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Comparative Effectiveness of Calcium Zincate Nanocomposite and Zinc Oxide Nanoparticles as Healing Agent Against Third Degree Burn Wound in Rabbits

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ABSTRACT

The aim of this study was to evaluate the comparative healing effects of calcium zincate nanocomposite and zinc oxide nanoparticles on 3rd degree burn wound in rabbits. For this purpose, 4 groups were made and each group contained 4 rabbits (10 to 12 weeks old and weighing about 1.5 to 2.0kg). Group A kept as a control group was treated by normal saline (T0). Group B and C were kept as experimental groups (T1, T2) and were treated with 1% calcium zincate nanocomposite and 1% Zinc oxide nanoparticles, respectively. While, group D named as standard group (T3) was given Quench cream® 15g (Silver sulfadiazine 1% ointment). Factors considered for the testing in this experimental work were wound contraction, healing time, hematology, thickness of epidermis and histopathology of the burn wound. The results indicated that wound contraction was maximum and rapid in case of 1% Calcium zincate nanocomposite than 1% zinc oxide nanoparticles and Quench cream® 15g. 1% calcium zincate nanocomposite took less time to completely heal the wound than 1% Zinc oxide nanoparticles. 1% Zinc oxide nanoparticles showed good healing than the Quench® cream. Moreover, 1% calcium zincate nanocomposite had the highest collagen percentage than 1% zinc oxide nanoparticles. Tissues treated with 1% calcium zincate nanocomposite exhibited better tensile characteristics than tissues treated with zinc oxide nanoparticles and 1% silver sulfadiazine. Furthermore, 1% calcium zincate nanoparticles exhibited faster re-epithelization at the wound site in comparison to zinc oxide nanoparticles and 1% silver sulfadiazine. So, it is concluded that 1% calcium zincate nanocomposite give better wound healing effects than 1% zinc oxide nanoparticles.

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INTRODUCTION

The skin acts as the first line of defense of body against environmental threats and injuries occur when this barrier fails to remain intact. There are two types of wounds: closed wounds have bruises and hematomas while open wounds have skin disruption. Burn burns are considered acute injuries with potential outcomes including tissue fluid loss, infection, discomfort and even death (Xu et al. 2019). There are several steps involved in healing burn injuries. Hemostasis (a few hours), inflammation (about 4 days), cellular proliferation (about 12 days), and remodeling (around 3–5 months) are the four phases of wound healing (Subramaniam et al. 2021). The wound healing can be delayed due to contamination with microbes, older age, immunosuppression, hormonal imbalance and dryness of tissue (Siddiqui and Bernstein 2010). Wound contamination is one of several variables that might affect how a wound heals. It causes a major delay in the healing process of wounds, which in turn causes pain, discomfort and drainage

at the wound site, which eventually culminates in death (Guo and DiPietro 2010).

Zinc is an essential metal found in trace amounts in the human body, and its significance in both health and disease is increasing. Zinc causes apoptosis in the epithelium by protecting it from reactive oxygen species (ROS) and bacterial toxins possibly via the anti-oxidant activity of cysteine-rich metallothioneins. Zinc deficiencies caused by nutrition or congenital abnormalities can lead to pathological changes that delay wound healing (Skoog et al. 2012).

Calcium ions are also important in wound healing because in addition to playing a crucial role in hemostasis as a coagulation factor, literature has demonstrated that the calcium ion serves as a basic cue that guides the biological processes of various cell types during wound healing. For keratinocytes and fibroblasts, calcium functions as an intracellular second messenger and an external signaling molecule. The effect of calcium concentrations on keratinocyte proliferation and differentiation has been investigated in earlier research (Souto et al. 2020). Silver sulfadiazine also gives best results in third degree wound healing (Levin et al. 2022).

Silver sulfadiazine (SSD) is a well-known bactericidal agent, commonly used as a topical treatment for second- and third-degree burns. While SSD is effective in managing bacterial infections in burn wounds, prolonged and excessive application may lead to systemic absorption and toxicity. Recent findings suggest that SSD could delay the wound healing process. However, SSD remains widely used due to its antimicrobial properties and ability to prevent opportunistic infections in burn wounds (Levin et al. 2022). Various other methods have been found to improve wound healing and prevent wound infection. In this regard, a number of studies are conducted in which the wound healing mechanism is described in detail. Various methods have also been invented to help the wound healing process (Dirwan et al. 2024; Chen et al. 2024). One of them is nanotechnology because of their best and quick results (Souto et al. 2020; Asif et al. 2023).

Nanotechnology has been used to solve complex problems in studying both living and nonliving subjects because of their nano size (Kausar et al. 2023; Mustafa et al. 2024) especially in wound healing. Nanotechnology has emerged as a promising field in the treatment of burn wounds, offering innovative solutions to enhance the healing process. Studies have shown the effectiveness of various nanomaterials in wound management, such as silver nanoparticles, cerium oxide nanoparticles and zinc oxide nanoparticles. These nanoparticles have demonstrated accelerated wound closure, reduced healing time and antibacterial properties, which are crucial in preventing infections and promoting tissue regeneration (Erdogan and Cevik 2023). It has been established that using nanoparticles to combat microorganisms has significant benefits, especially for the wound healing process. Furthermore, these are less expensive to treat than antibiotics that are typically found in pharmacies. Additionally, the prolonged presence of nanoparticles in the body leads to the continuous release of medication and accelerates the healing process (Huh and Kwon, 2011).

