



Research Article

Comparative Healing of full Thickness Cutaneous Wound in Rabbit by using Ointment of Nanoparticles Zinc Oxide and Commercial Available Ones

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ABSTRACT:

Background: The primary defensive line of body is skin when any discontinuity or violence occurs, lead to wound formation. These wounds majorly termed as close & open wounds. The skin automatically starts the healing process. But this process of healing may be delayed due to any infection or contamination. Many antiseptic agents available to prevent these infections, decrease-healing time when applied topically on wound site like zinc oxide. Objectives: The research was aimed to check, comparative therapeutic efficiency of ointment of nanoparticles of zinc oxide with the ointment of commercially available zinc oxide in the healing of full thickness cutaneous wound. Animals: 15 sound & healthy rabbits were selected for this research. **Methods:** For single experiment, dorsum of the single rabbit was selected as the site where the wound was inflicted. After that ointment of nanoparticles of ZnO, the ointment of ZnO (commercially available) & typical normal saline were used to treat the wound. Therapeutic efficacy of agents was checked by noting contraction rate of wound, time of healing & histopathological examination. To analyze the generated data of this experiment, ANOVA was used. **Results:** it was noted that ointment of nanoparticles of ZnO show better results than other 2 agents. **Conclusions:** The wounds treated by ointment of nanoparticles of ZnO show less time of healing, had superior content of collagen fibers, huge vascularization at the site of wound that justify the good efficiency of Zinc oxide nanoparticles ointment.

KEYWORDS:

Efficacy, *Oryctolagus cuniculus*, Nano particles, Zinc oxide.

1 | INTRODUCTION

Open & close wounds are 2 major classes of wound. If the wound only damage the underlying tissues of skin but continuity of the skin remain intact, is termed as close wound e.g. contusion, Hematoma & bruise. But if the wound also affect the continuity of skin is termed as open wound e.g. gunshot wound, perforating wound, penetrating wound, ulcerating wound & etc. Healing of the wound occur in following series of steps, 1st hemostasis, 2nd inflammation, 3rd proliferation and 4th or last one is resolution of the tissues of skin.¹ These steps always occur in a sequence within specified time during the healing process.² After the infliction of wound Re-generation process of healing starts within hours. This process firstly starts from the residual tissue lining & then move toward the granulation tissue.^{3,4} This process of healing of the wound should be affected by different type of factors like tissue dryness, status of health (immune compromised), more age, hormonal imbalance & infections due to pathogen. These factors also affect the stages of healing of wound & wound healing became delay.⁵ And it leads to different pathological issues & increase expanses to treat it so it also leads to the loss of economy.⁶ There are more type of complication may occur within the wound but the major is infection of the wound which leads to delay healing of wound. If the wound became infected, it leads to pain, inflammation & seepage of the site of the wound. It may prove hazardous for the life of living being. Contamination of the wound may also occur when we perform the

surgery.⁷ This contamination may be due to the hands of surgeon, airborne microorganisms or instrument (unsterilized).⁸

Many antiseptic agent's preparations are available in market to kill microbes & enhance the wound healing process.⁹ But topically applied agents show better result in healing the wound as it directly act on site of wound.¹⁰ The papyrus of Egypt give more information about the healing procedure of wounds like by using plant fiber or animal fat bandages or plasters of honey.¹¹ Many years ago, plants were used for the treatment of wound.¹² An ancient plant which can heal the wound is known as *aloe vera* by stimulating the growth factors of keratinocytes & collagen type-1.¹³ Another plant termed as Turmeric also used & act as an anti-inflammatory & also improve activity of fibroblast to boost up the collagen fiber's synthesis.¹⁴ And the natural honey (pure) is also used in healing of wound.¹⁵ Zinc is the trace (essential) element of the human body & constitutes 2g in adult & its major function is in the enzyme activity of the body which may lead to tissue repair. The most important enzymes which directly affect the protein synthesis & cell proliferation during mechanism of tissue repair are DNA & RNA polymerases.¹⁶ In skin incision the tensile strength & healing of the wound become reduced if deficiency of zinc is occurred while its supplements increase the tensile strength in incision wound & better healing in the wound.¹⁶ Zn₂O have property to absorb U.V Rays that's why it also used in lotion like sun block. And may also use as astringent in the patients of eczema & it heal the abraded skin & in humans it also use in treatment of hemorrhoids.¹⁷ In the cases of leg ulcers when Zinc topically applied, stops contamination of bacteria & it also reduces the inflammation & increases the re-epithelialization.¹⁶ Zinc oxide have anti-inflammatory & antibacterial properties so it enhance the healing of wounds (acute or chronic wounds) .¹⁸ We can use it to treat the open skin sores, dermatitis & blisters.¹⁹ To treat the fungal infections & acne Zinc oxide may use in creams & lotions. It also may act as bacteriostatic for *Staphylococcus aureus* which may contaminate the wound.²⁰

