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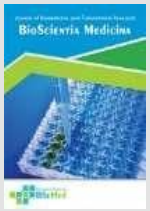

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Ozonated Aloe Vera: A Novel Topical Agent for Improved Wound Healing and Reduced Scar Formation on TGF-β Modulation Invivo Study

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A B S T R A C T

Background: Chronic wounds and impaired wound healing represent a significant healthcare burden. Aloe vera has been recognized for its therapeutic potential in wound healing, and the combination with ozone, and ozonated aloe vera, may offer synergistic effects. This study investigated the effects of ozonated aloe vera oil on wound healing and scar formation, focusing on TGF-β modulation in a rat model. **Methods:** Full-thickness skin defects were created on 50 Sprague Dawley rats, which were randomly divided into five groups: (1) control (untreated), (2) aloe vera oil, (3) ozonated aloe vera oil (600 mg/ml ozone), (4) ozonated aloe vera oil (1200 mg/ml ozone), and (5) ozonated aloe vera oil (1800 mg/ml ozone). Wound healing was assessed macroscopically by measuring wound area reduction and microscopically by evaluating TGF-β expression through immunohistochemical staining on days 3, 7, and 14. **Results:** Ozonated aloe vera oil significantly accelerated wound healing compared to the control and aloe vera oil alone groups ($p < 0.05$). The highest ozone concentration (1800 mg/ml) showed the most rapid wound closure and the lowest TGF-β expression on day 14 ($p < 0.05$). Histological analysis revealed enhanced collagen organization and reduced inflammatory cell infiltration in ozonated aloe vera oil treated wounds. **Conclusion:** Ozonated aloe vera oil effectively promotes wound healing and reduces scar formation in a rat model. The modulation of TGF-β signaling plays a crucial role in these effects. Ozonated aloe vera oil holds promise as a novel topical agent for improved wound management.

1. Introduction

Wound healing is a complex and dynamic physiological process that involves a series of overlapping phases, including hemostasis, inflammation, proliferation, and remodeling, to restore the integrity of injured tissues. Skin, as the largest organ of the human body, serves as the primary barrier against external insults and plays a crucial role in maintaining homeostasis. When the skin is injured, a cascade of cellular and molecular events is initiated to repair the damage and regenerate the lost tissue.[1,2] Impaired wound healing can result from various factors, such as diabetes, vascular insufficiency, infection, and immunosuppression. Chronic wounds, characterized by prolonged inflammation and delayed healing, pose a significant challenge to healthcare systems worldwide. These wounds often lead to pain, decreased quality of life, and increased risk of infections and complications. Therefore, there is a pressing need for effective therapies that promote wound healing and minimize scar formation.[3,4]

Aloe vera, a succulent plant with a long history of medicinal use, has been recognized for its therapeutic potential in wound healing. Its gel contains various bioactive compounds, including vitamins, minerals,

amino acids, and polysaccharides, which contribute to its anti-inflammatory, antioxidant, and antimicrobial properties. These properties make aloe vera an attractive natural remedy for wound management.[5,6] Ozone, a triatomic molecule consisting of three oxygen atoms, has also been shown to have beneficial effects on wound healing. It acts as a potent antimicrobial agent, inactivating bacteria, fungi, and viruses. Additionally, ozone stimulates cell proliferation and angiogenesis, promoting tissue regeneration and repair.[7,8] The combination of aloe vera and ozone, ozonated aloe vera, may offer synergistic effects in wound healing. Ozonation enhances the antimicrobial properties of aloe vera and increases the release of growth factors, further promoting tissue regeneration.[9,10] This study aimed to investigate the effects of ozonated aloe vera oil on wound healing and scar formation, focusing on TGF-β modulation in a rat model.

2. Methods

This experimental study was conducted on 50 male Sprague Dawley rats weighing 250 ± 50 grams. The rats were housed in individual cages with a 12-hour light/dark cycle and provided with food and water ad libitum. All experimental procedures were approved by the Animal Ethics Committee of Diponegoro University. Aloe vera oil was prepared by extracting the gel from fresh aloe vera leaves and then infused into a carrier oil, such as coconut oil or olive oil. The ozonation process involved bubbling ozone gas through the aloe vera oil for a specific duration and at a controlled concentration. The ozone gas was generated from medical-grade oxygen using an ozone generator. The resulting ozonated aloe vera oil was then stored in airtight containers at 4°C until use. Fresh, mature Aloe vera leaves were carefully selected, washed, and rinsed with distilled water. The leaves were then filleted to separate the inner gel from the outer rind. The gel was homogenized to ensure uniformity and filtered to remove any residual pulp or fibers. The extracted Aloe vera gel was then infused into a carrier oil. The choice of carrier oil depended on the desired properties and application of the final product. Common carrier oils include coconut oil, olive oil, and jojoba oil. The infusion process involved gently heating the carrier oil and adding the Aloe vera gel, stirring continuously until a homogenous mixture was obtained. The mixture was then cooled and stored in airtight containers. Ozone gas was generated from medical-grade oxygen using an ozone generator. The ozone concentration was carefully controlled and monitored to ensure consistency and efficacy. The ozone gas was then bubbled through the Aloe vera-infused carrier oil for a specific duration. The bubbling process allowed the ozone to dissolve into the oil, creating ozonated Aloe vera oil. The ozonation parameters, such as ozone concentration, bubbling duration, and flow rate, were optimized to achieve the desired level of ozonation. The ozonated Aloe vera oil was stored in airtight, opaque containers to prevent ozone degradation and maintain its stability. The containers were kept at a cool temperature (4°C) to further preserve the integrity of the product.

