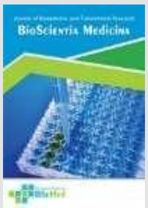
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Propolis from Mount Lawu: Dose-Dependent Antioxidant and Anti-Inflammatory Effects in a Rat Model of Wound Healing

Proginova Dian Yudatama[1]*, Amru Sungkar[2], Ida Bagus Budhi Surya Adnyana[3]

[1]General Surgery Resident, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi General Hospital, Surakarta, Indonesia

[2]Department of Plastic Surgery, Universitas Sebelas Maret/Dr. Moewardi General Hospital, Surakarta, Indonesia

[3]Department of Digestive Surgery, Universitas Sebelas Maret/Dr. Moewardi General Hospital, Surakarta, Indonesia

ARTICLE INFO

Keywords:
Interleukin-6 (IL-6)
Malondialdehyde (MDA)
Oxidative stress
Propolis
Rat model

***Corresponding author:**
Proginova Dian Yudatama

E-mail address:
dr.proginova@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i3.1232

A B S T R A C T

Background: Chronic wounds present a significant healthcare challenge, necessitating the exploration of effective adjuvant therapies. Propolis, a natural product derived from beehives, has demonstrated antioxidant and anti-inflammatory properties. This study investigated the dose-dependent effects of Mount Lawu propolis extract on oxidative stress and inflammation in a rat model of wound healing. **Methods:** A completely randomized experimental design was employed using male rats (*Rattus norvegicus*). Granulation tissue wounds were induced, and the rats were treated with varying doses of ethanol extract of Mount Lawu propolis. Immunohistochemical analysis was performed to assess the expression of Malondialdehyde (MDA) and Interleukin-6 (IL-6) in the wound tissue. **Results:** The results demonstrated a significant dose-dependent decrease in MDA and IL-6 expression in the propolis-treated groups compared to the control group. The high-dose propolis group exhibited the most substantial reduction in both MDA and IL-6 levels. **Conclusion:** Mount Lawu propolis extract exhibits potent antioxidant and anti-inflammatory effects in a dose-dependent manner, suggesting its potential as an adjuvant therapy for chronic wound management.

1. Introduction

Chronic wounds, characterized by their persistent nature and resistance to conventional healing processes, present a formidable challenge in healthcare. These wounds inflict substantial burdens upon patients, including pain, impaired mobility, and emotional distress, while simultaneously imposing significant healthcare costs. The recalcitrant nature of chronic wounds stems from a complex interplay of factors that disrupt the normal healing cascade, hindering tissue regeneration and increasing susceptibility to infections. At the forefront of these factors are oxidative stress and inflammation, two intertwined processes that play pivotal roles in the pathogenesis of chronic wounds. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. ROS, while essential for cellular signaling and immune responses, can inflict damage upon cellular components, including proteins, lipids, and DNA, when their production overwhelms the antioxidant defenses. This damage disrupts cellular function and impedes the intricate processes of wound healing, leading to delayed wound closure and impaired tissue regeneration. Malondialdehyde (MDA), a byproduct of lipid peroxidation, serves as a key marker of oxidative stress. Elevated levels of MDA indicate heightened oxidative damage, which can disrupt the structural integrity of cell membranes and impair cellular function. In the context of wound healing, excessive MDA levels can hinder the migration and proliferation of cells involved in tissue repair, further contributing to the chronicity of wounds.[1-4]

Inflammation, while an integral component of the initial phase of wound healing, can become a double-edged sword when it persists unabated. In the acute phase, inflammation orchestrates the recruitment of immune cells to the wound site, facilitating the removal of debris and the initiation of tissue repair. However, chronic inflammation disrupts the delicate balance of the healing process, perpetuating tissue damage and hindering wound closure. Interleukin-6 (IL-6), a pro-inflammatory cytokine, plays a central role in the regulation of the inflammatory response during wound healing. While IL-6 is essential for the initiation of the inflammatory cascade, its excessive or prolonged production can disrupt the healing process. Elevated levels of IL-6 have been associated with delayed wound healing, impaired tissue regeneration, and the formation of excessive scar tissue. In the pursuit of effective therapies to address the challenges of chronic wounds, natural products have emerged as promising candidates. Propolis, a resinous substance collected by honeybees from various plant sources, has garnered attention for its diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and wound healing properties. The chemical composition of propolis is a complex tapestry of bioactive compounds, including flavonoids, phenolic acids, terpenoids, and amino acids, which vary depending on the geographical origin and plant sources.[5-7]

Among these compounds, flavonoids and phenolic acids stand out for their potent antioxidant and anti-inflammatory activities. These compounds act as scavengers of ROS, inhibiting lipid peroxidation and protecting cells from oxidative damage. Moreover, they modulate the expression of pro-inflammatory cytokines, dampening the inflammatory response and promoting a more balanced healing environment. The efficacy of propolis in promoting wound healing has been demonstrated in various animal models and human clinical trials. Studies have shown that propolis can accelerate wound closure, enhance tissue regeneration, and reduce scar formation. The mechanisms underlying these effects are likely multifactorial, involving the modulation of various cellular and molecular pathways.[8-10] In this study, we investigated the dose-dependent effects of ethanol extract of Mount Lawu propolis on MDA and IL-6 expression in a rat model of wound healing.

