site of ulceration, which is evidenced by the development of Collagen and proliferative changes in the form of an increased number of Fibroblasts.

7) They enhanced the proliferation of Connective tissue with formation of granulation tissue at the local lesions caused by *Bhallataka Taila*.

8) When the dermal and epidermal sections of skin were examined for the infiltration of cells like Neutrophils as well as the Poly-morpho-nuclear cells which are indicative of an acute inflammatory condition, their number was found to be minimal in the lesions treated with either of these two *Lepa*; though this finding isn't statistically significant.

Thus, the results of present study lead us to reject the Null hypothesis and alternatively accept its rival hypothesis on the basis of statistical testing, which establishes the earlier assumption as a certain fact that "the experimental drug named *Daru-Sarshapa-Mustadi Lepa* shows significant efficacy against the local adverse effects of *Bhallataka Taila* in the experimental animals viz. Swiss albino mice".

DISCUSSION

The present experimental study to evaluate "efficacy of *Daru-Sarshapa-Mustadi Lepa* on local side-effects of *Bhallataka Taila*" is a pre-clinical study, which was aimed for the revalidation of ancient knowledge described in the text "*Anupan Manjari*". However, before we discuss about the experimental study, it's important to ponder upon the mechanism of toxic action of *Bhallataka (Bhallataka Visha Samprapti)* and the possible mechanism of pharmacological action of *Daru-Sarshapa-Mustadi Lepa* for its *Samprapti Bhedana*.

DISCUSSION OF AYURVEDIC ASPECTS

(A) *BHALLATAKA VISHA SAMPRAPTI* –

The toxicity of any poison is usually analyzed by using the modern principles of Toxicology. The modern science focuses on the presence of chemical constituents present in the toxin and evaluates its toxic effects in the body according to the mechanism of action of those chemical constituents. Though this is a proven and very effective methodology to analyze the toxicity of any poison, but from the viewpoint of *Ayurveda*, it proves insufficient as the poisons and their actions mentioned in *Ayurveda* cannot be assessed holistically using the modern principles. Therefore, it's important to analyze these poisons using *Ayurvedic* principles as well.

The "Principle of *Dravyaguna*" i.e. the principle of *Ayurvedic* Pharmacology is an important principle in *Ayurveda*, which explains the mechanism of action of any drug based on its *Guna* (Properties), *Rasa* (Taste), *Veerya* (Potency), *Vipaaka* (Post-digestive taste) etc. This principle is quite different from the principles of modern medical science, which tries to find the mechanism of action of a drug based on the Drug-Receptor mechanism. This principle has also been proven quite effective to analyze the pharmacological action of any drug; but from the viewpoint of *Ayurveda*, the mechanism of action of an *Ayurvedic* drug cannot be holistically assessed using the modern principles. Therefore, it's important to analyze the drugs of *Ayurveda* using the *Ayurvedic* principles as well.

Though *Ayurveda* doesn't prescribe any specialized principles for the analysis of *Visha Samprapti* only; however, the principles of *Ayurvedic* Pharmacology are very much efficient to serve for this purpose as well. These principles in *Ayurveda* are universal and are applicable to any substance in a broader sense. Therefore, the *Visha Samprapti* of *Bhallataka* can be explored using the "Principle of *Dravyaguna*" (*Ayurvedic* Pharmacology) and "*Pancha Mahabhuta Siddhanta*" (Principle of 5 basic elements), which reveals the mechanism of its toxicity from the *Ayurvedic* perspective.

***RASA PANCHAKA* (PROPERTIES) OF *BHALLATAKA* -**

- *Guna* - *Laghu* (Light), *Snigdha* (Unctuous), *Teekshna* (Sharp & Piercing)
- *Rasa* - *Madhura* (Sweet), *Kashaya* (Astringent) – By *Bhavprakash Nighantu*
  Katu (Pungent), *Tikta* (Bitter) – By *Dhanvantari Nighantu*, *Raj Nighantu*
- *Veerya* - *Ushna* (Hot)
- *Vipaaka* - *Madhura* (Sweet) – By *Bhavprakash Nighantu*
  Katu (Pungent) – By *Kaiyadeva Nighantu*
- *Prabhaava* - Not specific

MECHANISM OF TOXIC ACTION *(VISHA SAMPRAPTI)* -

Just like the Pharmacological action of any medicine, the Toxic action of all the poisons also depends upon their *Guna*, *Rasa*, *Veerya*, *Vipaka* and *Prabhaava*. When we study the common characteristics of *Bhallataka*, we find that it possesses the properties like *Laghu* (Lightness) & *Teekshna* (Sharp & Piercing) and its *Veerya* is *Ushna* (Hot). All these properties are similar to those of a poison.

According to *Acharya Sushruta*, *Laghu Guna* keeps the poison moving in the body from one place to another easily, thereby, making it *Asthira* (Unstable). Due to this, the therapeutic measures cannot catch up with the poison. As the poison keeps on escaping, the therapies don't produce desired effects and the poisoning becomes *Dushchikitsya* (Incurable). Even *Acharya Charaka* admits that, it is very difficult to find any therapeutic measures to treat the poison due to its *Laghu Guna* only.

Due to *Teekshna Guna*, the poison causes injury to *Marmas* (Vital sensitive areas), which can cause death or severe disfigurement; because *Marmas* are the vital junction points in the body which carry the subtle energy (*Prana*) and an injury to these points can be life threatening.

Though *Bhallataka* has a *Snigdha Guna* (Unctuousness or Oiliness) as well, it is always accompanied and undeniably nullified by the *Teekshna Guna* (Sharp & Piercing Property). Therefore, the *Snigdha Guna* doesn't show its soothing property like the other *Sneha* (*Ghee*, Oil etc.) and the toxic action is prominently exhibited by this *Teekshna Guna* only. However, this *Snigdha Guna* of *Bhallataka* seems to demonstrate its effects when utilized as medicine, as observed from its *Shukrala* (Aphrodisiac) action.

Bhallataka Phala has *Madhura* (Sweet) and *Kashaaya* (Astringent) *Rasa* (Tastes). According to *Acharya Charaka*, the taste of *Visha* (Poison) is *Anirdeshya Rasa* i.e. its taste cannot be described. Even *Acharya Vagbhata* has accepted that taste of *Visha* is *Avyakta Rasa* i.e. the taste can't be expressed. Therefore, it can be an argument whether *Rasa* plays any role in the toxic action of a poison, as *Acharyas* haven't bothered about specific composition of *Rasa* of the *Visha*. Even we find some *Visha* like *Vatsanabha* having *Madhura Rasa*, while some other poisons have acrid tastes like *Katu Rasa* of *Dhattura* (Dhatura metel) or *Tikta Rasa* of *Ahiphena* (Opium). However, the poisons of all *Rasa* appear to be toxic and fatal. Therefore, *Rasa* seems to play no role in the toxicity of the poison. At least we can say this in case of *Bhallataka Phala*; because *Madhura* and *Kashaaya Rasa* of *Bhallataka* don't seem to be pacifying *Pitta Dosha* & don't depress digestive fire also, exactly opposite to common rule. However, it definitely plays a vital role in its pharmacological action, as evident from *Shukrala* (Aphrodisiac) *Karma* of *Madhura Rasa* and *Vrana Shodhana-Ropana Karma* of *Kashaaya Rasa*.

Some other *Nighantu Grantha* like *Dhanvantari Nighantu* and *Raj Nighantu* have attributed even *Katu* (Pungent) and *Tikta* (Bitter) *Rasa* to *Bhallataka*. The presence of these two *Rasa* seems more acceptable, as the *Bhautika* composition of these two *Rasa* viz. *Katu Rasa* (*Vaayu + Teja*) and *Tikta Rasa* (*Vaayu + Akasha*) matches exactly with the active properties of *Bhallataka* i.e. *Laghu Guna* (*Teja + Vaayu + Akasha*) and *Teekshna Guna* (*Teja*). Also, the *Guna* of *Katu Rasa* are *Laghu*, *Ushna*,

Teekshna, which are prominently seen in *Bhallataka*. Therefore, *Katu Rasa* seems to play an important role in the toxic action of *Bhallataka*. However, *Tikta Rasa* isn't *Ushna-Teekshna*; but still, it is *Laghu* and its *Vipaaka* is *Katu Vipaaka*. Therefore, it expresses its *Karma* like *Krimighna, Kushtha-Kandughna, Agnideepana-Pachana, Shoshana* and *Vrana Shodhana*; and seems to play a major role in the Pharmacological action of *Bhallataka*, while its role in the toxic action appears trivial.

Ushna Veerya (Hot potency) aggravates *Pitta Dosha & Shonita (Rakta) Dhatu* in the body. *Ushna Veerya* specifically shows tendency of *Pachana* (Digestion). Therefore, it improves digestive fire (Agni) in the body. However, the excessive *Ushna Veerya* of *Bhallataka* causes *Dhatu Pachana* i.e. burning, inflammation and corrosion of the body tissues due to aggravation of *Pitta and Rakta*, thereby damaging the tissues.

Even though *Bhallataka* has a *Madhura Vipaaka* (Sweet after-taste), it predominantly acts by its *Ushna Veerya* (Hot potency) only. *Vipaaka* is the final biotransformation of any *Dravya*. However, the property of poison is *Apaaki* i.e. it is indigestible for the *Jatharagni* (Digestive fire). *Visha* also possesses properties like *Sukshma* (Fine), *Ashu* (Quick) and *Vyavayi* (Diffusible). Therefore, it quickly spreads in the whole body and there's no chance for its digestion by *Jatharagni* or *Tvak sthita Agni (Bhraajaka Pitta)* in case of local toxicity. This seems to be the reason that, *Madhura Vipaaka* of *Bhallataka* doesn't show any effect in its toxic action. However, this *Vipaaka* is very effective in its pharmacological action, as observed from its *Shukrajanana Karma*.

Kaiyadeva Nighantu and a contemporary scholar *Vaidya Go. Aa. Phadke* have described *Katu Vipaaka* of *Bhallataka*, as opposed to the traditional belief of *Madhura Vipaaka*. Even this argument stands good if we ponder upon the prominence of *Katu*, *Tikta*, *Kashaya Rasa* and *Laghu*, *Ushna*, *Teekshna Guna* in *Bhallataka*. Therefore, in the context of toxic action of *Bhallataka*, *Vipaaka* can be considered to be *Katu* and for explaining its pharmacological action, *Vipaaka* may be considered as *Madhura*.

Based on the properties of *Bhallataka*, we can contemplate about the *Panchabhautik* composition, *Dosha Prabhaava* and also the Toxic action of *Bhallataka Phala* as follows.

Table No. 70 – Assessment of *Bhallataka* according to its properties

Sr. No.	Properties of *Bhallataka Phala*	*Bhautika* Composition	*Dosha Prabhaava*	*Karma* (Toxic Action)
1	*Laghu Guna*	*Teja + Vaayu + Akasha*	*Vaatakara, Kaphaghna*	*Dushchikitsya*
2	*Snigdha Guna*	*Jala*	*Kaphakara, Vaata-Pittaghna*	**No role**
3	*Teekshna Guna*	*Teja*	*Pittakara, Vaata-Kaphaghna*	*Daaha-Paaka-Straavakara, Chhedana-Bhedana, Marmaghna*
4	*Madhura Rasa*	*Prithvi + Jala*	*Kaphakara, Vaata-Pittaghna*	**No role**
5	*Kashaaya Rasa*	*Prithvi + Vaayu*	*Vaatakara, Pitta-Kaphaghna*	*Laghu, Ruksha, Vishada, Vikashi*
6	*Katu Rasa*	*Vaayu + Teja*	*Vaata-Pittakara, Kaphaghna*	*Laghu, Ushna, Teekshna, Ruksha, Daaha-Straavakara, Chhedana-Bhedana*
7	*Tikta Rasa*	*Vaayu + Akash*	*Vaata-Pittakara, Kaphaghna*	*Laghu, Ruksha, Vishada, Katu Vipaaka, Chhedana*
8	*Ushna Veerya*	*Teja*	*Pittakara, Vaata-Kaphaghna*	*Laghu, Daaha-Sweda-Trusha-Moorchchhakara, Dhatu Pachana*
9	*Madhura Vipaaka*	*Prithvi + Jala*	*Kaphakara, Vaataghna*	**No role**
10	*Katu Vipaaka*	*Teja + Vaayu + Akasha*	*Vaatakara, Kaphaghna*	*Laghu, Ushna, Teekshna, Ruksha, Vishada, Daaha-Straavakara, Chhedana-Bhedana*

On the basis of these properties of *Bhallataka*, their *Panchabhautik* composition and individual *Dosha Prabhaava*, we can statistically assess the prominence of *Mahabhuta* in *Bhallataka* and its conspicuous effects on the body humors (*Dosha*). However, the 7 properties which have a role in toxic action have only been considered here for the analysis of *Bhallataka Visha Samprapti*.

Table No. 71 – Assessment of prominent *Mahabhuta* in Toxic action of *Bhallataka*

Sr. No.	Name of *Mahabhuta*	No. of properties indicating presence of specific *Mahabhuta* (Out of 7)	Percentage
1	*Akash*	3	**21.42 %**
2	*Vaayu*	5	**35.71 %**
3	*Teja*	5	**35.71 %**
4	*Jala*	0	0 %
5	*Prithvi*	1	7.14 %

From the above assessment of *Mahabhuta*, it is very evident that only *Teja*, *Vaayu* and *Akash Mahabhuta* are prominently responsible for toxic action of *Bhallataka*; while *Jala* and *Prithvi Mahabhuta* seem to be having their role in its pharmacological action only. Out of these three *Mahabhutas*, *Teja* and *Vaayu Mahabhuta* dominate the toxic action of *Bhallataka* due to their *Laghu, Ushna, Teekshna, Ruksha, Khara, Vishada, Sukshma Guna* and therefore cause *Daaha-Paaka-Straava-Santaapa-Bhedana and Dhatu Pachana* as well. On the other hand, *Akash Mahabhuta* seems to play a supportive role to aid in its *Laghu, Sukshma, Vishada Guna* and thereby increasing spread of the toxin due to *Ashukari Karma*, making it difficult to cure.

Table No. 72 – Assessment of *Dosha Prabhaava* during Toxic action of *Bhallataka*

Dosha	*Prabhaava* (Effect) on a *Dosha*	No. of properties indicating specific effect on a *Dosha* (Out of 7)	Percentage
Vaata	*Vriddhi*	5	**71.42 %**
	Kshaya	2	28.57 %
Pitta	*Vriddhi*	4	**57.14 %**
	Kshaya	1	14.28 %
	No Effect	2	28.57 %
Kapha	*Vriddhi*	0	0 %
	Kshaya	7	**100 %**

From the above assessment of *Dosha Prabhaava* (Effect on *Dosha*), it appears that *Bhallataka* has the potential for an increase in *Vaata* and *Pitta Dosha* in the body during its Toxic action. It's very obvious that *Pitta Dosha* can be seen increasing due to *Ushna*, *Teekshna Guna* of *Bhallataka* causing its toxicity. However, it's potential to increase the *Vaata Dosha* seems to be adjourned due to the *Ushna-Teekshna Guna*, which are so prominent and resilient that they seem to control *Vaata Dosha* very effectively in spite of other properties being favorable to *Vaata Vriddhi*. Therefore, *Bhallataka* shows the effect of *Ekaantika* (Solitary) *Pitta Vriddhi* only, while pacifying the *Vaata Dosha* due to its *Ushna-Teekshna* properties.

