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Research Article

Effect of Rosmarinic Acid in the Rat Experimental Wound Model

Hayat Ayaz, Ayfer Aktaş, Fırat Aşır*

Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakır, Turkey

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*Address for Correspondence:

Fırat Aşır, Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakır, Turkey

Abstract

Objective: The aim of this study was to investigate the effects of rosmarinic acid (RA) on wound healing using an experimental rat wound model, assessing collagen organization, inflammation, and epithelial regeneration.

Methods: Twenty-one rats were divided equally into three groups: Control, Wound, and Wound+RA. An wound was created on the dorsal skin of rats in the wound and wound+RA groups. Rosmarinic acid cream (10%) was applied daily to the wound+RA group for 14 days. Tissue samples were collected at the end of the experiment for histological (Masson Trichrome) and immunohistochemical (Cytokeratin 14, CK14) analyses.

Results: Histological evaluation showed significantly improved wound healing in the wound+RA group, characterized by organized collagen deposition, decreased inflammatory response, and enhanced epidermal regeneration compared to the wound group. Cytokeratin 14 immunostaining indicated increased CK14 expression, reflecting better epithelialization and keratinocyte differentiation in the RA-treated group.

Conclusion: Topical application of Rosmarinic Acid significantly improved wound healing by enhancing collagen organization, reducing inflammation, and promoting epithelial regeneration, suggesting potential clinical applications of Rosmarinic Acid in wound care management.

Keywords: rosmarinic acid, wound healing, CK14, immunohistochemistry

INTRODUCTION

The ability of an organism to repair or renew tissues is the basic principle of survival. The disruption or loss of normal anatomical structure and function of the tissue due to various reasons and the complete or temporary loss of its existing biological and physiological qualities are called wounds. The aim of the wound healing process is to correct the structure and function of the damaged tissue. The healing process begins at the time of injury and can continue for years. This dynamic process involves highly organized cellular, humoral and molecular mechanisms [1]. Wound healing consists of 4 physiological phases: hemostasis, inflammation, proliferation and remodeling. Fibroblast migration and proliferation, synthesis of the extracellular matrix (ECM) and granulation tissue formation are the distinguishing features of the proliferative phase of the dermal repair process [2]. Any disruption in this process leads to chronic or abnormal wound healing and serious morbidities [3]. Chronic wounds are considered to be a significant and growing economic burden on healthcare systems [4]. A study conducted in Denmark reported that chronic wounds cost €8 million in 2009 and will cost €224 million in 2020 [5]. Another study reported that 6.5 million patients in the United States suffer from chronic wounds, with an annual treatment cost of $25 billion. [4] Chronic wounds can be prevented and healed with appropriate treatments. However, in some cases, current treatments may not prevent complications of chronic wounds such as gangrene, bleeding, amputation, or even lead to death [6]. Many clinical and experimental studies have been conducted to examine the effects of herbal extracts on acute or pathological wound healing [7]. Rosmarinic acid (RA) is one of the most important water-soluble polyphenolic components of Sarcandra glabra leaf extract [8]. Due to the strong antioxidant and anti-inflammatory properties of RA and the acid extracts it contains; it has been used to protect the skin against ultraviolet (UV) rays and the negative effects of many external factors such as reactive oxygen species (ROS)[9-11].

Keratin intermediate filaments, one of the main protein components in epithelial cells, are encoded by a large family of 54 genes that are regulated depending on the tissue and differentiation [12]. Keratin and other intermediate filaments are involved in fundamental processes such as cell differentiation and tissue homeostasis. Keratin 14 has been reported to copolymerize to form the distinct intermedial filament apparatus that occurs in the progenitor basal layer of the epidermis and the associated complex epithelium [13]. In our study, we aim to examine and compare the effects of rosmarinic acid on wound healing in rats wound wounds using cytokeratin 14 (CK14) antibody immune staining.

MATERIALS AND METHODS

Study design

Rosmarinic acid (RA) (Carbosynth, cas no: 20283-92-3, Berkshire UK) was purchased commercially. 18 grams of Cold cream (Cerae albae, Olei Amygdalanum, Boracis, Aque rosae, Olei rosae) and 2 grams RA powder were used to obtain 10% rosmarinic acid cream [14].

