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Research Article

BURN WOUND HEALING POTENTIAL OF *RUBIA CORDIFOLIA* LINN ON WISTAR ALBINO RATS

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ABSTRACT

This study aimed to evaluate the wound healing property of *Rubia cordifolia* Linn in wistar albino rats in burn wound model where rats' dorsum were placed on wooden template and metallic screen. Both the template and screen were immersed in hot water bath for sixty seconds maintained at a temperature of 100^0C. Animals were divided into three groups comprising of six animals each. Control group received the vehicle, standard group received silver sulfadiazine and test group were treated with paste of *Rubia cordifolia* as a topical application respectively. The topical application of test drug has shown significant wound contraction (**P<0.01) in comparison to normal control group. This was supported by the histopathological findings with good epithelialisation of cells when compared to normal control group.

Key words: Wound healing, Burn, *Rubia cordifolia* Linn., Wound contraction, Collagenation, Epithelialisation

INTRODUCTION

Research on wound healing drugs is a developing area in modern biomedical sciences. Scientists who are trying to develop newer drugs from natural resources are looking for the safe and efficacious drugs. Most of these drugs are derived from plant origin and some of those plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and patients, but the potential of most remains unexplored.

Burn is a wound in which there will be coagulant necrosis of tissues and thus exerts a catastrophic influence on people in terms of human life, suffering, disability and financial loss. In India, a survey among 1,065,070,607 population reveals that around 1,411,637 patients of burns are being hospitalized annually.[1] Scalds are moist heat injuries, produced by the application to the body of a liquid at or near its boiling point, or in its gaseous form, such as steam. Water above 60^0C if in contact with skin for 10 seconds will result in partial skin loss & above70 ^{0}C will cause full thickness skin loss.[1]Treatment of major burns involves surgical debridement and frequent painful dressings. Topical antimicrobial agents are of peak importance in burn wound care. This can be achieved by some herbal drugs mentioned in the lexicons of *Ayurveda.* There are several treatments for minimizing the formation of scar tissue, infection and to reduce the amount of necrotic tissue produced during the healing process. But most of the drugs have either antimicrobial or anti-inflammatory properties which helps in the wound healing process. The herbal extracts which are considered being a collection of structurally diverse molecules possess wide therapeutic values such as flavonoids, alkaloids, taninins and phenols which has strong anti-oxidants, anti-inflammatory and anti-microbial properties. In such regards, *Rubia cordifolia* Linn, a perennial herbaceous climber,[2] possesses anti inflammatory[3], antibacterial [4], antioxidant, gastroprotective [5] and many more properties. The present study aimed to evaluate the efficacy of *Rubia cordifolia* as a single remedy to treat the burn wound healing.

MATERIALS AND METHODS

Chemical Material

Hydroxyproline, methyl red, chloramine – T, P-dimethyl amino benzaldehyde, citric acid monohydrate, sodium acetate trihydrate, methyl cellulose, perchloric acid (obtained from Sigma Traders, Dharwad, Karnataka, India) and concentrated hydrochloric acid, glacial acetic acid, toluene, sodium hydroxy pellets, (Visso Traders, Belgaum, Karnataka, India) were the chemicals used for the study.

Plant Material

Roots of *Rubia cordifolia*. Linn (Specimen num: CRL/2010/63) was collected from the natural habitat of Handibadanganath, Belgaum, Karnataka, India. The drug was authenticated at the department of Dravyaguna, KLE University's Shri B.M.K Ayurveda Mahavidhyalaya, Shahapur, Belgaum, Karnataka, India. The obtained roots were air-dried under sunlight and later stored in plastic air tight containers for further experimentation.

Preparation of drug

The dried root was taken and rubbed over a clean stone and made into paste with distilled water.

Experimental Animals

Healthy Male Wistar albino rats weighing between 200 ± 50g were selected for the experiment. They were obtained from animal house attached to KLE University's Shri B.M.K Ayurveda Mahavidhyalaya, Shahapur, Belgaum, Karnataka, India. The experimental protocol was approved by the institutional animal ethical committee under the reference no. BMK/IAEC/Res-05/2009. Animals were acclimatized for seven days in the standard laboratory conditions under naturally illuminated environment of 12:12h light and dark cycle, temperature of 25 ^{0}C and relative humidity of approximately 50%. The animals were fed with normal rat diet and water *ad libitum* throughout the study.

