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Comparative Efficiency of Zinc Oxide Nanoparticles Ointment and Zinc Oxide Ointment Commercially Available for the Healing of Wounds in Rabbit (Oryctolagus Cuniculus)

Shakeeb Ullah[1]
Faculty of Veterinary and Animal Sciences,
Gomal University, Dera Ismail Khan, Pakistan
Faiq Ahmad[2]
Department of Clinical Medicine and Surgery,
University of Agriculture, Faisalabad, Pakistan
Mohammad Arshad[3*]
Department of Clinical Medicine and Surgery,
University of Agriculture, Faisalabad-Pakistan
Muhammad Inamullah Malik[4]
Faculty of Veterinary and Animal Sciences,
Gomal University, Dera Ismail Khan, Pakistan
Ghulam Jelani[5]
Department of Clinical Medicine and Surgery,
University of Agriculture, Dera Ismail Khan
Muhammad Naeem[6]
Department of Physiology and Pharmacology,
University of Veterinary and Animal Sciences, Lahore.

Abstract

Skin is a protective layer of the body of all living organisms & saves the internal organs of the body from the external environment which may damage it. The damage in the continuity of skin is termed as wound & it causes exposure of internal body organs to the external environment which can be fetal for life. Skin automatically starts wound healing process. We can assist this process by keeping it germ, dust & moisture free with the help of topical applications of different agents e.g., zinc oxide. It controls the contamination of wound from bacteria e.g., Escherichia coli and Staphylococcus aureus, which can delay healing process. There is a hypothesis that

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when zinc oxide is converted to nanoparticles, it can increase the heeling efficiency of wound because these nanoparticles penetrate well in wound. The current study aimed to compare the efficacy of Zn2O nanoparticles ointment to commercially available for the healing of 2nd degree chemical burn cutaneous wounds. During this study, 3 wounds were inflicted on each rabbit group named as A, B & C (control group). Zinc oxide nanoparticles were used for Group A, commercially available Zn2O for group B & for group C normal saline. By checking healing time, histopathology and contraction rate, the result of different treatments were judged. To infer the decision, the overall efficacy of all medication on the respective wounds was assessed and comparison was done with each other.

Introduction

The injury of skin as a result of high radiation, chemical, heat and brushing is known as burn injury. Whereas the burn as a result of some alkali or acid is termed as chemical burn (Ward, 2012). Degree of the wound is measured on the basis of burned surface area & layers of damaged skin. On the basis of surface area involved, wound is classified into 6 classes which are termed as burn wound with scales, with chemical, with electricity, with radiation, bun wound with fire & last one due to contact. There are three major classes of wound based on involvement of skin layer. If only superficial layer is affected then it termed as 1st degree. If both 1st and 2nd layers are affected so termed as 2nd degree & If the burn wound effect the all 3 layers of skin so termed as 3rd degree (Hettiaratchy and Papini, 2004). Time of Healing wound depends on bacterial infection & severity of burn. Healing of wound becomes fast if wound is free of germs & vice versa (Forjuoh, 2006).

Healing process of burn wound involved a series of phases; firstly all inflammatory cells move towards site of burn that are followed by monocytes. That monocyte then stimulates the macrophage (Stipcevic *et al,* 2006). Then phagocytosis starts there & proteases are released which activate the release of growth factors and finally cytokines. The time of activation of these factors is different but purpose is to heal the wound (Waldorf and Fewkes, 1995). At last proliferation phase is start in which with the help of growth factors, fibroblasts and keratinocytes are stimulated & leads to formation of Granulation tissue containing blood and inflammatory cells, few

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fibroblasts & matrix proteins. Burn wound is a suitable media for the growth of microbes which is a major complication of the burn wounds, & this complication of wound by microbes may lead to loss of life (Nagoba *et al.,* 1998). Further also loss occurs due to compromised immune system of effected human or animal due to obstruction of circulation in underlying tissues (Norman, 2003).

Healing of burn wound prompts with the use of NSAIDS, dead skin's removal, antibiotics application, supportive therapy & gauze bandages (Hettiaratchy and Papini, 2004, Ward, 2012). This study aimed to enhance the burn wound healing through controlling bacterial contamination on wound site. The Zinc oxide is an agent which has both these properties. It promotes the wound healing as well as has antibacterial effect. So it helps to keep the wound clean, moist and prevent pus formation & dryness. Zinc oxide which is commercially available in the form of creams & lotions and it contain 10-20% w/v zinc oxide. This is used to treat burn injuries, prevent acne & fungus growth. It controls the bacterial growth especially *Staph. Aureus,* which cause main complication in the burn wound infection (Moezzi *et al*., 2012).