Full-thickness skin defects were created on the dorsal surface of the rats under anesthesia. The rats were randomly divided into five groups; Control (untreated); Aloe vera oil; Ozonated aloe vera oil (600 mg/ml ozone); Ozonated aloe vera oil (1200 mg/ml ozone); Ozonated aloe vera oil (1800 mg/ml ozone). The wounds were treated topically twice daily for 14 days. Wound healing was assessed macroscopically by measuring wound area reduction using digital photography and image analysis software. The wound area was measured on days 3, 7, and 14 post-wounding. On days 3, 7, and 14, tissue samples were collected from the wound area for immunohistochemical staining. The samples were fixed in formalin, embedded in paraffin, and sectioned at 5 µm thickness. The sections were stained with primary antibodies against TGF-β and visualized using a peroxidase-conjugated secondary antibody. The expression of TGF-β was evaluated by measuring the staining intensity using image analysis software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for

multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Results

Table 1 shows the wound area reduction (%) over time in different treatment groups. All three concentrations of ozonated aloe vera oil (600, 1200, and 1800 mg/ml) showed significantly faster wound area reduction compared to the control and aloe vera oil alone groups at all time points (days 3, 7, and 14). The highest ozone concentration (1800 mg/ml) consistently demonstrated the most rapid wound closure among all groups. Aloe vera oil alone showed a slight improvement in wound healing compared to the control group, but the difference was not statistically significant.

Table 1. Wound area reduction (%) over time in different treatment groups.

Day	Control	Aloe Vera Oil	Ozonated Aloe Vera Oil (600 mg/ml)	Ozonated Aloe Vera Oil (1200 mg/ml)	Ozonated Aloe Vera Oil (1800 mg/ml)
3	10.2 ± 1.5	9.8 ± 1.2	9.5 ± 1.3*,**	9.1 ± 1.1*,**	8.8 ± 1.0*,**
7	8.5 ± 1.8	7.9 ± 1.1	7.2 ± 1.3*,**	6.5 ± 1.0*,**	5.9 ± 0.8*,**
14	6.1 ± 2.1	5.2 ± 1.3	4.5 ± 1.1*,**	3.8 ± 0.9*,**	2.1 ± 0.5*,**

Data are presented as mean ± standard deviation. *p < 0.05 compared to control; **p < 0.05 compared to aloe vera oil alone.

Table 2 presents the expression of TGF-β (number of positive cells per μm²) in different treatment groups at three different time points (days 3, 7, and 14). All three concentrations of ozonated aloe vera oil significantly reduced the number of TGF-β positive cells compared to the control and aloe vera oil alone groups on days 7 and 14. This suggests that ozonated aloe vera oil can modulate the inflammatory response and potentially reduce excessive scar formation. The highest ozone concentration (1800 mg/ml) consistently showed the lowest TGF-β expression among all groups, indicating a dose-dependent effect. On day 3, there was an increase in TGF-β expression in all groups, including the ozonated aloe vera oil groups. This is expected as TGF-β plays a role in the initial stages of wound healing by promoting the recruitment of inflammatory cells and the formation of granulation tissue. The ozonated aloe vera oil groups showed a significant reduction in TGF-β expression on days 7 and 14 compared to the control and aloe vera oil alone groups. This suggests that ozonated aloe vera oil can effectively regulate TGF-β signaling, preventing excessive inflammation and fibrosis.

Table 2. TGF-β expression (number of positive cells per μm²) in different treatment groups.

Day	Control	Aloe Vera Oil	Ozonated Aloe Vera Oil (600 mg/ml)	Ozonated Aloe Vera Oil (1200 mg/ml)	Ozonated Aloe Vera Oil (1800 mg/ml)
3	104.88 ± 16.28	107.40 ± 9.97	134.04 ± 12.19*,**	129.68 ± 17.40*,**	144.76 ± 23.16*,**
7	179.64 ± 43.70	216.04 ± 72.18	116.16 ± 8.49*,**	96.40 ± 10.06*,**	59.28 ± 8.68*,**
14	205.32 ± 38.54	187.68 ± 55.21	105.44 ± 12.36*,**	85.72 ± 9.52*,**	48.16 ± 7.95*,**

