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ORIGINAL RESEARCH ARTICLE

AN EXPERIMENTAL STUDY TO EVALUATE EFFICACY OF DHATAKYADI YOGA LEPA IN BURN WOUND HEALING

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ABSTRACT

Background: Burn is coagulative necrosis of the tissues and Scalds are caused by contact of hot liquid with skin. The incidence in India is six to seven million; nearly 10% of them require hospitalization. Management of 1st degree and 2nd degree burn involves dressings with antibiotic ointments which reduce microbial load on the burn surface but potentially prolongs healing time and pain during repeated dressing. Sushruta mainly emphasizes the importance of lepa in DagdaVrana which reduces daha, sphota, vedana and does Vranaropana. Dhatakyadi yoga lepa is such preparation which meets all the above needs and mainly indicated in Agnidagda Vrana. **Objective:** To evaluate wound healing property of Dhatakyadi Yoga Lepa in moist heat induced burns in rats. **Material and methods:** Partial thickness burns were inflicted on three groups of rats (n=6 in each group) at back under ether anesthesia. Standard group was treated with Silverex ointment, test group with Dhatakyadi Yoga (DY) and control group with normal saline twice till complete healing. Study duration was for 21 days and the parameters observed were epithelialization period, hydroxyproline content, Scar area, wound contraction rate. Data was analyzed by one way ANOVA method. **Results:** There was significant increase in wound contraction rate, Hydroxyproline content and decreased period of Epithelization, Scar area in the group treated with Dhatakyadi yoga. **Conclusion** - Dhatakyadi yoga is effective in healing of burn wound.

Keywords – Burns, AgnidagdaVrana, Dhatakyadi Yoga, lepa, Silverex, Wound healing.

INTRODUCTION: Burn injuries are among the most devastating of all injuries and a major global public health crisis[1]. Burns are the 4th most common type of trauma worldwide, following traffic accidents, falls and inter personal violence[2]. In India about 700,000 people a year are admitted to hospital, though very few are looked after in specialist burn units[3]. About 90% of burns occur in the developing world and 70% of these are in children. Survival of injuries greater than 40% total body surface is rare in the developing

world, thousands of victims of burns are being mutilated, disfigured and handicapped every year[4]. The treatment protocol of 1st degree and 2nd degree partial thickness burn involves wound debridement, analgesics and dressings with antibiotic ointments[5]. Despite various current topical treatment regimens designed to eradicate the bacterial load within the burn wound, sepsis remains the leading cause of death in burn units around the world[6.] However, the use of these antimicrobial agents is associated with cellular toxicity and delayed healing[7].

Acharya Sushruta has given special importance to Agni DagdaVrana and explained its types and Chikitsa in detail. DagdaVrana is characterized by Vedana, Sphota and Daha due to Pitta and Raktadushana. Brahatrayi and laghutrayi have explained many lepa with taila and Ghrita in the management of Agni DagdaVrana which reduces pain, burning sensation and helps in promotion of wound healing.

Dhatakyadi yoga is a formulation specially mentioned by Yoga Ratnakar for the management of Agnidagda vrana[8]. Dhataki pushpa (*Woodfordia fruticosa*) Churna and Atasi Taila (Linseed oil) are the ingredients of the formulation. Linseed oil (*L. usitatissimum*) has been reported to exhibit significant anti-inflammatory, anti-arthritic, antiulcer properties[9]. *Woodfordia fruticosa* flowers are proved to have Anti bacterial, Antihelmithic, Anti ulcer, Styptic, Anti pyretic activity[10]. Hence the study is aimed to evaluate the efficacy of wound healing property of Dhatakyadi yoga in moist heat induced burns in Wister rats.

MATERIAL AND METHODS: The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee (Ref: BMK/IAEC/Res-07/2012).

Drug Collection and Authentication: *Woodfordia fruticosa* flowers were collected from natural habitat and authentication done in Ayush approved Central research faculty, KLEU's Shri BMK Ayurved Mahavidyalaya, Belgaum. Linseed oil was procured from available sources. Preliminary phytochemical and physico chemical analysis of *Woodfordia fruticosa* flower and *Linseed oil* were carried out in Central research faculty, Belgaum.

Preparation of Dhatakyadi yoga: Dhataki pushpa(*Woodfordia fruticosa* flower) – 1 part, Atasitaila *(Linseed oil* –Quantity Sufficient).