Human body contain minerals e.g., iron (most abundant) & the 2nd mineral is zinc which found in dermis & epidermis but its content in epidermis is 5-6 more than in dermis. In a lot of transcription factors, Zinc is found to be a co factor & it is a necessary element for metallo enzymes because metallo proteinases matrix is zinc dependent. So it plays the vital role in the migration of keratinocyte, auto-debridement & the wound restoration (Roston *et al.,* 2002; Agay *et al.,* 2005; Lansdown *et al.,* 2007).

Many researchers worked on it & proved that the zinc elements prevent the contamination of bacteria lead to enhancement of wound healing & formation of granulation tissue (Ehrlich, 1998, Roston *et al.,* 2002; Arslan *et al.,* 2012). But it is proved that the nanoparticles of Zinc oxide act as antimicrobials overcome bacterial resistance & reduce treatment cost as compared to antibiotics. It also proven that these nanoparticles remain in body for long time when compare with other conventional antibiotics (Jung and Kwon, 2011). Nanoparticles of Zinc oxide have become very famous due to its bacteriostatic effect on the colonies of *E. coli* and *Staph. aureus* on the wound & it also decreases the healing time of wound (Rao and Selvaraj, 2003).

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Burn wound contamination by microbes is a general problem occurred in both human & animals and it may cease or decrease the epidermal maturation and may leads to over formation of scar. These microbes penetrate deep into the tissue's layer and leads to chronic bacteremia & sepsis (Piotr *et al*., 2007). A survey shows that 75% mortality in burn wound cases, occur due to *Pseudomonas aeruginosa* or *Staphylococcus aureus* (methicillin resistant) but are very sensitive to nanoparticles of zinc oxide. So, burn wounds are effectively treated with nanoparticles of zinc oxide (Shokotihi *et al*., 2012).

Materials and Methods

For the progress of current study, we selected domestic rabbits. They were inflicted with second degree burn wound. To confirm our hypothesis, Drug under study & standard drug were applied topically to the site of wound.

Experimental Animals

From some villages of Faisalabad, we purchased ten locally reared rabbits. To reduce chances of mortality of rabbits during study period diseased free rabbits were purchased. The rabbits were kept in animal facility & Environment, feed, water, ventilation, light & housing management were according to need of rabbits & germ free or hygiene condition were produced in shed.

Clinical Examination

At CMS department, rabbits were kept in animal facility for 1 week to avoid stress before starting study. The light & temperature were 8-10 hours & 28-34 degree Celsius respectively & examined for any disorder. Deworming & medication of rabbits were done at the rate of 400 microgram per kg ivermectin (2 doses by subcutaneous route after a week) & 15 mg/kg amoxicillin (for 3 days) respectively, 1 week before initiating surgical intervention.

Preparation of Wound Site

Lateral thoracolumbar area of rabbits was sheared carefully by using razor blade, electrical clippers & scissor and tincture of iodine or methylated spirit was used as antiseptic at surgical site. On daily basis health parameters of rabbits e.g., pulse, heart & respiration rate & temperature were checked.

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Premedication, Anesthesia and Positioning

Chemical burn wounds were inflicted to rabbits during general anesthesia in regard of animal welfare. Before administering anesthesia rabbits were kept off-feed & atropine sulphate via S/C route was given to rabbits @ 0.035 mg/kg. Ketarol (Ketamine hydrochloride) was used @15-30 mg /kg. In sternal recumbency animals were placed then the area was sanitized & germ free by using iodine & swab of alcohol respectively. To give protection & keep dry the site of surgery covered by drapes of cloth.

1 cm^2 area was marked with a scale & permanent marker. From mid line, 2 cm apart, 2 wounds of 1 cm^2 were created laterally and on the central mid line of thoracolumbar region 1 wound was created. To differentiate the medication applied to wounds, these were named by alphabetics as A, B and C.

Burn Wound Infliction

For infliction of chemical burn wounds concentrated H_2SO_4 was purchased from a scientific store pH paper of dimension of 1cm^2 was soaked in conc. H_2SO_4 & placed at the marked area of rabbit body for 1 minute only & then picked up by help of forceps, to produce second degree burn wound. On lateral side of animal 2 wounds of 1 cm^2 were created at the 1 cm apart, each from mid line and on the central mid line of thoracolumbar region 1 wound was created. To differentiate the medication applied to wounds, these were named by alphabetic (A, B and C). Just after chemical burn wounds infliction, wound A, B & C (control group) were treated with ointment of Zn_2O nanoparticles, Zn_2O (commercially available) & normal saline respectively. All the rabbits were evaluated after treatment for their general body or health conditions.

