

Burn wound healing potential of *Plain gritha*, *Shatadhouta ghrita* and *Sahasradhouta ghrita* on wistar albino rats

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The cow *ghee* is commonly used in India for the treatment of burns and wounds. The *ghee* based formulation were useful for wounds, painful ulcers, insect bite wounds, herpes, leprosy, wounds caused by heat or fire and deep wounds by external application as per the classical texts of *Ayurveda*. The present study was carried out to evaluate the efficacy of three processed cow *ghee* in burn wound healing. Partial thickness burn wounds were inflicted upon 5 groups of 6 rats each. Group I served as control (No treatment), Group II- silver sulfadiazine ointment treated group served as reference standard, Group III- *Plain gritha* (PG), group IV- *Shatadhouta ghrita* (SDG) and Group V-*Sahasradhouta ghrita* (SHDG). All drugs were applied topically on the burn wounds for 28 days or till complete healing. The parameters observed were epithelisation period and percentage of wound contraction. The results were analysed using one way ANOVA followed by Dunnet's multiple 't' test. Epithelisation was hastened in all drug treated groups as compared to control group. The results of *Plain gritha* and *Shatadhouta ghrita* treated groups were comparable to that of the standard treatment drug silver sulfadiazine and found statistically significant in comparison to normal control group. *Plain gritha* and *Shatadhouta gritha* has burn wound healing potential comparable to silver sulfadiazine. Based on the analysis of the results it can be suggested that burn wound management can be initiated with *Shatadhouta ghrita* as the base for the formulation and mid way the management may be switched on to *Plain ghrita* based formulation. Further scientific investigation is required to provide basis for their efficacy and therapeutic claim.

Keywords: Burn wound, *Ghritha*, *Shatadhouta ghrita*, *Sahasradhouta ghrita*, Silver sulfadiazine

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The cow *ghee* is commonly used in India for the treatment of burns and wounds. The *ghee* based formulation were useful for wounds, painful ulcers, insect bite wounds, herpes, leprosy, wounds caused by heat or fire and deep wounds by external application as per the classical texts of *Ayurveda*¹. Burn can be defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight, or nuclear radiation². Wound healing is the process of repair that follows injury to the skin and other soft tissues. It involves a complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. Healing of

wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding. Healing process is broadly categorized into 3 stages, the inflammatory process consisting the establishment of inflammation, proliferative phase (consisting of granulation, contraction and epithelization) and finally the remodelling phase, which ultimately determines the strength and appearance of healing area³. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort and scarring. Improving the process of wound healing and tissue repair offers tremendous opportunities to enhance the quality of life for trauma and burn patients⁴. The present study was planned to

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evaluate the efficacy of three different preparation of cow *ghee*. Plain cow *ghee* (Cow *ghee* is a kind of liquid butter, made from the milk of cows and clarified by boiling (simmering). It is widely used for cooking in India. It is also used for preparing a type of Ayurvedic formulations known as '*ghrita*'), *Shatadhouta ghrita* (*Shata-dhauta-ghrita* is an Ayurvedic preparation, commonly prescribed for treatment of wounds, burns, and other skin lesions. It is prepared by washing clarified butterfat (cow *ghee*) one hundred times with water) and *Sahasradhouta ghrita* (*gritha* prepared by washing the clarified butter fat thousand times with water) with that of control and standard drug silver sulfadiazine on experimentally induced burn injury in rats.

Materials and method

Drugs and chemicals

Silver sulphadiazine 1% w/w (12gm) (Rexicin Pharmaceuticals), *Plain Ghrita* (PG), *Shatadhouta ghrita* (SDG) and *Sahasradhouta ghrita* (SHDG) were prepared at Rasashastra laboratory at SDM Ayurveda College Udupi, India as per the classical text *Astanga Hridayam Uttarasthana*⁵⁻⁶.

Experimental animals

Albino rats of wistar strains of either sex between 150 - 250 gm were obtained from animal house attached to department of Pharmacology, Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences Udupi. The experimental protocol was approved from the institutional ethical committee under the Ref. No: SDMCRA/IAEC-2011-12/BK02. The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for 2 weeks prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12hrs light and dark cycle, temperature of 25°C and relative humidity of approximately 50%.

Methodology

In the present study a partial thickness burn wound model was employed as per Bairy *et al.*⁷. The rats

were anaesthetised with ketamine at a dose of 100mg/kg and the hairs on dorsum were shaved. The burn wound were created by pouring hot molten wax at 80°C into a metal cylinder of 300 mm² circular opening placed on shaven back of the rat at the nape of the neck. After animals recovered completely from anaesthesia, they were kept individually in cages. During the experimental period all norms of good laboratory practice in caring the animals were followed.

