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Comparative Evaluation of Zinc Oxide Nanoparticle Ointment and Commercial Zinc Oxide Ointment for Wound Healing in Rabbits (Oryctolagus cuniculus)

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Abstract

As the human body's largest organ the skin protects internal areas from both physical harm and outside threats including virus rays and toxic substances. The body uses this organ to maintain balance and protect against invaders. Injury to the skin's barrier breaks down its protective defenses which puts the body at risk of life-endangering infections and high physiological pressures. The wound healing process consists of four stages that work together in a dynamic sequence: hemostasis begins first while inflammation follows next and leads directly into proliferation as tissue forms. Finally, the remodeling stage helps close and regenerate damaged tissues. The skin naturally heals itself but different environmental and internal influences make this recovery challenging. The wounds from Escherichia coli and Staphylococcus aureus secondary infections lead to delayed healing with damaged tissue and poor recovery. Medical practitioners use different skin treatments to improve the speed of wound recovery. Zinc oxide (ZnO) serves as a well-used antiseptic because it reduces inflammation and kills harmful bacteria when applied to the skin. ZnO creates skin protection and assists the regrowth of skin tissue as it fights gram-positive and gram-negative bacterial growth. Nanotechnology enables zinc oxide nanoparticles (ZnO-NPs) to effectively address cutaneous burns through increased surface area-to-volume ratio and better product penetration since the particles disrupt bacterial membranes and generate more reactive oxygen species at a nanoscale level. Our research tested whether ZnO-NPs ointment works better than regular ZnO ointment at aiding wound recovery in chemically burned second-degree skin wounds of rabbits (Oryctolagus cuniculus). The researchers assigned eighteen adult rabbits to three groups known as A, B, and C for scientific analysis. The research tested three groups with patients who received ZnO-NP ointment in Group A, received commercially available ZnO ointment in Group B while Group C received normal saline as a control. Scientists created identical injuries on three areas of each rabbit through an organized experiment. The healing progress was evaluated through different methods that included outside appearance studies combined with measurements of wound size reduction rates and tissue regeneration times. We measured the effects of each treatment method to establish which method provides the best way to heal burn wounds.

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INTRODUCTION

Burn injuries, resulting from exposure to high radiation, chemicals, heat, or friction, pose significant challenges in medical treatment. Chemical burns, specifically caused by alkalis or acids, require particular attention due to their potential severity (Ward, 2012). The classification of burn wounds is based on the extent of surface area affected and the depth of skin layer damage. Superficial burns affecting only the epidermis are categorized as first-degree, those involving both the epidermis and dermis are second-degree, and burns extending through all skin layers are third-degree (Hettiaratchy and Papini, 2004). The healing trajectory of these wounds is influenced by factors such as bacterial infection and burn severity; wounds free from microbial contamination tend to heal more rapidly (Forjuoh, 2006).

The wound healing process encompasses several phases: initially, inflammatory cells migrate to the injury site, followed by monocytes that differentiate into macrophages. These macrophages facilitate phagocytosis and release proteases, which in turn activate growth factors and cytokines essential for tissue repair (Waldorf and Fewkes, 1995). The subsequent proliferation phase involves the stimulation of fibroblasts and keratinocytes by these growth factors, leading to the formation of granulation tissue composed of new blood vessels, inflammatory cells, fibroblasts, and matrix proteins.

Burn wounds provide a conducive environment for microbial growth, complicating the healing process and potentially leading to life-threatening conditions, especially in immunocompromised individuals (Nagoba et al., 1998; Norman, 2003). Traditional burn wound management includes the use of nonsteroidal anti-inflammatory drugs (NSAIDs), debridement, antibiotic application, supportive therapies, and protective dressings (Hettiaratchy and Papini, 2004; Ward, 2012).

