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A clinical study on the efficacy of *Panchavalkala* cream in *Vrana Shodhana* w.s.r to its action on microbial load and wound infection

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Abstract

Background: The science of wound healing is advancing rapidly, particularly as a result of new therapeutic approaches. The wound healing effect of different herbal ointments have been enormous and are in wide practice these days. **Aim:** To evaluate the efficacy of *Panchavalkala* cream over wound debridement (wound infection and microbial load). **Materials and Methods:** *Ghanasatwa* (water extract) of the individual drugs of *Panchavalkala* was prepared and the extract formulated as herbal ointment. 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 Slough, swelling, redness, pain, discharge, tenderness, and malodor in wounds showed statistically significant 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 in reducing the microbial load and thus controlling infection, facilitating wound healing.

Key words: Chronic non healing wounds, infection, microbial load, *Panchavalkala*, *Vrana Shodhana*

Introduction

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[1] 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.[2,3]

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.[4-7] Hence, it becomes essential that a drug used to control the wound infection must necessarily reduce the microbial load.

In the Indian context, the formal descriptions of wound care have been vividly elaborated in the three great treatises (*Brahatrayi*) of Ayurveda viz. *Charaka Samhita*, *Sushruta Samhita* and *Astanga Sangraha*. These documents not only describe *Vrana* (various types of wounds) but they also present their systematic classification along with their management including various

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systemic and local drugs and preparations. Sushruta, the father of Indian surgery in 1000 BC has elaborated the concept of *Vrana*. He not only gave an elaborate description of various types of wounds, but also presented a descriptive etiopathogenesis of wounds along with their management. 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. His techniques are broadly classified as *Vrana Shodhana* and *Vrana Ropana*. He advocated external application of various drugs under these categories. One among them is the *Nyagrodhadi Varga* mentioned in *Vrana Ropana Kashaya* which includes *Panchavalkala*.[8] Clinically, *Panchavalkala* i.e. group of barks of five trees – *Vata* (*Ficus bengalensis* L.), *Ashwatha* (*Ficus religiosa* L.), *Udumbara* (*Ficus glomerata* Roxb.), *Plaksha* (*Ficus lacor* Buch-Ham.), *Parish* (*Thespesia populenea* Soland. ex corea.), is found to be very effective in controlling wound infection when used externally in different forms, which suggests its action on *Vrana Shodhana* as well. Bearing this in mind, in the present study, the effect of *Panchavalkala* cream over wound debridement with special reference to wound infection and the microbial load was estimated so as to prove its efficacy on *Vrana Shodhana*.

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 (Ref. No. IMS/I 43/2009/301906) and informed consent from each patient obtained.

Setting and sample

The study population consisted of 50 subjects with nonmalignant chronic wounds. 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.

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 by Sushruta on *Dushta Vrana* (non-healing ulcers). Patients diagnosed to have chronic non healing wounds which were infected, were randomly selected, irrespective of age, sex, associated disease etc., Patients without infection and malignant wounds were excluded from the study.

Test drug - *Panchavalakala* cream

Method of preparation of Ghanasatwa (dry aqueous extract) from Panchavalkala drugs

Fresh drugs were collected and cleaned with normal water and then dried for 10–15 days. They were then cut to small pieces (*Yawakuta Choorna*). Then decoction (*Kwatha*) of the individual drugs were prepared by adding 8 times of water, boiling at 100°C until the water reduced to ¼ of its initial volume, then it was filtered using a clean cloth and the liquid portion was separated from the *Yawakuta* of the drug. It was re-filtered to avoid presence of impurities. This liquid portion was kept in hot water bath for few hours and when it became concentrated; it was kept in incubator at 40°C for 4–5 days. When the drug dried up completely, it was powdered and stored in air tight containers. This powder is nothing but the *Ghana Satwa* or the aqueous extract of the drug. The yield of the individual drugs of *Panchavalkala* is depicted in Table 1. Each of the drugs was neatly labeled.

