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A traditional formulation of Shorea robusta resin found effective in excision wound model in rats

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Introduction

A large proportion of the population of developing countries still uses traditional medicines, either as a result of the high cost of Western pharmaceuticals and health care, or because the traditional medicines are more acceptable from a cultural and spiritual perspective(1). The WHO estimates nearly 80% of the population still depends upon herbal medicines due to their easy availability, low cost and possible less side effects as compared to allopathic system of medicines(2). These also cover healthcare systems that include beliefs and practices relating to diseases and health, which are products of indigenous cultural development and are not explicitly derived from a conceptual framework of modern medicine. Ingredients used in the preparation of those remedies may even provide attractive templates for the development of new pharmaceutical products(3).

All the people of Nepal have no access to allopathic medicine and health center because of illiteracy, poverty and unavailability. Thus, about 80% of the population in Nepal relies on traditional medicine(4). One such example is the use of formulation containing Shorea robusta resin, prepared by local practitioners themselves, for treating infected wounds and burns by some locals in Kathmandu valley. The ingredients used in traditional medicine, therefore, must be recognized and studied, not only as therapeutic agents with verifiable pharmacodynamic properties, but as agents of healing with beneficial effects, even when the precise mode of activity has not been properly understood(3).

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Shorea robusta Gaertn. is a tree commonly known as sal or shala tree, belonging to the family Dipterocarpaceae. The bark exudes an oleo-resin, which is whitish brown in color, bitter and acid taste. The resin is cooling but difficult to digest; bitter and acrid; astringent to the bowels; purifies the blood; lessens perspiration and fever; good for wounds, ulcers, burns, pains, itching, fractures, useful in dysentery; good for vaginal discharges (Ayurveda). It occurs in rough, brittle pieces having a faint resinous, balsamic odor and is widely used as incense in Indian religious ceremonies as it emits copious white fumes when burnt(5).

One of the very common diseases in day to day activities of the human is the wound, which may be due to physical, chemical, thermal, microbial or immunological insult to the tissue(6). According to the World Healing Society, wounds are physical injuries that result in an opening or break of the skin causing disturbance in the normal skin anatomy and function(7). The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration(8).

Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser-known hitherto unknown wild plants for treating wounds and burns(9). Traditional forms of medicine practiced for centuries in Africa and Asia may be being scientifically investigated for their potential in the treatment of wounds related disorders(9). Some medicinal plants have been employed in folk medicine for wound care (10-12). Many of these plants either possess pro-wound healing activities or exhibit antimicrobial and other related properties which are beneficial in overall wound care(13).

The wound healing efficacies of various herbal extracts can be evaluated in excision, incision, dead space, and burn wound models. *In vitro* and *in vivo* assays are stepping stones to well-controlled clinical trials of herbal extracts(14).

However, scientific validation and pharmaceutical supervision is necessary to generate medicinal preparations from plants and minimize some of the criticisms against the herbal medicine. Such re-evaluations would provide a rationale for the continued use of these medicinal products.

Hence, the aim of this study would be to investigate a traditional formulation based on Sal resin and assess its in-vivo wound healing potential in rat to scientifically validate it's use as well as to pave ways for development of an ideal herbal wound formulation based on Nepalese traditional practice.

MATERIALS AND METHODOLOGY:

Plant material and preparation:

All the *S.robusta* resin used in the study was bought from the local market and was identified by the botanists of Department of Pharmacy, Institute of Medicine, Tribhuvan University, Nepal.

The traditional formulation was prepared by the local practitioner during our observation with the above resin sample. The formulation contained 5 parts of the resin and 1 part of orange vermillion powder, triturated with mustard oil and finally diluted with water until an ointment-like consistency was achieved. It was done in a regular cleaned and dried kitchen mortar and pastel.

For the test ointments, the resin extracts were prepared by reflux condensation using ethanol at the temperature of 60-70˚C for 10 hours. Then the prepared resin extract was incorporated into simple ointment BP by trituration in 10% and 30% concentrations. The 10% and 30% ointments were consequently used as samples SRE-10 and SRE-30 respectively during the animal experiment. Simple ointment BP is an absorption ointment

base, prepared with the method as described in annex-2.

Animal Experiment:

Healthy albino rats of either sex, same age group, and approximately of similar weight (150-250g) were purchased from the Natural Plant Resource Laboratory (NPRL), Thapathali, Kathmandu. The experiment was conducted under the approval of the Institutional Animal Ethics Committee and in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes.

The animals were acclimatized for a period of 7 days before the experiment. They were maintained at a well-ventilated animal house under standard controlled conditions at temperature 22±1◦C to 30±1◦C, relative humidity 35±5 to 65±5%,and kept under 12/12h light/dark cycles with free access to food and water ad libitum. The animals were housed individually in clean, sterile polyvinyl cages containing paddy husk as bedding.