Zinc oxide nanoparticles have antibacterial and anti-inflammatory potential and speed up the healing of both acute and chronic wounds. These nanoparticles can manage redox imbalance following burn injuries, which aids in wound healing. Studies have also shown that rhamnolipid-coated zinc oxide nanoparticles demonstrate high wound healing efficacy and antibacterial activity (Asif et al. 2023). ZnONPs applied topically significantly accelerate the healing process of wounds. Because of its antibacterial properties, zinc oxide nanoparticles (ZnONPs) protect the wound site against bacterial contamination. ZnONPs prevent puss development at the site of injury. ZnONPs have been sold commercially as creams and ointments with percentages ranging from ten to twenty percent. Additionally, ZnONPs hinder the development of microorganisms like Propionibacterium acnes (Moezzi et al. 2012). Nanoparticles including zinc oxide have been investigated for their role in wound dressing formulations and tissue engineering to combat burn wound infections (Souto et al. 2020). Zinc oxide nanoparticles' efficacy in open-wound healing in rabbits has been evaluated, with promising clinical and histological results (Murali et al. 2021).

Rabbits have been widely used as a model for wound healing studies due to their physiological parallels to humans' wound healing systems. In animal models, nanoparticles like silver nanoparticles, cerium oxide nanoparticles, and zinc oxide nanoparticles have been found to have considerable wound healing effects (Kalashnikova et al. 2015). Furthermore, rhamnolipid-coated zinc oxide nanoparticles have demonstrated potential wound healing effectiveness and antibacterial activity in animal models (Malakar et al. 2023). Hence the rabbits were used as an experimental animal in this study.

The aim of this study is to reveal the comparative healing efficiency of 1% calcium zincate nanocomposite and 1% zinc oxide nanoparticles on 3rd degree burn wound in rabbits. For this purpose, wound contraction rate, healing time, hematology, and histopathology of the wound site are evaluated.

MATERIALS AND METHODS

All bioethical and biosafety parameters were strictly followed throughout the experimental phase of laboratory animal trails in Clinical Medicine and Surgery Department, University of Agriculture, Faisalabad Pakistan.

Animals and grouping

Sixteen healthy and active (10-12 weeks old and weighing about 1.5-2.0 kg) rabbits were purchased from market for experiment. Animals were divided into four groups: control, experimental and standard group as mentioned in Table 1. Aspect of animal welfare was kept in mind.

Table 1: Experimental design and grouping of rabbits

Groups	No of rabbits	Treatment applied	Group Description
A	4	Normal saline (T0)	Control group
B	4	1% Calcium zincate nanocomposite (T1)	Experimental group
C	4	1% Zinc oxide nanoparticles (T2)	Experimental group
D	4	Quench cream® 15g (Silver sulfadiazine 1% ointment) (T3)	Standard group

Temperature of 25-30 degree centigrade was maintained. Deworming of all rabbits was completed seven days before experiment by giving two dosages of ivermectin @ 400 microgram/kg subcutaneously. Fresh fodder was provided to animals twice a day. Bedding of wheat straw was provided to rabbits that also changed every day.

Preparation of nanoparticles

Preparation of Calcium zincate nanocomposite

1% Calcium zincate nanocomposite were prepared by microwave assisted sol gel method and obtained from Dr. Ambreen Ashar Associate Professor Department of chemistry, Government Graduate College Samanabad, Faisalabad. 1 % Calcium zincate nanocomposite ointment was made by adding petroleum jelly.

Preparation of Zinc oxide nanoparticles

Sodium hydroxide (NaOH) was used as a base while Zinc acetate dihydrate Zn (CH3COO)2.2H2O as Zn source for the preparation of ZnO nanoparticles. NaOH and Zn (CH3COO)2.2H2O were dissolved singly in deionized water in 1:1 ratio. Solution of Zn (CH3COO)2.2H2O was added in NaOH with robust stirring at room temperature until white precipitates were was obtained. Precipitates were filtered and placed in hot air oven for 2 hours at 90 °C to obtain the ZnO-NPs. Dried ZnO-NPs were grounded to get powder with help of electrical grinder. This process was repeated for 8 times (Osman and Mustafa 2015).

Characterization of Zinc oxide nanoparticles

At Government College University in Faisalabad's Central Hitech Laboratory, nanoparticle characterization was carried out. A Jeol JDX-3532 diffractometer (Japan) was used to assess the crystalline characteristics and purity of nano ZnO. Quanta 250 FEG has conducted electron microscopy (USA). Bruker IFS 125HR Japan's Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the composition of the manufactured products. Using a zeta particle sizer (Nano ZS-Melvern, USA), Dynamic Light Scattering was used to measure the particle size. The particle size distribution graph produced by the particle sizer shows that the synthesized ZnO particle sizes lie within a tight range.

Preparation of nanoparticles ointment

1% ointment of ZnO-NPS, calcium zincate nanocomposite was made in order to assess the study's hypothesis. First, 1gm ZnO-NPs were combined with 99gm of petroleum jelly to create a first ointment, and then 1gm Ca-NPs and zinc-NPs were added to 99gm of petroleum jelly to create a second ointment. After preparation, these ointments were kept in the plastic jar. Normal saline, acquired from the Care Pharmacy in Faisalabad, Pakistan, was used to treat the fourth group.

