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EFFECT OF PALASH KSHARA ON MICROBIAL LOAD IN INFECTED WOUND WITH SPECIAL REFERENCE TO WOUND BED PREPARATION

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ABSTRACT

Background: Healing of wound is a challenging task to surgeon in ancient time and even in today's era. Healing is protective mechanism of our body to invade against infection. In modern science many antibacterial formulations are available with their own limitations. non-surgical debridement modalities which were described by Sushruta in context of Vrana chikitsa. Kshar is one of them as effective debridement agent for wound bed preparation. The present study was undertaken to determine the effect of Palash Kshara (Butea monosperma (Lam.) on Microbial load in infected wound wsr to Wound Bed Preparation. This study was conducted for a period of two years from March 2017 to Feb 2019 in Department of Shalya Tantra, Sir Sundar Lal Hospital, IMS, BHU, Varanasi. **Aims & Objective:** To evaluate the effect of Palash Kshara in infected wound on the basis of Microbial load assessment. **Material & Methods:** Present study was carried out in total 20 patients of infected wound divided into 2 groups of 10 patients in each, according to drug application used on infected wound. In Group A (Treated Group) Palash Kshara pichu was applied on infected wound, while in Group B(Control Group) patients with infected wound were dressed daily with normal saline. 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. **Result:** *Palash Kshara pichu* as a dressing material is a better than normal saline for the management of Infected wound(Dusta Vrana). This is because the patients treated with Palash Kshara showed faster reduction in Edge, Necrotic Tissue type and amount and Exudate Type and amount, Skin colour surrounding tissue, Peripheral Tissue Edema. 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 in reducing the microbial load and thus controlling infection, facilitating wound healing.

KEYWORDS: Infected wound, Microbial load, Palash ,Kshara, Vrana shodhana.

INTRODUCTION

Human beings sustain wounds across their lifespan that range from a simple knee abrasion to a major surgical incision. With most acute wounds (abrasions, lacerations, or surgical incisions), no excessive concern is necessary since humans are "programmed" to heal and acute injury triggers the repair process. However, when an acute wound fails to heal normally or a wound develops as a result of a chronic condition (e.g., a venous or arterial ulcer), the patient's quality of life can be seriously affected, and the costs of care can increase substantively.

Wound is a discontinuity or break of the surface of the body. Wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular sequelae[1] Wounds may develop the deposition and multiplication of bacteria in tissue with an associated host reaction wherein they are called as infected wounds. Infection of a wound may be defined as invasion of organisms through tissues following a breakdown of local and systemic host defenses.[2] Wound infection has probably been a major complication of surgery and trauma.[3] SSI may be defined as invasion and multiplication of microorganisms in body tissue which may be clinically in apparent, or result in local cellular injury because of competitive metabolism, toxins, intracellular replication or antigen-antibody response.[4] 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 1×10^5 organisms per gram of tissue.[5,6,7,8] Hence, it becomes essential that a drug used to control the wound infection must necessarily reduce the microbial load.

In Sushruta Samhita a lot of description is available regarding the wound and its management under the

heading of Vrana (Su.Su. 21/40). If we ignore simple Vrana or do not manage it properly, then it can be converted into Dusta Vrana (Su.Su. 22/7). In management of Vrana, Acharya Sushruta has explained Shasti-Upakrama (the sixty types of wound management). Vrana Shodhana (wound bed preparation) and Vrana Ropana (wound healing) are the two main steps of wound management(su chi 1). Kshara karma has strong Shodhana and Lekhana property so it could be a better option for Vrana Shodhana. The main aim of Vrana Shodhana is to remove the dead tissue, keeping the wound bed free from unwanted and harmful material thus minimise the reactionary inflammation. Palash (Butea monosperma)[3] is one of the drug which give Kshara in sufficient quantity. At present time it has been found in studies that Palash has beneficial affect on wound healing in experimental and animal models (Suguna et al 2005, Muralidhar et al).[9,10] It has been found that Palash Kshara act as an excellent agent for debridement and it has healing property (Dr. Supriya Gupta 2014, Aarti Chaurasia 2018). Considering these facts the present work "Effect of Palash (Butea monosperma) Kshara on Microbial load in Infected Wound with special reference to Wound Bed Preparation" initiated to explore the nonsurgical debridement agent on local administration in infected/ chronic non healing wound.

