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Therapeutic Efficacy of Zinc Oxide Nanoparticles Ointment in Promoting Wound Healing in Dogs: A Clinical Study

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ABSTRACT

Cutaneous wounds in dogs, whether resulting from trauma or surgical procedures, present considerable healing challenges. These challenges include risks of infection, prolonged recovery times, delayed granulation, excessive inflammation, and impaired tissue regeneration. This preliminary study was carried out on eight clinical cases of dogs (five males and three females) with cutaneous wounds of varying severity. These wounds are located at the lateral abdomen (n=3), tarsal (n=2) and elbow (n=1) regions, neck (n=1), and nipple (n=1). The dog breeds included mongrel (n=4), Griffon (n=1), German Shepherd (n=1), Bullmastiff (n=1), and Golden Retriever (n=1). Each wound was washed with normal saline solution and treated once daily with topical application of a thin layer of zinc oxide nanoparticles (ZnO-NPs) ointment until complete healing. Follow-ups were conducted to monitor wound size and contraction, presence of infection, and potential adverse effects. Healing was assessed based on complete wound closure and the absence of infection. Results indicated that ZnO-NPs ointment was easily applied and caused no discomfort to the treated dogs. Moreover, ZnO-NPs ointment promoted wound healing in all treated dogs. Almost all treated wounds demonstrated complete healing after 30 days of treatment with substantial, fast return to the normal configuration and absence of infection. Clinical scores of clearances of wound infection revealed complete absence of infection after 7-14 days of treatment. In conclusion, ZnO-NPs enhanced the overall healing process and supported rapid as well as complication-free tissue repair in dogs with cutaneous wounds. Additionally, ZnO-NPs ointment was simple to apply and caused no discomfort to the dogs, making it suitable for routine veterinary use. Further studies are needed to optimize ZnO-NP formulations and evaluate their long-term safety and efficacy in different wound types.

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INTRODUCTION

Skin disorders, especially cutaneous injuries are a significant health concern in veterinary practice, especially in canines, and may be resulted from trauma, surgery, infections, or chronic conditions. Dogs, like other mammals, experience a complex healing process involving inflammation, tissue regeneration, and remodeling which are continuing without line of demarcation between them **(Stadelmann *et al.*, 1998; Sehn *et al.*, 2009).** However, factors such as infection, delayed healing, and scarring can complicate recovery **(Abbas *et al.*, 2023)**. Wound healing is particularly challenging in clinical cases where infections, such as *Staphylococcus aureus* or *Pseudomonas aeruginosa*, impede the healing process **(Metwally *et al.*, 2020)**.

Several agents have been applied to accelerate wound healing in dogs **(Agren, 1990; Abu-Seida,**

2015; Asif *et al.,* 2023). Topical zinc, as one of these agents, accelerates the healing of small and acute skin wounds **(Soderberg *et al.,* 2001)**, as well as stimulates re-epithelialization, reduces inflammation and bacterial growth **(Agren, 1990)**. Zinc oxide (ZnO) is characterized by photo-catalytic and photo-oxidizing capacity against chemical and biological species **(Sharma *et al.,* 2010).**

In response to the challenges in wound healing, nanotechnology has emerged as a potential solution in veterinary wound care. Locally administered nanoparticles usually remain in their local environment for a long period of time and, hence, produce therapeutic effect without causing any toxicity **(Metcalfe and Ferguson, 2007).**

ZnO-NPs, due to their unique physicochemical properties, including high surface area and small particle size, have demonstrated significant promise in improving wound healing outcomes. These nanoparticles possess powerful antimicrobial, anti-inflammatory, and regenerative properties, making them effective agents for promoting tissue repair and preventing infections in wounds **(Jamil, 2021; Asif *et al.,* 2023).** ZnO-NPs have a very strong antibacterial effect at a very low concentration against Gram-negative and Gram-positive bacteria **(Hazra *et al.,* 2013; Vimala *et al.,* 2013)**. Their enhanced penetration into the skin and deeper tissues allows for more effective treatment, particularly in the management of chronic and complicated wounds **(Saber *et al.,* 2024).**

The use of ZnO-NPs has been extensively studied in animal models, with positive results in various species. In donkeys, for instance, ZnO-NPs have been shown to markedly enhance wound healing by promoting collagen deposition and tissue regeneration **(Saber *et al.,* 2024)**. Similar results were recorded by **Ramachandran *et al.,* (2024)** in a zebrafish model. Moreover, studies in rabbits and goats have reported accelerated wound contraction and reduced infection when treated with ZnO-NPs **(Abbas *et al.,* 2023)**. These results underscore the potential for ZnO-NPs to be used as an alternative or complementary therapy to conventional treatments in veterinary practice.