Infliction of burn wound

The animals were denied food for six hours prior to the administration of anesthesia. xylazine (5 mg/kg body weight) and ketamine hydrochloride (35 mg/kg body weight) intramuscular were used as anesthesia (Henke et al. 2005). The experimental animals' lateral thoracolumbar site was surgically cut with a razor to inflict a burn wound. Methylated spirit antiseptic solution was utilized to clean the lateral thoracolumbar location. Metal bar heated on burner was applied perpendicularly to the skin for 15 seconds barely with gravitational pressure. Analgesia was injected by using Ketoprofen @ 1-3 mg/kg body weight after creating wounds to make sure pain free healing of the rabbits (Kulyar et al. 2022). Meanwhile, vital signs like temperature, heart rate, and respiration were monitored and documented on a regular basis.

Evaluation parameters for wound healing

Following parameters were used to evaluate the healing of burn wounds.

Wound contraction rate

Wound contraction rate is a measure of how much the initial wound shrank. Using a vernier caliper, the wound was measured on days 0, 7, 14, 21, and 28th of trial. It was contrasted with the pertinent wound's size on day 0. The following formula was utilized to determine the wound's percentage contraction;

$$\text{Contraction\%} = \frac{\text{100-(Wound area on specific day}}{\text{wound area on day 0)} \times 100}$$ (Teo et al. 2021).

Healing time

From the day of wound induction to the re-epithelization, healing time was taken into account. It was calculated by adding up all of the daily observations made until the scar disappeared. The estimation of healing time was based on the accumulation of daily observations from the day the wound was introduced until the scar fell off. This included both regeneration and re-epithelization.

Histopathology

Histopathology techniques consisting of fixation, washing, dehydration, clearing, Infiltration, embedding, sectioning, staining, mounting and observation of slides for used. For histopathological studies tissue samples were preserved in phosphate buffer formalin. Phosphate buffer formalin was prepared by using Buffered formalin100ml, distilled water 900ml and disodium hydrogen phosphate 6.5g and 4.0g. For fixation biopsy samples of the skin were cut in small fragments using the scalpel blade. Samples obtained after cutting were of 3mm size thickness from both sides of horizontal and vertical sides. Only properly sliced samples were further processed and washed with normal saline. After that these samples were fixed in 10% formalin. After fixation, washing of tissue samples was done in order to remove the fixating agents. For this purpose, tissue samples were placed in running tap water. After fixation and washing, tissue samples were dehydrated. To achieve this goal, different ascending graded alcoholics were used. Dehydration protocol was applied step by step, 70% alcohol (overnight), 80% alcohol (1 hour), 95% alcohol (1 hour), 95% alcohol (1 hour), 100% alcohol (1.5 hour), 100% alcohol (1.5 hour), 100% xylene + alcohol I (0.5 hour), xylene-I (1 hour), and xylene-II (1 hour). For the removal of dehydrating agent, clearing agent such as, Xylene 1 (30 minute), xylene II (15 minute), and xylene-III (15 minute) were used to achieve this goal. After the dehydration tissue samples were infiltrated and embedded using molted wax in form of blocks. Liquid paraffin is used for the infiltration at 67 °C. Paraffin-I, Paraffin-II, and Paraffin-III was used for 2

hours. Melted wax was poured in the steel mold after placing the tissue sample in the middle of the steel mold. Tissue sample was solidified at 4 °C. After the solidification of paraffin, block was taken out from the steel mold. Microtome was used to make the sections of the sample. A thin section of 5µm was cut. Tissue carrier was employed to grasp the block with help of clamps**.** Smooth ribbon sections were cut by placing the black in front of knife with help of microtome wheel. Slices were spread in water bath having temperature 60 °C. Slices were fixed on glass slides after picking the slides from water bath. These slides were treated with Meyer's egg albumin before fixing these slides. Extra paraffin was washed off the slides to remove wax from the slide by placing the slide on hot plate. All the slides were incubated in incubator remove paraffin fragments. for 30 minutes at 45-55 °C. Slides were labelled after sectioning and stained using Hematoxylin and Eosin stains. For staining, different staining agents were used like, xylene-I, II, absolute alcohol-I, II, 70% alcohol, hematoxylin, acid alcohol, ammonium alcohol for different time duration. For washing, water was used. DPX drop was dropped over the stained slide and cover slip. After this step slides were under microscope. At last, slides were observed under light microscope at 200X. Photomicrographs of all the slides were captured using Nikon Optiphot 2. Image J software was used to measure the dermis layer diameter of each slide.

Statistical Analysis

Data obtained was analyzed through ANOVA test, while mean will be measured by factorial and CRD test at 5% probability using SPSS version 25 of statistical computer program.