MATERIAL AND METHODS

The present study was planned to evaluate the effect of Palash Kshara on Microbial load in Infected Wound with special refrence to Wound Bed Preparation exclusively on clinical parameters.

Source of the drug: For preparation of Kshara, Stem bark of Palash were collected from peripheral area of Varanasi.

Methods of the study

Selection of cases

All patients with Infected Wound were registered from Shalya OPD/IPD of Sir Sundarlal Hospital IMS, BHU, Varanasi. Random selection was made irrespective of group, age, sex, religion, socio-economic status, inhabitancy, occupation, diet, addiction, type, site, onset, duration of wound, sign-symptom, including infected surgical wound were also registered. However, malignant, tubercular, diabetic ulcers with uncontrolled diabetes were excluded from the study.

Grouping of cases: The present study was carried out clinically. 20 patients were registered with infected wound, diagnosed after detail clinical history and investigatory finding as per designed proforma.
They were divided into two groups
Group A (Treated Group) - Dressing with *Palash Kshara Pichu*

Group B (Control group) - Dressing with Normal Saline

In both the group, Patients were clean & dressed daily & all the criteria were assessed daily. In every group, Tissue biopsy was taken from edges of Wound and Wound bed (before treatment day-0 and during treatment day-1, day-2, day-3, day-4, day-5, day-6) for the bacterial load assessment with patient's informed written consent.

Inclusion criteria – Mild to Moderate Infected wound.

Exclusion criteria - Malignant ulcer, Diabetic ulcer with uncontrolled diaabetes, Tubercular ulcer, Syphilitic ulcer, Leprotic ulcer, Actinomyecetes, HIV, HbsAg, Anti HCV (+) patient, CRF.

Method of Preparation of Palash kshara

Stem bark of Palash were taken from healthy plant, and then kept in shadow for drying. After proper drying in shadow it was burnt in a windless place. When the fire was extinguished the ash was collected and separated. Ashes were mixed in water (six times in volume) and the precipitate was allowed to settle down. Finally the supernatant fluid was collected in a separate vessel of stainless steel. The residual ashes were again mixed with four times of water and the same procedure was repeated at least twice in order to take away all the alkaline material from ashes. Ultimately the ashes remain as a neutral residue which should be thrown. The collected fluid was now filtered drop by drop through a double Whitman Filter Paper into a clean glass bottle. Finally, Kshara was a phytochemical substance obtained from evaporation of filtered solution of ash prepared by incineration of Palash.

Yield of Kshara

Part used- Stem bark of Palash (Butea monosperma)
Wt. of crude part-
Fresh - 30 kg
Dried - 10.8kg

	Natural burn
Ash	825 gm
Water taken	6 times
Yield	36 gm

Method of Palash Kshara pichu formati

A cotton gauze piece of 25 x 25 cm was mounted over the spherical hanger. 4g Guggulu (It was obtained from **Soxhlet Method** of extraction) was mixed with sufficient amount of alcohol (approximate 4ml) to make a solution. 4 layer of this solution were coated on prepared frame. Frame was allowed to dry hanging in hot air cabinate (at 40^0 C) for one day after each coating.

The prepared 4 gm Kshara was mixed with sufficient amount of alcohol (approximate 4ml) and this solution was used for coating on the prepared cotton frame. Frame was allowed to dry in hot air cabinate for one day after coating. After drying Small pieces of kshara pichu

(5×5 cm) were cut and packed in sterile dried test tubes to be used on the wound.

Application of Drug After culture of the discharge of the Dusta Vrana (Chronic non healing / infected wound), the Kshara was used for cleaning of the wound and applied (as Pichu form) over wound. The dressing was changed daily. Surgical debridement was also done in cases of excessive and extensive sloughing and necrosis was present to remove the dead necrosed tissues. This procedure was repeated every day and the sequential change in the wound were recorded from day 0 to day 6.

Microbiological study

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 appendorf 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.

Criteria for assessment

The primary study variables were the following:

1. Bates-Jensen Wound Assessment Tool[11] was used to assess the wound's status. All the items were assessed daily from day 0(Before Treatment) to day 6th. Rating against each item is done by picking the response that best describes the wound and entering that score in item score column for the appropriate date. When rated the wound on all items, total score was determined by adding together the 13-item score. The Higher the total score, the more severe is the wound status. This clinical study was conducted for 7 days only.

