

Evaluation of the Wound Healing Activity of Rhamnus Virgata Ointment in Experimental Burn Wound Models in Rats

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ABSTRACT

Background

Burn wounds are a major healthcare concern due to delayed healing, infection risk, and associated complications. Medicinal plants possessing antioxidant, antimicrobial, and anti-inflammatory properties have gained attention as potential wound healing agents. Rhamnus Virgata is a traditionally important medicinal plant with reported pharmacological activities.

Objective

The present study evaluated the wound healing potential of Rhamnus Virgata ointment in experimentally induced burn wound models in rats.

Materials and Methods

Burn wounds were induced in Wistar albino rats, which were divided into five groups (n = 6). Animals received ointment base, Silver sulfadiazine (1%), Rhamnus Virgata ointment 1%, or Rhamnus Virgata ointment 2.5% for 21 days. Wound healing activity was assessed by measuring wound area and percentage wound contraction on Days 0, 7, 14, and 21. Histopathological analysis was also performed.

Results

Rhamnus Virgata ointment significantly enhanced wound healing compared to control groups. Both formulations reduced wound area and increased percentage wound contraction, with the 2.5% formulation showing superior activity, indicating a dose-dependent effect. Histopathological findings supported enhanced tissue regeneration and re-epithelialization in treated groups. Although Silver sulfadiazine showed higher efficacy, the activity of Rhamnus Virgata was comparable.

Conclusion

The findings demonstrate significant wound healing activity of Rhamnus Virgata in burn wound models, supporting its traditional medicinal use and potential as a natural therapeutic agent for wound management.

Keywords: Rhamnus Virgata, Burn wound healing, Herbal ointment, Wistar rats, Silver sulfadiazine.

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INTRODUCTION

Wound healing is a highly coordinated physiological process involving four stages: hemostasis, inflammation, proliferation, and remodeling. Its primary objective is to restore damaged skin, reestablish structural integrity, and recover normal function as a protective barrier (1). During the hemostasis phase, platelets adhere to exposed subendothelial structures at the site of vascular injury and become activated. Simultaneously, the coagulation cascade is initiated, resulting in thrombin generation and conversion of fibrinogen to fibrin. The fibrin network, along with aggregated platelets, forms a stable clot that seals the wound site (2,3). Subsequently, during the inflammatory phase, immune cells such as neutrophils and macrophages are recruited to the wound site, where they eliminate pathogens and cellular debris through phagocytosis. These cells also release pro-inflammatory cytokines and growth factors that stimulate the activation and proliferation of fibroblasts, keratinocytes, and endothelial cells, thereby promoting wound bed preparation and granulation tissue formation (4,5). During the proliferative phase, granulation tissue forms, facilitating the replacement of damaged tissue. Subsequently, during the remodeling phase, connective tissue is reorganized, re-epithelialization is completed, and scar tissue is formed (6).

Chronic wounds and burn injuries represent a major healthcare challenge worldwide due to their prolonged healing time, high treatment costs, and increased risk of infection (7). Wounds are broadly classified into acute and chronic types based on the duration of healing. Acute wounds exhibit a well-regulated and predictable healing process and commonly result from surgical procedures or accidental injuries. Depending on the extent of epidermal and dermal damage, healing generally occurs within 8–12 weeks (8). Although an ideal healing process would result in complete regeneration of tissue closely resembling intact skin in both structure and function, true regeneration is rare, except in early fatal wound repair. Instead, wound healing generally produces tissue that is structurally and functionally adequate but not entirely identical to the original tissue (9,10).

However, disruption of this coordinated healing process impairs skin restoration, resulting in delayed healing and the development of chronic wounds. Furthermore, several factors can interfere with the normal healing process, leading to delayed wound repair, increased patient morbidity and mortality, and poor cosmetic outcomes. Such non-healing wounds are commonly characterized by persistent exudate, recurrent infections, tissue necrosis, impaired re-epithelialization, reduced angiogenesis, and excessive production of reactive oxygen species (ROS) (11,12). Conventional wound therapies are often associated with limitations such as high cost, antimicrobial resistance, allergic reactions, and delayed tissue regeneration (13). For thousands of years, medicinal herbs have played a significant role in both traditional and alternative systems of medicine. Their continued widespread use is largely attributed to the belief that herbal remedies are associated with fewer adverse effects compared to synthetic drugs. In recent years, traditional medicinal plants have attracted significant attention for their potential in promoting wound healing due to their diverse pharmacological properties and minimal side effects (14). The wound healing potential of medicinal plants is largely attributed to the presence of phytoconstituents such as flavonoids, tannins, phenolic compounds, and alkaloids, which possess antioxidant, antimicrobial, and anti-inflammatory properties. Among these, *Rhamnus Virgata* is an important medicinal plant traditionally valued for its therapeutic applications (15).