Methodology

The rats were anaesthetized with ketamine at a dose of 80mg/kg and the hair on dorsum were shaved to ensure even burn wounding. Each rat was then placed on its back in a template constructed of wood and a metal screen, so that its back was directly over the screen. Rat and template were immersed together into a 100^{0}C water bath for 60 seconds. Both should be far enough in the water so that the portion of the rat's back that is exposed by the screen is entirely in contact with the water, but so that no other part of the rat touches the water. Rats were removed from the template and placed on their back on absorbent bench paper to remove any remaining hot water and halt ongoing burning. Animals were then allowed to cool by turning over into their abdomen. Once the animals recovered completely from anesthesia, they were kept individually in cages.[6] 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 3 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 received topical treatment with paste of *Rubia cordifolia*. All drugs were applied topically once a day by sterile gauze on the burn wounds 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
percentage of wound contraction and period of epithelialization.

a) **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 formula:

% wound contraction = Initial wound size - specific day wound size x 100 / Initial wound size

Estimation of hydroxyproline concentration

Wound bed sample weighing 20mg was taken and the colorimetric assay was performed with known standards of hydroxyproline at 560 nm using double beam spectrophotometer. The hydroxyproline contents of individual samples were expressed as µg hydroxyproline/mg muscle wet weight. The amount of hydroxyproline in the final colorimetric reaction represents a proportion (1.5 ml/2.5 ml) of the total hydroxyproline in the final toluene extract. Multiply the result by (2.5/1.5) to obtain the total amount of hydroxyproline present in the final extract. Divide the result by the amount of muscle (wet weight) contained in the initial sample (0.5 mg) to obtain the hydroxyproline content (µg hydroxyproline/mg muscle).

Histopathology of skin

The full thickness skin from wound bed was dissected on last day of experimentation and then transferred to 10% formalin. The tissue was embedded in paraffin. The section was cut into 5µm thickness and stained with haematoxylin and eosin stain. Sections were qualitatively assessed under the trinocular light microscope. Stained slides were observed for assessing epithelialization, angiogenesis, collagen formation and for presence of leucocytes, these observations are compared with control group.

Statistical Analysis

The data was expressed in Mean ± SEM and statistically analyzed by employing one way ANOVA followed by Dunnet's multiple t- test as post hoc test. Graph pad prism Inst 3 was used for this purpose and $p<0.05$ and $p<0.01$ considered as statistically significant.

RESULT

The percentage of wound contraction was significantly increased in the topical *Rubia cordifolia* group compared to silver sulfadiazine and control groups.

The histopathological examination of skin tissue from full thickness burn wound bed revealed that there was marked dermal edematous changes in the control group. There was marked macrophages & neutrophilic infiltration and inflammatory changes and poor epithelialization. Whereas the reference standard and test drug administered groups showed moderate to good epithelialization and decreased cell infiltration and inflammatory changes as compared to control group.

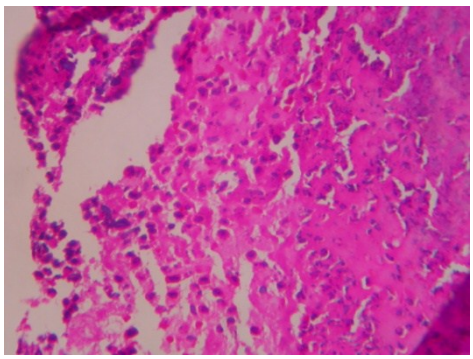
DISCUSSION

Hot liquid is the most common cause of burns. Hot water is easy to use for animal experiments. But, spilling of hot water limits the control of the area to produce burns in rats. Hence, special stages are to be followed to allow total control of area of burns. Dorsum of the rats is the choice of location for wound to produce burns perhaps due to its size and difficulty for the animals to provoke further injuries to the wound as it is very difficult for the animals to lick or scratch the back. Thus, in the present experimental study, dorsum of rats was exposed to hot water to produce burns.[7]

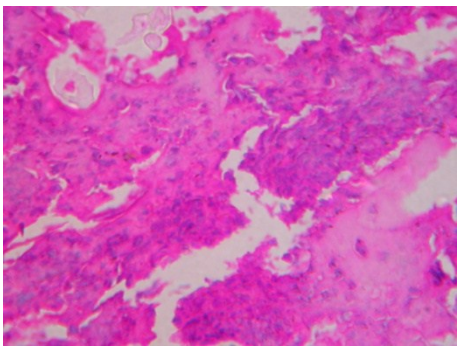
Table 1: Effect of test drug on percentage wound contraction recorded at different time interval

Group	Drug	4th day	8th day	12th day
Control	Distilled water	6.78 ± 1.60	16.65 ± 1.56	43.75 ± 2.89
Standard	Silver sulfadiazine	11.87 ±0.65	59.75 ± 3.55**	59.75 ± 3.55*
Test	*Rubia cordifolia*	2.16 ± 2.32	55.7± 3.82**	81.5 ± 4.26**

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to control group.