Due to the toxic effect of *Bhallataka*, there seems a very prominent decrease in *Kapha Dosha* inside the body, due to the *Kapha*ghna nature of all its properties responsible for its toxicity. All 100% properties of *Bhallataka* appear to bring out the pacification of *Kapha Dosha*. Even *Acharya Charaka* has identified *Bhallataka* as an amazing drug in diseases of *Kapha Dosha*. In this way, *Bhallataka* appears to demonstrate its toxic action on body humors.

At the level of *Dhaatu* (Tissues), it has been mentioned that any *Visha* initially causes *Shonita Dushti* (Defect in the Blood element). It can be prominently observed in case of local toxicity of *Bhallataka*, as all type of dermatological manifestations usually occur due to defect in the *Shonita (Rakta) Dhaatu*. Even *Maansa Dhaatu Dushti* (Defect in the Muscle tissue) and its *Upadhaatu* (Subsidiary tissue) *Shat-Tvacha Dushti* (Defect in the 6 types of skin) can be observed in *Bhallataka* toxicity, as the wound due to *Bhallataka* affects these elements.

Finally, the fatal action of any *Visha* occurs due to defect in *Oja* (The essence of all *Dhaatu*), as the 10 properties of *Visha* are exactly opposite to the 10 properties of *Oja*. However, the defect in *Oja* can be classified into three stages. The first stage is *Ojo Visransa* (Displacement), the second one is *Ojo Vyapad* (Derangement) and the last is *Oja Kshaya* (Diminution). The death due to *Visha* occurs in the terminal stage of *Oja Kshaya*. However, as we observe that the toxic action of *Bhallataka* usually occurs as a hypersensitivity reaction, it can be correlated to the stage of *Ojo Visransa*, where displacement of Body humors (*Dosha Chyavana*) can be observed. The defect in *Oja* is caused due to its displacement from *Hridaya* (Heart) due to *Teja* (*Pitta*) driven by *Vaayu*. As *Bhallataka* causes extreme vitiation of *Pitta Dosha*, it can displace the *Oja* and generate a hypersensitivity reaction due to *Ojo Visransa.*

Thus, the mechanism behind the toxic action *(Visha Samprapti)* of *Bhallataka* can be sought from the viewpoint of *Ayurveda* using the *Ayurvedic* Principles only. This exercise can help us to assess the pharmacological action *(Samprapti Bhedana)* by various antidotes of *Bhallataka* described in the *Ayurvedic* literature, purely from an *Ayurvedic* perspective.

(B) *SAMPRAPTI BHEDANA* BY INDIVIDUAL INGREDIENTS OF *LEPA* –

As we know that the Pharmacological action of any medicine depends upon its *Guna*, *Rasa*, *Veerya*, *Vipaaka* and *Prabhaava*, we need to study the characteristics of all the contents of *Daru-Sarshapa-Mustadi Lepa*. We find the drugs used in this *Lepa* carrying the *Ayurvedic* properties like –

1) DEVDARU (Cedrus deodara Roxb.) -

- *Guna* - *Laghu, Snigdha*
- *Rasa* - *Tikta*
- *Veerya* - *Ushna*
- *Vipaaka* - *Katu*
- *Doshaghnataa* - ***Vaataghna*** – Due to *Ushna Veerya* & *Snigdha Guna*.
  Pittakara – Due to *Ushna Veerya*.
  Kaphaghna – As *Tikta Rasa, Katu Vipaaka, Laghu, Ushna Veerya*

- *Baahya Karma* - *Shothahara, Vedanasthapana, Kushtha-Krimighna, Vrana Shodhana-Ropana*
- Mechanishm of Action –
  Vrana Straavahara – As it is *Tikta*, *Ushna* & *Laghu*.
  Vrana Durgandhihara – As it is *Krimighna* due to presence of Aromatic oil.
  Krimighna – As it has *Tikta Rasa* and *Ushna Veerya*.
  Shothahara – As it destroys *Kleda* & removes the *Strotorodha*.
  Vedanasthapana – As it is *Vaataghna*.
  Vrana Shodhana-Ropana – As it is *Krimighna* & *Rakshoghna.*
  Dhaatugata Dosha Pachana – As it's *Tikta Rasa*, *Katu Vipaaka* & *Ushna Veerya*
  Rakta Prasaadana – As it possesses *Tikta Rasa*.

- **Final Remarks –** *Devdaru* can be effective in local toxicity of *Bhallataka* due to its *Tikta Rasa*, as it is *Vishahara* and also due to its inherent *Baahya Karma*, which are beneficial for the skin manifestations.

***2) SARSHAPA* (MUSTARD) (Brassica campestris Linn.) -**

- *Guna* - *Teekshna, Snigdha* (*Sarshapa Taila – Laghu*)
- *Rasa* - *Katu, Tikta*
- *Veerya* - *Ushna*
- *Vipaaka* - *Katu*
- *Prabhaava* - -----
- *Doshaghnataa* - ***Vaataghna*** – Due to *Ushna Veerya* & *Snigdha Guna*.
  Pittakara – Due to *Ushna Veerya*.
  Kaphaghna – As *Katu-Tikta Rasa, Katu Vipaaka, Ushna Veerya*

- *Baahya Karma* - *Kushthaghna, Kandughna, Jantughna, Vedanahara* and *Varnya*.
- Mechanishm of Action –

  Kushthaghna – As it is *Kaphaghna, Kledaghna* & *Stroto Shodhana* due to *Ushna, Teekshna* & *Lekhana Guna*.

  Kandughna – As it pacifies *Kapha* & *Kleda* at the local area.

  Jantughna – As it has *Katu-Tikta Rasa, Ushna Veerya* and *Teekshna Guna*.

  Vedanahara – As it is *Vaataghna*.

  Varnya – As it causes *Twak Prasaadana* by increasing *Bhraajaka Pitta* & *Rakta Dhaatu* due to *Ushna Veerya*.

- **Final Remarks -** *Sarshapa* may prove effective in *Bhallataka* toxicity due to its *Katu* and *Tikta Rasa*, because both of these *Rasa* are *Vishaghna*. The inherent *Baahya Karma* of *Sarshapa* also appear to be beneficial for the skin manifestations, which might add to its effectiveness in the local toxicity of *Bhallataka*. However, the *Ushna Veerya* and *Teekshna Guna* of *Sarshapa* create a doubt about its efficacy, as these properties might also cause an increase in the local toxicity of *Bhallataka*, as these properties are similar to those of *Visha*.

3) MUSTAKA (NUT GRASS) (Cyperus rotundus Linn.) -

- *Guna* - *Laghu, Ruksha*
- *Rasa* - *Tikta, Katu, Kashaaya*
- *Veerya* - *Sheeta*
- *Vipaaka* - *Katu*
- *Prabhaava* - -----

- *Doshaghnataa* - ***Vaatakara*** – As all of its properties are *Vaata Vardhaka.*
  Pittaghna – Due to *Sheeta Veerya*.
  Kaphaghna – Due to its *Katu-Tikta-Kashaaya Rasa*.
- *Baahya Karma* - *Tvagdoshahara, Kandughna, Shothahara, Jantughna* and *Rakta Prasaadana*.

- Mechanishm of Action –

  Tvagdoshahara – As it is *Kaphaghna, Kledaghna* & *Stroto Shodhana* due to *Laghu, Ruksha* & *Lekhana Guna*. It also relieves *Rakta-Pitta Dushti* due to its *Tikta-Kashaaya Rasa* & *Sheeta Veerya*.

  Kandughna – As it pacifies *Kapha* at the local area and also relieves *Rakta-Pitta Dushti* due to its *Tikta-Kashaaya Rasa* & *Sheeta Veerya*.

  Shothahara – As it destroys *Kleda* & removes the *Strotorodha*.

  Jantughna – As it has *Katu-Tikta Rasa*.

  Rakta Prasaadana – As it possesses *Tikta Rasa*.

- **Final Remarks -** *Mustaka* can prove to be effective in *Bhallataka* toxicity due to its *Vishaghna Rasa* i.e. *Katu* and *Tikta Rasa*. Also, its *Sheeta Veerya* can pacify the *Ushna, Teekshna Guna* of *Bhallataka* and work as *Pittaghna*. The inherent *Baahya Karma* can also aid to cure the dermatological manifestations of local toxicity of *Bhallataka*.

4) NAVANEETA (Butter made up of Cow Milk) –

Navaneeta has been used as a base for this *Lepa*. Its specific properties like *Rasa, Veerya, Karma* have been mentioned in the *Ayurvedic Samhita* and *Nighantu* texts. However, other properties like *Guna, Vipaaka* haven't been entirely mentioned. Yet some of these properties can be inferred from its *Guna, Karma,* overall appearance and the properties of *Godugdha* (Cow Milk) as well.

- *Guna* - *Guru, Snigdha, Shlakshna, Pichchhila, Mridu*
- *Rasa* - *Madhura*
- *Veerya* - *Sheeta*
- *Vipaaka* - *Madhura*
- *Prabhaava* - -----

- *Doshaghnataa* - ***Vaataghna*** – As all of its *Guna* and *Rasa-Vipaaka* are *Vaatahara*.
  Pittaghna – Due to *Madhura Rasa-Vipaaka* and *Sheeta Veerya*.
  Kaphakara – As all of its properties are *Kapha Vardhaka.*
- *Baahya Karma – Varnya, Vaata-Pitta-Rakta Vikaara Naashaka, Vranaapaha.*

- Mechanishm of Action –
  Varnya – As it increases *Sapta Dhaatu* and ultimately does *Ojo Vardhana* due to its *Madhura Rasa-Vipaaka* and *Sheeta Veerya*.
  Vaata-Pitta-Rakta Vikaara Naashaka – Due to its *Vaataghna* and *Pittaghna* properties.
  Vranaapaha – As it does *Brihana* and *Dhaatu Vardhana* due to *Madhura Rasa*.

- **Final Remarks -** All the properties of *Navaneeta* like *Guna, Rasa, Veerya, Vipaaka* are opposite to those of *Bhallataka* and therefore can help in its anti-toxic action. Its *Madhura Rasa* can specifically be responsible for *Visha Prashamana* and the *Sheeta Veerya* can cause pacification of *Pitta*. Hence, it appears to aid in healing of the wounds caused by *Bhallataka* toxicity.

As perceived from the pharmacological properties of all these herbal raw drugs mentioned in the *Dravyaguna* literature as well as those of *Navaneeta* detailed in the *Ayurvedic Samhita* and *Nighantu* texts, the overall properties of all the ingredients of this *Lepa* appear to be beneficial for skin and its various manifestations, and therefore help in *Samprapti Bhedana* in case of local side effects of *Bhallataka*.

However, in order to assess the combined effect of these ingredient drugs in the form of *Lepa* preparation, a detailed comparative analysis of all these ingredients has been carried out further, based upon their *Ayurvedic* Pharmacological properties; so as to evaluate the mechanism of *Samprapti Bhedana* in local side effects of *Bhallataka*.

(C) *SAMPRAPTI BHEDANA* BY *"DARU-SARSHAPA-MUSTADI LEPA"* –

The contents of *Daru-Sarshapa-Mustadi Lepa* have been further analyzed to assess the mechanism of *Samprapti Bhedana* by all the individual ingredients as well as their collective mechanism as a whole formulation for its effectiveness in the local side effects of *Bhallataka*.

Though, all individual contents of Daru-*Sarshapa-Mustadi Lepa* possess some properties which show their potential to prove effective in the local toxicity of *Bhallataka*. However, many of the properties of these individual drugs appear to be similar to those of *Bhallataka* or in general, similar to *Visha Guna*. This creates a doubt whether these drugs can also enhance the action of *Bhallataka* as a *Visha*. But the ultimate fact is that, these individual drugs are not being used as a singular drug only and will be utilized as a mixture of all these drugs. When we use them collectively as a Drug compound, there must have to be a drug interaction which influences the overall properties of that Drug formulation. Therefore, the detrimental properties of some of the drugs can be nullified by the positive properties of the other drugs and the combined Drug formulation can demonstrate an anti-toxic action collectively.

Table No. 73 – *Samprapti Bhedana* by *Laghu-Guru Guna* against *Bhallataka*

Sr. No	Properties and their *Bhautika* Composition	Probable actions viz. *Vishaghna Karma* (Anti-toxic Action) and *Tvachya Karma* (Action on Skin) as per the properties			
		Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
1	*Laghu Guna = Teja + Vaayu + Akash*	√	√	√	-----
		Karma – Lekhana, Vrana Ropana			
2	*Guru Guna = Prithvi + Jala*	-----	-----	-----	√
		Karma – Sarva Dhaatu Vardhana			

From the above table, we can observe that all the herbal raw drugs viz. *Devdaru*, *Sarshapa* and *Mustaka* possess *Laghu Guna*, which is essentially a property of *Visha* and also present in *Bhallataka*. Therefore, use of all these three drugs can actually upsurge the *Laghu Guna* in total and can exaggerate the action of *Visha*. However, as all these drugs are mixed with *Navaneeta* while preparing the combined formulation, this *Laghu Guna* seems to get nullified by the *Guru Guna* of *Navaneeta* and therefore, it can aid in the anti-toxic action of this Drug formulation and can heal the wound by its *Sarva Dhaatu Vardhana Karma*. Even if *Laghu Guna* expresses itself, it can assist the healing of wound by its *Lekhana* and *Vrana Ropana Karma*.

Table No. 74 – *Samprapti Bhedana* by *Ruksha-Snigdha Guna* against *Bhallataka*

Sr. No	Properties and their *Bhautika* Composition	Probable actions viz. *Vishaghna Karma* (Anti-toxic Action) and *Tvachya Karma* (Action on Skin) as per the properties			
		Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
1	*Ruksha Guna = Vaayu + Prithvi*	-----	-----	√	-----
		Karma – Vrana Shodhana			
2	*Snigdha Guna = Jala*	√	√	-----	√
		Karma – Varna Prasaadana, Dhaatu Vardhana			

Out of these 4 ingredients of *Lepa*, only *Mustaka* possesses *Ruksha Guna*, which is similar to property of *Visha*. On the other hand, other 3 ingredients demonstrate the *Snigdha Guna*, which opposes the *Ruksha Guna* of *Visha*. Moreover, even though *Bhallataka* is a *Visha*, it expresses the *Snigdha Guna*, in contradiction to the general rule. Therefore, the *Ruksha Guna* of *Mustaka* doesn't appear to aid the toxic action of *Bhallataka*; conversely, it can help in healing of the local injury by *Bhallataka* due to its *Vrana Shodhana Karma*. On the other hand, the *Snigdha Guna* of other 3 ingredients can help with *Dhaatu Vardhana* and *Varna Prasaadana Karma* for healing the wound.

