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COMPARATIVE STUDY OF ZINC OXIDE OINTMENT AND ZINC OXIDE NANOPARTICLES AS A POTENTIAL WOUND HEALING SUBSTANCE IN RABBIT

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Abstract:
Nanotechnology has revolutionized medicine, with a focus on green nanoparticle production that is both friendly to the environment and cost effective. Biogenic nanoparticles have demonstrated great potential in wound healing, especially through nanotechnology drug delivery systems such as micelles, nanoparticles, nanoemulsions, and liposomes. These systems improve wound healing by lowering medication cytotoxicity, increasing skin penetration, and providing antimicrobial protection. Zinc oxide (ZnO) nanoparticles are particularly useful due to their antibacterial and anticancer characteristics. This study addressed the wound healing effects of ZnO nanoparticles in 18 rabbits separated into three groups: control (normal saline), ZnO nanoparticles, and ZnO ointments. Wound contraction size, hematological, and histology were evaluated on days 0, 5, 10, and 15. The results showed that ZnO nanoparticles significantly increased wound contraction and histological parameters such as angiogenesis and re-epithelization when compared to the control and ZnO ointment groups. The hematological study revealed no infection or harm. In conclusion, ZnO nanoparticles have better healing and therapeutic properties than ZnO ointment.

Keywords: Nanoparticles, Antibacterial, Anticancer, Ointments, Wound Healing

Introduction

Rabbits are small mammals that belong to the family of *Leporidae* with the order *Lagomorpha*. The scientific name of the rabbit is *Oryctolagus cuniculus* and is well known and used by wild predators as wild prey and is also domesticated as livestock and pets respectively. Rabbit has an extensive impact on ecological habitat and the environmental norms, and is found almost all around the world.

Rabbits have become prosaic and are gaining more importance in the field of experiments for research purposes (Gousalya *et al*., 2022).

The integumentary system is the basic system of the body which is a first line of defense against the environment (Jalil *et al*., 2020). The largest organ of the body is the skin, which plays various significant functions, it protects against infections and mechanical forces, hypo and hypervolemia, thermal irregularities, and myopathies. Skin is an external flexible and soft tissue that covers the body of all vertebrate animals. Skin regulates various joint movements due to its flexibility function in most of the regions of the body.

Skin plays a pivotal role in sensation to the external and internal stimuli. Many intrinsic and extrinsic reasons result in inadequate wound healing that demands medical intervention as the only remedy. Healing of a wound is a step-by-step process that consists of some phases to start healing; these main phases are i.e., Inflammation, Proliferation, Epithelialization, Angiogenesis, Remodeling, and Scarring. Biological regulation of wound healing of skin is a complicated procedure, that depends on various cell types and arbitrators that work in an exceptionally organized chronological succession (Sorg *et al*., 2017).

Nanotechnology is an emerging technology that is becoming more evident with versatile applications in the regeneration of skin. Nano fibers have achieved particular recognition in the regeneration of skin, because of their structural analogy to the connective tissue (extracellular matrix). A vast array of polymeric nano fibers with well-defined attributes have been evolved and tested as scaffolds for the regeneration of the skin. Apart from extending support to repairing tissue, nanofibrous materials also serve as carrier systems and deliver growth factors, drugs, proteins, and other molecules.

Furthermore, the morphological configuration, biodegradability, and other performances of nanofibrous substances can be controlled against specific states of wound healing. Moreover, nanostructured systems of drug delivery, like micelles, nanoparticles, Nano emulsions, and liposomes, are used to enhance the healing of wounds at different stages. These nanoscale delivery systems have exhibited various satisfactory results for the process of wound healing, along with debilitated drug cytotoxicity, inadequately water-soluble drug administration, enhanced skin infiltration, controlled delivering properties, antimicrobic activity, and safe guarding of drugs sensitive to light, temperature, pH and enzymes deterioration, besides activation of fibroblast proliferation, decreased inflammation (Alberti *et al*., 2017).

Nanomaterials are generally 1nm-100nmin in size. In modern medicine nanotechnology and the application of nanomaterial is growing and expanding rapidly, it includes the development of materials and molecules within a range of nanometer size. When the size of the substance is decreased to the nanoscale, it will eventually enhance the surface area, and surface area to volume ratios, which leads to progressive physiochemical characteristics (Rajendran *et al*., 2018).

The amount of manufacture and use of nanoparticles is increasing distinctly. Nanoparticles that are mostly used in sensors, catalysis, environmental remediation, and personal care products are metal oxide nanoparticles. Due to the antimicrobial and antitumor properties Zinc oxide (ZnO) amid Nano-sized metal oxides has been substantially used. Inorganic Zinc oxide (ZnO) has an antibacterial property which is more durable as compared to the organic agents. Zinc is a chemical element that is considered significantly important in wound healing, particularly in the belated healing of wounds and burns, and stays for a longer duration in the living cells. Topical administration of zinc reduces inflammation, improvement of re-epithelialization, and reduces the growth of bacterial in case of chronic wounds. Topical application is the most preferred choice of treatment for wounds because of their antiseptic properties and their direct action at the site of the wound. Zinc oxide is used generally for this purpose, although ZnO NPs hold superior properties as compared to conventional ZnO properties.

The development of granulation tissue to fill the wound bed, repair, and reinstatement of the epithelial barrier by re-epithelization and wound contraction are all necessary for wound healing by second intention. Poor wound healing and the diseases that impede it are the second most common reasons for patient death or euthanasia in clinical practice *(*Sparks *et al*., 2020).

REVIEW OF LITERATURE

A considerable number of Nanomaterials have been thoroughly investigated for the last several decades for biological applications that comprise fullerenes, liposomes, quantum dots, dendrimers, graphene, iron and titanium oxide, carbon nanotubes, and metal oxide NPs. Contemporarily, the field of nanotechnology is booming and growing expeditiously and pledges a brighter and safer future. To manufacture nanoparticles in a biodegradable and low-cost manner, substantial attempts are being made to substitute the present-day physical and chemical approaches that are currently in practice. The physical methods are very expensive and the chemical one is more toxic. Biological methods, besides being cost-effective, also deliver protein-capped nanoparticles that are more durable, have better dispersity do not get altered, and may be employed in numerous applications (Henglein 1989).
An animal's skin and flesh are particularly vulnerable to wounds, which are trauma-induced injuries. In medicine, the term "wound" refers to an injury to any part of the body caused by an agent because of external trauma, regardless of whether the surface is broken or not. In surgery, the term "wound" refers to a solution of continuity or interruption of the soft part of the body due to external trauma. The process of wound healing is dynamic and complex comprehending diseased processes requires first comprehending the events of typical wound healing. Animal and cellular models have shown to be highly helpful in this regard, even though humans and experimental animals repair skin wounds in very different ways (Graham, 2004).
In veterinary dermatology, punch biopsy is a common and effective tool for diagnostic investigations. To evaluate cutaneous healing, an experimental wound of standardized size and shape can also be made using a punch biopsy. When one considers the significant spontaneous contraction and remodeling properties of rabbit skin, the punch biopsy methodology alone can seem insufficient. It is possible to refine this approach and incorporate it into the natural healing equation by seeing wound contraction as a mathematical variable. The interpretation of intricate biological systems might be aided using a mathematical model. Additionally, the surgical protocol is made easier because it is now unnecessary to construct critical-sized wounds and wounds may be simply created with a little biopsy punch without stretching the rabbit skin (McDougall *et al*., 2006).
The exemplar for the treatment of delayed healing of a skin wound is autologous skin transplantation. This technique, nevertheless, might not be satisfactory occasions because of the unavailability of a donor site. On such occasions, engineered skin reserves a substitute for autologous transplantation of the skin. Undoubtedly, there is a requirement for the development of such approaches and strategies that encourage wound healing and prevention of scar formation.
The application of cell therapy in the presence and absence of growth factors during experimental demos has exhibited some favorable conclusions, but because of some complications in Scalable fabrication, Storage, high costs, governing issues, and standardization deficiencies have made it unable to reach the clinical settings. Furthermore, the effectiveness and safety protocols of this procedure have not been thoroughly studied (Atala *et al*. 2010).
ZnO NPs appear to have multiple morphological characteristics such as Nano wire, Nano rod, Nano belt, Nano flake, Nano flower (Paul kumar *et al*. 2013). Metal oxide NPs conception is implicit in the emergence of the nanotechnology world by virtue of their unique characteristics regarding a single species. The recent development of metal oxide NPs indicates that their physical and chemical characteristics are mainly associated with their absorbents and catalytic properties. For the synthesis of highly stabilized NPs, Green synthesis has been employed. Zinc oxide is deemed as a multi-task metal oxide that can be brought into play as a nano scale because of its distinctive physical, chemical, and biological properties (Salam *et al*. 2014).
The NPs are encased with a molecule known as a stabilizer and capping agent. NPs are arbitrarily divided into various classes established on their various properties such as Size, Structure, Morphology, etc. (Shirzad-Siboni *et al*. 2014). ZnO is an n-type semiconducting metal oxide. Zinc oxide NPs have gained immense attention in the last two-three years because of their broader compass of applicability in Optics, Electronics, and Biomedical Systems (Anbuvannan *et al*. 2015).
It is being researched how to increase the penetration of medications via the skin to address the different issues associated with conventional drug delivery systems. The goal of the innovative

