Study Protocol

Comparative assessment of tissue regeneration by Ayurveda medication (*AmalakiPatraMashi*ointment) and Silver Sulfadiazine in *DagdhaVrana* (Burn wound).

Abstract

Background: Skin is the largest organ in the body and performs a wide variety of different function. They ~~plays~~play an important role in the injury of the skin. Burns *(DagdhaVrana)* are significant health challenge and healing can result in scar formation. Within the herbal medicine tradition the concept of tonification and trophorestoration is well established. Concepts of regeneration and Trophorestoration in Ayurveda provide another dimension to the area of regenerative medicine.Acharya Charaka suggests to use drugs of*Amalaki* to prevent *Vrana*. *Amalaki*and Silver Sulfadiazine (SSD) are the two comparative drugs chosen to evaluate the Trophorestoration concept through *Dagdha Vrana* in Albino rabbits.

Materials and Methods: After identification and phytochemical study of test drugs 18 adult Albino rabbits will be divided into 3 groups with six Albino Rabbits in each group. The burn wound will be induced by usingmetal disc, three groups have been taken in which first is control group, second is standard control, third is experimental group. Before applying experimental drugs to respective group the tissue sample will be taken from each of sacrificed albino rabbits and from one of the healthy tissue sample of normal skin will be taken by Punch Biopsy for histopathalogical assay. Examination of wound area, swelling, redness, oozing will be observed. Macroscopic and Microscopic assessment of wound will be done on day 0th , 4th , 8th~~,~~ and $15^{th,}$ 21st day after burn. Simultaneously on 0th day and 21st day SOD and MDA test will be done. The remaining animals will be returned to the animal house for their reuse.

Expected Results: Whether *AmalakiPatraMashi*ointment has more effect on troporestoration property of *Twacha* in *DagdhaVrana*(Burn wound) than Silver Sulfadiazine.

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Keywords:*DagdhaVrana*, *SaptaTwacha*, Trophorestoration, *Amalaki*

1. INTRODUCTION-

Burns *(DagdhaVrana)* are significant health challenge and healing can result in scar formation.[1].Burn is defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight or nuclear radiation.The most common are burns caused by scalds, building fires and flammable liquids and gases. Thermal burn and related injuries have remained a major cause of death and disability. Although small burns are not usually life threatening, they need the same attention as large burns, in order to achieve functional and cosmetic outcome. [2].Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight [3].

Wound closure represents a primary goal in the treatment of very deep large wounds, for which mortality rate is particularly high. However, the spontaneous healing of skin eventually results in the formation of epithelized scar and scar contractures (repair) whichmight distort the tissues and cause lifelong deformities and disabilities. This clinical evidences suggest that wound closure attained by means of skin regeneration instead of repair, should be the true goal of burn wound management.[4].

Mashi Kalpana is an important pharmaceutical preparation mentioned in Ayurveda Pharmaceutics. Mashi Kalpana is the form of medicine which can be prepared by heating herbal or animal content upto transfer into carbonized form. Itis used externally as well as internally. It is cost effective, less time consuming preparation and having quick result.[5]. Rasa Tarangini, Rasashastra treatise 20th century enumerated various types of *MalaharKalpana* based on Yogratnakar mentioned *MalaharKalapana*, it removes *Mala* (residue) etc. from wounds etc. this is similar to ointments in modern pharmaceutics *MalaharKalpana.Malahar* has a property like *Snehan* (oleation), cleansing, *Ropan* (Healing), *Lekhan* (Scarping) and *Varnya* (beautifying).[6]. It has its own therapeutic advantages like easy pharmaceutical procedure ease of mode of application and higher shelf life period.[7].