Table No. 75 – *Samprapti Bhedana* by *Teekshnaadi Guna* against *Bhallataka*

<table>
<tr><th rowspan="2">Sr. No</th><th rowspan="2">Properties and their Bhautika Composition</th><th colspan="4">Probable actions viz. Vishaghna Karma (Anti-toxic Action) and Tvachya Karma (Action on Skin) as per the properties</th></tr>
<tr><th>Devdaru</th><th>Sarshapa</th><th>Mustaka</th><th>Navaneeta</th></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Teekshna Guna = Teja</td><td>-----</td><td>√</td><td>-----</td><td>-----</td></tr>
<tr><td colspan="4">Karma – Lekhana, Stroto Shodhana</td></tr>
<tr><td rowspan="2">2</td><td rowspan="2">Mridu Guna = Akash + Jala
Shlakshna Guna = Teja
Pichchhila Guna = Jala</td><td>-----</td><td>-----</td><td>-----</td><td>√</td></tr>
<tr><td colspan="4">Karma –
Mridu Guna – Tvak Maardavakar, Daaha-Paaka-Straavahar
Shlakshna Guna – Vrana Ropana, Dhaatu Vardhana
Pichchhila Guna - Vrana Ropana, Dhaatu Vardhana, Sandhaanakar</td></tr>
</table>

In this *Lepa* formulation, only *Sarshapa* demonstrates the *Teekshna Guna*, which is the most important property of *Bhallataka* as well as any *Visha*. Therefore, *Sarshapa* appears to aid the toxic action of *Bhallataka*. However, it may or may not be the case, as it is not used alone and a *Lepa* mixture is prepared by combination of other 3 ingredients. In this combination, specifically *Navaneeta* carries the properties like *Mridu*, *Shlakshna* and *Pichchhila*; which acts opposite to the *Teekshna Guna* and nullify it. Therefore, this complete formulation might not show the *Teekshna Guna* of *Sarshapa* to much extent and can overall demonstrate the anti-toxic and wound healing properties of *Mridu*, *Shlakshna* and *Pichchhila Guna* against *Bhallataka* toxicity.

Table No. 76 – *Samprapti Bhedana* by *Rasa* of *Dravyas* against *Bhallataka*

Sr. No	Properties and their *Bhautika* Composition	Probable actions viz. *Vishaghna Karma* (Anti-toxic Action) and *Tvachya Karma* (Action on Skin) as per the properties			
		Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
1	*Madhura Rasa = Prithvi + Jala*	-----	-----	-----	√
		Karma –Varnya, Tvachya, Sandhaanakara, Sarva Dhaatu Vardhana, Daaha Shamana, ***Visha Prashamana***			
2	*Katu Rasa = Vaayu + Teja*	-----	√	√	-----
		Karma – Lekhana, VranaShodhana, Kushthaghna, Krimighna, ***Visha Prasaadana***			
3	*Tikta Rasa = Vaayu +Akash*	√	√	√	-----
		Karma – Lekhana, Kledopashoshana, Vrana Shodhana, Kushtha-Kandughna, Tvak-Maasa Prasaadana, Krimighna, ***Vishahara***			
4	*Kashaaya Rasa = Prithvi + Vaayu*	-----	-----	√	-----
		Karma – Sandhaanakara, Lekhana, Vrana Peedana, Vrana Ropana, Kledopashoshana, Tvak Prasaadana			

Taking the *Rasa* (Taste) of all the ingredients into consideration, it's observed that most of the contents are of *Tikta Rasa* and *Katu Rasa*. Both of these *Rasa* are *Vishaghna* (Anti-toxic). Even *Madhura Rasa* of *Navaneeta* is *Visha Prashamana* (Pacifier of Toxins). Therefore, all these *Rasa* can act in the anti-toxic action of this *Kalpa* against *Bhallataka* toxicity. Also, all these 3 *Rasa* as well as the *Kashaaya Rasa* of *Mustaka* can demonstrate many such *Karma* (Actions) which are beneficial for skin and wound healing. Therefore, all these 4 *Rasa* present in this *Lepa* appear to have an important role in its effectiveness against local toxicity of *Bhallataka*.

Table No. 77 – *Samprapti Bhedana* by *Veerya* of *Dravyas* against *Bhallataka*

Sr. No	Properties and their *Bhautika* Composition	Probable actions viz. *Vishaghna Karma* (Anti-toxic Action) and *Tvachya Karma* (Action on Skin) as per the properties			
		Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
1	*Ushna Veerya = Teja*	√	√	-----	-----
		Karma – Vrana Paachana			
2	*Sheeta Veerya = Jala*	-----	-----	√	√
		Karma – Sarva Dhaatu Vardhana, Daaha Shamana			

Out of the four contents of this *Lepa*, two ingredients have *Ushna Veerya* (Hot Potency) while the other two have *Sheeta Veerya* (Cold Potency). Therefore, they seem to compensate for each other and the *Ushna Veerya* is nullified, so that it doesn't exaggerate the *Ushna Guna* of *Visha*. Even if *Ushna Veerya* somehow shows its action, it can help in healing of wound by its *Vrana Paachana Karma*. On the other hand, *Sheeta Veerya* can give a symptomatic relief by *Daaha Shamana* and complete relief from *Visha* by *Sheeta Guna* and heal the wound by *Sarva Dhaatu Vardhana Karma*.

Table No. 78 – *Samprapti Bhedana* by *Vipaaka* of *Dravyas* against *Bhallataka*

Sr. No	Properties and their *Bhautika* Composition	Probable actions viz. *Vishaghna Karma* (Anti-toxic Action) and *Tvachya Karma* (Action on Skin) as per the properties			
		Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
1	*Katu Vipaaka = Teja + Vaayu + Akasha*	√	√	√	-----
		Karma – Lekhana, Kledopashoshana, Vrana Shodhana-Ropana			
2	*Madhura Vipaaka = Prithvi + Jala*	-----	-----	-----	√
		Karma – Varnya, Tvak Prasaadana, Vrana Ropana, Sarva Dhaatu Vardhana, Sandhaanakara			

As we have already discussed that, *Visha* quickly spreads in the whole body and there's no chance for its digestion by *Jatharagni* (Digestive Fire) or *Tvak sthita Agni (Bhraajaka Pitta)* in case of local toxicity; therefore, *Vipaaka* of *Bhallataka* doesn't seem to have any effect in its toxic action, because it is the final biotransformation after metabolism of any *Dravya*. Similarly, in case of the *Lepa* formulation, the locally applied drugs also might not develop a full-fledged *Vipaaka* (Final transformed state) as in case of orally ingested *Dravya*; because the digestion by *Tvak sthita Agni (Bhraajaka Pitta)* might not be as profound as in case of *Jatharagni* (Digestive Fire).

However, if we consider *Madhura Vipaaka* or *Katu Vipaaka* of *Bhallataka*, the combination of both *Madhura Vipaaka* and *Katu Vipaaka* drugs in *Daru-Sarshapa-Mustadi Lepa* can nullify the effect of *Vipaaka* of *Bhallataka*. Both these *Vipaaka* can aid in wound healing as well by their *Vrana Ropana Karma*.

Table No. 79 – Assessment of *Panchabhautika* composition of ingredients of *Lepa*

	Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
Overall *Bhautika* Composition (Percentage)	***Akash* – 30 %** ***Vaayu* – 30 %** ***Teja* – 30 %** *Jala* – 10 % *Prithvi* – Nil	***Akash* – 23.07** ***Vaayu* – 30.76** ***Teja* – 38.46** *Jala* – 7.69 *Prithvi* – Nil	***Akash* – 20 %** ***Vaayu* – 40 %** ***Teja* – 20 %** *Jala* – 6.66 % *Prithvi* – 13.33 %	*Akash* – 8.33 *Vaayu* – Nil *Teja* – 8.33 ***Jala* – 58.33** ***Prithvi* – 25**

Taking the *Bhautika* composition of all these 4 ingredients into consideration, we can observe that all 3 herbal components show predominance of *Akash*, *Vaayu* and *Teja Mahabhuta* only, which are similar to those present in *Bhallataka*. Therefore, these 3 can actually aid in the toxic action of *Bhallataka* at the *Bhautika* level. However, all these 3 herbal components are intermixed with *Navaneeta* to form the *Lepa* preparation, which is dominantly rich with *Jala* and *Prithvi Mahabhuta*. Therefore, the whole formulation appears to be an even combination of all the 5 *Mahabhutas* and this *Panchabhautika Kalpa* can be used to reduce the dominance of *Akash*, *Vaayu* and *Teja Mahabhuta* caused by local toxicity of *Bhallataka*.

Table No. 80 – Assessment of *Doshaghnata* of ingredient drugs of *Lepa*

	Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
Doshaghnata	*Vaataghna* ***Pittakara*** *Kaphaghna*	*Vaataghna* ***Pittakara*** *Kaphaghna*	***Vaatakara*** *Pittaghna* *Kaphaghna*	*Vaataghna* *Pittaghna* ***Kaphakara***

If we assess this formulation at the level of *Dosha*, we find that 3 of the ingredients are *Vaataghna*, which can pacify the *Vaata Vriddhi* if any, though *Bhallataka* itself is a good *Vaatahara Dravya* and therefore, doesn't cause a rise of *Vaata Dosha*. Two of the ingredients of *Lepa* are *Pittakara*, while the other two are *Pittaghna*, which can compensate for each other and can also compensate for the *Pitta Vriddhi* caused by *Bhallataka Visha*. *Navaneeta* present in this *Kalpa* is specifically *Kaphakara*; and therefore, it can cure the *Kapha Kshaya* caused by toxic action of *Bhallataka*.

Table No. 81 – Assessment of *Karma (Action)*of ingredient drugs against *Bhallataka*

	Devdaru	***Sarshapa***	***Mustaka***	***Navaneeta***
Karma (Action)	*- Vrana Straavahara* *- Vrana Durgandhihara* *- Krimighna* *- Shothahara* *- Vedanaa Sthapana* *- Vrana Shodhana-Ropana* *- Rakta Prasaadana*	*- Kushthaghna* *- Kandughna* *- Jantughna* *- Vedanahara* *- Varnya*	*- TvagDoshahara* *- Kandughna* *- Shothahara* *- Jantughna* *- Rakta Prasaadana*	*- Varnya* *- Vaata-Pitta-Rakta Vikaara Naashaka* *- Vranaapaha* *- Ojo Vardhak*

The *Karma* (Actions) of all these 4 ingredients appear to provide either a symptomatic relief from the pain, edema of the injury or can aid in the healing of the wound. The *Ojo Vardhak* property of *Navaneeta* can specifically compensate for the *Ojo Visransa* caused due to *Bhallataka*. Therefore, all the contents of this *Lepa* seem to be beneficial for the dermatological manifestations of local toxicity of *Bhallataka* and in this way, the overall *Kalpa* i.e. *Daru-Sarshapa-Mustadi Lepa* seems to achieve the *Bhedana* of *Bhallataka Visha Samprapti*. Therefore, in order to establish this mechanism of *Samprapti Bhedana* scientifically, the present experimental research has been carried out.

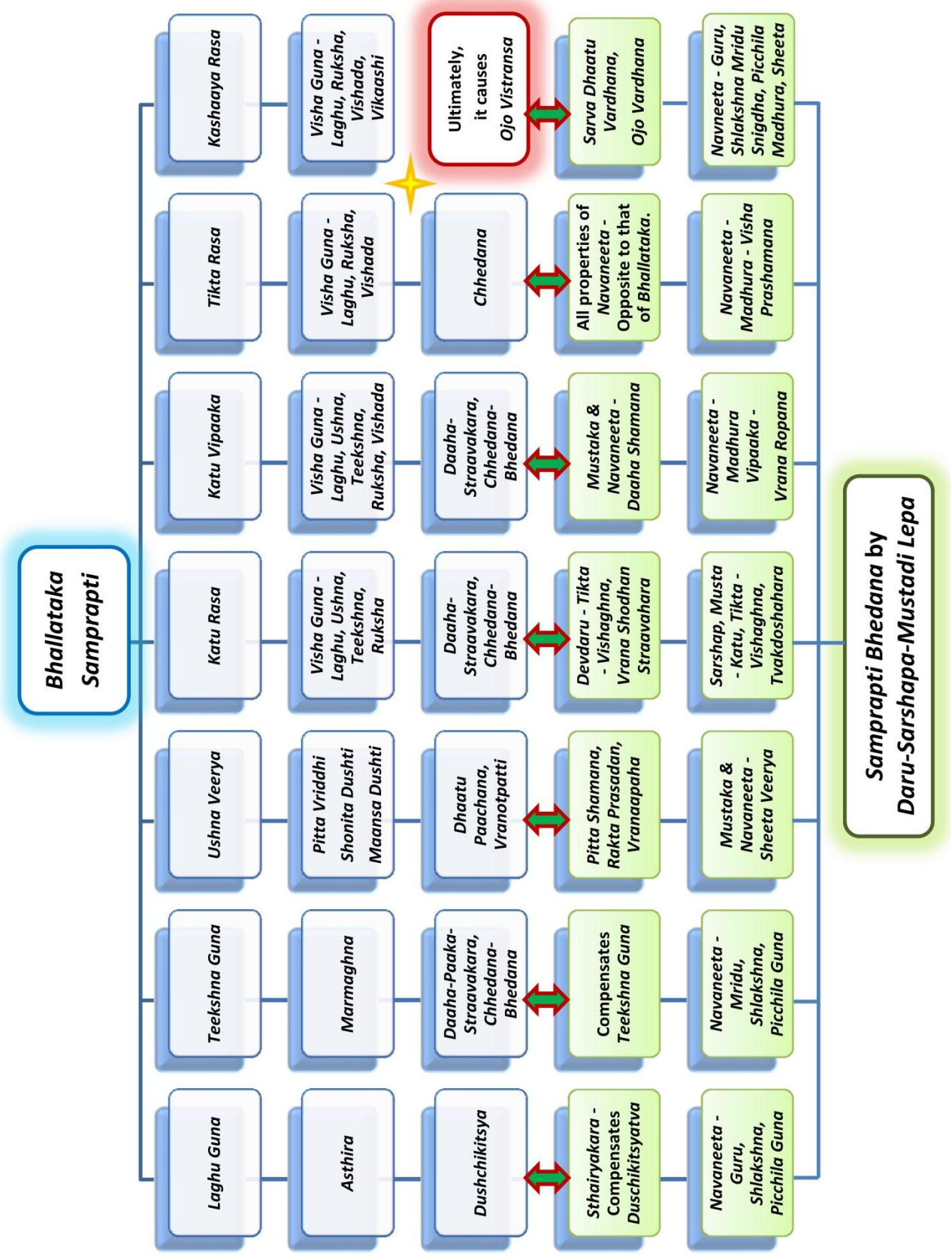


Figure 19 – Flow Chart about Mechanism of *Bhallataka Visha Samprapti Bhedana*

DISCUSSION OF EXPERIMENTAL ASPECTS

After completion of the research work, it's important to discuss some practical difficulties encountered during the study and also their remedial measures. The important results also need to be interpreted, along with the discussions about shortcomings of the study and future directions for further advancement in this area.

