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Comparing the effectiveness of honey with *Rubus fruticosus* plant powder from the Algerian farm on wounds and the resultant oxidative stress

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Abstract. The use of traditional remedies remains a common therapeutic option for treating burns. In this study, we investigated the healing effect of honey and *Rubus fruticosus* plant powder, and the combination of these two products in burn treatment and accompanied oxidative stress regulation. Over 30 days, Wistar rats with dorsal burns were divided into five groups. Burns were induced on the dorsal region, and treatments were applied topically: honey, blackberry powder, and a honey-plant powder mixture. Two groups served as controls, while treatments were administered daily. Initially, a morphological study was conducted by following the different stages of wound healing and assessing the most effective treatment. At the end of the treatment, histological sections of the wound sites were made, along with an evaluation of oxidative stress by monitoring the activity of Glutathione (GSH), Glutathione-S-Transferase (GST), Glutathione-Peroxydase (GPX), and Catalase (CAT) enzymes, biomarkers at the hepatic level. The results indicate that the natural products used are highly effective in treating burns compared to those treated with the marketed healing cream. The combination of honey and blackberry powder showed a synergistic effect in burn healing, and the histological observations support the findings from the planimetric study. The study of biomarkers shows a state of oxidative stress in the control rats through a decrease in GSH levels and an increase in the activity of hepatic GST, GPX, and CAT. In conclusion, the natural products used in our study demonstrate significant effectiveness in treating burns and oxidative stress regulation, particularly when used in combination.

Keywords: honey, *Rubus fruticosus*, mixture, oxidative stress, wound healing

Introduction

The quest for new antioxidant, anti-inflammatory, and antimicrobial compounds derived from natural resources has become a significant pursuit in the pharmaceutical, cosmetic, and polymer industries (Bouacha *et al.,* 2024). This interest natural medicine from public perception and the growing demand for naturally derived products, as well as the desire for better resource utilization and lower production costs. Additionally, the need to address pathogens developing resistance to conventional antibiotics and the substitution of synthetic compounds that may pose risks to public health has further driven this interest (Neiva *et al.,* 2020). Dressings provide an environment conducive to wound healing while protecting wounds from damage and infection. However, their absorbency can lead to drying out, causing adhesion to the wound. In addition, some dressings lack antimicrobial agents, antioxidants, and other bioactive compounds (Yasin *et al.,* 2023).

Used for centuries, honey is recognized for its effectiveness in treating skin infections, wounds, burns and ulcers. It creates a moist environment that acts as a protective barrier against infections, while its bioactive components ensure antimicrobial, anti-inflammatory and antioxidant properties (Bouacha *et al.,* 2023). In addition, its acidic pH stimulates the activity of macrophages and fibroblasts at wound sites (Minden-Birkenmaier and Bowlin, 2018). Honey may exert antioxidant effects through the synergistic interactions of various active compounds, particularly phenolic and flavonoid constituents (Boudiar *et al.,* 2022). It is relevant to ensure that a dressing incorporates the properties of honey, or another product known for its effects against infections and inflammations related to burns, and the combined characteristics/properties would enhance the efficacy.

Herbal medicine is a major approach in treatment of burns because of its effectiveness, low cost, limited risk of toxicity, and the great variety offered by the diversity of the plant world. Over 600 species of medicinal and aromatic plants in Algeria are commonly utilized in traditional medicine. However, only a limited number have been studied for their phytochemical compounds and biological activities (Meziti *et al*., 2018). Our ancestors traditionally used bramble leaves for dressing and treating external wounds and injuries (Zia-Ul-Haq *et al*., 2014). Subsequent studies have revealed that they exhibit antibacterial and antifungal properties, as well as significant anti-inflammatory and antioxidant activities (Meziti *et al*., 2018).

This study aims to evaluate the therapeutic capacity of two natural products, honey and blackberry (*Rubus fruticosus*) powder, both individually and in combination, in the treatment of burns and the regulation of associated oxidative stress.

Materials and methods

Sample processing

Our samples (honey and *Rubus fruticosus* leaves) were sourced from an unpolluted forest region (Fig. 1). The honey was of multifloral origin from *Apis mellifera* species. The harvesting location was abundant in *Eucalyptus globulus, Erica arborea* plant species and wild lavender (*Lavandula stoechas*). Immediately after harvesting, the healthy leaves were carefully rinsed with water and subjected to a desiccation process in an environment protected from any light exposure. To preserve the optimal integrity of the molecules, the plant was then rudimentarily ground using an electric mill.