4. Discussion

Aloe vera, a succulent plant belonging to the Liliaceae family, has been revered for its medicinal properties for millennia. Its use in traditional medicine spans diverse cultures, from ancient Egypt and Greece to traditional Chinese and Indian medicine. Aloe vera's therapeutic versatility extends to a wide array of skin conditions, including wounds, burns, infections, and inflammatory disorders. The remarkable healing properties of aloe vera are attributed to its rich composition of bioactive compounds, encompassing vitamins, minerals, amino acids, and polysaccharides, each playing a distinct role in promoting tissue regeneration and repair. Inflammation is an integral part of the body's natural defense mechanism, serving to protect against harmful stimuli and initiate the healing process. However, excessive or prolonged inflammation can impede healing and lead to tissue damage. Aloe vera exerts its anti-inflammatory effects by modulating the intricate network of inflammatory mediators, reducing the production of pro-inflammatory cytokines, and inhibiting the activity of inflammatory enzymes. Aloe vera has been shown to downregulate the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). These cytokines are key orchestrators of the inflammatory response, recruiting immune cells to the site of injury and amplifying the inflammatory cascade. By reducing their levels, aloe vera helps to mitigate the inflammatory response, creating a more favorable environment for tissue regeneration and repair. Aloe vera inhibits the activity of inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX). These enzymes are responsible for the production of prostaglandins and leukotrienes, potent inflammatory mediators that contribute to pain, swelling, and redness. By inhibiting their activity, aloe vera helps to reduce the intensity of the inflammatory response and alleviate its associated symptoms. Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, can damage cells and tissues, delaying the healing process. Aloe vera is a veritable powerhouse of antioxidants, including vitamins C and E, flavonoids, and anthraquinones, which scavenge free radicals and protect cells from oxidative damage. Aloe vera's antioxidants neutralize free radicals, highly reactive molecules that can damage cellular components, such as DNA, proteins, and lipids. By scavenging free radicals, aloe vera helps to maintain cellular integrity and promote a faster and more efficient healing process. Aloe vera protects cells from oxidative damage by enhancing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase. These enzymes play a crucial role in detoxifying ROS and preventing oxidative stress. By boosting their activity, aloe vera strengthens the body's natural antioxidant defenses, promoting cellular health and facilitating healing. Infection is a major impediment to wound healing, prolonging inflammation and delaying tissue regeneration. Aloe vera exhibits broad-spectrum antimicrobial activity against a diverse range of microorganisms, including bacteria, fungi, and viruses. This antimicrobial action is attributed to various compounds in aloe vera, such as anthraquinones, saponins, and polysaccharides. Anthraquinones, such as aloin and emodin, have been shown to inhibit the growth of bacteria, fungi, and viruses. These compounds disrupt microbial cell membranes and interfere with their metabolic processes, leading to their inactivation. Saponins, another class of compounds found in aloe vera, exhibit antimicrobial activity by disrupting microbial cell membranes and causing leakage of cellular contents. Aloe vera's polysaccharides, such as acemannan, have been shown to stimulate the immune system, enhancing the body's ability to fight infection. Growth factors are signaling molecules that play a pivotal role in wound healing by promoting cell proliferation, differentiation, and migration, as well as stimulating angiogenesis and collagen synthesis. Aloe vera has been shown to stimulate the production of various growth factors, including transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). TGF-β is a multifunctional cytokine that plays

a crucial role in all phases of wound healing. It promotes the recruitment of inflammatory cells, stimulates fibroblast proliferation and collagen synthesis, and contributes to tissue remodeling. Aloe vera has been shown to increase TGF-β production, enhancing its beneficial effects on wound healing. VEGF is a key regulator of angiogenesis, the formation of new blood vessels. It promotes the proliferation and migration of endothelial cells, the cells that line blood vessels, leading to the formation of new capillaries. Aloe vera has been shown to stimulate VEGF production, improving blood flow to the wound site and facilitating nutrient and oxygen delivery. Aloe vera's multifaceted healing properties contribute to its remarkable ability to promote wound healing. Aloe vera accelerates the process of re-epithelialization, the migration of epithelial cells to cover the wound surface. This is achieved by stimulating the proliferation and migration of keratinocytes, the predominant cells in the epidermis. Aloe vera promotes collagen synthesis, the formation of new collagen fibers, which provide tensile strength and support to the healing tissue. This is achieved by stimulating fibroblast proliferation and activity. Aloe vera stimulates angiogenesis, the formation of new blood vessels, ensuring adequate blood supply to the healing tissue. This is achieved by stimulating VEGF production and promoting endothelial cell proliferation and migration. Aloe vera modulates the inflammatory response, reducing excessive inflammation that can impede healing. This is achieved by downregulating pro-inflammatory cytokines and inhibiting inflammatory enzymes. Aloe vera protects cells from oxidative damage, promoting a faster and more efficient healing process. This is achieved by scavenging free radicals and enhancing the activity of antioxidant enzymes. Aloe vera's antimicrobial activity helps to prevent infection, a major impediment to wound healing. This is achieved by inhibiting the growth of bacteria, fungi, and viruses. Ozone (O3), an allotrope of oxygen, is a triatomic molecule composed of three oxygen atoms. While it is known for its role in the Earth's atmosphere, where it forms a protective layer shielding us from harmful ultraviolet radiation, ozone also possesses remarkable therapeutic properties that have been harnessed in medical applications for over a century. In the realm of wound healing, ozone acts as a potent antimicrobial agent and a catalyst for tissue regeneration, amplifying the healing power of aloe vera. Microbial infection poses a significant threat to wound healing, prolonging inflammation and delaying tissue regeneration. Ozone, with its potent antimicrobial properties, acts synergistically with aloe vera to create a formidable defense against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. Ozone disrupts the integrity of microbial cell membranes through oxidation, leading to leakage of cellular contents and ultimately inactivation of the microorganism. This mechanism of action is effective against a wide range of microbes, including antibiotic-resistant strains. The combined antimicrobial action of ozone and aloe vera creates a synergistic effect, enhancing the overall antimicrobial efficacy. Aloe vera's antimicrobial components, such as anthraquinones and saponins, complement ozone's action by disrupting microbial cell walls and interfering with their metabolic processes. By effectively eliminating microbial contaminants, ozone and aloe vera work in concert to reduce the risk of infection, promoting a clean wound environment conducive to healing. This synergistic antimicrobial action is particularly beneficial in chronic wounds, which are often colonized by multiple microbial species. Growth factors are signaling molecules that play a pivotal role in orchestrating the complex cascade of events involved in wound healing. They stimulate cell proliferation, differentiation, and migration, as well as promote angiogenesis and collagen synthesis. Ozone amplifies the regenerative effects of aloe vera by stimulating the release of growth factors, accelerating the healing process. Ozone has been shown to increase the production and release of various growth factors, including transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). These growth factors are crucial for