Woodfordia fruticosa flowers were dried and made into powder form and sieved in 120 number mesh and it was mixed with Atasi taila *(Linseed oil).*

Preparation of Animals: Healthy male Wister rats weighing between 180-200 g were used in the study. The animals were acclimatized to the laboratory conditions for at least five days prior to the start of the study with room temperature of 22±3ºC and relative humidity at least 30% and preferably not exceed 70% with artificial lighting, the sequence being 12 hours light, 12 hours dark. They were housed individually and maintained on normal diet and water ad libitum. Approximately 24 hours before the study, 20 % of the total body surface area was made clear for the induction of burn by depilating fur from the dorsal area of the trunk. Overnight starved animals were inflicted with full thickness burn wound under ether anesthesia by placing the dorsal area of animal into boiled water (100^0C) for 60 sec[11].

Study design: The animals were randomly allocated into three groups of six animals each.Group-I control (normal saline) Group-II Dhatakyadi yoga, Group-III standard (1% silver sulfadiazine ointment).

Dosing schedule: Drugs were applied twice daily from day 0 to the day of complete

healing or the 21st postoperative day, whichever was earlier.

The parameters studied are as follows:

a) Epithelialization period: It was monitored by noting the number of days required for the scab to fall off from the burn wound surface without creating a new wound.

b) Wound contraction rate: It was noted by following the progressive changes in wound area planimetrically, excluding the day of the wounding. The size of the wounds was traced on a transparent paper every third day, throughout the monitoring period. The tracing were then transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated. The evaluated surfacearea was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm^2, as 100%, by using the following equation:

Percentage of wound contraction

$$= 1 - \frac{\text{Wound area on corresponding day}}{\text{Wound on zero day}} \times 100$$

c) Hydroxyproline content estimation in granulation tissue- Hydroxyproline is produced by hydroxylation of the amino acid proline by the enzyme prolyl hydroxylase following protein synthesis. Estimation of Hydroxyproline helps us to understand progress rate of healing process in connective tissue of the wound[12,13].d) Histopathology of healed skin was done to assess the rate of wound healing.

Statistical Analysis: All results are expressed as the mean ± SD. One way ANOVA was applied for testing the significant difference between three groups at each time point.

RESULTS: In the present study, the significant promotion of burn wound-healing activity was observed in test drug treated animals. In test drug treated animals wound contraction was to the extent of 16%, 46%, 62%, and 76% by day 4, 8, 12, 16 respectively. These animals took 18 days for complete epithelization. While, topically applied Dhatakyadi yoga shortened the period of epithelization significantly (*P=0.001) by 3 days (Table.2). Besides, it also promoted the wound contraction throughout the study (Table.1) as compared to the control and standard groups. There was a statistically significant (*P=0.001) reduction of scar area in Dhatakyadi yoga group as compared to that of control (Table.2). Significant increase in Hydroxyproline content was present in DY treated group when compared to that of control (Table.3). The percentage of change in the healing of burn wounds from 4th to 16th postwounding days was compared in all the three groups and it was observed significant changes in all the three treated groups. It is evident from these findings that the topical application of Dhatakyadi yoga enhances the healing of burn wounds.

Table No.1 - Comparison of three groups (Control, Standard, and Test) with respect to burn wound healing in different time points

Groups (n= 6) in each	Summary	Wound Closure rate(%)				% of changes of healing from 4th day		
		4th	8th	12th	16th	4-8th	4-12th	4-16th
C	Mean	7.65	17.4	42.3	59.2	12.1	30.1	59.7
	±S.D	3.95	3.18	4.79	3.53	2.22	3.70	3.91
T	Mean	16.0	46.0	62.7	76.0	31.0	62.4	96.4

	±S.D	4.03	8.93	7.32	5.40		5.29	7.88	10.2
S	Mean	13.0	30.7	60.7	71.7		25.4	57.2	86.7
	±S.D	3.78	5.78	8.25	9.44		5.56	10.8	11.7
F Value		7.03	30.0	15.7	10.5		26.8	28.0	30.0
P Value		0.001 *	0.001*	0.001*	0.001*		0.001*	0.001*	0.001*

Graph No.1 Comparison of Three Groups (Control, Test, Standard) with Respect To Burn Wound Healing At Different Points

