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CASE STUDY

The Antimicrobial Effect of *Tutthakadi Malahara* Topically in *Dushtavrana*: A Case Report

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ABSTRACT

Non-healing or chronic ulcers are characterized by their resistance to initial treatment or persistence despite proper care, failing to progress towards healing within an expected timeframe, often due to an underlying cause. Infection by microorganisms is a primary factor hindering the healing process. Typically, non-healing ulcers harbor a diverse range of microorganisms, known as polymicrobial flora. The bacteria present in these infected wounds release inflammatory mediators, which impede the various stages of the healing process. Hence, development of bioactive naturally derived antimicrobial drugs which are cost effective, without adverse effect is the need of the present scenario.

Dushtavranas are the *vranas* with bad odor, abnormal color with profuse discharge, intense pain and which is difficult to heal or takes long duration to heal which can be considered as chronic non healing ulcer. Susrutha has described *Shashtirupakramas* (sixty measures) for the treatment of ulcer, and *lepana* is one among them.

Malahara is a modified ointment formulation with *ghrita* or *siktha taila* as its base. *Tutthakadi malahara* is a herbomineral formulation from *RasaTarangini*. The properties of this formulation like *vrana samshodhana*, *pooya nisaraka* and *vividha vrana nashana* helps in keeping ulcer bed clean, free from microbes. In the present case study *Tutthakadi malahara mentioned in 21st Taranga- Upadhathwadivignaniya of Rasatarangini* is used in the form of topical application in *dushtavrana* for 28 days and its antimicrobial effect is assessed by pus culture and sensitivity test, discharge and pain. Based on the study it was found that *Tutthakadi malahara* is an effective formulation in reducing microbial load, discharge and pain in chronic non healing ulcer. Hence it can be used as an naturally derived cost effective medicine in the treatment of chronic non healing ulcers.

Key Words *Dushta Vrana, Shodhana ,Tutthakadi malahara, Rasatarangini*

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INTRODUCTION

A discontinuity of the surface epithelium or its molecular death is known as ulcer[1]. Chronic ulcers or non healing ulcers are defined as spontaneous or traumatic lesions, that are unresponsive to initial therapy or that persist despite appropriate care and do not proceed towards healing in a defined time period[2]. According to Indian epidemiological data chronic non healing ulcer was reported as 4.5 per 1000

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population[3]. It is estimated that almost 10% of the population would develop a chronic wound in the course of a lifetime with wound related mortality rate of 2.5%[4].Infection of a wound with a large number of bacteria delays the healing process[5]. Bacteria in an infected wound produce inflammatory mediators that inhibit the inflammatory phase as well as epithelization phase of wound healing. It cause cell death which will lead to an increase in local inflammation response and prolonged inflammatory phase. The clinical signs and symptoms evaluated in infectious ulcer includes increasing pain, increase in wound exudate, presence of slough or nonviable tissue at wound base and unpleasant odour. The conventional treatment for non healing ulcers includes wound cleansing, necrotic tissue debridement, treatment of infection by topical antiseptic or antimicrobial agents and systemic antibiotics ,management of blood glucose levels, hyperbaric oxygen therapy, skin grafting ,vaccum assisted closure and reconstructive surgery as needed .Acharya Susrutha has mentioned detailed management of *Vrana*. *Lepa* is one of the major procedure in *Vrana Chikitsa* while describing the *Shashtirupakrama*. *Lepa* act as both *Sodhana* and *Ropana*[6] . The process of *shodhana* aims at keeping the wound bed free from microbial load, reduces exudation, removes foul odour, eliminates pathological course locally and the process of *ropana* maintains a nutritional reserve in the site[7].

The contents of *Tutthtakadi Malahara* are *Tuttha*(Copper sulphate), *Tankana* (Borax), *Khatika* (Calcium carbonate),*Kaparda bhasma* (Cyprae moneta Linn), *Rala choorna* (Oleoresin of Shorea robusta) and *goghrita*(cowghee)[8]. Both gram positive and negative bacteria,including antibiotic resistant bacteria, fungus and viruses, when exposed to high copper concentrations are killed due to its biocidal action[9].*Tankana* ,*Shala* and *Kapardika bhasma* has antiseptic, anti inflammatory and anti microbial properties[10,11,12,13].

