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Healing Efficiency of Zinc Oxide Nanoparticles in Various Concentrations on the Full Thickness Wounds in Rabbits (Oryctolagus Cuniculus)

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Abstract

Integumentary system is the basic system of the body that acts as the first line of defense against the environment. Any type of attack result in the damage of skin known as wound, depending on nature and magnitude of attack. For proper functions of skin healing of wound is compulsory and depends on many factors. Many natural and synthetic products are responsible for quick healing of clinical wounds. Zinc is one the major agent appropriate for this purpose especially when topically used in Nano Zinc oxide form. This study was carried out to find out the healing capacity of Nano Zinc Oxide ointment on surgical wounds in various concentrations as 0.25, 0.5, 0.75 and 1%. For this purpose 10 healthy mature rabbits were selected, as the ten experimental models. Four surgical wounds were developed on lateral side of each rabbit and each wound was treated with one of four different concentrations of ZO NPs as 0.25, 0.5, 0.75 and 1% respectively. The healing efficiency of wounds and ointment was measured on days 5, 10, 15, 20 and 25th through healing time, tissues tensile strength, histopathology, and contraction rate of the wound by randomized recording method. Obtained data was analyzed with the help of ANOVA and results showed that wound healing time was the minimum as 16th day for 1.00% and maximum 21st day for 0.25% concentration of NPs ZO. Same is the case for rate of wound contraction and histopathology that results are in support of 1.00% concentration and not encouraging for

0.25%. This study appeals for the use of ZO NPs ointment for the healing of surgical wounds but more study of ZO NPs in the field of surgery at more molecular level is need of the day.

Key words: Histopathology. Nano zinc oxide, *Oryctolagus cuniculus,* wound,

Introduction

The wound is described as the interruption and damage in the defense mechanism of skin resulting in its instability may or may not include internal organs and connective tissue (1). Wounds are basically and majorly of two types open and close. When skin is disrupted and protective mechanism is breached it is open type otherwise close one. Many complex and sequential physiological mechanisms like inflammation, hemostasis, proliferation and remodeling are responsible for wound healing (2, 3). Healing of wound leads to many complications such as infection, which is major factor in delayed healing process. Infection of wound results in inflammatory condition. That is ultimately dangerous for life.

A number of agents and factors are responsible for process of wound healing during different phases of healing. These include the health condition, age and hormonal stability of patient, microbial contamination level, tissue integrity and dryness and many other pathological conditions are responsible for delayed healing of wound and ultimately lead to serious health and economic crises (4, 5). Topical application of the antiseptic preparations, germicides and healing agents is the ideal one because this is the way in which direct action is possible and quick too (6, 7). From a long period ago many chemicals and plants are in use of wound healing (8).

Among the number of major metal oxides nanoparticles, zinc is one of the most important because of its specific chemical and physical properties and employed in many fields. The USA Food and Drug Administration (FDA) graded ZnO as Generally Recognized as Safe (GRAS) (9). ZnO NPs are comparatively less toxic and inexpensive than the other metal oxide nanoparticles and have biomedical applications like wound healing, anti-inflammatory, and antimicrobial, bio imaging, drug delivery and as anticancer (10-13). Nanotechnology is the study of materials having size not more than 100nm. Nanoparticles are used in a wide range of processes and fields such as medical, diagnostic, cosmetic, food industry, and agriculture and material science (14-18).

Nanoparticles have good antimicrobial activity, decline their poisonous severity and protection against microbial contamination during process of wound healing. These are also

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economical than traditional antibiotics and have long half-life than commercial products in body so long term remedial results (19). ZnO NPs have a four steps mechanism of microbes killing, first of all attract microbes and then produce hydrogen per oxide that causes microbial cell injury. In third phase react with microbial compounds having Sulphur and phosphorus like DNA of bacterium and finally disturb the metabolic rate of cells and lead to bacterial cell death (20-23).

