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Antioxidant and Anti-Inflammatory Potential of *Brassica oleracea* Accelerates Third-Degree Burn Healing in Rats

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Abstract: Burns account for more than 265,000 deaths per year in the world, mainly associated with infections. Therefore, the shorter the healing time, the better the prognosis. Based on this, the objective of the present study was to investigate the effect of an ointment based on *Brassica oleracea* var. *capitata* extract in the third-degree healing process. Twenty-five male Wistar rats (335 ± 16 g, three months of life) were individualized in cages with food and water *ad libitum*. After anesthesia, two circular third-degree burn wounds (12 mm in diameter) were made on the animals, which were randomly separated into five treatments (n = 5/group), i.e., SAL: saline solution 0.9%; OV: ointment vehicle; SS: silver sulfadiazine 1%; PB1: 10% *B. oleracea* extract; and PB2: 20% *B. oleracea* extract. The animals were treated with the ointment daily for eight days. Every four days, the area and the wound contraction index were evaluated. Tissue samples were taken for histopathological analysis (cellularity, blood vessels, and extracellular matrix components) and analysis of oxidative/nitrosative status (antioxidant enzymes, lipid, and protein oxidation markers, as well as nitric oxide (NO) and hydrogen peroxide (H_2O_2)). The ointment based on *B. oleracea* var. *capitata* at 10 and 20% concentrations increased the number of cells, blood vessels, and fibrous components of the extracellular matrix and the activity of antioxidant enzymes, promoting a fast and efficient cutaneous repair in third-degree burn wounds.

Keywords: inflammation; oxidative stress; natural compounds; burn wounds

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1. Introduction

The skin, the largest organ in the body, consists of the epidermis and dermis and is responsible for maintaining the body's homeostasis, providing chemical, physical, and bacterial barriers [1]. Wounds caused by burns, mechanical trauma, cuts, tumors, poor blood circulation, and surgeries can compromise the structure and function of the skin [2], exposing individuals to the risk of further ulceration and infection [3].

Burn injuries affect more than 11 million people annually worldwide [4] and can be caused by heat, chemicals, electricity, and radiation, among other things. They are classified as first-degree burn (superficial, reaches the epidermis), second-degree burn (partial thickness, reaches the epidermis and a part of the dermis), and third-degree burn (total thickness, reaches the epidermis, dermis, hypodermis, and muscles) [5,6]. The depth of the burn is related to the time of exposure to the aggressive agent, temperature, and pressure under the tissue, and these factors usually determine the prognosis after injury [7].

The histopathological analyses of the tissue can be used to confirm the degree of the lesion. However, the cellular and parenchymal elements of the skin are compromised after an injury, making it difficult to assess the depth of the burn [8].

The cutaneous repair process is essential for the survival of all higher organisms [9] and occurs in three phases: inflammatory, proliferative, and remodeling [10]. During the first phase, the release of mediators derived from platelets, bacterial byproducts, and chemoattractants promotes tissue hemostasis [11]. In addition, leukocytes are recruited and act by eliminating leftover tissue and infections, allowing the next step to occur harmoniously and effectively [12]. In the next phase, cells and blood vessels proliferate, forming the granulation tissue, rich in blood vessels and type III collagen [13,14]. The granulation tissue, although fragile, is essential for tissue maturation and will provide the scaffold for type I collagen synthesis in the wound [15]. In the remodeling phase, type III collagen is replaced by type I, thicker and organized into bundles of fibers, providing the scar strength and resistance [16]. The end of this process involves the interaction between cells, matrix, and cytokines and results in a rapid and effective wound closure [17].

During the inflammatory phase, the recruitment of immune cells occurs in burn lesions, mainly macrophages and neutrophils, and reactive oxygen species (ROS) are formed by a process known as respiratory explosion [18]. To neutralize oxidative stress, there may be an increase in the antioxidant defense consumption, reducing the tissue's antioxidant capacity. As a result, there is an exaggerated increase in ROS, exceeding the antioxidant system's capacity to neutralize and eliminate them [19]. To protect the organism from oxidative damage, antioxidant enzymes are produced, such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) [20–22]. These enzymes act by neutralizing or preventing the formation of free radicals that can damage vital biomolecules and, consequently, the body's tissues [23]. The decrease in antioxidant enzymes and the degradation of cellular constituents compromise the cicatricial process, leading to ineffective tissue repair [24].

Severe burns must be treated quickly to prevent infection, the leading cause of death in burn patients [25]. Therefore, adequate drugs must be available for this purpose [26] as most of the current drugs have shown antimicrobial rather than cicatrizing effects [27]. The use of medicinal plants has been increasing, since several phytochemical compounds can act synergistically to effectively inhibit microbial proliferation and stimulate wound closure [28,29]. It is also a simple and inexpensive treatment with fewer side effects [30].