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A 33 yrs old male participant presented with a non-healing ulcer over anterior aspect of right foot associated with pain and pus discharge since 3 months. He consulted a local doctor and was treated but no improvement was noticed. He had previous history of recurrent chronic ulcer on right foot associated with varicose vein since 2 years . The patient had no prior medical history of hypertension, diabetes mellitus, or other significant health conditions. The family history was also not significant with the patient disorder.

LOCAL EXAMINATION

Location: Anterior aspect of right foot

Size of ulcer: 5x8x0.5 cm

Discharge: Thick Purulent discharge present

Floor: Covered with slough

Margin : irregular

Edges: Inflamed

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Tenderness grade 2 and localized raised temperature present.

THERAPEUTIC INTERVENTION

Name of the drug-

Tutthakadi malahara

Malahara is a modified ointment formulation with *ghrita* or *siktha taila* as its base. *Tutthakadi malahara* is a herbomineral formulation from *RasaTarangini 21st Taranga-Upadhathwadivignaniya of Rasatarangini* which is used in the form of topical application in *dushtavrana.*

Preparation of the drug-

Goghrita 2 *tola* (24 g) was melted and to it *Rala churna*- 1/8 *tola*(1.5g) was added until it became an uniform mixture.Then powdered *Shodhitha Tuttha*-1\8 *tola* (1.5g) ,*Khatika*-1 *tola* (12g), *kapardika bhasma*(1 *tola*) and purified *tankana*(1 *tola*) were added and triturated .Then water was added and rubbed several times till the water was clear, the resultant mixture was *Tutthakadi Malahara*. It was stored in an airtight glass container containing sterile water and water was changed everyday.The medicine was prepared each time with 240 gm of ghee as base and it was used within 2-3 weeks of preparation.

Details Of Intervention

The ulcer is cleaned thoroughly with sterile distilled water. After drying with sterile gauze, *Tutthakadi malahara* is applied over sterile gauze pad and bandaging done with sterile gauze. Dressing is done once daily and is removed next day and ulcer is cleaned again with sterile distilled water.

.The intervention was done for a period of 28 days. Based on outcome parameters assessment was done on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day for microbial load, pain and discharge.

OUTCOME MEASUREMENTS

Subjective Parameter

a. PAIN- It was assessed by Verbal Rating Scale (Table 1)

The pain was assessed on the 0^{th},7^{th}, 14^{th} ,21^{st} and 28^{th} day using verbal rating scale

Table 1 Verbal Rating Scale

Objective Parameter

a. MICROBIAL LOAD- Assessed by pus culture and grading on 0^{th},7^{th},14^{th},21^{st} and 28^{th} day. (Table 2)

Table 2 Pus Culture -Microbial Loading

MICROBIAL GRADING	EXPLANATION
0	Absent
1	Mild
2	Moderate
3	Severe

b. DISCHARGE-

Amount of Discharge was assessed on 0^{th},7^{th}, 14^{th}, 21^{st} and 28^{th} day. (Table 3)

Table 3 Assessment of Amount of Discharge

0	No discharge
1	Mild-If ulcer wets 1 sterile pad of 4x4cm size.
2	Moderate-If ulcer wets 2 sterile pads of 4x4cm size.
3	Profuse discharge-If ulcer wets more than 2 sterile pads

Type of Discharge was assessed on 0^{th},7^{th},14^{th},21^{st} and 28^{th} day. (Table 4)

Table 4 Assessment of Type of Discharge

0	Serous drainage: clear or light yellowish
1	Sanguinous drainage: bright red
2	Serosanguinous drainage: pink
3	Purulent drainage: thick/thin and yellow or pale green

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OBSERVATION AND RESULTS

The outcome parameters were assessed on the 0th day before the treatment ,7th,14th,21st and 28th day at the end of study period.The following observations and results are enlisted below in the table. (Table 5).

