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Histological investigation of α-lipoic acid in the wound healing process of diabetic lesions *in vivo*

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ABSTRACT. α-Lipoic acid (ALA) is a significant therapeutic agent for inflammation. Understanding the histological parameters involved in the healing process influenced by inflammatory activity is crucial for developing new technologies to treat wounds caused by diabetes mellitus. This study involved diabetic Wistar rats, divided into groups based on treatment: a control group (distilled water and cellulose), a 100 mg.kg^{-1} dose of α-lipoic acid, and a 200 mg kg^{-1} dose. Diabetes was experimentally induced, and lesions were created on the rats' backs. The α-Lipoic acid groups received oral doses of 100-200 mg kg^{-1}, while the control group received distilled water with cellulose. Wounds were later excised and subjected to histological analysis. The 100 mg kg^{-1} dose exhibited 'mild' to 'absent' chronic inflammatory responses, while the 200 mg kg^{-1} dose showed similar results for acute treatments, based on all analyzed parameters. α-Lipoic acid reduced all parameters examined, with the lower dose demonstrating beneficial effects on healing during continuous treatment. In contrast, the 200 mg kg^{-1} dose yielded results indicating effectiveness in acute treatments. Further studies are required to explore the absorption process of ALA *in vivo* models.

Keywords: inflammation; lesions; natural product; tissue regeneration.

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Introduction

Diabetes mellitus (DM) is a chronic degenerative disease that significantly impacts public health. According to the World Health Organization [WHO] (2024), approximately 422 million people worldwide have diabetes, with 62 million of them living in the Americas (Pan American Health Organization [PAHO], 2022). Chronic skin lesions can arise from excessive inflammatory activity caused by macrophages in the presence of hyperglycemia (Lv et al., 2023). While inflammation is a crucial part of the healing process, its persistence can hinder tissue repair (Gois et al., 2021). In this context, research must focus on identifying substances that can reduce inflammation and promote tissue regeneration.

α-Lipoic acid (ALA; C8H14O2S2), also known as 1,2-dithiolane-3-pentanoic acid or lipoic acid, is a promising therapeutic agent due to its ability to modulate various pathways and mechanisms, including metabolic processes (Abdullah et al., 2024) and endothelial effects (Skibska et al., 2022). At the intracellular level within the inflammation pathway, ALA can: (i) inhibit or reduce the activity of inflammatory modulators such as TLR4, NLRP3, IL-1β, and IL-18 (Li et al., 2023); (ii) eliminate reactive oxygen species (ROS), thereby mitigating cellular damage caused by oxidative stress (Reis et al., 2023); (iii) enhance the glycolysis pathway, reducing factors that induce cell apoptosis (Li et al., 2021). Additionally, ALA may increase the biosynthesis and levels of antioxidant enzymes, such as glutathione peroxidase (GSH), in fatty tissues. ALA can also reverse protein oxidation, carbonylation, and glycation in insulin-resistant mice, suggesting a promising role in the wound healing process (Brzezik-Dajnowicz et al., 2022).

There is a need for histological research on the effects of ALA to confirm its role in promoting cell proliferation within the skin layers of wounds (Choi et al., 2021). Such research would provide scientific data on the structures involved in the skin healing process and validate the efficacy of natural products with therapeutic potential to accelerate tissue repair and enhance the re-epithelialization rate in the treatment of chronic wounds (Rezvanian et al., 2021).

The present study aimed to analyze histological parameters (e.g., congestion, edema, inflammatory infiltrate, and margination) following the oral administration of ALA in cutaneous lesions of alloxan-induced diabetic rats. The study focused on investigating the wound healing process, inflammation reduction, and efficient tissue repair.

Material and methods

Animals and experimental groups

Experiments were conducted at the *Laboratório de Tecnologias e Inovações Farmacológicas* (LATIF) of *Universidade Regional do Cariri* (URCA) and were approved by the Commission for Experimentation and Use of Animals (CEUA/URCA) under process number 00167/2018.1.