Rhamnus Virgata is a *deciduous* shrub or small tree distinguished by simple, elliptic leaves exhibiting characteristic arcuate venation. *Rhamnus Virgata* is a large, thorn-bearing shrub or small tree that is extensively found in Pakistan (notably Swat and Kashmir), China, and India (16,17). Ethnobotanical studies and literature reports further highlight the medicinal significance of *Rhamnus Virgata*, suggesting its considerable therapeutic potential (18). Previous studies have reported antioxidant and antimicrobial activities of *Rhamnus Virgata*, which may contribute to its wound healing potential (19).

Despite its traditional medicinal importance and reported pharmacological activities, scientific investigations evaluating the wound healing efficacy of *Rhamnus Virgata* ointment remain limited. Therefore, the present study was undertaken to systematically evaluate the wound healing potential of *Rhamnus Virgata* ointment using experimentally induced burn wound models in rats. The study aimed to assess its effect on wound contraction, tissue regeneration, and overall healing progression in order to explore its potential as a natural therapeutic agent for burn wound management.

1. Material and methods

1.1. Chemicals and reagents

Leaves of *Rhamnus Virgata* were collected from the local region of Dehradun, Uttarakhand, and the plant material was authenticated by the Botanical Survey of India, Dehradun. The collected leaves were further used for the preparation of the ointment formulation. Silver Sulfadiazine and Povidone Iodine were purchased from SRL Pvt Ltd India and TCI, Japan. All the other chemicals used for the experiment were of analytical grade.

1.2. Animal studies

10-12 weeks old Wistar albino rats were procured from animal house facility of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. Rats were acclimatised in propylene cages for 1 week prior to conduction of the experiments. Controlled environmental conditions of 12-hour light/dark cycle and temperature of 24°C were maintained. The rats were fed with standard laboratory chow and water *ad libitum*. The experimental design was reviewed and approved by the Institutional Animal Ethics Committee, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The experimental study was executed in accordance with the guidelines of Committee for the Control and Supervision of Experiments on Animals (CCSEA).

1.3. Induction of Burn wound

Prior to wound induction, animals were anesthetized with an appropriate dose of thiopental sodium to ensure adequate sedation and to minimize pain and distress during the procedure. The dorsal surface of each animal was carefully shaved and cleaned to remove hair and contaminants, thereby providing a uniform site for wound creation.

A cylindrical metal rod of 10 mm diameter was heated to a predetermined temperature and gently applied to the prepared dorsal skin for a fixed duration to induce a reproducible partial-thickness burn wound. Care was taken to maintain consistency in temperature, pressure, and contact time across all animals to ensure uniformity of the injury model. Immediately following burn induction, Ringer lactate solution (1 ml/kg) was administered via the intraperitoneal route to support physiological recovery, maintain fluid balance, and reduce the risk of dehydration. The animals were then closely monitored during the post-procedural period for signs of recovery, distress, or complications.

Throughout the experimental duration, animals were observed regularly for any indications of infection, such as redness, swelling, pus formation, or abnormal behaviour. Animals exhibiting such signs were excluded from the study and replaced to ensure the accuracy, reproducibility, and reliability of the experimental results.

1.4. Grouping and treatment protocol

Following burn wound induction, animals were randomly divided into five groups comprising six animals each (n = 6). Group I served as the negative control and received 0.5% Na CMC (1 mL/kg, p.o.). Group II served as the positive control and received plain ointment base. Group III served as the standard treatment group and received Silver Sulfadiazine 1% ointment. Group IV comprised animals treated with *Rhamnus Virgata* ointment 1%, whereas Group V comprised animals treated with *Rhamnus Virgata* ointment 2.5%. All treatments were administered topically once daily for 21 consecutive days. Respective doses were selected from previous studies. Wound healing parameters were assessed periodically throughout the study period.

After 21 days blood sample was collected by retro-orbital puncture. Animals were humanely euthanized using carbon dioxide (CO₂) as per CCSEA guidelines and the skin tissue from each rat was excised and collected under sterile conditions for further biochemical and histological evaluation.

1.5. Physical examination

Wound healing progression was assessed at predetermined time intervals (Day 0, 7, 14, and 21) by measuring the wound area using an appropriate method. The initial wound area recorded on Day 0 was considered as the baseline for comparison. Subsequent measurements were taken at each time point to monitor the rate of wound closure throughout the experimental period.

