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Evaluation of antibacterial, anti-inflammatory, antioxidant, and wound-healing properties of multifloral honey

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Abstract

In recent years, there has been a renewed interest in using honey produced by the *Apis mellifera* bee to treat a variety of diseases. Honey is considered one of the most widely used natural products. The purpose of the present work was to assess the antibacterial, anti-inflammatory, anti-oxidative stress, and wound-healing properties of honey harvested from a semi-arid region of Algeria. The antibacterial activity was performed against 11 bacterial species isolated from burn wound infections, including *Escherichia coli*, *Klebsiella oxytocae*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Enterococcus faecalis*. The anti-inflammatory effect was achieved by denaturing albumin and stabilising the membrane. The wound-healing effect was tested on dorsal skin burns in Wistar rats. The treatments were administered daily for 32 days. The liver was analysed for glutathione, glutathione-S-transferase, glutathione peroxidase, and catalase. The skin sections were examined under a light microscope to determine the effect of the treatment on cells. Honey effectively inhibited the growth of ten pathogenic bacteria, with inhibitory diameters ranging from 11.5 ± 0.33 for *K. pneumoniae* to 30.83 ± 0.78 for *E. faecalis*. The anti-inflammatory effect was demonstrated by a percentage inhibition of albumin denaturation ranging from 13.86 to 74.40%, as well as a percentage membrane stabilisation ranging from 11.38 to 83.80%. The honey-treated burns healed completely by the 28th day, outperforming both untreated burns and those treated with Cicatryl-Bio. Honey treatment resulted in earlier scar formation, reduced inflammation, and decreased cell necrosis, demonstrating its superior efficacy in burn healing. In rats treated with honey, oxidative stress parameters were consistently close to the standards. Honey is an ideal dressing because due to its humidity and composition, it can provide a better environment for wound healing, while also acting as an anti-inflammatory, antibacterial, and stimulating protection against infections.

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Introduction

Burns are a global public health problem, accounting for approximately 180,000 deaths each year. They are characterised by Systemic Inflammatory Response Syndrome (Kuznetsova *et al.*, 2022). Burn is a vulnerable site for opportunistic colonisation by pathogenic microorganisms of both endogenous and exogenous origins (Pruitt *et al.*, 1998; Magnet *et al.*, 2013). In some cases, microorganisms develop resistance to antibiotics used in the treatment of infected burns, making management more difficult. The use of natural honey produced by *Apis mellifera* bees to address this issue is highly valuable, and it is regarded as an important component of traditional medicine.

Honey has been used since antiquity to treat a wide range of diseases, particularly burns and infectious diseases. Honey is a natural substance with numerous therapeutic properties, such as healing activity (Subrahmanyam, 1996), antibacterial (Bouacha *et al.*, 2018), anti-inflammatory (Kassim *et*

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al., 2010b), antioxidant (Fabunmi *et al.*, 2021), antitumoral (Saranraj and Sivasakthi, 2018), and antimutagenic effects (Bouacha *et al.*, 2022b). Many studies have proved that honey is a natural source of bioactive compounds. Honey contains approximately 200 compounds, including sugars, proteins, vitamins, minerals, organic acids, flavonoids, phenolic acids, enzymes, and other phytochemicals (Goetz, 2009; Boudiar *et al.*, 2022a).

Honey also has antibacterial properties against the most pathogenic bacteria, yeasts, moulds, and viruses (Mandal and Mandal, 2011; Bouacha *et al.*, 2018; Boudiar *et al.*, 2022a). Although the exact mechanism of action of honey is unknown, the factors responsible for its antimicrobial activity include high osmolarity caused by high sugar content, which causes significant dehydration of the bacteria. Additionally, honey has a pH of 3 to 4 at which bacteria cannot abundantly multiply. Some honey samples, however, have a much higher pH of 5 to 6 (*e.g.*, chestnut and honeydew honey). These types of honey still have antibacterial properties. It also contains an enzyme called glucose oxidase, which is secreted by the honey bees' feeder gland, and is responsible for its high antibacterial activity by producing hydrogen peroxide (H_2O_2), a bacterial growth inhibitor (Saranraj and Sivasakthi, 2018). These beneficial functions are parts of honey's healing effect; it protects wounds from microbial contamination, deodorises, and reduces inflammation, oedema, and exudation (Williams, 2020).

