Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2021 Month; 4 (2s): 594 - 597

A clinical study on the efficacy of Lakshadi Gana Taila in Vrana Shodhana w.s.r to its action on microbial load and wound infection

Gond Pushpa[1], Singh Lakshman[2]

[1]Lecturer, Government Ayurvedic College and Hospital, Banda (U.P.), India.

[2]Professor, Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P. India.

Article Received: 01 October 2021 Article Accepted: 10 October 2021 Article Published: 09 November 2021

ABSTRACT

Background

Wounds are a significant cause of morbidity worldwide. The science of wound healing is advancing day by day, particularly as a result of new therapeutic advancement. The wound healing effect of different herbal preparation have been described in Sushruts Samhita..

Aim

To evaluate the efficacy of Lakshadi Gana Taila over wound debridement (wound infection and microbial load).

Materials and methods

Lakshadi gana (Laksha , Nimba, Haridra and Amaltas) drug was collectively prepared to make a taila. This was used to treat patients of infected chronic non healing wounds. The signs and symptoms of infection were graded before and during the course of treatment. Tissue biopsy to estimate the microbial load prior to and during the course of treatment was done.

Results

The clinical symptoms like Pain, Discharge, Tenderness and Malodor in wounds showed statistically reduction following treatment. The microbial load of the wounds was also reduced significantly.

Conclusion

In most of the cases, there was a progressive reduction in the microbial load with time, during the course of treatment indicating the efficacy of the formulation (Lakshadi Gana Taila)in reducing the microbial load and thus controlling infection, facilitating wound healing.

Keywords: Infected Wound, Dhushta Vrana, Lakshadi Gana , Vrana Shodhana , Vrana Ropana.

INTRODUCTION

A wound is a break in the integrity of skin or tissue often, which may be associated with disruption of the structure and function.1 During the course of healing, following complications may occur: Infection of wound due to entry of bacteria delays the healing Infected wound (Dushta Vrana) is a common and often encountered problem faced in surgical practice. The presence of Infected wound (Dushta Vrana) worsens the condition of the patient with different complications (eg; Implantation cyst, pigmentation, deficient scar formation, hypertrophied scar, keloid formation etc.

The effective management of traumatic, surgical, and chronic wounds remains a significant challenge in clinical practice due to the high rates of infection and complications that can negatively impact patient outcomes and healthcare costs. Wound care, whether addressing open fractures, surgical site infections (SSIs), or injuries from trauma, requires comprehensive therapeutic approaches that incorporate advanced technologies, meticulous surgical techniques, and innovative strategies to ensure optimal healing and minimize complications.2

Certain factors that influence wound healing include bacterial infection, nutritional deficiency, drugs, site of wound etc., All chronic wounds intrinsically contain bacteria, and the process of wound healing can still occur in their presence. It is, therefore not the presence of bacteria but their interaction with the host that determines the organisms' influence on chronic wound healing. However, the relative number of micro-organisms and their pathogenicity, in combination with host response and factors, such as immunodeficiency, dictate whether a chronic wound becomes infected or shows signs of delayed healing.

Wound infection is defined as the presence of replicating microorganisms within a wound with a subsequent host response that leads to delayed healing. Because of this it is important that infection is recognized as early as possible. The signs and symptoms of local infection are redness (erythema), warmth, swelling, pain, and loss of function. Foul odor and pus may accompany this. Eventually, the local bacterial burden will increase further and become systemically disseminated resulting in sepsis, which if not actively treated could progress to septicemia and

multi-organ failure.

There are several factors known to affect the bacterial burden of chronic wounds and increase the risk of infection. These include the number of microorganisms present in the wound, their virulence, and host factors. Experimental studies have demonstrated that regardless of the type of microorganism, impairment of wound repair may occur when there are more than 1×105 organisms per gram of tissue. Hence, it becomes essential that a drug used to control the wound infection must necessarily reduce the microbial load.3

Vrana (Wound) in Ayurveda has stated as " Vranagatravichurne" i.e of tissue distrucion and discoloration of viable tissue to various etiology. In Ayurveda Infected wound or nonhealing ulcer may be correlated with Dushta vrana . The Lakshanas of Dushta Vrana by Maharshi Sushruta, Charak , Vagbhata and Madhavakar are clearly mentioned as foul smelling, continuously flowing pus, along with blood, with cavity, since long time etc. Maharshi Sushruta has scientifically classified in a systemic manner a wealth of clinical material and the principles of management which are valid even today.