(A) PRACTICAL DIFFICULTIES DURING THE STUDY –

1) The first and foremost difficulty was the extraction of *Taila* (Oil) from *Bhallataka*. Initially, the *Bhallataka Taila* (Oil) was proposed to be extracted by heating the *Bhallataka Beeja* directly over a flame. However, the yield of oil seemed lesser by this method, when undamaged nuts were exposed to the flame. Therefore, a few pricks were made in the marking nuts with the help of a large-bore needle and then they were held over the flame. After using this technique, there was a remarkable improvement in the yield of the oil. Such minor modification in the initial technique was proven very beneficial for extraction of *Bhallataka Taila* (Oil), which can provide a guideline for the further researchers by simplifying the process of oil extraction.

   Though there was another method available, that was used in the earlier research i.e. steaming the *Bhallataka* for one minute in *Swedana Yantra*, then cutting it into two pieces and squeezing it to extract the sap. Also, there's another standard method for the oil extraction of *Bhallataka* making use of *Paataala Yantra*, which is mainly used in the *Ayurvedic* pharmacies for extracting *Taila* for the manufacturing of pharmaceutical products. However, as the quantity of *Bhallataka Taila* required was very small (Only 0.1 ml per patch; therefore, 4.2 ml for 42 patches in 21 mice), the simple method of extraction of *Bhallataka Taila* (Oil) over the flame was well sufficient for the purpose of this research work.

2) As there is no mention of *Pramaana* (proportion) of contents of '*Daru-Sarshapa-Mustadi Lepa*' in *Anupan Manjari*, all the contents were mixed in equal quantity by considering "*Anukta Pramaana Grahana Vidhi*" suggested by *Acharya Sharangdhar*. But it was observed that, as the dry *Churna* (Powder) of *Devdaru*, *Sarshapa* and *Mustaka* absorb the unctuousness of *Navaneeta*, the mixture appears dry, brittle and non-sticky at the beginning and cannot be retained on the skin as a *Lepa* for a longer duration. However, when the *Lepa* was subjected to the vigorous activity of *Mardana Sanskaara*, it was observed that the particles of all the *Churna* become finer, which makes it smoother. As all the three *Churna* contain some proportion of oil as well, it gets released from the powdered particles, which makes the mixture more slimy and sticky. Therefore, in order to improve the consistency of *Lepa*, vigorous activity of *Mardana* is very essential and the time required for this activity can be decided by assessing the consistency of *Lepa* only.

3) The schedule for application of *Lepa* was planned in such a way that, the lesion would not be continuously covered by *Lepa* only. The *Lepa* was kept applied for 8 hours before washing it away with plain water. Usually the *Lepa* is removed when it dries up; however, the '*Daru-Sarshapa-Mustadi Lepa*' would always remain moist and won't dry up unlike the other *Lepa*, as it contains *Navaneeta*. Therefore, if this *Lepa* is continuously kept applied over the lesion, then the moisture will lead to the putrefaction of skin. Even *Ayurveda* has described this phenomenon of formation of *Kleda* due to continuous dampness. Therefore, in view of this possible occurrence of *Klinna Vrana* (Moist wound), lesions were kept open to dry up for the next 4 hours.

4) As swiss albino mice are animals having fur, the removal of hairs from their back was essential before the local application of *Bhallataka Taila*. The simplest method for hair removal is making use of depilatory cream. However, the residual effect of the chemicals present in the depilatory cream like Barium sulphide, Thallium etc. would have interfered with the local action of *Bhallataka Taila*, as we needed to assess the dermal side-effects of *Bhallataka*. Even as a rule of thumb in dermal toxicity studies, use of chemicals should be stopped 7 days before the initiation of the experiment.

Therefore, this method was avoided for removal of hairs and mechanical methods were preferred. Out of the two mechanical methods viz. trimmer and shaving with Blade Razor, trimmer was also avoided, as it wouldn't have been able to provide a clean surface, as some little stalks of hairs are left behind on the skin. Therefore, in order to obtain a clear surface of skin without any chemical interference, the mechanical method of shaving with Blade Razor was utilized. However, more time and skill was required to shave off the soft hairs of the mice, even in the anesthetized animals.

5) The anesthesia was induced to the mice by the method of inhalation of ether in a glass chamber. It is a standard method, which is practiced in most of the animal experiments. However, during the induction of anesthesia, one mouse was found dead inside the glass chamber only, probably due to the overdose of ether. It was replaced with another healthy mouse. Therefore, ether should always be used in a proper dose, as the overdose can prove fatal for the animals. On the other hand, an insufficient dose will lead to pain and sufferings to the animals and it will be very difficult to handle the mice.

6) *Shatadhauta Ghrita* is a popular formulation among the *Ayurvedic* community, and is a very good remedy for the treatment of burns, wounds and various skin diseases caused due to aggravation of *Pitta Dosha*. The local side-effects of *Bhallataka* also seem to manifest as chemical burns, open wounds and other dermal manifestations caused by *Pitta Vriddhi*. Therefore, *Shatadhauta Ghrita* appears to possess a great amount of potential to pacify the local side-effects of *Bhallataka Taila*.

In the earlier research work titled "An experimental study to evaluate the efficacy of *Shatadhauta Ghrita* application in contact poisoning caused by *Bhallataka Beeja*" conducted by *Dr. Sruthy Das*, *Shatadhauta Ghrita* was found effective in the healing of wound caused by *Bhallataka*. In this study, the efficacy of *Shatadhauta Ghrita* was found to be even more superior to the reference standard drug "Silver nitrate". Therefore, *Shatadhauta Ghrita* was selected for the present study.

(B) INTERPRETATION OF IMPORTANT RESULTS -

1) **Mortality -** During the present study, 100% mortality was observed in Group 1, where all the 3 Albino Mice died. As no treatment was given to these animals, local application of *Bhallataka Taila* might have induced its toxicity at the systemic level through absorption, resulting in the mortality; though period of survival was variable.

   Even 1 Mouse out of the 3 in Group 7 (Only *Navaneeta Lepa*) was found dead after 5 days. As it was observed that, the solitary use of *Navaneeta* in these animals led to unhealed *Klinna Vrana* (Moist wound); it appears to have somehow failed to counterbalance the local toxicity of *Bhallataka Taila*, the exact mechanism for which may not be possible to suggest.

   Bhallataka is a well-known toxin and has the potential to cause death due to its local toxicity; as observed in the cases reported during literary review, though the cases are very rare in human beings. However, the dose applied in the present study i.e. two patches of 0.1 ml *Bhallataka Taila* in each Albino Mouse might have proven fatal in the untreated and complicated groups of mice.

   Another observation in the expired mice was that, 3 out of the 4 mice which died were females. So, it can be a possibility that females might be more susceptible to the local toxicity of *Bhallataka* or probably the females could not tolerate the toxicity as good as the males; because the male mouse died on the 7^{th} day, while the female mice died much earlier within first 5 days only. However, there's no concrete data to prove this observation and it can be considered as a speculation only.

2) **Erythema -** Among the signs of local side-effects, erythema i.e. redness of skin was the most prominent sign developed in the mice and was most consistent in almost all the experimental animals. It was observed as the earliest sign due to increased blood flow, because of increased capillary permeability at the site of acute inflammation. However, erythema could be observed only at the borders of lesions, as the visibility was obstructed due to dark discoloration caused by *Bhallataka Taila* at the beginning and dry brown scab/eschar formation after deposition of exudates at the later stages.

3) **Edema -** The swelling was observed as an increased thickness of skin and was perceived by visualization as well as palpation. The formation of papules, vesicles (Small blisters) and bullae (Large blisters) were also visible only at the borders of the lesions due to the dark discoloration and scab/eschar formation. Therefore, a careful observation was required, with the help of a magnifying glass, in order to identify the details of these lesions.

4) **Formation of Blisters and Ulceration -** The ulcers were visible only when the scab fell off, because the exudates from the ulcers covered and protected them during the initial period. However, once the scab fell off, the raw area under the scab/eschar was visible in the form of open wounds. The exudates again formed another scab obstructing the visibility of the wound and only papules, vesicles or bullae were visible again at the border of lesion. Therefore, there was waxing & waning of the visibility of ulcers & wounds; and therefore, score for formation of blisters & ulceration was also seen fluctuating between 1-2-3 and 4.

5) **Other Symptoms -** Apart from the above three signs, other symptoms like Pruritus (Itching), Burning sensation and Pain cannot be practically recorded, as the available resources prove insufficient for the assessment and measurement of these symptoms. Though these symptoms can be inferred from the increase in writhing and irritability of the animals and also by using some standardized systems like Mouse Grimace Scale, which is used to assess the post-operative pain in the animals on the basis of Orbital Tightening, Nose Bulge, Cheek Bulge, Ear position and Whisker Change.

However, it's not easy to recognize and quantify the pain suffered by mice, as these animals are always frightened due to the fear of falling prey to other animals, and therefore show very little signs of pain, sufferings or deformities. They usually hide the signs of mild or moderate pain during the animal experiments in the presence of human beings. Also, mice have a well-developed stress-induced analgesic system, which lowers their response to the pain suffered.[101] Therefore, the symptoms like Pruritus (Itching), Burning sensation and Pain were not assessed in the present study.

6) **Efficacy of Constituents of Experimental *Lepa* -** It was observed during the present study that the Group 4 (*Devdaru + Navaneeta Lepa*) has better results even than the Group 3 (*Daru-Sarshapa-Mustadi Lepa*), although all the ingredients of Group 4 are present in Group 3 as well. This might be due to the presence of *Sarshapa* having *Ushna-Teekshna Guna*, which could have resulted in contradiction to the healing action of all the other ingredients.

As *Sarshapa* possesses *Ushna Veerya* (Hot potency) and *Teekshna Guna* (Sharp and Piercing attributes) similar to those of *Bhallataka*, the Group 5 (*Sarshapa + Navaneeta Lepa*) has shown lesser efficacy than the other groups of constituent drugs viz. Group 4 (*Devdaru + Navaneeta Lepa*) and Group 6 (*Mustaka + Navaneeta Lepa*). Moreover, it has also shown an increase in the formation of Blisters and Ulceration due to its *Ushna* and *Teekshna Guna.*

7) **Efficacy of *Shatadhauta Ghrita Lepa* -** The Group 2 (*Shatadhauta Ghrita Lepa*) of the present experiment has been proven less effective than the experimental drug i.e. '*Daru-Sarshapa-Mustadi Lepa*' in the quest to reduce the local side-effects of *Bhallataka Taila*. During some observations, it was found that, even though *Shatadhauta Ghrita Lepa* reduces some signs like Edema and heals the Blisters and Ulceration, the results are not statistically significant.

So, these results might raise a question about the efficacy of *Shatadhauta Ghrita* as a Control, which was advocated by the results of an earlier research work titled "An experimental study to evaluate the efficacy of *Shatadhauta Ghrita* application in contact poisoning caused by *Bhallataka Beeja*" conducted by *Dr. Sruthy Das*.

However, it should be noted that the above-mentioned research was an extensive experiment for 24 days, which had shown good results of *Shatadhauta Ghrita* application in contact poisoning of *Bhallataka Beeja* with complete healing of wound after 21 days. As the present research work was an experiment of 7 days only, it seems very acceptable that the *Shatadhauta Ghrita Lepa* couldn't demonstrate as good results as seen in the earlier research work.

8) **Efficacy of Only *Navaneeta Lepa* -** As the possible occurrence of *Klinna Vrana* (Moist wound) was expected, the lesions were not covered by *Lepa* continuously and were kept open to dry up for 4 hours. However, even after taking such precautions, the lesions of mice in the Group 7 (Only *Navaneeta Lepa*) appeared to be wet. These lesions did not form a hard, dry, brown scab; but only formed light, yellowish crust over the lesions, which kept on shedding and wound remained clammy and unhealed.

The reason behind this appeared to be the *Klinnatva* caused due to *Navaneeta*, which delayed the healing of the wound. *Ayurveda* describes the phenomenon of formation of *Kleda* due to continuous dampness, which leads to the putrefaction of skin. It's a scientifically established fact that, the excessively wet skin exposes it to the risk for developing maceration as well as infections. Therefore, the skin should be subjected to an optimum level of moisture only.[102]

The extremely moist conditions like application of moist *Lepa*, such as the use of *Navaneeta* alone has been observed to be interfering with the healing of the wounds, as evidenced in the Group 7. However, *Lepa* of *Navaneeta* accompanied with *Churna* of other herbal drugs, as applied in the Groups 3, 4, 5, and 6 did not produce *Kleda*, as these *Lepa* were not excessively moist. The dry *Churna* (Powder) of other herbs absorbed the *Klinnatva* of *Navaneeta*; and therefore, all these four *Lepa* were in a moderately moist form, which didn't generate *Kleda* and healing of the lesions was found good in these four groups. On the other hand, use of *Navaneeta* alone, being excessively moist, was found to cause a *Klinna Vrana* (Moist wound).

(C) SHORTCOMINGS OF THE STUDY –

1) **Small Sample Size -** As per rules and regulations of SPCA (Society for Prevention of Cruelty to Animals), only six animals should be included in each group. The same regulations have also been reiterated by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and IAEC (Institutional Animal Ethics Committee) of IDRAL (Indian Drug Research Association & Laboratory) Pune as well, while approving the present study vide Research Project no. 294 T-17.

These rules and regulations have been formulated to ensure that a minimum number of animals should undergo the experiment and they shouldn't be subjected to any pain and sufferings unnecessarily. With a view to help in this good cause, it was planned to induce 2 lesions on the back of each mouse. Thus, only 3 animals were required for every group and the total number of animals was substantially reduced to 21 rather than 42. However, due to a smaller sample size, there might be some chances of error, as the case is in almost all the animal studies.

(D) FUTURE DIRECTIONS FOR FURTHER RESEARCH –

1) During the present research work, 100% mortality was observed in Group 1, where all the 3 Mice died without treatment & 1 Mouse in Group 7 (Only *Navaneeta Lepa*) was also found dead after 5 days. As the fatal effect of local toxicity of *Bhallataka Taila* has been witnessed during the present study, further LD50 study of local toxicity of *Bhallataka Taila* should be performed to find out the median lethal dose.

2) As the efficacy of '*Daru-Sarshapa-Mustadi Lepa*' has been proven against the local side-effects of *Bhallataka Taila* in the experimental animals through the present research work, further clinical trials can be conducted in order to investigate its effects in human participants.

   During this experimental study, no harmful effects of '*Daru-Sarshapa-Mustadi Lepa*' were noted. Thus, the safety profile of this drug formulation has also been established through the present study and therefore, it can be safely utilized for further researches in human beings.