RESULTS

Wound Contraction Rate

Wound measurement was carried out using Vernier caliper at days 0, 7th,14th, 21st and 28th. It was compared with the size of relevant wound at day 0. At day 28th, wound was completely removed by using 1% calcium zincate nanocomposite and Zinc Oxide nanoparticles as shown in Table 2. while, at day 21th wound contraction was 1.78% and 3.08% by using 1% calcium zincate nanocomposite and zinc oxide nanoparticles, respectively. Wound contraction readings of all the groups were clearly showing that wound contraction was rapid and maximum in case of using 1% calcium zincate nanocomposite. Results were highly significant showing that the group treated with 1% calcium zincate nanocomposite was best for healing. Zinc oxide nanoparticles (ZnONPs) also showed good results than that of using Quench® cream.

Wound contraction rate by wound contraction by zinc oxide nanoparticles (ZnONPs) and 1% calcium zincate nanocomposite is shown in Fig. 1 and 2.

Graphically comparison of means of wound contractions at day 0, 7, 14, 21 and 28th is given in Fig. 3. 1% calcium zincate gave faster wound contraction rate than zinc oxide nanoparticles.

Healing time

Wound healing was measured of all the groups was observed on daily basis. Mean healing time of all groups described that wound healing time of 1% calcium zincate nanocomposite was minimum. It took less time to completely heal the wound and showed outward healing pattern leaving no scar. Zinc oxide nanoparticles showed good healing than the Quench® cream. Mean values of healing time of different groups is given in Table 4.

According to this (Fig. 4) graph, 1% calcium zincate give faster results than zinc oxide nanoparticles.

Thickness of epidermis

Epidermal thickness values were recorded and compared. Mean thickness of epidermis of all groups are describing

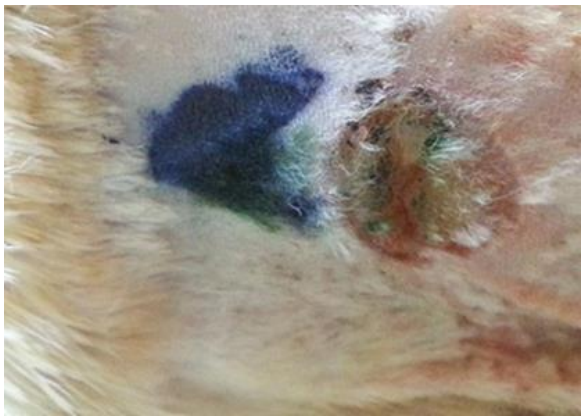

Fig. 1: Wound contraction by ZnONPs.

Fig. 2: Wound contraction by 1% Ca zincate nanocomposite.

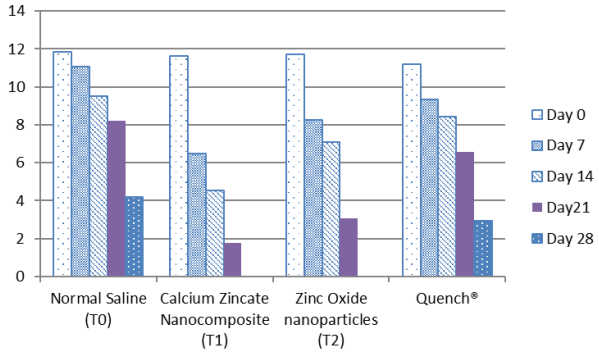

Fig. 3: Graphically comparison of means of wound contractions.

Table 2: Mean value and standard deviation of wound contraction of all treatments (% age+SE)

Treatments	Day 0	Day 7	Day 14	Day 21	Day 28	Means
Normal Saline (T0)	11.82±0.162A	11.06±0.367A	9.52±0.129B	8.21±0.507CD	4.21±0.881J	8.96±0.305
1 % Calcium Zincate Nanocomposite (T1)	11.6±0.542A	6.46±0.72E	4.52±0.48F	1.78±0.139I	0±0J	4.87±0.297
Zinc Oxide Nanoparticles (T2)	11.71±0.638A	8.26±0.379CD	7.1±0.372DE	3.08±0.099GH	0±0J	6.03±0.253
Quench® cream (T3)	11.2±0.862A	9.35±0.407BC	8.42±0.089BC	6.57±0.177E	2.95±0.457HI	7.7±0.301
Means	11.58±0.292	8.78±0.168	7.39±0.188	4.91±0.187	1.79±0.423	0±0

Table 3: ANOVA for means of wound contraction rates

Source	DF	SS	MS	F	P
Treatment	3	195.24	65.078	314.63	0
Days	4	893	223.25	1079.34	0
Treatment* Days	12	67.45	5.621	27.17	0
Error	60	12.41	0.207		
Total	79	1168.09			
Grand mean	6.8939				
CV	6.6				

Table 4: Mean healing times of the treatments used

Treatment	Mean
Normal saline (T0)	32.75
1% Calcium zincate nanocomposite (T1)	27
Zinc oxide nanoparticles (T2)	28.75
Quench® cream (T3)	30.5
Observations per Mean	4
Standard Error of a Mean	0.4677
Std Error (Diff of 2 Means)	0.6614

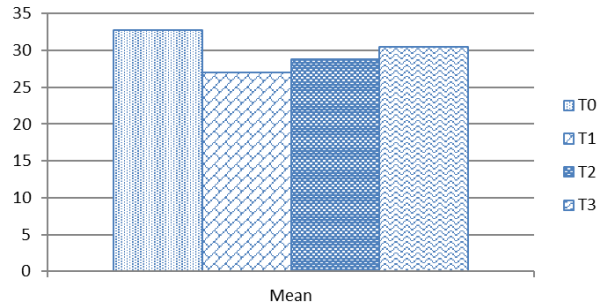

Fig. 4: presents healing times of different groups. T0=Normal saline, T1= 1% calcium zincate nanocomposite, T2= zinc oxide nanoparticles, T3= Quench® cream.