A 1



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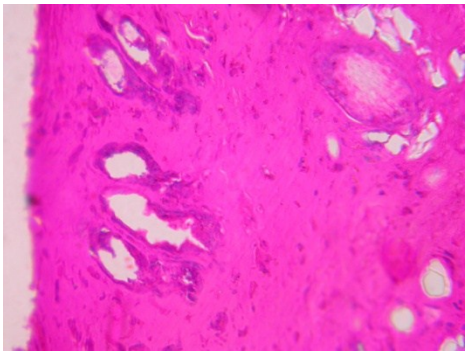

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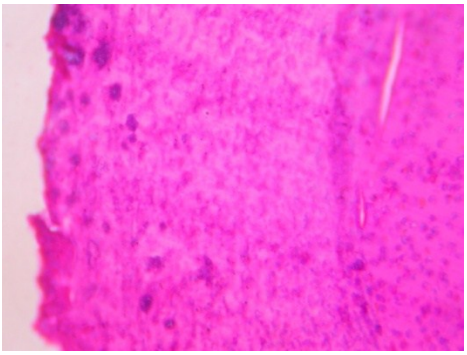

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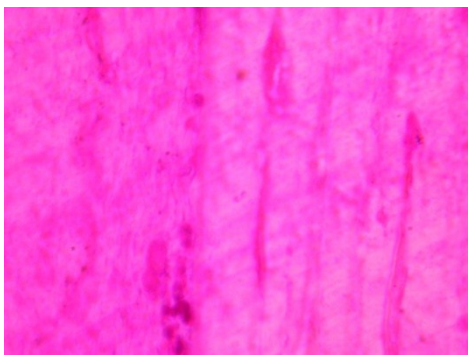
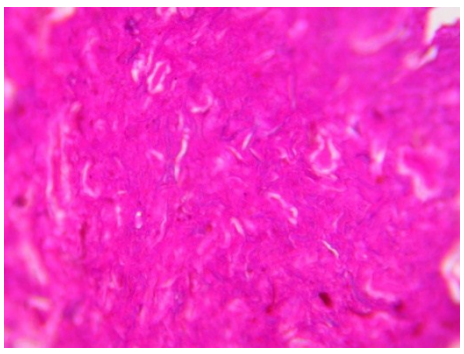


C 1



C2

Histology of skin; A1 & A2 – control group, B1 & B2 –standard group, C1 & C2 – test group

Inflammation is the immediate response to tissue injury mediated by damaged cells along the wound site. This response is to counteract microbial wound infection so as to enhance wound closure. This initial phase of inflammation aims to re-establish tissue integrity. Further, fibroblasts are infiltrated at the wound site initiating proliferative phase. There is increased production of collagen and fibronectin within the first three days after tissue injury caused due to burns. Collagen gets deposited by fibroblasts in the dermal wound area modulating healing process. Fibroblasts also secrete cytokines which release keratinocyte cells to the site of injury. Keratinocytes function in re-epithelialising the wound. Concurrently, angiogenesis (formation of new blood vessels) occurs with fibroblast and keratinocyte migration; thereby wound healing is enhanced due to continuous supply of oxygenated blood. Re-epithelialisation stage of wound repair follows the proliferative phase. Imbalance in either excessive collagen synthesis or decreased collagen catabolism leads to keloid and hypertrophic scar formation. Newly formed blood vessels continue to mature in this stage forming a functional vascular network.[8] Thus, wound contraction, collagenation and epithelialisation become the crucial phases of wound healing.[9] In the present investigation,

from the percentage of wound contraction and histopathological findings, topically applied paste of *Rubia cordifolia* Linn significantly accelerated wound healing and improved the quality of vascularity of granulation tissue possibly by increasing fibroblast proliferation, maturation of collagen content on one hand and decreasing collagenase activity on the other .[10] This could be possibly related to anti-inflammatory, anti-oxidant and antimicrobial properties of the drug.

CONCLUSION

Based on the result we could conclude that the test drug *Rubia cordifolia* Linn possess significant wound healing potential. And supports its folklore therapeutic claim, however, the further experimental exploration on wound healing potential need to be confirmed for its better therapeutic effect in clinical set up.

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