3) The use of *Sarshapa* in the '*Daru-Sarshapa-Mustadi Lepa*' has been observed to intervene the healing action of other ingredients of *Lepa* due to its *Ushna* and *Teekshna Guna*. Therefore, only "*Daru-Musta-Navaneeta Lepa*" can be tried and tested in future by omitting *Sarshapa*, which can give better results in local side-effects of *Bhallataka*.

4) Recently, newer advancements of technology have created a paradigm shift in the process of drug discovery and development. A Reverse Pharmacology (RP) approach is now-days used to evolve the New Chemical Entities (NCE) from traditionally prescribed treatment remedies.

Network pharmacology (NP) is another novel tool, which is used to understand the putative actions, indications and mechanisms of botanical bio-actives. It tries to establish the safety and efficacy of the *Ayurvedic* drugs, with an aim to put forth an evidence-based science of *Ayurveda*.[103]

Therefore, there's an immense scope for further research to be carried out in this direction and the effects of '*Daru-Sarshapa-Mustadi Lepa*' can be further revealed and its exact mode of action can be explored using the newer advances of Network Pharmacology and Reverse Pharmacology approach.

5) As *Anupan Manjari* has described some very brief formulations for treatment of various *Visha* (Poison), which are extremely simple and practically useful; the other formulations mentioned in the text of *Anupan Manjari* can also be tried and tested on the same lines by undertaking the animal experiments. This can result into a great database about the evidence-based simplistic antidotes against various poisons.

6) During the present experimental study, '*Daru-Sarshapa-Mustadi Lepa*' was prepared fresh every day for application to the lesions inflicted by *Bhallataka Taila* in albino mice. Even according to the principles of *Ayurveda*, *Lepa* should be prepared fresh every time before application.

However, in today's era of busy life schedule, the patients seem to refrain from *Ayurvedic* medicines for the issue of time-consuming preparation methods. To solve this issue, the *Ayurvedic* pharmaceutical companies have come up with many *Ayurvedic* ointments, which are preserved for a longer period by using preservatives like Sodium benzoate, Stearic acid, Methyl paraben, Propyl paraben etc. Therefore, the *Ayurvedic* ointments can now be packaged and marketed to the consumers easily.

The same marketing principle can be utilized for the propagation of '*Daru-Sarshapa-Mustadi Lepa*' as well and it can be packed and traded for the convenience of the patients. However, there will be a need to find a suitable method of preservation for this particular *Lepa*, as every ointment needs different kind of preservatives according to its specific ingredients. Therefore, from the viewpoint of the marketing strategy, the research about suitable preservatives can be a future addition to the present research work.

7) Urushiol is the main chemical constituent present in all the plants of Anacardiaceae family, which is responsible for the appearance of contact dermatitis caused due to these plant products. As '*Daru-Sarshapa-Mustadi Lepa*' has been proven effective in the Urushiol-induced local side-effects of *Bhallataka Taila* in the present study, these results can be further extended and its efficacy against the Urushiol-induced allergic rashes caused due to other plants of the same family like Poison oak, Poison ivy etc. which are more prevalent in some areas like United states, can also be studied.

CONCLUSION

CONCLUSION

The present in-vivo study validates the claim of the classical text named *"Anupan Manjari"* about the efficacy of the experimental drug "*Daru-Sarshapa-Mustadi Lepa*" to be effective against the locally expressed adverse effects of the *Taila* (Oil) of *Bhallataka* (Semecarpus anacardium) in the experimental conditions.

As a result, the topical use of the experimental drug "*Daru-Sarshapa-Mustadi Lepa*" can be recommended for further investigations through clinical trials, in order to study its effects in the human population as well.

SUMMARY

SUMMARY

The present Experimental research work, which was performed to ascertain the efficacy of the experimental drug "*Daru-Sarshapa-Mustadi Lepa*" against the locally expressed adverse effects of *Taila* (Oil) of *Bhallataka;* has been divided into Introduction, Review of Literature, Aims and Objectives, Research Methodology, Observations, Analysis, Interpretation, Discussion, Conclusion and Summary in the end.

The aim and objectives of the study were determined in order to firstly assess and then correlate the locally expressed adverse manifestations of *Taila* (Oil) of *Bhallataka.* The study was mainly intended to investigate the efficacy of the experimental drug i.e. *Daru-Sarshapa-Mustadi Lepa* and every single component of this *Lepa* as well against these locally expressed adverse effects of *Taila* (Oil) of *Bhallataka* (Semecarpus anacardium) in albino mice.

The review of literature was done regarding the toxin "*Bhallataka*", especially for its local side effects, the experimental drug formulation "*Daru-Sarshapa-Mustadi Lepa*" along with its all four ingredient drugs and the *Lepa Kalpana* as mentioned in the *Ayurvedic* literature. Various ancient *Samhita Grantha*, contemporary textbooks, journals and websites related with these topics have been reviewed for this purpose.

All the herbal raw drugs were procured from a well-known Herbal drugs provider and later authenticated and standardized at a research laboratory. *Navaneeta* was purchased from a renowned Dairy and Standardization certificate was obtained. *Shatadhauta Ghrita* was obtained from a popular Pharmacy and its Standardization certificate was also obtained.

The *Bhallataka Taila* (Oil) was extracted by heating it over a flame. '*Daru-Sarshapa-Mustadi Lepa*' was prepared by mixing all ingredients in equal proportions and utilizing the standard *Lepa* preparation method. Both the *Taila* and *Lepa* were standardized in the research lab before their use in the experimental study.

21 Swiss albino mice were selected for the study and were divided into 7 groups of 3 mice each. The hairs were removed from their back mechanically, in the form of two patches. On next day, 0.1 ml *Bhallataka Taila* was applied on each patch of 1 cm diameter and the Swiss albino mice were monitored over a day (Twenty-Four hours) to study the pattern of occurrence of adverse manifestations locally.

The first group (Normal group) was spared from any treatment, while the second group (Control group) was treated with '*Shatadhauta Ghrita Lepa*'. The third group was tested with '*Daru-Sarshapa-Mustadi Lepa*' and next four groups were experimented with the four contents of the *Lepa* separately. The treatment was given 12 hourly, keeping the *Lepa* for 8 hours and keeping the lesion open for next 4 hours. Daily observations were made for 7 days to assess erythema, edema and formation of blisters and ulceration. Histopathological examination was also performed at the end.

All observed data was arranged in statistical tables and was subjected to statistical analysis in order to compare all the seven groups and to draw the conclusion. Further, diagrams and charts were drawn to signify the important findings. As the efficacy of all the seven groups has been found to be variable, the significant results have been compiled under the "Results" section.

The discussion has been put forth from two angles, the *Ayurvedic* aspects and the Experimental aspects. Firstly, the *Visha Samprapti* of *Bhallataka* has been investigated to find the mechanism of its toxicity from an *Ayurvedic* perspective. Later, the mechanism of pharmacological action *(Samprapti Bhedana)* by the experimental drug i.e. *Daru-Sarshapa-Mustadi Lepa* as well as every single component of this *Lepa* has been ascertained against the local side-effects of *Bhallataka*. In the second half of discussion, some practical difficulties encountered during the study were discussed along with the adopted counter-actions to solve these difficulties. The significant results were deduced to draw key inferences and critical discussions were propounded to discourse the inadequacies and limitations of the present research work. Also, the future directions for further advancement in this area have been discussed.

Finally a conclusion was drawn that "the experimental drug named *Daru-Sarshapa-Mustadi Lepa* shows significant efficacy against the locally expressed adverse effects of the *Taila* (Oil) of *Bhallataka* (Semecarpus anacardium) in the experimental animals viz. Swiss albino mice".

However, "*Devdaru* + *Navaneeta Lepa*" has shown better results than the complete formulation, as *Sarshapa* added as an ingredient in '*Daru-Sarshapa-Mustadi Lepa*' was noticed to cause a hindrance in the curative effect of other three constituents, which can be specifically attributed to the *Ushna Veerya* as well as the *Teekshna Guna* of *Sarshapa Beeja Churna*. For this reason, further researches can be conducted by omitting *Sarshapa*, using "*Daru-Musta-Navaneeta Lepa*" only.

APPENDICES

- ***REFERENCES***
- ***BIBLIOGRAPHY***

REFERENCES

[1] Dr. Achal AP. Agadtantra (Ayurvediya Visha Vijnana). 4th ed. Varanasi: Chaukhamba Surbharati Prakashan. 1998. p. 1.

[2] Acharya Shri Vishram. Anupan Manjari. 1st Edition. Jamnagar: Sahitya Sanshodhana Vibhagiya Prakashan (GAU). 1972. p. 14.

[3] Prof. Sharma PV. Dravyaguna Vijnana - Volume II. Reprint ed. Varanasi: Chaukhamba Bharti Academy. 2005; p. 75, 152, 166, 370.

[4] Chunekar KC, Pandey GS. Bhavaprakasha Nighant of Shri Bhavamishra. 10th ed. Varanasi: Chaukhamba Bharati Academy. 2010. p. 774.

[5] Singhal SK. Singhal's Toxicology at a glance. 8th ed. Mumbai: National Book Depot. 2014; p. 17.

[6] Illan chezhian R, Roshy Joseph C, Rabinarayan Acharya. Urushiol-induced contact dermatitis caused during Shodhana (Purification process) of *Bhallataka* (Semecarpus anacardium Linn) fruit. AYU 2012; Vol-33, Issue-2; p. 270-3. Available at http://www.ayujournal.org accessed on 4/4/2016.

[7] Dr. Reddy K.S.N. The Essentials of Forensic Medicine and Toxicology. 31st ed. New Delhi: Jaypee Brothers. 2012. p. 517.

[8] Article in 'The Hindu' Newspaper (Andhra Pradesh) dated 27th Sept 2009. Available at http://www.thehindu.com/todays-paper/tp-national/tp-andhrapradesh/expert-cautions-on-use-of-marking-nut/article192350.ece Accessed on 1/4/2016

[9] Dr. Anantram Sharma, Sushruta Samhita - Volume I. Reprint ed. Varanasi: Chaukhamba Surbharati Prakashan. 2012. p. 412.

[10] Bhatia K, Kataria R, Singh A, Safderi ZH, Kumar R. Allergic contact dermatitis by Semecarpus anacardium for evil eye: A prospective study from Central India. Indian J Basic Appl Med Res 2014; 3:122-7.

[11] Parikh CK. Parikh's Textbook of Jurisprudence, Forensic Medicine & Toxicology Reprint ed. New Delhi: CBS Publishers & Distributors; 2002. p. 9.35.

[12] Pandit K. Shastri, Dr. G. Chaturvedi. The Charaka Samhita of Agnivesha – Volume II. 22nd ed. Varanasi: Chaukhambha Bharati Academi. 1996. p. 1030.

[13] Dr. Anantram Sharma, Sushruta Samhita - Volume II. Reprint ed. Varanasi: Chaukhamba Surbharati Prakashan. 2012. p. 509.

[14] Kashinath Shastri. Rasa Tarangini of Sadananda Sharma. 11th ed. New Delhi: Motilal Banarasidas Publication; 2004. p. 648-649.

[15] Malik V. Drugs and Cosmetics Act 1940 with Drug and Cosmetic Rules. 18th ed. Lucknow: Eastern Book Co; 2005. p. 282.

[16] Dr. Anantram Sharma, Sushruta Samhita - Volume I. Reprint ed. Varanasi: Chaukhamba Surbharati Prakashan. 2012. p. 412.

[17] Panini. Ashtadhyayi (Panini Sutra) (Pa.Un.Bho. 2/2/42) [Internet] Available at https://avg-sanskrit.org/texts/astadhyayi/ accessed on 07/08/2018.

[18] Valmiki. Ramayana – Ayodhya Kaanda - II. (Chapter 56/Sutra 7) [Internet] Available on 07/08/2018 at http://www.valmikiramayan.net/utf8/ayodhya/sarga56/ayodhyaroman56.htm

[19] Dr. D. Sarvade et al. *Bhallataka* (Semecarpus Anacardium Linn.F.) - A Comprehensive Ayurveda Review. Int J of Adv Res, 4(6), 2016, 445-457.

[20] Definitions of *Bhallataka* [Internet]. Available on 14/08/2018 at https://www.wisdomlib.org/definition/*Bhallataka*

[21] R. Shamasastry. Kautilya Arthashaastra – Volume II – Book V-XV. Reprint ed. Varanasi: Chowkhamba Vidya Bhavan, 2010.

[22] Nene AS, Presentation about "Sustainable Materials and Construction Techniques of Ancient India" [Internet]. Available at https://www.slideshare.net/ashoknene/sustainable-building-materials-of-ancient-india accessed on 04/09/2018

[23] Yadavji Trikamji Acharya. Charaka Samhita. Reprint ed. Varanasi: Chowkhamba Krishnadas Academy; 2006. p. 382-383.

[24] Yadavji Trikamji Acharya. Sushruta Samhita. 1st ed. Varanasi: Chowkhamba Krishnadas Academy; 2004. p. 206.

[25] Athavale Anant Damodar. Ashtanga Sangraha (Indu Vyakhya). 1st ed. Pune: Sreemad Aatreya Prakashanam; 1980. p. 916.

[26] Harishastri Paradkar. Ashtanga Hridayam. 9th ed. Varanasi: Chaukhamba Orientalia. 2005. p. 928.

[27] JLN Shastri. Dravyaguna Vijnana - Volume 2. Reprint ed. Varanasi: Chaukhambha orientalia. 2008, Page 136.

[28] Uday Pawade, A Toxicological Review of *Bhallataka* (Semecarpus anacardium Linn), International *Ayurvedic* Medical Journal, 3(12), 2015, p. 2480.

[29] Illan chezhian R, Roshy Joseph C, Rabinarayan Acharya. Urushiol-induced contact dermatitis caused during Shodhana (Purification process) of *Bhallataka* (Semecarpus anacardium Linn) fruit. AYU 2012; Vol-33, Issue-2; p. 270-3.

[30] Chopra RN et al. Indigenous Drugs of India. 2nd ed. Kolkata: Academic publishers. 1994. p. 408.

[31] Singhal SK. Singhal's Toxicology at a glance. 8th ed. Mumbai: National Book Depot. 2014. p. 86.

[32] Dr. Anantram Sharma, Sushruta Samhita - Volume I. Reprint ed. Varanasi: Chaukhamba Surbharati Prakashan. 2012. p. 412.

[33] Illan chezhian R, Roshy Joseph C, Rabinarayan Acharya. Urushiol-induced contact dermatitis caused during Shodhana (Purification process) of *Bhallataka* (Semecarpus anacardium Linn) fruit. AYU 2012; Vol-33, Issue-2; p. 270-3.

[34] Article in 'The Hindu' Newspaper (Andhra Pradesh) dated 27th Sept 2009. Available on 1/4/2016 at http://www.thehindu.com/todays-paper/tp-national/tp-andhrapradesh/expert-cautions-on-use-of-marking-nut/article192350.ece

[35] Dr. Reddy K.S.N. The Essentials of Forensic Medicine and Toxicology. 31st ed. New Delhi: Jaypee Brothers. 2012. p. 517.