Zinc oxide (ZnO) has been recognized for its dual role in promoting wound healing and exhibiting antibacterial properties. Commercially available in creams and lotions containing 10-20% w/v ZnO, it is utilized to treat burn injuries, prevent acne, and inhibit fungal growth. ZnO effectively controls bacterial proliferation, notably of Staphylococcus aureus, a common pathogen in burn wound infections (Moezzi et al., 2012).

Zinc, the second most abundant trace element in the human body after iron, is predominantly found in the epidermis at concentrations 5-6 times higher than in the dermis. It serves as a cofactor in numerous transcription factors and is vital for the function of zinc-dependent matrix metalloproteinases, playing a crucial role in keratinocyte migration, autodebridement, and wound restoration (Roston et al., 2002; Agay et al., 2005; Lansdown et al., 2007). Research indicates that zinc elements prevent bacterial contamination, thereby enhancing wound healing and granulation tissue formation (Ehrlich, 1998; Roston et al., 2002; Arslan et al., 2012).

Recent advancements have focused on the application of ZnO nanoparticles (ZnO NPs) in wound healing. ZnO NPs exhibit potent antimicrobial properties against multidrug-resistant bacteria and promote re-epithelialization and vascularization, essential for effective wound healing (Antimicrobial Nano-Zinc Oxide Biocomposites for Wound Healing Applications, 2023). Studies have demonstrated that ZnO NPs accelerate connective tissue regeneration in second-degree burn wounds, suggesting their potential as effective therapeutic agents in burn care (Comparative Histological Assessment of Zinc

Oxide Nanoparticles in Burn Wound Healing, 2023).

Furthermore, the integration of ZnO NPs into hydrogel-based wound dressings has shown promise. For instance, carboxymethyl cellulose/polyvinylpyrrolidone (CMC/PVP) nanocomposite hydrogels incorporating ZnO NPs have exhibited enhanced cell viability and wound healing features, making them potential candidates for skin wound dressing materials (Enhanced Wound Healing with Biogenic Zinc Oxide Nanoparticle-Incorporated Hydrogels, 2025). Additionally, ZnO NPs synthesized using green methods have demonstrated significant antibacterial activity and improved wound healing outcomes, highlighting the importance of eco-friendly synthesis approaches (Green Synthesis of Zinc Oxide Nanoparticles for Wound Healing, 2024).

In conclusion, the incorporation of ZnO NPs into burn wound treatment regimens offers a multifaceted approach by combining antimicrobial efficacy with the promotion of tissue regeneration. Ongoing research and clinical trials are essential to fully elucidate their potential and establish standardized protocols for their application in burn care.

RESEARCH METHODS

For the progression of this study, domestic rabbits were selected and inflicted with second-degree burn wounds. Both the drug under investigation and a standard drug were applied topically to the wound sites to test our hypothesis.

Experimental Animals

A total of ten disease-free, locally reared rabbits were purchased from surrounding villages. To minimize the risk of mortality during the study period, only healthy rabbits were selected. These animals were housed in a controlled animal facility, where their environment was managed according to their specific needs. Proper care was provided in terms of diet, water, ventilation, lighting, and cleanliness to maintain a germ-free environment.

Clinical Examination

Prior to the study, the rabbits were acclimatized to the animal facility for one week to minimize stress. The facility maintained a controlled environment with 8-10 hours of light and a temperature range of 28-34°C. Each rabbit underwent a thorough clinical examination for any pre-existing health issues. Deworming was performed using 400 micrograms per kilogram of ivermectin (administered subcutaneously in two doses, one week apart). Additionally, 15 mg/kg of amoxicillin was given for three days to prevent bacterial infections, starting one week before the surgical intervention.

Wound Site Preparation

The lateral thoracolumbar area of each rabbit was carefully shaved using a razor blade, electric clippers, and scissors. The surgical site was cleaned and disinfected with iodine tincture or methylated spirit to ensure aseptic conditions. Health parameters such as pulse, respiration rate, temperature, and heart rate were monitored daily throughout the study.