Objectives

- To find out the healing efficacy of Zinc oxide Nanoparticles in different concentrations.
- To Measure the efficacy of ZO NPs on the basis of wound healing parameter like, time of healing, histopathology, tensile strength and contraction rate of wound.

Material and Methods:

Infliction of surgical wound

Before surgery sideways of trunk (thoracic-blunder area) region of each rabbit was disinfected completely and shaved. All the rabbits were anesthetized with the help of xylazine (5mg/kg) and ketamine (35mg/kg) in sternal prostration. Five full thickness surgical wounds of same measure were made at appropriate distance from the mid line and marked with indelible marker.

Treatment protocol

All experimental concentrations 0.25, 0.50, 0.75 and 1.00% ZnO NPs were utilized on all 10 rabbits and on the each same model but different wound then healing was observed on regular basis.

Parameter recorded

Following parameters were recorded to check the healing process of wound on days 5^{th}, 10^{th}, 15^{th} and 20^{th}.

Histopathology, time of healing, contraction rate of wound.

Statistical Analysis

The obtained data was statistically analyzed by using ANOVA to check out the significance of the results [24].

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Results

Evaluation parameters

Wound contraction rate

Wound contraction rates of different concentration of ZnO NPs were measured for each wound and every rabbit. Contraction completed on day 16th in 1% ZnO Nps, on day 17th for 0.75%, 19th for 0.50% and 21st for 0.25%. While 5th one control group treated with Normal Saline was recover on day 25th. Mean contraction rates were maximum for wounds treated with maximum concentration of ZnO NPs showed its positive significance.

Table. 1. Comparison Among means of ZnO NPs contractions

Rate of ZnO NPs Concentrations	Days						
	4th	8th	12th	16th	20th	24th	28th
N.S	0.931	2.374	3.919	5.807	8.074	9.92	10
0.25 %	1.087	3.071	5.829	8.658	9.969	10	10
0.5 %	1.692	3.607	6.662	9.308	10	10	10
0.75 %	1.888	3.988	6.919	9.878	10	10	10
1 %	2.046	4.453	7.165	10	10	10	10