methods is to deliver controlled drug release, which lowers the need for repeated medication administration, lowers the likelihood of unpleasant side effects, and safeguards against the degradation of encapsulated pharmaceuticals. Site-specific skin targeting is made possible by drug delivery systems powered by nanotechnology, which may lead to higher drug retention at the target site. Due to the negative charge on the surface of skin epithelial cells, it is anticipated that drug delivery systems bearing a positive charge will easily interact with the cells, increasing drug permeability and prolonging the pharmacological effect (Firooz *et al*., 2015).

Impaired wound healing in people and animals is currently a major problem in the medical and veterinary industries. Although wound healing is a normal process, the development of a chronic wound (such as diabetes mellitus, venous stasis ulcers, skin ulcers, etc.) can happen because of the persistence of chronic diseases. In recent years, the number of patients with chronic wounds has significantly increased. An enormous amount of work has gone into the wound. Given the skin is more susceptible to several external influences and can sustain different types of skin injury, the likelihood of developing cutaneous wounds is higher. The existing conventional remedies typically entail expensive, protracted treatments with an ulcer relapse rate of greater than 70% (Garcia-Orue *et al*., 2016).

When compared to their bulk counterparts, nanoparticles display unique features that make them very desirable for a wide range of biomedical applications. Metal oxide nanoparticles (MONPs) have proven adaptable platforms for therapeutic and diagnostic interventions. Inorganic nanoparticles have garnered a great deal of interest in biology and medicine. The ability of MONPs to stimulate the production of reactive oxygen species (ROS) by cells in the presence and absence of irradiation under a variety of circumstances has received extensive research. Due to oxidative stress, ROS has been found to cause a variety of pathological outcomes, such as genotoxicity and fibrosis (Augustine *et al*., 2017).

Skin damage and wound healing are extensive and complex biological processes that require intercellular route activation, coordination of tissue integrity, and homeostasis. The type and extent of the injury will determine whether the wound is acute or chronic. To speed up the healing of wounds, a range of dressing and topical materials are available. An ideal dressing should function as a three-dimensional template that can simulate an extracellular matrix, be biologically stable, flexible, and able to eliminate wound exudate by creating a moist environment at the wound site. To shield the wound from dangers outside, it ought to create a protective bed (Basu *et al*., 2017).

The wound occurs due to the discontinuity of the normal anatomical epithelial tissue barriers. Its disruption is caused by injury, tissue resection, or burns. Sometimes wound delays healing in due course, rather turns into chronic because of accompanying anomalies such as Peripheral Arterial Disease, Diabetes, etc. Delay in healing also occurs due to improper post-operative care and infections. Chronic wounds are reluctant to heal resulting in a growing health and economic burden and are also accompanied by an increase in morbidity that eventually amplifies the cost of animal care and management (Naderi *et al*. 2018).

Due to their peculiar characteristics, NPs can be used as a substitute method of wound treatment. Employment of plants through a safer and green eco-friendly method was a time of need and scientists much needed that procedure. Plant-based NP production is quicker, cheaper, and has less hazardous effects. As a result, nanoparticles can be formulated with unique sizes and variable shapes (Ovais *et al*. 2018).

The US Food and Drug Administration has designated ZnO as "Generally Recognized as Safe" (GRAS) (21, CFR 182, 8991). Reduced granulation tissue deposition, decreased tensile strength, and delayed wound closure rates have all been noted in zinc-deficient animals. When administered to open wounds, zinc has been found to hasten the healing process. Additionally, zinc applied topically seems to be more therapeutic than zinc taken internally (Ågren *et al*., 1991). Due to its exceptional antibacterial, anti-inflammatory, and angiogenic characteristics, nano ZnO has recently also been the subject of extensive research as a powerful chemical for biological applications (Kaushik *et al*., 2019).

Nanostructured materials with dimensions between 1 and 100 nm are now widely used in bio nanotechnology, basic and applied sciences, and other fields. Modern fabric compounds, food processing, agricultural production, and advanced pharmaceutical treatments are only a few of the revolutionary applications that can be provided by nanotechnology, a research hotspot in innovative materials science (De and Goswami, 2022).

In veterinary practice, wound management is a common event in both general and specialty practice. Wound healing involves a complex series of events. Healing requires cytokines and growth factors for the commencement of the process and to guide and carry healing overall. Generally, wound healing usually occurs in three phases, the inflammatory phase, the repair phase, and the maturation phase (Lux 2022).

MATERIALS AND METHODS

3.1 Experimental Station:

The study was conducted at the University of Veterinary and Animal Sciences (UVAS), Lahore, in the department of veterinary surgery. The experimental animals were also kept in UVAS. The operations were carried out in accordance with the guidelines and instructions approved by UVAS Lahore's Ethical Review Committee for the Use of Laboratory Animals.

3.2 Ethical Consideration:

The University of Veterinary and Animal Sciences (UVAS), Lahore's Office of Research Innovation and Commercialization approved the study with Institutional Guidelines of Ethical Review Committee, vide No.: DR/ 791 Dated: 26-12-2022.

3.3 Experimental animal:

18 adult, healthy rabbits of either sex or breed, aged six months to two years, with body weights ranging from 1.5 to 3.5 kg, served as the subjects for the current study. Animals were kept with suitable feed and access to water during the study period. The area where the animals were housed was ventilated. Regular clinical examinations were conducted to assess the animals' overall health. Any systemic infection before the study was ruled out through proper clinical examination.

3.4 Wound standards:

Hair from the shoulder area of rabbits was clipped and processed aseptically for this study. Under lignocaine, local anesthesia, a punch biopsy was used to remove about 8mm of skin.

3.4 Chemicals:

- ZnO nanoparticles were prepared and characterized by the Department of Pathology at Agriculture University Faisalabad and GCU Faisalabad using Zeta potential(Kumar and Dixit, 2017).
- Commercially available ZnO ointment was purchased.

3.6 Preparation of Nanoparticles for topical use:

For ointment, 01 g zinc oxide nanoparticles were mixed in 99 g of petroleum jelly to make 100 g preparation (Bhutta *et al*., 2021).

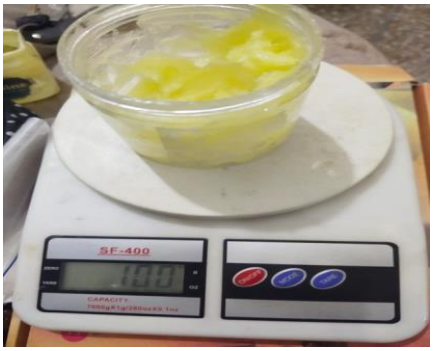

Figure No. 3.1: 100 g preparation of Zinc oxide Nanoparticles

In Group C, Zinc Oxide ointment was used as the treatment protocol for wound healing.

3.7 Experimental Design

A total of 18 animals were used in this study. Animals were randomly assigned to three groups according to different treatments.

(i) GA-control, treated with normal saline
(ii) GB-formulated zinc oxide nanoparticles (ZnO NPs) treated group and
(iii) GC- formulated zinc oxide ointment treated group.