[36] Bhatia K, Kataria R, Singh A, Safderi ZH, Kumar R. Allergic contact dermatitis by Semecarpus anacardium for evil eye: A prospective study from Central India. Indian J Basic Appl Med Res 2014; 3:122-7.

[37] Pandit K. Shastri, Dr. G. Chaturvedi. The Charaka Samhita of Agnivesha – Volume I. 22nd ed. Varanasi: Chaukhambha Bharati Academi. 1996. p. 238.

[38] Kashinath Shastri. Rasa Tarangini of Sadananda Sharma. 11th ed. New Delhi: Motilal Banarasidas Publication; 2004. p. 474-476.

[39] Venkatachalam K, Asha S, Subhashini C, et al. Allergic contact dermatitis due to Semecarpus anacardium. J Evolution Med Dent Sci 2017; 6(92):6655-6657.

[40] Ghorpade A. Contact dermatitis caused by Indian marking nut juice used to relieve ankle pain. Int J Dermatol 2014; 53:117-9.

[41] Parikh CK. Parikh's Textbook of Jurisprudence, Forensic Medicine & Toxicology Reprint ed. New Delhi: CBS Publishers & Distributors; 2002. p. 9.35.

[42] Verma Prashant, Chhabra Namrata, Sharma Reena. Severe Marking-Nut Dermatitis. Dermatitis 2012; Vol-23, Issue-6; p. 293–294.

[43] Bhushan Patwardhan, MN Saraf, SB David. Toxicity Of Semecarpus Anacardium Extract. Anc Sci of Life 1988; Vol-8, No-2; p. 106-9.

[44] Livinghood CS, Rogers AM, Fitz-Hugh T. Dhobi mark dermatitis. J Am Med Assoc 1943; 123:23-6.

[45] Ghorpade A. Contact dermatitis caused by Indian marking nut juice used to relieve ankle pain. Int J Dermatol 2014; 53:117-9.

[46] Dr. Vanarase Mithila, Dr. Gadekar Jayant. Allergic Contact Dermatitis (ACD) Presenting as Cellulitis an Unusual Presentation Secondary to Marking Nut Application. Int J of Sci and Res 2019; Vol-8, Issue-4; p. 1321-3.

[47] Verma Prashant, Chhabra Namrata, Sharma Reena. Severe Marking-Nut Dermatitis. Dermatitis 2012; Vol-23, Issue-6; p. 293–294.

[48] Herath HM, Wazil AW, Ratnatunga NV, Badurdeen AS, Weerakoon KG. Could the latex of Semecarpus anacardium (Kiri Badulla) cause Nephrotic syndrome. Ceylon Medical Journal 2012; 57: 92-93.

[49] Matthai TP, Date A. Renal cortical necrosis following exposure to sap of the marking-nut tree. Am J Trop Med Hyg 1979; 28(4):773-4.

[50] Norman R. Goldsmith. Dermatitis from Semecarpus anacardium (Bhilawanol or Marking Nut) spread by contaminated mail. JAMA 1943; Vol-123, No-1; p. 27.

[51] Sateesh Kumar Reddy, Ushasree P, Shanmuga Kumar SD, Vijay Raghavendra NC, Faheemuddin MD. A Case Report on Semecarpus anacardium Induced Extensive Irritant Contact Dermatitis. J Basic Clin Pharma 2018; 9:106-108.

[52] Shankar DS. Contact urticaria induced by Semecarpus anacardium. Contact dermatitis 1992; 26:200.

[53] Cade A, Nelson CS. Semecarpus anacardium-induced Facial edema. Brit J of Dermatol 1996; 135:330-45.

[54] Ghorpade A. Contact dermatitis caused by Indian marking nut juice used to relieve ankle pain. Int J Dermatol 2014; 53:117-9.

[55] Ghorpade A. Tense bullae after Toxicodendron treatment for a twisted ankle. Clin Exp Dermatol 2010; 35:24-25

[56] Ghorpade A. Targetoid skin lesion with marking nut hair dye. Int J Dermat 2015; 54:1414-5.

[57] Singh SN, Bhattamishra AB, Gardin HK, et al. Irritant dermatitis to marking nut used as a hair dye. Indian J Dermatol 2000; 45:32-33.

[58] Ghorpade A. Voodoo dermatitis after an attempted voodoo cure for marking nut dermatitis. Int J Dermatol 2009; 48:663-5.

[59] Ghorpade A. Nutty dermatitis-marking-nut dermatitis after use as a home remedy. Clin Exp Dermatol 2009; 34:997-8.

[60] Hafejee A, Coulson IH, Lakshiminarasimhan P, Leon CJ. Traditional tattoo treatment trauma. Clin Exp Dermatol 2008; 33:536-7.

[61] Verma G et al. Airborne contact dermatitis apparently acquired as a result of using pericarp juice from Indian marking nut as a home remedy to treat patchy hair loss. Contact dermatitis 2019; Vol-81, Issue-3; p. 211-3.

[62] Jie Shen Fok, Anita Christine Kral, John Hayball, William B Smith. Marking nut anaphylaxis. AP Allergy 2016; 6:192-4.

[63] Modi NJ. Modi's Textbook of Medical Jurisprudence and Toxicology. 20th ed. Bombay: N.M. Tripathi Private Ltd; 1977. p. 608-9.

[64] Illan chezhian R, Roshy Joseph C, Rabinarayan Acharya. Urushiol-induced contact dermatitis caused during Shodhana (Purification process) of *Bhallataka* (Semecarpus anacardium Linn) fruit. AYU 2012; Vol-33, Issue-2; p. 270-3.

[65] Parikh CK. Parikh's Textbook of Jurisprudence, Forensic Medicine & Toxicology Reprint ed. New Delhi: CBS Publishers & Distributors; 2002. p. 9.35.

[66] Kaviraj Ambikadatta Shastri. Bhaishajya Ratnavali of Vd. Rajeshwar Datta. 17th ed. Varanasi: Chaukhamba Sanskrit Sansthan. 2004. p. 584.

[67] Pandit Dattaram Choube. Brihad Rasa Raj Sundar (Apurva Rasagrantha). 5th ed. Bombay: Dnyansagar printing press. 1979. p. 188.

[68] Prof. KR Srikantha Murthy. Sharangdhar Samhita. 1st ed. Varanasi: Chaukhambha Orientalia. 1984. p. 273.

[69] Chunekar KC, Pandey GS. Bhavaprakasha Nighant of Shri Bhavamishra. 10th ed. Varanasi: Chaukhamba Bharati Academy. 2010. p. 137.

[70] Verma Prashant, Chhabra Namrata, Sharma Reena. Severe Marking-Nut Dermatitis. Dermatitis 2012; Vol-23, Issue-6; p. 293–294.

[71] Swami Narayan. Narayan Chikitsa. [Internet] Available on 21/06/2019 at https://epustakalay.com/book/2499-narayan-chikitsa-by-swami-narayan/

[72] Chunekar KC, Pandey GS. Bhavaprakasha Nighant of Shri Bhavamishra. 10th ed. Varanasi: Chaukhamba Bharati Academy. 2010. p. 329-31.

[73] Nirmal Saxena. Vangasena Samhita - Volume II. 1st ed. Varanasi: Chaukhambha Sanskrit Series Office; 2004. p. 483.

[74] A. Kumar et al., Principle of Dravyaguna (*Ayurvedic* Pharmacology), Biomedical & Pharmacology Journal, 4(1), 2011, 147-152

[75] Deodar Cedar (Cedrus deodara) - Description and Uses. Available on 03/10/2018 at https://www.kalliergeia.com/en/deodar-cedar-cedrus-deodara-description-and-uses/ and Prachi Singh et al. Devadaru (Cedrus Deodara (Roxb.) Loud.): A Critical Review on Medicinal Plant. Int. J. Ayur. Pharma Res, 2014; 2(1): 1-10.

[76] *Ayurvedic* Pharmacopoeia of India Part- I Volume – IV. Govt of India. [Internet] Available at http://www.ayurveda.hu/api/API-Vol-4.pdf accessed on 12/10/2018.

[77] Vaidya Gogate VM. *Ayurvedic* Pharmacology and Therapeutic Uses of Medicinal Plants. Reprint ed. Varanasi: Chaukhambha Publications. 2016. p. 667.

[78] Zeng WC et al. Antibacterial activity of water-soluble extract from pine needles of Cedrus deodara. Int J Food Microbiol. 2012 Feb 1;153(1-2):78-84.

[79] Gogate VM. Dravyaguna Vijnana. 2nd ed. Nagpur: Pimpalapure Publishers. 1997. and Deshpande AP et al. Dravyaguna Vijnana. Reprint ed. Pune: Anmol Prakashan. 2002.

[80] Mustard. [Internet] Available on 12/10/2018 at https://www.drugs.com/npp/mustard.html

[81] Dr. Neethu P et al. A review on anti-toxic effect of Shweta *Sarshapa*a. The Pharma Innovation Journal 2019; 8(1): 261-264.

[82] Dr. Tripathi Indradeva, Dr. Tripathi Dayashankar. Yoga Ratnakara. 1st ed. Varanasi: Krishnadas Academy. 1998. p. 870.

[83] Prof. Dr. Ali Esmail Al-Snafi. A review on Cyperus rotundus - A potential medicinal plant. IOSR Journal of Pharmacy. Volume 6, Issue 7 - Version 2 (July 2016). p. 32-48

[84] *Ayurvedic* Pharmacopoeia of India Part- I Volume – III. Govt of India. [Internet] Available at http://www.ayurveda.hu/api/API-Vol-3.pdf accessed on 12/10/2018.

[85] Soumaya Kilani et al. Chemical Composition, Antibacterial and Antimutagenic Activities of Essential Oil from (Tunisian) Cyperus rotundus. Journal of Essential Oil Research. Nov-Dec 2005. 17:6, p. 695-700.

[86] Nagarajan et al. A review of pharmacology of Ativisha, Musta and their substitutes. J Ayurveda Integr Med. December 2014. p. 1-13.

[87] Prof. Dr. Ali Esmail Al-Snafi. A review on Cyperus rotundus - A potential medicinal plant. IOSR Journal of Pharmacy. Volume 6, Issue 7 - Version 2 (July 2016). p. 32-48

[88] UG Course- Dairy Technology- Lesson 17- Composition and Classification of Butter [Internet] Available on 30/01/2019 at http://ecoursesonline.iasri.res.in/mod/page/view.php?id=5759

[89] Hae-Soo Kwak, Palanivel Ganesan, Mohammad Al Mijan. Book: Milk and Dairy Products in Human Nutrition: Production, Composition and Health. Chapter: Butter, Ghee, and Cream Products. Publisher: John Wiley & Sons Ltd. p. 412-434. [Internet] Available on 30/01/2019 at https://www.researchgate.net/publication/284481060_Butter_Ghee_and_Cream_Products

[90] Yogitha Bali. *Lepa*s - Topical application of Ayurveda. Int. Res. J. of Pharmacy. 2012, 3(7). p. 8-9.

[91] Prof. KR Srikantha Murthy. Sharangdhar Samhita. 1st ed. Varanasi: Chaukhambha Orientalia. 1984. p. 24.

[92] Prof. KR Srikantha Murthy. Sharangdhar Samhita. 1st ed. Varanasi: Chaukhambha Orientalia. 1984. p. 354.

[93] Dissertation "An experimental study to evaluate the efficacy of Shatadhauta Ghrita application in contact poisoning caused by *Bhallataka* Beeja (Semecarpus anacardium Linn.)" By - Dr. Sruthy Das; submitted to RGUHS, Karnataka in 2015.

[94] Draize-FHSA Scoring system [Internet], Available on 7/4/2016 at http://www.rhc.org/suppdata/sm/c1/c1sm05249g/c1sm05249g.pdf

[95] Draize-FHSA Scoring system [Internet], Available on 7/4/2016 at http://www.rhc.org/suppdata/sm/c1/c1sm05249g/c1sm05249g.pdf

[96] David J. Gawkrodger, Michael R. Ardern-Jones. Dermatology. 6th ed. UK: Elsevier Publishers. 2016. p. 14.

[97] Article "Cytology, Histopathology, Biopsies" [Internet], Available at http://www.vcahospitals.com/main/pet-health-information/article/animal-health/cytology-histopathology-biopsies/251 accessed on 10/09/2016.

[98] Article "Skin Biopsy" [Internet], Available at https://en.m.wikipedia.org/wiki/Skin_biopsy accessed on 10/09/2016.

[99] Article “Pathology and Histopathology” [Internet], Available on 10/09/2016 at http://vet.uga.edu/dlab/user_guide/pathology_histopathology

[100] OECD Draft Guidance Document No. 116 [Internet], Available on 20/01/2017 at http://www.oecd.org/chemicalsafety/testing/44135709.pdf

[101] Margarete Arras. Improvement of Pain Therapy in Laboratory Mice. ALTEX 24, Special Issue 2007. Available on 29/01/2019 at http://www.altex.ch/resources/AltexSupl006008.pdf

[102] Article “7 Factors that Affect Wound Healing”. Available on 29/01/2019 at https://www.woundcareinc.com/resources/factors-that-affect-wound-healing

[103] Barapatre NB, Joglekar VP. Innovative Approaches in Drug Discovery: Ethnopharmacology, Systems Biology and Holistic Targeting. J Ayurveda Integr Med. 2019 Apr-Jun; 10(2): 143–145.