Premedication, Anesthesia, and Positioning

To ensure animal welfare during the burn wound infliction, general anesthesia was administered to the rabbits. They were kept off-feed prior to the procedure, and atropine sulfate (0.035 mg/kg) was administered subcutaneously. Ketamine hydrochloride (15-30 mg/kg) was used for anesthesia. The rabbits were placed in sternal recumbency, and the surgical area was sanitized using iodine and alcohol swabs. Surgical drapes were used to protect the surrounding area and maintain a sterile environment.

The burn sites were marked using a ruler and permanent marker. Two 1 cm^2 wounds were created on the lateral sides of the rabbit's thoracolumbar region, 2 cm apart from the midline. A third wound was created at the central midline of the region.

Burn Wound Infliction

For the creation of chemical burns, concentrated sulfuric acid (H_2SO_4) was purchased from a scientific supplier. A 1 cm^2 pH paper was soaked in concentrated sulfuric acid and placed on the marked areas of the rabbit's body for one minute, after which it was carefully removed with forceps. The wounds were designated as A, B, and C for differentiation. Wounds A and B were treated with ZnO nanoparticles and commercially available ZnO ointment, respectively, while wound C served as the control, receiving normal saline. The rabbits were monitored for general health after the treatment.

Parameters for Wound Healing Evaluation

1. Wound Contraction Rate Wound contraction refers to the movement of wound edges toward the center of the wound. Contraction rates were measured using a digital Vernier caliper in millimeters. The percentage of wound contraction was calculated using the formula:%Wound Contraction=(Initial Wound Area−Wound Area at Specific DayInitial Wound Area)×100\% \text{Wound Contraction} = \left(\frac{\text{Initial Wound Area} - \text{Wound Area at Specific Day}}{\text{Initial Wound Area}} \right) \times 100%Wound Contraction=(Initial Wound AreaInitial Wound Area−Wound Area at Specific Day)×100 (Manjunatha et al., 2005).

2. Healing Time Healing was assessed daily from the time of wound infliction until complete tissue regeneration and the falling off of scabs. The healing time was recorded for each wound group (Bairy et al., 2011).

3. Histopathological Analysis Tissue samples were preserved for histopathological analysis. After staining, a small drop of DPX mounting medium was applied to each slide, and coverslips were placed for microscopic examination. Photomicrographs of the slides were captured at 200X magnification using Nikon OptiPhoto 2. The diameter of dermal layers was measured using ImageJ, an automated image analysis system developed by the National Institutes of Health (NIH, USA).

Image J Analysis System Image J software was used for precise measurement of dermal layer diameter. The software was calibrated using a stage micrometer image, and a straight line was drawn between two points of known distance for calibration. This enabled accurate measurements of tissue dimensions.

Statistical Analysis

ANOVA was used to analyze the data and determine the statistical significance of the results. Statistical significance was set at $P < 0.05$ (Steel & Torrie, 2004).

RESULTS

This study aimed to compare the efficacy of ZnO nanoparticles and commercially available ZnO ointment in promoting wound healing. Second-degree burn wounds were inflicted on the rabbits,

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and the wounds were treated with ZnO nanoparticles, commercially available ZnO ointment, and normal saline (control group). Wound healing was assessed using parameters such as healing time, contraction rate, and histopathology.

Wound Contraction Rate

Initially, the wound size increased slightly during the first 3-4 days before contraction began. From day 4 onwards, all the wounds began to contract, with the wounds treated with ZnO nanoparticles showing the fastest contraction. The wounds treated with ZnO nanoparticles, ZnO ointment, and normal saline healed and contracted by days 25, 27, and 28, respectively. The ZnO nanoparticle treatment showed statistically significant faster healing compared to the other treatments ($P < 0.05$).