Finally 40 g of each drug was taken and mixed to prepare a homogenous *Panchavalkala* extract.

Method of preparation of the cream

Composition of the test drugs were depicted in Table 2.

Step 1

Take light liquid paraffin, glycerine, butylated hydroxy toluene, methyl paraben and propyl paraben in stainless steel (SS) container. Gently heat while stirring until butylated hydroxy toluene, methyl paraben and propyl paraben fully dissolve.

Step 2

Take water in separate SS container and add Sodium Lauryl Sulphate (SLS). Heat gently until SLS dissolve. Add the required extract into the water and heat gently till it get fully dissolved. Filter the solution with 100# filter cloth.

Step 3

Heat hard paraffin and cetostearyl alcohol in separate container (at temp 80°C) to melt.

Step 4

Attach step 2 solution with homogenizer and mix properly. Add the step 1 solution into the container with step 2. After

Table 1: Yield of *Ghanasatwa* of *Panchavalkala*

Drug	Part used	Weight of crude part (kg)	Water taken (times)	Yield (g)
Vata	Bark	2	8	65
Udumbara	Bark	2	8	45
Ashwatha	Bark	2	8	50
Parish	Bark	2	8	40
Plaksha	Bark	2	8	45

Table 2: Composition of the *Panchavalkala* cream

Ingredient	Quantity
Dried extract of the drugs	As per required percentage for all the variants
Base	
Light liquid paraffin	12%
Butylated hydroxy toluene	0.1%
Propylene glycol	5%
Propyl paraben	0.02%
Sodium lauryl sulfate	0.5%
Hard paraffin	3%
Cetosteryl alcohol	8%
Methyl paraben	0.2%
Glycerine	5%
Water	QS

QS: Quantity sufficient

thoroughly mixing, mix the step 3 (melted part) into step 2. Solution will become viscous and formation of cream will start. After cooling smooth cream will form. Total mixing time is 12 min.

Thus prepared *Panchavalkala* cream in ointment form was packed in tubes.

Posology

Panchavalkala cream was administered by local application once daily for dressing of the wound until complete debridement. Surgical debridement was done as per the need. Internally, analgesics were administered whenever necessary.

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 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.

Criteria for assessment

The primary study variables were the following:

1. Clinical signs and symptoms of infection

A clinical signs and symptoms checklist was developed to measure the presence or absence of chronic wound infection (that is- slough,[9] swelling,[10] redness, discharge, malodor,[11] pain and tenderness[12]). A grading system for each sign and symptom was prepared under the guidance of wound experts.

The grades were noted before and after the treatment. Weekly assessment was done in all the patients and the grades of each of the above mentioned signs and symptoms were assessed during the course of treatment.

2. Culture findings based on viable wound tissue specimens.

Wound culture using tissue biopsy to assess the microbial load was done thrice during the course of treatment. The biopsy was taken at the first visit, which is prior to the treatment and the load assessed. The procedure was followed after 2 weeks and 4 weeks of treatment. The change in the microbial load prior and during the course of treatment was assessed.

For statistical evaluation, the above readings were divided into three grading - mildly infected (values with 10^5 and 10^6 dilutions), moderately infected (values with 10^7 and 10^8 dilutions) and severely infected (values > 10^8) given with Grades 1–3 respectively.

The statistical values were then inferred using Student's *t*-test (paired).