Figure 1. Sampling location in the forest area of the municipality of Berrahal (Djebel El Idough), Annaba Province, Algeria.

For the mixture, we triturated honey and the fine powder of blackberry plant leaves with a spoon in a sterile glass flask. The latter was added in small quantities until a homogeneous and creamy blend was achieved, presenting a texture that is easy to apply.

Experimental animals

Male Wistar rats weighing 220.2g (±10g) were used to create the burns in this study. The experiment was conducted in accordance with the specified ethical guidelines for animal care and use. The study design consisted of five groups (Tab. 1) of five rats each , housed individually and acclimatized in our animal facility for one week before the start of the 30-day experimental period. Anesthesia was administered before the procedures, and the animals were humanely sacrificed at the end of the experiment.

Table 1. Distribution of experimental groups.

Group 01(C-)	Without any treatment
Group 02(C+)	Treated with the commercially available cream (Cicatryl Bio®)
Group 03(H)	Treated with honey
Group 04(P)	Treated with plant powder
Group 05(HP)	Treated with the honey-plant mixture

The experimental study was conducted following previously described methods (Abdullahi et al, 2014), with an experimental protocol adhering to ethical recommendations and good practices according to the Guide for the Care and Use of Animals and the Manual of CCP Guidelines (National Research Council, 2010).

After general anesthesia (1.5 mg/kg acepromazine maleate combined with 100 mg/kg ketamine hydrochloride), burn injuries were induced in the middle of the back of each animal. The burns were created using a sterile round brass piece with a diameter of 22 mm. Our treatments were applied topically once every 24 hours. Immediately following the induction of burns, animals in the treated groups received an application of the product corresponding to each group.

Evaluation of healing through digital planimetry

Photographs were captured using a high-resolution Canon ultraSonic camera every 6 days, starting from Day 0 up to Day 30. Subsequently, the images were processed using ImageJ® (image processing software 2019" General Public License") (Chang *et al.,* 2011), ensuring consistent height and angle parameters at regular intervals for each capture.

The percentage of wound contraction was calculated using the following equation (Bouacha et al, 2024):

$$\text{Contraction percentage} = \frac{(Initial\ Wound\ Size\ (Day\ 0) - Wound\ Size\ at\ Day\ n}{Initial\ Wound\ Size} \times 100$$

Histological study

For each group, two samples from the burn sites were collected and processed accordin previuosly described protocols (Preece, 1972; (Martoja et Martoja, 1967). Tissue sampleswere fixed in 10% formalin for structure preservation and specimen hardening. Following paraffin embedding, 6μm thick sections were cut from using a Leica microtome. Hematoxylin-eosin staining was applied, and as the dyes were in aqueous solution, the slides needed to be deparaffinized before rehydration

Following another dehydration process withtwo baths of 95°C alcohol followed by 100°C, and three baths of toluene), the stained slides were mounted between glass slides and coverslips using synthetic resin. The observation of histological sections of the tissu was conducted with a microscope (Leica DM500) equipped with a camera (Leica ICC50 E) allowing image capture with digital imaging software (Leica LAS EZ).

Biochemical assays

At the end of the experiment, the rats were sacrificed, and liver dissection was performed. One gram of tissue was crushed and homogenized in phosphate buffer (pH 7.4; 0.1 M; stored at 4°C). The tissue homogenates were centrifugated (9000 rpm, 4°C, 15 min), and the resulting supernatant was used for various biochemical assays.

Glutathione (GSH) assay. The GSH assay was performed according to Weckbeker and Cory (1988), which relies on measuring the optical absorbance of 2-nitro-5-mercapturic acid resulting from the reduction of 5,5-dithio-bis-2-nitrobenzoic acid by the (-SH) groups of glutathione, at λ = 412 nm.

Glutathione S-Transferase (GSTs) activity assay. The measurement of GST activity is determined following the method described by Habig *et al.* (1974). The reaction is based on the interaction between GST and 1-Chloro2,4-dinitrobenzene in the presence of glutathione, which leads to the formation of 1-S-Glutathionyl 2-4 Dinitrobenzene molecule, allowing the measurement of GST activity at 340 nm within five minutes.