Cabbage (*Brassica oleraceae* var. *capitata*) belongs to the family Brassicaceae (Cruciferae) [31] and is a plant of great economic importance due to its use in food worldwide [32]. Cabbage leaves are used in traditional medicine for wound healing and joint pain relief [33,34]. Phytochemical components of *Brassica*, such as glucosinolates, sulforaphans, tannins (phenolic acids), and flavonoids, are already known for their healing potential [31,35–38] and antimicrobial properties [39,40]. Based on the importance of understanding the therapeutic effects of this plant, this study aims to evaluate the effect of *Brassica oleracea* extract at 10 and 20% concentrations on the healing process of third-degree burns in Wistar rats.

2. Materials and Methods

2.1. Extract Preparation, Phytochemical Prospection, and Ointment Preparation

The leaves of *Brassica oleracea* var. *capitata* were collected from a preserved Atlantic Forest area in the Minas Gerais State, Brazil (20°43′00″ S and 42°29′10″ W). Extraction was carried out by maceration with ethyl alcohol (95%). Phytochemical triage for the presence of secondary metabolites was carried out according to procedures described by Harborne [41]. The ointment formulation was prepared as follows: (1) *B. oleracea* var. *capitata* ethanolic extract (10% or 20% by weight) was prepared; this concentration was chosen after preparing a systematic review and checking that the best concentration was between 10 and 20% [42]. (2) Ointment vehicle was prepared with cetostearyl alcohol, polyol–fatty acid esters, carbamide (5% by weight), ethoxylated sorbitan monooleate, petroleum jelly, methylparaben, propylparaben and butylated hydroxytoluene (10% by weight), anhydrous lanolin and demineralized water (15% by weight), hydrogenated castor

oil (5% by weight). (3) Finally, the extract was incorporated into the ointment until the complete homogenization of the ointment was achieved [43].

2.2. Animals and Production of the Skin Burn

Twenty-five male Wistar rats (*Rattus norvegicus*; 3 months old; 335.4 ± 16 g) were housed in individual cages, and the ethical procedures were approved by CEUA/UFV (registration number: 90/2017). The rats were anesthetized with an intraperitoneal injection of Pentobarbital (70 mg/kg) to produce the burns. The dorsolateral region of each animal was shaved, and two circular 12 mm diameter third-degree burn wounds were made simultaneously. The material was collected for the first and second burn after seven and fourteen days, respectively. Using an iron cylinder, which was heated in boiling water (100 °C) and pressed onto the animals' backs for 30 s [22] (adapted from Partoazar et al., 2016) [44]. The wound area was measured with an analog caliper (Mitutoyo Sul Americana Ltda®, São Paulo, Brazil) [45].

2.3. Wound Area and Wound Contraction Index

The wound area and the wound contraction index were evaluated every 4 days using images scanned with 320 × 240 pixels (24 bits/pixel). The wound area was calculated by the formula, $A = \Pi \times (r)^2$, where r = radius. The ratio calculated the wound contraction index (WCI): initial wound area (Ao)—area on a given day (AI)/initial wound area (Ao) × 100 [46]. The healing process was analyzed in the sample collected on the last day of the experiment.

2.4. Experimental Design

The animals were randomly separated into five groups (n = 5/group): wounds treated with saline solution 0.9% (SAL); wounds treated with the ointment vehicle (OV); wounds treated with silver sulfadiazine 1% (SS); wounds treated with 10% *B. oleracea* extract (PB1); and wounds treated with 20% *B. oleracea* extract (PB2). The daily amount of ointment applied to each wound was 0.1 g.

The wounds were cleaned daily with 0.9% saline solution before ointment application. All treatments were initiated 24 hours after the wounds were created and repeated daily for eight days. The samples of regenerating tissue were collected on days zero (wound 0 = F0), four (wound 1 = F1), and eight (wound 2 = F2), repeating the previously reported anesthetic procedure for the withdrawal of the samples (Pentobarbital at 70 mg/kg). The fragments contained tissue from the center of the lesion and a part of the uninjured tissue adjacent to the edge of the lesion. The animals were euthanized at the end of the treatment by cardiac puncture exsanguination after anesthesia.

2.5. Histological Analysis

The samples collected from the wounds were fixed in 10% formaldehyde solution buffered in 0.1 M sodium phosphate (pH 7.2), dehydrated in ethyl alcohol, diaphanized in xylol, and immersed in paraffin. Histological sections (4 µm thick) were obtained by Leica Multicut® 2045 rotatory microtome (Leica Biosystems, Deer Park, IL, USA) and stained with Hematoxylin and Eosin for the analysis of fibroblasts and blood vessels [47], modified Sirius Red for the study of collagen fibers (type I and type III collagen fibers) and observed under polarized light microscopy (Sigma, Saint Louis, MO, USA) [48], Verhoeff for the elastic fiber differentiation [49], and toluidine blue for the evidence of mast cells [50]. One in ten cuts was used to avoid the repeated analysis of the tissue. Histological images were visualized and captured in a BX–60® light microscope (Olympus, São Paulo, Brazil) coupled to a QColor-3® digital camera (Olympus, São Paulo, Brazil). Five images were obtained by random cutting, with a 2592 × 1944 pixel resolution and 200× magnification. Fibroblasts and blood vessels were counted in scanned images with a grid of 300 intersections, and elastic and collagen fibers with a grid of 256 intersections, associated with a software system for image analysis Image Pro-Plus 4.5 (Media Cybernetcs®, Silver Spring, MD, USA). Mast cells were analyzed at 40× magnification in 10 random histological sections to obtain a

total area (TA) of 3.11 × 106 μm^2. The number of mast cells per unit of histological area was calculated according to the formula, QA = Σ mast cells/TA [51].