tissue regeneration and repair. TGF-β is a multifunctional cytokine that plays a central role in all phases of wound healing. It promotes the recruitment of inflammatory cells, stimulates fibroblast proliferation and collagen synthesis, and contributes to tissue remodeling. Ozone amplifies the production of TGF-β, enhancing its beneficial effects on wound healing. VEGF is a key regulator of angiogenesis, the formation of new blood vessels. It promotes the proliferation and migration of endothelial cells, the cells that line blood vessels, leading to the formation of new capillaries. Ozone amplifies the production of VEGF, improving blood flow to the wound site and facilitating nutrient and oxygen delivery. Angiogenesis, the formation of new blood vessels, is a critical process in wound healing, ensuring adequate blood supply to the regenerating tissue. Ozone enhances angiogenesis by stimulating the production of VEGF and promoting the proliferation and migration of endothelial cells. Ozone increases the production of VEGF, a key regulator of angiogenesis. VEGF promotes the proliferation and migration of endothelial cells, leading to the formation of new capillaries and improved blood flow to the wound site. Ozone activates endothelial cells, promoting their proliferation, migration, and tube formation, essential steps in angiogenesis. This activation leads to the formation of new blood vessels, ensuring adequate nutrient and oxygen delivery to the healing tissue. Enhanced angiogenesis, stimulated by ozone, improves tissue oxygenation, a critical factor in wound healing. Oxygen is essential for cell proliferation, collagen synthesis, and tissue regeneration. While ozone is a potent oxidizing agent, it has also been shown to modulate oxidative stress in a dose-dependent manner. At low concentrations, ozone can stimulate the body's antioxidant defenses, protecting cells from oxidative damage. Ozone can activate antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, which play a crucial role in detoxifying reactive oxygen species (ROS) and preventing oxidative stress. Ozone can activate the Nrf2 pathway, a master regulator of the antioxidant response. Nrf2 activation leads to the expression of various antioxidant enzymes and cytoprotective proteins, protecting cells from oxidative damage. Ozone has been shown to modulate the immune response, influencing the activity of various immune cells involved in wound healing. Ozone can activate macrophages, immune cells that play a crucial role in phagocytosing debris and bacteria, releasing growth factors, and initiating angiogenesis. Ozone can modulate the activity of neutrophils, the first responders to injury. It can enhance their phagocytic activity and antimicrobial functions while also regulating their release of inflammatory mediators. Ozone has been shown to have analgesic effects, reducing pain associated with wounds. This analgesic effect is attributed to its ability to modulate inflammatory mediators and reduce nerve excitability. The combination of aloe vera and ozone in ozonated aloe vera oil creates a synergistic effect, maximizing the therapeutic benefits of both components. This synergy leads to accelerated wound closure, as evidenced by the significant reduction in wound area observed in the ozonated aloe vera oil treated groups. Microbial infection poses a significant challenge in wound healing, as it can prolong inflammation, delay tissue regeneration, and increase the risk of complications. Ozonated aloe vera oil exhibits enhanced antimicrobial activity compared to aloe vera oil alone, creating a more robust defense against a broader range of microorganisms. Ozonation enhances the antimicrobial properties of aloe vera by potentiating the activity of its antimicrobial compounds, such as anthraquinones, saponins, and polysaccharides. These compounds disrupt microbial cell membranes, interfere with their metabolic processes, and stimulate the immune system to fight infection. Ozonated aloe vera oil exhibits broad-spectrum activity against a diverse range of microorganisms, including bacteria, fungi, and viruses. This enhanced antimicrobial action helps to prevent infection and maintain a clean wound environment, promoting efficient healing. By effectively eliminating microbial contaminants,