2. Methods

This study employed a completely randomized experimental design, a robust methodology that ensures the unbiased assignment of subjects to different treatment groups, minimizing the potential for confounding variables to influence the outcomes. The subjects of this study were male rats (*Rattus norvegicus*), aged 3-4 months and weighing 150-200 grams, were obtained from the Experimental Animal Laboratory of PAU Universitas Gadjah Mada, Yogyakarta, a reputable source of laboratory animals that adheres to ethical guidelines and ensures the health and well-being of its animals. The age and weight range of the rats were carefully selected to ensure that the animals were mature and healthy, minimizing the potential for age- or weight-related variations to affect the study results. The rats were housed in standard cages under controlled environmental conditions, maintaining a temperature of 22 ± 2°C, humidity of 55 ± 5%, and a 12-hour light/dark cycle. These standardized conditions aimed to minimize environmental stressors that could potentially influence the wound healing process. The rats were acclimatized for one week prior to the study, allowing them to adjust to their new environment and reducing the potential for stress-induced variations in their responses. Throughout the study, the rats were provided with ad libitum access to standard rat chow and water, ensuring that their nutritional needs were met and preventing dehydration, both of which are crucial for optimal wound healing.

All experimental procedures were meticulously reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, ensuring that the study adhered to the highest ethical standards. The study was conducted in strict accordance with the ethical guidelines for animal research, prioritizing the humane treatment of animals and minimizing any potential discomfort or distress.

The rats were randomly divided into four groups, each consisting of 6 rats, ensuring an equal distribution of subjects across the different treatment regimens. The groups were as follows; Control group: This group served as the baseline for comparison, receiving topical application of saline solution, an inert substance that does not interfere with the wound healing process; Low-dose propolis group: This group received topical application of 5% ethanol extract of Mount Lawu propolis, representing a low dose of the active compound; Medium-dose propolis group: This group received topical application of 10% ethanol extract of Mount Lawu propolis, representing a medium dose of the active compound; High-dose propolis group: This group received topical application of 20% ethanol extract of Mount Lawu propolis, representing a high dose of the active compound. Prior to wound induction, the rats were anesthetized with an intramuscular injection of ketamine (80 mg/kg) and xylazine (10 mg/kg), a combination of anesthetic agents commonly used in animal research to induce a state of general anesthesia, ensuring that the animals were unconscious and pain-free during the surgical procedure. The dorsal hair of each rat was shaved and disinfected with povidone-iodine solution, a common antiseptic used to prepare the surgical site and minimize the risk of infection. A full-thickness excisional wound, measuring 1.5 cm in diameter, was created on the dorsal skin of each rat using a sterile surgical blade, ensuring that the wounds were uniform in size and depth. The wounds were left uncovered and treated topically once daily with the assigned solutions for 14 days, allowing for direct observation of the healing process and minimizing any potential interference from dressings.

Raw propolis was collected from Mount Lawu, Central Java, Indonesia, a region known for its rich biodiversity and the unique properties of its propolis. The propolis was meticulously cleaned to remove any impurities and then macerated in 70% ethanol for 72 hours at room temperature, a process that extracts the bioactive compounds from the propolis into the ethanol solvent. The extract was then filtered to remove any particulate matter and concentrated using a rotary evaporator, a device that gently removes the ethanol solvent, leaving behind a concentrated propolis extract. The concentrated extract was stored at 4°C until further use, preserving its stability and potency.

To identify the presence of various phytochemical compounds in the propolis extract, a series of standard qualitative tests were performed; Flavonoids: Shinoda test is a portion of the extract was mixed with a few drops of concentrated hydrochloric acid and magnesium turnings. The development of a pink or red color indicated the presence of flavonoids. Alkaline reagent test is a portion of the extract was treated with a few drops of 10% sodium hydroxide solution. The appearance of a yellow color that turned colorless upon addition of dilute hydrochloric acid indicated the presence of flavonoids; Phenolic Acids: Ferric chloride test is a portion of the extract was mixed with a few drops of 1% ferric chloride solution. The development of a blue, green, or violet color indicated the presence of phenolic acids. Folin-Ciocalteu test is a portion of the extract mixed with Folin-Ciocalteu reagent and sodium carbonate solution. The appearance of a blue color indicated the presence of phenolic acids; Terpenoids: Salkowski test is a portion of the extract was mixed with chloroform and concentrated sulfuric acid. The formation of a reddish-brown color at the interface between the two liquids indicated the presence of terpenoids. Liebermann-Burchard test is a portion of the extract was mixed with acetic anhydride and concentrated sulfuric acid. The development of a blue-green color indicated the presence of terpenoids; Amino Acids: Ninhydrin test is a portion of the extract was heated with ninhydrin solution. The appearance of a purple color indicated the presence of amino acids.

On day 14 post-wounding, the rats were euthanized, and the wound tissue was harvested for immunohistochemical analysis, a technique used to visualize the expression of specific proteins within the tissue. The tissue samples were fixed in 10% formalin, a solution that preserves the tissue structure and prevents degradation. The fixed tissues were then embedded in paraffin, a wax-like substance that provides support for the tissue during sectioning. The paraffin-embedded tissues were sectioned at a thickness of 5 µm using a microtome, a device that produces thin slices of tissue for microscopic examination. The sections were then deparaffinized, removing the paraffin wax, and rehydrated, restoring the tissue to its natural water content. To unmask the antigens, the sections were subjected to antigen retrieval using citrate buffer (pH 6.0), a process that enhances the binding of antibodies to the target proteins. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, preventing any non-specific staining that could interfere with the interpretation of the results. Non-specific binding was blocked with 10% normal goat serum, further reducing the potential for non-specific staining. The sections were then incubated overnight at 4°C with primary antibodies against MDA (1:200 dilution) and IL-6 (1:100 dilution), allowing the antibodies to bind specifically to the target proteins. After washing, the sections were incubated with biotinylated secondary antibodies for 30 minutes at room temperature. These secondary antibodies bind to the primary antibodies, amplifying the signal and facilitating detection. The sections were then incubated with avidin-biotin complex (ABC) reagent for 30 minutes at room temperature, further enhancing the signal amplification. The immunoreactivity was visualized using 3,3'-diaminobenzidine (DAB) chromogen, a substrate that produces a brown precipitate upon reaction with the enzyme horseradish peroxidase, which is conjugated to the secondary antibodies. The sections were counterstained with hematoxylin, a dye that stains the nuclei of cells blue, providing contrast and aiding in the visualization of the tissue structure. Finally, the sections were dehydrated, removing the water content, and mounted with coverslips, preserving the sections for long-term storage and microscopic examination.