Table 5 Observations and Result

PARAMETER	0th DAY	7th DAY	14th DAY	21st DAY	28th DAY
Microbial load	3	3	3	2	0
Amount of Discharge	3	3	3	1	1
Type of Discharge	3	3	3	2	0
Pain	3	3	2	1	1

DISCUSSION

DISCUSSION ON SELECTION OF TOPIC

In daily clinical practice, *Dushta Vrana*, is a major challenge faced by the Ayurvedic medical practitioners. Wound healing is impeded by various factors and is a concern in surgical practice. To aid better healing, it must be free from *dosha dushti* (vitiated factors) and microorganisms that can disrupt the natural healing process. There have been new therapies developed by modern sciences but the traditional remedies remain most effective due to emerging antibiotic resistance due to indiscriminate use of antibiotics and other antimicrobial agents. The signs and symptoms of chronic non-healing ulcers closely resemble those of *Dushta Vrana* described in Ayurvedic texts, indicating the relevance of ancient wisdom in understanding and management of chronic non healing ulcers.

Wound debridement is a vital step in managing non-healing ulcers, as it allows medications to reach healthy tissue and promote the healing process."*Tutthakadi malahara* is an ointment based preparation described in *Rasatarangini*. The contents of *Tutthakadi Malahara* are *Tuttha* (Copper sulphate), *Tankana* (Borax),*Khatika* (Calcium carbonate),*Kaparda bhasma* (Cyprae moneta), *Rala choorna* (oleoresin of Shorea robusta) and *goghrita*(cow ghee)·It's properties include *Vrana samshodhanam param* (best in ulcer cleansing), *pooyanisaranam* and *vividhavrana nashana* (alleviates different types of wound). Most of the drugs are having *katu,tiktha ,kashaya rasa, lekhana,krimighna* properties which enhances the wound debridement making the ulcer bed clean and free from microbial load. As the infection get controlled there will be reduction in pain and discharge associated with it. Herbomineral based dressings are gaining popularity for their effective antimicrobial properties. So herbomineral formulation *Tutthakadi malahara* from *Rasatarangini* was selected to assess the antimicrobial effect on non healing ulcer.

PROBABLE MODE OF ACTION OF DRUG BASED ON PROPERTIES OF INGREDIENTS

Tuttha possess *Katu,Kashaya rasa, ushna virya, katu vipaka. Katu rasa* has *VranaShodhana, Kushthaghna, Krimighna* and *Lekhana Karma. Kashaya rasa* has *Vrana Shodhaka, Vrana*

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Ropaka and *Kleda Shoshaghna Karma*. *Tuttha* is *Kaphapittaghna*, it has *lekhana,krimighna and vranashodhana* property which results in reduction of *srava* and antimicrobial action. "Modern studies show copper sulfate salts has significant antibacterial activity even against multi-drug resistant pathogens. The study confirmed the effective activity (bactericidal or bacteriostatic) of the copper sulfate salts."[14] "Both gram positive and gram negative bacteria, including antibiotic resistant bacteria,fungus and viruses,when exposed to high copper concentrations are killed by biocidal action of copper ions. It is suggested that copper nanoparticles release their ions by creating electrochemical potential onto the cell membrane of bacteria that leads to the disruption of the bacterial cell due to oxidative stress."[9]

Tankana possess *Katu rasa, Tikshna,rooksha guna,ushna virya, katu vipaka* and has *kaphavishleshaka* property which results in decreasing *srava*. *Tankana* comes under *kshara varga, Ksharakarma* is one of the best treatment modality for *Dushtavrana* described by *Acharya Sushrutha*. *Vrana* with *Utsanna Katinamamsa, Chirothita Dushoddhya and Kandu* should be treated by *Ksharakarma*. Due to its *Ushna Teekshna, Chedana, Bhedana, Dharana, Lekhana* properties, it helps in wound debridement and makes the ulcer bed clean and free from microbes. It also possess *vatasamana* property which result in reduction of pain and is *vrananashana* which aids in better wound healing.Latest studies on Tankana shows its effectiveness against several bacterial strains like P.aeruginosa,Ecoli,S.pyogenes,S.aureus and fungal strains C.albicans,A.clavus and A. niger"[11].