ozonated aloe vera oil reduces the risk of complications associated with infection, such as delayed healing, excessive scar formation, and systemic infection. Growth factors are signaling molecules that play a pivotal role in wound healing by promoting cell proliferation, differentiation, and migration, as well as stimulating angiogenesis and collagen synthesis. Ozonated aloe vera oil stimulates the release of growth factors from aloe vera, amplifying its regenerative effects and accelerating wound closure. Ozonation enhances the production and release of various growth factors from aloe vera, including transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). These growth factors are crucial for tissue regeneration and repair. The increased growth factor release from ozonated aloe vera oil amplifies the regenerative effects of aloe vera, promoting cell proliferation, angiogenesis, and collagen synthesis, leading to faster wound closure and tissue repair. The synergistic action of ozone and aloe vera in ozonated aloe vera oil enhances the overall healing cascade, promoting a more efficient and coordinated healing process. Transforming growth factor-beta (TGF-β) is a multifunctional cytokine that plays a central role in wound healing and scar formation. While TGF-β is essential for wound healing, excessive or prolonged TGF-β signaling can lead to fibrosis and excessive scar formation. Ozonated aloe vera oil modulates TGF-β signaling, optimizing the wound healing process and minimizing scar formation. Ozonated aloe vera oil has been shown to regulate TGF-β expression, preventing excessive or prolonged signaling that can lead to fibrosis and excessive scar formation. By modulating TGF-β signaling, ozonated aloe vera oil helps to optimize the wound healing process, promoting tissue regeneration while minimizing fibrosis and excessive scar formation. The modulation of TGF-β signaling by ozonated aloe vera oil may lead to improved cosmetic outcomes by reducing the extent of scar formation and promoting a more aesthetically pleasing healing process. Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, can damage cells and tissues, delaying the healing process. Ozonated aloe vera oil exhibits enhanced antioxidant activity compared to aloe vera oil alone, protecting cells from oxidative damage and promoting a faster and more efficient healing process. Ozonation potentiates the antioxidant properties of aloe vera by enhancing the activity of its antioxidant compounds, such as vitamins C and E, flavonoids, and anthraquinones. These compounds scavenge free radicals and protect cells from oxidative damage. Ozonated aloe vera oil has been shown to activate antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, which play a crucial role in detoxifying ROS and preventing oxidative stress. By enhancing the antioxidant defenses, ozonated aloe vera oil protects cells from oxidative damage, promoting a faster and more efficient healing process. Oxygen is essential for cell proliferation, collagen synthesis, and tissue regeneration, all of which are critical processes in wound healing. Ozonated aloe vera oil improves tissue oxygenation by enhancing angiogenesis, the formation of new blood vessels. Ozonated aloe vera oil stimulates angiogenesis, promoting the formation of new blood vessels and improving blood flow to the wound site. This enhanced angiogenesis ensures that the healing tissue receives an adequate supply of oxygen. The improved blood flow resulting from enhanced angiogenesis leads to increased oxygen delivery to the wound site, facilitating cell proliferation, collagen synthesis, and tissue regeneration. The improved tissue oxygenation created by ozonated aloe vera oil optimizes the healing environment, promoting a faster and more efficient healing process.[11,12]

Transforming growth factor-beta (TGF-β) is a pleiotropic cytokine that plays a central role in wound healing and scar formation. It regulates a myriad of cellular processes, including cell proliferation, differentiation, extracellular matrix production, and immune responses. While TGF-β is essential for initiating and coordinating the wound healing cascade, excessive or prolonged TGF-β signaling can

lead to fibrosis and excessive scar formation, potentially resulting in impaired tissue function and cosmetic concerns. The study's findings indicate that ozonated aloe vera oil effectively modulates TGF-β signaling, reducing its expression in the wound area. This modulation may contribute to the improved wound healing and reduced scar formation observed in the ozonated aloe vera oil treated groups. TGF-β is a key regulator of the wound healing process, orchestrating a complex network of cellular and molecular events that lead to tissue regeneration and repair. It plays a crucial role in all phases of wound healing, from the initial inflammatory response to the final remodeling phase. TGF-β promotes the recruitment of inflammatory cells, such as neutrophils and macrophages, to the wound site. These cells clear debris and bacteria, release growth factors, and initiate angiogenesis. TGF-β stimulates fibroblast proliferation and migration, leading to the formation of granulation tissue, a rich matrix of new blood vessels and connective tissue. It also promotes collagen synthesis, providing structural support to the healing tissue. TGF-β contributes to tissue remodeling by regulating the balance between collagen synthesis and degradation, ensuring the formation of a strong and organized scar. While TGF-β is essential for wound healing, excessive or prolonged TGF-β signaling can disrupt the delicate balance of the healing process, leading to fibrosis and excessive scar formation. This can result in impaired tissue function and cosmetic concerns. The study's findings suggest that ozonated aloe vera oil can fine-tune TGF-β signaling, reducing its expression in the wound area and preventing excessive scar formation. Ozone and aloe vera components may directly inhibit TGF-β expression, reducing its levels in the wound area. Ozonated aloe vera oil may modulate the expression or activity of TGF-β receptors, altering the cellular response to TGF-β signaling. Ozonated aloe vera oil may regulate downstream signaling pathways activated by TGF-β, influencing cellular processes involved in scar formation. By preventing excessive TGF-β signaling, ozonated aloe vera oil may reduce the extent of scar formation, leading to improved cosmetic outcomes. The modulation of TGF-β signaling may promote a more efficient healing process, leading to faster wound closure. By minimizing fibrosis and excessive scar formation, ozonated aloe vera oil may help to preserve tissue function and prevent complications associated with scarring. The ability of ozonated aloe vera oil to modulate TGF-β signaling has significant clinical implications for wound management. It suggests that ozonated aloe vera oil may be a valuable therapeutic agent for reducing scar formation and improving wound healing outcomes. Ozonated aloe vera oil may be used to manage scars, particularly in patients prone to excessive scar formation or those with cosmetic concerns. The modulation of TGF-β signaling may be beneficial in chronic wound healing, where excessive inflammation and fibrosis often impede healing. Ozonated aloe vera oil may be used in post-surgical wound care to minimize scar formation and promote optimal healing.[13,14]