Assessment Parameters

1. Size 2.Depth 3.Edge 4.Undermining (5,6)Necrotic Tissue Type and Amount, 7,8. Exudate Type and Amount 9. Peripheral Skin Color 10,11. Peripheral Tissue Edema and Induration 12. Granullation Tissue 13. Epithelization

2. Culture findings based on viable wound tissue specimens. Wound culture using tissue biopsy to assess the microbial load was done before treatment (day0) and during the course of treatment (from day1 to day6). The biopsy was taken and bacterial load was assessed in microbiology. The change in the microbial load prior treatment and during the treatment was assessed daily. For statistical evaluation, **Scoring of Bacterial load was done to study which is as follows**

Score	No of micro-organism	Microbial status of wound
0	Less than 10^5	No infection
1	10^5	Mild infection
2	10^6	Mild infection
3	10^7	Mild to Moderate infection
4	10^8	Moderate infection
5	10^9	Moderate infection
6	$>10^9$	Severe infection

For statistical analysis, Mann Whitney Test and Unpaired t Test was used for comparing results between the groups. Paired t Test and Wilcoxon signed ranks Test was used for within the group comparison during follow-ups.

OBSERVATION AND RESULTS

The present study out of 20 patients, maximum number i.e. 15 (75%) were from the age group of 31-60 years followed by > 60 years i.e. 3(15% and <30 years i.e. 2 (10%). More number of patients were male i.e. 17 (85%), maximum number of patients were hindu (95%) followed by 5% muslim. Incidence of wound infection was maximum in worker, Farmer (about 30%) and Businessman (about 30%) a followed by Serviceman (20%), Housewife (15%) and student (5%).Incidence of infected wound was 65% in rural area than that compared to urban area(35%). About 65 patients was addicted to multiple type of addiction. Maximum no. of patients (65%) was found from rural area. Maximum no of patients (55%) were taking mix diet. maximum number i.e. 10 (50.00%) were of Nija Vrana (ulcers due to systemic diseases). Major finding of this study showed that 10(50%) of patients were had history of wound of fall between < four weeks of duration on their first visit. This clearly indicates that wound in acute condition and high bacterial bioburden.

Following observations were made on the 20 subjects based on their grading. No singnificant change was found in size and depth, peripheral tissue induration, undermining of infected wound. This study reveled that on intragroup (within the group) comparison.

Edge

In **Group A** at day-0= 3,5,2 patients have score 2,3,5 respectively which gradually reduces to 1,6,2,1 patients having score 1,2,3,4 respectively at day-6

In **Group B** at day-0=3,5,1,1 patients have score 2,3,4,5 respectively which gradually reduces to6,3,1 patients having score 2,3,4 respectivelyat day-6.

Group-A (Treated Group) has statistically significant change in score for edge (P <0.05) while in Group B (Control Group) statistically non-significant change. (P >0.05).

Necrotic Tissue Type
In **Group A** at day-0= 1,1,2,6patients have score 2,3,4,5 respectively which gradually reduces to 3,7patients having score 1,2 respectively at day-6.

In **Group B** at day-0=3,4,3 patients have score 2,3,4 respectively which gradually reduces to 5,4,1 patients having score 1,2,3 respectively at day-6.

Group-A (Treated Group) as well as Group B (Control Group) have statistically significant change present in score for necrotic tissue type from day-0 to day-6 ($P < 0.05$).

Necrotic Tissue Amount
In **Group A** at day-0= 1,2,7patients have score 2,4,5 respectively which gradually reduces to 3,5,2patients having score 1,2,3 respectively at day-6.

In **Group B** at day-0=1,2,5,2patients have score 1,2,3,4respectively which gradually reduces to 7,3 patients having score 1,2,respectively at day-6.

Group-A (Treated Group) as well as Group B (Control Group) has statistically significant change present in score for necrotic tissue amount from day-0 to day-6 ($P < 0 .05$).

Exudate Type
In **Group A** at day-0= 3,7patients have score 3,5 respectively which gradually reduces to 2,5,3patients having score 1,2,3 respectively at day-6.