BIBLIOGRAPHY

Sr. No	Name of Author	Publisher & Address	Title of Book	Edition & Year
1	Acharya Shri Vishram	Sahitya Sanshodhana Vibhageeya Prakashan, GAU, Jamnagar	Anupan Manjari	1st Edition, 1972
2	Dr. Ayodhya Prasad Achal	Chaukhamba Surbharati Prakashan, Varanasi	Agadtantra (Ayurvediya Visha Vijnana)	4th Edition, 1998
3	Pandit K. Shastri, Dr. G. Chaturvedi	Chaukhambha Bharati Academy, Varanasi	The Charaka Samhita of Agnivesha	22nd edition, 1996
4	Yadavji Trikamji Acharya	Chowkhamba Krishnadas Academy, Varanasi	Charaka Samhita	Reprint Edition, 2006
5	Dr. Anant Ram Sharma	Chaukhamba Surbharati Prakashan, Varanasi	Sushruta Samhita of Maharshi Sushruta	Reprint, 2012
6	Yadavji Trikamji Acharya	Chowkhamba Krishna Das Academy,Varanasi	Sushruta Samhita	1st Edition 2004
7	Dr. Ravi Dutt Tripathi	Chaukhamba Sanskrit Pratishthan, Delhi	Ashtang Sangraha of Shrimad Vriddha Vagbhata	Reprint, 2006
8	Anant Damodar Athavale	Sreemad Aatreya Prakashanam, Pune	Ashtang Sangraha (Indu Vyakhya)	1st Edition 1980
9	Hari Shastri Paradkar	Chaukhamba Orientalia, Varanasi	Ashtanga Hridayam	9th Ed, 2005

10	Dr. N. J. Modi	N. M. Tripathi Private Ltd, Bombay	Modi's Textbook of Medical Jurisprudence and Toxicology	20th edition, 1977
11	Dr. C. K. Parikh	CBS Publishers & Distributors, New Delhi	Parikh's Textbook of Jurisprudence, Forensic medicine & Toxicology	Reprint, 2002
12	S. K. Singhal	The National Book Depot, Mumbai	Singhal's Toxicology at a glance	8th Edition, 2014
13	Dr. K. S. Narayan Reddy	Jaypee Brothers, New Delhi.	The Essentials of Forensic Medicine and Toxicology	31st Edition, 2012
14	Prof. K.R. Srikantha Murthy	Chaukhamba Orientalia, Varanasi	Sharangdhar Samhita	1st Edition, 1984
15	Prof. P. V. Sharma	Chaukhamba Bharati Academy, Varanasi	Dravyaguna Vijnana- Volume 2	Reprint, 2005
16	J. L. N. Shastri	Chaukhamba Orientalia, Varanasi	Dravyaguna Vijnana- Volume 2	Reprint, 2008
17	Prof. K. C. Chunekar, Prof. G. S. Pandey	Chaukhamba Bharati Academy, Varanasi	Bhavaprakasha Nighantu of Shri Bhavamishra	10th Edition, 2010
18	Pandit Kashinath Shastri	Motilal Banarasidas, New Delhi	Rasa Tarangini of Sadananda Sharma	11th Ed, 2004
19	V. Malik	Eastern Book Company, Lucknow	Drug & Cosmetic Act 1940, Drug & Cosmetic Rules	18th Edition, 2005

20	Kaviraj Ambikadatta Shastri	Chaukhamba Sanskrit Sansthan, Varanasi	Bhaishajya Ratnavali of Vd. Rajeshwar Datta	17th edition, 2004
21	Pandit Dattaram Choube	Dnyansagar printing press, Bombay	Brihad Rasa Raj Sundar (Apurva Rasagrantha)	5th Edition, 1979
22	David Gawkrodger, Michael Ardern-Jones	Churchill Livingstone Elsevier	Dermatology	5th Edition 2012
23	R. Shamasastry	Chowkhamba Vidya Bhavan, Varanasi	Kautilya Arthashaastra – Volume II	Reprint Edition, 2010
24	Colonel R. N. Chopra	Academic publishers, Kolkata	Indigenous Drugs of India	2nd Edition 1994
25	Nirmal Saxena	Chaukhambha Sanskrit Series Office, Varanasi	Vangasena Samhita-Volume 2	1st Edition, 2004
26	Vaidya V. M. Gogate	Chaukhambha Publications, Varanasi	*Ayurvedic* Pharmacology and Therapeutic Uses of Medicinal Plant	Reprint, 2016
27	Vaidya V. M. Gogate	Pimpalapure Publishers, Nagpur	Dravyaguna Vijnana	2nd Edition 1997
28	A. P. Deshpande, R. R. Javalagekar, S. Ranade	Anmol Prakashan, Pune	Dravyaguna Vijnana	Reprint Edition, 2002
29	Indradeva Tripathi, Dayashankar Tripathi	Krishnadas Academy, Varanasi	Yoga Ratnakara	1st Edition 1998

ANNEXURE- 1 - Authentication Certificate from Drug Supplier

MANAKARNIKA AUSHADHALAYA

(Center for Quality Ayurvedic Medicines)

***Chinchwad** - Shedge Bldg., Padwal Lane, Chinchwadgaon,
Pune - 411 033. Ph. 9921625384 E-mail: manakarnikapc@gmail.com
***Pune City** - 'Vedant', 1015, Sadashiv Peth, Nr. Dr. Ranade Hosp.,
Nagnath Par, Pune - 411 030 Ph. (020) 24493953.

Out ward No.: AD/159/18 Date: 15/12/2018

To
Dr. Nishant B. Barapatre
T.M.V. Gultekdi, Pune.
Mob.: 8149694530

Resp. Sir,

We certify that raw herbs supplied by us to you by Invoice no. CA-3052, Dated 15/12/2018 are authentic and genuine product are purchased from authentic supplier and are original in nature.

S.N.	Raw herbs	Pack	Batch No.
1)	Devdar Akhand *(Cedrus deodara)*	1 x 250 gm	03/18
2)	Bibba Akhand *(Semecarpus anacardium)*	1 x 250 gm	03/18
3)	Nagarmotha Akhand *(Cyperus rotundus)*	1 x 250 gm	04/18

Thanking you,
With regards
Yours Faithfully

Girish D. Gandhi
Manakarnika Aushadhalaya

Manakarnika Aushadhalaya
Vedant Aprt., 1015,
Sadashiv Peth, Nagnath Par,
Pune – 411 030 (M.S.), India
Mob.: +91 9011001633

ANNEXURE- 2 - Authentication Certificate of *Sarshapa Beeja*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. _____ Date _____

Report No. 69. 31-12-2018

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.,
Your Ref No:	dt. 17-12-2018.
Type of the Sample:	Sharshap Beeja.
Date of Receipt:	17-12-2018.
Batch No:	-.
Quantity Received:	1 X 250 gm.in Pl.bag.

Sample Drawn by Party

Authentification Certificate

The given sample was critically studied by Macroscopic, Microscopic and Organoleptic characters. We hereby authenticate that the given sample of dried seeds of Brassica campestris Linn. family Brassicaceae.

This certificate is issued on request vide sample given for analysis. It is to be used only for academic purpose.

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 3 - StandardizationCertificateof *Navaneeta*- 1

Date : 14 Feb.2019

DESHI (COOKING) BUTTER TEST REPORT

Sr. No.	Parameter	TEST RESULTS
1	Name and Address of the Manufacturer	M/S B.G.CHITALE, BHILAWADI STATION- 416 303, Tal. Palus, Dist. Sangli (Maharashtra) INDIA
2	Name of the Product	Deshi Cooking Butter
3	MFG Date	30/01/2019
4	Batch No.	C296
	CHEMICAL ANALYSIS	
5	Moisture %	14.84
6	Milk fat %	83.55
7	Curd %	1.61
8	RM Value	28.68
9	PV Value	1.58
10	Acidity % (as lactic acid)	0.023
11	FFA	0.29
12	Boudouing test	Negative
13	Colour added Test	Negative
	MICROBIOLOGICAL ANALYSIS	
14	Total Plate Count / g Max.	540
15	Coliform Count / g	ABSENT
16	Yeast and Mould Count / g	ABSENT
17	E. Coli / g	ABSENT
18	Salmonella / 25 g	ABSENT
19	Shigella / 25 g	ABSENT
20	S. Aureus / g	ABSENT
21	Anaerobic Spore Count / g	ABSENT
22	Listeria monocytogens/ g	ABSENT

CHEMIST

MICROBIOLOGIST

QUALITY ASSURANCE OFFICER

M/S. B. G. CHITALE
BHILAWADI STATION - 416 303
Tal. Palus, Dist. Sangli (Maharashtra)
Ph. (02346) 283112 (Five Lines)

ANNEXURE- 4 - StandardizationCertificateof *Navaneeta* - 2

Nutritionl information of Deshi (Cooking) Butter

*Nutritional Information	
Amount per serving	
Energy 759.6 kcal	From Fat 747.0 kcal
	%DV
Total Fat 83.0	127.7
Saturated Fat 64.1 g	320.5
Monounsaturated Fatty Acid 16.4 g	- - -
Polyunsaturated Fatty Acid 2.5 g	- - -
Trans Fat 0 g	- - -
Cholesterol 161.0 mg	53.7
Sodium 8.0 mg	0.33
Total Carbohydrate 0.7 g	0.23
Total Sugars 0 g	- - -
Added Sugar 0 g	- - -
Dietary Fiber <0.5 g	- - -
Protein 0.7 g	1.4
Vitamin A 741.0 mcg	49.35
Vitamin C 4.3 mg	7.2
Calcium 22.7mg	2.27
Iron 0.8mg	4.4
Percent Daily Values are based on 2000 Calorie diet. Your daily values may be higher or lower depending on your calorie needs.	

* Approximate Values

Contains milk solids

M/S. B. G. CHITALE
SHIRAWADI STATION - 416 303
Tal. Palus, Dist. Sangli (Maharashtra)
(02346) 233112 (Five Lines)

ANNEXURE- 5 - AuthenticationCertificateof *Bhallataka Beeja*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. _____ Date 31-12-2018

Report No. 71.

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune., dt. 17-12-2018.
Your Ref No:	
Type of the Sample:	Bhallatak Beeja.
Date of Receipt:	17-12-2018.
Batch No:	-
Quantity Received:	1 X 250 gm.in Pl.bag.

Sample Drawn by Party

Authentification Certificate

The given sample was critically studied by Macroscopic and Microscopic characters. We hereby authenticate that the given sample of dried fruits of Semicarpus anacardium Linn. from the family Anacardaceae.

This certificate is issued on request vide sample given for analysis. It is to be used only for academic purpose.

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 6 - Deposition Certificate of Voucher specimens

Mahila Utkarsha Pratishthan's, Risod

Ayurved College, Hospital & Research Center,

Pundlik Nagar, Degaon (Phata), Post - Linga (K)
Tq. Risod, Distt. Washim (MAH.) Pin 444 506
Tel Ph. (07251) College - 227511, Hosp - 227505, Fax No. : 227512
• Web site : www.mupachrc.com • Email : principalbams@gmail.com

Recognised by Central Council of Indian Medicine, New Delhi, Govtl of Maha. & Affiliated to Maharashtra University of Health Science, Nashik

Founder Chairperson - **Hon'ble Bhavanatai Gawali** (M.P. Yeo-Wsm, Maharashtra)

DEPARTMENT OF AGADTANTRA

Outward No. – MUP/AG/001/2019 Date – 18/02/2019

CERTIFICATE

This is to certify that, **Dr. Nishant Bhimraj Barapatre,** a Ph.D. Scholar of Tilak Maharashtra Vidyapeeth, Pune and currently working as a Lecturer in our department, has submitted the below mentioned Herbal Drugs/Poisons to the Agadtantra Museum maintained by our department. These samples have been archived in properly preserved condition and have been deposited in "Poisons & Antidotes" section as Voucher Specimens.

Hence, certified.

Sr. No.	Name of Specimen	Latin Name	Category	Voucher No.
1	Bhallataka Beeja	Semicarpus anacardium Linn.	Poison	01/2019
2	Devdaru Kaand	Cedrus deodara Roxb.	Antidote	02/2019
3	Sarshap Beeja	Brassica campestris Linn.	Antidote	03/2019
4	Mustaka Kanda	Cyperus rotundus Linn.	Antidote	04/2019

Ayurved College Hospital & Research Degaon Risod

विभाग प्रमुख
अगद तंत्र व व्यवहार आयुर्वेद
म.उ.प्र.आयुर्वेद कॉलेज, हॉस्पिटल व रिसर्च सं.
पुंडलिक नगर, देगांव (फाटा), ता.रिसोड, जि.वाशिम

ANNEXURE- 7 - StandardizationCertificateof *Shatadhauta Ghrita*

न्यू युनायटेड फार्मसी, पुणे
शुध्द आयुर्वेदिक औषधांचे निर्माते आणि विक्रेते

स्थापना १९८६

११४१, सदाशिव पेठ, गीता भवन, १ ला मजला, पेरुगेट, पुणे – ३०. फोन: (०२०) २४४८ ९२७७ मो.: ०९८२३० ५८३८० E-mail: gujar999@gmail.com

Outward No. – 01/2019

Date – 06/02/2019

To,
Dr. Nishant B. Barapatre
PhD Scholar, TMV, Pune
Mob. - 8149694530

Resp. Sir,

We certify that the "Shatadhauta Ghrita" supplied by us to you by Invoice no. 2596, Dated 06/02/2019 is an authentic and genuine product. It is manufactured according to the Standard Manufacturing Processes in our GMP certified Pharmacy and is original in nature.

Product supplied –

1)	Shatadhauta Ghrita	Pack - 4 x 24 gm	Batch No. - 1902
			MFG Date – 02/2019

Thanking you,
With regards

New United Pharmacy, Pune
1141, Sadashiv Peth, Geeta Bhavan,
1st Floor,Perugate,Near Hotel Tarang,
Pune-411 030 Call - 9823058380
E-mail : gujar999@gmail.com

Vd. Shailesh Gujar
New United Pharmacy, Pune

GMP Certified by Govt. of Maharashtra

पुण्यात स्थापन झालेली एक अग्रगण्य आयुर्वेदिक औषध उत्पादक संस्था आहे. आयुर्वेद शास्त्राच्या नियमांप्रमाणे व सिद्धान्तानुसार गुणवान आयुर्वेदिक औषध निर्मिती हेच आमचे ध्येय आहे. शुध्द कच्चा माल, कुशल व तज्ज्ञ आयुर्वेदिक डॉक्टर/वैद्याचे मार्गदर्शन. शास्त्रीय निर्मिती व परीक्षणे (Test), उत्कृष्ट पॅकिंग व कार्यक्षम वितरणव्यवस्था यामुळे न्यू युनायटेड फार्मसी, पुणे यांची उत्पादने संपूर्ण देशभर व परदेशात लोकप्रिय झाली आहेत. आपले आशीर्वाद व सहकार्याची अपेक्षा !
न्यू युनायटेड फार्मसी, पुणे यांची उत्पादने औषधांच्या प्रत्येक दुकानात मिळतात.

ANNEXURE- 8 - AuthenticationCertificateof *Devdaru Kaand*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. _____ Date _____

Report No. 68. 31-12-2018

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.,
Your Ref No:	dt. 17-12-2018.
Type of the Sample:	Devdar Kand.
Date of Receipt:	17-12-2018.
Batch No:	-.
Quantity Received:	250 gm.in Pl.bag.

Sample Drawn by Party

Authentification Certificate

The given sample was critically studied by Macroscopic, Microscopic and Organoleptic characters. We hereby authenticate that the given sample of dried heart wood of *Cedrus diodara* (Roxb.) Loud. family Pinaceae.

This certificate is issued on request vide sample given for analysis. It is to be used only for academic purpose.

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 9 - AuthenticationCertificateof *Mustaka Kanda*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. Report No. 70. Date 31-12-2018

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.,
Your Ref No:	dt. 17-12-2018.
Type of the Sample:	Mustak Kand (Rhizome).
Date of Receipt:	17-12-2018.
Batch No:	-.
Quantity Received:	1 X 250 gm.in Pl.bag.

Sample Drawn by Party

Authentification Certificate

The given sample was critically studied by Macroscopic, Microscopic and Organaleptic characters. We hereby authenticate that the given sample of dried rhizome of *Cyperus rotundas* Linn. family Cyperaceae.

This certificate is issued on request vide sample given for analysis. It is to be used only for academic purpose.

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 10 - StandardizationCertificateof *Devdaru Churna*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. ~~Report~~ No. 72. Date 31-12-2018

CERTIFICATE OF ANALYSIS
CONFIDENTIAL.

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.
Your Ref No:	dt. 18-12-2018.
Type of the Sample:	Devdar Churna.
Date of Receipt:	18-12-2018.
Batch No.	-
Quantity Received:	1 X 250 gm.in Poly bag.