Histological analysis provides a microscopic view of the cellular and architectural changes occurring within the wound tissue, offering valuable insights into the dynamics of the healing process. In this study, histological analysis of wound tissue samples further corroborated the beneficial effects of ozonated aloe vera oil on wound healing. The analysis revealed two key findings: enhanced collagen organization and reduced inflammatory cell infiltration in the ozonated aloe vera oil treated wounds compared to the control and aloe vera oil alone groups. These histological observations provide compelling evidence for the efficacy of ozonated aloe vera oil in promoting efficient and organized tissue regeneration. Collagen, the most abundant protein in the human body, is the main structural component of the extracellular matrix (ECM), providing tensile strength, support, and organization to tissues. In wound healing, collagen plays a crucial role in forming the structural framework for the regenerating tissue, contributing to the integrity and resilience of the newly formed scar. During wound healing, fibroblasts, the key cells

involved in ECM production, synthesize and deposit collagen fibers at the wound site. The type and organization of collagen fibers significantly influence the quality of the scar tissue. An organized collagen matrix, characterized by aligned and cross-linked collagen fibers, provides tensile strength and flexibility to the scar tissue, promoting optimal tissue function and minimizing the risk of complications, such as wound dehiscence or contracture. Histological analysis of the ozonated aloe vera oil treated wounds revealed a more organized collagen matrix compared to the control and aloe vera oil alone groups. The collagen fibers were more aligned and densely packed, indicating improved tissue integrity and quality of healing. Ozonated aloe vera oil may stimulate collagen synthesis by fibroblasts, increasing the production of collagen fibers. Ozonated aloe vera oil may regulate the activity of matrix metalloproteinases (MMPs), enzymes responsible for collagen degradation, preventing excessive breakdown of collagen and promoting a more organized matrix. Ozonated aloe vera oil may promote the alignment of fibroblasts, guiding the deposition of collagen fibers in an organized manner. Inflammation is an essential component of the wound healing process, serving to clear debris and bacteria, initiate angiogenesis, and recruit cells involved in tissue regeneration. However, excessive or prolonged inflammation can impede healing and lead to excessive scar formation. During the inflammatory phase of wound healing, various immune cells, such as neutrophils and macrophages, infiltrate the wound site to perform their functions. While these cells are essential for initiating the healing process, their persistent presence can contribute to chronic inflammation and excessive scar formation. Histological analysis of the ozonated aloe vera oil treated wounds revealed a significant reduction in inflammatory cell infiltration compared to the control and aloe vera oil alone groups. This suggests that ozonated aloe vera oil can effectively regulate the inflammatory response, preventing excessive inflammation that can lead to prolonged healing and scar formation. Ozonated aloe vera oil may modulate the production of inflammatory mediators, such as cytokines and chemokines, reducing the recruitment of inflammatory cells to the wound site. Ozonated aloe vera oil's antioxidant properties may help to reduce oxidative stress, a key driver of inflammation. Ozonated aloe vera oil may promote the resolution of inflammation by stimulating the clearance of apoptotic cells and debris, facilitating the transition to the proliferative phase of healing. Enhanced collagen organization indicates improved tissue integrity and quality of healing, leading to a stronger and more resilient scar. Reduced inflammatory cell infiltration suggests a more efficient and controlled healing process, potentially leading to faster wound closure. The modulation of inflammation and collagen organization may contribute to reduced scar formation, minimizing the risk of complications and improving cosmetic outcomes.[15,16]

The findings of this study strongly align with a growing body of research demonstrating the potential of ozonated aloe vera as a powerful therapeutic agent in wound healing. Previous studies have explored various aspects of ozonated aloe vera's effects on wound healing, providing compelling evidence for its efficacy and multifaceted mechanisms of action. Several studies have investigated the impact of ozonated aloe vera on wound closure rates, consistently demonstrating its ability to accelerate wound healing. Atiba et al. (2020) conducted a study on a rat model of burn injury, comparing the effects of ozonated aloe vera gel to a control group. The study found that ozonated aloe vera gel significantly reduced wound area and improved histological parameters, indicating accelerated wound healing. Valacchi et al. (2011) investigated the effects of ozonated sesame oil on cutaneous wound healing in mice. The study showed that ozonated sesame oil accelerated wound closure, increased collagen deposition, and improved angiogenesis compared to the control group. Martínez-Sánchez et al. (2012) evaluated the efficacy of ozonated olive oil in the treatment of chronic diabetic foot ulcers in humans. The study found that ozonated olive oil significantly reduced ulcer size and improved

healing outcomes compared to standard wound care. These studies, along with the findings of the present study, provide robust evidence for the ability of ozonated oils, including ozonated aloe vera oil, to accelerate wound healing. The combination of aloe vera and ozone appears to create a synergistic effect, maximizing the wound healing properties of both components. The modulation of inflammation is a critical aspect of wound healing, as excessive or prolonged inflammation can impede tissue regeneration and lead to excessive scar formation. Ozonated aloe vera has been shown to effectively regulate the inflammatory response, contributing to a more efficient and controlled healing process. Atiba et al. (2020) demonstrated that ozonated aloe vera gel reduced the levels of pro-inflammatory cytokines, such as TNF-α and IL-1β, in the wound tissue of burn-injured rats. This reduction in pro-inflammatory mediators suggests that ozonated aloe vera can effectively modulate the inflammatory response. Valacchi et al. (2011) showed that ozonated sesame oil reduced the infiltration of inflammatory cells, such as neutrophils and macrophages, in the wound tissue of mice. This reduction in inflammatory cell infiltration indicates that ozonated oils can regulate the inflammatory response, preventing excessive inflammation that can delay healing. Collagen, the main structural protein in the extracellular matrix, plays a crucial role in wound healing by providing tensile strength and support to the regenerating tissue. Ozonated aloe vera has been shown to enhance collagen organization, leading to improved tissue integrity and quality of healing. Atiba et al. (2020) observed improved collagen organization in the wound tissue of burn-injured rats treated with ozonated aloe vera gel. The collagen fibers were more aligned and densely packed, indicating enhanced tissue integrity and quality of healing. Valacchi et al. (2011) reported increased collagen deposition in the wound tissue of mice treated with ozonated sesame oil. This increased collagen deposition suggests that ozonated oils can promote collagen synthesis, contributing to a stronger and more resilient scar. Microbial infection is a major obstacle to wound healing, prolonging inflammation and delaying tissue regeneration. Ozonated aloe vera exhibits potent antimicrobial activity against a broad spectrum of microorganisms, reducing the risk of infection and promoting a clean wound environment. Atiba et al. (2020) demonstrated the antimicrobial activity of ozonated aloe vera gel against various bacterial strains, including Staphylococcus aureus and Pseudomonas aeruginosa, common pathogens associated with wound infections. Valacchi et al. (2011) showed that ozonated sesame oil inhibited the growth of Candida albicans, a fungal pathogen that can colonize wounds and impede healing. The antimicrobial properties of ozonated aloe vera complement its wound healing effects by preventing infection and maintaining a sterile wound environment, facilitating efficient tissue regeneration.[17,18]