The extent of healing was quantitatively evaluated by calculating the percentage of wound contraction, which represented the reduction in wound size relative to the original wound area. This parameter served as an indicator of the effectiveness of the treatment in promoting tissue repair and regeneration. A progressive decrease in wound area over time indicated enhanced wound healing activity in the treated groups compared to the control group.

1.6. Histopathological analysis

On day 21, the animals were euthanized, and the experiment was terminated. The wound tissue was carefully excised from the surviving animals for histological examination. The collected tissue samples were fixed in an appropriate fixative (such as 10% neutral buffered formalin) and processed according to standard histological procedures, including dehydration, clearing, and paraffin embedding. Sections of approximately 5 µm thickness were then prepared using a microtome and stained with hematoxylin and eosin, a routine stain widely employed in histopathological studies for general tissue evaluation.

The stained sections were examined microscopically for key histological parameters, including the extent of re-epithelialization, the degree of maturation and organization of epidermal squamous cells, the thickness of the granular cell layer, and the overall extent of tissue regeneration

and remodeling. Additional observations such as collagen deposition, fibroblast proliferation, and inflammatory cell infiltration were also considered where applicable.

1.7. Statistical analysis:

Data were expressed as the mean ± S.E.M. For a statistical analysis of the data, group means were compared by one-way ANOVA with post hoc analysis. The Tukey-Karmer post hoc test was applied to identify significance among groups. P<0.05 was considered to be statistically significant. Also, some of the data were expressed by two-way ANOVA considering multiple comparisons followed by Bonferroni analysis. For nerve conduction velocity, Student's t test was used to compare the difference between means for quantitative variables. Least squares regression was used for model building and hypothesis testing.

2. Results

2.1. Effect of *Rhamnus Virgata* on wound area (mm²) in the burn wound model

As shown in **Table 1**, there was initially no significant difference in wound area among all experimental groups on Day 0, indicating uniformity in wound induction. As the study progressed, the negative control group showed a slow and gradual reduction in wound area over the observation period. The positive control group (ointment base) demonstrated a moderate improvement in wound contraction compared to the negative control.

The standard treatment group treated with Silver sulfadiazine (1%) exhibited a highly significant reduction (P < 0.001) in wound area on Days 7, 14, and 21 compared to the control groups, indicating rapid wound healing.

Animals treated with *Rhamnus Virgata* ointment (1% and 2.5%) showed a significant decrease (P < 0.05–0.01) in wound area compared to the control groups. Among the test groups, the 2.5% formulation demonstrated a greater reduction in wound area than the 1% formulation, suggesting a dose-dependent effect. By Day 21, both test

groups exhibited marked wound contraction, approaching the healing profile of the standard-treated group.

2.2. Effect of *Rhamnus Virgata* on

wound contraction in the burn wound model

As shown in **Table 2**, a progressive increase in the percentage of wound contraction was observed in all experimental groups throughout the study period. The negative control group exhibited a slow rate of wound contraction, indicating delayed healing. The positive control group (ointment base) showed a moderate improvement in wound contraction compared to the negative control. The standard-treated group (Silver sulfadiazine) demonstrated a highly significant increase ($P < 0.001$) in percentage wound contraction on Days 7, 14, and 21 when compared to the control groups, indicating enhanced wound healing activity.

Animals treated with *Rhamnus Virgata* ointment (1% and 2.5%) showed a significant increase ($P < 0.05-0.01$) in wound contraction compared to the control groups. Among the test groups, the 2.5% formulation exhibited a greater percentage of wound contraction than the 1% formulation, suggesting a dose-dependent response. By Day 21, both test groups showed substantial wound closure, approaching the healing efficacy of the standard-treated group.

Table 1- Effect of *Rhamnus Virgata* on wound area (mm²) in the burn wound model

Group number	Name of group	Day 0	Day 7	Day 14	Day 21
<i>I</i>	NC	100 ± 0.0	88 ± 2.5	70 ± 3.0	50 ± 2.8
<i>II</i>	PC	100 ± 0.0	80 ± 2.3*	60 ± 2.7*	35 ± 2.5**
<i>III</i>	Std	100 ± 0.0	55 ± 2.0***	25 ± 1.8**	8 ± 1.5**

<i>IV</i>	Test group A	100 ± 0.0	65 ± 2.2**	35 ± 2.0**	12 ± 1.8**
				*	*

<i>V</i>	Test group B	100 ± 0.0	60 ± 2.1***	28 ± 1.9**	10 ± 1.6**
				*	*

Data are expressed as mean ± SEM (n = 6). * $p < 0.05$, * $p < 0.01$, ** $p < 0.001$ as compared with the negative control group. **. NC: Normal control group; PC: Positive control group; Std: Standard group treated with Silver Sulfadiazine 1%; Test group A: Test group treated with *Rhamnus Virgata* ointment 1%; Test group B: Test group treated with *Rhamnus Virgata* ointment 2.5%.