The various *Dravyas*are used for applications to cure *DagdhaVrana*. Bhavaprakash and AcharyaCharak suggested that Out of all *RasaKashayRasatmakDravyas* are useful for *VranaRopan*.[8].*Amalaki* (*Emblicaofficinalis*) (EO) is a rich source of vitamin C, which is a potent antioxidant. [9]. It is foremost amongst the anti-aging drug (*Vayasthapan*) or best amongst the rejuvenating herbs; it has properties like *Rasayana* (adaptogenic), ajara (usefulness in pre-mature aging), *Ayushprada* (prolongs cell life), *Sandhana karaka*(improves

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cell migration and cell binding), *Kantikara* (improves complexion).[10]. According to Acharya Charaka, *Kashay Rasa*also having the properties of *Sanshaman* (palliative), *Sangrahi, Sandhan* (to hold), *Pidan* (Pain killer), *Ropan*(Healing property of *Vrana*), *Shoshan* (to absorb), *Kledan* (to provide moisture). It also pacifies the *Pitta* and *KaphaDosha*.[11]. Many researches are found the extractions of the *Amalaki* leaves are used for wound healing purpose.Leaves containsgallic acid, chebulic acid, ellagicacid, chebulinicacid, chebulagicacid, amlicacid, alkaloids phyllantine and phyllantidineetc. These phyto-cChemicals having capacity of biological activities like antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antitissuive, anti-radio protective, chemo preventive, wound healing activities and so on.–[12].Traditional system of medicine like Ayurveda which are known for their healing capabilities can offer a lot more to the science of regenerative medicine. Trophorestaration is the path to repair and regeneration. Within herbal medicine tradition notion of tonification and trophorestoration are well established.[13]. The present study will be carried out to explore the concept of trophorestoration of tissuesby the test ointment with and without the treatmentin second degree thermal burns and compare it with ointment Silver Sulfadiazine.

Comment [PM1]: Table please; compounds and properties

2. MATERIALS AND METHODS -

1.Materials:-

This study will be conducted under following headings.

1.[1.1]Experimental Animal:

All 18albino rabbits weighing 3 to 3.5 kgwill be used in this study. All albino rabbits will kept in a temperature-controlled (25 ± 1°C) environment with a 12-h light/dark cycle and kept in individual cages. They will fed with fresh hay, water, and fresh vegetablesand given water *adlibitum*[14]will be obtained from Central Animal House of DMIMS. All experimental protocols involving the use of animals will be conducted in accordance with the CPCSEA guidelines after the approval of institutional animal ethical committee.

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1.[1.2]Selection of material/Drug : The plant of *Amalaki*and other material will be identified authenticated and from Dravyaguna Department of Mahatma Gandhi Ayurved College Hospital and Research centre, Wardha.

1.[1.3] The material/Drug: (Both the experimental drugs will be freshly prepared)

- *AmalakiPatra* in the form of*Mashi*
- *Coconut oil*- 100% standard virgin pure oil

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- Silver sulphadiazine1% 25 gmswill be used which is procured from AVBRH pharmacy.

2. Methods:-

Experimental ~~study :~~study:

2.[2.1] Preparation of Animal models-The albino rabbitswill be acclimatized to laboratory conditions for one week prior to the experiment. The albino rabbits will be anesthetized with single intramuscular injections, xylazine 2.5-10 mg/kg IM,ketamine 22-50 mg/kg IM ~~.~~[47].

2.[2.2]Thermal injury:

- The area on the back of the rabbit was shaved and animal kept for fasting overnight. The next day the animal were anaesthetized using Ketamine in the dose of 50 mg/Kg of body weight I.M. (1 ml/kg of body weight) .A metal disc of wt 50 gm, diameter 2.5 cm (25mm), thickness 1.1cm (11mm) and area 4.910 sq. cm (491.07 sq. mm) was heated in the blue portion of the flame of spirit lamp for 5 minute and then immediately kept on the shaved part for 30 seconds with minimal pressure. This method was found to be more accurate and convenient in producing the second degree burns in comparison with the molten wax method. The scientific paper in this regard was presented in 56th annual national conference of physiologist and pharmacologist of India, APPICON 24th December 2010, JNMC, Sawangi, Maharashtra.
- **Administration and Application of Drugs ~~-:~~**Standard Ointment Silver Sulfadiazine and New Herbal Ointment was applied daily on the burn wound.
- **Fluid resuscitation -**All animals will be immediately resuscitated with lactated Ringer's solution (2 ml/100 g body weight) applied intraperitoneally to prevent dehydration.
- **Cooling effect-** After Burn the area of burn will immediately cooled by Running water and cold water~~.~~(2 to 15°C).
- **Local anaesthesia-** to prevent pain Nevocaine 5 % ointment will be used
- The burn should subsequently be covered with a sterile, occlusive, non-adherent dressing to reduce pain, limit contamination and prevent further trauma.