Table 5: ANOVA for the means of healing times

Source	DF	SS	MS	F	P
Treatment	3	72.5	24.1667	27.62	0
Error	12	10.5	0.875		
Total	15	83			
Grand mean	29.75				
CV	3.14				

that thickness of epidermis of the wound treated with 1% calcium zincate nanocomposite is maximum (166.65 µm) as shown in Table 6. Zinc oxide nanoparticles (144.8 µm) showed good strength of epidermis than the Quench® cream (133.82 µm).

The findings of this study showed that wounds treated with calcium zincate nanocomposite had a thickest epidermis. while wounds treated with zinc oxide nanoparticle ointment showed better results than normal saline. Hence, zinc oxide nanoparticles ointment demonstrated statistically significant ($P<0.05$). Fig. 5 explain means of epidermal thickness of wounds of different groups.

Table 6: Mean thickness of epidermis of all groups (µm)

Treatments	Means
Normal saline (T0)	100.96
1% Calcium zincate nanocomposite (T1)	166.65
Zinc oxide nanoparticles (T2)	144.8
Quench® cream (T3)	133.82
Observations per Mean	4
Standard Error of a Mean	2.3403
Std Error (Diff of 2 Means)	3.3097

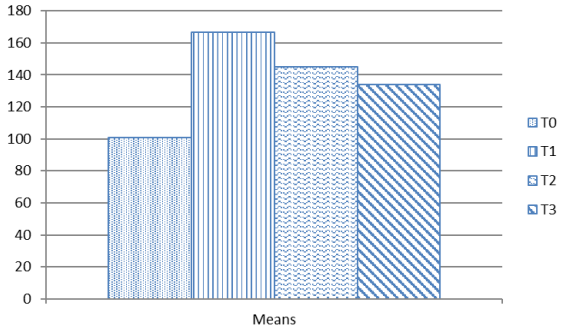

Fig. 5: Graphically comparison of means of epidermal thickness of wounds. T0=Normal saline, T1= 1% calcium zincate nanocomposite, T2= zinc oxide nanoparticles, T3= Quench® cream

Hematological Examination (CBC)

In CBC firstly there was increase in white blood cells counts (WBCs) and then according to the use of treatments its gradually moved towards normal in healing as shown in Fig. 6 and 7.

Fig. 6: CBC during trial of CaZincate

Histopathology of skin samples after wound healing

Histopathology of skin samples treated with different treatment groups are shown in Fig. 8-12.

Histopathology of the skin samples revealed that 1% calcium zincate nanocomposite had improved epidermis and dermis growth. Increased angiogenesis was observed in the histology examination of wounds treated with Ca zincate nanocomposite ointment as compared to other groups.

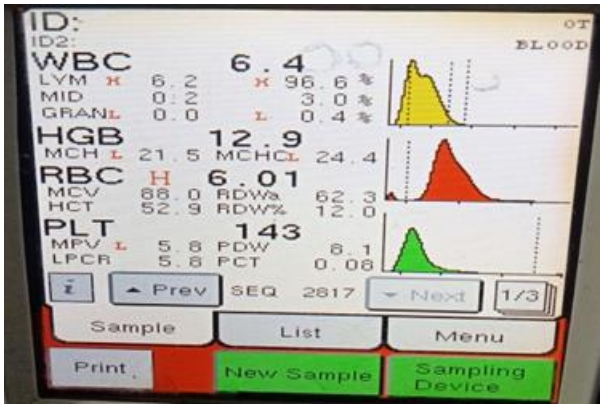

Fig. 7: CBC after trial of CaZincate.

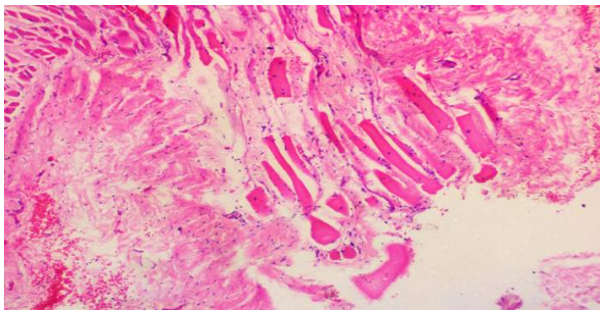

Fig. 8: Histopathology of wound treated with calcium zincate nanocomposite

Neutrophils and macrophages presence indicates ongoing inflammation and debris clearance. New capillaries indicate angiogenesis and are seen as small, rounded structures filled with red blood cells. Fibroblasts are active during the proliferative phase, laying down collagen. collagen fibers are initially disorganized but become more structured during the remodeling phase. Epithelial cells are indicating re-epithelialization and restoration of the skin barrier.