In addition, ZnO-NPs also exhibit significant antibacterial activity against a wide range of bacteria, including antibiotic-resistant strains such as *methicillin-resistant Staphylococcus aureus (MRSA)* **(Asif *et al.,* 2023)**. Their ability to eradicate bacteria, combined with their capacity to stimulate wound healing processes such as cell proliferation and migration, collagen formation, and angiogenesis, makes them highly beneficial in preventing infections and promoting faster recovery **(Khan *et al.,* 2021; Al-Timimi *et al.,* 2025; Pangprasit *et al.,* 2025)**.

Although therapeutic effects of ZnO-NPs are well-documented in different animal species, their application in dogs is still an emerging area of research. Recent studies highlight the growing interest in using ZnO-NPs for treating cutaneous wounds, especially in cases involving chronic or infected wounds **(Asif *et al.,* 2023 and Jabeen *et al.,* 2024).** Despite the promising results from animal studies, there are several challenges in the clinical application of ZnO-NPs for veterinary use. These include optimizing the formulation, determining the ideal dosage, and assessing the long-term safety and efficacy of repeated applications **(Hashemi *et al.,* 2023)**. Therefore, further studies are necessary to nderstand the clinical implications of ZnO-NPs, especially in the context of wound healing in dogs, and to establish protocols for their safe and effective use in veterinary practices. This study aimed to evaluate the therapeutic efficacy of ZnO-NPs ointment in promoting wound healing in dogs by assessing its impact on wound contraction, infection prevention, and overall tissue regeneration in clinical cases.

MATERIALS AND METHODS

Ethical approval

The current study was approved by the Institutional Animal Use and Care Committee at Faculty of Veterinary Medicine, Cairo University, Egypt (Approval number: **Vet CU 08072023704**). All procedures were done after oral consent from animals' owners for ointment applications and pictures.

Animals

The study was carried out on eight dogs of both sexes (five males and three females). The age of these animals ranged between three months and nine years. They were admitted to the Veterinary Teaching Hospital, Cairo University, Egypt, during 2024. All animals underwent a thorough health and care management. Three dogs presented with old and septic wounds located on the lateral abdomen, while three other dogs had old and septic wounds, two at the lateral aspect of the tarsal region and one at the elbow region. One dog exhibited traumatized wounds on the neck region, and one dog had an old necrotic and septic wound on the nipple as shown in **Table (1).**

Preparation of ZnO-NPs powder

To prepare the ZnO-NPs ointment, an amount of 1.5 g of zinc acetate was added to 0.9 g of sodium hydroxide, and the mixture was mechanically ground. Afterward, 0.9 g of sodium alginate was introduced, and the mixture was blended and ground for 5 minutes at room temperature. To isolate pure ZnO-NPs powders, which were stabilized or coated with sodium alginate, the resulting powder was washed with distilled water and subjected to a centrifugation process. Finally, the wet ZnO-NPs powder was dried using a freeze-drying technique **(Siddiqui *et al.,* 2022).**

Table 1: Demographic and clinical characterization of the eight injured dogs treated with ZnO-NPs ointment enrolled in the study.

Case	Age (Year/ month)	Sex	Weight (Kg)	Breed	Origin/ localization of the wound	Characteristics of the wound
1	9 (y)	Female	5	Griffon	Ruptured abscess/ lateral abdomen	Infected wound (3–4 days)
2	1 (y)	Female	18	Mongrel	Biting wound/lateral abdomen	Infected wound (5 days)
3	3 (m)	Male	5	German Shepherd	Biting wound/lateral abdomen	Infected wound (3 days)
4	2 (y)	Male	23	Mongrel	Accidental wound/ lateral aspect of tarsal joint	Chronic infected wound with bone exposure and septic arthritis (10 days)
5	1 (y)	Male	20	Mongrel	Accidental wound/ lateral aspect of tarsal joint	Chronic infected wound (5 days)
6	3 (y)	Male	24	Mongrel	Accidental wound/ lateral aspect of the elbow joint	Chronic infected wound (7 days)
7	9 (m)	Male	35	Bullmastiff	Wound from wearing Elizabethan collar/ neck	Infected wound (3 days)
8	8 (y)	Female	30	Golden Retriever	Wound from excessive licking/ nipple	Necrotic tissue infected wound (>7 days)