A total of 21 animals were taken without applying the experimental procedure and divided into 3 groups as control, wound, wound+RA. At the beginning of the experiment, general anesthesia was applied to the animals in the wound group and wound+RA groups with 75 mg/kg Ketamine HCL (Ketalar; Pfizer, Istanbul, Turkey) and 8 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey).

1. **Control group:** No procedure was applied.
2. **Wound group:** After anesthesia was provided, a 1 cm diameter wound was made on the dorsal surface of the animals' back skin corresponding to the lumbar spine [15, 16]. After the surgical procedure, the animals were taken to cages.
3. **Wound+RA group:** After the wound procedure, 10% Rosmarinic acid cream was applied locally to the wound area every day for 14 days [14].

At the end of the 14-day experiment, tissue was taken from the area corresponding to the wound area in the groups and fixed with 10% formalin.

Histological Tissue Embedding

Tissues to be taken from the wound wound area were subjected to routine histological follow-up after 10% formalin fixation and paraffin blocks were prepared. 5 µm thick sections from paraffin blocks were stained with Masson trichrome. Sections were passed through xylene and decreasing alcohol series to perform antigen retrieval and then primary antibody cytokeratin 14 (CK14) (catalog no: sc-53253, Santacruz Biotech, US) was applied and kept overnight at +4 °C. Then secondary antibody and streptavidin peroxidase were applied. After DAB application, sections were examined for protein expression levels under a light microscope [17].

RESULTS

Masson Trichrome staining revealed distinct histological differences among the experimental groups (Figure 1). In the control group (Figure 1A), the tissue maintained a normal histological structure with well-organized collagen fibers (stained blue) and an intact epidermal layer (stained red) with keratin layer. In the wound group (Figure 1B), significant tissue disruption was observed, with an increased inflammatory response, loosely arranged collagen fibers, and an incomplete epidermal layer, indicating impaired wound healing. In contrast, the wound+RA group (Figure 1C) group exhibited improved wound healing characteristics, including a more organized collagen network, reduced inflammation, and better epidermal regeneration compared to the wound group. These findings suggest that RA promotes more effective wound healing by enhancing collagen organization and reducing inflammation.

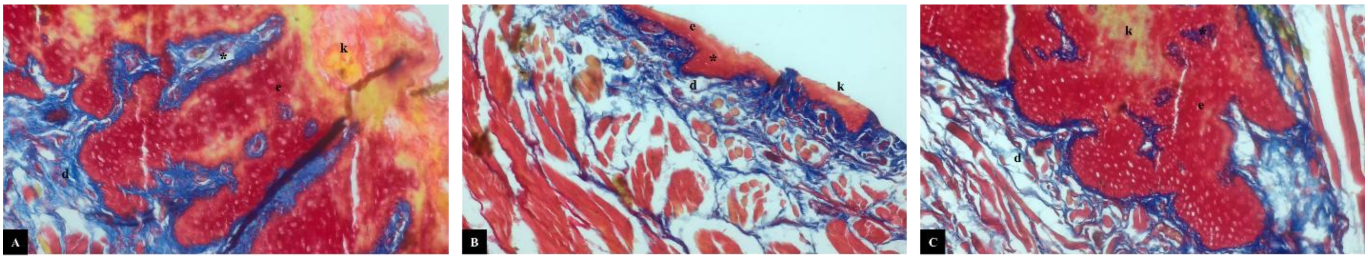

Figure 1: Cross sections of skin tissue. A) control, B) Wound, C) Wound+RA group. k: keratin, e: epidermis, d: dermis, *: dermal papillae, Trichrome Masson, Scale bar: 50 µm, Magnification: 20X

Cytokeratin 14 (CK14) immunostaining demonstrated variations in epithelial regeneration among the experimental groups (Figure 2). In the control group (Figure 2A), CK14 expression was observed mainly in the basal layer of the epidermis, with a uniform and well-organized distribution. In the wound group (Figure 2B), CK14 expression appeared reduced and scattered, indicating delayed epithelial regeneration and structural disorganization due to the wound. In contrast, the wound+RA (Figure 2C) group exhibited a stronger and more continuous CK14 expression pattern in the epidermis, suggesting enhanced epithelialization and improved wound healing. These findings indicate that Rosmarinic Acid may contribute to better wound recovery by promoting epithelial regeneration and maintaining cytokeratin integrity.