Furthermore, honey has healing properties that regenerate skin tissue. This action is due to honey's high osmolarity, which attracts water, and drains lymph and plasma to the outside, removing debris and cleansing the wound. It also promotes wound healing by stimulating angiogenesis, granulation, and epithelialisation. In addition, honey, as an antioxidant, may control the formation and elimination of reactive oxygen species (ROS), which are consistently generated at a basal level incapable of causing damage under optimal conditions (Kocyigit *et al.*, 2019; Fabunmi *et al.*, 2021). In some cases, an overproduction of ROS or a decrease in antioxidants disrupts the delicate balance between ROS generation and removal, causing destructive actions on DNA and proteins, as well as tissue damage through lipid peroxidation (Besnaci *et al.*, 2019). Research has shown that honey has been shown to significantly strengthen the antioxidant defence system, reducing the negative effects of ROS on cell function (Fabunmi *et al.*, 2021). Honey may induce antioxidant effects through the synergistic effects of several active components, particularly its phenolic and flavonoid contents.

Several factors influence honey's effectiveness, including soil type, bee breed and physiological state, floral origin, environmental climate, harvesting season, and conservation condition (Bouacha *et al.*, 2022a). Algeria is located in northern Africa, and has a variety of climates (humid, sub-humid, semi-arid, arid, and Sahara). It has significant floral resources (more than 3,000 species), 15 of which are endemic, with many medicinal benefits. As a result, Algerian honey production capacity is extremely high; however, most Algerian honey varieties have not been published or studied scientifically. The purpose of the present work was, therefore, to assess the antibacterial, anti-inflammatory, anti-oxidative stress, and burn-healing properties of multifloral honey harvested in a semi-arid region (Djelfa).

Materials and methods

Honey sample

Dark brown honey was collected in March 2022 from a semi-arid region in the centre of northern Algeria. We collected and stored honey in sterile bottles at room temperature (24 ± 2). The honey had a pH of 4.12 ± 0.14, a moisture content of 16.27 ± 0.16 (g/100 g), and an electrical conductivity of 0.23 ± 0.03 (mS/cm). Honey's melissopalynology quantitative analysis revealed that it is multifloral (*Ziziphus lotus*, *Xanthium strumarium*, *Artimitia herba alba*, *Teucrium polium*, and *Erica arborea*). Dilutions (5, 10, 20, 40, 60, and 80% (v/v)) were freshly prepared in distilled water, as well as undiluted honey.

Antibacterial effect

The antibacterial activity of honey was assessed using the well diffusion and microdilution methods in 96 microwell plates. Several authors have already described these methods (Al-Kafaween *et al.*, 2019; Boudiar *et al.*, 2022b; Bouacha *et al.*, 2023). The antibacterial effect was assessed using ten multidrug-resistant bacteria isolated from burn wound infections (Table 1). Briefly, 6 mm diameter wells were created in Mueller Hinton agar plates. Plates were inoculated with 10^6 colony-forming units

Table 1. Susceptibility of pathogenic bacteria against antibiotics.

Pathogenic bacteria		Susceptibility to antibiotic														
		Penicillins			Cephalosporins			Fluoroquinolones			Aminoglycosides			Others		
		OX	AM	AMC	CN	CF	CX	OF	NA	CIP	AM	GM	TM	NIT	FOS	SXT
Gram-negative bacteria	*E. coli*	ND	R	R	R	R	R	S	S	S	R	S	R	R	R	R
	E. aerogenes	ND	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	C. koseri	ND	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	K. oxytocae	ND	R	R	R	R	R	S	R	R	R	S	R	R	R	R
	K. pneumoniae	ND	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	P. mirabilis	ND	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	P. vulgaris	ND	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	P. aeruginosa	ND	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Gram-positive bacteria	*E. faecalis*	R	ND	ND	R	R	R	S	S	S	R	S	R	R	R	R
	S. aureus	R	ND	ND	R	R	R	R	R	R	R	R	R	R	R	R
	S. saprophyticus	R	ND	ND	R	R	R	S	S	S	R	S	S	R	S	R

OX: oxacillin; AM: amoxicillin; AMC: amoxicillin-clavulanic-acid; CN: cefazolin; CF: cefixime; CX: cefotaxime; OF: ofloxacin; NA: nalidixic acid; CIP: ciprofloxacin; AM: amikacin; GM: gentamicin; TM: tobramycin; NIT: nitrofurantoin; FOS: Fosfomycin; SXT: sulfamethoxazole-trimethoprim; R: resistant; S: susceptible; and ND: not determined.

(CFU/mL) of bacteria, followed by 50 µL of honey in each well. After 24 h of incubation at 37°C, the inhibition diameters around the wells were measured in triplicate. The mean ± standard deviations (SD) were then calculated and reported.

The minimum inhibitory concentrations (MICs) were determined using sterile 96-well microtiter plates from Fisher Scientific (UK). Each well received 100 µL of the tested bacteria inoculum mixed with 100 µL of honey. Plates were incubated at 37°C for 24 h. The lowest honey dilution that inhibited bacterial growth was reported as the MIC. The remaining wells with no visible turbidity were inoculated on nutrient agar plates to determine the minimum bactericidal concentration (MBC). The plates were incubated at 37°C for 24 h. The MBC represents the lowest dilution of honey, which does not allow bacterial growth. The MBC/MIC ratio was calculated to determine whether the honey was bactericidal or bacteriostatic.