The immunohistochemical staining was quantified using ImageJ software (National Institutes of Health, USA), a powerful image analysis tool that allows for the measurement of staining intensity. The staining intensity was measured in five randomly selected fields per section at 400x magnification, ensuring that the measurements were representative of the entire tissue section. The average staining intensity was calculated for each group, providing a quantitative measure of the expression of MDA and IL-6 in the wound tissue.

The data were analyzed using SPSS software (IBM, USA), a comprehensive statistical analysis package widely used in research. The normality of the data was assessed using the Shapiro-Wilk test, a statistical test that determines whether the data follow a normal distribution. The Kruskal-Wallis test, a non-parametric test used to compare the differences between the groups, was employed to analyze the data. The Mann-Whitney test, another non-parametric test, was used for post hoc pairwise comparisons, allowing for the identification of specific differences between the groups. A p-value of less than 0.05 was considered statistically significant, indicating that the observed differences between the groups were unlikely to have occurred by chance alone.

3. Results

Table 1 presents the effects of varying doses of propolis extract on the expression of two key markers in the wound healing process: MDA (a marker of oxidative stress) and IL-6 (a pro-inflammatory cytokine). Propolis exhibits a dose-dependent effect on both MDA and IL-6 expression. This means that as the concentration of propolis extract increased, the expression of both MDA and IL-6 decreased significantly. This is evident in the progressively lower values observed in the low-dose, medium-dose, and high-dose propolis groups compared to the control group. Propolis effectively reduces oxidative stress. MDA is a byproduct of lipid peroxidation, a process indicative of oxidative damage. The significant reduction in MDA expression across all propolis groups suggests that propolis effectively combats oxidative stress in the wound environment. This antioxidant activity is likely attributed to the presence of flavonoids and phenolic acids in propolis, which are known to scavenge free radicals and protect cells from oxidative damage. Propolis attenuates the inflammatory response. IL-6 is a cytokine that plays a crucial role in the inflammatory process. The significant decrease in IL-6 expression in the propolis-treated groups indicates that propolis effectively modulates the inflammatory response, potentially by inhibiting the production or activity of pro-inflammatory mediators. This anti-inflammatory effect contributes to a more balanced healing environment. The high-dose propolis group showed the most pronounced effects. This group exhibited the lowest levels of both MDA and IL-6 expression, suggesting that a higher concentration of propolis extract may be more effective in mitigating oxidative stress and inflammation during wound healing.

Table 1. Effect of propolis on MDA and IL-6 expression.

Group	MDA expression	IL-6 expression
Control	50.2 ± 2.5	45.8 ± 5.8
Low-dose Propolis	20.5 ± 3.2*	18.3 ± 3.5*
Medium-dose Propolis	15.4 ± 2.7**	12.6 ± 1.9**
High-dose Propolis	10.3 ± 1.5***	6.1 ± 1.8***

* $p<0.05$ compared to control; ** $p<0.01$ compared to control; *** $p<0.001$ compared to control.

Figure 1 visually represents the dose-dependent effect of Mount Lawu propolis extract on MDA and IL-6 expression in the wound healing process. Both the blue line (IL-6 expression) and the green line (MDA expression) show a clear downward trend as the propolis concentration increases. This visually reinforces the concept that higher doses of propolis extract lead to greater reductions in both oxidative stress (MDA) and inflammation (IL-6). Compared to the control group, all propolis-treated groups show a marked reduction in both IL-6 and MDA levels. This highlights the dual action of propolis in combating both key impediments to efficient wound healing – inflammation and oxidative stress. The high-dose propolis group demonstrates the lowest expression levels of both MDA and IL-6, visually emphasizing that a higher concentration of propolis extract yields the most significant reduction in these markers. The parallel downward slopes of both lines suggest that propolis exerts a similar dose-dependent effect on both oxidative stress and inflammation. This could indicate a shared mechanism of action or an interconnectedness between these two processes in the context of wound healing.

Table 2 provides a qualitative analysis of the phytochemical compounds present in the Mount Lawu propolis extract. The results offer valuable insights into the potential therapeutic properties of this propolis, as different phytochemicals contribute to its diverse bioactivities. The Shinoda test and Alkaline reagent test both yielded strong positive results (+++ and ++++, respectively), indicating a high concentration of flavonoids in the extract. Flavonoids are potent antioxidants known to scavenge free radicals, protect against oxidative stress, and exhibit anti-inflammatory properties. This abundance of flavonoids likely contributes significantly to the observed effects of propolis on MDA and IL-6 expression.