Albino male Wistar rats, weighing between 200 and 300 g, were obtained from the URCA vivarium. Animals were housed under controlled conditions, with a temperature of 23°C ± 1°C, a 12-hour light/dark cycle, and free access to water and food. They were subjected to forced oral administration and evaluated at three different time points: 1, 7, and 14 days (Table 1).

Table 1. Distribution of experimental groups of Wistar rats submitted to different treatment conditions without and with ALA.

Group	Treatment	Code
1	Distilled water and cellulose	Control
2	ALA at 100 mg kg^{-1}	A-100
3	ALA at 200 mg kg^{-1}	A-200

Anesthesia and diabetes induction

Animals were weighed and then returned to their original cages. Based on their weights, anesthesia was administered intraperitoneally by using a mixture of xylazine (Ceva, BR) at 10 mg.kg^{-1} and ketamine (Dechra, BR) at 100 mg kg^{-1}. After an average latency period of three minutes, the animals displayed complete prostration, confirmed by spontaneous diuresis and the total abolition of eyelid reflexes, as commonly observed under these conditions (Sampaio et al., 2018).

To induce diabetes, animals were fasted for 24 hours. They were then anesthetized with xylazine (Ceva, BR) at 10 mg kg^{-1} and ketamine (Dechra, BR) at 100 mg kg^{-1}. Alloxan (Sigma-Aldrich, USA) was then administered at 50 mg kg^{-1} via the dorsal penile vein. Six hours after the alloxan injection, a 10% glucose solution was provided for 24 hours. Blood glucose levels were measured 72 hours after alloxan administration and again on the day of euthanasia to confirm diabetes. Animals with blood glucose levels below 250 mg dL^{-1} were excluded from the study (Sampaio et al., 2021). Blood samples were obtained by collecting blood from the tip of the anesthetized animal's tail and placing it onto Accu-Chek Active® reagent strips (Osang Healthcore, KOR). Readings were taken using an Accu-Chek Active® device (Osang Healthcore, KOR).

Cutaneous wound production and treatment protocol

After confirming diabetes and administering anesthesia, the animals were placed in the prone position. The dorsal area was manually trichotomized, followed by antisepsis of the operative field with povidone-iodine. A surgical excision of a skin fragment, measuring approximately 7 mm, was performed by using a punch. Wound margins were standardized to ensure the complete removal of all skin layers except the underlying musculature (Sampaio et al., 2021). Wounds were then measured, analyzed, and photographed. Following the procedure, the animals were returned individually to their respective cages. After surgery, the animals were divided into three groups: a control group receiving oral distilled water and cellulose, and two treatment groups receiving α-lipoic acid (ALA) at doses of 100 mg kg^{-1} or 200 mg kg^{-1}. Each group was further subdivided into three subgroups, with five animals each, based on treatment durations of 1, 7, or 14 days, totaling 45 animals.

Histological evaluation and data analysis

Tissue samples from the lesions were excised and preserved in 10% formaldehyde. The solution was prepared with the following components: 0.01M potassium phosphate (7786-77-0, Scientific Exodus, BR), 0.138M NaCl (7647-14-5, Scientific Exodus, BR), and 0.0027M KCl (7447-40-7, Scientific Exodus, BR), adjusted to a pH of 7.4. Histopathological studies were conducted using archived paraffin blocks, which were sectioned with a Minot American Optical rotary microtome. Sections were cut to a thickness of 5 µm. The

samples were then stained using hematoxylin/eosin and Giemsa, following the AFIP protocol (Prophet et al., 1993). Histological analysis focused on observing changes in the epidermis and dermis. Additionally, qualitative observations of relative and absolute parameters were conducted, evaluating congestion, edema, margination, and inflammatory exudate.

Results

Figure 1 presents the results obtained from the analysis of the histological slides. The treatments are represented as follows: Figure 1A: Control; Figure 1B: A-100; Figure 1C: A-200.