Anti-inflammatory effect

The anti-inflammatory activity was assessed *in vitro* using albumin denaturation and membrane stabilisation methods similar to those described by Ali *et al.* (2017b) with minor modifications. In test tubes, 1% aqueous solution of bovine serum albumin fraction was mixed with honey at various dilution levels. The tubes were incubated at 37°C for 15 min, then heated to 70°C for 10 min. The absorbances were measured using a spectrophotometer at 660 nm. The negative control was distilled water, while the positive control was aspirin (100 µg/mL). The percentage inhibition of albumin denaturation was calculated as: $\left(\frac{1-A1}{A2}\right) \times 100$, where, A_1 = honey absorbance, and A_2 = control absorbance (distilled water).

For the membrane stabilisation method, 10 mL of human blood was centrifuged at 3,000 rpm for 10 min, and washed with 0.9% saline solution (pH 7.0). A mixture of 1 mL of honey at various dilutions and 1 mL of 10% human red blood cell suspension was prepared and incubated for 30 min at 56°C. The supernatant was obtained by centrifugation at 2,500 rpm for 5 min, and the absorbances were measured at 560 nm. Aspirin (100 µg/mL) served as a positive control, while saline solution served as a negative control. The percentage membrane stabilisation was calculated as: $\left(\frac{1-A1}{A2}\right) \times 100$, where A_1 = honey absorbance, and A_2 = control absorbance (normal saline solution).

Wound-healing effect

The wound-healing effect was achieved using the procedure previously described by Abdullahi *et al.* (2014). It followed the ethical and good practice recommendations outlined in the Guide for the Care and Use of Animals. The experiment was conducted with 30 Wistar rats weighing between 200 and 250 g.

i) Housing and care

The animals were randomly assigned into three groups: one treated with honey, one treated with a marketed healing cream called Cicatryl-Bio® (positive control), and one left untreated (National Research Council, 2010; Abdullahi *et al.*, 2014). The rats were kept in individually ventilated cages with controlled temperature (22 ± 2°C), humidity (55 ± 10%), and a 12-h light/dark cycle. They were fed with standard laboratory chow, and given unlimited access to water *ad libitum*.

ii) Blinding

To eliminate bias, the individuals responsible for measurements were blinded to the group assignments throughout the study.

iii) Anaesthesia and procedure

Rats underwent general anaesthesia *via* intramuscular injection of 1.5 mg/kg acepromazine maleate combined with 100 mg/kg ketamine hydrochloride. Each animal received four skin burns in the dorsal region after having their hair removed with a round metal plate, 22 mm in diameter. The metal plate, which had previously been immersed in boiling water (100°C) for 5 min, was applied to the chosen area for 30 s without pressure.

iv) Treatment

The treatment was administered every 24 h for 32 d. The wounds were left uncovered to simulate a more natural healing environment, and determine the direct effects of the treatments used.

v) Outcome measures

At the end of the experimental period, the rats were sacrificed by decapitation under anaesthesia to ensure humane treatment. The results were expressed as mean burn surface area ± SD.

Inhibition of oxidative stress

The inhibition of stress oxidative activity was measured using the method described by Besnaci *et al.* (2019). The liver was collected for the following measurements: glutathione (GSH) after deproteinisation with sulfosalicylic acid, glutathione-S-transferase (GST), glutathione peroxidase (GPX), and catalase (CAT). The burned skins were immediately treated with 10% formalin for 24 h. Skin samples were dehydrated in alcohol baths, and washed with deionised water before being immersed in a kerosene bath for microscopic analysis. The tissues were cut into 5 µm slices with a Leica microtome (Leitz, Germany), immersed in kerosene, and stained with eosin-haematoxylin. The sections were examined and photographed using a light microscope (Leica DM500).

Statistical analysis

Data were expressed as mean ± SD and calculated using GraphPad Prism 7.00 (GraphPad Software, La Jolla, California, USA). A One-way analysis of variance (ANOVA) was used to compare the groups. A p-value of ≤ 0.05 was considered significant (approx. 95% confidence level).

Results

Antibacterial effect

Figure 1 and Table 2 show the results of honey's antibacterial activity against pathogenic bacteria. The susceptibility of pathogenic bacteria to honey varied significantly; Gram-positive bacteria were more susceptible than Gram-negative bacteria. Table 2 shows that the MIC values range from 10 to 60% (v/v), while the MBC values range from 10 to 100%. Additionally, the MBC/MIC ratio was between 1.00 and 2.50, indicating that the honey tested had a bactericidal effect. An antibacterial agent's effectiveness is proportional to its MBC/MIC ratio. If it is greater than four, the substance is classified as a bacteriostatic agent, which means it prevents bacterial growth rather than killing it. However, if the MBC/MIC ratio is less than or equal to four, it is classified as a bactericidal agent, which means that it reduces the viability of microorganisms. As a result, tested honey had a good bactericidal effect on the pathogenic bacteria found in infected burns.