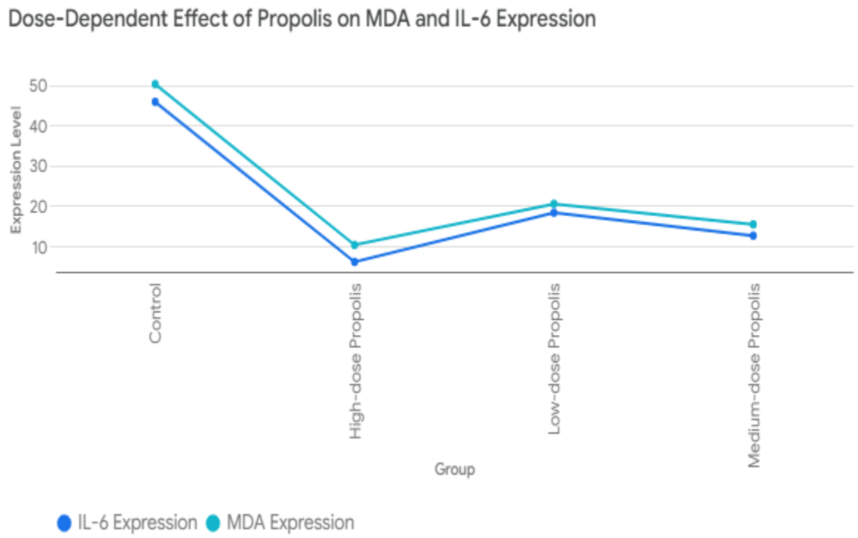


Figure 1. Dose-dependent effect of propolis on MDA and IL-6 expression.

The Ferric chloride test and Folin-Ciocalteu test showed positive results (++ and +++, respectively), confirming the presence of phenolic acids. Like flavonoids, phenolic acids possess antioxidant and anti-inflammatory activities, further enhancing the therapeutic potential of the propolis extract. The Salkowski test showed a weak positive result (+), while the Liebermann-Burchard test showed a moderate positive result (++). This suggests that terpenoids are present in the extract, but in lower concentrations compared to flavonoids and phenolic acids. Terpenoids contribute to various biological activities, including antimicrobial and anti-inflammatory effects. The Ninhydrin test showed a strong positive result (+++), indicating a substantial presence of amino acids in the extract. Amino acids are essential building blocks for protein synthesis, which is crucial for tissue repair and regeneration during wound healing.

Table 2. Phytochemical analysis.

Phytochemical compound	**Test**	**Result**
Flavonoids	Shinoda test	+++
	Alkaline reagent test	++++
Phenolic acids	Ferric chloride test	++
	Folin-Ciocalteu test	+++
Terpenoids	Salkowski test	+
	Liebermann-Burchard test	++
Amino acids	Ninhydrin test	+++