For management of infected (Dushta Vrana), Maharshi Sushruta has described number of drugs and classified in detail into processes Vranashodhana and Vranaropana. Both the processes had been therapeutically described under seven heading: Kashaya, Varti, Kalka, Taila. Raskriya and Avachoornana and sixty different procedures for the management of wounds along with numerous herbal drugs, which he had used as local applicant for curing them, have been described.

A lot of research work has been done in Shalya Tantra department related to Dushta Vrana management but still there is demand of time to search new drug to make more availability which is cost effective. This needs of search of Ayurvedic formulation forced to mind of researcher to taken Lakshadi Gana from the 37 Dravya Gana described in Sushruta Samhita4 which have properties as: Kaphapittatinashak (relieves the diseases of Kapha and Pitta), Dushta Vrana Shodhak (purifies bad ulcers), Kustha nashak (cures leprosy and some other skin diseases) and Krimi Nashak (having antimicrobial property). Four commonly available drugs from this Lakshadi Gana have been included in the formulation of new drug according to Sushruta description. It's have been discussed and named it Laksadi Gana Tail. In this Lakshadi Gana, four drugs on the basis of earlier work such drug contents have been found effective.

Table No.1. Ingredients of Lakshadi Gana Taila and their general Information

Sr. No.	Name	English name	Scientific Name	Family	Used Part
1.	Laksha	lac	Laccifera Indica	Lacciferidae	Resin of Insect origin
2.	Nimba	Neem	Azadirachta indica	Meliaceae	Leaves
3.	Haridra	Turmeric	Curcuma longa	Zinziberaceae	Rhizome
4.	Amaltas	Purging Cassia	Cassia fistula	Leguminosae	Fruit Pulp
	Til(Base oil)	Sesame	Sesamum indicum	Pedaliaceae	tail

MATERIALS AND METHODS

The study employed a single arm before-after clinical trial design. Patients who met entry criteria with chronic wounds were assessed for signs and symptoms of localized infection, and viable wound tissue specimens were obtained for quantitative microbiological analyses prior to and during the course of the treatment. The approval from the Institutional Ethical Committee (IEC) was taken and informed consent from each patient obtained.

Setting and sample

The study population consisted of 61 subjects with chronic infected wound or non-healing wound and randomly divided into two groups. The outpatient department of the Department of Surgery in the Ayurvedic and modern wings, "Wound Clinic" under the modern wing and the Department of Microbiology in the same campus served as settings for the study.

Groups

Group I (Trial Group) – 30 patients were taken in trial group and per day dressing were done by Ayurvedic formulation (Lakshadi Gana Tail).

Group II (Control Group) – 31 patients were treated with local application of

Povidone Iodine solution (5%).

Inclusion and exclusion criteria

The criteria of selection of cases of wounds were based on the symptomatology presented by the patients in accordance to description of mild to moderate infected chronic wound were randomly selected age 18-60 years irrespective of sex. Patient excluded Patient without infection, Leprotic wound, Syphilitic ulcer, Tubercular wound, Malignant wounds, Immune Compromised Patient, Hepatitis Positive Patient, Pregnancy condition, Patients with disorders like septicaemia etc, Uncontrolled Diabetic Patients, Severe Infected Wound.