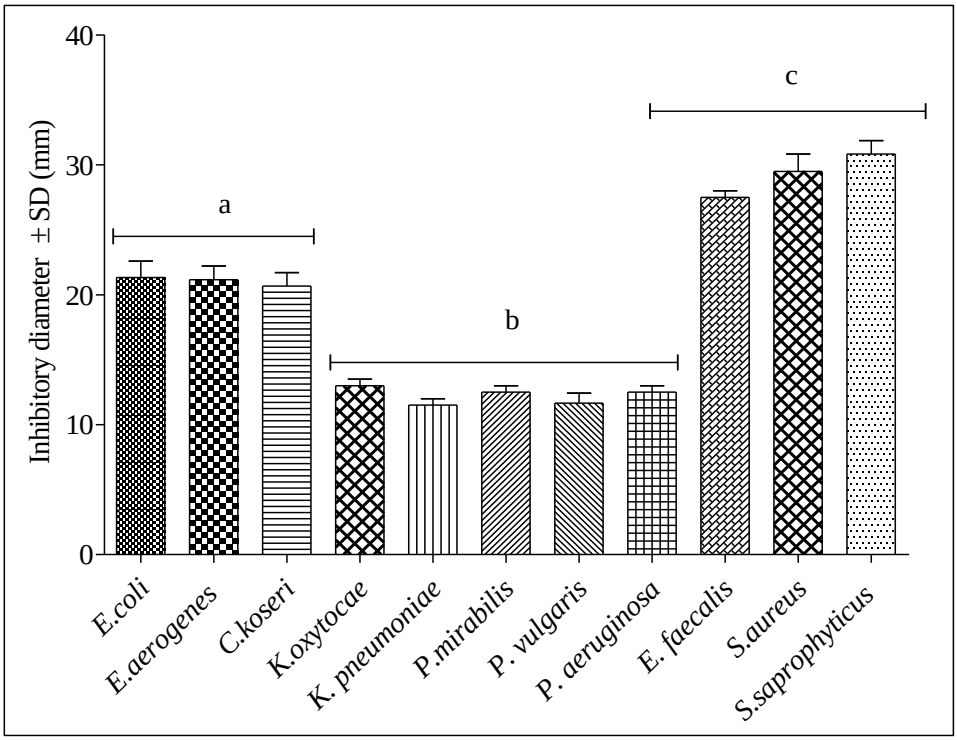

Figure 1. Mean of inhibitory diameters (± SD) of honey against pathogenic bacteria from burn wound infections. Values are means of three independent experiments ± SD. Means with similar lowercase letter are not significantly different ($p > 0.05$). Means with different lowercase letters are significantly different ($p < 0.01$).

Table 2. MIC and MBC values of honey against pathogenic bacteria from burn wound infections.

Pathogenic bacteria	MIC% (v/v)	MBC% (v/v)	MBC/MIC ratio
E. coli	40	40	1.00
E. aerogenes	40	40	1.00
C. koseri	40	40	1.00
K. oxytocae	40	80	2.00
K. pneumoniae	60	100	1.67
P. mirabilis	40	100	2.50
P. vulgaris	40	60	1.50
P. aeruginosa	60	80	1.33
E. faecalis	10	10	1.00
S. aureus	10	10	1.00
S. saprophyticus	10	10	1.00

Anti-inflammatory effect

Figure 2 illustrates the results of honey's anti-inflammatory activity, as determined by membrane stabilisation and albumin denaturation. It was observed that honey had a strong anti-inflammatory effect that was statistically equivalent to aspirin, particularly at dilutions ranging from 60 to 100% (v/v). The results in Figure 2 suggest that honey inhibited protein denaturation, and protected the lysosome membrane.