Wound contraction was monitored by measuring wound area planimetrically, on every 4 days till the wounds completely healed. Time taken for full epithelization was measured by recording the days required for fall of scab leaving no raw wound behind. Apart from the drugs under investigation no local/systemic chemotherapeutic cover was provided to animals. The animals were randomly divided into 5 groups of 6 rats each. Group I did not receive any treatment and served as control. Group II received topical treatment with Silver sulfadiazine served as a reference standard; Group III, IV & V received topical treatment with PG, SDG and SHDG respectively. All drugs were applied topically once a day by sterile gauze on the burn wounds for 21 days or till complete healing whichever were earlier.

Assessment of burn wound healing

Animals were inspected daily and the healing was assessed based on the physical parameters like epithelization period and percentage of wound contraction.

- a) **Epithelization period:** It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.
- b) **Wound contraction:** It was assessed by noting the progressive changes in wound area planimetrically, excluding the day of the wounding. The sizes of the wounds were traced on a transparent paper every 4 days, throughout the monitoring period. The tracing was then superimposed on a 1 mm 2 graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction,

taking the initial size of the wound, as 100%, by using the following formula⁸.

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{specific day wound size} \times 100}{\text{Initial wound size}}$$

Statistical analysis

The experimental data were expressed as Mean ± SEM. Statistical analysis was carried out by one way analysis of variance followed by Dunnet’s multiple comparison ‘t’ test as post hoc test and p value < 0.05 implied statistical significance of results obtained.

Results

The SDG showed an initial remarkable and consistence percentage wound contraction in comparison to normal control group. The results were comparable with that of reference standard group. The PL and SHDG showed initially decreased till 12 days but later on there is a remarkable increase in the percentage of wound contraction while compared with that of normal control group. The results are presented in Table 1.

There was significant reduction in the duration of epithelization in the *Plain ghrita* and silver sulfadiazine ointment administered groups. The results seen with PL and reference standard was highly significant (p<0.01) when compared to control. There is an increase in the duration of epithelization with SDG which was found to be significantly high in comparison to control and all the other groups. SHDG has been taken days equal to normal control group and it is found to be statistically non significant. The result has been shown in the Table 2. The different stages of wound healing represented in Figs. 1-5.

Discussion

The data obtained show a complex wound healing promoting effect with different types of *ghrita*. *Plane ghrita* was less active during the inflammatory phase but produced better wound healing property. The possible mechanisms can be that in cow’s *ghee* several types of fatty acids exist, but in terms of wound treatment, the most important ones are the linoleic and linolenic acids⁹. They are referred to as essential fatty acids. Studies showed that Linoleic acid (LA) plays an important chemotaxic role for microphages, and it is fundamental in the expression of the components of the fibrinolytic system (regulates the production of collagenase). It favours autolytic debridement in the wound bed because it contributes to the production of metalloproteins, inducing granulation and accelerating the healing process¹⁰. It has been observed that LA is capable of inhibiting the growth of *Staphylococcus aureus*, affecting protein synthesis, cell wall, nucleic acids, and cellular membranes during cell division¹¹. So the presence of these essential fatty acids in *ghee* may

Table—2 shows duration of epithelization of burn wound in days

Group	No. of days
Control	30.00 ± 1.96
silver sulfadiazine ointment	27.50 ± 1.52**
<i>Plain ghrita</i> (PL)	27.00 ± 1.37**
<i>Shatadhouta ghrita</i> (SDG)	36.83 ± 2.34 **
<i>Sahasradhouta ghrita</i> (SHDG)	30.33 ± 0.95

Each value represented in Mean ± SEM, *p<0.05 in comparison with control group (one way ANOVA). PG- *Plain gritha*, SDG- *Shatadhauta gritha*, SHDG- *Sahasradhauta Ghritha*

Table 1—Percentage of wound contraction after drug treatment on day 4, 8, 12, 16, 20 & 24

Group	4 th day	8 th day	12 th day	16 th day	20 th day	24 th day
Control	0.125 ± 10.41	30.86 ± 4.25	32.16 ± 8.33	39.51 ± 09.77	60.11 ± 4.93	87.15 ± 6.27
Silver sulfadiazine ointment	5.67 ± 10.52	26.47±4.32	40.47 ± 3.94	55.46 ± 10.12	72.18 ± 9.85	89.83 ± 7.41
<i>Plain ghritha</i> (PL)	-27.02 ± 11.17	-0.83 ± 12.43*	13.72 ± 9.79*	22.9 ± 11.88	77.54 ± 5.62	93.70 ± 2.49
<i>Shatadhauta ghritha</i> (SDG)	20.35 ± 4.70	25.30 ± 8.31	34.93 ± 5.15	42.67 ± 3.41	54.05 ± 3.81	68.97 ± 6.01
<i>Sahasradhauta ghritha</i> (SHDG)	- 12.51 ± 2.50	-3.35 ± 2.67 **	3.74 ± 4.58 *	13.28 ± 4.51**	43.50 ± 10.69	79.09 ± 5.01

Each value represented in Mean ± SEM, *p<0.05 in comparison with control group (one way ANOVA).