2.6. Antioxidant Enzymes

Fragments of the injured area were collected from each wound, immediately frozen in liquid nitrogen (−196 °C), and stored in a freezer at −80 °C. The samples were homogenized in phosphate-buffered saline (PBS) and centrifuged at 5 °C [52]. The activity of superoxide dismutase (SOD) was determined by the method using the reduction of the superoxide (O-2) and hydrogen peroxide, thereby decreasing the auto-oxidation of pyrogallol [53]. SOD activity was calculated as a unit per milligram of protein, with one unit (U) of SOD defined as the amount that inhibited the rate of pyrogallol autoxidation by 50%. Catalase activity (CAT) was determined with the Aebi method [54] using H_2O_2 as the substrate. The CAT activity was calculated as a unit per milligram of protein (U), where one unit of CAT activity is defined as the amount of enzyme that decomposes one mmol of H_2O_2 for 1 min. The glutathione S-transferase activity (GST) activity was measured using the method of Habig et al. [55] GST activity was analyzed according to the formation of glutathione-conjugated 2,4-dinitrochlorobenzene (CDNB). One unit of GST was defined as the amount of enzyme that catalyzed the formation of one μmol of product/min/mL. GST activity was expressed as U per mg of protein.

2.7. Markers of Oxidative Stress

2.7.1. Malondialdehyde Assay

Lipid peroxidation (LPO) content was estimated with the total malondialdehyde (MDA) levels [56]. The concentration of MDA was determined by using the standard curve of known concentrations of 1, 1, 3, and 3-tetramethoxypropane (TMPO). The results were expressed as μmol/L per mg of protein [57].

2.7.2. Protein Oxidation

Protein carbonyl content was measured using 2,4-dinitrophenylhydrazine (DNPH) [58] based on the carbonyl groups reaction with DNPH. The pellets resulting from the tissue homogenates from the previous extractions were used for quantification. The results were expressed as nmol per mL of protein.

2.7.3. Nitric Oxide Production

Nitric oxide (NO) was indirectly quantified through the detection of nitrite/nitrate (NO_2^-/NO_3^-) levels using the standard Griess reaction [59]. A 50 μL volume of supernatants were incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-Naftil) etilenodiamina, and 2.5% H_3PO_4) and kept at room temperature for 10 min. The conversion of absorbance into the micromolar concentrations of NO was obtained from a sodium nitrite standard curve (0–125 μM) and expressed as NO concentrations (μmol/L) [60].

2.7.4. Hydrogen Peroxide Production

The hydrogen peroxide (H_2O_2) production was measured in the supernatants of homogenates of tissues. A 50 μL volume of supernatants was incubated with 50 μL of αPhenylenediamine dihydrochloride (OPD) and an equal volume of peroxidase type II (15 mmol/L). The conversion of absorbance into the micromolar concentrations of H_2O_2 was obtained from a standard curve using a known concentration of H_2O_2. The results were expressed as μmol/L.

2.8. Statistical Analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) software (version 9.5.1). The data were reported as mean and standard deviation of the mean (mean ± SD). The results were subjected to an analysis of variance (ANOVA) test from GraphPad Software Inc., San Diego, CA, USA. followed by the Student–Newman–Keuls test q. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Phytochemical Prospection and Wound Area

The phytochemical prospection of *B. oleracea* extract revealed the presence of alkaloids, flavonoids, saponins, steroids/triterpenes, and carotenoids. The wound area was smaller on day 4 (F1) and day 8 (F2) day in the groups treated with 10 and 20% *B. oleracea* compared to the other groups. The wound contraction index was higher in the groups treated with 20% *B. oleracea* compared to the other groups on day 8 (F2) (Table 1).

Table 1. Area (mm^2) and rate of wound contraction (RWC) (mm^2/day) in animals treated with *Brassica oleracea* var. *capitata* extract at different concentrations, after 4 and 8 days.

		SAL	OV	SS	PB1	PB2
F0	Area	156.6 ± 20.1	161.6 ± 29.4	141.3 ± 15.7	126.9 ± 13.3	134.9 ± 13.5
	RWC	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
F1	Area	153.1 ± 33.4	178.4 ± 18.8	138.4 ± 32.3	87.4 ± 19.7 *	97.7 ± 22.4 *
	RWC	10.3 ± 6.9	3.3 ± 20.6	2.4 ± 18.4	31.4 ± 10.8	27.8 ± 13.3
F2	Area	142.2 ± 30.0	155.4 ± 34.7	133.9 ± 50.4	67.6 ± 20.9 *	66.4 ± 21.4 *
	RWC	15.9 ± 14.3	10.4 ± 19.8	28.1 ± 18.0	47.2 ± 12.2	51.4 ± 12.5 *

SAL: saline solution 0.9%; OV: ointment vehicle; SS: silver sulfadiazine 1%; PB1: *B. oleracea* extract (10%); and PB2: *B. oleracea* extract (20%). F0 = intact tissue and F1, F2 = scar tissue after 4 and 8 days, respectively. Data represented as mean ± standard deviation of the mean. * Statistical difference between treatments, SAL, OV, and SS (Student–Newman–Keuls test).