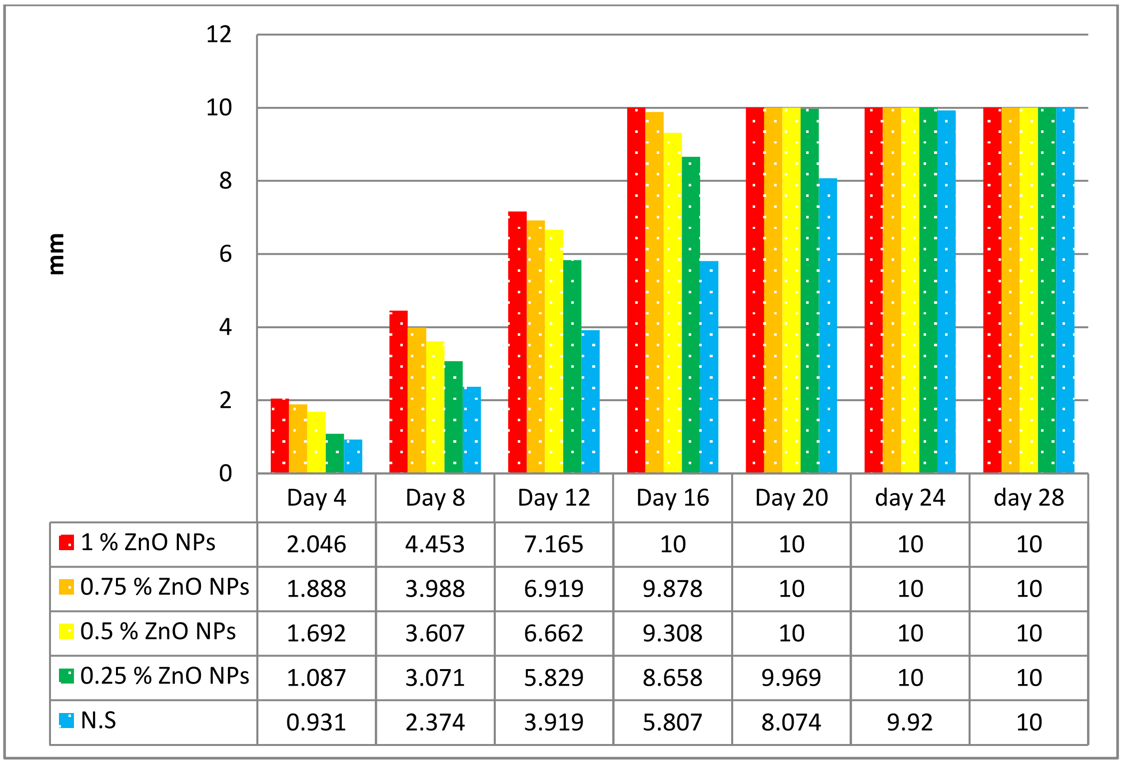

Fig. 1. Comparison among the means of different concentrations of ZnO NPs.

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Time of Healing

For time of healing required for wound complete healing result was also best for more concentration than lower concentration. Healing time is indirectly related to concentration percentage of ZnO NPs as 0.25% ZnO NPs have maximum healing time of 21st days.

Table. 2. Effects of ZnO NPs in Different concentration on wounds healing

Rate of Concentration	Means healing time in days
N.S	25.2
0.25% ZnONPs	20.8
0.5% ZnONPs	18.8
0.75% ZnONPs	17.6
1% ZnO NPs	16.4

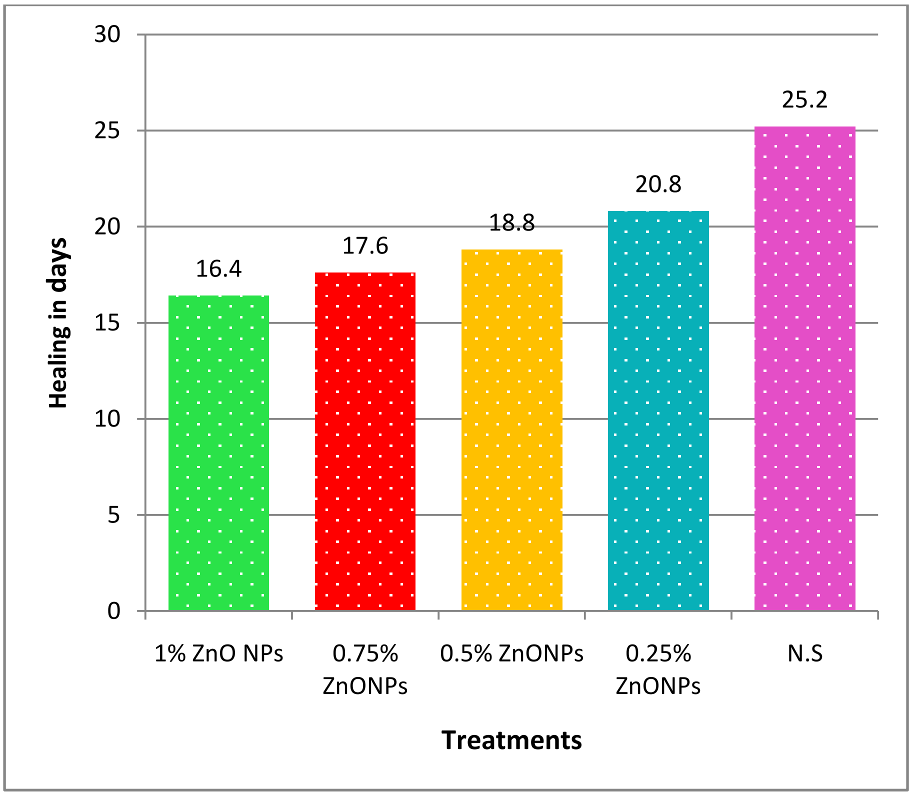

Fig. 2. Effects of ZnO NPs in Different concentration on wounds healing

Histopathological examination

For histopathology of healed wound the tissue samples were collected from the same region to examine these parameters as thickness of dermis, epidermis and collagen substance.