G1 A	G2 B	G3 C
The group treated with normal saline	Group treated with Zinc Oxide nanoparticles	Group treated with zinc oxide ointment

3.7.1 Group A

A1, A2, A3, and so forth were the names of the rabbits in group A. The biopsy-punched wound in the rabbits in Group A was treated with Normal Saline as a control. The wound site was adequately cleaned after the hair around it was shaved off. The wounds were then treated directly with Normal Saline every day until they had fully healed.

3.7.2 Group B

B1, B2, B3, and so on were the names of the rabbits in group B. Zinc Oxide Nanoparticles were employed to treat the wound in Group B, which was a second group. In addition to adequately cleaning the wound site with Normal Saline, the surrounding hair was shaved off. Once the wounds were created, ZnO nanoparticles were put directly into them after some time.

3.7.3 Group C

Group C's rabbits were designated as C1, C2, C3, and so forth. A common method of treating wounds in Group C was using Zinc Oxide ointment. In addition to adequately cleaning the wound site with Normal Saline, the surrounding hair was shaved off. After that, until the wounds were created, ZnO ointment was applied.

3.7.4. Treatment Plan:

Table 3.1 Treatment Plan

Sr. No	Groups	No. of Animals	Treatment Protocol	Application	Frequency
1.	Group A	6 Rabbits	Normal Saline	Direct application with gauze	After 24 hours
2.	Group B	6 Rabbits	Zinc Oxide Nanoparticles	Direct application with gauze	After 24 hours
3.	Group C	6 Rabbits	Zinc Oxide ointment	Direct application with gauze	After 24 hours

3.8 Post-treatment management:

Daily ointment application was done after 24 hours until complete wound healing.

3.9 Parameters of Study:

a) Macroscopic Evaluation

I. Wound Contraction size (mm):

Wound healing was evaluated by measuring the wound contraction size at days 0, 5, 10, and 15 with a Vernier caliper.

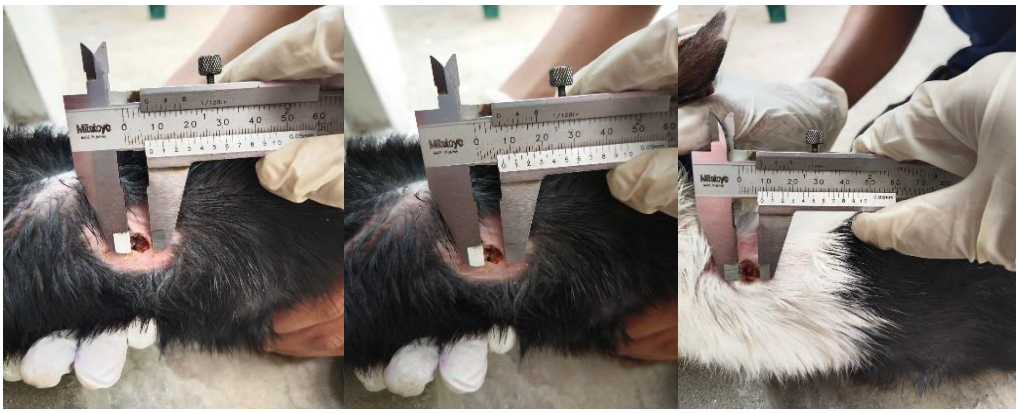

Figure No.3.2: Measuring contraction size with vernier caliper

II. Wound Contraction Rate (%):

Wound healing was evaluated by measuring the wound contraction rate at days 0, 5, 10, and 15. The wound contraction rate was calculated by the following formula;

$$\%Wound\ Contraction = \frac{initial\ wound\ size\ -specific\ day\ wound\ size}{initial\ wound\ size} \times 100$$

b) Microscopic Evaluation (Histopathology):

Histology of wound samples of groups was done to observe and compare the re-epithelization, granulation, angiogenesis, neutrophils, and fibrous connective tissue of the skin. Samples for histology were taken by biopsy punch (8mm), before the start of treatment (day 0) and on the 15th day. The tissue samples were fixed in 10% formalin for 48 hours. After rinsing and dehydration, samples were embedded in paraffin. These 4-7µm thick sections were stained with hemotoxylin-eosin (H&E) stain and then photographed under 100X magnification.

c) Hematological Parameters:

a) White blood cells or leukocytes (WBCs)
b) Platelets count
c) Hemoglobin (Hb) levels.

Blood sampling will be done through the Jugular vein on days 0,5,10 and 15th day

3.10. Procedure:

3.10.1-Preparation of Site

The hair surrounding the surgical site was shaved off. The surgical site was prepared aseptically and after complete scrubbing animal was ready for further procedures.

Figure No 3.4: Surgical site is ready

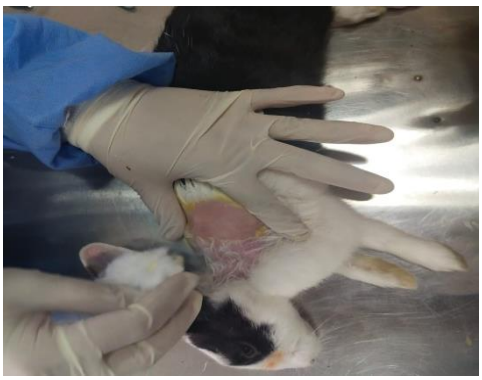

Figure No. 3.3: Shaving the surgical site

3.10.2- Anesthesia

Local anesthesia (Lignocaine 2%) was used at the surgical site before creating and collecting the biopsy sample for histopathology.

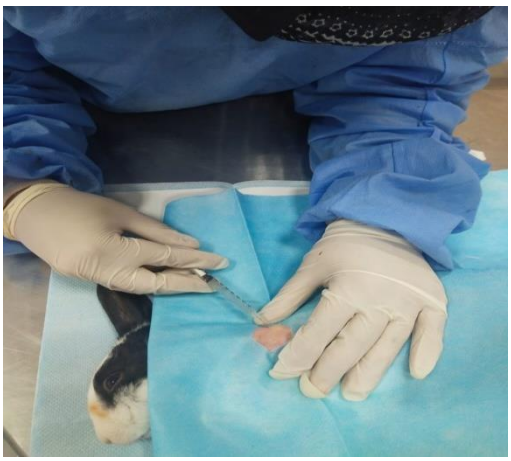

Figure No. 3.5: Local Anesthesia infiltration

3.10.3. Punch wound creation:

Under lignocaine, local anesthesia, a punch biopsy was used to remove about 8mm of skin.

Figure No. 3.6: Biopsy punch 8mm

Punch biopsy is the most widely utilized biopsy method in veterinary dermatology. Punch biopsies are easy procedures that only need basic equipment. The double punch is a biopsy procedure used in veterinary dermatology. For big subcutaneous tumors with minimal epidermal involvement that are being biopsied for both histology and culture, this technique is highly helpful (Logas and Dermatology, 2021). The rabbits were placed into three groups, with six rabbits each in groups A through C. Each rabbit received an 8mm punch wound from this biopsy punch. Punch wound biopsy formation involves pressing the punch and gently rotating it until a circular patch separates from the remaining lining.

Group A gets the application of normal saline, Group B gets Zinc oxide Nanoparticles and Group C gets zinc oxide

RESULTS

This study was conducted to access the comparative study of Zinc oxide ointment and Zinc oxide nanoparticles as potential wound-healing substances in rabbits. A total of eighteen rabbits with biopsy punch wounds on the shoulder were included in the study. These rabbits were subjected into three groups, A, B, and C, each comprised of six rabbits. Rabbits in Group A were treated with Normal

Saline while those in Group B were treated with Zinc oxide nanoparticles and those in Group C were treated with Zinc oxide ointment. The following parameters were studied on each Rabbit:

4.1-Evaluation Parameters

I. Macroscopic Evaluation

a) Wound Size (mm)
b) Wound Contraction Rate (%)

II. Hematological evaluation:

a) WBCS
b) Hb count
c) Platelets count

III. Microscopic Evaluation

a) Histopathological Findings
b) Inflammation
c) Granulation
d) Fibrous Connective Tissue
e) Re-epithelization
f) Angiogenesis

4.1.1-Wound Size (mm)

The treatment protocol of the respective groups was applied from day 0 till the 15th day. Wound size and contraction in each rabbit were measured by Vernier Callipers and the readings were taken on days 0, 5th, 10, and 15th.