3.2. Histopathological Results

The number of total cells and mast cells was increased in wounds treated with PB1 on day 4 (F1) compared to the SAL, OV, and SS groups. On day 8 (F2), the total cellularity and mast cells were higher in groups PB1 and PB2 than those in SAL (Figure 1A,D–F). The number of blood vessels was higher in PB1 compared to SAL, OV, and SS on day 4. On day 8, the number of blood vessels was higher in the PB2 group compared to SAL and SS (Figure 1B,C).

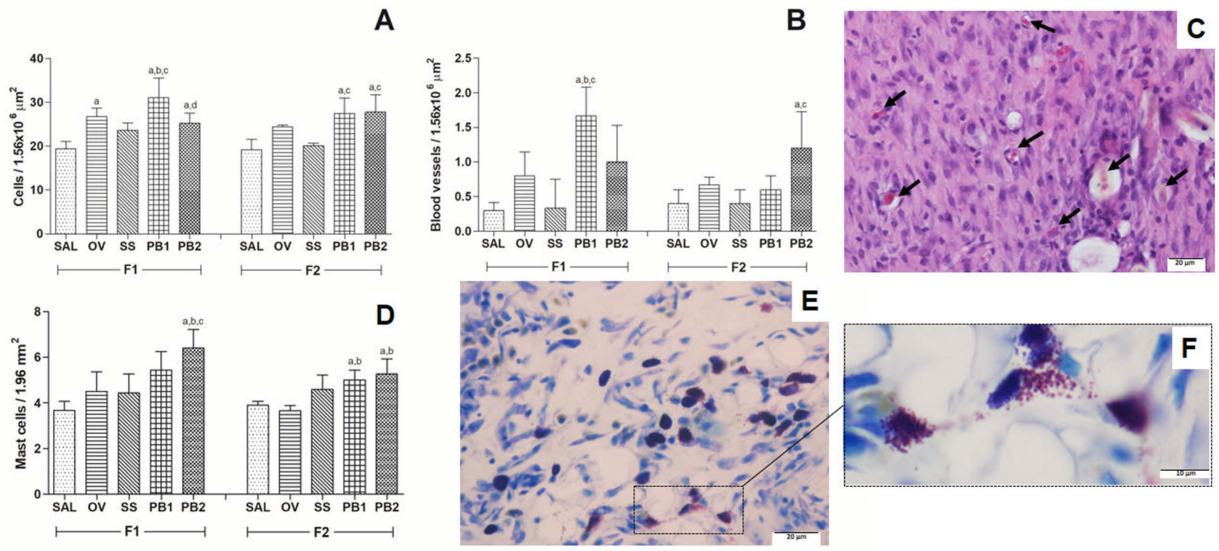

Figure 1. Proportion of cells (**A**), blood vessels (**B**,**C**), and mast cells (**D**–**F**) in the scar tissue of rats treated with *B. oleracea* extract. (**E**,**F**): photomicrograph of mast cells after treatment with *B. oleracea* extract (20%) (toluidine blue, E (20 µm = 40× magnification) and F (10 µm = 100× magnification)). F1, and F2 = scar tissue after four and eight days, respectively. (**C**,**E**,**F**) were obtained in F2 (eight days). SAL: saline solution 0.9%; OV: ointment vehicle; SS: silver sulfadiazine, PB1: *B. oleracea* extract (10%); and PB2: *B. oleracea* extract (20%). Arrows show the blood. Data represented as mean ± standard deviation of the mean. $^{a, b, c, d}$ represent the statistical difference between treatments, SAL, OV, SS, and PB1, respectively ($p < 0.05$) (Student–Newman–Keuls test).

The proportion of type III collagen fibers was higher in the groups treated with OV and SS compared to SAL, PB1, and PB2 on day 4. On day 8, the proportion of these fibers was lower in the groups treated with 10% *B. oleracea* extract (PB1) and 20% (PB2) compared to that of the other groups. The group treated with silver sulfadiazine (SS) had a lower proportion when compared to SAL (Figure 2B,E).

The proportion of type I collagen fibers was higher after treatment with 20% *B. oleracea* extract (PB2) than in all the other groups on day 4. On day 8, this proportion was higher in the groups treated with *B. oleracea* extract (10% and 20%) and SS compared to that of SAL and OV (Figure 2B). In addition, the proportion of fibers in the group treated with the highest dose of *B. oleracea* (PB2) was higher than that in the group receiving the lowest dose (PB1) (Figure 2A,D).

On day 4, the percentage of elastic fibers showed no statistical difference between treatments. On day 8, the proportion was higher after treatment with 20% *B. oleracea* extract (PB2) compared to that of SAL and OV groups (Figure 2C,F). PB1 and SS groups showed a higher proportion of elastic fibers compared to OV.