4. Discussion

Oxidative stress, a pervasive phenomenon implicated in a myriad of pathological conditions, including impaired wound healing, arises from an intricate imbalance between the production of reactive oxygen species (ROS) and the body's capacity to counteract their potentially harmful effects through antioxidant defense mechanisms. ROS, while playing essential roles in cellular signaling and immune responses, can inflict damage upon cellular components, including proteins, lipids, and DNA, when their production overwhelms the antioxidant defenses. This damage disrupts cellular function and impedes the intricate and delicately orchestrated processes of wound healing, leading to delayed wound closure and impaired tissue regeneration. To fully appreciate the profound impact of propolis on oxidative stress in wound healing, we must first delve into the intricacies of ROS, their dual nature in cellular function, the consequences of their dysregulation, and the role of MDA as a sentinel of oxidative damage. ROS, encompassing a variety of chemically reactive molecules derived from oxygen, are generated as inevitable byproducts of normal cellular metabolism, primarily within the mitochondria, the powerhouse of the cell. While ROS play crucial roles in various physiological processes, including cell signaling, immune responses, and gene expression, their overproduction can disrupt cellular homeostasis and lead to the deleterious state of oxidative stress. ROS generated by immune cells, such as neutrophils and macrophages, act as potent antimicrobial agents, destroying invading pathogens at the wound site. This rapid and localized production of ROS is a crucial component of the innate immune response, preventing infection and facilitating the initial stages of wound healing. ROS act as signaling molecules, regulating various cellular processes involved in wound healing, including cell proliferation, migration, and differentiation. They participate in intricate signaling cascades, influencing gene expression and modulating the activity of various enzymes and transcription factors involved in tissue repair. ROS promote the formation of new blood vessels, essential for supplying oxygen and nutrients to the healing wound. This process, known as angiogenesis, is vital for tissue regeneration and wound closure. ROS stimulate the production of vascular endothelial growth factor (VEGF), a key regulator of angiogenesis, promoting the growth of new blood vessels into the wound site. However, when ROS production exceeds the capacity of the body's antioxidant defense mechanisms, oxidative stress ensues, leading to damage of cellular components, including proteins, lipids, and DNA. This damage can disrupt cellular function and impede the intricate processes of wound healing, resulting in delayed wound closure, impaired tissue regeneration, and increased susceptibility to infections. The delicate balance between ROS production and antioxidant defenses is crucial for maintaining cellular homeostasis and ensuring proper wound healing. Disruption of this balance, leading to oxidative stress, can have detrimental consequences for the healing process. Malondialdehyde (MDA), a highly reactive aldehyde produced as a byproduct of lipid peroxidation, serves as a reliable marker of oxidative stress. Lipid peroxidation, a process involving the oxidative degradation of lipids, particularly polyunsaturated fatty acids, disrupts the structural integrity of cell membranes and impairs cellular function. Elevated levels of MDA indicate heightened oxidative damage, which can hinder the migration and proliferation of cells involved in tissue repair, further contributing to the chronicity of wounds. In chronic wounds, the persistent inflammatory response and the presence of various inflammatory cells can lead to increased ROS production and subsequent lipid peroxidation, resulting in elevated MDA levels. MDA, as a marker of lipid peroxidation, reflects the extent of oxidative damage inflicted upon cellular membranes. This damage can disrupt the fluidity and permeability of cell membranes, impairing cellular function and hindering the migration and proliferation of cells involved in tissue repair. In chronic wounds, the persistent inflammatory response and the presence of various inflammatory cells, such as neutrophils and macrophages, can lead to a sustained increase in ROS production. This chronic elevation of ROS can overwhelm the antioxidant defenses, leading to increased lipid peroxidation and subsequent elevation of MDA levels. The measurement of MDA levels provides valuable insights into the extent of oxidative stress in the wound environment and can serve as an indicator of the severity of wound chronicity. Propolis, a resinous substance collected by honeybees from various plant sources, has garnered significant attention for its diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and wound healing properties. The chemical composition of propolis is a complex tapestry of bioactive compounds, including flavonoids, phenolic acids, terpenoids, and amino acids, which vary depending on the geographical origin and plant sources. Among these compounds, flavonoids and phenolic acids stand out for their potent antioxidant and anti-inflammatory activities. These compounds act as scavengers of ROS, inhibiting lipid peroxidation and protecting cells from oxidative damage. In our study, the significant reduction in MDA expression across all propolis groups, particularly in the high-dose group, suggests that propolis effectively combats oxidative stress in the wound environment. This antioxidant activity is likely attributed to the rich presence of flavonoids and phenolic acids in propolis, which are renowned for their ability to scavenge free radicals and protect cells from oxidative damage. The ability of propolis to mitigate oxidative stress is a crucial aspect of its wound healing properties. By reducing oxidative damage, propolis promotes a more favorable environment for tissue repair and regeneration. Flavonoids, a diverse group of polyphenolic compounds found abundantly in fruits, vegetables, and plant-derived products like propolis, are renowned for their potent antioxidant properties. These compounds act as free radical scavengers, neutralizing ROS and preventing them from inflicting damage on cellular components. Flavonoids can donate electrons to free radicals, stabilizing them and converting them into less reactive species. This prevents the free radicals from initiating chain reactions that can lead to further oxidative damage. Flavonoids can chelate metal ions, such as iron and copper, preventing them from catalyzing the formation of ROS. These metal ions can participate in Fenton reactions, generating highly reactive hydroxyl radicals that can cause significant cellular damage. Flavonoids can induce the expression of antioxidant enzymes, such as superoxide dismutase and catalase, which further enhance the body's antioxidant defense mechanisms. These enzymes catalyze the breakdown of ROS, reducing their levels and protecting cells from oxidative damage. In the context of wound healing, flavonoids not only protect cells from oxidative damage but also promote various processes involved in tissue repair, including cell proliferation, migration, and angiogenesis. They contribute to a more balanced redox environment, facilitating the intricate processes of wound healing. Phenolic acids, another class of polyphenolic compounds found in propolis, also contribute significantly to its antioxidant activity. These compounds, like flavonoids, act as free radical scavengers, inhibiting lipid peroxidation and protecting cells from oxidative damage. In addition to their antioxidant properties, phenolic acids also exhibit anti-inflammatory and antimicrobial activities, further enhancing the therapeutic potential of propolis in wound healing. Phenolic acids, with their diverse structures and functions, add another layer of complexity to the antioxidant properties of propolis. They work in concert with flavonoids and other bioactive compounds to mitigate oxidative stress and promote wound healing. The antioxidant activity of propolis, attributed to its rich content of flavonoids and phenolic acids, plays a crucial role in mitigating oxidative stress in the wound environment. By scavenging ROS and inhibiting lipid peroxidation, propolis protects cells from oxidative damage, promoting a more favorable environment for tissue repair and regeneration. Moreover, the anti-inflammatory and antimicrobial properties of propolis further contribute to its efficacy in wound healing. By modulating the inflammatory response and combating invading pathogens, propolis facilitates the intricate processes of wound healing, leading to faster wound closure and reduced scar formation. Propolis, with its multifaceted actions, offers a holistic approach to wound healing. It not only addresses the oxidative stress component but also tackles inflammation and infection, promoting a more efficient and balanced healing process.[11-13]