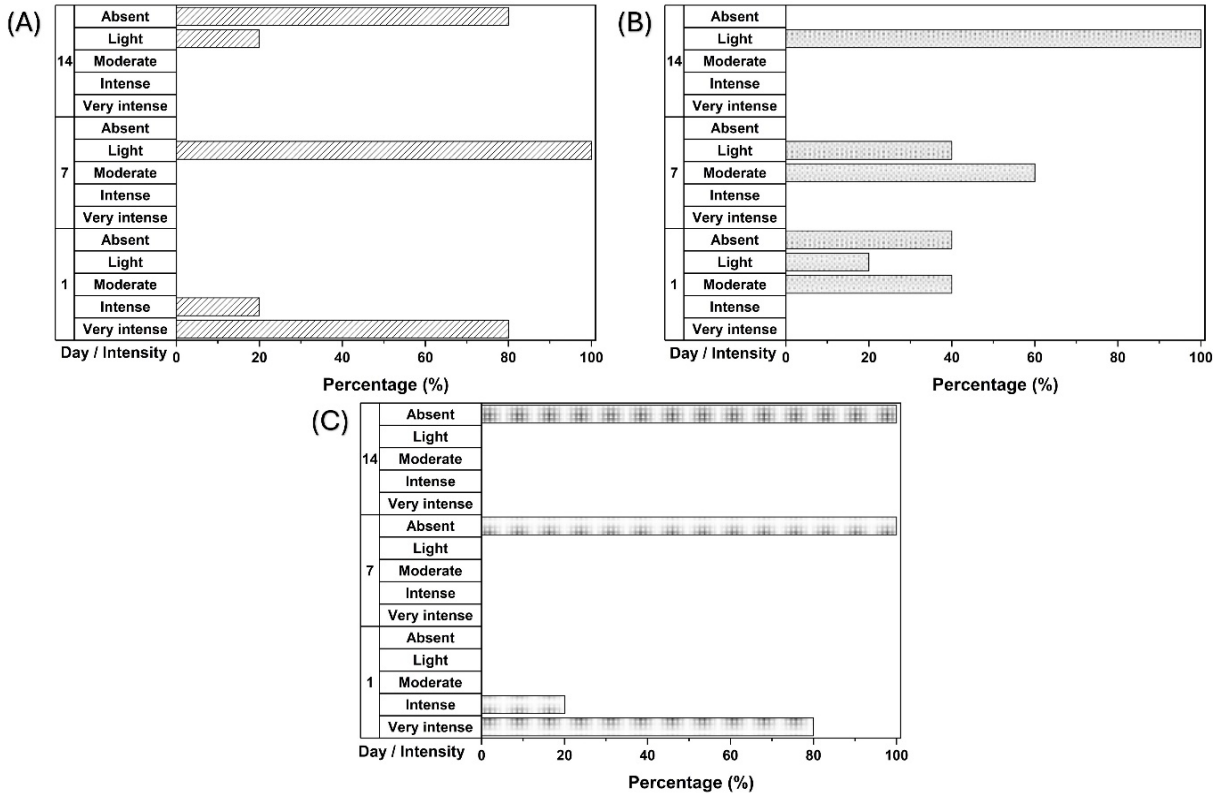

Figure 1. Histological analysis of the effect of α-lipoic acid (ALA) on congestion in diabetic rat lesions, by day of treatment. Figure 1A: Control, Figure 1B: A-100, Figure 1C: A-200. (*) Indicates no qualitative profile for accounting.

Figure 1 illustrates the results for congestion in diabetic rat lesions across treatment groups. The control and A-100 groups exhibited 'very intense' and 'moderate' congestion on the first day, accounting for 80% and 20%, respectively. By the 7th day, both groups reached the 'absent' level at 100%, indicating a chronic action. In contrast, the A-200 group demonstrated a progression from 'moderate' to 'absent' congestion on the first day, suggesting an acute activity. This behavior persisted through the 14th day, with 100% of the slides classified as 'mild'.