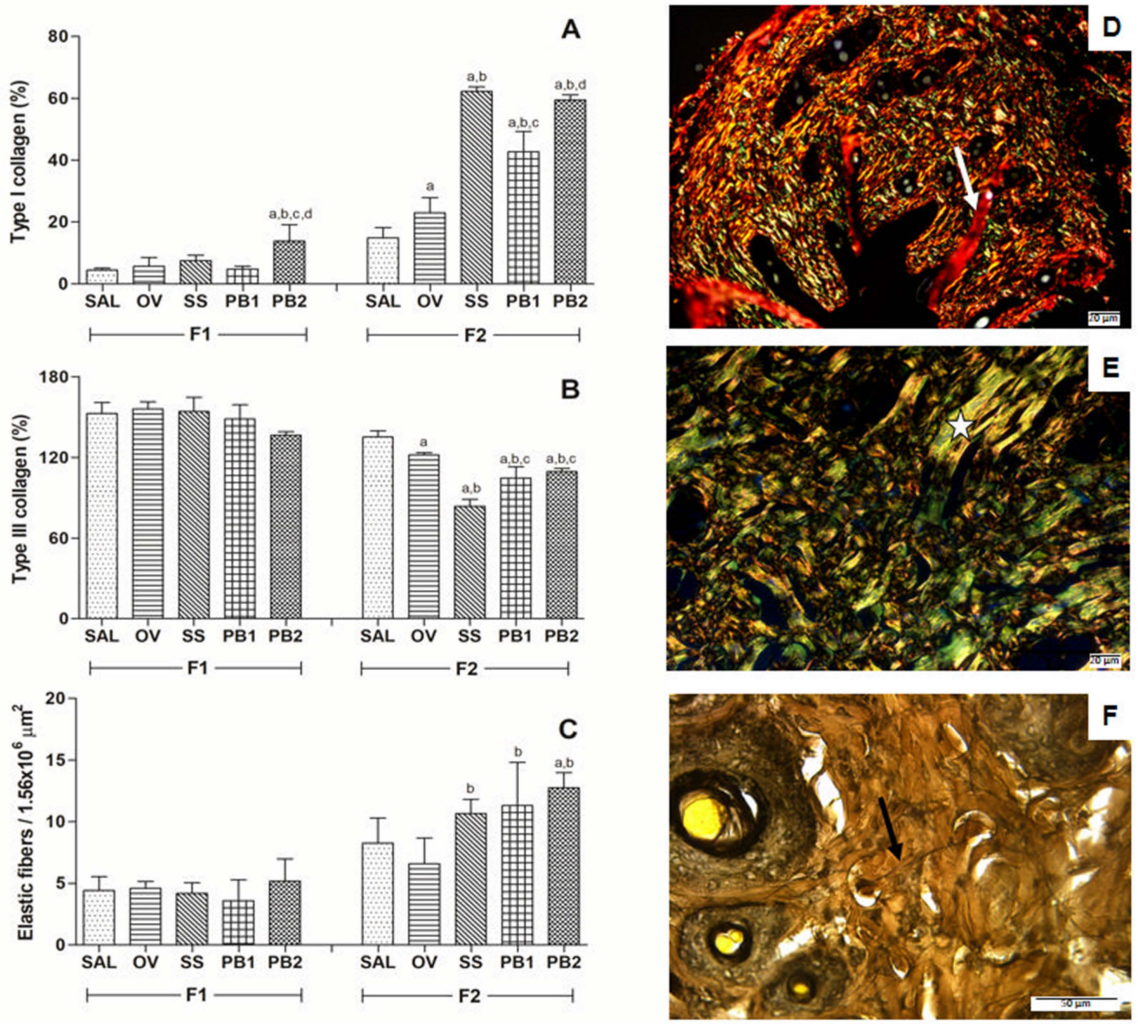

Figure 2. Proportion of type I (**A**) and type III (**B**) collagen fibers and elastic fibers (**C**) in the scar tissue of rats treated with *B. oleracea* extract. F1, and F2 = scar tissue after four and eight days, respectively. (**D**–**F**): photomicrograph on day 8. (**D**,**E**): type I (white arrow) and type III collagen fibers (white star) (20 µm). (**F**): elastic fibers (black arrow) (50 µm). SAL: saline solution 0.9%; OV: ointment vehicle; SS: silver sulfadiazine 1%; PB1: *B. oleracea* extract (10%); and PB2: *B. oleracea* extract (20%). Data represented as mean ± standard deviation of the mean. $^{a, b, c, d}$ represent statistical differences between treatments, SAL, OV, SS, and PB1, respectively ($p < 0.05$) (Student–Newman–Keuls test).

3.3. Antioxidant Enzymes

The levels SOD and CAT enzymes were higher after treatment with PB2 compared to all other groups on day 4. On day 8, the levels of these enzymes were higher than those of SAL, OV, and SS (Figure 3A,B). GST levels were higher in the PB1-treated group than in the SS group on day 4, and higher in both PB1 and PB2 groups than in the SAL and OV groups on day 8 (Figure 3C).