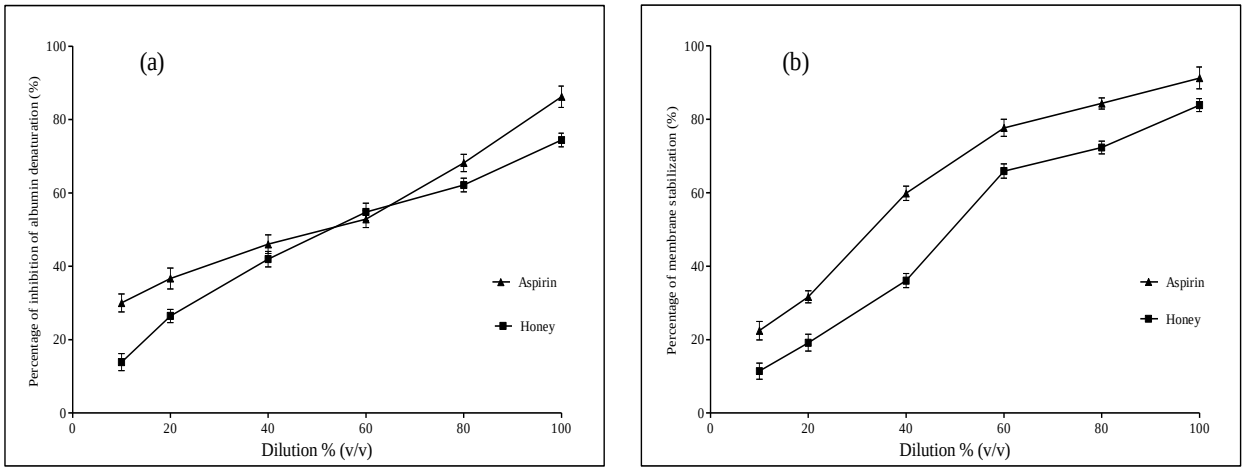

Figure 2. Anti-inflammatory effect of multifloral honey compared to aspirin: **(a)** percentage of inhibition of albumin denaturation; and **(b)** percentage of membrane stabilisation.

Wound-healing effect

Figure 3 shows the results of treating burns with honey *versus* Cicatryl and untreated burns. The burns treated with honey healed completely (before the 28th day). Data analysis revealed significant differences between the different treatments and the negative control (untreated burns). Significant differences in wound surface reduction were observed between honey treatment and the other groups beginning on the 20th day. Thus, it could be concluded that honey treatment was more effective than Cicatryl treatment and the untreated groups. As illustrated in Figure 4, there is a decrease in inflammation and cell necrosis. The histopathological analysis of the tissue revealed that scar formation in wounds treated with honey began much earlier than in control groups.

Inhibition of oxidative stress

Figure 5 displays the results of determining GSH, GST, GPX, and CAT, which are oxidative stress parameters. Results show that the honey-treated group had higher CAT and GPX activities but lower GSH activity, indicating a strong antioxidant

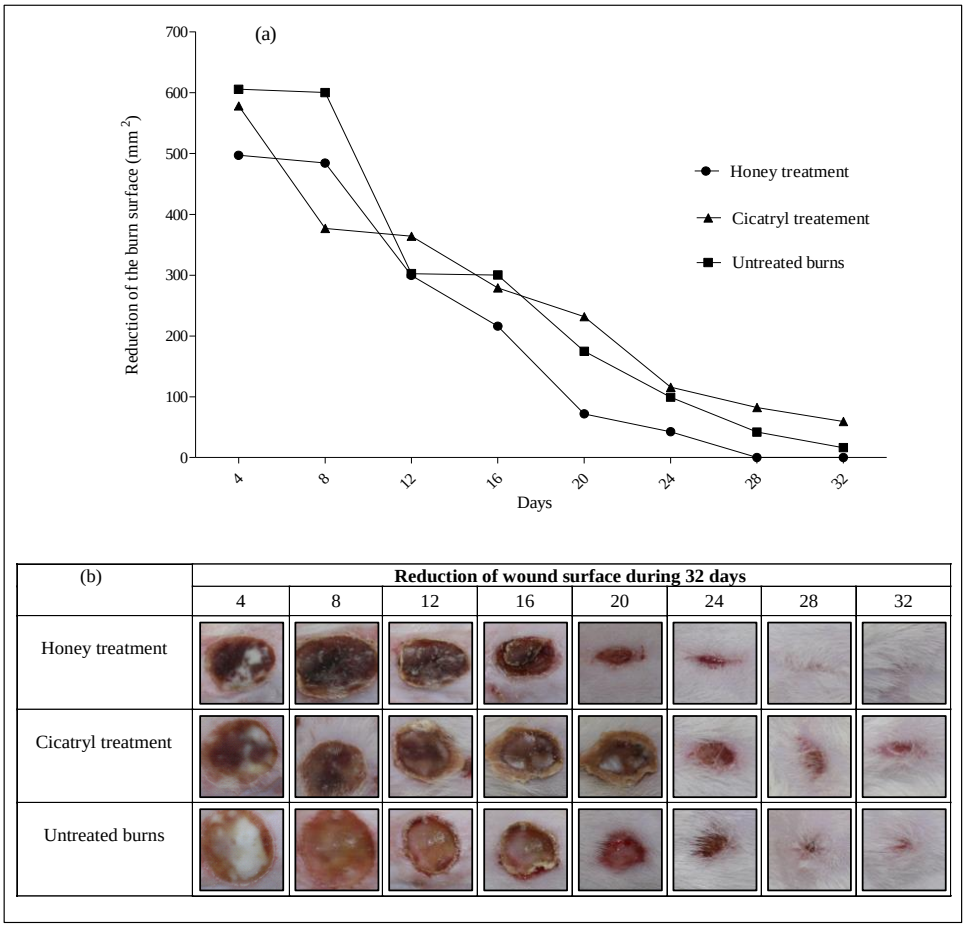

Figure 3. Wound healing effect of multifloral honey compared to Cicatryl and untreated burns: **(a)** mean of wound surface (mm^2); and **(b)** reduction of wound surface using "ImageJ®" (image processing software 2019) and a high-resolution camera (Canon ultra-Sonic) every four days for 32 days.