For the experiment, the animals were randomly grouped into numbers of 5 each with 3 rats (n=3). Group I was a control group which received simple ointment base as treatment after the wound creation, group II was a reference control that received standard drug (Mupirocin 2% ointment), group III was a test group that received SRE (10%), group IV was a test group that received the formulated ointment SRE (30%) and the last group V was a test group that received the Sal resin traditional formulation.

The method of Thakur et al was followed for the excision wound healing model(14). All the procedures were carried out under sterile conditions under general anesthesia. The predetermined area for wound infliction at the back of the animal was prepared for surgery by removing hairs with razor. The animals were anaesthetized intraperitoneally with anesthetic drug 100mg/kg body weight ketamine sulphate. They were maintained under standard husbandry conditions and on a uniform diet and managed throughout the experimental period. Animals were closely observed for any infection; those who showed signs of infection were separated and excluded from the study. They were periodically weighed before and after the experiments.

Excision wounds were inflicted on the dorsal thoracic region 1–1.5cm away from the vertebral column on either side and 5cm away from the ear. After wound area preparation with 70% alcohol, using a surgical blade, the circular skin from the predetermined area on the depilated back of the animal is excised to its full thickness to obtain a wound area of about 200–500mm^2 diameter and 2mm depth. Hemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline. The respective therapeutic treatment was administered topically to the animals of respective groups until complete epithelialization starting from the day of operation. Percentage wound contraction and period of epithelialization parameters were studied.

Application of test formulations to the wound area was done once a day at the group-dependent time intervals after cleaning with sterile surgical cotton wool in the experimental group animals for a period of n days, respectively. The ointments according to the animal groups were applied at a dose of 100mg/kg/day to 500mg/kg/day with a gauze sponge lasted for 10 min. The wound area was then covered with a fresh sterile bandage which was secured by adhesive tape. The standard drug ointment, containing 2% mupirocin in the same quantity was applied daily to wounds of respective group animals.

Evaluation parameters:

Percentage Wound Contraction:

The progressive reduction in the wound area was monitored planimetrically by tracing the raw wound boundaries initially on a transparency paper sheet in mm^2 without causing any damage to the wound area, and

then, the wound area recorded was measured using a graph paper every day.

Percentage wound contraction was calculated as:

$$\text{Wound Contraction (\%)} = \frac{\text{Initial wound size- specific day wound size} \times 100\%}{\text{Initial wound size}}$$

Epithelialization period:

The period of epithelialization is expressed as the number of days required for falling of the eschar (dead-tissue remnants) without any residual raw wound is considered as the end point of complete epithelialization (15).

Qualitative Phytochemical Screening of the S. robusta resin:

The extract was subjected for tests of glycosides, alkaloids, essential oil, terpenoids, steroids, flavonoids, reducing sugar, saponins, phenols and tannins.

Statistical analysis of the data:

Data obtained from experiments were expressed as Mean ± S.E. using SPSS version 20. The Mean ± S.E. was calculated for each parameter. Total variations present in a set of data were estimated by one-way analysis of variance (ANOVA) by post hoc Tukey test. The P-value < 0.05 was considered as statistically significant when compared to the control.

Table.1. Effect of ethanolic extract of *S.robusta* resin on wound contraction in rats

Animal group	Treatment	Percentage wound contraction (%)										
		Day 1	3	5	7	9	11	13	15	17	19	20
Control	Simple ointment BP	12.45	31.6	52.94	63.75	77.55	90.84	96.03	98.99	99.04	99.58	100
Standard*	Mupirocin	28.6	48.4	63.84	77.42	93.42	97.98	99.58	99.99	100		
SRE-10	10% w/w SRE	23.8	34.3	65.81*	78.01*	94.13*	98.79*	99.86*	100*			
SRE-30*	30% w/w SRE	36.1	52.6	75.91	86.60	97.94	99.79	100				
Traditional*	Traditional ointment	26.2	40.7	74.86	90.12	97.80	99.56	100				