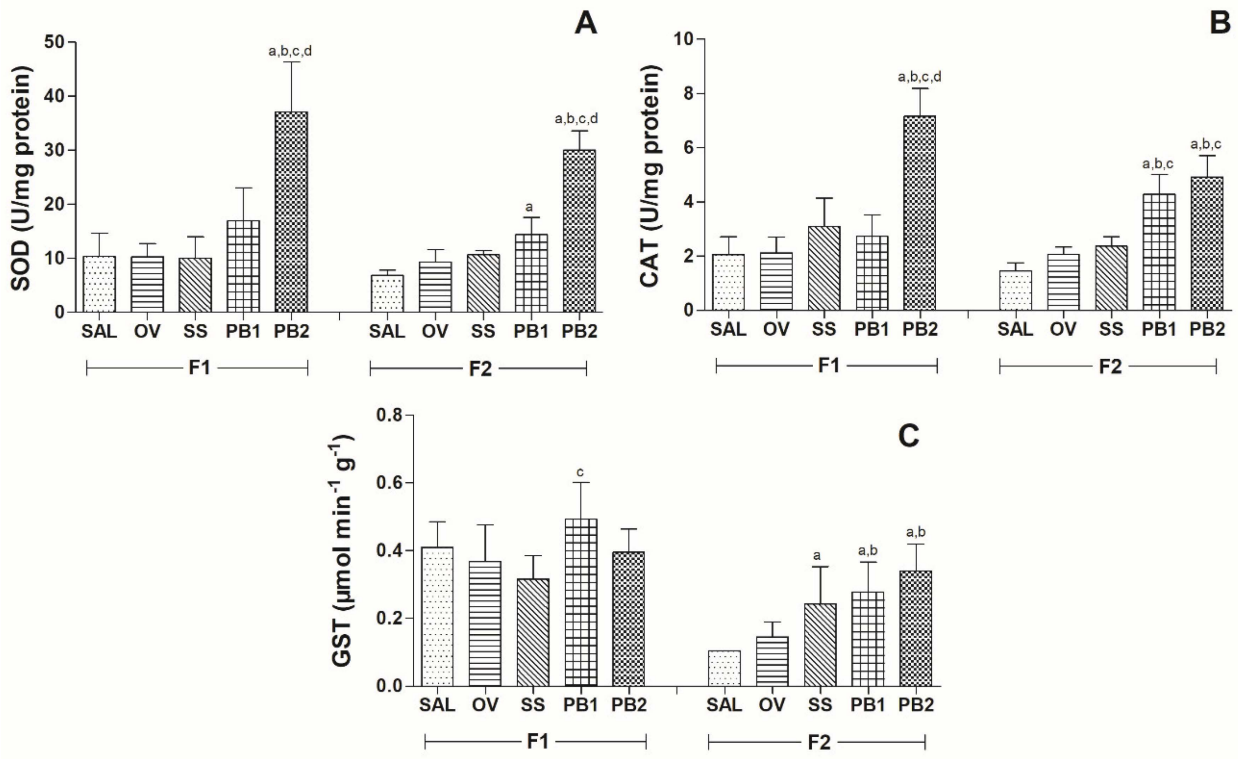

Figure 3. Levels of superoxide dismutase (SOD) (**A**), catalase (CAT) (**B**), and glutathione S-transferase (GST) (**C**) in the scar tissue of rats treated with *B. oleracea* extract. F1, and F2 = scar tissue after four and eight days, respectively. SAL: saline solution 0.9%; OV: ointment vehicle; SS: silver sulfadiazine 1%; PB1: *B. oleracea* extract (10%); and PB2: *B. oleracea* extract (20%). Data represented as mean ± standard deviation of the mean. $^{a, b, c, d}$ represent statistical differences between treatments, SAL, OV, SS, and PB1, respectively ($p < 0.05$) (Student–Newman–Keuls test).

Oxidative Markers

MDA levels were lower in the PB1, PB2, and SS treated groups compared to the OV group on day 4 (Figure 4A). The concentration of carbonylated proteins (PCN) was lower in the PB1 and PB2 groups compared to that of SAL on day 4 (Figure 4B). On day 8, the production of nitrite and nitrate (NO_2/NO_3) was higher in the PB1 and PB2 groups than in the SAL group (Figure 4C). On day 8, H_2O_2 levels were higher in the groups treated with 10% and 20% *B. oleraceae* extract (PB1 and PB2) and SS compared to SAL and OV (Figure 4D).