Figure 2 shows the effect of ALA at doses of 100 mg.kg^{-1} and 200 mg kg^{-1} on edema in diabetic lesions. Figure 2A: Control; Figure 2B: A-100; Figure 2C: A-200.

Figure 2 shows that the control group and A-100 exhibited a progression from 'very intense' to 'intense' edema on the 1st day. In contrast, A-200 demonstrated a transition from 'moderate' to 'absent' edema on the 1st day, indicating an acute action. From the 7th to the 14th day, A-200 maintained a 'moderate' behavior during chronic treatment. Meanwhile, the control and A-100 groups showed 'mild' to 'intense' edema behavior on the 7th and 14th days of the lesion treatment.

Figure 3 shows the results of ALA administration on the margination of diabetic rat lesions.

Figure 3 shows that the A-200 group exhibited 'absent' to 'mild' behavior in 40% of the slides during acute treatment. For the treatment period from the 7th to the 14th day, A-200 showed 'moderate' behavior in 100% of the slides analyzed regarding wound margins. In contrast, the control group displayed a progression from 'moderate' to 'absent' behavior, while the A-100 group showed a shift from 'intense' to 'mild' behavior.

Figure 4 shows the analysis of the wound exudate process treated with ALA. The following results were obtained from the evaluation of this marker.