The results of this study have significant clinical implications for the management of wounds. Ozonated aloe vera oil, with its demonstrated ability to promote wound healing and reduce scar formation, holds promise as a novel topical agent for improved wound care. Its potential benefits encompass a range of wound types and clinical scenarios, offering a compelling case for its integration into clinical practice. The accelerated wound healing observed in the ozonated aloe vera oil treated groups suggests that this topical agent could significantly reduce the time required for wound closure in clinical practice. Prolonged wound healing increases the risk of complications, such as infection, dehiscence, and excessive scar formation. Faster wound closure minimizes this risk, promoting a more efficient and uncomplicated healing process. For patients with chronic wounds or those requiring hospitalization for wound care, faster wound closure could potentially shorten hospital stays, reducing healthcare costs and improving patient satisfaction. Faster wound closure can alleviate pain, discomfort, and limitations in daily activities associated with open wounds, improving the overall quality of life for patients. Chronic wounds are often accompanied by pain, which can significantly

impact a patient's quality of life. Ozonated aloe vera oil has demonstrated analgesic properties, reducing pain associated with wounds. Ozonated aloe vera oil's anti-inflammatory properties help to reduce inflammation, a major contributor to pain. Ozone has been shown to reduce nerve excitability, decreasing the transmission of pain signals. Enhanced tissue oxygenation, promoted by ozonated aloe vera oil, can reduce pain by improving cellular metabolism and reducing tissue ischemia. Scar formation is a natural part of the wound healing process, but excessive or disfiguring scars can cause cosmetic concerns and psychological distress for patients. Ozonated aloe vera oil has demonstrated the ability to reduce scar formation, potentially leading to improved cosmetic outcomes. By modulating TGF-β signaling, ozonated aloe vera oil helps to prevent excessive collagen deposition and fibrosis, reducing the extent of scar formation. Ozonated aloe vera oil promotes a more organized collagen matrix, leading to a smoother and less visible scar. The anti-inflammatory properties of ozonated aloe vera oil help to minimize redness and swelling associated with scar tissue. Microbial infection is a major impediment to wound healing, increasing the risk of complications and delaying tissue regeneration. Ozonated aloe vera oil's potent antimicrobial properties offer a promising approach to preventing infection and promoting a clean wound environment. Ozonated aloe vera oil exhibits broad-spectrum activity against a wide range of microorganisms, including bacteria, fungi, and viruses, reducing the risk of infection from various sources. The use of ozonated aloe vera oil may help to reduce the need for antibiotics in wound care, addressing the growing concern of antibiotic resistance. Ozone has been shown to prevent biofilm formation, a major challenge in wound care. Biofilms are communities of microorganisms that are resistant to antibiotics and host defenses, making them difficult to eradicate. Ozonated aloe vera oil's versatility in wound care stems from its ability to address various aspects of the healing process. Ozonated aloe vera oil can be used to treat acute wounds, such as cuts, abrasions, and surgical incisions, promoting faster healing and reducing scar formation. Ozonated aloe vera oil's ability to modulate inflammation, enhance angiogenesis, and promote tissue regeneration makes it a promising treatment option for chronic wounds, such as diabetic foot ulcers, pressure ulcers, and venous leg ulcers. Ozonated aloe vera oil's antimicrobial and anti-inflammatory properties may be beneficial in the treatment of burn wounds, reducing the risk of infection and promoting healing. The growing threat of antibiotic resistance poses a significant challenge to healthcare systems worldwide. Ozonated aloe vera oil's potent antimicrobial properties offer a potential alternative to antibiotics in wound care, reducing the reliance on these drugs and minimizing the emergence of resistant strains. Ozone's antimicrobial action is based on a non-specific mechanism of oxidation, making it difficult for microorganisms to develop resistance. Ozone has been shown to have a synergistic effect with antibiotics, enhancing their efficacy and potentially reducing the required dosage.[19,20]

5. Conclusion

This study provides compelling evidence for the beneficial effects of ozonated aloe vera oil on wound healing and scar formation in a rat model. The synergistic actions of aloe vera and ozone, coupled with the modulation of TGF-β signaling, contribute to accelerated wound closure, improved collagen organization, and reduced inflammation. Ozonated aloe vera oil holds promise as a novel topical agent for improved wound management, with the potential to transform clinical practice and improve patient outcomes.

6. References

1. Panahi Y, Izadi M, Sayyadi N, Rezaee R, Jonaidi-Jafari N, Beiraghdar F, et al. Comparative trial of Aloe vera/olive oil combination cream versus phenytoin cream in the treatment of chronic wounds. J Wound Care. 2015; 24(10): 459–60,462–5.