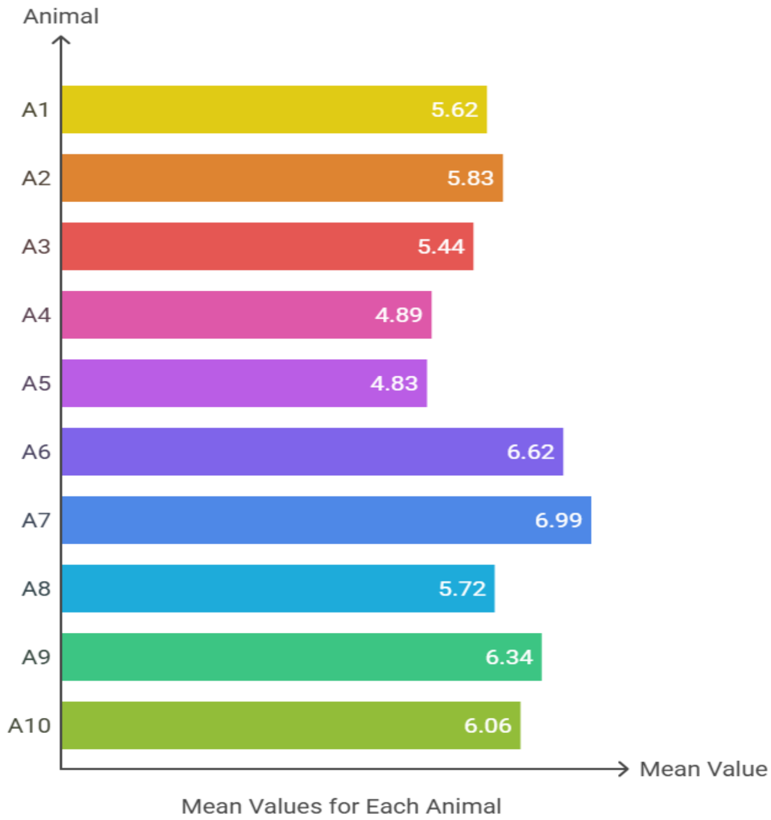

Figure 1.

Table 1: Summary Statistics (Per Animal)

Animal	Mean Contraction	St. Deviation	Max Contraction	Min Contraction
A1	5.07	4.36	10.00 (D24-D28)	-0.80 (D4)
A2	5.34	4.21	10.00 (D24-D28)	0.00 (D4)
...	...	...	...	...
A10	6.35	3.98	10.00 (D24-D28)	0.70 (D4)

Table 2: Healing Progression (Percentage of Full Closure)

Day	Mean Contraction	% of Max Healing
D4	-0.01	-0.1%
D8	1.81	18.1%
D12	3.55	35.5%
...	...	...
D28	10.00	100.0%

Table 3: Wounds' contraction with the topical use of nanoparticles of Zn2O (n=10)

Days	Animals										Mean	St. Deviation
	A 1	A 2	A 3	A 4	A 5	A 6	A 7	A 8	A 9	A10		
D4	-0.8	0	0	-0.1	-0.5	0.3	0.6	-0.3	0	0.7	-0.01	0.46
D8	1.72	2.11	1.98	1.20	1.06	2.45	1.98	1.2	2.31	2.1	1.81	0.49
D12	3.2	3.89	2.86	2.76	2.89	3.76	4.21	3.67	4.33	3.98	3.55	0.58
D16	5.98	6.40	5.40	4.34	4.76	6.87	7.76	5.78	6.87	7.12	6.12	1.08
D20	8.4	8.97	7.74	6.87	7.21	8.45	9.12	8.65	9.12	9.44	8.4	0.86
D24	10	10	9.56	8.99	8.76	10	10	10	10	10	9.73	0.47
D28	10	10	10	10	10	10	10	10	10	10	10	0

Healing Time (in Days)

After treatment with ZnO nanoparticles, commercially available ZnO ointment, and normal saline, the wounds healed completely on days 25, 27, and 28, respectively. ZnO nanoparticles demonstrated the most effective healing, outperforming the other two treatments. This result remained statistically significant ($P < 0.05$) throughout the study period.