Micromorphology of ZnO-NPs powder

The micromorphological structure of ZnO-NPs was done by using Scanning Electron Microscope (SEM). Images were obtained using (ZEISS FE-SEM ULTRA Plus) equipped with an EDX analyzer microscope with Philips CM20 microscope, operating at an accelerating voltage of 200 kV (**Muhammad *et al.*, 2019).**

Preparation of ZnO-NPs ointment

To prepare the ZnO-NPs ointment, 2 grams of ZnO-NPs powder were first dissolved in 8 mL of N-Methyl-2-pyrrolidone (NMP) solvent. The mixture was placed in an appropriate container, and sonication was applied to ensure complete dissolution. In a separate container, 90 grams of lanolin were weighed and heated in a sonicator or water bath until it became a uniform liquid. Once both the lanolin and ZnO-NPs solution were prepared, the melted lanolin was carefully combined with the ZnO solution, and the mixture was stirred thoroughly to ensure even distribution of the nanoparticles in the lanolin base. The resulting mixture formed an ointment, which was then transferred to storage containers. To improve the texture and help the ointment solidify, the containers were frozen for approximately five minutes. The finished product was stored in a cool and dry place and away from direct sunlight to maintain its stability and effectiveness.

Study Design

Prior to treatment, the dogs were sedated with one mg/kg xylazine HCl 2% (Xylamed®, Bimeda Animal Health, Dublin, Ireland) intramuscularly. The area around the wounds was clipped and shaved. The wounds were debrided and disinfected with normal saline solution to ensure proper application of the ointment. The wounds were treated with a topical thin layer of ZnO-NPs ointment once daily until complete healing. Whenever possible a thin layer of sterile gauze was placed over the wound surface. All treated animals wore an Elizabethan collar to prevent self-trauma, ensuring that the treatment area remained undisturbed.

To prevent infection, a daily systemic antibiotic was also administered intramuscularly for one week (Ceftriaxone®, Sandoz Company, Basel, Switzerland) at a dose of 50 mg/kg. The animals were closely monitored throughout the treatment period, which lasted for three weeks.

Evaluation of wound infection

The wound infection was evaluated and classified according to **Metwally *et al.*, (2020)** as follows:

-: Absence of signs of wound infections.
+: Presence of mild signs of wound infections.
++ Presence of moderate signs of wound infections.
+++ Presence of severe signs of wound infections **(Table 2).**

Table 2: Visible signs of wound infection in the treated dogs:

Case	Wound site	Clinical score of clearance of infection				
		Before treatment	After 7 days of treatment	After 14 days of treatment	After 21 days of treatment	After 30 days of treatment
1	Lateral aspect of abdomen	+++	-	-	-	-
2	Lateral aspect of abdomen	++	-	-	-	-
3	Lateral aspect of abdomen	+	-	-	-	-
4	Lateral aspect of hind limb	+++	+	-	-	-
5	Lateral aspect of hind limb	+	-	-	-	-
6	Elbow joint	++	+	-	-	-
7	Neck	-	-	-	-	-
8	Nipple	+++	+	-	-	-

(-) indicates absence of signs of wound infections. (+) indicates presence of mild signs of wound infections. (++) indicate presence of moderate signs of wound infections. (+++) indicate presence of severe signs of wound infections **(Metwally *et al.,* 2020)**.

Evaluation of wound healing

The evaluation of wound healing was performed using both subjective and objective methods. The objective method involved planimetry evaluation, where the size of each wound was measured with a digital caliper on day zero, when animals were first admitted to the clinic. Measurements were then repeated on days 7, 14, 21 and 30 post-treatments. Macroscopic images of the wounds were captured using a digital camera at the same intervals, with a standardized ruler included in each photograph for calibration. Wound size was estimated using standardized techniques by taking photos and measuring the wound using the software "Digimizer" **(Sardari *et al.,* 2007)**. The subjective evaluation depended on the macroscopic findings of each wound and the visible signs of wound infection across the period of treatment **(Metwally *et al.,* 2020).**

Statistical Analysis

Wound size and contraction percentages were expressed as mean ± standard deviation (SD) for each time point.