Table 2- Effect of *Rhamnus Virgata* on wound contraction in the burn wound model

Group number	Name of group	Day 7	Day 14	Day 21
<i>I</i>	Negative control	12 ± 2.5	30 ± 3.0	50 ± 2.8
<i>II</i>	Positive control	20 ± 2.3*	40 ± 2.7*	65 ± 2.5**
<i>III</i>	Standard	45 ± 2.0***	75 ± 1.8***	92 ± 1.5***
<i>IV</i>	Test group A	35 ± 2.2**	65 ± 2.0***	88 ± 1.8***
<i>V</i>	Test group B	40 ± 2.1***	72 ± 1.9***	90 ± 1.6***

Data are expressed as mean ± SEM (n = 6). * $p < 0.05$, * $p < 0.01$, ** $p < 0.001$ as compared with the negative control group. **. NC: Normal control group; PC: Positive control group; Std: Standard group treated with Silver Sulfadiazine 1%; Test group A: Test group treated with *Rhamnus*

Virgata ointment 1%; Test group B: Test group treated with *Rhamnus Virgata* ointment 2.5%.

Standard

Test A

Te

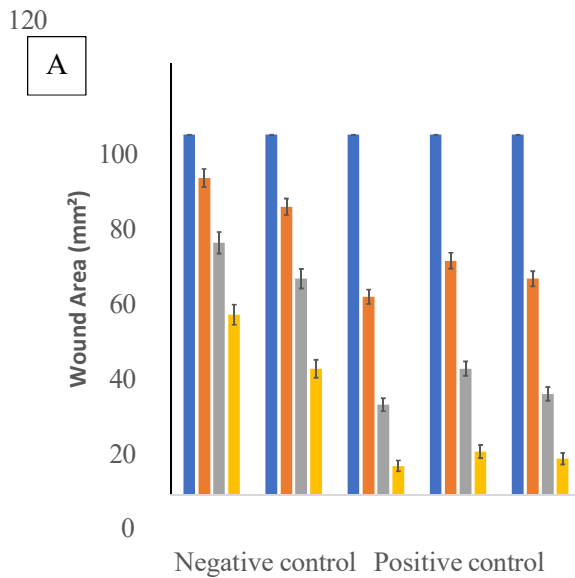


Fig.1. Effect of *Rhamnus Virgata* on (A) wound area (mm²) in the burn wound model (B) wound contraction in the burn wound model; Standard group treated with Silver Sulfadiazine 1%; Test group A: Test group treated with *Rhamnus Virgata* ointment 1%; Test group B: Test group treated with *Rhamnus Virgata* ointment 2.5%.

2.3. Effect of *Rhamnus Virgata* on histopathological analysis of skin tissue

3. Discussion

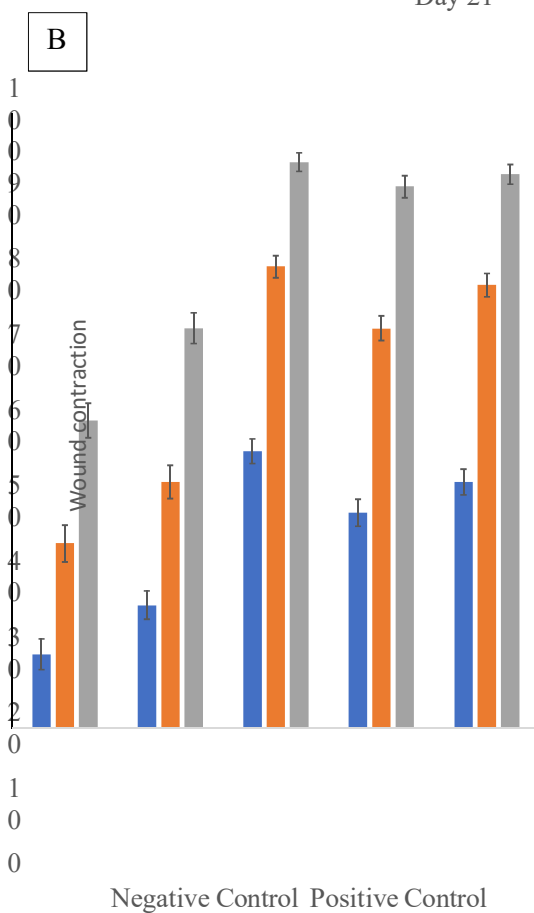
Wound healing is a complex and dynamic biological process involving sequential phases of inflammation, proliferation, and remodeling (20). It includes various cellular events such as fibroblast proliferation, collagen synthesis, angiogenesis, and re-epithelialization, which collectively contribute to restoration of tissue integrity (21). Any imbalance in these processes, often due to oxidative stress, infection, or excessive inflammation, can delay healing. Therefore, agents with antioxidant, anti-inflammatory, and antimicrobial properties are known to enhance wound repair (22).