Grade	Evaluation
Evaluation of slough (0-5 scale)	
0	No slough
1	20% wound surface covered with slough
2	40% wound surface covered with slough
3	60% wound surface covered with slough
4	80% wound surface covered with slough
5	100% wound surface covered with slough
Grading for swelling	
0	No swelling
1	Mild swelling <2 cm
2	Moderate swelling with tenderness
3	Swelling >5 cm with tenderness
Grading for redness	
0	No redness
1	Slight red, tender and hot with painful movement and without indurations
2	More red, having painful movement, with more local temperature and with indurations
3	Angry look, hot, resist to touch and with more indurations
Grading for pain: Visual analogous scale	
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 and little wet dressing
2	Often discharge and 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 and rigidity
Grading for malodour	
0	No smell
1	Bad smell
2	Tolerable unpleasant
3	Foul smell which is intolerable

Observations

Among the 50 subjects, males outnumbered the females (71.4%), majority of them were found in the age

group of 41–50 years (40%). Almost half the subjects belonged to the labor class (50%). In 64.3% of the subjects, the mode of onset was sudden and in 35.7% the duration of wound was between 3 and 6 months. About 83.3% of subjects complained of pain out of which 26.2% had mild pain, 35.7% moderate pain and 21.4% had severe pain. Nearly 88.1% of the subjects observed discharge out of which 52.4% had serous discharge, 19.0% sero-sanguineous and 16.7% had purulent discharge. The shape of the wound was irregular in 47.6% and 54.8% of the wounds were in the foot. 88.1% of the subjects had single wound and 11.9% had multiple wounds. About 14.3% observed glossy red and edematous areas surrounding the wound, whereas 11.9% observed eczematous and pigmented surroundings. The remaining 73.8% were either normal or in other forms. Based on the type of the wound 38.1% of the subjects had diabetic wound where as 35.7% had traumatic. 2.4% had tropical, 9.5% each had venous and arterial wounds. The remaining 4.8% of the subjects had leprotic wound.

Following observations were made on the 50 subjects based on their grade of slough - Grade 0–7.1%, Grade 1: 11.9%, Grade 2: 14.3%, Grade 3: 14.3%, Grade 4: 11.9% and Grade 5: 40.5%. More than half of the 50 subjects (59.5%) were classified under the Grade 0 category of swelling, where as 28.6% were under Grade 1 category. 4.8% of the subjects were classified under Grade 2 category and 7.1% under the Grade 3 category. 85.7% of the 50 subjects had Grade 0 category of redness around the wound, 11.9% had Grade 1 category of redness, and 2.4% had Grade 2 redness. There were no subjects with Grade 3 redness. Since an average of 16.7% of the 50 subjects did not complain of pain, out of the rest, 26.2% had mild pain, 35.7% had moderate pain whereas 21.4% had severe pain. Similarly, since an average of 11.9% of the 50 subjects did not complain of discharge, out of the rest, 52.4% had serous discharge, 19.0% had sero-sanguineous discharge whereas 16.7% had purulent discharge. Nearly about half of the 50 subjects (47.6%) had no tenderness. Of the rest, 35.7%belonged to Grade 1 category, 2.4% to Grade 2 category and remaining 14.3%to Grade 3 category. Of the 50 subjects, 19.0% did not have any malodor, 40.5% had Grade 1 malodor, 26.2% had Grade 2 and 14.3% had Grade 3 malodor.

Results

Slough, swelling, redness, pain, discharge, tenderness, and malodor in wounds showed statistically significant reduction following treatment [Table 3].

Effect of therapy on microbial load

The average microbial load of the wounds was reduced from 1.80 to 0.32 showing marked improvement with mean difference of 1.48 ± 0.71 and *t* value 10.362, which is statistically significant ($P = 0.001$) [Table 4].

From the above observations, it is clear that *Panchavalkala* cream 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.