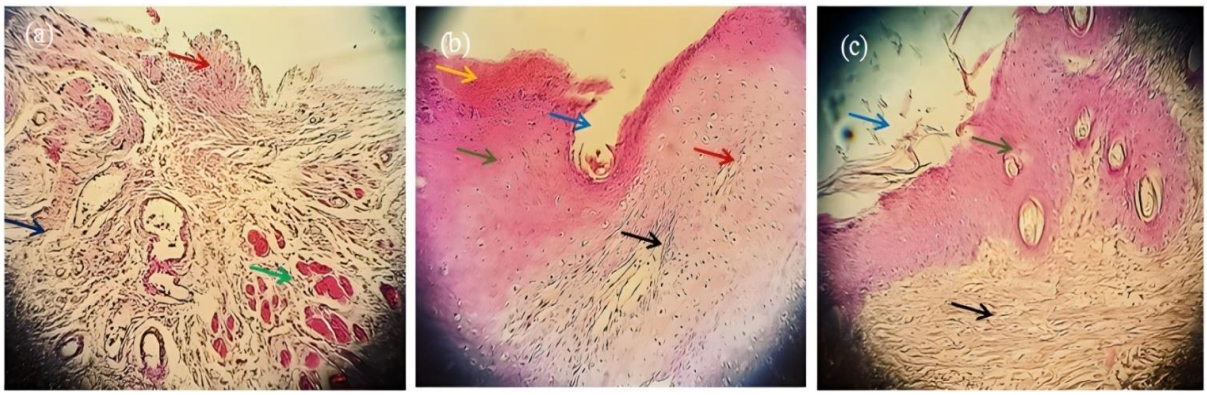

Figure 4. Representative photomicrograph of wound tissue section (400× magnification): **(a)** honey-treated; **(b)** Cicatryl-treated; and **(c)**: untreated burns.

: Low number of young fibroblasts with dense inflammation
: High number of fibroblasts with simple inflammation
: Vessel full of inflammatory elements
: Debris cell
: Newly formed vessels "slightly dense fibrosis"
: Normal dermis
: Tissue necrosis

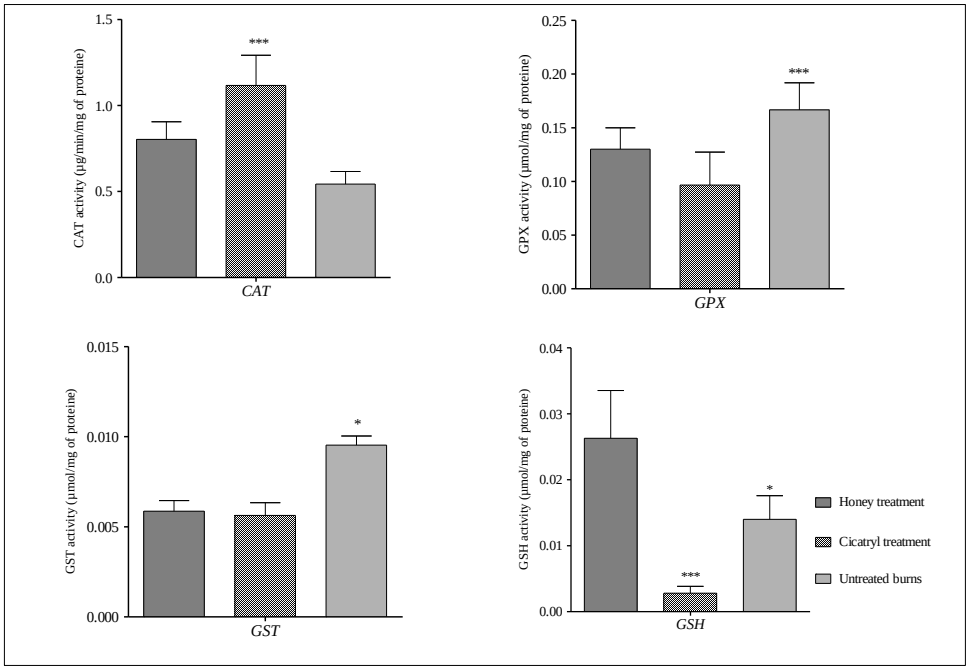

Figure 5. *In vivo* antioxidant effect of multifloral honey compared to Cicatryl treatment and untreated wounds. GPX: glutathione peroxidase; GSH: glutathione; GST: glutathione-S-transferase; CAT: catalase. *significant differences between groups.

response. The Cicatryl-treated group exhibited increased CAT, GST, and GSH activity, indicating distinct but significant antioxidant responses. These suggested that both honey and Cicatryl treatments influenced oxidative stress parameters in burnt rats, albeit in different ways. Honey appeared to significantly increase CAT and GPX activities, whereas Cicatryl significantly increased CAT and GST activities.