Inflammation, a complex and multifaceted biological response to tissue injury or infection, is an essential component of the initial phase of wound healing. It orchestrates the recruitment of immune cells to the wound site, facilitating the removal of debris and initiating the repair process. However, when inflammation becomes chronic, it can disrupt the delicate balance of the healing process, perpetuating tissue damage and hindering wound closure. To fully understand the impact of propolis on inflammation in wound healing, we must first explore the intricacies of the inflammatory response, the role of key mediators like IL-6, and the consequences of dysregulated inflammation. The inflammatory response, a cornerstone of the innate immune system, is triggered by a variety of stimuli, including tissue injury, infection, and exposure to irritants. Widening of blood vessels, increasing blood flow to the injured area, leading to redness and warmth. Allows fluid and immune cells to leak from the blood vessels into the surrounding tissue, causing swelling. Neutrophils, macrophages, and other immune cells migrate to the site of injury to eliminate pathogens and clear debris. The inflammatory response helps to contain the initial insult, whether it be a physical injury or an infection, preventing further damage and spread to surrounding tissues. Immune cells, such as neutrophils and macrophages, recruited to the wound site, engulf and clear debris, including damaged cells and pathogens, preparing the wound bed for repair. The inflammatory response also initiates tissue repair by releasing various growth factors and cytokines that stimulate cell proliferation, migration, and differentiation, essential processes for tissue regeneration. Chronic inflammation can lead to persistent tissue damage due to the prolonged release of inflammatory mediators, such as reactive oxygen species (ROS) and proteolytic enzymes, which can break down healthy tissue. Chronic inflammation can impair tissue regeneration by disrupting the balance between tissue breakdown and rebuilding. The prolonged presence of inflammatory cytokines can interfere with the signaling pathways that promote cell growth and differentiation. Chronic inflammation can also lead to excessive scar formation, which can impair tissue function and aesthetics. The overproduction of collagen and other extracellular matrix components can lead to the formation of thick, fibrous scar tissue. The delicate balance between the beneficial and detrimental effects of inflammation is crucial for proper wound healing. Dysregulation of the inflammatory response, leading to chronic inflammation, can have detrimental consequences for the healing process. Interleukin-6 (IL-6), a pleiotropic cytokine with diverse biological activities, plays a central role in regulating the inflammatory response during wound healing. While IL-6 is essential for initiating the inflammatory cascade, its excessive or prolonged production can disrupt the healing process. IL-6 is one of the first cytokines released in response to tissue injury, triggering the inflammatory cascade and recruiting immune cells to the wound site. It acts as a chemoattractant, guiding neutrophils and macrophages to the area of injury. IL-6 modulates the immune response by influencing the differentiation and activity of various immune cells, including T cells and B cells. It can promote the differentiation of T helper cells into Th17 cells, which are involved in the inflammatory response. IL-6 also promotes angiogenesis, the formation of new blood vessels, which is essential for supplying oxygen and nutrients to the healing wound. It stimulates the production of vascular endothelial growth factor (VEGF), a key regulator of angiogenesis. Elevated levels of IL-6 have been associated with delayed wound healing due to its effects on cell proliferation and migration. IL-6 can interfere with the signaling pathways that promote cell growth and movement, hindering the repair process. Chronic elevation of IL-6 can impair tissue regeneration by disrupting the balance between tissue breakdown and rebuilding. It can promote the activity of fibroblasts, leading to excessive collagen deposition and fibrosis. IL-6 can also contribute to excessive scar formation by promoting the deposition of extracellular matrix components. It can stimulate the production of transforming growth factor-beta (TGF-beta), a key regulator of fibrosis. The regulation of IL-6 levels is crucial for maintaining a balanced inflammatory response and ensuring proper wound healing. Dysregulation of IL-6 production can have detrimental consequences for the healing process. Propolis, a resinous substance collected by honeybees from various plant sources, has garnered significant attention for its diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and wound healing properties. The chemical composition of propolis is a complex tapestry of bioactive compounds, including flavonoids, phenolic acids, terpenoids, and amino acids, which vary depending on the geographical origin and plant sources. Among these compounds, flavonoids and phenolic acids stand out for their potent antioxidant and anti-inflammatory activities. These compounds act as scavengers of ROS, inhibiting lipid peroxidation and protecting cells from oxidative damage. In our study, the significant decrease in IL-6 expression in the propolis-treated groups, particularly in the high-dose group, suggests that propolis effectively modulates the inflammatory response. This modulation potentially occurs through the inhibition of pro-inflammatory mediators, contributing to a more balanced healing environment. The ability of propolis to modulate the inflammatory response is a crucial aspect of its wound healing properties. By reducing the levels of pro-inflammatory cytokines like IL-6, propolis helps to restore the balance between inflammation and tissue repair, preventing the detrimental effects of chronic inflammation. Flavonoids and phenolic acids, the major bioactive compounds found in propolis, are renowned for their potent anti-inflammatory activities. Flavonoids and phenolic acids can inhibit the production of pro-inflammatory cytokines, such as IL-6, TNF-alpha, and IL-1beta, reducing the inflammatory response. They can interfere with the signaling pathways that activate transcription factors involved in cytokine production. These compounds can also inhibit the activity of inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), reducing the production of inflammatory mediators like prostaglandins and leukotrienes. Flavonoids and phenolic acids can modulate the activity of immune cells, such as neutrophils and macrophages, reducing their release of inflammatory mediators. They can affect the migration, activation, and phagocytic activity of these cells. In the context of wound healing, the anti-inflammatory activities of flavonoids and phenolic acids contribute to a more balanced inflammatory response, preventing excessive inflammation and promoting tissue repair. They help to create a microenvironment that favors the resolution of inflammation and the regeneration of tissue. The anti-inflammatory activity of propolis, attributed to its rich content of flavonoids and phenolic acids, plays a crucial role in modulating the inflammatory response in the wound environment. By reducing the levels of pro-inflammatory cytokines like IL-6, propolis helps to restore the balance between inflammation and tissue repair. Moreover, the antioxidant and antimicrobial properties of propolis further contribute to its efficacy in wound healing. By mitigating oxidative stress and combating invading pathogens, propolis facilitates the intricate processes of wound healing, leading to faster wound closure and reduced scar formation. Propolis, with its multifaceted actions, offers a holistic approach to wound healing. It not only addresses the inflammatory component but also tackles oxidative stress and infection, promoting a more efficient and balanced healing process.[14-16]