RESULTS

Results of micromorphology of ZnO-NPs

The ZnO-NPs dispersed in a coarse manner and interconnected to form a ZnO flower-like structure **(Fig. 1).** No agglomeration of the individual components was observed, confirming the nano structural properties of the synthesized materials.

Findings of wound healing

Macroscopic findings

The macroscopic evaluation of the treated dogs showed a marked improvement in wound healing following the topical application of ZnO-NPs ointment.

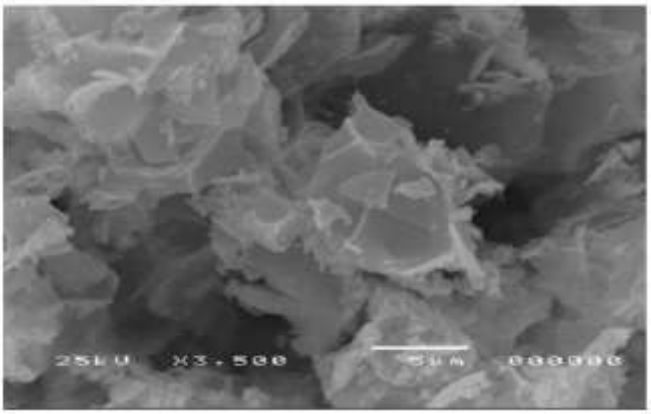

Fig. 1: SEM image of the prepared ZnO-NPs.

Case 1

A 9-year-old female Griffon was presented with an old wound at the lateral abdomen caused by a ruptured abscess. The wound was large, infected, and showed necrotic tissue upon admission. Following three weeks of daily ZnO-NPs ointment application, the wound exhibited a significant reduction in size, granulation tissue formation, and improved epithelialization. The necrotic areas were resolved, and

no secondary infections were observed throughout the healing period **(Fig. 2).**

Case 2

A one-year-old female mongrel dog was presented with an infected biting wound at the lateral abdomen that had been worsening for five days before treatment. The wound was deep, inflamed, and infected, with visible exudate and necrotic areas. After three weeks of ZnO-NPs ointment application, the wound showed significant contraction, absence of infection, and formation of healthy granulation tissue. The surrounding inflammation subsided, and epithelialization was well-advanced **(Fig. 2).**

Case 3

A three-month-old male German Shepherd was presented with an infected biting wound at the lateral abdomen, which had been worsening for three days before treatment. The wound exhibited visible inflammation, exudate, and early necrotic changes. Following three weeks of ZnO-NPs ointment application, the wound showed complete contraction, full re-epithelialization, and hair regrowth in the affected area. No infection or residual scarring was observed **(Fig. 2).**

Case 4

A two-year-old male mongrel dog was presented with an accidental wound at the lateral aspect of the tarsal joint ten days ago. The wound was chronically infected and associated with bone exposure and septic arthritis. The wound exhibited necrotic tissue and extensive inflammation at the time of admission. After four weeks of treatment with ZnO-NPs ointment, the wound showed a notable reduction in size, with the formation of healthy granulation tissue and complete coverage of the previously exposed bone. The surrounding tissue underwent re-epithelialization, and no signs of secondary infection were observed throughout the healing process **(Fig. 2).**

Fig. 2: Macroscopic photographs of the first four dogs with chronic wounds before and after treatment with ZnO-NPs ointment. D: day.

Case 5

A one-year-old male mongrel dog suffered from an accidental wound at the lateral aspect of the tarsal joint, presenting with severe inflammation, tissue exposure, and infection. The wound was deep with exposed tissue and scabbing at the margins. After three weeks of ZnO-NPs ointment application, there was a remarkable improvement, with wound contraction, re-epithelialization, and significant reduction in inflammation. The wound was almost completely healed, with minimal scarring and no signs of infection **(Fig. 3).**

Case 6

A three-year-old male mongrel dog was presented with an accidental wound at the elbow joint. The wound exhibited severe tissue exposure, inflammation, and infection before treatment. It showed poor healing progression at the time of admission and significant tissue necrosis. After two weeks of ZnO-NPs ointment treatment, there was marked wound contraction, epithelialization, and healing. The treated site demonstrated no residual infection or inflammation. But the owner discontinued the treatment at day 14 **(Fig. 3).**