Effect on Wound Area

In the present study, wound area was measured at different time intervals to assess the rate of healing. Initially, there was no significant difference in wound area among all experimental groups on Day 0, indicating uniform wound induction. Over the study period, the negative control group showed a slow reduction in

wound area, representing the natural healing process. The positive control group treated with ointment base exhibited moderate improvement, suggesting a supportive role of the base formulation.

The standard-treated group receiving Silver sulfadiazine (1%) demonstrated a highly significant reduction in wound area at all observed time points, indicating rapid and



effective wound healing.

Animals treated with *Rhamnus Virgata* ointment (1% and 2.5%) showed a significant decrease in wound area compared to the control groups. Among the test groups, the 2.5% formulation exhibited a greater reduction in wound size than the 1% formulation, suggesting a dose-dependent effect. By Day 21, the wound area in the treated groups was markedly reduced, approaching the healing profile of the standard group.

Effect on Percentage Wound Contraction

The percentage of wound contraction was used as a quantitative measure of wound healing efficiency. A progressive increase in wound contraction was observed in all groups over time. The negative control group showed the lowest percentage of wound contraction, indicating delayed healing, while the positive control group exhibited moderate improvement.

The standard-treated group (Silver sulfadiazine) showed a highly significant increase in wound contraction at all time points, confirming its effectiveness in promoting wound closure.

Treatment with *Rhamnus Virgata* ointment resulted in a significant increase in percentage wound contraction compared to the control groups. The 2.5% formulation demonstrated higher wound contraction than the 1% formulation, indicating enhanced efficacy with increased concentration. By Day 21, both test groups exhibited substantial wound closure, closely comparable to the standard-treated group. Overall Interpretation

The enhanced wound healing activity observed with *Rhamnus Virgata* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and phenolic compounds, which possess antioxidant, anti-inflammatory, and antimicrobial properties. These constituents likely promote collagen synthesis, fibroblast proliferation, and angiogenesis, thereby accelerating tissue repair and re-epithelialization.

Overall, the results suggest that *Rhamnus*

Virgata exhibits significant wound healing potential, with the higher concentration (2.5%) showing superior efficacy, thereby supporting its traditional use in wound management. SUMMARY The present study was undertaken to evaluate the wound healing potential of *Rhamnus Virgata* using a burn wound model in experimental animals. The plant material was collected, authenticated, and processed for preparation of ointment formulations at different concentrations (1% and 2.5%). The wound healing activity was assessed by measuring wound area and percentage wound contraction at predetermined intervals (Day 0, 7, 14, and 21).

The results demonstrated that treatment with *Rhamnus Virgata* ointment significantly enhanced wound healing compared to control groups. Both test formulations showed a marked reduction in wound area and a corresponding increase in percentage wound contraction over the study period. The 2.5% formulation exhibited greater efficacy than the 1% formulation, indicating a dose-dependent response. The standard-treated group receiving Silver sulfadiazine showed the highest wound healing activity.

Overall, the findings suggest that *Rhamnus Virgata* possesses significant wound healing properties, which may be attributed to its bioactive phytoconstituents with antioxidant, anti-inflammatory, and antimicrobial effects.

4. CONCLUSION

In conclusion, the findings of the present study demonstrate that *Rhamnus Virgata* possesses significant wound healing activity in the experimental burn wound model. Topical application of the ointment formulation promoted wound contraction and enhanced tissue regeneration in a dose-dependent manner, with the 2.5% formulation exhibiting the most pronounced effect. Although the standard treatment showed comparatively higher efficacy, the activity observed in the test groups was found to be comparable. These results support the traditional medicinal significance of *Rhamnus Virgata* and highlight its potential as a promising natural agent for wound healing. Further pharmacological and clinical studies are warranted to elucidate its underlying mechanisms and therapeutic

applicability.

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Institutional Review Board Statement:

The Institutional Animal Ethics Committee (IAEC) Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana examined and approved all animal experimentation protocols, in accordance with CCSEA guidelines, Government of India.

Informed Consent Statement: Not relevant to the current research.

Data and material accessibility: Not applicable

Consent for publication: The final work has been reviewed, approved, and submitted and published with the approval of all authors.

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