Sample Drawn by Party.

Description: Light Yellowish White powder, insoluble in water having characteristic odour.

TEST	RESULT
1. Total ash:	0.42 % w/w.
2. Acid Insoluble ash:	0.12 % w/w.
3. Water Soluble Extractive:	1.62 % w/w.
4. Alcohol Soluble Extractive:	7.55 % w/w.

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 11 - StandardizationCertificateof *Sarshapa Churna*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎ : (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. Report No. 73. Date 31-12-2018

CERTIFICATE OF ANALYSIS
CONFIDENTIAL.

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.,
Your Ref No:	dt. 18-12-2018.
Type of the Sample:	Sarshap Churna.
Date of Receipt:	18-12-2018.
Batch No.	-
Quantity Received:	1 X 100 gm.in Poly bag.

Sample Drawn by Party.

Description: Yellowish brown churna having characteristic odour. Insoluble in water.

TEST	RESULT
1. Total ash:	4.86 % w/w.
2. Acid Insoluble ash:	0.39 % w/w.
3. Water Soluble Extractive:	18.55 w/w.
4. Alcohol Soluble Extractive:	21.71 % w/w.
5. Fixed Oil Content:	36.70 % w/w.

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 12 - StandardizationCertificateof *Mustaka Churna*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. Report No. 74. Date 31-12-2018

CERTIFICATE OF ANALYSIS
CONFIDENTIAL.

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.
Your Ref No:	dt. 18-12-2018.
Type of the Sample:	Mustak Churna.
Date of Receipt:	18-12-2018.
Batch No.	-
Quantity Received:	1 X 250 gm.in Poly bag.

Sample Drawn by Party.

Description: Light Brownish colour churna having characteristic odour.Insoluble in water.

TEST	RESULT
1. Total ash:	6.72 % w/w.
2. Acid Insoluble ash:	3.70 % w/w.
3. Water Soluble Extractive:	12.22 % w/w.
4. Alcohol Soluble Extractive:	6.22 % w/w.

For I.D.R.A. & L.Pune.

ANNEXURE- 13 - StandardizationCertificateof *Bhallataka Taila*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Report No. 67. 31-12-2018

Ref. No. _____ CERTIFICATE OF ANALYSIS Date _____

CONFIDENTIAL.

Name of the Party	Dr Nishant B.Barapatre, Tilak Maharashtra Vidyapeeth, Gultekadi-Pune.,
Your Ref No:	dt. 18-12-2018.
Type of the Sample:	Bhallataka Taila.
Date of Receipt:	18-12-2018.
Quantity Received:	1 X 50 gm.(appx).
	Sample Drawn by Party
1. Description:	Black, thick Viscous (Liquid) Oil.
2. Weight/ml:	0.9993 gms/ml.
3. Refractive Index:	1.583
4. Viscosity:	431.78 Centistokes/Sq.cm.
5. Iodine Value:	115.45
6. Saponification Value:-	222.77
7. Peroxide Value(Rancidity):	17.89
8. Acid Value:	15.20
9. Thin Layer Chromatography:	
Methanolic Extract:	Taken for analysis:
Solvent System:	Toluene:Ethyl Acetate(4:1).
Adsorbent Used:	Silica gel $G_{60}f_{254}$
Observations:	
1. Visidual	Five spots. Rf:-0.108(Blue),0.55(Grey), 0.683(Blue), 0.833, 0.95 (Brown).
2. 254 nm:	Four spots. Rf:- 0.316(Blue), 0.55,0.66(Grey Blue), 0.916 (Blue).
3. 365 nm:	Five spots. Rf:- 0.116(Fluorescent Yellow), 0.55 (Brown), 0.683(Grey Blue), 0.775 (Fluorescents Greenish Yellow), 0.933 (Brown).
4. Iodine Vapours:	Six spots. Rf:- 0.141(Yellow), 0.31(Brown), 0.525 (Blue Grey), 0.66 (Blue), 0.816 (Brown), 0.833 (Brown).

For I.D.R.A. & L.Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 14 - StandardizationCertificateof *Daru-Sarshapa-Mustadi Lepa*

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎ : (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. _____ Date 13-02-2019.

Report No. 296.

CERTIFICATE OF ANALYSIS
CONFIDENTIAL

Name of the Party.	Dr Nishant B. Barapatre, Tilak Mahaarashtra Vidyapeeth, Gultekadi-Pune.
Your Ref.No:	Dated 08/02/2019.
Type of the Sample:	Daru-Sarshap-Mustadi Lepa.(Fresh).
Date of Receipt:	08/02/2019.
Batch No:	MFG Dt. 08/02/2019.
Quantity Received:	2 X 15 gm in pl.Container.

Sample Drawn by Party.

Description: Yellowish brown oily lepa having characteristic odour. Insoluble in water.

TEST	RESULT
1. pH at RT (as is):	6.50.
2. Rancidity Test:	Complires (No colour development).
3. Total Fatty Matter:	58.35 % w/w.
4. Loss on Drying:	7.55 % w/w.
5. Total Ash:	2.31 % w/w.
6. Acid Insoluble Ash:	0.86 % w/w.

7. Microascopy : Brownish-Yellow colour fragments, oil globules. Fragments of reddish brown cells. Yellowish Oil globules. Pigmented cells filled with reddish brown content. Thin walled pieces of paranchymatous cells.

For I.D.R.A. & L. Pune.

ESTD. 1945 : MORE THAN SIXTY YEARS OF SERVICE & RESEARCH

ANNEXURE- 15- Permission of IAEC and Completion of Experimental Study

Indian Drugs Research Association & Laboratory

561-B, Shivajinagar, Behind Congress Bhavan Lane, Pune - 411 005. ☎: (020) 25534018 / 25537875
E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. 120/2019

Date 15-02-2019

CERTIFICATE

This is to certify that, Vd. Nishant Bhimraj Barapatre, student of Faculty of Ayurved, Tilak Maharashtra Vidyapeeth, Mukund Nagar, Gultekadi, Pune - 37, under the Guidance of Prof. Vd. Prashant A. Suru, has satisfactorily conducted his Animal Experiment (on Albino Mice) as a part of experimental work, for the Thesis of Ph.D. (Agadtantra), in this institute as per the protocol provided by researcher and approved by IAEC of IDRA&L through Research Project No. 294 T-17.

The title of his Thesis is **"An Experimental study of Efficacy of Daru-Sarshap-Mustadi Lepa on Local Side Effects of Bhallataka Taila"**.

Mr. S.U. Deshmukh
Study Director

ANNEXURE- 16- Master Chart of Experimental Data

			Erythema							Edema							Formation of Blisters & Ulceration						
Day -			0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
Group 1	A	Lt.	4	4	*	*	*	*	*	*	2	2	*	*	*	*	*	*	3	3	*	*	*	*	*	*
		Rt.	4	4	*	*	*	*	*	*	3	3	*	*	*	*	*	*	3	3	*	*	*	*	*	*
	B	Lt.	4	4	4	4	4	4	4	4	2	2	3	3	3	3	3	3	2	3	3	3	3	3	3	3
		Rt.	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	C	Lt.	4	4	4	4	*	*	*	*	3	3	3	3	*	*	*	*	2	3	3	3	*	*	*	*
		Rt.	4	4	4	4	*	*	*	*	3	3	3	3	*	*	*	*	3	3	3	3	*	*	*	*
Group 2	A	Lt.	4	4	3	3	3	3	3	2	3	3	2	2	2	2	2	2	2	2	2	2	2	2	4	4
		Rt.	4	4	4	4	3	3	3	2	3	2	2	2	2	2	2	2	3	3	2	2	2	2	2	4
	B	Lt.	4	4	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1
		Rt.	4	4	3	3	3	3	3	2	2	3	2	2	2	2	2	2	2	3	3	3	3	2	2	1
	C	Lt.	4	4	3	3	3	3	3	2	3	3	2	2	2	2	1	1	3	3	3	2	2	2	2	1
		Rt.	4	4	4	3	3	3	3	2	3	3	2	2	2	2	2	2	2	3	3	3	3	2	2	1
Group 3	A	Lt.	3	3	2	2	2	2	1	1	2	2	2	2	2	2	1	0	2	2	2	2	2	2	1	1
		Rt.	3	3	2	2	2	2	2	1	2	3	3	2	2	2	2	0	2	3	3	2	2	2	2	1
	B	Lt.	4	4	3	3	3	2	2	1	2	2	2	1	1	1	1	0	2	2	2	2	2	2	4	1
		Rt.	4	4	4	4	3	3	3	1	3	2	2	2	2	2	1	0	3	3	2	2	2	2	4	1
	C	Lt.	4	4	3	2	2	2	2	2	3	2	2	1	1	1	1	1	3	3	4	4	4	4	4	4
		Rt.	4	4	4	2	2	2	2	2	3	2	2	1	1	1	1	1	3	2	2	2	2	2	4	4
Group 4	A	Lt.	4	4	4	3	2	2	2	2	3	3	3	2	2	2	1	1	3	3	3	2	2	2	4	1
		Rt.	4	4	3	3	3	2	2	2	2	3	2	2	2	2	2	1	2	3	3	2	2	2	2	1
	B	Lt.	4	4	3	3	2	2	2	1	2	2	2	2	1	1	1	0	3	2	2	2	1	1	4	1
		Rt.	4	3	3	3	3	3	2	1	2	2	2	2	2	2	2	0	3	2	2	2	2	2	2	1
	C	Lt.	4	4	3	3	2	1	1	1	3	2	2	2	2	1	1	0	2	2	2	2	2	1	1	1
		Rt.	4	3	3	3	2	1	1	0	3	2	2	2	2	1	1	0	2	2	2	2	2	1	1	0

			Erythema									Edema									Papules							
Day -			0	1	2	3	4	5	6	7		0	1	2	3	4	5	6	7		0	1	2	3	4	5	6	7
Group 5	A	Lt.	4	4	3	2	2	2	2	2		2	3	1	1	1	1	1	1		2	2	4	4	4	4	4	4
		Rt.	4	4	4	3	3	2	2	2		2	2	2	2	1	1	1	1		2	2	2	2	4	4	4	4
	B	Lt.	4	4	3	3	3	3	2	2		2	2	2	2	2	2	1	1		2	2	2	2	2	2	1	1
		Rt.	4	4	3	3	3	3	2	2		2	3	2	2	2	2	1	1		2	3	2	2	2	2	1	1
	C	Lt.	4	4	3	3	3	3	2	2		3	3	2	2	2	2	2	2		3	3	3	3	2	2	4	4
		Rt.	4	4	4	3	3	3	2	2		3	3	2	2	2	2	2	1		3	3	2	2	2	2	4	4
Group 6	A	Lt.	4	4	4	3	3	3	3	2		2	2	2	2	1	1	1	1		3	3	4	4	2	1	1	1
		Rt.	4	4	4	4	3	3	3	2		2	2	2	2	1	1	1	1		2	2	2	4	2	1	1	1
	B	Lt.	3	3	3	3	3	3	2	2		2	2	2	2	2	2	1	0		3	2	2	2	2	2	4	1
		Rt.	4	4	4	3	3	2	2	2		2	3	2	2	2	1	1	0		3	3	2	2	2	1	4	4
	C	Lt.	4	4	3	3	3	3	3	2		2	2	2	2	2	2	1	0		2	2	2	2	2	2	4	1
		Rt.	4	4	4	3	3	2	2	2		2	2	2	2	2	1	1	0		3	3	2	2	2	1	4	2
Group 7	A	Lt.	4	4	4	3	3	3	3	3		2	2	2	1	1	1	1	1		2	2	4	2	2	4	2	4
		Rt.	4	4	4	3	3	3	3	3		2	2	2	1	1	1	1	1		2	2	4	2	2	2	2	4
	B	Lt.	4	4	4	4	4	3	3	3		3	3	3	2	2	2	2	2		2	3	3	3	3	2	2	2
		Rt.	4	4	4	4	3	3	3	3		3	3	3	3	2	2	2	2		2	3	3	3	3	2	2	2
	C	Lt.	4	4	4	4	4	4	*	*		3	3	3	2	2	2	*	*		3	3	3	3	3	3	*	*
		Rt.	4	4	4	4	4	4	*	*		2	3	3	2	2	2	*	*		3	3	3	3	3	3	*	*

ANNEXURE- 17- Histopathological Examination Report

Report of Histopathological observations of SKIN tissue

Study Code: 294 T-17 Month: July 2019

Referred by: Dr. Nishant B. Barapatre c/o- IDRA&L, Pune

Details of Organs: Total 36 Skin Tissue samples

During Histopathological examination, the changes occurring in the skin tissues at the histological level have been assessed for the following parameters:

Criteria A - Scab tissue formation in Epidermal layer
Criteria B -Formation of new Epidermal skin Epithelial tissue as a Healing process
Criteria C -Healing of skin by Collagen formation and proliferation of Fibroblast tissue in Dermis layer and Subcutaneous tissue
Criteria D -Connective tissue proliferation with Granulation tissue formation
Criteria E -Infiltration of Acute Inflammatory Cells (Neutrophils and Polymorphonuclear Cells – PMN) in the skin section (Dermis and Epidermis)

Code of Slide	Criteria A	Criteria B	Criteria C	Criteria D	Criteria E
1-B-R	1	1	1	1	4
1-B-L	1	1	1	1	2
2-A-R	2	2	2	2	2
2-A-L	3	3	3	3	1
2-B-R	2	2	2	2	3
2-B-L	1	2	3	3	2
2-C-R	3	3	2	2	2
2-C-L	3	3	2	3	1
3-A-R	3	3	3	3	1
3-A-L	3	2	4	4	1
3-B-R	3	4	4	3	2
3-B-L	4	4	4	4	1
3-C-R	3	4	4	3	2
3-C-L	4	3	3	3	1
4-A-R	3	3	3	4	2
4-A-L	3	3	4	3	1
4-B-R	4	3	3	3	1
4-B-L	4	4	4	4	2
4-C-R	4	4	4	2	1
4-C-L	3	4	4	4	1

Code of Slide	Criteria A	Criteria B	Criteria C	Criteria D	Criteria E
5-A-R	3	3	3	3	1
5-A-L	3	2	3	2	2
5-B-R	3	2	3	3	1
5-B-L	3	2	3	3	3
5-C-R	3	3	4	2	2
5-C-L	3	2	2	2	1
6-A-R	3	3	3	3	1
6-A-L	3	2	4	3	2
6-B-R	3	3	2	3	3
6-B-L	4	2	4	2	2
6-C-R	3	3	4	3	1
6-C-L	2	4	3	3	1
7-A-R	2	3	2	3	2
7-A-L	2	2	3	2	1
7-B-R	3	2	3	2	2
7-B-L	2	2	2	2	2

Note: The scores assigned for each criterion have been assessed as per the Scoring System for Histopathological Assessment described in the Protocol of this Research Study.

Dr. Chandrashekhar Mote
MVSc, PhD *(Vet Pathology)*
Consulting Vet. Pathologist