Case 7

A nine-month-old male Bullmastiff dog developed multiple skin wounds on the neck caused by prolonged use of an Elizabethan collar, leading to skin abrasions and inflammation. The affected area exhibited redness,

lacerations, and scabbing, causing discomfort. After three weeks of ZnO-NPs ointment application, the wound completely healed, with re-epithelialization and significant hair regrowth. The treated area showed no residual inflammation, infection or scarring, indicating effective tissue repair and cosmetic healing **(Fig. 3).**

Case 8

An eight-year-old female Golden Retriever was presented with a necrotic, infected wound around the nipple due to excessive licking. The wound exhibited severe tissue necrosis, inflammation, and infection with bad odor and pus formation at the time of admission. Following daily application of ZnO-NPs ointment for four weeks, a remarkable improvement was observed. The wound area showed reduced inflammation, re-epithelialization, formation of healthy granulation tissue, and significant contraction of the wound. Additionally, there were no signs of secondary infection, pus formation, or foul odor, indicating effective antibacterial action of ZnO-NPs **(Fig. 3).**

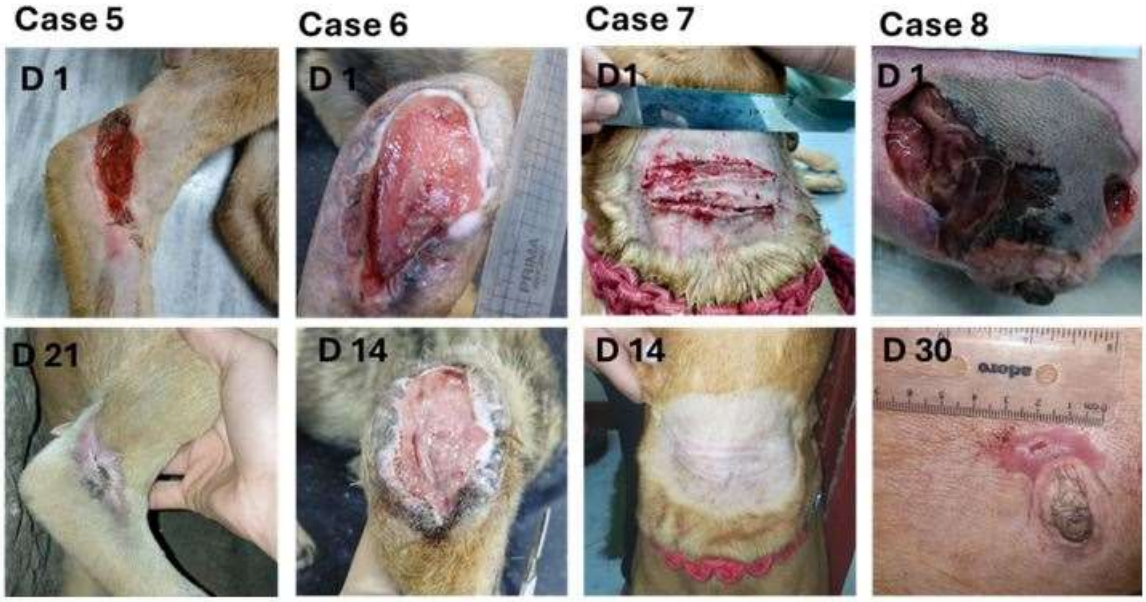

Fig. 3: Macroscopic photographs of the second four dogs with chronic wounds before and after treatment with ZnO-NPs ointment. D: day.

Planimetry findings

Wound size percent

All cases showed a marked wound size reduction, with most cases reaching full closure by day 30. Case 7 showed the fastest healing (fully healed by day 14), while case 8 showed the slowest healing. Cases 4 and 8 exhibited slower contraction than other wounds, with case 4 healed partially (13.78%) by day 30. The wound size percent for all cases is represented in the graph line **(Fig. 4a),** and the mean ± SD values are shown in **Table 3.**