PG- *Plain gritha*, SDG- *Shatadhauta gritha*, SHDG- *Sahasradhauta ghritha*



Figs. 1-5—Different stages of burn wound healing; 2—Standard group; 3—Plain *gritha*; 4—*Shatadhauta gritha*; 5—*Sahasradhauta gritha*

help it for a better wound healing activity. It seems that *Plain ghrita* contains factor which hasten maturation and remodelling of burn wounds including early eschar fall off.

Role of antioxidant property of *ghee* in wound healing during cutaneous thermal injury, several factors contribute to further tissue damage, among which oxygen free radicals are important. The relationship between the amount of products of oxidative metabolism and natural scavengers of free radicals determines the outcome of local and distant tissue damage and further organ failure in burn injury. In normal body conditions there exists a balance between free radicals and the natural scavengers of the body, while in a traumatic state the balance is lost and reactive oxygen metabolites are greater in number. An increased generation of oxygen free radicals in the extracellular space is seen in inflammatory states, in which the relatively low concentrations of enzymatic and non-enzymatic antioxidants increase the susceptibility of extracellular components to oxygen radical injury. The level of these free radicals in the plasma of patients with thermal skin injury increases in the first few hours post burn, reaching peak level on day 3 post-burn, i.e., during the inflammatory phase of wound healing¹². At this stage the therapeutic application of antioxidants becomes essential. The Vitamins, which are present in *ghee* like Vitamin A and Vitamin E are antioxidants and are helpful in reducing keton bodies, helpful in preventing oxidative injury to the body. Studies have shown that, besides EFA, vitamins A and E also contribute to the process of tissue repair. Vitamin A keeps epithelial tissue of the body intact¹³. Though *ghee* was not much active during the initial phase of wound healing, where usage of anti oxidants are recommended, probably the effect of free radicals were overcame by *ghee* during later phase of wound healing. Over all effective wound healing capacity of *ghee* was noted in this study.

***Shatadhouta ghrita*:** This *ghrita* was very effective in first phase of burn wound healing. At the same time it delayed the wound healing in later phase. Exact mechanism of its activity may not be possible to suggest, but it can be speculated that *Shatadhouta*

ghrita is different form of *ghrita* prepared by washing in water for hundred times. As mentioned earlier it is an oil water emulsion. The moisture value of used *Shatadhouta ghrita* was 49%. So changes in wound closure happened after the application of *Shatadhouta ghrita* can be considered as kind of moist wound healing. Over the centuries many publications have pointed out that a moist environment enhances epithelialization in the wound healing process¹⁴. Controlled experimental and clinical data have in recent times supported the suggestion that a moist environment enhances wound healing in the form of an occlusive dressing compared with a dry environment. The moist environment may provide a medium for cell migration and supporting healing processes. This may be the probable reason why in later phases *Shatadhouta ghrita* delayed the wound healing, moist healing with *Shatadhouta ghrita* may not work in proliferative and remodelling phases. In such condition *Plain ghrita* may produce better effect. When compared to *Plain ghrita*, *Shatadhouta ghrita* probably contains less or very least amount of fatty acid like linoleic and linolenic acids. These fatty acids might have been washed off from the *ghee* due to several washings.

***Sahasradhouta ghrita*:** Though *Sahasradhouta ghrita* was not effective in wound healing; it was effective in debriding effect in the form of eschar fall. One of main objective of topical application in any wound is debriding effect. In burn wound capillary permeability is greatly increased. Due to this, plasma rich in protein pours out continuously in large amount. These exudates collect in blisters or begin to form a dry brown crust known as eschar which protects the wound during initial days. Early separation of eschar will help better blood circulation in wound area, as it exerts pressure on the blood vessel. The quality of the eschar reflects in the regeneration of the new epithelium, this may lead to a better quality of scar formation. So, *Plain ghrita* group and *Sahasradhouta ghrita* group have shown early eschar fall. The eschar fall can occur mainly in two forms, i.e., soft and easy to rub off and hard and solid form. The appeared eschar in all the groups was soft and was easily separated except in control group

where it was comparatively hard. The advantage of *Sahasradhouta ghrita* was though the moisture content was less compared to *Shatadhouta ghrita* (29%) it could adhere for more duration over the skin surface. This property could prolong the moisture atmosphere by that eschar which was formed was soft and easy to get separated. So, this *ghrita* can be considered a good base for topical application. Healing property was absent probably due to escape of all major fatty acids during washing.

Conclusion

Based on the analysis of the results it can be suggested that burn wound management can be initiated with *Shatadhouta ghrita* as the base for the formulation and mid way the management may be switched on to *Plain ghrita* based formulation. Further scientific investigation is required to proof its efficacy and therapeutic claim.

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