Glutathione Peroxidase (GPx) activity assay. The enzymatic activity of GPx was measured using the method by Flohe and Gunzler (1984) at 412 nm within five minutes, which is based on the reduction of hydrogen peroxide in the presence of reduced glutathione. Under the influence of GPx, the latter is transformed into the oxidized glutathione (GSSG).

Catalase activity assay. Catalases are tetrameric enzymes, with each unit carrying a heme molecule and an NADPH molecule. These enzymes eliminate reactive species by accelerating the spontaneous reaction of the reactive hydrogen peroxide (H_2O_2) hydrolysis, into water and oxygen at 240 nm within five minutes (Aebi, 1984).

Statistical analysis

Statistical analysis of the data was performed using MINITAB software (Version 16). The results were presented as the mean ± standard deviation (M±SD). The statistical treatment of the results involved a one-way analysis of variance (ANOVA) (AV1) and a two-way analysis of variance (AV2), followed by Fisher's *post hoc* comparison test. Differences were considered significant when $p \leq 0.05$ (*), highly significant when $p \leq 0.01$ (**), and very highly significant when $p \leq 0.001$(***).

Results

Evolution of wound healing

According to the obtained results , the wound size evolution of the treated batches did not record any reduction in their surfaces until the 12th day in case of the groups treated with honey and the mixture and from 24th day for the group treated with the plant powder (Fig. 2a). The statistics confirm these observations (Fig. 2b). According to the percentage of contraction (Fig. 2), we can put the following order of healing of the wounds of the different groups: HP, H, P, C-, C+ (Fig. 3).