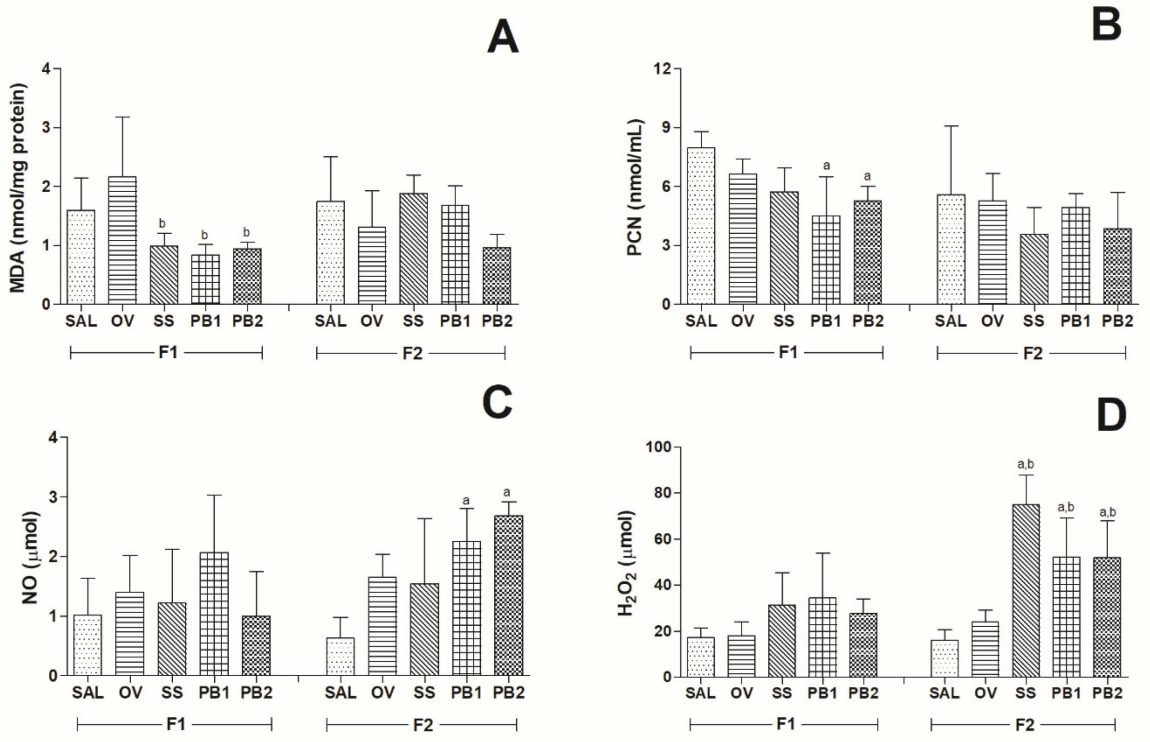

Figure 4. Levels of malondialdehyde (MDA) (**A**), carbonylated proteins (PCN) (**B**), nitrite and nitrate (NO_2/NO_3) (**C**), and hydrogen peroxide (H_2O_2) (**D**) in the scar tissue. F1 F2 = scar tissue after four and eight days, respectively. SAL: saline solution 0.9%; OV: ointment vehicle; SS: silver sulfadiazine 1%; PB1: *B. oleracea* extract (10%); and PB2: *B. oleracea* extract (20%). Data is represented as the mean ± standard deviation of the mean. $^{a, b}$ describes the statistical difference between treatments, SAL and OV, respectively ($p < 0.05$) (Student–Newman–Keuls test).

4. Discussion

Burns occur when the skin is exposed to chemical, physical, or biological agents, resulting in tissue destruction, infection, pain, and even death [61]. For this reason, recent research has focused on finding compounds that enhance the skin repair process [62]. In the present study, we used the extract of *Brassica oleracea* var. *capitata* and evaluated its effect on the healing of skin wounds caused by third-degree burns. *Brassica* is a plant widely used in Brazilian cuisine, popularly known as cabbage [63], as an anti-inflammatory, antioxidant, and anticancer agent [31]. The main phytochemical compounds of *B. oleracea* and the best extractor of these compounds have been described previously. Ahmed et al. [64] observed that ethanolic solvents contain higher amounts of phenolic compounds and consequently have higher antioxidant activity than other extractors such as petroleum ether, ethyl acetate, chloroform, and aqueous extracts. In addition to phenolics, other compounds such as alkaloids, saponins, glycosides, steroids, terpenoids [64], and carotenoids [65] are also found in *Brassica* extract. Our results showed a significant reduction in burns in the groups treated with 10% and 20% *B. oleraceae* extract. Sarandy et al. [66] demonstrated that *B. oleracea* var. *capitata* accelerated the closure of second-degree wounds in Wistar rats and increased the rate of wound contraction, preventing the development of infection. We believe that the acceleration of wound closure is probably due to the high presence of flavonoids and anthocyanins in *B. oleracea* extract [67,68]. These compounds have a high healing power because, in addition to their high antioxidant activity, they stimulate the formation of new blood vessels and ensure better tissue nutrition, promoting rapid recovery of damaged tissue [69–71].

Our results showed an increase in cells and vessels in the groups treated with *B. oleracea* extract, especially on the fourth day. This result was already expected, since at this stage the inflammatory process is usually accelerated and intense [72]. The chemical media-

tors released in the inflammatory phase stimulate collagen synthesis and consequently the formation of granulation tissue rich in vessels and cells [73,74]. High cellularity and vascularization may be related to increased cellular metabolism and possibly tissue nutrition, which are important for tissue closure [75]. Potentiation of the inflammatory process at the beginning of repair may lead to more rapid recovery of the lesion area [76]. The findings of cellularity and vascularization corroborate the wound contraction data, as in a well-vascularized tissue, cellular metabolism is high, leading to rapid and effective closure that as observed in the *B. oleracea* extract treated groups.