Kapardika Bhasma possess *Katu, tiktha rasa, ruksha,Tikshna guna,ushna veerya* and *katu vipaka*.It has *kaphapittaghna,lekhana,sravahara* property which results in reduction of discharge and is *Vatasamaka* which results in reduction of pain. "Modern studies also shows significant antimicrobial,antiseptic,anti-inflammatory and wound healing effect of *Kapardika"*[12,13].

Khatika exhibits sweet and bitter tastes, oily properties, and cooling potency, and possesses properties that mitigate *pitta*, purify blood, promote wound healing, and reduce discharge and pain. Calcium carbonate is used in wound dressing owing to its antibacterial, hematostatic and hygroscopic properties. Its hemostatic action and rapid fluid absorption facilitate reduced discharge from wounds. Recent research demonstrates that topical calcium carbonate effectively treats chronic ulcers by enhancing angiogenesis and epithelialization. Furthermore, in vitro studies reveal that calcium carbonate particles exhibit antimicrobial effects against both gram-positive and gram-negative bacteria[15,16].

Rala possess *Kashaya rasa, ruksha guna, ushna veerya, katu vipaka*,it has got *kaphapittahara, vranashodhana, ropana,raktadoshahara* and *krimighna* properties."Modern research work shows the oleoresin of Shorea robusta possess analgesic and stronger and broaderspectrum of

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antimicrobial action which aids in faster wound healing"[17],[18],[19].It contains flavonoids,polyphenols and triterpinoids which possess antibacterial,antioxidant, anti inflammatory and wound healing properties."[20]

GoGhrita (cow ghee) possess *Madhura rasa, snigdha guna,seetaveerya, Madhura vipaka* .It is *tvachya, Vatapittasamana* and *vishahara* which results in *vedanaprasamana* and *kriminashana*. "Cow ghee possess antiseptic,antibacterial,anti inflammatory properties.Cow ghee is antioxidant rich due to the presence of beta carotene and fat soluble vitamins.The lipophilic action of cow ghee makes easier drug delivery to the cellular level. The cow ghee-based ointment bases also showed good spreadability, extrudability and solubility, indicating their effectiveness as carriers for active components."[21] "cow ghee has antibacterial bioactive components and are potential sources of antibacterials due to their ability to generate radical oxygen species which are harmful to bacterial cells moreover finding from research studies states that cow Ghee is active against E.coli and notable against Staphylococcus aureus[22]."

DISCUSSION ON OUTCOME PARAMETERS

Effect on Microbial load:

The microbial load in pus culture on 0th day before treatment revealed heavy mixed growth of Streptococcus sp and Proteus mirabilis. (Fig 1).The consecutive 2 swab tests on 7th and 14th day showed persistent heavy growth of organisms which reduced to moderate growth on 21st day and no definite organism after 2 days of incubation on 28th day at the end of study period (Fig 2). Most of the drugs in *Tutthakadi malahara* possess *krimighna* property which has resulted in reducing microbial load. The drugs also possess *kshara,lekhana , shodhana* properties.By *vrana shodhana* devitalised tissue ,slough is removed from the ulcer bed making it free from microbes.