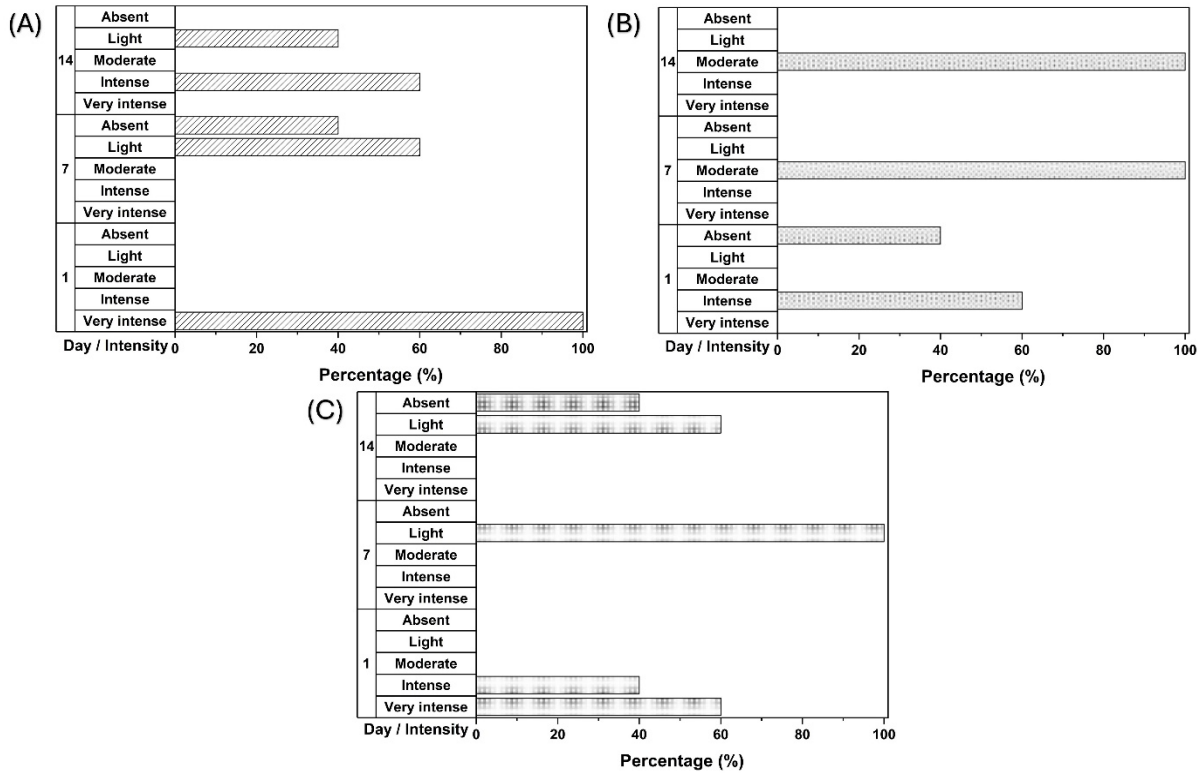

Figure 2. Histological analysis of the effect of α-lipoic acid (ALA) on edema in diabetic rat lesions, by day of treatment. Figure 2A: Control; Figure 2B: A-100; Figure 2C: A-200. (*) Indicates no qualitative profile for accounting.

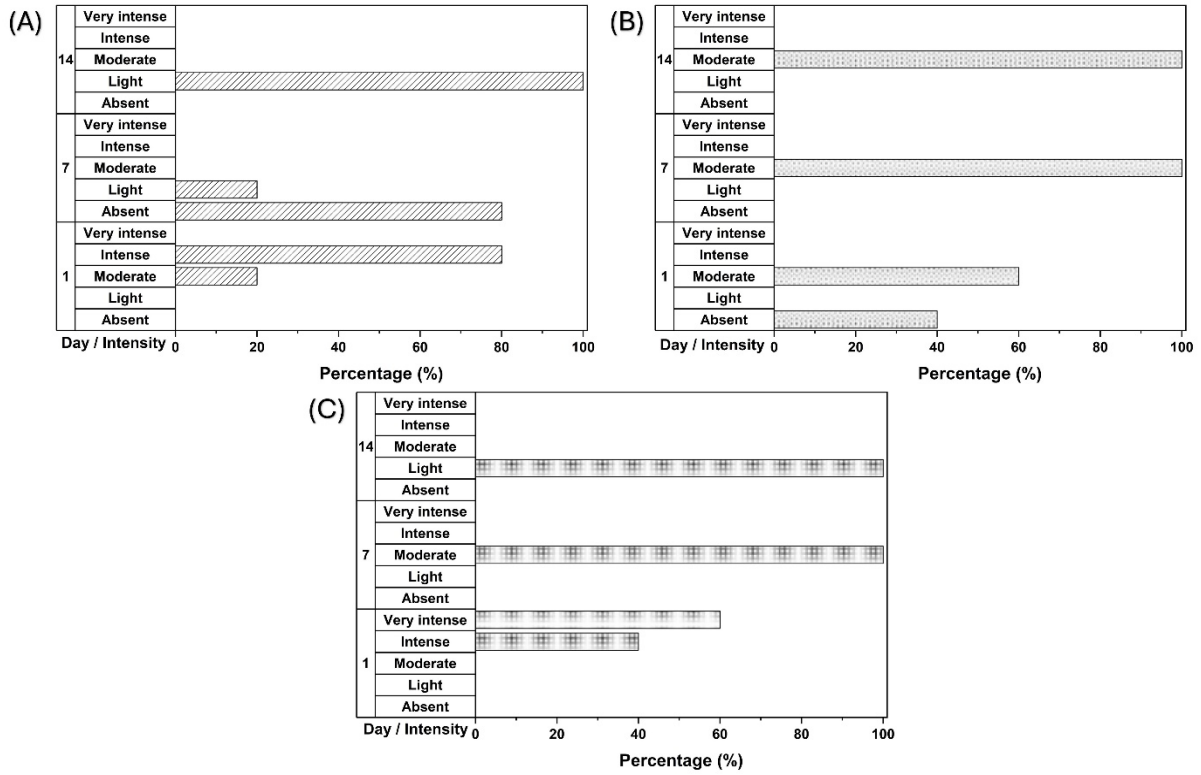

Figure 3. Histological analysis of the effect of α-lipoic acid (ALA) on margination in diabetic rat lesions, by day of treatment. Figure 3A: Control; Figure 3B: A-100; Figure 3C: A-200. (*) Indicates no qualitative profile for accounting.