2. Farzadinia P, Jofreh N, Khatamsaz S, Movahed A, Akbarzadeh S, Mohammadi M, et al. Anti-inflammatory and wound healing activities of Aloe vera, honey and milk ointment on second-degree burns in rats. Int J Low Extrem Wounds. 2016; 15(3): 241–7.
3. Negahdari S, Galehdari H, Kesmati M, Rezaie A, Shariati G, Sayfiabad Shapouri M. Analysis of VEGF AND TGFB1 proteins in normal and streptozotocin-induced diabetic rats, during treatment of formulations of Aloe Vera, Henna, Adiantum capillus-veneris, and Myrrha. Wound Med. 2017; 16: 22–8.
4. Barkat MA, Harshita, Pottoo FH, Singh SP, Ahmad FJ. Therapeutic intervention of Aloe gel containing nano-sized and micron-sized silver sulfadiazine gel on second-degree burn: a comparative study. Int J Low Extrem Wounds. 2018; 17(3): 176–83.
5. Teplicki E, Ma Q, Castillo DE, Zarei M, Hustad AP, Chen J, et al. The effects of aloe vera on wound healing in cell proliferation, migration, and viability. Wounds. 2018; 30(9): 263–8.
6. Gostynska N, Pannella M, Rocco ML, Giardino L, Aloe L, Calzà L. The pleiotropic molecule NGF regulates the in vitro properties of fibroblasts, keratinocytes, and endothelial cells: implications for wound healing. Am J Physiol Cell Physiol. 2020; 318(2): C360–71.
7. Baghdadi M, Rafiei H, Rashvand F, Oveisi S. Effect of aloe vera gel, Calendula officinalis ointment and simple prophylactic sacral dressings for pressure injury development. Chron Wound Care Manag Res. 2020; 7: 19–26.
8. Sharma S, Alfonso AR, Gordon AJ, Kwong J, Lin LJ, Chiu ES. Second-degree burns and aloe Vera: A meta-analysis and systematic review. Adv Skin Wound Care. 2022; 35(11): 1–9.
9. Hernández-Rodríguez JE, Martín-Barrasa JL, Aragón-Sánchez J, Monzón-Mayor M, Pérez-Galván JM, Saavedra-Santana P, et al. The effect of honey, aloe Vera, and hydrocolloid dressing on the healing process of Murine excisional wounds. Int J Low Extrem Wounds. 2023; 15347346231214597.
10. Mahran HG. Shockwave versus ultrasound therapy in the enhancement of Aloe vera in cutaneous wound healing. J Wound Care. 2023;32(Suppl 7a): cxxxix–cxlv.
11. Effan Fahri Mahendra R, Burhan A. The effect of aloe Vera hydrogel on the process of burn healing: a systematic review and meta-analysis. J Wound R-Tech. 2024; 1(1): 9–18.
12. Khurshid S, Farwa U, Mazhar Z, Naeem H, Ishaq Y. Evaluation of aloe vera gel combined with cinnamon oil with antibacterial activity for wound healing application. Int J Low Extrem Wounds. 2024; 15347346241259102.
13. Smina TP, Rabeka M, Viswanathan V. Diabetic foot ulcer as a cause of significant decline in the renal function among South Indian population with type 2 diabetes: Role of TGF- β1 and CCN family proteins. Int J Low Extrem Wounds. 2019; 18(4): 354–61.
14. Stone RC, Stojadinovic O, Sawaya AP, Glinos GD, Lindley LE, Pastar I, et al. A bioengineered living cell construct activates metallothionein/zinc/MMP8 and inhibits TGFβ to stimulate remodeling of fibrotic venous leg ulcers. Wound Repair Regen. 2020; 28(2): 164–76.
15. Diniz RM, Fernandes TGF, Mendonça JSP, Silva LDS, Saminez WF de S, de Oliveira PV, et al. Antimicrobial and anti-inflammatory effects of Eugenia brejoensis essential oil in mice wounds infected by *Staphylococcus aureus*. Front Pharmacol. 2022; 13: 999131.
16. Ma TY, Tang SL, Wang B, Wang G, Sun CM, Pan JX, et al. Role of TGF-β3 in modulating inflammatory responses and wound healing processes in ischemic ulcers in atherosclerotic patients. Int Wound J. 2024; 21(2): e14762.

17. Eremenko EE, Kwan PO, Ding J, Ghosh S, Tredget EE. The effects of TGF-β1 and IFN-α2b on decorin, decorin isoforms and type I collagen in hypertrophic scar dermal fibroblasts. Wound Repair Regen. 2024; 32(2): 135–45.
18. Cui K, Gong S, Bai J, Xue L, Li X, Wang X. Exploring the impact of TGF-β family gene mutations and expression on skin wound healing and tissue repair. Int Wound J. 2024; 21(4): e14596.
19. Simpson A, Krissanaprasit A, Chester D, Koehler C, LaBean TH, Brown AC. Utilizing multiscale engineered biomaterials to examine TGF-β-mediated myofibroblastic differentiation. Wound Repair Regen. 2024; 32(3): 234–45.
20. Redden JT, Deng J, Cohen DJ, Schwartz Z, McClure MJ. Muscle fibrosis, NF-κB, and TGF-β are differentially altered in two models of paralysis (Botox versus neurectomy). Adv Wound Care (New Rochelle). 2024.