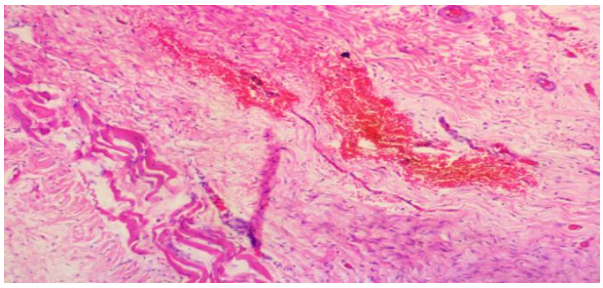

Fig. 9: Histopathology of wound treated with calcium zincate nanocomposite

It shows a fully regenerated, stratified epidermis with a normal basal layer and organized keratinocytes. The dermis exhibits dense, well-organized collagen fibers, indicating robust extracellular matrix formation. Minimal inflammatory cells are present, reflecting the resolution of inflammation. Mature, well-formed blood vessels are visible, indicating successful angiogenesis. Overall, the tissue architecture resembles that of uninjured skin with restored functionality and appearance.

The dermis shows increased numbers of fibroblasts, which are responsible for producing collagen and other extracellular matrix components. Collagen Deposition is significant collagen deposition in a more organized manner compared to the chaotic collagen seen in early healing stages. Inflammatory cells such as neutrophils and macrophages are significantly reduced or absent, indicating that the acute inflammatory phase has passed.

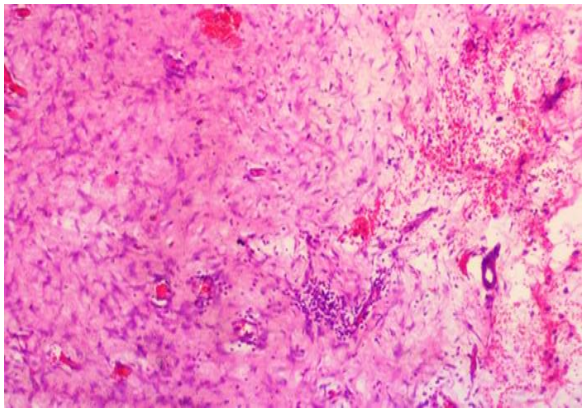

Fig. 10: Histopathology of wound treated by zinc oxide nanoparticles

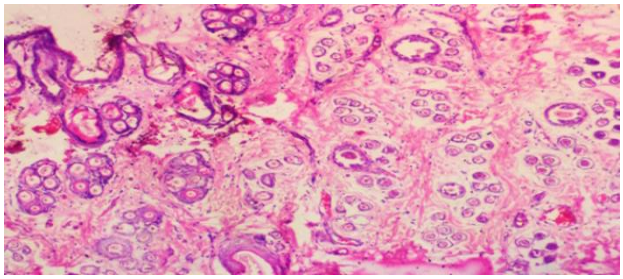

Fig. 11: Histopathology of wound treated with Quench® Cream

Collagen fibers in the dermis appear sparse, disorganized, and immature, lacking the normal, dense, and parallel arrangement. A significant presence of inflammatory cells such as neutrophils and macrophages, indicating ongoing inflammation and delayed healing. The epidermis is not fully re-formed, with noticeable gaps or thinning, indicating a failure of keratinocyte migration and proliferation.

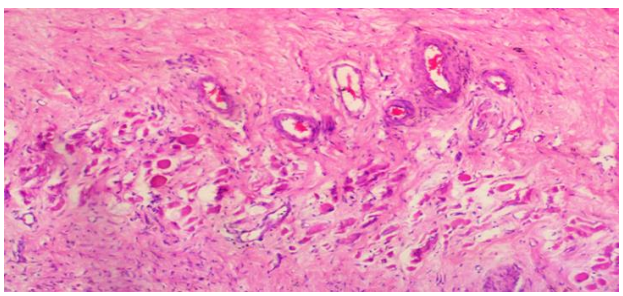

Fig. 12: Histopathology of wound treated with normal saline

Incomplete re-epithelialization with gaps in the epidermis, disorganized and sparse collagen deposition in the dermis, and persistent inflammatory cells. There is absence of mature blood vessels, indicating insufficient angiogenesis.

Fibroblasts and myofibroblasts are sparse, reflecting inadequate fibroplasia and wound contraction. The overall tissue architecture is appearing disorganized and not fully regenerated.

Collagen Content percentage

Compared to wounds treated with commercially available zinc oxide and regular saline ointment, collagen fibers in wounds treated with zinc oxide nanoparticle ointment were found to be more compacted. The zinc oxide nanoparticle ointment was shown to be statistically significant ($p<0.05$). Wounds treated with regular saline did not heal completely. Comparing the mean value of normal saline, it was found that the proportion of collagen was higher in wounds treated with zinc oxide nanoparticle ointment. 1% calcium zincate nanocomposite had highest %age of collagen than 1% zinc oxide nanoparticles as shown in Table 7. This is due to the antioxidant qualities of calcium and zinc which, together with their tiny surface areas, work in concert to enhance the migration of fibroblasts and myofibroblasts towards the wound site.