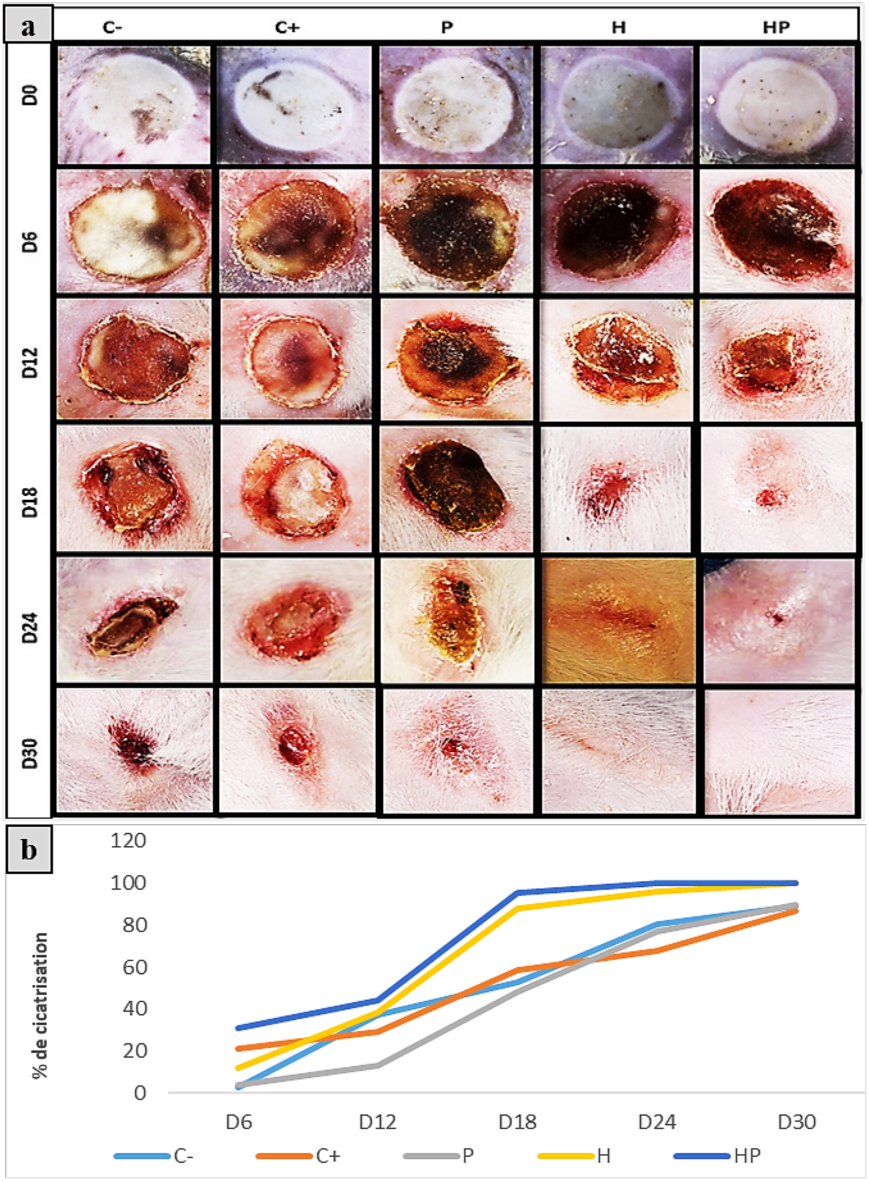

Figure 2. Wound healing effect of the different treatment. (a): Chronology of burn healing with a high-resolution camera (Canon ultra-Sonic), (b): Contraction percentages of wounds in treated and untreated burns. D: day, C-: negative control group, C+: positive control group, P: group treated with plant powder, H: group treated with honey, HP: group treated with honey-plant powder mixture.

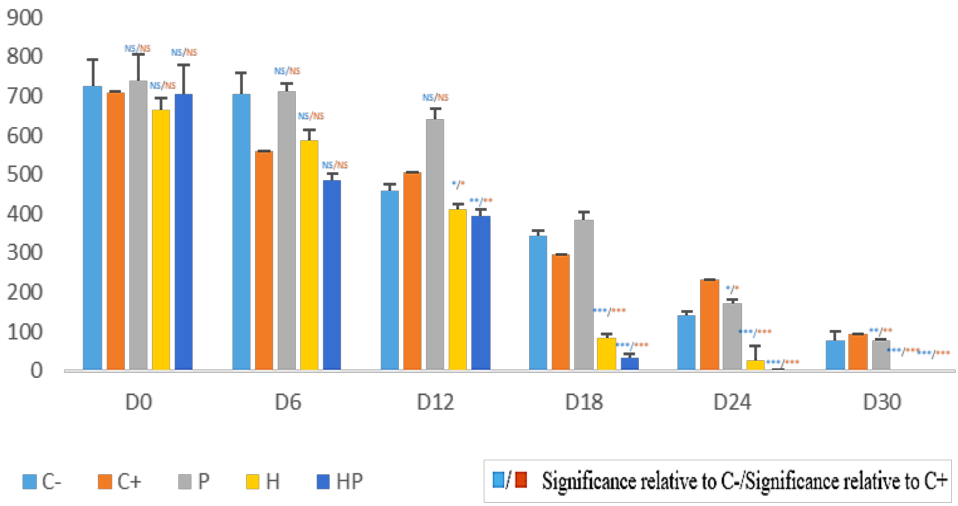

Figure 3. Evolution of the average burn surfaces of the five groups during the experimental period (30 days). D: day, C-: negative control group, C+: positive control group, P: group treated with plant powder, H: group treated with honey, HP: group treated with honey-plant powder mixture. (*)Differences were considered significant, (**) highly significant, and (***) very highly significant.

Histological sections

In general, the processes of inflammation and repair were observed in all examined sections, but to varying degrees among the batches. The inflammatory process was particularly pronounced in the control batches, while the intensity of inflammation decreased to become very low, almost absent, in the batches treated with honey and the mixture, and moderate reduction of inflammation(?) in the batch treated with the plant powder. The repair process, assessed by the intensity of fibrosis, was moderate in the control batches, while the batches treated with natural products (honey, plant powder and mixture) showed a higher intensity of fibrosis (Fig. 4).