Figure 4 shows that the A-200 group exhibited ‘absent’ to ‘moderate’ behavior, indicating acute action. In contrast, the control group demonstrated ‘moderate’ to ‘intense’ behavior, while the A-100 group showed ‘intense’ to ‘very intense’ behavior. During chronic treatment from the 7th to the 14th day, the A-200 group showed 100% ‘moderate’ behavior, whereas the A-100 and control groups presented ‘mild’ exudate results.

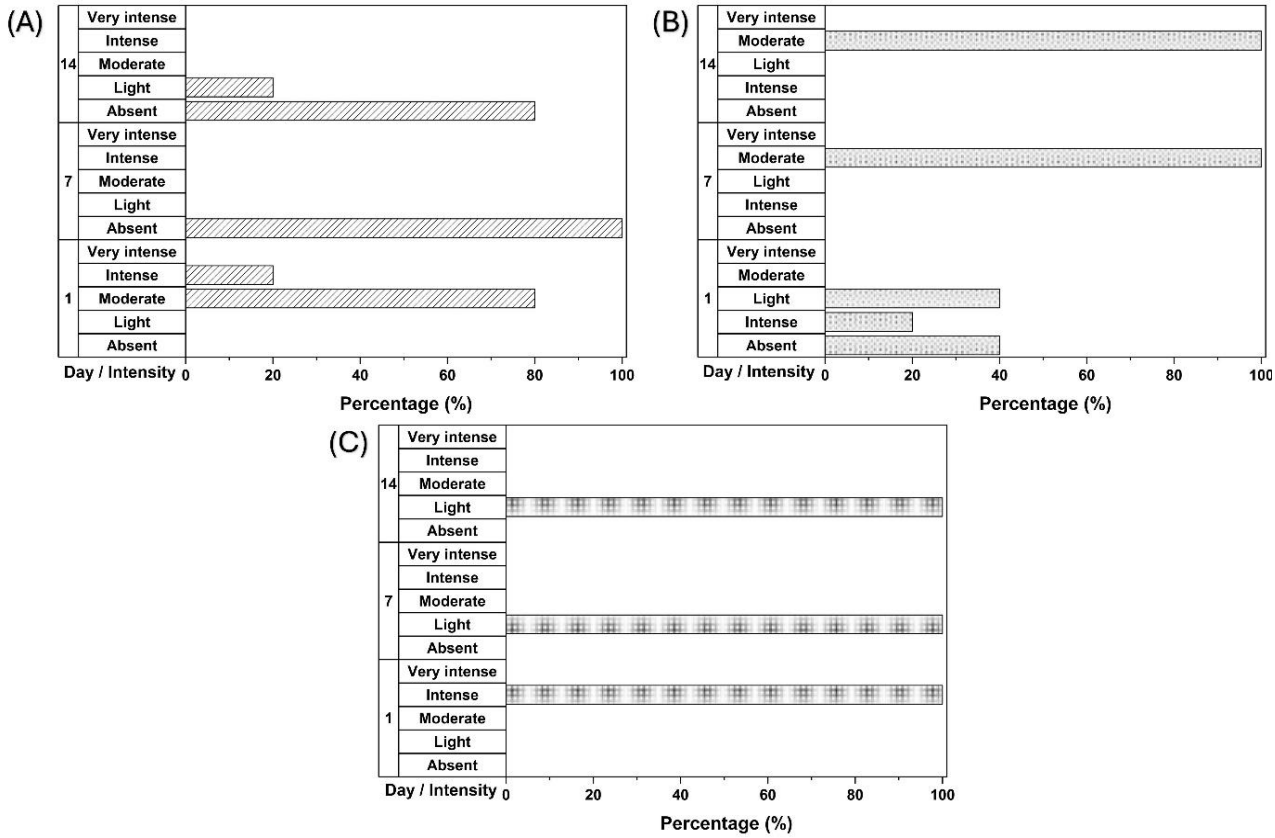

Figure 4. Histological analysis of the effect of α-lipoic acid (ALA) on inflammatory exudate in diabetic rat lesions, by day of treatment. Figure 4A: Control; Figure 4B: A-100; Figure 4C: A-200. (*) Indicates no qualitative profile for accounting.