Discussion

Honey is a natural product that shows promise as a therapeutic strategy for a variety of diseases. Figure 1 and Table 2 show that the susceptibility of pathogenic bacteria to honey varied significantly; Gram-positive bacteria were more susceptible than Gram-negative bacteria. Several authors have also previously reported similar findings (Mandal and Mandal, 2011; Bouacha *et al.*, 2018). Indeed, honey's effect on bacterial growth is determined by the structure of the bacterial cell wall. Gram-negative bacteria have a thick outer wall that prevents antibacterial agents from entering the bacterial cell, limiting their effectiveness.

The efficiency of honey, on the other hand, is determined by its bioactive substance composition, floral origin, bee species, and storage conditions (Bouacha *et al.*, 2022a). Numerous studies have found a strong link between some honey's floral sources and its antimicrobial properties (Cooper *et al.*, 2002; Bouacha *et al.*, 2018). According to Molan (1992), honey's botanical origin is critical to its many beneficial properties, particularly its antimicrobial activity. The MIC, MBC, and MBC/MIC ratios in Table 2 indicate that the tested honey had a bactericidal effect. Bacterial species with smaller inhibitory diameters had higher MIC and MBC values, indicating reduced susceptibility to honey. This resulted in a higher MBC/MIC ratio, which means that a much higher concentration of the antimicrobial is required to kill the bacteria rather than simply inhibiting their growth. Smaller inhibitory zones indicate the bacteria's resistance, necessitating higher concentrations for effective inhibition and killing. As a result, interpreting antimicrobial efficacy requires taking into account both MIC and MBC values, as well as the size of the inhibition zones.

Although honey has long been used as a natural remedy for skin infections, no pathogenic microorganisms have developed resistance to it. This is due to the combination of various antimicrobial factors that work synergistically to increase its overall antibacterial activity, lowering the risk of bacteria developing resistance to all components at the same time (Henriques *et al.*, 2011; Maddocks and Jenkins, 2013; Boudiar *et al.*, 2022a). Furthermore, honey is

becoming more widely recognised for its prophylactic properties in a variety of medical settings. Recent research has demonstrated its efficacy in preventing infections, particularly in wound care and veterinary medicine. Honey has been shown to have broad-spectrum antimicrobial activity, which reduces the risk of infection from surgical wounds. Similarly, Mandel *et al.* (2020) and Gustafsson *et al.* (2021) reported that honey can prevent postoperative complications, and promote healing in horses. These findings all point to honey as a promising prophylactic agent due to its antimicrobial properties (Mandel *et al.*, 2020; Gustafsson *et al.*, 2021; Bocoum *et al.*, 2023).

As shown in Figure 2, the honey had a potent anti-inflammatory effect at dilutions ranging from 60 to 100% (v/v), comparable to that of aspirin. Similar findings have been reported previously (Manukumar and Umesha, 2015). Although inflammation occurs in the early stages of wound healing (Harti *et al.*, 2018), it remains a significant issue in the treatment of burns, chronic wounds, and microbial diseases. Indeed, protein denaturation and the release of lysosome constituents are well-known inflammatory factors. On the other hand, erythrocyte membranes are similar to lysosome membranes (Ali *et al.*, 2017a). Thus, honey may inhibit protein denaturation while also protecting the lysosome membrane. However, previous research has suggested that honey inhibits the synthesis of prostaglandins, which are frequently responsible for the heat, itching, and pain associated with inflammation (Kassim *et al.*, 2010a). Honey has also been linked to reduced ROS production, which reduces inflammation, and promotes wound healing (Kassim *et al.*, 2010b). Honey may influence the production of pro-inflammatory cytokines like TNF-α (Hadagali and Chua, 2014).

Figures 3 and 4 show a decrease in inflammation and cell necrosis. This indicated that honey may have an anti-inflammatory effect, which promotes tissue growth and repair. Following honey treatment, epithelial thickness and fibroblast count increased significantly, resulting in increased connective tissue regeneration and accelerated healing. As a result, the wound closed, and epidermal cells re-epithelised and proliferated, resulting in faster burn healing in the honey-treated group. Histopathological tests revealed that scar formation in wounds treated with honey began much earlier than in control groups. Thus, it can be concluded that the honey's restorative activity was primarily determined by the concentration of naturally active substances found in it.