Nowadays nanoparticles used excessively to treat wound due to its bactericidal effectiveness.²¹ In sector of livestock the use of these nanoparticles increases because of its effective properties.¹⁷ Synthetic & natural form of nanoparticles exist but synthetic nanoparticles of metal oxide have effective antibacterial activity e.g Ca₂O, Mg₂O & Zn₂O.²¹ They are used as sensor, in care products of individuals & for remediation of environment .²² In veterinary medicine, Zn₂O nanoparticles are vastly utilized because of its unique features like angiogenic agent, antibacterial, wound healing agent. And these nanoparticles effectively control the intracellular pathogen as compare to therapeutic agents which contain macromolecules.¹⁷ It also used in the anti-cancer medicine due to its anti-neoplastic properties.²³ It also used to treat the meningitis & that diseases which are caused with intracellular microbes like viruses, fungus (*Cryptococcus neoformans*, *Histoplasma capsulatum*) & bacteria (Chlamydia, Rickettsia, Brucella etc). So these nanoparticles as compare to therapeutic agents (traditionally available) are much beneficial for use.²⁴ And nanoparticles of zinc oxide also possess antimicrobial activity.²⁵ Bactericidal activity of nanoparticles of Zinc oxide occur in 4 phases. 1stly development of affinity occurs between Zn₂O & bacterial cell.²⁶ Then cell injury occur due to production of hydrogen per oxide.²⁷ Then these nanoparticles adhere to Sulphur & phosphorus containing compounds of bacteria like DNA.²⁸ Then finally death of bacterial cell occur when these nanoparticles arrest the cellular metabolism & bind to the molecules of protein of bacterial cells.²⁹ Good antimicrobial activity of Zn₂O Nanoparticles are because of their small size accumulate in the cytoplasm & plasma membrane of cell of bacteria.³⁰ In high productive dairy animals mastitis is common disease & caused by bacteria (*E.coli*, *Streptococcus* & *Staphylococcus*). Bacteria form biofilm in udder which make it impervious for physical, innate immune & chemical response & also decreases milk production. But nanoparticles of Zn₂O can penetrate in cell of staphylococcus & easily treat mastitis. All the above information stated that nanoparticles of Zn₂O used in the treatment of many diseases. But In the treatment of surgical wound, the use of nanoparticles of Zn₂O is not reported yet. So the current study carried out to evaluate the objectives which are given below:

- To check the effect of nanoparticles of zinc oxide in healing of the wound in rabbits.
- To evaluate the comparative efficacy of nanoparticles of Zn₂O with ointment of Zn₂O (Commercially available) in the healing of wound.

2 | MATERIAL AND METHODS

2.1 | Feeding, Housing, Prophylactic Treatments & Clinical Examination

Rabbits were placed Animal Facility laboratory on the department of clinical surgery & medicine. All animals were managed uniformly for a time period of 3 weeks. Aspect of animal welfare was kept in mind. They were provided clean bedding and were given proper ventilation. Light duration of 8-10 hours was provided to animals. Room temperature was maintained at 25-30 degree Celsius. Deworming of animals was done a week before experiment by

giving two doses of ivermectin @ 400 microgram per kg subcutaneously. Fresh water was provided to animals ad libitum. Fresh fodder was provided to animals 2 times in 1 day. Bedding by straw of wheat was provided to animals, replaced every day.

2.2 | Grouping & Procurement of Rabbits for Experiment

15 rabbits were chosen for this research & weighed them which varies between 2.3 - 3.0 kg. These rabbits were bought from the village of Faisalabad & were sound & healthy. For infliction of wound, the area of trunk region of the rabbits was chosen & cleaned by electrical shaving machine from hair to prevent the cuts on skin & 3 wounds of 1cm * 1 cm were inflicted here. Then topically apply the drugs on 3 wounds on daily basis. To treat 10 animals, nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline were used. While other 5 rabbits were treated with the gauze containing nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline.