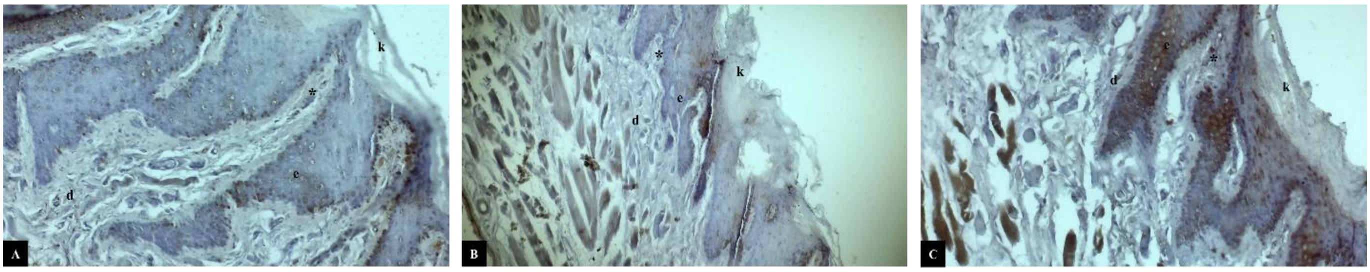

Figure 2: Cross sections of skin tissue. A) control, B) Wound, C) Wound+RA group. k: keratin, e: epidermis, d: dermis, *: dermal papillae, CK14 immune staining, Scale bar: 50 µm, Magnification: 20X

DISCUSSION

Wound healing is a complex biological process that involves multiple cellular and molecular mechanisms, including inflammation, proliferation, and tissue remodeling[18]. In this study, we investigated the effects of Rosmarinic Acid (RA) on wound healing in a rat wound model by evaluating histological changes through Masson Trichrome staining and epithelial regeneration using Cytokeratin 14 (CK14) immunostaining. Our findings demonstrate that RA promotes collagen organization, reduces inflammation, and enhances epithelial regeneration, suggesting a beneficial role in wound healing.

Masson Trichrome staining revealed that the wound + RA group exhibited a more organized collagen network and reduced inflammation compared to the wound group, indicating improved dermal repair. These findings align with previous studies demonstrating that RA enhances collagen synthesis and fibroblast activity, both of which are essential for effective wound healing[9]. Similarly, a study by Küba et al reported that topical RA application significantly accelerated wound closure and improved granulation tissue formation in a rat wound model, further supporting our results[14].

Furthermore, CK14 immunostaining showed that RA-treated wounds exhibited stronger CK14 expression, indicating enhanced epithelial regeneration. CK14 is a critical marker of basal keratinocytes involved in re-epithelialization, and its upregulation suggests improved epithelial repair[12]. Previous studies have also reported that RA has potent anti-inflammatory and antioxidant effects, which contribute to faster epidermal renewal and protection against oxidative damage[10]. The increased CK14 expression in our study suggests that RA promotes keratinocyte proliferation and differentiation, which is essential for wound closure.

Comparing our findings with the existing literature [19-21], our study confirms that RA positively influences both the dermal and epidermal phases of wound healing, reducing inflammation and enhancing tissue regeneration. While some studies have focused on RA's antioxidant and anti-inflammatory properties, fewer have directly evaluated its effects on epithelial markers such as CK14. Our study contributes to this gap by demonstrating that RA facilitates epidermal repair through increased CK14 expression, making it a promising candidate for wound healing therapies.

CONCLUSION

In conclusion, our study demonstrates that topical application of Rosmarinic Acid enhances wound healing by promoting collagen organization, reducing inflammation, and facilitating epithelial regeneration. The findings suggest that RA accelerates both dermal and epidermal repair, as evidenced by improved collagen structure in Masson Trichrome staining and increased CK14 expression in immunostaining. Given its strong antioxidant and anti-inflammatory properties, RA may be a potential therapeutic agent for wound healing applications. Future studies should focus on elucidating the molecular mechanisms underlying RA's effects on keratinocyte proliferation and its potential use in clinical wound care treatments.

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Ethical approval: This study was approved by Dicle University Animal Experimentation Local Ethics Committee (data: 27/01/2022 and issue: 2022/04).

Conflict of Interest: All authors declare there is no conflict of interest.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Informed Consent Statement: Not applicable.

Author Contributions: All authors have equal contribution in the preparation of manuscript and compilation.

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