*** -** Significant at p value < 0.05

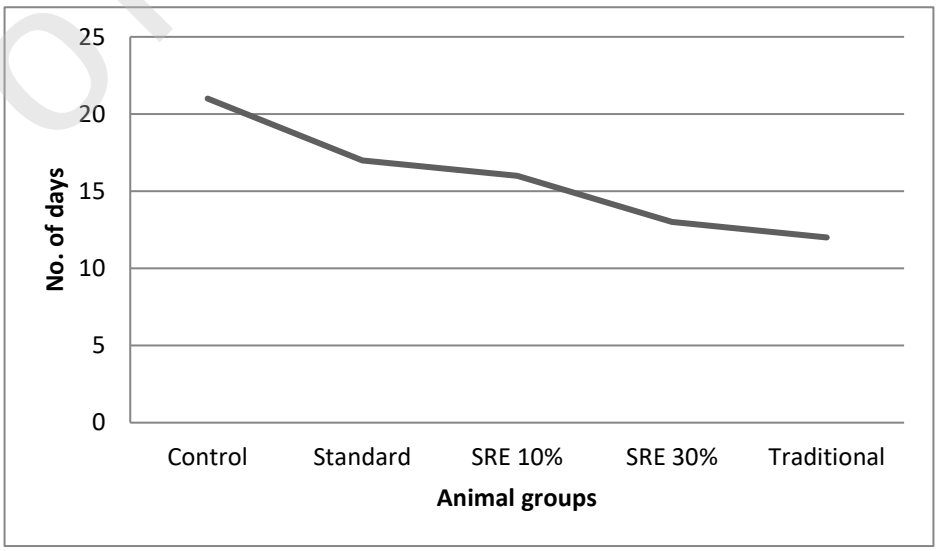

Fig.1. Epithelization periods of different animal groups

Results and discussion:

Qualitative phytochemical screening:

Phytochemical screening of the resin showed the presence of tannin, diterpenes, triterpenoids, carbhohydrates, saponin, glycoside by Borntrager test and flavonoid.

Wound healing activity:

All the treatments, i.e., SRE (10% w/w), SRE (30% w/w), traditional Sal resin and standard drug were found to cause significant increase in wound contraction (p-value < 0.01) as compared to the control group, by using ANOVA.

Furthermore, post hoc Tukey test was used to calculate the significance of mean difference at level of significance p-value < 0.05 among the different groups. The mean difference was found to be negative and significant for SRE 30% and traditional ointment as compared to control for day 2 and day 4.

The mean difference for SRE 30% group was positive and significant as compared to all other groups for each day. This means that SRE 30% produced significant increase in wound contraction in all days as compared to SRE 10%, hence SRE has dose dependent effect on wound contraction.

For traditional ointment, the mean difference was positive and significant as compared to SRE 10% and standard group in day 2, negative and significant as compared to standard and SRE 30% group as shown in the table 1.

Percentage wound contraction was calculated as:

$$\text{Wound Contraction (\%)} = \frac{\text{Initial wound size- specific day wound size} \times 100\%}{\text{Initial wound size}}$$

Epithelization period:

It is taken as the number of days required for the complete epithelization of the wound. The maximum epithelization period was seen in control group (n = 21), followed by standard group (n = 17), SRE 10% (n = 16), traditional group (n = 14) and SRE 30% (n = 13). Hence, the wound was completely healed in SRE 30% group at first (Fig. 1).

Discussion:

The ethanolic extract of the resin was formulated into a suitable topical formulation, i.e. ointment in two different concentrations 10% w/w and 30% w/w for the study of wound healing activity. Simple ointment BP was used as ointment base for the formulation using simple trituration method. The thus prepared ointments were used topically on the wounds inflicted on rats. The rats of different groups of treatment were observed for the whole period of wound healing and the area was measured every day. The effect of the ethanolic extract of resin on increase in wound contraction was studied and it was found to be significant p < 0.05 and dose dependent. Along with it, another traditional ointment whose main ingredient is *S.robusta* resin was also studied for its claimed wound healing activity and it was found to be significant as compared to the control group. This result is in concordance with another study conducted by Wani et al. The study demonstrated that the ethanolic extract of *S.robusta* resin may be capable of promoting wound healing activity due to its ability to accelerate wound contraction, increase tensile strength and increased hydroxyproline content and suggested its therapeutic potential in wound healing(16).

Another study conducted by Khan et al indicated that the triterpene-rich fraction and essential oil of *S. robusta* have the highest wound healing activities and confirm the traditional claims on this plant for healing of wounds(17).

Dutta et al suggested that a combination of flax seed oil, *Shorea robusta* resin and *Yashada bhasma* can be useful in wound contraction, improvement of tensile strength and augmentation in hydroxyproline content or collagen content. These properties together make this combination a potential candidate for anti-aging activities especially for better skin health(18).

Excision wounds are akin to open wounds and require considerable fibroplasias to fill the defect by formation of more collagen deposits. Inflammation is an integral part of wound-healing process. In the initial stages, wound contraction is independent of myofibroblast involvement. Later, growth factors facilitate differentiation of fibroblasts into myofibroblasts which are required for wound contraction. Fibroblasts lay down collagen to reinforce the wound as myofibroblasts contract. Hence, the current study provides ground for further investigation of wound healing activity of *S.robusta* resin using various other in-vitro and in-vivo models.

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