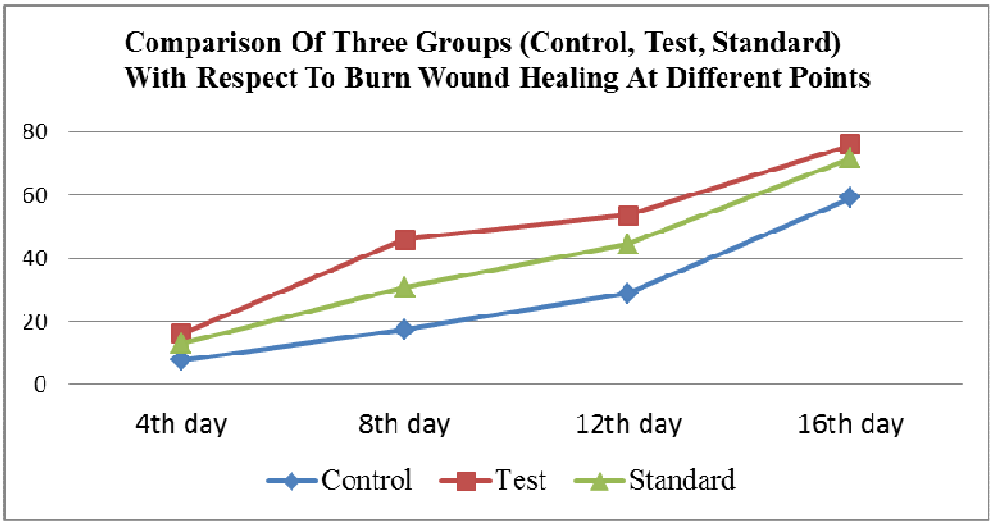


Table: 2 - Comparison of Three Groups (Control, Standard, and Test) with Respect To Epithelization Time (Days) and Scar Area (Cms)

Groups	Summary	Epithelization time(days)	Scar Area(cms)
Control	Mean ± SD	21.00 ± 0.8944	5.778 ± 0.6953
Test	Mean ± SD	18.33 ± 0.5164	3.487 ± 1.102
Standard	Mean ± SD	20.00 ± 1.169	11.59 ± 2.345
	F- value	13.77	43.61
	P –value	0.001*	0.001*

Note: *p<0.05

Graph No.2- Epithelization Time (Days)

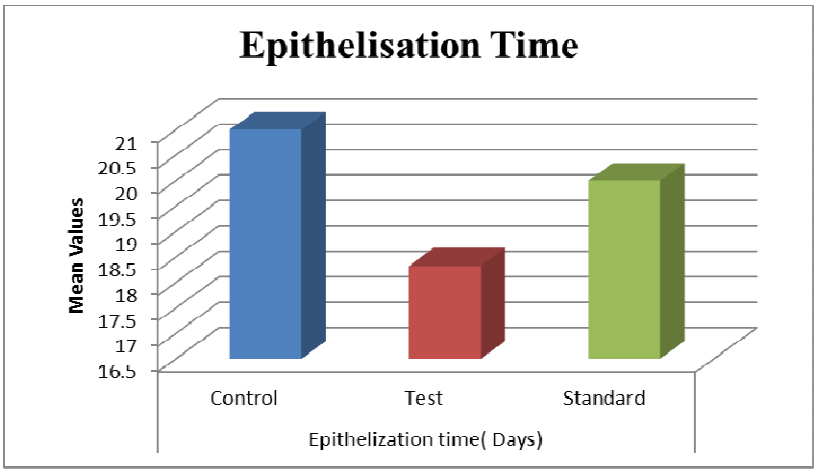
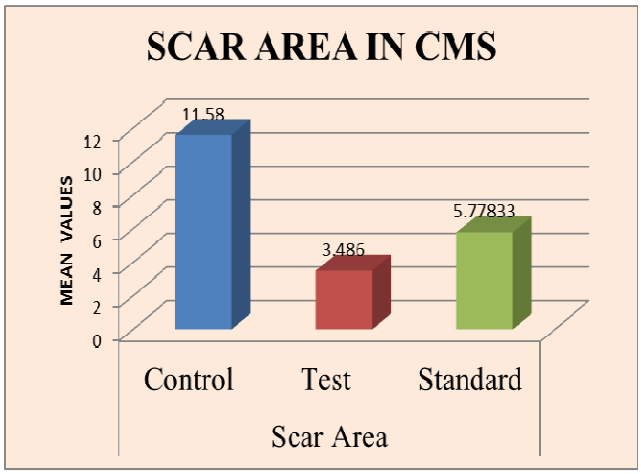