Figure 5 displays histological slides illustrating qualitative characteristics associated with the tissue repair process, focusing on the four parameters studied: congestion, edema, margination, and inflammatory exudate. These parameters play crucial roles in the healing process, reflecting reductions in pro-inflammatory properties. The analysis revealed that treatments with A-100 and A-200 doses accelerated the resolution of inflammation and promoted tissue regeneration compared to the control. Among the treatments, the A-200 dose demonstrated superior histological results, showing a greater reduction in the inflammatory process and better tissue organization. However, after 14 days, the tissue exhibited a distinct organization.

Discussion

The analysis of histological slides during acute treatments highlights A-200 as superior to the control and A-100 groups due to its significant reduction in parameters such as congestion (Figure 1), edema (Figure 2), margination (Figure 3), and inflammatory exudate (Figure 4). During the initial inflammatory phase, there is a decrease in defense cell activity, leading to reduced phagocytic function and increased levels of cytokines and inflammatory mediators. These changes can impair collagen synthesis and delay epithelial regeneration (Gois et al., 2021). The use of A-200 appears to promote healing by mitigating the effects of excessive inflammation in diabetic wounds, making it a potentially effective acute therapeutic intervention. In contrast, A-100 demonstrated positive effects characteristic of a chronic intervention.

Costa et al. (2020) evaluated the potential of α-lipoic acid (ALA) in modulating the inflammatory response in intestinal lesions of Swiss mice induced by irinotecan, using doses of 100 mg kg^{-1} and 200 mg kg^{-1} of ALA. The authors observed that during the 4-day treatment period, histological findings in the duodenum showed intense inflammatory cell infiltration, vacuolization, and edema in the 100 mg kg^{-1} ALA group and the control group (saline solution). Conversely, the 200 mg kg^{-1} ALA dose resulted in reduced inflammatory cell infiltration, although vascularization and edema remained unchanged. Similarly, Skibska et al. (2022) found that ALA reduces the release of inflammatory cytokines in the epithelial cells of rat kidneys. These findings suggest that ALA can reduce the production of pro-inflammatory cytokines in injured tissue, contributing to the control of inflammation intensity during the initial phase.