However, as earlier mentioned, very little research has been conducted on Algerian honey. Moreover, to the best of our knowledge, this is the first study to look at the efficacy of Algerian honey in treating burns. The results observed in the present work were consistent with other studies that examined the healing effect of various types of honey on burn wounds (Robson *et al.*, 2009), including studies on Gelam-honey (Tan *et al.*, 2009), pectin-honey (Giusto *et al.*, 2017), and honey gel (Febriyenti *et al.*, 2019). Several factors contribute to honey's healing properties. It protects the wound, reduces infections and pain, removes necrotic tissue, and promotes the formation of granulation tissue (Naik *et al.*, 2022). In addition, honey's viscosity acts as a protective barrier against microbial infection, and promotes wound healing. The production of H_2O_2 aids wound debridement, and protects against bacterial infections (Subrahmanyam, 1996; Lusby *et al.*, 2002). The pH of honey may also provide an ideal environment for fibroblast activity (migration, proliferation, and collagen organisation), which requires slightly acidic wound conditions (Subrahmanyam, 1996).

Honey's nutrient content, which includes levulose and fructose, promotes epithelisation on the wound's upper surface. Honey's high osmolarity also allows for rapid absorption of oedema fluid from weeping burns (Subrahmanyam, 1996; Lusby *et al.*, 2002). As a result, honey is an ideal dressing because its humidity and composition create a better environment for wound healing by involving various steps such as cell migration, cell differentiation, angiogenesis, matrix formation, granulation tissue formation, and re-epithelialisation. It can absorb exudates from the wound's surface (Sipos *et al.*, 2004; Lu *et al.*, 2013). Wound contraction is an important process in healing that leads to wound closure, and honey may increase contraction and promote the deposition of fibroblasts and collagen, both of which are required for healing (Saikaly and Khachemoune, 2017). It has been shown that the inflammatory response to skin degenerative processes is primarily mediated by the overproduction of ROS, and activation of the antioxidant system (Aquino *et al.*, 2002).

According to Oryan *et al.* (2019), honey can protect stem cells applied to burn wounds from oxidative stress, and improve their regeneration

ability. The type of honey used to treat burns is critical, with medical-grade honey standing out due to its strict quality standards, which ensure both safety and efficacy in wound management (Hermanns *et al.*, 2020). It has also revealed significant differences in therapeutic effects between different types of honey, implying that supplements containing honey may provide additional benefits, possibly through additive or synergistic effects (Pleeging *et al.*, 2020; Boekema *et al.*, 2024).

As shown in Figure 5, both honey and Cicatryl treatments had a significant effect on oxidative stress parameters, indicating different mechanisms in burn recovery. GSH participates in several vitamin metabolism reactions, including those involving vitamins A, C, and D, all of which play an important role in skin protection. It also helps protect cell membranes from free radicals. Fonseca *et al.* (2010) found that GSH levels increased after UVB-induced burns in mice. They explained that it is an important strategy for preventing GSH depletion caused by UVB irradiation while also protecting against skin damage. Schreck *et al.* (1992) proposed that high levels of GSH could stabilise rather than scavenge radicals in the cell.

On the other hand, GST is an enzyme that plays an important role in detoxification. GST is best known for its ability to catalyse conjugation reactions between glutathione and harmful substances (Besnaci *et al.*, 2019). The high rate of ROS necessitates a high rate of GSH, and a low rate of GST, resulting in a well-organised antioxidant system (detoxification) under the influence of honey. Furthermore, CAT and GPX are two enzymes that contribute to the primary defence mechanism against ROS by catalysing the conversion of H_2O_2 to water (Franco *et al.*, 1999; Hirrlinger *et al.*, 2002). Antioxidants like CAT and GPX promote wound healing by eliminating free radicals (Honnegowda *et al.*, 2015). The negative control group exhibited the highest GPX activity. This increase was due to oxidative stress (late detoxification), as the organism has begun to produce GPX to catalyse the maximum reduction of H_2O_2. Catalase activity was significantly higher in the positive control than in the burn treated with honey; this could be due to the overproduction of this enzyme to regulate the dismutation of H_2O_2 during wound healing, which occurred late in this batch. The observed changes in oxidative stress markers indicated that the body was undergoing a metabolic adjustment in response to the burns, including an increased antioxidant response as part of the repair and recovery mechanisms.

Conclusion

Results indicated that the honey possessed various therapeutic properties, including its ability to inhibit the growth of the pathogenic bacteria most prevalent in infected wounds. In addition, the honey possessed anti-inflammatory properties that effectively reduced the discomfort associated with a skin burn. It has also been shown to have antioxidant and healing properties. Therefore, the honey can be used in the treatment of burns contaminated by bacteria that are resistant to many drugs. Nevertheless, further research is needed to elucidate the mechanism of action, and identify the specific factor responsible for honey's therapeutic effects.

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