Discussion

Effect of therapy on individual symptoms:

Pain

Considering the mode of action by the *Rasa*, *Panchavalkala* must have been *Vatakara* and hence increase the *Ruja* (pain) which is predominantly due to *Vata*. But the effect of the drug on *Ruja* is found to be highly significant. This might due to the action of the *Guna* (Property). Having *Guru* (heavy) *Guna* it is supposed to be *Vatahara* and thus might have decreased the *Ruja*.[13]

Discharge

Panchavalkala is a drug with *Kashaya Rasa* (astringent taste) and by the action of the *Rasa*; it acts as a *Stambhaka* (arresting) and *Grahi* (that holds).[14] It also must be *Atitwak Prasadaka* (cleanses the skin and removes all the dirt from here).[15]

Due to all these properties, it must have reduced the *Srava* (discharge). The *Stambhana* effect might also be attributed to the *Sheeta Veerya* (cold in potency) of the drug.[16]

Redness

Panchavalkala are considered to be *Pittaghna*, that is both by the action of *Rasa* (taste) and *Veerya* (potency) they are *Pittahara* and therefore they must decrease the *Raga* (redness), which is mainly due to *Pitta*. By virtue of its *Kashaya Pradhana Rasa*, it must have acted as *Rakta Shodhaka* (blood purifier). *Pitta Shamana*, *Varnya* (giving color) and *Twak Prasadana* (purity/brightness of skin) actions aided to improve the skin color by improving the local blood circulation.

Swelling

In case of *Panchavalkala*, which is considered to be good *Shothahara* (that which reduces swelling), due to the *Kashaya Rasa* of the drug it acts with *Peedana* (act of squeezing), *Ropana* (heal) and *Shodhana* (curative effect) property. Due to these properties, it destroys or liquefies the accumulated substances and hence minimizes the swelling. Furthermore, the drug is *Rooksha* (dry) and *Kaphahara*. Even due to

Table 3: Effect of therapy on different signs and symptoms

Symptoms	Mean score		% relief	SD	SE	*t*	*P*
	BT	AT					
Slough	3.33	0.43	87.09	1.72	0.26	10.93	0.001
Swelling	0.60	0.22	63.33	0.69	0.10	3.54	0.001
Redness	0.17	0.00	100	0.43	0.06	2.47	0.018
Pain	1.62	0.50	69.14	0.96	0.14	7.49	0.001
Discharge	1.40	0.31	77.86	0.85	0.78	8.35	0.001
Tenderness	0.83	0.43	48.19	0.62	0.09	4.18	0.001
Malodor	1.36	0.31	77.21	0.82	0.12	8.23	0.001

BT: Before treatment, AT: After treatment, SD: Standard deviation, SE: Standard error

Table 4: Effect of therapy on microbial load

Mean		Mean difference	Paired *t*-test		
BT	AT		SD	*t*	*P*
1.80	0.32	1.48	0.71	10.362	0.001

BT: Before treatment, AT: After treatment, SD: Standard deviation

this, *Shopha*, which is *Kaphaja*, gets reduced. Moreover the *Lekhana* (scraping), *Kledahara* (arresting Dampness), *Chedana* (destroying/removing) *and Raktashodhaka* (blood purifier) properties of *Kashaya Rasa* also will facilitate the debridement of the slough.

Pharmacological action of *Panchavalkala*

Pharmacological action of *Panchavalkala* proves that all the five drugs of *Panchavalkala* are found to have anti-inflammatory, analgesic, antimicrobial, and wound healing properties.[17-22] The pharmacodynamic properties of *Panchavalkala* are stated in Table 5.

Chemical constituents of the trial drug-*Panchavalkala* cream are explained in Table 6. Tannins are known antioxidants and blood purifiers with anti-inflammatory actions.[23]

Table 5: Pharmacodynamic properties of *Panchavalkala*

Drug	*Rasa*	*Guna*	*Veerya*	*Vipaka*
Vata	*Kashaya*	*Guru-Rooksha*	*Sheeta*	*Katu*
Udumbara	*Kashaya*	*Guru-Rooksha*	*Sheeta*	*Katu*
Ashwatha	*Kashaya Madhura*	*Guru-Rooksha*	*Sheeta*	*Katu*
Parisha	*Kashaya*	*Laghu-Rooksha*	*Sheeta*	*Katu*
Plaksha	*Kashaya*	*Guru-Rooksha*	*Sheeta*	*Katu*