2.3 | Preparation of Ointment of Nanoparticle of ZnO

To check the hypothesis of our study 0.5% w/w ointment of Zn₂O nanoparticles was generated. 0.5 gram of zinc oxide nanoparticles were mixed to 99.5 gram of paraffin as it is considered as inert carrier of medicine and its absorption is also good. After preparation it was packed in plastic containers.

2.4 | Preparation of Zinc Oxide Nanoparticle Gauze

In addition to check our hypothesis by using Zinc oxide nanoparticles ointment, gauzes were also prepared in which zinc oxide nanoparticles were incorporated. They were prepared from National Textile University. Their results regarding wound healing were compared to that of ointment of nanoparticles of Zn₂O but this drug delivery was far better in the wounds which were cured with the help of ointment of Zn₂O nanoparticles.

2.5 | Infliction of the Wounds (excision)

To inflict the wounds, specific area then shaved properly by the help of electrical clipper. Thoraco lumber region of animals was shaved firstly by shaver and then by cleansing agent. At the site of wound Antiseptics were also applied. For this purpose methylated spirit and tincture of iodine was used. Keeping regard of the aspect of welfare of animal, surgical wounds were inflicted to rabbits in general anesthesia. No feed was given to animals before the administration of anesthesia & the Ketamine HCL (known as Ketarol in market) @ 15-30 mg / kg B.W was used. Animals were placed in ventral recumbency. For making the skin germ free firstly we use iodine then use alcohol swabs before surgery. To protect the skin from contamination & to keep skin dry Cloth drapes were used on the site of surgery. The 1 cm * 1cm area was marked by scale & a permanent marker. On the lateral side 2 wounds of 1 cm² were inflicted & the distance of them from mid line was 2 cm & 1 wound created on the central line of that region. To differentiate the treatment of wounds which applied to it, wound were named as A, B and C.

2.6 | Treatment

Randomly applied treatments on excision wounds were following:

- 0.5 % ointment of nanoparticles of Zn₂O (Prepared by Healers pharmacy limited)
- Gauzes of nanoparticles of Zn₂O (Prepared by national Textile University)
- 10 % ointment of nanoparticles of Zn₂O (prepared by Healer's pharmacy limited)
- Normal saline N/S (PAKSOL NS or M.S Enterprises Limited)

Out of 15 rabbits, the wound A, B & C of 10 rabbits was cured by ointment of nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline respectively. And the wound A, B & C of remaining 5 rabbits were cured by the gauze containing nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline respectively. Allocated drug was applied to the injuries from day 1 & their healing was observed on daily basis & proper care of injuries was performed after infliction of injuries by kept creatures in separate cages to prevent the injuries from scratching.

2.7 | Parameters Evaluated for Healing of Wound

Following parameters were used to check the healing of injuries.

2.7.1 | Contraction Rate of the Wound

Withdrawal rate of wound is characterized as "the rate lessening in unique injury measure". To take the measurements of injury we used advance Vernier caliper because it gave exact perusing, which was taken in millimeter.

2.7.2 | Time of Healing of Injuries

Recuperating begins ideal just after the infliction of injures & continue upto the time when injuries become fully healed. Healing capacity & time is noted on daily basis just after 1 day of the infliction of injury. Day by day perceptions of evaluated every day recuperating time was included upto that day when the scar on the injury removed naturally.²²

2.7.3 | Histopathology of the Injuries

The samples of tissues were taken for Histopathological studies.

2.7.4 | Slides Staining

After sectioning the slides were properly labeled & got stained by the help of H&E stain.

2.7.5 | Procedure for Mounting

We preped the slide to check in the microscope by using the drop of DPX on the recoulered slide then mount a glass slide on it.

2.7.6 | Histometrical Methods

These slide were seen under microscope at lens power about 200X. The micrograph of that slides was captured by using the Nikon Opt iPhoto 2. Dermis layer measure ments were examined by the Image J.

2.7.7 | System of Image Analysis

Image J is a system which is used to analyze the images & this system is developed by USA in the National Institute of health. It is the software which is the world leading system of analysis of image. Speed of processing in 1 second is about 40 million pixels. Its more information is also available at URL: <http://rsb.info.nih.gov.ij/download.html>. By capturing the images at 40X on stage micrometer this software was calibrated. In image J the image then was opened. For the purpose of calibration, between 2 points whose distance is known, we draw a straight line. The distance was put on a set scale option of software.