In **Group B** at day-0=2,6,2patients have score 2,3,5respectively which gradually reduces to 6,4 patients having score 1,2, respectively at day-6.

Group-A (Treated Group) as well as Group B (Control Group) has statistically significant change present in score for exudates type from day-0 to day-6 ($P < 0 .05$).

Exudate Amount
In **Group A** at day-0= 2,2,,2,4patients have score 2,3,4,5 respectively which gradually reduces to 2,6,2patients having score 1,2,3 respectively at day-6**.**

In **Group B** at day-0=4,4,2patients have score 2,3,4 respectively which gradually reduces to 5,5 patients having score 1,2, respectively at day-6.Group-A (Treated Group) as well as Group B (Control Group) has statistically significant change present in score for exudate amount from day-0 to day-6 ($P < 0 .05$).

Peripheral Skin Color
In **Group A** at day-0= 3,1,4,2patients have score 1,2,3,4 respectively which gradually reduces to 6,2,1,1patients having score 1,2,3,4 respectively at day-6**.**

In **Group B** at day-0=4,4,2patients have score 1,2,4 respectively which gradually reduces to 8,2 patients having score 1,2, respectively at day-6.

Group-A (Treated Group) has statistically significant change present in score for skin color of surrounding skin from day-0 to day-6 ($P < 0.05$).

While in Group-B (Control Group), statistically non-significant change present from day-0 to day-6 ($P>0.05$)

Perpheral Tissue Edema
In **Group A** at day-0= 4,4,1,1patients have score 1,2,4,5 respectively which gradually reduces to 8,1,1patients having score 1,2,4 respectively at day-6**.**

In **Group B** at day-0=7,2,1patients have score 1,2,4 respectively which gradually reduces to 10 patients having score 1respectively at day-6.

Group A (Treated Group) has significant change in score for peripheral tissue edema ($P <0.05$) while Group B, statistically non-significant change present from day-0 to day-6($P>0.05$)

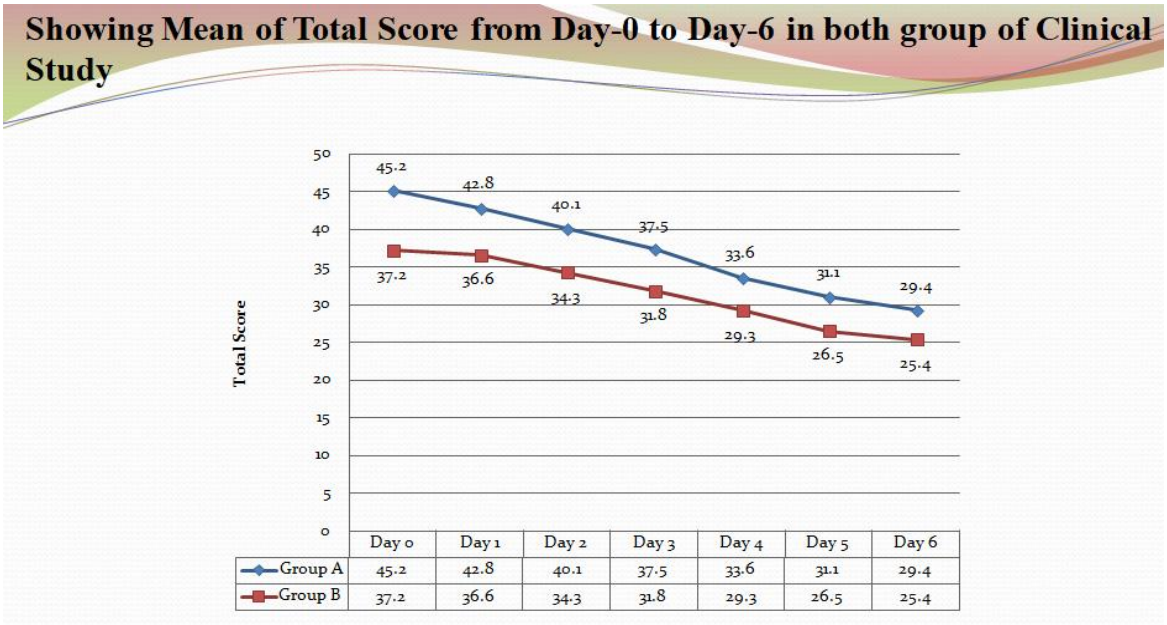
Granulation Tissue
In **Group A** at day-0= 1,2,7patients have score 3,4,5 respectively which gradually reduces to 1,4,5patients having score 1,2,3 respectively at day-6**.**

In **Group B** at day-0=2,3,4,1patients have score 2,3,4,5 respectively which gradually reduces to 2,7,1 patients having score 1,2,3 respectively at day-6.