Table 7: Means of the collagen content percentages

Treatment	Mean
Normal saline (T0)	72.183
1% calcium zincate nanocomposite (T1)	94.15
zinc oxide nanoparticles (T2)	90.815
Quench® cream (T3)	88.25
Observations per Mean	4
Standard Error of a Mean	0.6049
Std Error (Diff of 2 Means)	0.8555

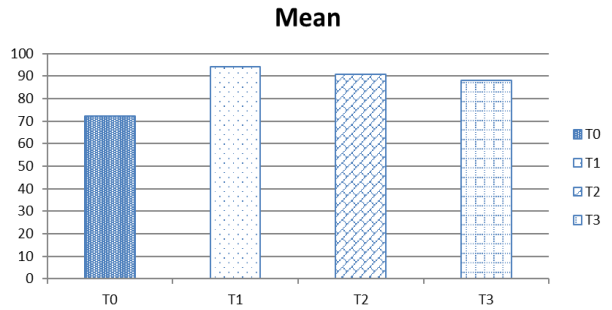

Fig 13: Graphical comparison of means of the collagen content (%). T0=Normal saline, T1= 1% calcium zincate nanocomposite, T2= zinc oxide nanoparticles, T3= Quench® cream

Table 8: ANOVA for collagen (%).

Source	DF	SS	MS	F	P
Treatment	3	1140.42	380.139	259.69	0
Error	12	17.57	1.464		
Total	15	1157.98			
Grand mean	86.349				
CV	1.4				

DISCUSSION

Healing of wound is a dynamic process that follows a sequence of different stages. Such stages include biochemical, physiological, cellular events. They include remodeling of tissues hemostasis, inflammation, and proliferation. If any of the stage is disturbed than healing of wound is disturbed (Subramaniam et al. 2021). This time taking wound healing can cause many serious problems for rabbit's health. Many of the features contributed in overdue curing process of wound. These best good characteristics includes damaged skin barrier than microorganism can easily be attacked the wound bed. Because immune responses of animals are weak and also weaker than biological attacks caused by various infections also delay healing speed. Stress, age and the state of hypoxic infection can also important for wound healing (Guo and DiPietro 2010; Siddiqui and Bernstein 2010; Manuja et al. 2020; Subramaniam et al. 2021; Demyashkin et al. 2024). As animals and people suffered from wounds, methods and materials were found to improve wound healing and prevent wound infection. In this regard, a number of studies were conducted in which the wound healing mechanism was described in detail. Various materials and methods have also been invented to help the wound healing process (Dirwan et al. 2024; Chen et al. 2024). The purpose of this study was to check the healing efficacy along comparison of 1% calcium zincate nanocomposite ointment with ointments of 1% zinc oxide nanoparticles ointment and 1% silver sulfadiazine, that were applied topically on the third degree burn wound in rabbit model.

According to our study, the wound treated with 1% calcium zincate nanocomposite ointment did not have any pus or exudate because the *Staphylococcus aureus* bacterial population that causes pus was absent from the wound site. Because zinc has antibacterial qualities, pus does not form at the site of the lesion. Zinc oxide nanoparticles also have best antibacterial properties (Manuja et al. 2020; Metwally et al. 2020; Ndlovu et al. 2023). Variation in particle size and surface area of zinc oxide nanoparticles, responsible for the noteworthy higher antimicrobial activity (Lim et al. 2011; Oyarzun-Ampuero et al. 2015). Effective control of bacterial infections is critical in the treatment of third-degree burns, as these injuries are highly susceptible to infections that can complicate healing and lead to severe complications (Ahmed et al. 2022; Ndlovu et al. 2023).

In this study, wound contraction rate was measured by the vernier caliper at 0, 7^{th},14^{th}, 21^{st} and 28^{th} days. These results showed that at 21^{th} day, mean values of wound contraction rate of 1% calcium zincate nanocomposite and zinc oxide nanoparticles was 1.78 ± 0.139I and 3.08 ± 0.099. It showed that 1% calcium zinc nanocomposite have faster and maximum wound contraction rate that 1% zinc oxide nanoparticles. This can be due to antibacterial and anti-inflammatory characteristics that speeds up the healing of both acute and chronic wounds (Asif et al. 2023b). Calcium zincate nanocomposite modulate the immune response more effectively, reducing the risk of chronic inflammation and promoting a balanced healing process. Moreover, the use of calcium zincate nanocomposite results in reduced scar formation compared to zinc oxide nanoparticles. This is beneficial for both functional and aesthetic reasons, ensuring better long-term outcomes for the treated animals. This study has shown that calcium zincate nanocomposite leads to more consistent and quicker healing of third-degree burns compared to zinc oxide nanoparticles. This includes better re-epithelialization, collagen deposition, and overall

wound closure rates.

Further, according to this study, the following mean values were found for the collagen contents: 72.183%, 94.15%, 90.815%, and 88.25% for normal saline, 1% calcium zincate nanocomposite ointment, 1% zinc oxide nanoparticle ointment, and 1% silver sulfadiazine ointment (Quench cream®), respectively. Collagen percentage was found to be higher in wounds treated with 1% calcium zincate nanocomposite as compared to other groups. This is because of antioxidant properties of zinc and calcium according to their small surface area which acts synergistically enhancing the fibroblast and myofibroblast migration towards wound site (Xie et al. 2011). That's why, it resulted in the faster re-epithelization at the wound site. Results obtained from this study suggest that the 1% Calcium Zincate nanocomposite can be used for the treatment of third degree burn wounds because it has stronger tissue regeneration as compared to 1% zinc oxide nanoparticles and silver sulfadiazine.