Collected data regarding wound size and wound contraction were analyzed through one-way-ANOVA using PROC GLM in SAS software (Version 9.1). Significant treatment means were compared through the DMR test. Statistical analysis showed that data is significant at $p \leq 0.05$.

1) Wound size of rabbits in Group A (Control)

In Group A, 6 rabbits with punch wounds were treated with normal saline. The wounds were treated and observed till the 15th day. The mean wound size of rabbits on day 0 was 8.0 ±0.00, while on day 5 it was 5.95 ± 0.52, till day 10the wound size was reduced up to 3.33 ± 0.41. On day 15 size of the wound was found to be 1.08 ± 0.24.

Table No. 4.1: Individual Wound Size (mm) of Rabbit Control Group (GroupA)

Individual Wound Contraction Size (mm) of Rabbit Control Group							
Time (Days)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**Mean ± SD**
0	8	8	8	8	8	8	8.0±0.00
5	6.5	5.2	5.5	6.5	6	6	5.95±0.52
10	3	3.5	3.5	4	3	3	3.33±0.41
15	1	1	1.2	1.5	0.8	1	1.08±0.24

2) Wound size of rabbits in Group B (ZnO Nanoparticles)

In Group B, 6 rabbits having punch wounds of 8mm were treated with the zinc oxide nanoparticles. The mean wound size of rabbits on day 0 was 8.00 ±0.00, while on day 5 it was 4.27 ± 0.5. Till day 10, out of 6 total wounds 5 wounds were completely healed, and the mean wound size was found to be 0.08 ± 0.20. When measurements were taken on day 15, the mean wound size was 0.0 ± 0.00, with all wounds completely healed when treated with Zinc oxide nanoparticles in Group B.

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Table No. 4.2: Individual Wound Contraction Size (mm) of Rabbit NanoParticle treated Group (GroupB)

Individual Wound Contraction Size (mm) of Rabbit NanoParticle treated Group (Group B)							
Time (Days)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean ±STD**
0	8	8	8	8	8	8	8.0±0.00
5	5	4.6	4.5	3.5	3.8	4.2	4.27±0.55
10	0.5	Healed	Healed	Healed	Healed	Healed	0.08±0.20
15	Healed	Healed	Healed	Healed	Healed	Healed	0.0±0.00

3) Wound size of rabbits in Group C (ZnO ointment)

In Group B, 6 rabbits having punch wounds of 8mm were treated with the zinc oxide ointment. The mean wound size of rabbits on day 0 was 8.00 ±0.00, while on day 5 it was 5.43 ± 0.85. Till day 10, out of 6 total wounds 3 wounds were completely healed, and the mean wound size was found to be 1.00 ± 1.10. When measurements were taken on day 15, the mean wound size was 0.15 ± 0.18, with 3 wounds completely healed when treated with Zinc oxide nanoparticles in Group B.

Table No. 4.3: Individual Wound Size(mm) of Rabbit Zincoxide treated Group (Group C)

Individual Wound Size(mm) of Rabbit Zincoxide treated Group (Group C)							
Time (Days)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**Mean ± SD**
0	8	8	8	8	8	8	8.0±0.00
5	6	6.5	4.3	4.8	6	5	5.43±0.85
10	1.8	2	0	0	2.2	0	1.00±1.10
15	0.2	0.3	0	0	0.4	0	0.15±0.18

1) Comparison of Mean Wound Size of all groups

The mean wound size of Groups A, B, and C on day 0 was 8.00±0.00 respectively. It shows wounds included in all Groups were of the same size as 8mm. The mean wound size of Groups A, B, and C on day 5 was 5.95 ± 0.52, 4.27±0.55 and 5.43 ± 0.85 respectively. On day 10, the mean wound size of group A was 3.33 ± 0.41 while in group B it was 0.08 ± 0.20 and in group C the mean wound size was 1.00±1.1. Reaching the 15^{th} day, the mean wound size of group A was 1.08 ± 0.24 while the mean wound size of group B showed that all the wounds were healed with a mean wound size of 0.00 ± 0.00 while in group C majority of the wounds healed with mean wound size of 0.15±0.18.The number of individuals mentioned were beings compared accordingly, by applying one-way- ANOVA using PROC GLM in SAS software, and data is significant at $p \leq 0.05$.

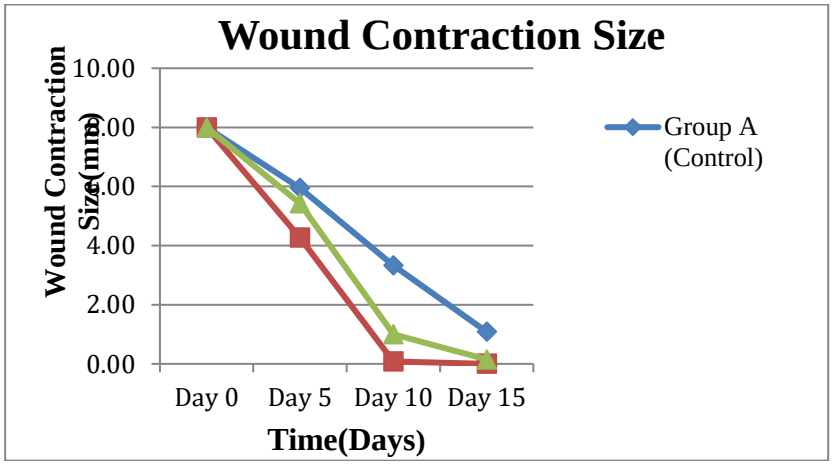
Table No. 4.4: Mean± S.D Wound Size (mm) of Rabbit and P-Value

Mean± S.D Wound Size (mm) of Rabbit and P-Value				
Time (Days)	**GROUP-A**	**GROUP-B**	**GROUP-C**	
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**P-Value**
0	8.0±0.00	8.0±0.00	8.0±0.00	0.1
5	5.95±0.52	4.27±0.55	5.43±0.85	0.02
10	3.33±0.41	0.08±0.20	1.00±1.10	0.00
15	1.08±0.24	0.0±0.00	0.15±0.18	0.00

2) Graphical Representation of Wound Contraction size of all groups

The wound size of Groups A, B, and C was the same. The comparison of wound contraction rates of all treatment protocols is represented in Figure 4.1. In the graphical representation, Blue lines denote the wound contraction size of Group A (control), red lines denote the wound contraction size of Group B (ZnO nanoparticles) while green lines show the wound contraction size of group C (ZnO ointment) at regular intervals. It is visible from the graph that wounds that were treated with zinc

oxide nanoparticles in group B were contracted faster and healed more effectively as compared to the wounds that were treated with zinc oxide ointment and normal in groups A and C.



Graph No. 4.1: Comparison of Mean Wound Contraction size of all groups

4.1.2. Wound Contraction Rate (%)

Wound Contraction was measured in percentage and evaluated by the formula given in Materials and Methods (Chapter 3).

1) Wound Contraction Rate of Group A (control)

In group A (normal saline), the mean wound contraction rate of all 6 wounds on day 0 was 0.0 ± 0.00, while on day 5, the mean wound contraction percentage was 26.67 ± 6.69. Till day 10, wounds of group A were 58.33 ± 5.10 percent healed. While, till day 15, with a mean contraction rate of 86.46± 3.00 percent no wound completely healed in Group A.

Table No. 4.5: Individual Wound Contraction Rate (%) of Rabbit Control Group (GroupA)

Individual Wound Contraction Rate (%) of Rabbit Control Group							
Time (Days)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**Mean ± SD**
0	0	0	0	0	0	0	0.0±0.00
5	18.75	37.5	31.25	22.5	25	25	26.67±6.69
10	62.5	56.25	56.25	50	62.5	62.5	58.33±5.10
15	87.5	87.5	85	81.25	90	87.5	86.46±3.00

2) Wound Contraction Rate of Group B (ZnO nanoparticles)

In group B the wounds were treated with zinc oxide nanoparticles. The mean wound contraction rate of all 6 wounds on the day was 0.0 ± 0.00, while on day 5, the mean wound contraction percentage was 46.67 ± 6.88. It means that till day 10, all the wounds were 90% healed when zinc oxide nanoparticles were used as a treatment protocol with a mean contraction percentage of 97.92±5.10. Till day 15, all the wounds are 100% healed with a mean contraction percentage of 100.0 ± 0.00.