Laboratory investigation

Hematological investigation, Complete Blood Count (CBC), Erythrocyte Sedimentation Rate (ESR), Biochemical Investigations,Blood Glucose – Fasting & Post Prandial, Liver Function Test (LFT), Renal Function Test (RFT), Lipid Profile, Serological Investigation, HIV (Human Indeficiency Virus), Australia Antigen (HBsAg), Venereal Disease Research Laboratory (VDRL) Urine-Routine & Microscopic,Others were Radiography if needed and Monteux test

Trial Drug: Lakshadi Gana Tail

Collection and identification of plant materials

The fresh and healthy pods of plants Aragvadha, leaves of Nimba and Rhizome of Haridra were collected from various areas of Banaras Hindu University Campus. Laksha was purchased from local market of Varanasi, U.P. The plant specimens were identified and deposited in the Dravyaguna Department, Faculty of Ayurveda, IMS, BHU, Varanasi. For further analysis trail drug and its ingredients were sent for analysis to Karnataka, S.D.M. Centre for Research in Ayurveda and Allied Sciences (AYUSH Centre for Excellence and Recognized Scientific and Industrial Research Organizations (SIROS) by the Department of Scientific and Industrial Research (DSIR).

Preparation of Drug (Lakshadigana Taila)

Identified ingredients Laksha (resin of insect origin(Laccifera lacca), Nimba patra (Azadirachta indica, Rhizome of Haridra (Curcuma longa) and Amalatas Pulp (Cassia fistula) of Lakshadi Gana was prepared to make kwath and after then Lakshadi Gan taila was made according description as classical method Sneha pak kalpana (Kalka, Sneha ,Til Tail (Sesamum indicum) and Kwath-Ratio 1:6:24) according to Sharangadhar Samhita Madhyam Khand 9/6 and madhyam pak lakshana used according to Sushruta Chikitsasthan 31/ 8-12. Then, Lakshadi Gana Tail was kept in air tight container.

GC-MS Identification of Lakshadigana Tail done to know the compound present in
Lakshadigana Tail.

Assessment Criteria for Healing

A.Subjective Criteria-Pain

B. Objective Criteria-Discharge,Tenderness, Malodor,Granulation Tissue, Size Measurement – Length, Width, Depth, Area, Unit Healing Time

Biochemical Parameter-Bacterial load

Application of Drug and Advice

After culture of tissue of the wound, Normal Saline was used for cleaning of the wound and then polyherbal preparation i.e. Lakshadi Gana Tail was applied over wound with sterile cotton gauge piece. The dressing was changed daily. Culture of wound tissue was done after two and four weeks to know bacteriological status of the wound. Once Biopsy of wound was done before treatment and once after 3 weeks of treatment after noticing the granulation tissue. Surgical debridement was also done in cases of some moderate infected wound cases to remove the dead necrosed tissues followed by daily application of drug.

Advice and Supportive Drugs

Advice

To take- regular light, warm, protein-rich diet, To avoid- spicy food, keep wound surrounding clean etc. Supportive drugs - Analgesic, Anti-Hyperglycaemic, or Anti-Hypertensive drugs for respective patient.

Duration of Treatment

Duration of treatment was upto complete healing of wound or upto 8 weeks whichever is earlier. A well-planned grading system was followed to assess different signs and symptoms of the patient and the grade were assessed on the first visit and weekly henceforth i.e. the above-mentioned symptoms and signs were noted at subsequent intervals i.e. 0th day, 1st week, 2 nd week, 3 rd week,……8 th week or upto complete healing whichever is earlier.

Criteria for assessment

The grading of the above was done as follows

Grading for Pain: Using Visual analog scale (VAS) (0-10)