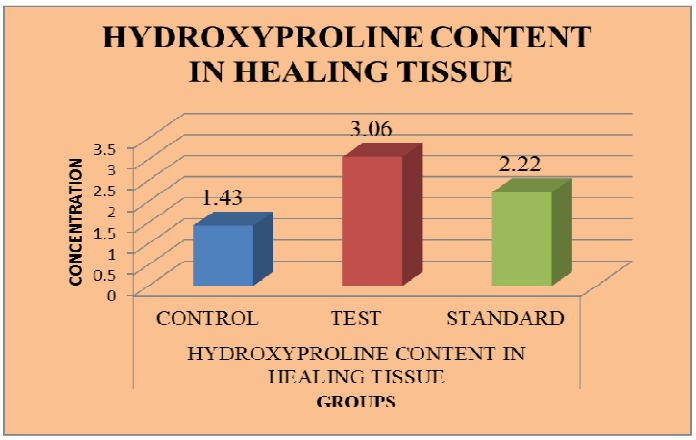
Table No. 3 Hydroxyproline Content In Healing Tissue

Groups	Summary	Hydroxyproline content
Control	Mean± SD	1.43 ± 0.1599
Test	Mean ±SD	3.06 ± 0.1036
Standard	Mean± SD	2.22 ± 0.08877
	F- value	45.58
	P –value	0.001*

Graph No.3 Scar Area



Graph No.4 –Hydroxyproline content in healing tissue



DISCUSSION: The present study was designed to evaluate the efficacy of Dhatakyadi yoga on experimentally induced burn wound in Wister rats. The result showed Significant changes and confirms the efficacy of DY on burn wound healing which was previously explained by ancient Acharyas. Wound healing is a complex multifactorial process that results in the contraction and closure of the wound and restoration of a functional barrier[14]. Repair of injured tissues occurs as a sequence of events, which include inflammation, proliferation, and migration of different cell types [15]. In burn wounds, there is an extensive loss of cells and tissues compared to an incision wound, and this makes the repair process more complicated[16]. The Dhatakyadi yoga was found to be effective at mainly three main phases of wound healing, i.e. Collagenation, wound contraction and epithelization.

Wound contraction- is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast[17]. Results of the present study on burn wound, clearly indicate that DY has promoted the healing of burn wound and reduced the scar features. Since, DY enhanced wound contraction; it would have either enhanced contractile property of myofibroblast or increased the number of myofibroblasts recruited into the wound area.

Epithelialization time- DY hastened the period of epithelialization significantly as compared to that of control and standard. It appears that DY was able to promote epithelialization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells. Previous study reveals Dhataki pushpa (*Woodfordia fruticosa*) has increased tensile strength, Hydroxyproline content in granulation tissue and wound healing activity[18].

Wound Infection- is one of the most common complications of burns which interfere with healing of wounds[19]. Infection in wound is due to the presence of necrotic tissue and disruption of protective barrier to microorganisms. The agents possessing the antimicrobial activity are expected to reduce the bacterial load of a wound and facilitate wound healing by attenuating local inflammation, tissue destruction and also by stimulating immune activity[20]. DY has an antimicrobial activity against various microbes including those causing burn and wound infections like pseudomonas aeruginosa and staphylococcus aureus[21]. Consequently, it

would be expected to produce pro-healing effect on infective burn wounds.

Hydroxyproline estimation – Hydroxyproline is an amino acid essential for collagen synthesis. For this reason, Hydroxyproline content has been used as a marker to determine the collagen content[22]. Estimation of Hydroxyproline content revealed significantly increase in the test drug treated animals then control group and standard group.

Histopathology of skin – Marked increase in the dermal fibroblast, dermal granulation tissue and re-epithelisation in test group shows that the drug has promoted collagen synthesis, neoangiogenesis and matrix modelling[23].

CONCLUSION: It was observed from the results that the burn wounds treated with Dhatakyadi yoga showed significant healing of burn wound when compared to the control and standard groups. Dhatakyadi yoga hastened wound contraction, reduced epithelization time, scar area and increased hydroxyproline content in collagen tissue.

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