Parameters Evaluated for Wound Healing

On the basis of following parameters, wound healing was evaluated.

Rate of contraction of wound

Contraction means movement of the edges of the wound towards wound's Centre. For more accurate readings (in millimeter), Digital Vernier caliper was used. Formula for calculating wound contraction rate in percentile is presented below: (Manjunatha *et al.,* 2005).

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$$\%\text{wound contraction} = \frac{\text{(Initial wound area – Specific day wound area)}}{\text{Initial wound area}} \times 100.$$

Healing Time

Just after the time of wound infliction healing starts & continues up to complete regeneration of damaged tissue. Time of healing was evaluated daily. After wound infliction, daily observed the wound to estimate the time of healing up to the dropping of scars (Bairy *et al.,* 2011).

Histopathology

For histopathology, samples of the tissues were preserved.

Mounting Procedure

After tissue samples' staining, DPX's small drop was dispensed on the slide & covered with slip, now examined it under microscope.

Histometrical Methods

Observed slide under microscope at 200 X & by using Nikon Opt iPhoto 2, Photomicrographs of slide was captured. To determine the diameter of dermis layers, Image J (automated image analysis system) was used.

Image J Analysis System

It is an image analysis system discovered by the National Institute of health, USA & Its processing speed in pixel is 40 million/sec. At 40X images of stage micrometer is capture & by that image this Software was calibrated. Then image was opened in image J & for calibration purpose, a line was drawn straight between 2 points of known distance. In software this distance was put on a set scale option.

Statistical Analysis

ANOVA was used for the evaluation of all data at the end of study to confirm that either the results were significant or not (Steel and Torrie, 2004).

Results

The current study was aimed to compare the efficacy of Zn_2O (nanoparticles) with Zn_2O (commercially available) ointment in term of wound healing. To progress this study, burn wounds were inflicted on rabbits and were treated by nanoparticles of zinc oxide, zinc oxide (commercially available) and solution of normal saline. The parameters like healing time, contraction rate and histopathology of wound were noted to compare better agent for healing among all three. All rabbits were placed in

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sound environmental condition, free of any disease or toxicity for one and a half month.

Parameters of Study

Wound Contraction Rate

Contraction means edges of wound move to center & it was noticed that initially wound expanded for first 3 to 4 days. After this all the wounds started contracting and this trend was calculated as the percent decline of wound's size from 4th day to onwards.

The wounds which were treated by nanoparticles of zinc oxide, by zinc oxide (commercially available) ointment & Normal saline, were healed & contracted by day 25, 27 & 28 respectively. So nanoparticle of zinc oxide took least time in contraction of wound as compare to zinc oxide (commercially available) ointment and solution of normal saline & found statistically significant at each study. During all study period the same trend was found ($P<0.05$).

		Animals											
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Mean	St. Deviation
Days	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

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Table 4.1: wounds' contraction with the topical use of nanoparticles of Zn_2O (n=10)

Healing Time (in days)

After treatment with nanoparticles of Zn_2O, commercially available Zn_2O ointment & Normal saline, wounds were completely healed on 25, 27 & day 28 respectively. So nanoparticle of zinc oxide was better in the regard of wound as compare to other 2 agents or treatments, Up to the termination of study it was significant ($P<0.05$)

Histopathological Examination

Samples of wound scar were taken for histopathological study and fixed in 10% buffered formalin, then by graded alcohols dehydration was done, by xylene & chloroform samples were cleaned and by paraffin wax samples were impregnated. Stained of Specimens by haematoxylin and eosin stain and by using light micros-copy were examined. Parameters like thickness of epidermis, percentage of collagen content and thickness of dermis.

Thickness of Epidermis

The results showed that treatment with nanoparticles of Zn_2O have better thickness of epidermal as compare to ones treated by commercially available Zn_2O ointment & Normal saline. Statistically nanoparticles of Zn_2O showed significantly positive results ($P<0.05$).

Collagen Content percentage

The wounds treated with nanoparticles of Zn_2O have compact form of Collagen fibers as compare to normal saline and commercial Zn_2O ointment treated wounds, which were healed incompletely. Statistically during all study period Zn_2O nanoparticles ointment was found significant ($P<0.05$).

Thickness of Dermis

After conducting histopathology dermal thickness was noted & incurred that wounds treated with nanoparticles of Zn_2O have better dermal thickness relative to Zn_2O ointment commercially available. While normal saline treated wounds had least thickness of dermis.