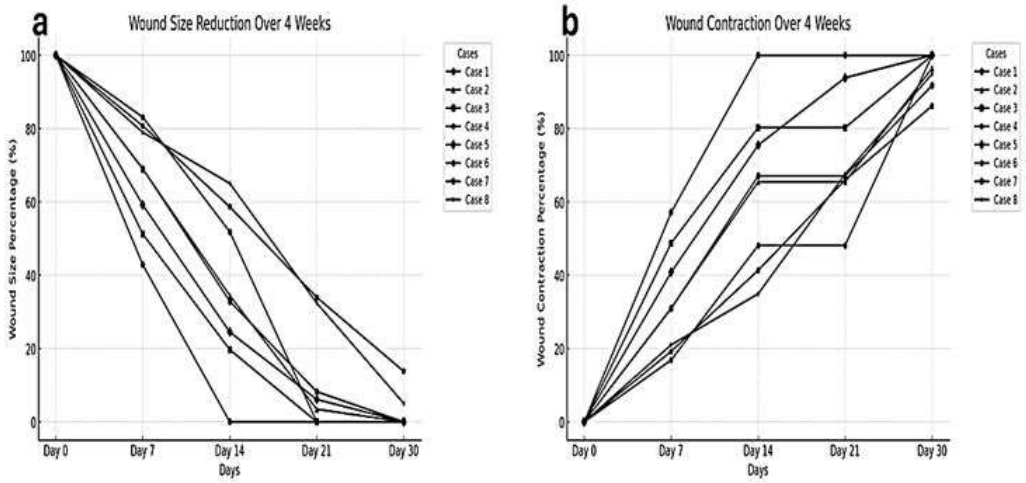

Fig. 4: Wound size percentage **(a)** and wound contraction percentage **(b)** over 4 weeks in all dogs treated with ZnO-NPs ointment.

Wound contraction percent

Almost all cases showed complete contraction by day 30, with case 7 being the quickest to fully close. Cases 4 and 8 showed slower contraction in the initial weeks but achieved a noticeable closure by day 30. Case 2 had a slower progression initially but achieved nearly complete healing by day 30. The wound contraction percent for all cases is represented in the graph line **(Fig. 4b),** and the mean ± SD values are shown in **Table 3.**

Table 3: Mean ± SD of wound size and contraction % in the treated dogs.

Day	Mean wound size (%) ± (SD)	Mean wound contraction (%) ± (SD)
Day 0	100.00%	0.00%
Day 7	66.78% ± 14.61%	33.22% ±14.61%
Day 14	35.89% ± 21.76%	64.11% ±21.76%
Day 21	10.52% ± 14.32%	73.57% ±16.91%
Day 30	2.36% ± 4.95%	96.19% ± 5.05%

DISCUSSION

This study evaluated the wound-healing potential of ZnO-NPs in dogs, and the results provide strong evidence for their effectiveness in promoting cutaneous wound healing. The findings are consistent with a growing body of literature indicating the broad therapeutic potential of ZnO-NPs in both veterinary and human medicine. The ZnO-NPs markedly accelerated wound contraction, reduced infection, and promoted tissue regeneration as well as wound healing with minimal scar tissue formation, validating their role as an innovative treatment for wound care.

ZnO-NPs exhibit multi-faceted mechanisms that contribute to their wound-healing properties. As noted by **Khan *et al.,* (2021)**, ZnO-NPs have excellent antibacterial properties, which are crucial in controlling wound infections, especially those caused by *Staphylococcus aureus* and *Escherichia coli*, which are common in animal wounds. This antibacterial property is further supported by the green synthesis of ZnO-NPs, which has been shown to reduce microbial load in infected wounds **(Moalwi *et al.,* 2024)**. This study observed similar results, with treated wounds showing minimal pus and edema, consistent with the antibacterial action of ZnO-NPs.

Furthermore, ZnO-NPs play an essential role in reducing inflammation at the wound site. This anti-inflammatory action facilitates faster healing by preventing prolonged inflammatory responses, which can hinder the regeneration of new tissue. This finding aligns with the work of **Abbaszadeh *et al.,* (2023)** who observed a reduction in inflammatory cytokines and oxidative stress at wound sites treated with ZnO-NPs. Such reductions in inflammation promote a more favorable environment for granulation tissue formation and epithelialization.