0 = No pain

1 = 1-3 in the VAS, Mild pain

2 = 4-7 in the VAS, Moderate pain

3 = 8-10 in the VAS, Severe pain

Grading for Discharge

0 = No discharge/dry dressing

1 = Scanty occasional discharge & little wet dressing

2 = Often discharge & with blood on dressing
3 = Profuse, continuous discharge which needs frequent dressing
Grading for Tenderness
0 = Tolerance to pressure
1 = Little response on sudden pressure
2 = Wincing off face on super slight touch
3 = Resists to touch & rigidity
Grading for Malodour
0 = No smell
1 = Bad smell
2 = Tolerable unpleasant
3 = Foul smell which is intolerable
Evaluation of Granulation tissue: (Evaluated on 0-5 scale)
0 = No granulation
1 = 20% wound surface represented by red granulation tissue
2 = 40% wound surface represented by red granulation tissue
3 = 60% wound surface represented by red granulation tissue
4 = 80% wound surface represented by red granulation tissue
5 = 100% wound surface represented by red granulation tissue
Measurement of wound
a. Linear measurement – Length, Width, Depth, Area
b. Unit healing time
a) Wound size measurements
Two types of wound measurements that track the change in the wound size over time were followed:
I. Surface area (SA) measurements (length by width)
II. Depth
The SA length and width of the wound were measured from wound edge to wound edge. It was measured across the diameter of the greatest length and the greatest width. Then the length was multiplied by the width, which gave the estimated square area of the wound or SA.
b) Unit healing time
Unit healing time was calculated from the total number of days during treatment divided by initial area-last area of wound in square centimeter. So it can be
represented as:
UHT = Total number of days taken during treatment
Initial area – Last area of wound (sq. cms.)
Biochemical Parameter
Bacterial load
Tissue biopsy was taken for the quantitative and qualitative estimation of the bacterial load before treatment, after 2 weeks and 4 weeks of treatment patients. Method followed in the laboratory Procedure Wound biopsies were processed at a single microbiology laboratory. The tissue specimens were collected under strict sterile conditions. Tissue biopsy was done which is removal of a piece of tissue with a scalpel or by punch biopsy. Before performing a tissue biopsy for wound culture, the area was cleansed with sterile solution which did not contain antiseptic. The biopsy was performed and pressure applied to the area to control bleeding. The biopsy tissue was promptly transported to the laboratory where it was weighed, ground and homogenized, serially diluted in test tubes and plated onto blood agar. Plates were incubated under anaerobic conditions at 40°C for 24 h. Because dilutions were based on weight of tissue, the plate count multiplied by the dilution factor yielded the number of organisms per gram of tissue.
Table No.7.3: Scoring of microbiological system (Bhatt Shobha K et al, 2014)
Score Number of micro-organisms Microbial status of wound
0= Less than 105 No infection
1= 105 to 10 6 Mild infection
2 =107 to 108 Moderate infection
3= More than 108 Severe infection.

Follow Up
The subjects were asked to visit the OPD or the wound clinic once in 15 days upto one month. For statistical evaluation, the above readings were divided into three grading - mildly infected (values with 105 and 106 dilutions), moderately infected (values with 107 and 108 dilutions) and severely infected (values > 108) given with Grades 1–3 respectively.5

OBSERVATION

Table No.2: Statistical analysis of microbial load

Group	Mean ± SD of log of microbial load			Within the group comparison paired 't'Test	
	BT	AT2	AT4	BT-AT2	BT-AT4
Group I	7.22±1.07	4.52±1.34	1.93±1.02	d= 2.70±1.39 t = 10.623 p < 0.001	d=5.16±1.44 t = 15.184 p < 0.001
Group II	7.24±1.16	4.56±1.13	2.28±0.75	d= 2.68±1.31 t = 11.359 p < 0.001	d=4.64±1.26 t = 15.131 p < 0.001
Between the group unpaired 't' Test	t = 0.071 p = 0.944	t = 0.140 p = 0.889	t = 0.135 p = 0.265		

This statistical study shows that microbial load was decreased in both group at the end of 2 nd week and 4 th week in both group and this decrement of microbial load was statistically highly significant (p<0.001) in both groups. Higher decrement of microbial load was in group I than group II but statistically insignificant (p>0.05).

Table No.3: Statistical analysis of UHT according to Microbial Load

Microbial Load	Mean ± SD of UHT		Between the Group Comparison Unpaired 't'Test
	Group I	Group II	
Mild	3.07±1.17	3.62±1.14	t = 1.285, p = 0.210
Moderate	8.37±2.84	6.15±1.26	t = 2.792, p = 0.009
Within the Group mild v/s moderate Comparison Unpaired 't' test	t = 6.305 p < 0.001	t = 5.851 p <0.001	

This statistical analysis shows that mild infected wound showing better Unit Healing (p < 0.001) than moderate infected wound in both groups. Better unit healing time of mild infected wound of group I than Group II but statistically insignificant (p= 0.210). Statistically better unit healing time of moderate infected wound of group II than group I (p=0.009).