Table No. 4.6: Individual Wound Contraction Rate (%) of Rabbit Nano Particle treated Group (Group B)

Individual Wound Contraction Rate (%) of Rabbit Nano Particle treated Group B)							
Time (Days)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean ±STD**
0	0	0	0	0	0	0	0.0±0.00
5	37.5	42.5	43.75	56.25	52.5	47.5	46.67±6.88
10	87.5	100	100	100	100	100	97.92±5.10
15	100	100	100	100	100	100	100.0±0.00

3) Wound Contraction Rate of Group C (ZnO ointment)

In group C the wounds were treated with zinc oxide nanoparticles. The mean wound contraction rate of all 6 wounds on the day was 0.0 ± 0.00, while on day 5, the mean wound contraction percentage was 32.1 ± 10.68. Till day 10, all the wounds were 80% healed with a mean contraction percentage of 87.5±13.78 when zinc oxide ointment was used as a treatment protocol. Till day 15, half of the wounds are healed with a mean contraction percentage of 98.2 ± 2.16.

Table No. 4.7: Individual Wound Contraction Rate (%) of Rabbit Zinc oxide treated Group (Group C)

Individual Wound Contraction Rate (%) of Rabbit Zinc oxide treated Group (Group C)							
Time (Days)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**Mean ± SD**
0	0	0	0	0	0	0	0.0±0.00
5	25	18.75	46.25	40	25	37.5	32.1±10.68
10	77.5	75	100	100	72.5	100	87.5±13.78
15	97.5	96.5	100	100	95	100	98.2±2.16

4) Comparison of Mean Wound Contraction Rate of all the groups

The mean wound contraction of Groups A, B, and C on day 0 was 0.0 ± 0.00. Till day 5, wounds of group A were contracted up to 26.67± 6.69%, while wounds of group B were contracted up to 46.67± 6.88 % and wounds of group C contracted up to 32.1±10.68 %. The wound contraction rate in group A till the 10^{th} day was 58.33± 5.10, while in group B it was 97.92± 5.10%. The wound of Group C shows a contraction rate of 87.5±13.78%. Reaching the 15^{th} day wounds of group A were healed up to 86.46± 3.00, while the wounds included in group B were efficiently healed up to 100.0 ± 0.00.

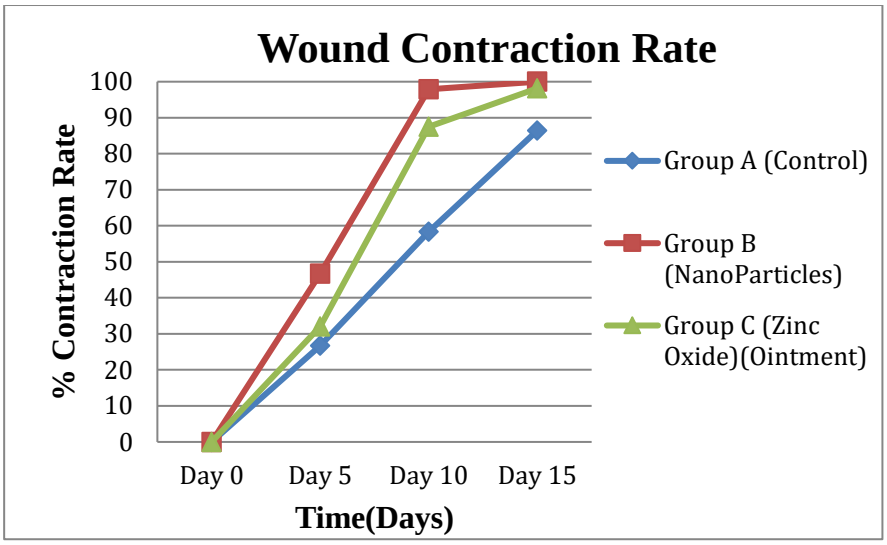
Wounds treated with zinc oxide ointment in group C healed up to 98% with a mean contraction rate of 98.2±2.16%. The number of individuals mentioned in the superscripts were beings compared accordingly, by applying Factorial ANOVA using PROC GLM in SAS software, and data is significant at $p \leq 0.05$.

Table No. 4.8: Mean ± S.D Wound Contraction Rate (%) of Rabbit and P-Value

Mean ± S.D Wound Size(mm) of Rabbit and P-Value				
Time (Days)	**GROUP-A**	**GROUP-B**	**GROUP-C**	
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**P-Value**
0	0.0±0.00	0.0±0.00	0.0±0.00	0.1
5	26.67±6.69	46.67±6.88	32.1±10.68	0.02
10	58.33±5.10	97.92±5.10	87.5±13.78	0.00
15	86.46±3.00	100.0±0.00	98.2±2.16	0.00

5) Graphical Representation of Wound Contraction Rate of all groups

The wound size of Groups A, B, and C was the same. The comparison of wound contraction rates of all treatment protocols is represented in Figure 4.1. In the graphical representation, Blue lines denote the wound contraction size of Group A (control), red lines denote the wound contraction size of Group B (ZnO nanoparticles) while green lines show the wound contraction size of group C (ZnO ointment) at regular intervals. It is visible from the graph that wounds that were treated with zinc oxide nanoparticles which is group B were contracted faster and healed more effectively as compared to the wounds that were treated with zinc oxide ointment and normal in groups A and C.



Graph No. 4.2: Comparison of Mean Wound Contraction Rate of all groups

4.2. Hematological count:

4.2.1-WBC Count

1. WBC's count of Rabbits(Group A)

WBCs of all Group A rabbits were monitored on days 0, 5, 10, and 15. On day 0 the mean WBC's count of all rabbits was 6.81 ± 0.02. Mean ± S.D, on day 5 it was increased up to 8.72± 0.16. Mean ± S.D while on day 10 WBCs were recorded 8.58 ± 0.10. Mean ± S.D and on day 15 it was recorded as 8.46 ± 0.11.

Table No. 4.9: Individual WBC's Count of Rabbit Control Group (Group A)

Individual WBC's Count of Rabbit Control Group (Group A)							
Time (Days)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**Mean ± SD**
0	6.81	6.78	6.83	6.82	6.79	6.84	6.81±0.02
5	8.52	8.67	8.56	8.82	8.94	8.78	8.72±0.16
10	8.46	8.59	8.48	8.64	8.74	8.57	8.58±0.10
15	8.32	8.45	8.36	8.52	8.63	8.46	8.46±0.11

2. WBC's count of Rabbits(Group B)

WBCs of all Group B rabbits were monitored on days 0, 5, 10, and 15. On day 0 the mean WBC count of all rabbits was 6.82 ± 0.03 Mean ±S.D, on day 5 it was increased to 7.43± 0.16 Mean ± S.D while on day 10 WBCs were recorded at 6.93 ± 0.27 Mean ± S.D and on day 15th it was recorded as 6.79 ± 0.55 Mean ± S.D.

Table No. 4.10: Individual WBC's Count of Rabbit Nano Particle treated Group (Group B)

Individual WBC's Count of Rabbit Nano Particle treated Group (Group B)							
Time (Days)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean ±STD**
0	6.82	6.83	6.79	6.84	6.85	6.78	6.82±0.03
5	7.23	7.51	7.64	7.27	7.38	7.52	7.43±0.16
10	6.92	7.17	7.17	6.98	6.45	6.87	6.93±0.27
15	6.56	6.75	6.56	6.5	7.89	6.45	6.79±0.55

3. WBC's count of rabbits (Group C)

WBCs of all Group C rabbits were monitored on days 0, 5, 10, and 15. On day 0 the mean WBC count of all rabbits was 6.81 ± 0.05 Mean ± S.D, on day 5 it was increased to 7.42± 0.13 Mean ±

S.D while on day 10 WBCs were recorded 7.30 ± 0.31 Mean ± S.D and on day 15th it was recorded as 6.95 ± 0.04 Mean ± S.D.