2.7.8 | Statistical Analysis

The data that was generated was analyzed by statistics. We used ANOVA for this purpose. ^{.31} The purpose for applying statistical design was to check if the results were significant or non-significant.

3 | RESULTS AND DISCUSSION

To check the comparative therapeutic efficacy of ointment of nanoparticles of Zn₂O with the ointment of Zn₂O (commercially available) in the healing of full thickness cutaneous wound of rabbit. According to our hypothesis the injuries which were cured by the nanoparticles of Zn₂O may effectively decrease the time of healing of wound & prevent it from the contamination of microbes due to having the activity of antibacterial agent. To precede our hypothesis, 10 sound rabbits were selected & this study was proposed for 1.5 month. Every animal selected as 1 experimental model.

3.1 | Parameters evaluated for healing of wound

3.1.1 | Rate of contraction of Injury/wound

Contraction of Injury is defined as the edges around the injury move toward the center of surface of the wound. From the day 4 up to the closing of edges, percent reduction in wound size is known as Contraction. On the interval of 4 days the reading from the wound were noted & by these readings measurement of rate of contraction was determined. The rate of contraction of all wounds treated by all agents was determined. The wounds treated by ointment of nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline were healed on day 16, day 24 & day 26 respectively. So, the wounds treated by ointment of nanoparticles of Zn₂O show least time to heal the wounds but other 2 agents take more time to heal the wounds. Statistically ointment of nanoparticles of Zn₂O were found significant because its results were much better than other 2 agents & these findings was found ($p < 0.05$) in overall study period

Table 1: Therapeutic effects of topically applied nanoparticles of Zn₂O on rate if contraction of Injuries (n=10) in rabbits (n=10) in (mm)

		Animals											
Days		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Mean	SD
Days	D4	1.71	1.89	1.32	2.45	0.96	1.18	2.55	1.92	1.62	2.84	1.84	0.61
	D8	3.34	3.97	3.12	3.56	3.05	3.21	4.46	3.98	3.09	5.36	3.71	0.74
	D12	5.42	7.06	5.19	7.26	4.97	5.12	8.39	6.40	5.48	8.76	6.41	1.40
	D16	8.63	10	8.56	10	7.84	8.65	10	7.89	9.11	10	9.07	0.88
	D20	10	10	10	10	10	10	10	10	10	10	10	0
	D24	10	10	10	10	10	10	10	10	10	10	10	0
	D28	10	10	10	10	10	10	10	10	10	10	10	0

3.1.2 | Time of Healing (Days)

The wounds treated by ointment of nanoparticles of Zn₂O showed least time to heal the wounds but Zn₂O ointment (commercially available) & normal saline take more time to heal the wounds. So the ointment of nanoparticles of Zn₂O was much better than other 2 agents with reference to time of healing of wound & up to termination of study, it was found significant ($p > 0.05$).

3.1.3 | Histopathological Examination

After the completion of healing of wounds, from the site of wound tissue samples were taken for the histopathological examination of tissues & following Parameters were checked, thickness of skin layers like dermis, epidermis & also collagen contents. To determine the collagen fibers arrangement & compactness, %age of content of Collagen was evaluated. In addition to appendages of skin & beds of capillaries, the inflammatory cells were also noted.

3.1.4 | Epidermis Layer of Skin's Thickness

The injuries treated by ointment of nanoparticles of Zn₂O, were show the thickness of epidermis about 128.76-micron meters. This thickness is much more than the thickness which were obtained by Zn₂O ointment (commercially available) & normal saline. So statistically ointment of nanoparticles of Zn₂O were found significant because its results were much better than other 2 agents & these findings was found in overall study period ($p < 0.05$).

3.1.5 | Collagen Content percentage

After the evaluation of samples of skin on the slides in laboratory it was found that the wounds treated by ointment of nanoparticles of Zn₂O had superior quality collagen fibers which were compact & there was a good vascularization of that wound. But wounds that were treated by Zn₂O ointment (commercially available) had inferior quality of collagen fibers which were less compact & low vascularization. And the wounds treated by normal saline other 2 agents with reference to percentage of content of collagen fibers & up to termination of study, it was found significant (p<0.05).