DISCUSSION

Clinical Study focused mainly on studying of efficacy of drug on three basic properties that an ideal dressing must have i.e. debridement, antibacterial and healing. Effect of Lakshadi Gana Taila was compared with povidone iodine. For this, clinical study completed on 61 patients by randomly allocated them into two equal groups as Group I (treated by Lakshadi Gana Taila) and Group II (treated by povidone iodine 5% solution). The effect of drug was observed on clinical parameters such as pain, discharge, tenderness, malodor, granulation tissue, surface area, depth and UHT. Antibacterial effect of drug was analyzed on the basis of microbial load of wound.

Pharmacological properties of ingredients (Laksha, Nimba, Haridra and Amaltas) of Lakshadi Gana Tail, according

to Ayurvedic and Modern parameters along with criteria for drug selection, and forms for use of drug were also reviewed individually. The authenticity of the raw drugs is confirmed by the macro-microscopic examination. Medicated oil was examined for pH and compositional analysis by methylation of fatty acids. Gas chromatography mass spectrometry identification of Lakshadi Gana Taila was performed. GCMS showed that there is 14 compounds were present out of which 5 new compounds detected in Lakshadi Gana Taila. These 9 compounds are – Tetradecane, Heptadecane, 2-Methy Pentadecane, Hexadecane, 2, 6, 10- Trimethyl Dodecane, Heneicosane, Octadecane, Octacosane and Squalene.

This study revealed that occurrence of infected wound (Dushta Vrana) is more common in hindu, male patients of 31-60 years age group having > 6 weeks – 12 weeks duration of wound, labor with lower socioeconomic status belonged to rural habitat having mixed diet pattern, Nija (Endogeneous) aetiological factor with gradual onset. Infected wound (Dushta Vrana) occurrences is more in weaker section of society living in rural areas, doing strenuous physical work as labors and so are more prone to trauma results in Dushta Vrana.

In majority of cases, Dushta Vrana was found in perianal (in 30 subjects out of 61 subjects) and lower limb (26 out of 61 subjects) site of wound. Staphylococcus aureus species was the commonest microorganism causing wound infections in this study.

Average unit healing time was found minimum in 31-60 years age, mostly males, opted mixed diet pattern, having 6-12 weeks of duration of wound of Agantuja etiology in both Groups, middle socio-economic status in Group I and high in Group II. These results based on observing mean of analysis but statically insignificant within and between the groups. The wound treated with trial drug, healed faster (having minimum UHT) in comparison to wounds treated by control group. Surface area was markedly reduced in treated group. This result on observing median but statistically analysis was found insignificant. Depth was markedly reduced in treated group. This result on observing mean but statistically analysis was found insignificant. Vranashodhana (Wound debridement) was assessed based on the following parameters in both the groups- A. Clinical signs and symptoms of infection including pain, discharge, tenderness and malodor. In this study it was noticed that statistically significant values there in both group ($p<0.001$) on all sign and symptoms and between the group comparison showed insignificant p value.

Thus, it can be concluded that both the group drug is almost equally effective in wound debridement. Microbial load estimation supported further the clinical finding too as trial drug Lakshadi Gana Taila showed significant value in reducing the microbial load and by comparison between groups it was showed insignificant result. In this way, it can be concluded from observation of clinical observation and microbial load both that trial drug used for wound debridement is good.

Median value of the surface area was decreased in every assessment in both groups which is statically highly significant ($p<0.001$) in both group. Wound healing is better in group I than II but statically not significant ($p>0.05$). On intergroup comparison, the trial group (Group I) was having better healing response than control group (Group II) by observing mean but the difference between group I and group II found statistically insignificant. There was no local or systemic side effect observed after application of trial drug.

CONCLUSION

There is a marked reduction of pain, tenderness discharge, malodor, surface area, depth, microbial load & improvement in granulation tissue. It can be concluded that Lakshadi Gana Taila is effective Ayurvedic formulation in healing mild to moderate infected wound. From the above clarifications, it can be concluded that Lakshadi Gana Taila efficiently decreases the microbial load, clinically controls infection, hastens wound healing and can be recommended in the management of chronic infected wound.

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