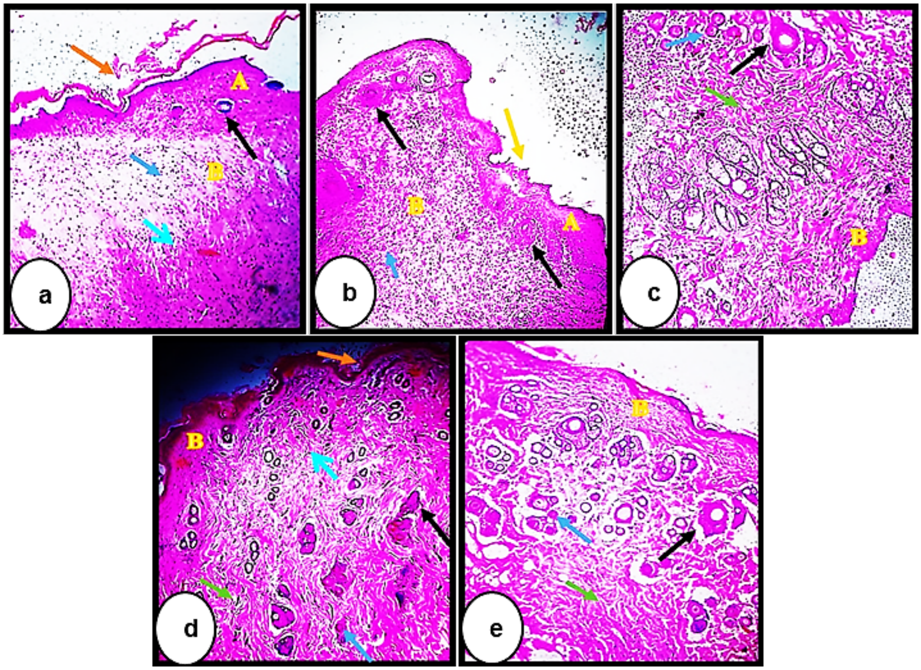

Figure 4. Histological sections of wound tissue (GX400). a: untreated control group (C-), b: treated control group (C+); c: group treated with honey (H), d: group treated with plant powder (P), e: group treated with mixture (HP). A: young fibroblasts with dense inflammation, B: a high number of fibroblasts with simple inflammation, Dark blue arrow: Vessel full of inflammatory elements, orange arrow**:** Debris cell, Light blue arrow: newly formed vessels "Slightly dense fibrosis", Green arrow: Normal dermis, Black arrow: tissue necrosis

Oxidative stress parameters

An significant increase of GSH, GST, GPX and CAT levels/expression was observed in the positive control and the treated groups compared to the untreated control (Fig. 5).

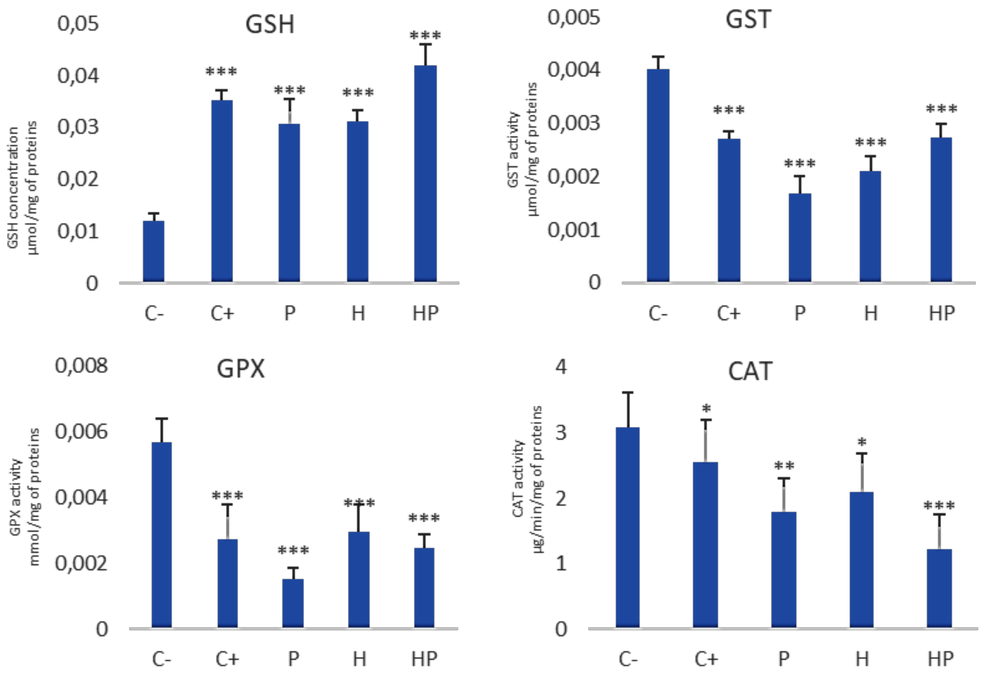

Figure 5. Hepatic enzymatic levels and activities of the oxidative stress parameters of the experimental groups. (*)Differences were considered significant, (**) highly significant, and (***) very highly significant.