Table No. 4.11: Individual WBC's Count of Rabbit Zinc oxide treated Group (Group C)

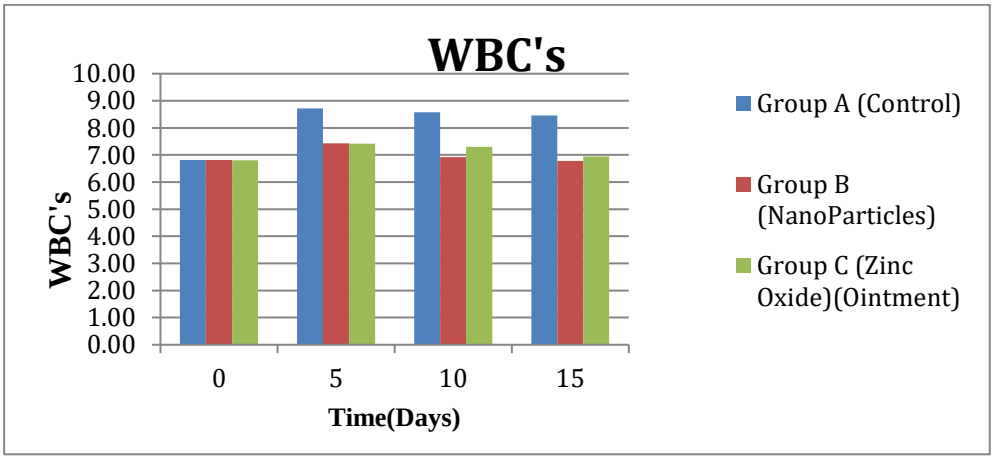
Individual WBC's Count of Rabbit Zinc oxide treated Group (Group C)							
Time (Days)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**Mean ± SD**
0	6.76	6.82	6.78	6.82	6.76	6.89	6.81±0.05
5	7.31	7.29	7.54	7.4	7.62	7.38	7.42±0.13
10	7.45	6.74	7.43	7.14	7.54	7.48	7.30±0.31
15	6.89	6.94	6.96	6.99	6.98	6.93	6.95±0.04

4. WBC's count Comparison of Group-A, B, and Group-C with P-value:

All groups were analyzed and compared on a significant level $p < 0.05$. All groups were non-significant on day 0 with P=0.812 while significant on day 5, 10, and 15 with P=0.00 respectively.

Table No. 4.12: Mean ± S.D WBC's Count of Rabbit and P-Value

Mean ± S.D WBC's Count of Rabbit and P-Value				
Time (Days)	**GROUP-A**	**GROUP-B**	**GROUP-C**	
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**P-Value**
0	6.81±0.02	6.82±0.03	6.81±0.05	0.812
5	8.72±0.16	7.43±0.16	7.42±0.13	0.00
10	8.58±0.10	6.93±0.27	7.30±0.31	0.00
15	8.46±0.11	6.79±0.55	6.95±0.04	0.00



Graph No. 4.3: Comparison of Mean WBC's Count of all Groups

4.2.2-Hemoglobin

1. HB level of rabbits (Group A)

Hemoglobin of group A rabbits was monitored on days 0, 5, 10, and 15. On day 0 the mean hemoglobin of all rabbits was 13.20 ± 0.67 Mean ± S.D, on day 5 it was decreased up to 12.08 ± 0.74 Mean ± S.D while on day 10 it was recorded as 10.60 ±1.02 Mean ± S.D and on day 15 it was 11.34 ±0.87 Mean ± S.D.

Table No. 4.13: Individual HB of Rabbit Control Group (Group A)

Individual HB of Rabbit Control Group (Group A)							
Time (Days)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**Mean ± SD**
0	13	13.36	12.29	12.68	13.88	13.98	13.20±0.67
5	11.86	11.92	11.97	10.94	12.86	12.95	12.08±0.74
10	10.89	9.76	9.75	9.65	11.83	11.74	10.60±1.02
15	12.59	10.69	10.64	10.54	11.43	12.16	11.34±0.87

2. HB level of rabbits (Group B)

Hemoglobin of group B rabbits was monitored on days 0, 5, 10, and 15. On day 0 the mean hemoglobin of all rabbits was 12.84 ±0.42 Mean ± S.D, on day 5 it was decreased up to 12.45± 0.52 Mean ± S.D while on day 10 it was recorded as 12.56 ±0.54 Mean ± S.D and on day 15 it was 12.85 ± 0.33Mean ± S.D.

Table No. 4.14: Individual HB of Rabbit Nano Particle treated Group (Group B)

Individual HB of Rabbit Nano Particle treated Group (Group B)							
Time (Days)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean ±STD**
0	13.12	12.16	13.06	12.54	12.87	13.29	12.84±0.42
5	12.98	11.94	12.87	12.01	11.99	12.92	12.45±0.52
10	12.96	11.72	12.93	12.16	12.47	13.1	12.56±0.54
15	13.41	12.78	12.98	12.83	12.49	12.58	12.85±0.33

3. HB level of Rabbits (Group C)

Hemoglobin of group C rabbits was monitored on days 0, 5, 10, and 15. On day 0 the mean hemoglobin of all rabbits was 13.03 ± 0.20 Mean ± S.D, on day 5 it was decreased up to 12.05± 0.07 Mean ± S.D while on day 10 it was recorded as 12.56 ± 0.33 Mean ± S.D and on day 15 it was 12.69 ± 0.24 Mean ± S.D.

Table No. 4.15: Individual HB of Rabbit Zinc oxide treated Group (Group C)

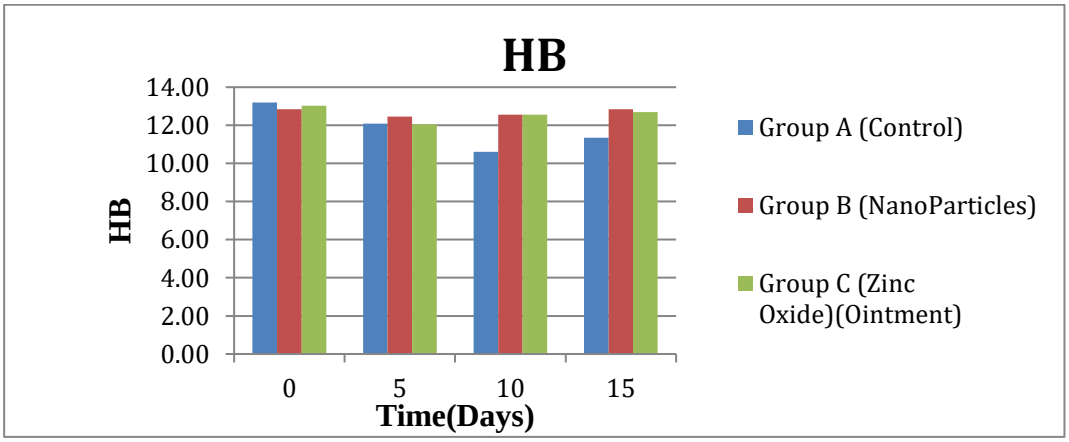
Individual HB of Rabbit Zinc oxide treated Group (Group C)							
Time (Days)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**Mean ± SD**
0	13.26	13.19	12.89	12.86	13.17	12.78	13.03±0.20
5	12.01	11.98	12.06	12.12	12.15	11.99	12.05±0.07
10	12.71	12.86	12.7	12.79	12.1	12.18	12.56±0.33
15	12.89	12.89	12.78	12.76	12.51	12.3	12.69±0.24

4. HB Comparison of Group-A, B, and Group-C with P-value :

All groups were analyzed and compared on a significant level of $p < 0.05$. All groups were non-significant on days 0 and 5 with P=0.440 and 0.365 while significant on day10 and 15 with P=0.00 respectively.