3.1.6 | Thickness of Dermis

After the evaluation of samples of skin on the slides in laboratory it was found that the wounds treated by ointment of nanoparticles of Zn₂O show superior dermal thickness as compared to the Zn₂O ointment (commercially available) & normal saline. It was found that the results of ointment of nanoparticles of Zn₂O were (p<0.05) statistically as compare to the results of Zn₂O ointment (commercially available) & typical normal saline were (p>0.05) non-significant. So the ointment of nanoparticles of Zn₂O was much better than other 2 agents with reference of dermal thickness.

4 | DISCUSSION

Healing of the wound occur in following series of steps, 1st hemostasis, 2nd inflammation, 3rd proliferation and 4th or last one is resolution of skin tissues. These steps always occur in proper sequence within specific time during the healing process. If any step got disturbed cause delay wound healing which may be fatal for animal's health. The process of the wound healing should be affected by different type of factors like tissue dryness, immune compromised health status due to metabolic diseases, hypoxic conditions, stress, old age, hormonal imbalance & infection by foreign agents. But by applying therapeutic agents all of these factors may prevented. Animals and human both can get wound by any accident. So, researchers are searching for new method & agents which prevent the infection & perform early healing of that wound. Many methods & agents were described to support the process of healing of wound. The process of wound healing is exist naturally & starts automatically after wound infliction. But we want to enhance this process & prevent the infection of wound.

The present research was aimed to check the comparative therapeutic effectiveness of ointment of nanoparticles of Zn₂O with the ointment of Zn₂O (commercially available) in the healing of full thickness cutaneous wound of rabbit. The parameters like rate of contraction of wound, time of healing of wound & Histopathological examination were evaluated in the current study. Rate of wound contraction was noted from day 3 up to the complete healing of wound but other 2 parameters were noted after the complete healing of wound. After the study the results shows that application of ointment of nanoparticles of Zn₂O directly on skin improve the content of collagen fibers, thickness of dermis & epidermis layer, also decrease the time of contraction of healing of the wound, no pus or bacteria (*staphylococcus aureus*) or other inflammatory cells were found at the site of the wound and increased vascularization. It means the ointment of nanoparticles of Zn₂O has effective antibacterial activity & show better healing of site of wound as compare to Zn₂O ointment (commercially available) & normal saline show exudate formation at site of wound & pus was also seen. With reference to percentage of content of collagen, the wounds treated by ointment of nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline had collagen content percentage about 92.12%, 83.55 % & 76.93 % respectively. So the results shows that ointment of nanoparticles of Zn₂O had superior quality collagen fibers which was compact & there was a good vascularization as compare to Zn₂O ointment (commercially available) & normal saline. The wounds treated by ointment of nanoparticles of Zn₂O, Zn₂O ointment (commercially available) & normal saline were healed on day 16, day 24 & day 26 respectively. So, the wounds treated by ointment of nanoparticles of Zn₂O show least time to heal the wounds but other 2 agents take more time to heal the wounds. It is due good angiogenesis & the compact content of collagen fibers.^{32, 33}

Healing of the injury is directly related to the generation of content of collagen fibers which may the important components of healing process like proliferation.³⁴ By performing histopathological examination of the tissues it is proved that the wounds treated by ointment of nanoparticles of Zn₂O had superior quality collagen fibers which was compact, there was a good vascularization, no pus or bacteria or other inflammatory cells were found at the site of the wound, enhance thickness of dermis & epidermis layer as compare to other 2 agents.

Research was conducted to evaluate process of healing of wound & the factors which may affects it & they said that time of healing of wound can reduce it more extent by the expression of collagen regulation at site of wound.³⁵ And by histopathology of the current study it was proved that ointment of nanoparticles of Zn₂O had superior quality collagen fibers which was compact & there was a good vascularization as compare to Zn₂O ointment (commercially available) & normal saline. So, it is concluded that the wound healing is enhance by these agents more effectively than other 2 agents.

5 | CONCLUSION

In after the evaluation of all the above results, it was noted that ointment of nanoparticles of Zn₂O show better efficiency of healing as compare to Zn₂O ointment (commercially available) & normal saline. The wounds treated by ointment of nanoparticles of Zn₂O show less time of healing, had superior content of collagen fibers, huge vascularization at the site of wound that justify the good efficiency of ointment of nanoparticles of Zn₂O.

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