Group A (Treated Group) as well as Group B (Control Group) has statistically significant change in score for Granulation tissue from day-0 to day-6 ($p <0.05$).

Total Score of All 13 parameter in both group from day 0 to day 6.

Group	Mean ± SD							Mean Diff.	Within the Group Paired t test
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6		
A	45.20± 6.339	42.80± 5.750	40.10± 5.087	37.50± 4.577	33.60± 4.502	31.10± 4.332	29.40± 4.671	15.800± 4.158	t =12.016 p=0.000
B	37.20± 6.179	36.60± 5.892	34.30± 5.755	31.80± 5.432	29.30± 5.417	26.50± 5.061	25.40± 5.016	11.800± 5.808	t =6.425 p=.000
Between the Group, Unpaired t test	t = 2.858 P=.010	t =2.381 P=.028	t =2.388 p=.028	t =2.538 p=.021	t =1.931 p=.069	t =2.184 p=.042	t =1.845 p=.081		



Analysis of total score in both the group were carried out initially at day-0 and then after from day-1 to day-6.

In group A , initially mean total score was 45.20±6.339 and first, second, third, fourth, fifth, sixth, it was 42.80±5.750, 40.10±5.087, 37.50±4.577, 33.60±4.502, 31.10±4.332, 29.40±4.671. Mean difference in total score between day-0 and day-6 was 15.800±4.158.

In group B, initially mean total score was 37.20±6.179 and first, second, third, fourth, fifth, sixth, it was 36.60±5.892, 34.30±5.087, 31.80±5.432, 29.30±5.417, 26.50±5.061, 25.40±5.016. Mean difference in total score between day-0 and day-6 was 11.800±5.808.

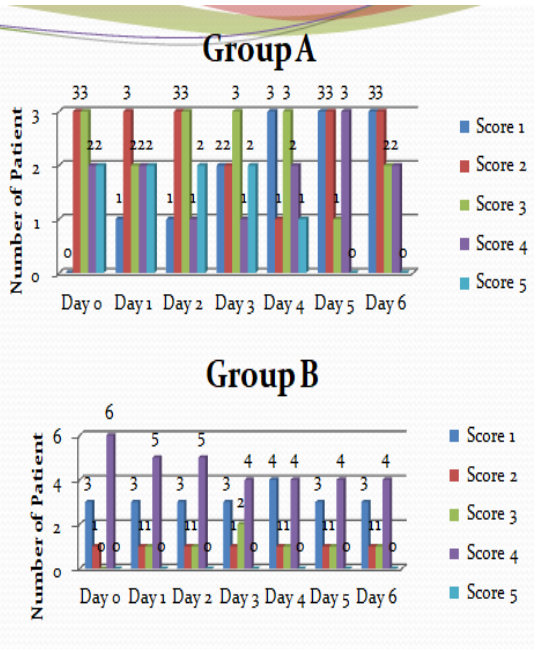
The results reveals that on intragroup (within group) comparison, Group A (Treated Group) as well as Group B (Control Group) has significant changes present (Because P value < .001) from day-0 to day-6. Mean difference in Group–A was (Treated Group) was 15.800±4.158 while in Group B (Control Group) was 11.800±5.808. This mean difference shows that Group A (Treated Group) is more active in comparison to Group – B (Control Group).

Thus Palash kshara pichu is more effective as compare to Normal saline in preparing wound bed preparation.