Table No. 4.16: Mean ± S.D HB of Rabbit and P-Value

Mean ± S.D HB of Rabbit and P-Value				
Time (Days)	**GROUP-A**	**GROUP-B**	**GROUP-C**	
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**P-Value**
0	13.20±0.67	12.84±0.42	13.03±0.20	0.440
5	12.08±0.74	12.45±0.52	12.05±0.07	0.365
10	10.60±1.02	12.56±0.54	12.56±0.33	0.00
15	11.34±0.87	12.85±0.33	12.69±0.24	0.00



Graph No. 4.4: Comparison of Mean HB of all Groups

4.2.3-Platelets count:

1. Platelets count of rabbits (Group A)

The platelet count of group A rabbits was monitored on days 0, 5, 10, and 15. On day 0 the mean of all rabbits was 363.50 ± 26.82 Mean ± S.D, on day 5 it was decreased up to 398.17 ± 26.29 Mean ± S.D while on day 10 it was recorded as 393.0 ± 19.66 Mean ± S.D and on day 15 it was 373.5 ±11.59 Mean ± S.D.

Table No. 4.17: Individual Platelet Count of Rabbit Control Group (Group A)

Individual Platelet Count of Rabbit Control Group (Group A)							
Time (Days)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**Mean ± SD**
0	410	345	363	343	342	378	363.50±26.82
5	435	396	420	374	366	398	398.17±26.29
10	396	362	404	405	377	414	393.0±19.66
15	361	382	384	366	362	386	373.5±11.59

2. Platelets count of rabbits (Group B)

The platelet count of group B rabbits was monitored on days 0, 5, 10, and 15. On day 0 the mean of all rabbits was 355.50 ± 3.90 Mean ± S.D, on day 5 it was decreased up to 349.83 ± 83.38 Mean ± S.D while on day 10 it was recorded as 340.33 ± 4.46 Mean ± S.D and on day 15 it was 332.33 ±22.03 Mean ± S.D.

Table No. 4.18: Individual Platelet Count of Rabbit Nano Particle treated Group B

Individual Platelet Count of Rabbit Nano Particle treated Group (Group B)							
Time (Days)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**Mean ±STD**
0	348	356	355	359	354	358	355.00±3.90
5	314	302	325	336	304	518	349.83±83.38
10	340	341	343	341	332	345	340.33±4.46
15	313	304	352	355	348	322	332.33±22.03

3. Platelets count of rabbits (Group C)

The platelet count of group B rabbits was monitored on days 0, 5, 10, and 15. On day 0 the mean of all rabbits was 361.3 ± 38.09 Mean ± S.D, on day 5 it was decreased up to 394.67 ± 50.82 Mean ± S.D while on day 10 it was recorded as 352.00 ± 22.01 Mean ± S.D and on day 15 it was 341 ±39.00 Mean ± S.D.

Table No. 4.19: Individual HB of Rabbit Zinc oxide ointment treated Group (Group C)

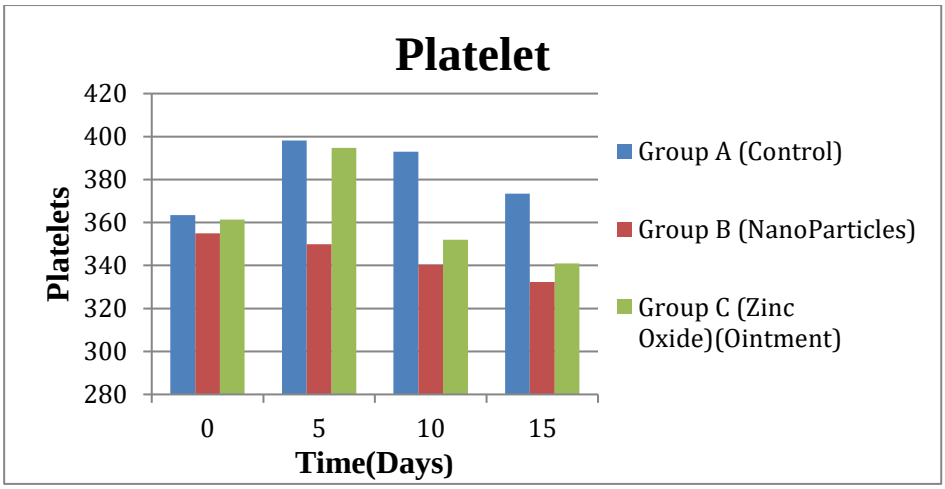
Individual Platelet Count of Rabbit Zinc oxide ointment treated Group (Group C)							
Time (Days)	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**Mean ± SD**
0	386	302	346	361	415	358	361.3±38.09
5	342	386	356	371	443	470	394.67±50.82
10	358	311	355	354	356	378	352.00±22.01
15	310	364	312	325	325	410	341±39.00

4. Platelets count comparison of Group-A, B, and Group-C with P-value:

All groups were analyzed and compared on a significant level of $p < 0.05$. All groups were non-significant on days 0 and 5 with P=0.853 and 0.307 while significant on days 10 and 15 with P=0.00 and p =0.042 respectively

Table No. 4.20: Mean ± S.D Platelet Count of Rabbit and P-Value

Mean ± S.D Platelet Count of Rabbit and P-Value				
Time (Minutes)	**GROUP-A**	**GROUP-B**	**GROUP-C**	
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**P-Value**
0	363.50±26.82	355.00±3.90	361.3±38.09	0.853
5	398.17±26.29	349.83±83.38	394.67±50.82	0.307
10	393.0±19.66	340.33±4.46	352.00±22.01	0.000
15	373.5±11.59	332.33±22.03	341±39.00	0.042



Graph No. 4.5: Comparison of Mean Platelets count of all Groups

4.3-Histopathological Findings:

Histology was done to evaluate the granulation, inflammation, angiogenesis, and re-epithelisation of wounds, before and after the treatment protocol of the respective groups (Table 4.2). Hematoxylin and Eosin staining were used for the histology of tissue samples.

4.3.1 Group A

Histopathological findings showed an influx of neutrophils on day 0 as evidence of acute inflammation and dead tissue was also seen microscopically. Following the treatment protocol of normal saline, wounds were contracted up to day 15. Histology was done on the 15th day. Those wounds that were in the process of healing had neutrophil and polymorph nuclear cells seen during histology, and epidermal outgrowth on the wound edge was in progress**.**

4.3.2 Group B

Histopathological findings showed an influx of neutrophils on day 0 as evidence of acute inflammation and dead tissue was also seen microscopically. Following the treatment protocol of Zinc oxide nano-particles, 1 out of 6 wounds were incompletely healed while the other 5 were completely healed on the 10th day, and all wounds were completely healed before the 15th day. Histology was done on the 15th day. Those wounds which were completely healed before the 15th day, all had a wound bed fully covered by new epidermal cells with scar formation.

On histology, Group B seemed to have a higher number of fibroblast cells in the epidermal layer as compared to Group A. The wound healing process in Group B reached the third phase in almost 10 days (from inflammation to proliferation to maturation), while Group A's wounds were in transition from inflammation to proliferation in the same period of time.

4.3.3. Group C

Histopathological findings showed an influx of neutrophils on day 0 as evidence of acute inflammation and dead tissue was also seen microscopically. Following the treatment protocol of Zinc oxide ointment, 3 out of 6 wounds were incompletely healed while on the 10th day, all wounds

were towards healing. Histology was done on the 15th day. Those wounds which were completely healed before the 15th day, had a wound bed fully covered by new epidermal cells with scar formation.

Table 4.21: Comparison of histological findings of Group A, B, and Group C

Treatment Groups	Inflammation	Granulation	Fibrous Connective tissue	Re-epithelization	Angiogenesis
Normal Saline	+++	+++	++	++	++
ZnO Nano-particles	++	+++	++	+++	+++
ZnO Ointment	+++	++	+++	++	+++

DISCUSSION

The current experimental study was conducted on Rabbits *(Oryctolagus cuniculus)*. The objective of the study was to evaluate the formulation and characterization of the biogenic zinc oxide nanoparticles, determine of effects and comparison of zinc oxide ointment and zinc oxide nanoparticles on skin wound healing, and investigate the enhancing effect of ZnO ointment and Nanoform as clinical use for the topical treatment of skin wound healing in rabbits. A total of 12 rabbits were subjected to three equal groups. The groups were as follows:

Group A: Application of normal saline on punch wounds to achieve wound healing in rabbits.