2.[2.3]Preparation of the Material/Drug ~~-:~~ Preparation o~~O~~f *AmalakiPatraMashi*will be done on the basis of classical methods given the text of Ayurveda.

- ***AmalakiPatraMashi ointment-***

  Preparation of the ointment will be done as per *Malhar Kalpana*.

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2[2.4]AnatomicalAssessment Criteria for burn wound :-

Microscopic Assessment of *DagdhaVrana*:–

1.According to Ayurveda parameters and Grades

-Varna (colour)

-Strav (secretions)

-Gandha (smell)

-Akriti (Floor & Granulations)

2.AssessmentAccording to Vrana on the day 0th , 4th ,8th ,15th and 21st

3.wound contraction rate will be calculated according to formula-

Wound contraction rate =Original wound area - specific day wound area ×100

Original wound area

4.Criteria for Grading of Epithelial Regeneration

MicroscopicAssesmentof *DagdhaVrana*-

1.Histopathology: Punch Biopsy[16]

2.Parameters of Histological assessment of wound

3.Parameters of Histological assessment of Ayurvediya*Twacha*

4.Blood investigations -SOD and MDA levels.

Grouping :- (Table ~~no.~~ 1)

Groups	Intervention	Drug	Quantity For local application	Sample Size	Duration Day	Route
Group A Placebo	No intervention will be done	No drug	-	6	21 ~~day~~	LA
Group B Standard control	Standard Drug	Silver Sulfadiazine (SSD)		6	21 ~~Days~~	LA
		APM (*AmalakiPatraMashi*oint				

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GroupC Experimental	Study Drug	ment)	1 Angula i.e 2 Cm thick (Sha. (Sa.UK.74-75)	6	21~~Days~~	LA

2.9 Methods of Data Collection -Data will be collected by laboratory reports and histology reports

Statistical analysis:

The thickness of granulation tissue was examined at the center of each wound and recorded. The data were expressed as means ± standard errors (SEM). Differences between group means and between days ~~four~~4, ~~nine~~9, and 14 were estimated using a one-way analysis of variance (ANOVA) and a Duncan test was performed for multiple comparisons using the SPSS 12.0 for Windows. Results were considered as statistically significant at $P < 0.001$.

2.11 Expected Results-To find out better drug *DagdhaVrana* (Burn wound) though Trophorestoration concept of Ayurveda wrt*TwachaSharir*.

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3. DISCUSSION-The healthy regeneration of tissues are based on the fundamental concepts of ~~Ayurveda which~~Ayurveda, which encourages tissue regeneration *Santarpan* (nourishing), *Jivaniya* (life promoting), *Bruhan* (Bulk promoting), *Ropan* (Healing), *Sandhan* (Unifying), *Tarpan*(Nutrient Provider), *Preenan* (Nutrient Provider).*Amalaki* acts as a both *Vayasthapan* and *Rasayan* Herb which having restoration property~~.~~[17].The concept of Trophorestoration which is the path to repair and regeneration is well established in Ayurveda through herbal medicine through herbal medicine~~.~~[18].Modern medicine is yet to explore conceptual aspects of the regenerative medicine. Hence it is need to explore Trophorestoration concept of Ayurveda based on fundamental modalities in case of *DagdhaVrana* compare with modern medicine.

4. CONCLUSION-Conclusion will be made on the basis of results of Statistical Analysis and histopathology reports.

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5. CONSENT -It is not applicable

6.ETHICAL APPROVAL -Protocol is approved by institutional animal ethics committee

NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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Comment [PM8]: must be improve

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