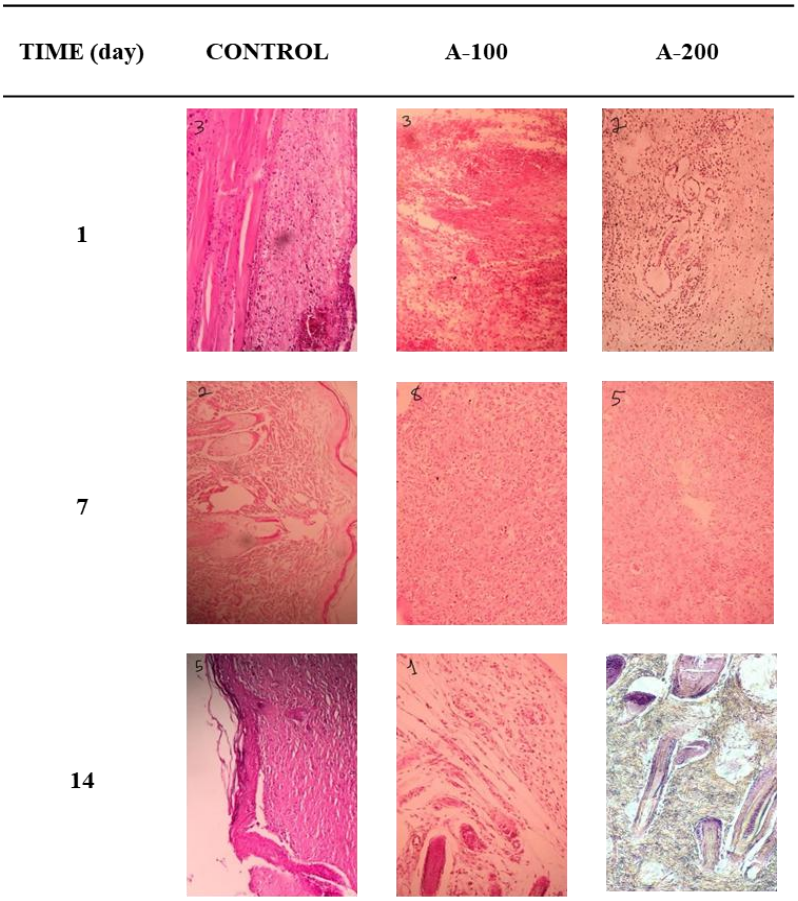

Figure 5. Illustrative images of tissue samples from lesions induced in rats, showing a qualitative analysis of the wound healing process. The figure relates exposure time (first column) and treatment applied: control, A-100, and A-200 (first row).

Over the 14-day period, the A-100 and control groups exhibited 'absent' to 'mild' behavior, while the A-200 group maintained 'moderate' to 'absent' effects across the four studied parameters (Figures 1-4). These findings suggest variations in chronic therapeutic responses. This phenomenon could be related to the toxicological potential of the higher dose used in continuous treatment, as lipoic acid treatment has been shown to reduce the weight of treated animals (Lucarini et al., 2020).

Control of reactive oxygen species (ROS) is crucial during the wound healing phases. Reduced ROS levels can mitigate external damage that might compromise diabetic lesions by influencing the inflammatory process and facilitating tissue repair (Deng et al., 2021). Sampaio et al. (2021) emphasized the effectiveness of ALA at 200 mg kg^{-1} in acute treatments, highlighting its antioxidant effect on diabetic wounds. They observed a decrease in malondialdehyde (MDA) and nitrate levels and an increase in glutathione (GSH) levels, which inhibited oxidative stress and demonstrated the antioxidant effect of ALA in delaying healing. Maciejczyk et al. (2022) further noted that reducing pro-inflammatory cytokines is directly linked to a decrease in oxidative stress. Their results showed that reduced brain manifestations caused by diabetes were associated with the anti-inflammatory effects of ALA, particularly through the synthesis of IL-10 in the hypothalamus. Consequently, α-lipoic acid may prevent the activation of nuclear factor kappa-B (NF-κB), the key regulator of the inflammatory response.

The activation of pro-inflammatory cells (e.g., NF-κB) is a key process in triggering inflammation pathways, which can result in multicellular injury. In individuals with diabetes, the accumulation of advanced glycation end products contributes to the increased expression of NF-κB. This elevated expression impairs tissue repair and hinders the activation of anti-inflammatory cellular mechanisms (Li et al., 2022).

Conclusion

This study demonstrated the efficacy of ALA as an anti-inflammatory agent in diabetic lesions through histological analysis. At the lower dose of 100 mg kg^{-1}, ALA showed positive outcomes in the chronic treatment of congestion, edema, margination, and inflammatory exudate. Conversely, the higher dose of 200

mg.kg^{-1} was particularly effective in the acute treatment phase. These findings highlight the potential of ALA in the development of new therapeutic approaches for the treatment of diabetic wounds. However, limited research has investigated the anti-inflammatory effects of ALA at doses of 100 to 200 mg kg^{-1} or its topical application. Further studies considering different treatment durations and parameters are needed for a comprehensive evaluation (Lucarini et al., 2020).

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