Effect on Pain:

On the 0th day participant presented with severe pain before the treatment which persisted as severe pain on 7th day which reduced to moderate severity on 14th day and pain reduced significantly to mild on 21st day and it persisted as mild at the end of the study period.Pain is an important feature of *Vata*. Contents of *Tutthakadi malahara* such as *Tuttha,Tankana,Rala* were *Ushna virya* thus *Vedanasthapana* by pacifying *Vata.*

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Accredited by National Accreditation Board for Hospitals and Healthcare Providers (NABH) & Quality and Accreditation Institute (QAI)

Investigation Report

Name :
Age/Gender : 33-Year(s)/Male
Client Name : GAC TVM
Ref.Dr :

Pat.Id : MLS38407
Visit No. : GHT2455260
Registered On : 03-05-2024 12:13:00
Collected On : 03-05-2024 13:23:00
Reported On : 05-05-2024 11:45:20

MICROBIOLOGY

Pus Culture & Sensitivity

Specimen	:	PUS
GRAM STAIN	:	2 - 3 Pus cells/OIF,Gram Negative bacilli seen
Organism Growth	:	Streptococcus sp and Proteus mirabilis
Comments	:	Routine culture yielded heavy mixed growth of Streptococcus sp and Proteus mirabilis.Evaluate clinically.

Antimicrobial Susceptibility Test Result
Streptococcus sp

Antimicrobial Agent	Interpretation	Zone Size (mm)
Amoxicillin / Clavulanic Acid	Sensitive	26mm
Ampicillin	Sensitive	26mm
Cephalexin	Sensitive	24mm
Ciprofloxacin	Sensitive	26mm
Cloxacillin (Cox)	Sensitive	25mm
Cotrimoxazole	Resistant	--
Erythromycin	Resistant	--
Gentamycin	Sensitive	19mm
Linezolid	Sensitive	26mm
Ofloxacin	Sensitive	19mm
Penicilin	Sensitive	26mm
Telcoplanin	Sensitive	21mm
Vancomycin	Sensitive	--
Doxycycline Hydrochloride(DO)	Sensitive	19mm

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Figure 1 Microbial load in pus culture on 0th day Before Treatment

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Investigation Report

Name : [redacted]
Age/Gender : 33-Year(s)/Male
Client Name : GAC TVM
Ref.Dr :
Ex.Patient No :

Pat.Id : MLS76167
Visit No. : GHT2459341
Registered On : 31-05-2024 11:50:28
Collected On : 31-05-2024 12:08:00
Reported On : 02-06-2024 14:54:52

MICROBIOLOGY

Pus Culture & Sensitivity

Specimen	:	PUS SWAB
GRAM STAIN	:	1-2 cells /OIF , No definite organism seen.
Comments	:	No growth after 2 days of incubation.

-- End of Report --

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DISCLAIMER

] end of study period

Effect on Vrana Srava:

The participant had profuse pus discharge before the treatment on 0th day (Fig 3) which persisted as profuse discharge during 7th and 14th day which reduced to mild discharge from 21st day (Fig 4) and persisted as mild at end of study period on 28th day (Fig 5). There was significant reduction in type of discharge indicating healing of the wound.Purulent discharge persisted for 0th, 7th and 14th day which reduced to serosanguineous discharge on 21 st day and serous discharge on 28th day at the end of study period. *Tutthakadi malahara* have *shodhana ,pooyanisaraka* property. The contents in *Tutthakadi malahara* like Copper sulphate, Borax and Calcium carbonate are hygroscopic in nature thereby absorbing moisture from the ulcer, moist environment of the ulcer enhances autolytic debridement and promotes healing. This action is due to *Lekhana, shoshana,sravahara,kshara* properties present in *Tutthakadi malahara*.

Figure 3 Ulcer on 0th day Before Treatment

Figure 4 Ulcer on 21st day During Treatment

Figure 5 Ulcer on 28th day at the end of study period

CONCLUSION

The present case study was targeted to evaluate

the antimicrobial effect of *Tutthakadi Malahara* in *Dushtavrana*. It is found effective in the management of *Dushta Vrana* by decreasing microbial load,discharge and pain thereby controlling the infection. It helps avoid the painful procedure of surgical debridement of slough in those who are unable to tolerate pain. It also has properties like v*ividha vrana nashana* including *vranashodhana and vranaropana*. It removes only the unhealthy granulation tissue, and due to its *shodhana* property it makes the ulcer bed clean and aids in better healing. Thus *Tutthakadi malahara* with antiseptic,anti-inflammatory and antimicrobial property is an effective formulation in treating *dushtavrana*.

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