Bacterial load

Group	Score	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	1	0	1	1	2	3	3	3
	2	3	3	3	2	1	3	3
	3	3	2	3	3	3	1	2
	4	2	2	1	1	2	3	2
	5	2	2	2	2	1	0	0
B	1	3	3	3	3	4	3	3
	2	1	1	1	1	1	1	1
	3	0	1	1	2	1	1	1
	4	6	5	5	4	4	4	4
	5	0	0	0	0	0	0	0
Between the Group Comparison, Mann Whitney Test		Z=.512 P=.609	Z=.468 P=.640	Z=.233 P=.816	Z=.233 P=.816	Z=.236 P=.813	Z=.039 P=.969	Z=.156 P=.876

Within the Group Comparison Wilcoxon signed ranks Test Day 0 Vs Day 6	Group A	Group B
	Z=2.640 P=.008	Z=1.890[a] P=.059

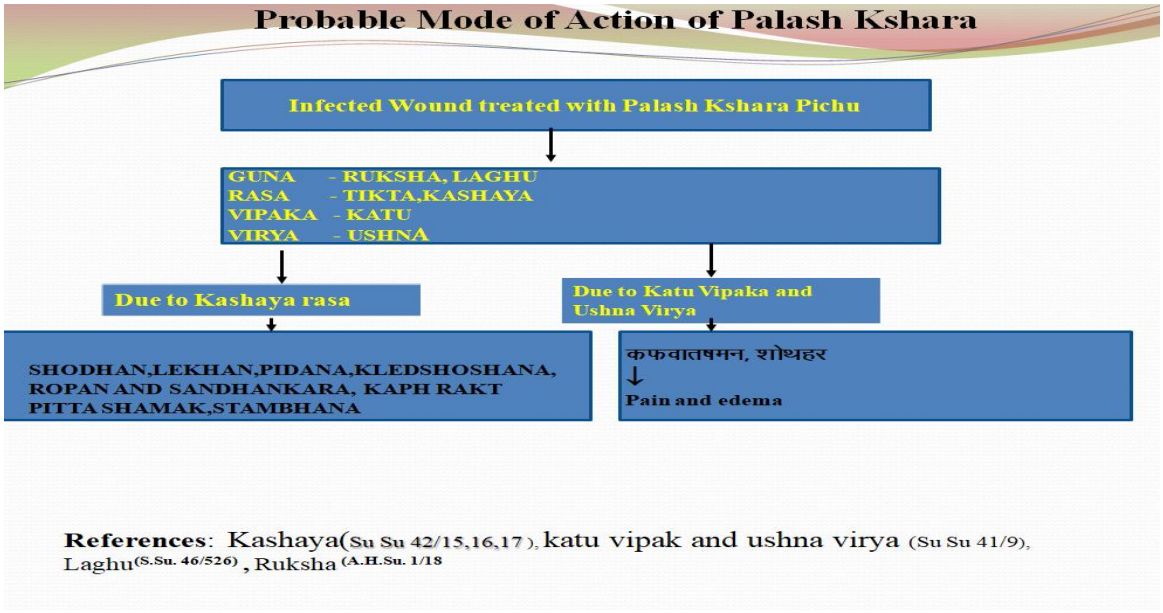


In Group A, at day-0 = 3,3,2,2 patients have score 2,3,4,5 respectively which gradually reduces to 3, 3,2, 2 patients having score 1,2,3 ,4 respectively at day-6

In Group B, at day-0 = 3, 1, 6, patients have score 1,2,4 respectively which gradually reduces to 3,1,1,4 patients having score 1,2,3,4 respectively at day-6.

The result reveals that on intragroup (within group) comparison, Group A (Treated by Palash Kshara) has highly significant changes present in score($p<0.01$) while Group B (Control Group) has non-significant changes present in score for bacterial burden from day-0 to day-6 ($p >0.05$)

From the above observations, it is clear that *Palash kshara* is effective in reducing the microbial load of the chronic non healing wounds. Anyhow the action on individual variety of pathogens cannot be explained as only quantitative assessment of the load was included in the study.



SUMMARY AND CONCLUSION

- *Palash Kshara* was far superior in between two groups in terms of reduction of edges and surrounding skin color, peripheral tissue edema and necrotic tissue type and amount, exudate type and amount and granulation tissue score along with bacterial load score. All of which helps and promote faster healing of wounds. But its application appears to be most effective in a preparing wound bed, which essentially is provided by debridement.

- Palash kshara has significant properties like fast debridement, Promote healthy granulation tissue formation along with removal of necrotic tissue and exudates amount and antimicrobial properties, all of which are helpful in early and easy preparation of wound bed.

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