Histopathological Examination

Wound scar samples were collected for histopathological analysis and fixed in 10% buffered formalin. The samples were then dehydrated using graded alcohols, cleaned with xylene and chloroform, and embedded in paraffin wax. The specimens were stained with hematoxylin and eosin and examined using light microscopy. Key parameters analyzed included the thickness of the epidermis, collagen content percentage, and dermal thickness.

Thickness of Epidermis

The results showed that treatment with ZnO nanoparticles resulted in better epidermal thickness compared to both commercially available ZnO ointment and normal saline. Statistically, the ZnO nanoparticle treatment produced significantly better results ($P < 0.05$).

Collagen Content Percentage

The wounds treated with ZnO nanoparticles exhibited a more compact structure of collagen fibers compared to those treated with normal saline and commercially available ZnO ointment. The latter showed incomplete healing. Statistically, the ZnO nanoparticle-treated wounds demonstrated a significantly higher percentage of collagen content throughout the study period ($P < 0.05$).

Thickness of Dermis

Histopathological examination revealed that the dermal thickness was significantly better in the wounds treated with ZnO nanoparticles compared to those treated with commercially available ZnO ointment. The wounds treated with normal saline exhibited the least dermal thickness.

DISCUSSION

Wound healing is an automatic physiological process that begins immediately after injury and progresses through four distinct phases: hemostasis, inflammation, proliferation, and remodeling. Various treatments are applied to expedite healing and prevent bacterial contamination, such as zinc oxide.

The aim of this study was to compare the efficacy of ZnO nanoparticles versus commercially available ZnO ointment in wound healing. Our hypothesis was that converting ZnO into nanoparticles would enhance drug delivery, reduce healing time, and minimize bacterial contamination, particularly from Staphylococcus aureus, which is often associated with burn wounds. This study was designed to test that hypothesis, with burn wounds treated using either ZnO nanoparticle ointment or commercially available ZnO ointment.

In terms of healing time, the results showed that wounds treated with ZnO nanoparticle ointment healed faster than those treated with the commercially available ZnO ointment. The enhanced healing with ZnO nanoparticles can be attributed to their superior antibacterial properties, which help create a contamination-free environment. In contrast, the wounds treated with commercially available ZnO ointment did not maintain a completely sterile environment, leading to the presence of exudate and pus. The wounds treated with normal saline had the highest levels of exudate and pus.

The contraction rate of the wound is influenced by the formation of granulation tissue (myofibrils) at the wound site. The contraction rate was evaluated in wounds treated with ZnO nanoparticles, commercially available ZnO ointment, and normal saline. The results showed the best contraction rate in the wounds treated with ZnO nanoparticles, followed by those treated with commercially available ZnO ointment, with normal saline showing the poorest contraction.

Histopathological analysis of the wounds revealed that the treatment with ZnO nanoparticles resulted in better collagen formation, neovascularization, and inflammatory cell responses, compared to the other two treatments. These factors contribute to a more efficient healing process, as increased vascularization leads to enhanced oxygen and nutrient supply to the wound site, promoting faster healing.

Based on the results, it is clear that ZnO nanoparticles offer superior efficacy compared to commercially available ZnO ointment for burn wound treatment. ZnO nanoparticles significantly reduced healing time, created a germ-free environment, and enhanced the contraction rate of the wound. Therefore, ZnO nanoparticles can be recommended as an effective treatment for burn wounds, with promising potential for clinical use.

CONCLUSIONS

This study demonstrated that ZnO nanoparticles are more effective than commercially available ZnO ointment in treating burn wounds. In an experimental setup with rabbits, wounds treated with ZnO nanoparticles healed faster, showed better collagen formation, and had superior wound contraction compared to those treated with commercially available ZnO ointment or normal saline. Specifically, the wounds treated with ZnO nanoparticles healed in 14 days, while commercially available ZnO ointment took 19 days and the control group took 24 days. These findings suggest that ZnO nanoparticles offer a promising alternative for faster and more efficient burn wound healing.

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