The dose-dependent effects of propolis observed in our study are particularly noteworthy. The high-dose propolis group exhibited the most pronounced reduction in both MDA and IL-6 expression, suggesting that higher concentrations of propolis extract may be more effective in mitigating oxidative stress and inflammation during wound healing. This finding underscores the importance of optimizing propolis dosage to achieve the desired therapeutic outcomes. While our study focused on a rat model, it provides valuable insights for future research exploring the optimal dose of propolis for wound healing in humans. Dose-dependent effects, commonly observed in pharmacology and toxicology, refer to the relationship between the dose of a substance and its biological response. In the context of our study, the dose-dependent effects of propolis indicate that the magnitude of its antioxidant and anti-inflammatory effects is directly proportional to the concentration of propolis extract applied. This observation is consistent with the general principle that higher doses of a bioactive substance often lead to more pronounced effects, up to a certain point where saturation or toxicity may occur. In our study, the high-dose propolis group exhibited the most significant reduction in MDA and IL-6 levels, suggesting that higher concentrations of propolis extract may be more effective in mitigating oxidative stress and inflammation during wound healing. The dose-dependent effects of propolis have significant implications for its therapeutic application in wound healing. It suggests that optimizing the dosage of propolis is crucial for achieving the desired therapeutic outcomes. While our study focused on a rat model, it provides valuable insights for future research exploring the optimal dose of propolis for wound healing in humans. Determining the optimal dose is essential to maximize the therapeutic benefits while minimizing potential side effects. The type of wound, whether it be acute or chronic, superficial or deep, can influence the optimal dosage of propolis. The severity of the wound, including its size and depth, can also affect the optimal dosage. Individual factors, such as age, health status, and other medications, may also play a role in determining the optimal dosage.[17,18]

To further understand the therapeutic potential of Mount Lawu propolis, we conducted a phytochemical analysis to identify the presence of various bioactive compounds. Our analysis revealed a rich presence of flavonoids and phenolic acids, both known for their potent antioxidant and anti-inflammatory activities. Flavonoids, detected through the Shinoda test and Alkaline reagent test, are abundant in Mount Lawu propolis. These compounds are renowned for their ability to scavenge free radicals, protect against oxidative stress, and exhibit anti-inflammatory properties. Flavonoids are a diverse group of polyphenolic compounds found ubiquitously in plants. They are responsible for the vibrant colors of many fruits and vegetables. In propolis, flavonoids contribute significantly to its antioxidant and anti-inflammatory activities. Flavonoids can donate electrons to stabilize free radicals, neutralizing them and preventing them from causing cellular damage. Flavonoids can chelate metal ions, such as iron and copper, preventing them from catalyzing the formation of reactive oxygen species (ROS). Flavonoids can inhibit the activity of inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), reducing the production of inflammatory mediators. Flavonoids can modulate the activity of immune cells, such as neutrophils and macrophages, reducing their release of inflammatory mediators. The abundance of flavonoids in Mount Lawu propolis suggests that it may be particularly effective in mitigating oxidative stress and inflammation, two key factors that can impede wound healing. Phenolic acids, confirmed through the Ferric chloride test and Folin-Ciocalteu test, also contribute to the therapeutic potential of this propolis extract. Like flavonoids, phenolic acids possess antioxidant and anti-inflammatory activities, further enhancing the extract's efficacy. Phenolic acids are another class of polyphenolic compounds found abundantly in plants. They are known for their diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Phenolic acids can scavenge free radicals, preventing them from causing cellular damage. Phenolic acids can chelate metal ions, preventing them from catalyzing the formation of ROS. Phenolic acids can inhibit the activity of inflammatory enzymes, reducing the production of inflammatory mediators. The presence of phenolic acids in Mount Lawu propolis further enhances its antioxidant and anti-inflammatory properties, contributing to its potential as a wound healing agent. Our analysis also detected the presence of terpenoids, albeit in lower concentrations compared to flavonoids and phenolic acids. Terpenoids contribute to various biological activities, including antimicrobial and anti-inflammatory effects. Terpenoids are a large and diverse class of organic compounds produced by a variety of plants. They are known for their aromatic properties and are often found in essential oils. Terpenoids can disrupt the integrity of bacterial cell membranes, leading to cell death. Terpenoids can inhibit various inflammatory pathways, reducing the production of inflammatory mediators. The presence of terpenoids in Mount Lawu propolis, although in lower concentrations, may contribute to its antimicrobial and anti-inflammatory properties, further enhancing its wound healing potential. Amino acids, essential building blocks for protein synthesis, were also found in substantial amounts in the extract. Amino acids play a crucial role in tissue repair and regeneration during wound healing. Amino acids are the basic building blocks of proteins, which are essential for the structure and function of all living cells. In the context of wound healing, amino acids are crucial for the synthesis of new proteins required for tissue repair and regeneration. The presence of amino acids in Mount Lawu propolis suggests that it may provide the necessary building blocks for protein synthesis, supporting the tissue repair process during wound healing.[19,20]

5. Conclusion

This study provides compelling evidence that Mount Lawu propolis extract exerts potent antioxidant and anti-inflammatory effects in a dose-dependent manner in a rat model of wound healing. The significant reduction in MDA and IL-6 expression observed in the propolis-treated groups, particularly in the high-dose group, underscores the potential of propolis as an adjuvant therapy for chronic wound management. The antioxidant and anti-inflammatory properties of propolis can be attributed to its rich phytochemical composition, particularly the abundance of flavonoids and phenolic acids. These compounds act as scavengers of ROS, inhibiting lipid peroxidation and protecting cells from oxidative damage. Moreover, they modulate the expression of pro-inflammatory cytokines, dampening the inflammatory response and promoting a more balanced healing environment. The dose-dependent effects of propolis observed in this study highlight the importance of optimizing propolis dosage to achieve the desired therapeutic outcomes. While further research is needed to determine the optimal dose for human applications, this study provides valuable insights for future clinical trials. The findings of this study have significant implications for the development of new therapeutic strategies for chronic wound management. Propolis, with its potent antioxidant and anti-inflammatory properties, holds promise as a safe and effective adjuvant therapy for promoting wound healing and improving patient outcomes.