The decrease in Type III collagen and the increase in Type I collagen and elastic fibers in the groups treated with *B. oleracea* extract on day 8 are indicative of a high rate of tissue maturation. Type III collagen fibers, which are less thick and robust, should generally predominate at the beginning of the process during the inflammatory and proliferative phases [77], as they serve as a scaffold and support for the deposition of type I collagen, which provides greater tissue strength and resistance [45,62]. Both types of collagen are critical to a healthy healing process [78]. The higher proportion of type I collagen may be associated with a high number of cells and vessels [79], indicating the intense metabolic activity of the tissue after treatment with *B. oleracea.* The higher concentration of collagen in the tissue indicates high fibroblastic activity and faster wound closure [80]. These findings may also be related to the predominance of flavonoids in the extract of *B. oleracea,* as these compounds influence collagen synthesis and are associated with increased protein translation in the process of skin wound healing [81,82].

The decrease in malondialdehyde (MDA) and carbonylated proteins (PCN) levels in the groups treated with *B. oleraceae* extract indicates the high antioxidant power of this extract, which reduces tissue damage caused by lipid and protein oxidation. MDA is a byproduct of lipid peroxidation resulting from the breakdown of polyunsaturated fatty acids [83]. Intense tissue injury occurs during the burning process, and it is therefore common to find an excess of free radicals and consequently MDA in the tissue. The increase in this compound usually indicates damage to the cell membrane, often associated with a local increase in oxidative stress [84]. The level of PCN is another marker of tissue injury and indicates the level of protein oxidation [85]. The higher the level of this marker, the greater the destruction of the tissue proteins by free radical activity. Sarandy et al. [46] reported reduced MDA and PCN levels in the second intention, cutaneous wounds of Wistar rats treated with *S. pseudoquina*. The authors report the action of flavonoids as potent antioxidants, confirming our results with *B. oleracea* extract treatment.

The results shown above for oxidative stress markers corroborate our results for antioxidant enzymes, since the groups that received *B. oleracea* extract showed an increase in the main enzymes responsible for tissue protection, such as SOD, CAT, and GST. We can suggest that the antioxidant potential of *B. oleracea* extract is due to its ability to inhibit lipid and protein oxidation by stimulating the action of antioxidant enzymes, contributing to the faster and more effective tissue repair of burn wounds. The enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), are responsible for protecting tissues from oxidative damage caused by reactive oxygen species (ROS) [86,87]. These enzymes have been previously studied and their importance in the skin repair process is well stablished [51,88,89]. Al-Roujayee [90] demonstrated that after treatment of burned Wistar rats with the phenolic compound Naringenin, there was an increase in SOD, CAT, and GST enzymes and a consequent reduction in lipid peroxidation and an increased matrix synthesis, making the scar tissue more resistant.

When a tissue is injured, there is an increase in ROS, such as superoxide anion, which scavenges tissue nitric oxide (NO), reducing its effective concentrations and signaling effects in cells [91]. Normally, NO levels in burned tissue are low due to the high concentrations of ROS and low levels of antioxidant enzymes [92]. In our study, there was an increase in NO in the groups treated with *B. oleracea*, which may indicate a decrease in ROS in the tissue, demonstrating the protective and antioxidant effects of the extract. NO is a molecule that regulates several physiological functions, and in some cases, elevated levels of this marker

may signal tissue homeostasis [93]. Kim et al. [94] report in a literature review that the increase in NO production improves the healing process by promoting angiogenesis and assisting in the transition of the wound from the inflammatory to the proliferative healing process.

Hydrogen peroxide (H_2O_2) is generated in vivo by the dismutation of the superoxide anion radical (O^{2-}) by enzymes such as superoxide dismutase (SOD) [95]. H_2O_2 is rapidly generated in lesions and is an essential promoter of the inflammatory response of wound healing [96]. When H_2O_2 levels are elevated in a tissue, there is an increase in antioxidant enzymes such as CAT and thioredoxin peroxidases [97]. Increases in H_2O_2 levels have been reported under conditions of stress, such as in burn wounds [98]. In general, high levels of this molecule indicate tissue stress; however, they may also indicate an active and effective way of detoxifying cells with antioxidant enzymes. Considering the results observed in our study, we agree with the second hypothesis, and since the defense systems are overestimated, the stress markers are reduced after treatment with *Brassica* extract. These findings could also justify the increase of CAT in the tissues, since this enzyme uses H_2O_2 as a substrate. Therefore, an increased CAT may indicate the need to eliminate excess hydrogen peroxide to maintain tissue homeostasis [99].

5. Conclusions

Our results indicate that *Brassica oleracea* var. *capitata*, at 10 and 20% concentrations, accelerates the wound area closure and the proliferation of cells and blood vessels. There was a modulation of the extracellular matrix, resulting in the formation of solid and resistant scar tissue in third-degree burns. In addition, *B. oleracea* showed a high antioxidant potential since it is widely used in human food and has a low potential for toxicity, making it a safe treatment alternative. This study should add to the scientific knowledge of the efficacy of *Brassica oleraceae* in the healing process. It may help to reduce the risk of complications and in benefit patients suffering from burn injuries.

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