Fu et al. (2023) suggested that for healing of the wound, basic constituent is collagen content production and the healing time. Collagen content production can be observed by the process of histopathology. After observation in our study, we find denser collagen contents in 1% calcium zincate nanocomposite ointment wound cured wounds comparison with other three treated groups. Wounds treated with 1% calcium zincate nanocomposite ointment and 1% zinc oxide nanoparticle ointment had minimum inflammatory cells., As we know, if wound has minimum inflammatory cells, then the process of healing is boosted up (Elhabal et al. 2024).

According to this study, mean values of healing times were noted for the treatments, like 27^{th} for 1% calcium zincate nanocomposite ointment, 28.75^{th} for 1% zinc oxide nanoparticles, 30.5^{th} for 1% silver sulfadiazine and 32.75^{th} for normal saline. It indicates that the 1% calcium zincate nanocomposite ointment showed significant result in wound healing statistically. This is because of development of dense collagen contents and angiogenesis (Fu et al. 2023). Moreover, Calcium zincate nanocomposite has superior capacity in scavenging oxygen radicals, which are typically elevated in burn wounds. This property helps in reducing oxidative stress and further tissue damage, thereby promoting a healthier healing environment. According to Mahmood et al. (2020), zinc oxide nanoparticles give better healing of the wound. Manuja et al. (2020) also discovered wound healing capacity of zinc oxide nanoparticles. While, green zinc oxide nanoparticle gel eradicates infections more quickly and sped up wound healing than chemical zinc oxide nanoparticle gel (Metwally et al. 2020). Biosynthesis of nanoparticles using extracts of therapeutic plants, fungi, bacteria, algae, etc., improves their stability and biocompatibility in many biological settings, and its bio fabrication alters its physiochemical behavior, contributing to biological potency (Metwally et al. 2020; Huston et al. 2021; Chopra et al. 2022; Shnawa et al. 2022).

As the blood supply to the wound site is increased than the process of wound healing is boost up (Chen et al. 2022). 1% calcium zincate nanocomposite ointment also had capacity to increases blood supply towards the wound place with nutrient and oxygen. Studies have shown that dense collagen content boost up the process of healing (Bhutta et al. 2021). This Study shows that calcium zincate nanocomposite promotes better cell proliferation and migration, which are essential processes for tissue regeneration and wound healing (Addis et al. 2020). The accelerated proliferation of keratinocytes and fibroblasts helps in faster wound closure and recovery (Addis et al. 2020; Chen et al. 2022). Zinc oxide nanoparticles ointment was recognized to inspire the wound-healing mechanism, especially for the reason of their harshness and antimicrobial potential. It can also be observed prominent wound shrinkage and an amplified proportion in epithelialization.

These results declared that the tensile characteristics for the tissues treated with 1% calcium zincate nanoparticles were superior as compared to the tissues treated with zinc oxide nanoparticles and 1% silver sulfadiazine. On the other hand, zinc oxide nanoparticles and silver sulfadiazine showed better properties than the tissues treated with normal saline. Along with faster re-epithelization at wound site took place in Calcium zincate nanoparticles than Zinc oxide nanoparticles and 1% silver sulfadiazine. The histopathological study explained that tissues achieved after applying 1% calcium zincate nanocomposite were efficient and having good tensile strength.

In this study, 1% calcium zincate nanocomposite has shown higher biocompatibility compared to zinc oxide nanoparticles. This means that calcium zincate nanocomposite is less likely to cause adverse reactions when applied to burn wounds, ensuring a safer healing process for the affected tissues. The ability of calcium zincate nanocomposite to enhance angiogenesis, the formation of new blood vessels, is superior to that of zinc oxide nanoparticles. As we know, enhance angiogenesis ensures better oxygenation and nutrient supply to the healing tissue, thereby speeding up the recovery process (Jiang et al. 2024).

In accordance to the outcomes of this research work, it can be concluded that 1% Calcium zincate nanocomposite ointment shows better healing characteristics for the third degree burn wounds. While zinc oxide nanoparticles give comparatively moderate healing efficiency and slow healing of burned wounds is seen in 1% silver sulfadiazine. The healing time for the wounds treated with 1% calcium zincate nanocomposite exhibited faster healing in very short time period. The nanocomposite actually generates a larger passageway bed at site of generated wound and also distended collagen fiber percentage, originated at wound region. All these results show that 1% calcium zincate nanocomposite ointment gives enhance efficacy of wound healing.

Conclusion

It is concluded that wounds healed with 1% calcium zincate nanocomposite have thick layer, high rate of healing, more amount of collagen content, more thickness of dermis and epidermis. So, 1% calcium zincate nanocomposite gives better third-degree wound healing than 1% zinc oxide nanoparticles. Combination of calcium and zinc in calcium zincate nanoparticles provides synergistic effect that enhances their overall therapeutic potential.

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Author Contribution
AR, SM, BH wrote the manuscript, MIS, AR, TS, FZ methodology, data curation, formal analysis, AA, KZ, AA, AAB managed references, figures, A ur R, SFH, MKS, reviewed and edited the manuscript. QMK, MWS, SKDS, AM overall read and improve the manuscript.

Competing interest
The authors declare that they have no relevant financial or non-financial interests to disclose.

Ethical Statement
No Ethical permissions were required for this article.

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