Discussion

To replace synthetic molecules, the healing of burns is achieved through the use of conventional products made from natural ingredients. These products have been used in traditional medicine since times to treat various pathologies, as they are considered safer compared to pharmaceuticals (Markiewicz-Gospodarek *et al.,* 2022). They also offer significant advantages in terms of availability, safety, and reduced costs (Jayakumar, 2015). The use of honey as a treatment for burns is well-documented in several scientific studies that have reported its ability to protect wounds, reduce infections, alleviate pain, debride necrotic tissue, and promote granulation tissue formation (Bouacha *et al.,* 2022; Naik *et al.,* 2022; Bouacha *et al.,* 2024). The application of honey to a wound creates a favorable moist environment due to its hygroscopic properties and optimal pH (Tashkandi, 2021),which facilitates faster healing compared to a dry dressing, as it prevents damage to newly formed epithelial tissues. Furthermore, its hyperosmolarity absorbs exudates and promotes a reduction in tissue edema, thereby indirectly improving local microcirculation (Benhanifia *et al.,* 2011).

Phytotherapy is the oldest method of healing in the world, found in all civilizations through various preparations using fresh or dried plants. Phenolic compounds and flavonoids have the ability to inhibit the production of pro-inflammatory mediators such as leukotrienes and prostaglandins (Tangney and Rasmussen, 2013). The richness of the *Rubus fruticosus* extract in these compounds contributes to its anti-inflammatory effect.

Honey is very rich in anti-inflammatory molecules (Saikaly and Khachemoune, 2017), as is blackberry (Tangney and Rasmussen, 2013).

In our study, the natural treatments we selected resulted in complete wound healing, albeit to varying healing degrees, allowing us to compare the healing effects of the products used.

According to the results obtained, wounds treated with the honey-R. fruticosus powder mixture showed better healing progress (Fig. 2 "a and b"). This outcome can be explained not only by the richness and chemical composition of honey but also by the properties of the blackberry. Furthermore, a synergistic effect may exist between honey and *Rubus fruticosus* powder. According to Spoială *et al.,* (2022), the efficacy of a mixture may be related to the synergy of the anti-inflammatory, antioxidant, and antibacterial activities of its components (honey and blackberry in our case).

The effects of our products were manifested in the histological sections, thereby confirming the morphological results and indicating the efficacy of these products at the tissue level. It was observed that the inflammatory process was still present in the negative control group, with an early formation of vessels, while in the treated control group, there was an increase in the number of these vessels, along with cleansing inflammatory vesicles. The treated groups show good re-epithelialization due to the significant formation of neovessels, promoting the emergence of healthy tissue. However, the combination of these two natural products yielded excellent outcomes for wound healing compared to controls, by eliminating inflammation and promoting the renewal of skin cells (fibroblasts). This confirms the biological activities and, especially, the healing properties present in these natural products.

Morphological and physiological changes always subject living organisms to a state of stress (Lagadic *et al.,* 1997). Glutathione is a non-enzymatic antioxidant that also plays a protective role for the skin by participating in the metabolic synthesis of several vitamins, such as vitamin D and vitamin A. The low GSH level observed in the negative control group indicated the mostlyintensified oxidative stress levels during the treatment period, which may explain the absence of wound healing. GST is an essential enzyme for the detoxification process. Our results show a decrease in GST activity across all treated groups. A high level of GSH requires low GST activity, as the enzyme is responsible for the transformation of GSH(Besnaci *et al.,* 2019; 2022). In the antioxidant system, GPX and CAT hydrolyze hydrogen peroxide into water and subsequently reduce the levels of free radicals. The high activities of GPX and CAT indicate elevated levels of H_2O_2 and free radicals, which explains the increased stress state in the control group, while oxidative markers were alleviated/reduced in the treated groups. The observed variations in oxidative stress markers suggested that the body was undergoing metabolic adaptation in reaction to the burn injuries. This process involved an enhanced antioxidant response, reflecting the activation of repair and recovery mechanisms aimed at restoring homeostasis and promoting tissue healing.

Conclusion

In conclusion, we find that the efficacy of these products encourages the use of natural remedies, which represent a better solution for the treatment of burns and wound healing. Honey and blackberry exhibit good anti-inflammatory properties and an excellent antioxidant effect, while their combination provides optimal results due to the synergy between the bioactive molecules of the two products.

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