Table 6: Chemical constituents of the trial drug-*Panchavalkala* cream

Chemical constituents	Ingredients	Pharmacological actions	Effects on clinical features
Tannins	*Vata, Udumbara*	Anti-inflammatory	Reduces swelling
Phytosterols, β-sitosteryl-d-glucoside	*Vata, Ashwatta*	Analgesics	Helps to reduce pain and tenderness, reduces redness by vasoconstriction
Tannins	*Vata, Udumbara, Ashwatta, Pareesha, Plaksha*	Anti-microbial	Reduces discharge
Flavonoids	*Ashwatta, Plaksha*	Anti-inflammatory	Reduces swelling
Glycosides, phytosterols	*Vata, Udumbara*	Promote healing	Reduces wound size, approximates wound margin
Tannins	*Vata*	Ability to increase the collagen content	Promotion of wound healing and increases tensile strength
Vitamin A, K	*Vata*	Epithelialization	Scar formation, maturation

As the oxidation process hampers the wound healing, antioxidants protect the tissue from the oxidative damage. The flavonoids rich fraction of the bark of *Pareesha*, *Vata*, *Ashwatha* and *Plaksha* has proven to possess good *in vitro* antioxidant property. Tannins, phytosterols and flavonoids are anti-inflammatory; hence they prevent the prolongation of the initial phase. They also reduce the pain, tenderness, redness, swelling like features and thus help to control the infection.[24] Tannins and phytosterols promote the healing process by wound contraction with increased capillary formation. Tannins have been reported to possess ability to increase the collagen content, which is one of the factors for promotion of wound healing.[25] Vitamin A and K are essential for epithelialization promoting the healing.

The statistical data as stated above has revealed highly significant results in reducing the slough, swelling, redness, pain, discharge, tenderness, and malodour. Moreover, the tissue biopsy taken for the estimation of the microbial load supported the clinical study showing highly significant results for reduction of the load prior to and after treatment.

Conclusion

From the above clarifications, it can be concluded that *Panchavalkala* cream efficiently decreases the microbial load, clinically controls infection, hastens wound debridement and can be recommended in the management of chronic non healing wounds.

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हिन्दी सारांश

पञ्चवल्कल लेप का व्रणशोधन मे चिकित्सात्मक प्रभाव–व्रणदुष्टि के विशेष सन्दर्भ में

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व्रण कर्म का विज्ञान आज अत्यन्त तेजी से आगे बढ रहा है । व्रण रोपणार्थ कार्य करनेवाले अनेक वानस्पतिक द्रव्यों के मलहरों का ज्ञान आज भलीभांति प्रख्यात है और कई जगहों पर इनका उपयोग देखने को मिलता है । प्रस्तुत अध्ययन मलहर स्वरूप में उपयुक्त आयुर्वेदीय द्रव्य का व्रणपर ऍन्टिमाइक्रोबियल कार्य देखकर उसे चिकित्सा स्वरूप में योग्य प्रकार से उपयोग में लाने के लिये है । इस अध्ययन में पंचवल्कल के हर द्रव्य का घनसत्व बनाकर उनका मलहर बनाया गया । इस मलहर का उपयोग चिरकारी, अरूढ एवं दूषित व्रणों के रुग्णों पर किया गया । चिकित्सा के पहले एवं चिकित्सा के दौरान इन्फ़ेक्शन के लक्षणों के ग्रेड स्थापित किये गये । मायक्रोबियल लोड देखने के लिये चिकित्सा के पूर्व तथा चिकित्सा के दौरान टिश्यु बायॉप्सी किया गया । ज्यादातर अवस्थाओं में समयानुसार चिकित्सा के कारण मायक्रोबियल लोड क्रमशः कम होता देखा गया जो द्रव्य को इन्फ़ेक्शन और मायक्रोबियल लोड पर नियंत्रण प्रमाणित करता है ।