The most striking finding from our study was the rapid wound contraction observed in most cases, with case 7 achieving complete closure by day 14. This result is consistent with the results of earlier authors **(Metwally *et al.,* 2020)** who reported that ZnO-NPs markedly accelerated wound contraction in equine models, with green ZnO-NPs showing faster healing and infection clearance than their chemical counterparts. ZnO-NPs stimulate fibroblast proliferation and migration, key cells responsible for producing collagen and contracting the wound edges. In this study, the ZnO-NPs ointment promoted faster wound regeneration and repair. The promotion of these processes is in line with previous authors **(Irfan *et al.,* 2022)** who demonstrated that ZnO-NPs stimulate collagen synthesis and enhance the fibroblast activity necessary for wound healing.

Moreover, the nanoparticles' small size and high surface area increase their penetration capacity, allowing them to reach deeper layers of tissue and provide therapeutic effects more efficiently than bulk ZnO **(Khan *et al.,* 2021)**. In our study, this was evident as the ZnO-NPs ointment successfully penetrated deep into the wound beds, where they likely promoted collagen cross-linking and tissue regeneration at the cellular level.

When compared to other treatments, ZnO-NPs proved superior in enhancing wound healing **(Abbas *et al.,* 2023).** The authors compared ZnO-NPs with plant extracts for wound healing in rabbits and reported that ZnO-NPs provide faster closure and more effective contraction. Our study similarly found that wounds treated with ZnO-NPs ointment showed faster healing. **Metwally *et al.,* (2020)** also confirmed that ZnO-NPs outperform other agents, including green ZnO-NPs gels, in terms of wound closure and infection prevention in equine wounds, reinforcing our finding that ZnO-NPs are a potent therapeutic tool in infected wounds. **Erdogan and Cevik (2022)** added that ZnO-NPs synthesized using *Saccharomyces cerevisiae* aqueous lysate enhanced wound closure in L929 fibroblast cells in a dose-dependent manner, with significant effects observed at 10, 100, and 1000 µg/mL. These findings suggest their potential role in promoting cell migration and tissue repair.

Moreover, exploring the synergistic effects of ZnO-NPs with other therapeutic agents, such as plant extracts, growth factors, or antimicrobial peptides, could enhance their healing efficacy. **Irfan *et al.*, (2022)** demonstrated that combining ZnO-NPs with plant-derived compounds could further accelerate wound healing and improve antibacterial activity.

Limitations

One of the key limitations of this study is the inclusion of animals with varying ages, sexes, and breeds. These factors may have introduced variability in the healing process. Specifically, younger animals tended to experience faster wound closure compared to older animals, as evidenced by the quicker healing times in younger subjects. This age-related difference in healing rates could be attributed to the higher regenerative capacity typically found in younger animals.

Additionally, the heterogeneity in wound characteristics, such as size, shape, location, and severity, influenced the rate of healing. Larger, more complex wounds may have taken longer to heal, while smaller or simpler wounds responded more quickly to treatment. The presence of infection, as well as variations in wound location and depth, further affected the speed of wound healing and the period of complete recovery. These factors, though important for real-world application, introduce a level of variability that may limit the generalizability of the results. Future studies with more standardized subjects and wound characteristics could provide more consistent and reliable data on the effects of ZnO-NPs in wound healing.

Another limitation observed in our study was the variation in healing times among cases. For example, case 4 exhibited a slower healing process, with the wound size reduced to 13.78% by day 30, suggesting that larger or more chronic wounds may require longer treatment times or higher doses of ZnO-NPs for optimal healing. Further research on the optimal concentration and formulation of ZnO-NPs for different wound types is necessary to address this variability.

Another challenge lies in the potential for ZnO-NP accumulation in tissues over time, which may lead to toxicity if used excessively. While our study did not observe any significant adverse effects, it is essential to continue monitoring the long-term safety of ZnO-NPs in clinical settings. Recently Fujihara and Nishimoto (2024) cautioned about the potential for nanoparticle aggregation and subsequent toxicity when used in different concentrations, highlighting the need for further toxicity testing and safety evaluations.

CONCLUSION

ZnO-NPs are an effective treatment for chronic infected wound healing in dogs. They offer numerous benefits, including antibacterial, anti-inflammatory, and regenerative properties. ZnO-NPs markedly accelerated wound contraction, promoted tissue regeneration, and prevented infection, making them a promising alternative to conventional wound treatments in veterinary medicine. Further studies are needed to optimize ZnO-NP formulations and evaluate their long-term safety and efficacy in different wound types.

Conflict of interest

The authors declare that they have no competing interests.

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