Discussion

Healing of wound is automatic physiological process, starts after injury & has 4

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phases. These phases follow a series of one after the other. First phase is hemostasis in which inflammation occur & leading to granulation tissue' proliferation there. Then finally remodeling of the tissue occurs. Different medications are implied to reduce the time of healing & to prevent bacterial contamination e.g., zinc oxide.

The aim of current study was to compare the efficacy of healing of wound with nanoparticles zinc oxide and commercial Zn_2O ointment. It was a hypothesis that drug delivery is enhanced when Zn_2O is converted to its nanoparticles & it reduce the healing time & population of *Staphylococcus auerus* which mostly effect the burn wound & healing time reduces by reducing bacterial contamination. To search out this hypothesis, this study was conducted & burn wounds were treated by ointment of nanoparticles of zinc oxide, zinc oxide (commercially available) ointment. Parameters that were noted during the study were contraction rate, histopathology & time of healing of wound.

In regard of time of wound healing, it is reported that healing was quick when treatment of wounds was done with nanoparticles of Zn_2O ointment than the (commercially available) Zn_2O ointment. The reduction in time of healing in case of nanoparticles of zinc oxide its antibacterial effect which produce area free of contamination as compare to the wounds that were treated with commercially available Zn_2O, free of contamination area was not maintained but this wound contain exudate & pus. But the wound treated by normal saline contained more exudate & pus. So, antibacterial activity of zinc oxide increased, when it changed into nanoparticles because its drug delivery increased by it.

Contraction rate of the wound refers to the granulation tissue's formation (myofibrils) at site of wound. We use 3 agent refer to Zn_2O nanoparticles, Zn_2O commercially available ointment and normal saline, to boost up the myofibrils migration & contraction rate at site of wound. Contraction rate of wound treated by 3 agents was excellent, good & bad respectively. So, the maximum contraction rate of wound was noted in that wound which was treated by nanoparticles of zinc oxide. Study about histopathology of wound treated with 3 agents, was conducted to note the collagen content' formation, neovascularization & inflammatory cells in healed wound. The results shows that the wounds treated with 3 agents according to dermis

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layer, collagen content' formation & vascular bed which lead to increased supply of oxygen and nutrients & promote healing, was excellent, good & bad respectively. All the above data proved that nanoparticles of zinc oxide have better efficacy than the commercially available zinc oxide because it decreased the healing time, produce germ free area, promote contraction rate of wound. So it can be recommended as good treatment for burn wound.

Summary

Now-a-days we are mostly exposed to number of electrical appliances, chemical, radiations & heat resources & these agents inflicts harmful effects like burn injuries on skin of any living organisms nearby. Burn injuries have highly occurrence & can lead to fatal conditions of effected living organisms. Their treatment should be specific which have to prevent the bacterial contamination, enhance formulation of granulation tissue & promote the healing of wound or injury & we mostly use zinc oxide for this purpose which is commercially available in different concentrations in market. Moreover, if we change this zinc oxide into form of nano-particle, its results are much better. To check & compare the efficiency of Zn_2O nanoparticles and Zn_2O (commercially available) ointment for treatment of burn wound, the present study was designed. Ten healthy rabbits were selected & all were anaesthetized & on each rabbit, 3 chemical wounds were inflicted by the help of conc.H_2So_4. These wounds were named as A, B and C & treatment was done with ointment of zinc oxide nanoparticles, zinc oxide (commercially available) and solution of normal saline respectively after the infliction of burn wound up to wound healing on daily basis. Environment, feed, water, ventilation, light & housing management were according to need of rabbits & germ free.

The comparative effect of commercially available zinc oxide & zinc oxide nanoparticles ointment was evaluated by noting contraction rate of wound, histopathology & time of healing of wound. Contraction rate of wound was checked on the interval of 3 days by the help of digital Vernier caliper till the completion of healing of wound. A tissue mass was picked form the site of wound for histopathology & to note the quality of collagen contents in healed wound. The time of healing also checked on daily basis. The above parameters positive results about

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zinc oxide nanoparticles ointment. Contraction rate of wound was fast in that wound treated by nanoparticles ointment of zinc oxide & collagen content of this wound was of superior quality. In Regard of healing time of wound, the wounds treated with Zn_2O nanoparticles ointment & Zn_2O commercially available, healed completely in 14 & 19 days respectively & control group took 24 days.

All the above data proved that nanoparticles ointment of zinc oxide have better efficacy than the commercially available Zn_2O for healing of burn wound.

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