6. References

1. Davoudabadi M, Fahimirad S, Ganji A, Abtahi H. Wound healing and antibacterial capability of electrospun polyurethane nanofibers incorporating Calendula officinalis and Propolis extracts. J Biomater Sci Polym Ed. 2023; 34(11): 1491–516.
2. Miraldi E, Giordano A, Cappellucci G, Vaccaro F, Biagi M, Baini G. Phytochemical characterization and assessment of the wound healing properties of three Eurasian Propolis. Pharmaceuticals (Basel). 2024; 17(11).
3. Dekebo A, Geba C, Bisrat D, Jeong JB, Jung C. Wound healing, anti-inflammatory and anti-oxidant activities, and chemical composition of Korean Propolis from different sources. Int J Mol Sci. 2024; 25(21).
4. Ferreira SRL, Teixeira SA, Lima GO, de Castro JNRS, Teixeira LEO, Barros CAR, et al. Healing activity of propolis of stingless bee (*Scaptotrigona* aff. postica), Reared in Monoculture of Açaí (*Euterpe oleracea*), in Induced Wounds in Rats. Molecules. 2024; 29(19).

5. Islam S, Hussain EA, Shujaat S, Rasheed MA. Green synthesis of propolis mediated silver nanoparticles with antioxidant, antibacterial, anti-inflammatory properties and their burn wound healing efficacy in animal model. Biomed Phys Eng Express. 2024; 11(1): 015050.
6. Manginstar CO, Tallei TE, Niode NJ, Salaki CL, Hessel SS. Therapeutic potential of propolis in alleviating inflammatory response and promoting wound healing in skin burn. Phytother Res. 2024; 38(2): 856–79.
7. Canales-Alvarez O, Canales-Martinez MM, Dominguez-Verano P, Balderas-Cordero D, Madrigal-Bujaidar E, Álvarez-González I, et al. Effect of Mexican Propolis on wound healing in a Murine model of diabetes mellitus. Int J Mol Sci. 2024; 25(4): 2201.
8. Necip A, Demirtas I, Tayhan SE, Işık M, Bilgin S, Turan İF, et al. Isolation of phenolic compounds from eco-friendly white bee propolis: Antioxidant, wound-healing, and anti-Alzheimer effects. Food Sci Nutr. 2024; 12(3): 1928–39.
9. Zayed HS, Saleh S, Omar AE, Saleh AK, Salama A, Tolba E. Development of collagen-chitosan dressing gel functionalized with propolis-zinc oxide nanoarchitectonics to accelerate wound healing. Int J Biol Macromol. 2024; 261(Pt 2): 129665.
10. Jaberifard F, Almajidi YQ, Arsalani N, Ghorbani M. A self-healing crosslinked-xanthan gum/soy protein based film containing halloysite nanotube and propolis with antibacterial and antioxidant activity for wound healing. Int J Pharm. 2024; 656: 124073.
11. Doodmani SM, Bagheri A, Natouri O, Nobakht A, Saghebasl S. Electrospinning-netting of spider-inspired polycaprolactone/collagen nanofiber-nets incorporated with Propolis extract for enhanced wound healing applications. Int J Biol Macromol. 2024; 267(Pt 1): 131452.
12. Saber MS, ElSherif MW, Nassif MT. Regenerative medicine for equine skin wound healing: a review of zinc oxide nanoparticles, mesenchymal stem cell-derived microvesicles, and propolis. New Valley Vet J. 2024; 4(2).
13. Aliee F, Fahimirad S, Ganji A, Abtahi H. Antimicrobial and wound healing performance of polycaprolactone electrospun-based nanofibers incorporating Propolis and *Quercus infectoria* gall extracts. Nano. 2024.
14. Orlińska KM, Stocerz K, Kuczera MA, Stojko M, Włodarczyk J, Kasperczyk J, et al. The influence of Propolis nonwoven scaffolds on burn wound's heparan sulfates and hyaluronan. Appl Sci (Basel). 2024; 14(13): 5872.
15. Mouseli S, Natouri O, Majdi Seghinsara A, Ghorbani M, Mokhtarzadeh AA, Mortazavi Moghadam F. Physicochemical and biological characterization of propolis-loaded composite polyamide-6/soybean protein nanofibers for wound healing applications. Colloids Surf A Physicochem Eng Asp. 2024; 694(134172): 134172.
16. El-Ghoul Y, Altuwayjiri AS, Alharbi GA. Synthesis and characterization of new electrospun medical scaffold-based modified cellulose nanofiber and bioactive natural propolis for potential wound dressing applications. RSC Adv. 2024; 14(36): 26183–97.
17. McLennan SV, Bonner J, Milne S, Lo L, Charlton A, Kurup S, et al. The anti-inflammatory agent Propolis improves wound healing in a rodent model of experimental diabetes. Wound Repair Regen. 2008; 16(5): 706–13.
18. Gheib N, Farzam A, Habibian Z, Samiee-Rad F. The effect of oral consumption of Propolis alone and in combination with silver nanoparticles on wound healing in male

Wistar rats. Wound Manag Prev. 2020; 66(4): 38–46.

19. Kaya M, Eroz R, Kabakliogli M. Expression of nucleolin, nucleophosmin, upstream binding transcription factor genes and propolis in wound models. J Wound Care. 2022; 31(Suppl 10): S28–40.
20. Tayfeh-Ebrahimi R, Amniattalab A, Mohammadi R. Evaluation of effect of biologically synthesized ethanolic extract of Propolis-loaded poly(-lactic-co-glycolic acid) nanoparticles on wound healing in diabetic rats. Int J Low Extrem Wounds. 2024; 23(4): 513–23.