Group B: Application of zinc oxide nano-particles on punch wound to achieve wound healing in rabbits.

Group C: Application of zinc oxide ointment on punch wound to achieve wound healing in rabbits.

To manufacture nanoparticles in a biodegradable and low-cost manner, substantial attempts are being made to substitute the present-day physical and chemical approaches that are currently in practice. The physical methods are very expensive and the chemical one is more toxic. Biological methods, besides being cost-effective, also deliver protein-capped nanoparticles that are more durable, have better dispersity do not get altered, and may be employed in numerous applications (Henglein, 1989). The factors that affect the healing of wounds in rabbits include contraction of wound surface area, its epithelization process, granulation tissue formation, and contamination. Any hindrance in wound healing process not only delays the wound healing process but may increase the chances of infection (Dart *et al*., 2009). Deficiency of deep supportive tissue, more bony prominence, and frequent joint motion cause a delay in the healing of wounds because of the extended preparatory phase (Celeste *et al*., 2011). Also, the imbalanced collagen synthesis and degradation of collagenase may lead to the formulation of exuberant granulation tissue which ultimately delays wound healing in rabbits (Schwartz *et al*., 2002).

The wound healing process in rabbits may be improved by averting exuberant granulation tissue formation, promoting epithelization, and increasing the contraction rate. It is essential to fasten the contraction rate, ultimate cosmetic attendance, and diminish the scar tissue which as a result enhances the chances of a return to complete athletic effectiveness (Jørgensen *et al*., 2021).

ZnO NPs appear to have multiple morphological characteristics such as Nano wire, Nano rod, Nano belt, Nano flake, and Nano flower. The NPs are coated with a molecule known as a stabilizer and capping agent. NPs are arbitrarily divided into various classes established on their various properties such as Size, Structure, Morphology, etc. (Shirzad-Siboni *et al*., 2014).

All three above-mentioned groups were treated daily and then evaluated based on wound contraction size, rate, hematology, and histopathology, and then inferences were evaluated statistically as well. After punching wound creation, all animals were treated with normal saline, zinc oxide nanoparticles, and zinc oxide ointment. By using one-way ANOVA utilizing SAS software's PROC GLM, the numbers of animals stated were compared appropriately, and the data was found to be significant at $p < 0.05$. The wound contraction size was the same in all the groups and on day 0, the average wound size for Groups A, B, and C was 8.00±0.00. It demonstrates that the 8mm wounds included in each Group were all of the same size. All group results are statistically non-significant at a p-value greater than 0.05 as $P = 0.1$. On day 5, the mean wound sizes for Groups A, B, and C were 5.95 ± 0.52, 4.27 ± 0.55, and 5.43 ± 0.85. Wound size contraction in group B Zinc oxide nanoparticles treated group was faster as compared to the other two groups. All group's results are statistically significantly

different with P = 0.02. On day 10, group A mean wound size was 3.33 ± 0.41, group B was 0.08 ± 0.20, and group C was 1.00 ± 1.1, with results of all groups were statistically significantly different with P= 0.00. On day 15 group A mean wound size was 1.08 ± 0.24, and group B mean wound size was 0.00 ± 0.00, which indicates better wound healing in group B. In contrast, the majority of the wounds in group C were also healed, with a mean wound size of 0.15±0.18. So, the results were also statistically significantly different when compared with a p-value 0.05 as P= 0.00.

The wound contraction rate was measured with the above-mentioned formula and this parameter was used in this study to compare all groups and to check whether the results were significantly different or not. On day 0, Groups A, B, and C had a mean wound contraction percentage of 0.0 ± 0.00, because the wound size was the same in all the groups. And the results when evaluated based on p-value showed that all groups were statistically non-significant with P= 0.1. On the 5th day, Group A wound size decreased to 26.67 ± 6.69%, Group B wound size to 46.67 ± 6.88 %, and Group C wounds to 32.1± 10.68 %. All the results were statistically significantly different with P= 0.02. Group A experienced a wound contraction rate of 58.33 ± 5.10 until the 10^{th} day, but group B showed at a contraction rate of 97.92 ± 5.10%. A contraction rate of 87.5±13.78% was seen in Group C wounds, all the groups were statistically significantly different with P= 0.00. As the 15th day approached, the wounds in Group A had effectively healed to 86.46 ± 3.00, whereas Group B wounds had healed to 100.0 ± 0.00. Moreover, Group C zinc oxide ointment-treated wounds healed up to 98% of the time, with a mean contraction rate of 98.2 ±2.06. All the results were statistically significantly different with P= 0.00.

The current study's hematological analysis of the zinc oxide ointment and nanoparticle treatment showed a substantial difference in the levels of hemoglobin, platelets, and white blood cells on days 5, 10, and 15. In WBC count the results of every group were examined and evaluated at the significant level ($p < 0.05$). On day 0, P=0.812, all groups were non-significant but, on days 5, 10, and 15, P=0.00, they were, accordingly. The platelet count was examined and evaluated at a significant level ($p < 0.05$). On days 0 and 5, all groups showed statistically non-significance (P = 0.853 and 0.307), however on days 10 and 15, P = 0.00 and P = 0.042, respectively, all groups showed significant results. All groups were analyzed and compared on a significant level $p < 0.05$. All groups were non-significant on days 0 and 5 with P=0.440 and 0.365 while significant on days 10 and 15 with P= 0.00 respectively.

Comparing zinc oxide nanoparticles with zinc oxide ointment, zinc oxide ointment showed less wound contraction and more scar and scab formation, while zinc oxide nanoparticles demonstrated scar formation with significant wound contraction, better re-epithelialization, and significant keratinocyte migration. At day 15, the zinc oxide nanoparticles treated group which is Group B exhibited a good dermal skin layer and mixed collagen pattern. Moreover, this group has been shown to form nuclei and blood vessels (Masood *et al*., 2019). The zinc oxide ointment-treated group showed a significant number of granulation tissues, oriented collagen fibers vertically, and a large number of inflammatory infiltrates. Group B demonstrated a high rate of proliferating cell body maturation and good dermal layer formation. As a result, the findings validated our hypothesis.

The biosynthesis of nanoparticles turned out to be relatively cheaper and eco-friendly when compared to physical and chemical methods. Plants and nanotechnology are associated with the approach of green chemistry due to the Plant-mediated synthesis of nanoparticles. Metal nanoparticles perform a significant role in the field of research as a result of large surface energies and, a specific large surface area to volume ratio as compared to bulk material (Pitout, 2012).

Metal oxide NPs conception is implicit in the emergence of the nanotechnology world by virtue of their unique characteristics regarding a single species. The recent development of metal oxide NPs indicates that their physical and chemical characteristics are mainly associated with their absorbents and catalytic properties. For the synthesis of highly stabilized NPs, Green synthesis has been employed. Zinc oxide is deemed as a multi-task metal oxide that can be brought into play as a nano scale because of its distinctive physical, chemical, and biological properties (Salam *et al*., 2014).

The exemplar for the treatment of delayed healing of a skin wound is autologous skin transplantation. This technique, nevertheless, might not be satisfactory in particular occasions as a result of the

unavailability of a donor site. On such occasions, engineered skin reserves a substitute for autologous transplantation of the skin. Undoubtedly, there is a requirement for the development of such approaches and strategies that encourage wound healing and prevention of scar formation. The application of cell therapy in the presence and absence of growth factors during experimental demos has exhibited some favorable conclusions, but as a result of some complications in Scalable fabrication, Storage, high costs, governing issues, and standardization deficiencies have made it unable to reach the clinical settings. Furthermore, the effectiveness and safety protocols of this procedure have not been thoroughly studied (Atala *et al.*, 2010).

Conclusion:

It was concluded from the study that zinc oxide nanoparticles have more therapeutic and healing efficacy than zinc oxide ointment for wound healing purposes. Better and quick healing of wounds in animals can be achieved with the use of nano-form of metal oxides hence, zinc oxide nanoparticles should be used for wound healing purpose in animals because it has high therapeutic and healing efficacy.

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