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To find the pressure level, collagen strands and rate of collagen content were determined. In treated samples fiery cells were seen too.

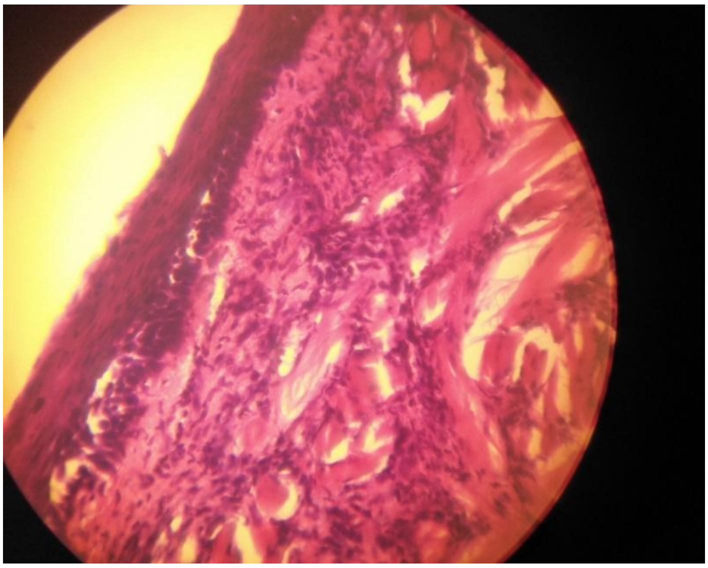

Plate: Demonstrating highest collagen fibers in the dermis, thin epidermis and healed wound tissue containing Blood vessels

Discussion

Wound healing is a complex process containing sequence wise steps and have dynamic procedure. These stages are combination of physiological, biochemical and cellular events that are overlapping and mutual to each other. Broadly these stages are hemostasis, inflammation, proliferation and remodeling. If these stages are disturbed then whole process of wound healing is delayed. This delay in healing process leads to complexity of wound and lethal for survival of individual. A number of factors are responsible for this postponement of healing process like as hypoxic condition, age, stress and overall health condition of the individual. Ultimately open or breached skin is one of the major facilitating factors for the entrance of infectious agent into the body.

For this purpose, research is continued to find out the techniques and materials or agents that could enhance the wound healing and mending process. Same level of work is in progress to stop and decline the contamination of wound and ultimately delayed healing. In this regard a number of techniques and materials have been developed to help the wound healing process.

In case of Collagen substances, ZnO NPs concentration was directly proportional to the thickness of collagen substance as maximum result was obtained for 1.00% concentration of ZnO NPs than the other concentrations i.e. 76.93% for Normal saline, 83.55 % for 0.25 %

ZnO NPs treatment, 87.54% for 0.5%, 92.12 % for 0.75%, and 94.52 % for 1% ZnO NPs treatment. The data showed that wound treated with 1% ZnO NPs have better collagen substance than the remaining concentrations.

Conclusion

Wounds are one of the major risk factors of the body. Proper and timely healing of wound is necessary otherwise might lead to severe problematic conditions. A number of techniques and products are utilized for the enhancement of the healing activity. Different metal oxides and their nanoparticles are utilized in the different fields of research and medications. ZnO nanoparticles are of unique importance due to their peculiar characteristics and properties. These are also used in living being as their toxicity level I much lower than the other metal oxides. The have proved as one of the best anti-cancer agents, anti-inflammatory and anti-microbial agents. In the present research focus was made on the healing capacity of the ZnO NPs in various concentrations and results proved their healing efficacy directly proportional to their concentration rate on various healing and wound parameters. It is concluded from the whole research that at this level of study 1.00% concentration of ZnO NPs is better than the lower concentration rates. But further study and research are recommended to make sure its efficacy and develop more advanced products.

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