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The Role of γ-Irradiated Zinc oxide Nano-particles as a Treatment to Improve Burn Healing in Male Albino Rats

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Abstract: Chemical Burn accidents happen on a daily basis, especially when it comes to chemical cleaners. Most chemical used for cleaning are extremely irritant or even sometime dangerous to the user's skin. Zinc oxide is well known remedy for skin burns as well as a component in an array of other cosmetics. Nanoparticulation is a growing technology that aims at, in part, to improve drug kinetics and to target its content to the coveted organ. The main aim of the present study is to investigate the impact of nanoparticulation of zinc oxide followed by γ- irradiation on its ability to promote healing of partial-thickness burns model in male rats. Male Albino rats were categorized into four groups; G1 were the control group while partial thickness burns were induced in G2, G3 and G4. G2 were topically treated with pure paraffin, whereas G3 and G4 were topically treated with ordinary zinc oxide ointment, and irradiated nano-zinc oxide ointment respectively. Burn healing was examined over seven weeks of burn induction in all groups. The data from the present study showed that topical application of γ- irradiated nano-zinc oxide is superior to non-nano particulate zinc oxide ointment in the recovery from partial burn thickness in albino male rats.

Keywords: Partial thickness Burns – Zinc oxide nanoparticles – Rats – Healing process – Gamma irradiation.

1 Introduction

Burns are life-threatening events that affect the quality of life and puts a strain on international healthcare systems. Burn is a complex pathophysiological lesion causes structural and functional damage in many organs. animal models of burns have been created to discuss various characteristics of burns, to study the pathophysiology, and to explore possible treatments [1].

Zinc is found in all body tissues including the brain, muscles, bones and skin. Zinc shares in the body's metabolism, protein and nucleic acid synthesis, hematopoiesis and neurogenesis [2;3; 4and5]. At the World Union of Wound Healing Societies meeting, it has been hypothesized that zinc has a potential use in wound healing [6]. Zinc has an importance in healing burns through the process of wound matrix remodeling; Zinc in the skin represents 20% of the total zinc content in the body in the form of zinc metalloenzymes [7;8].

Zinc oxide recognized as a safe substance by the US Food and Drug Administration (FDA) [9]. Zinc oxide heals post-surgical wounds [10] ; improves the removal of wound debris from the skin, reduces the initial stage of bleeding, and helps to regrow damaged skin and hair.

Nanotechnology improves the bioavailability and efficacy of many therapeutic agents: through improving uptake into target cells, targeting of drugs to specific tissues, refining of drug profile, and pharmacokinetics; and reducing toxicity [11]. Nanoparticles is a promising technology for drug delivery to the brain; through improving their passage through the blood-brain barrier [12].

Zinc oxide nanoparticles are inexpensive and less toxic, making them promising for biomedical research and their anticancer effects [13 and 14]. Zinc oxide nanoparticles are a potent and stable preservative against pathogens in topical treatment. Gamma irradiation proved to promote the antibacterial effects of zinc oxide nanoparticles [15].

Our investigation tried to examine the advantage of

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irradiated nano-zinc oxide ointment in healing partial thickness burns compared to ordinary zinc oxide ointment.

2 Materials and Methods

Animals: This study was carried out according to the recommendations in the Guide for the Care and Use of Laboratory Animals approved by the Research Ethics Committee of National Center for Radiation, Research and Technology, Egypt. Male Wistar albino rats (200 ± 10 g) were purchased from the Animal house of Hot Laboratories Center, Egyptian Atomic Energy Authority, Egypt. Animals were housed in plastic cages with stainless-steel grid tops and kept at a room with standard conditions (a 12-h light/dark cycle; temperature maintained 23 ± 2 °C). The animals received standard diet, water and libitum.

Chemicals: Zinc oxide was purchased from Sigma Aldrich, Egypt; Paraffin was purchased from the pure company, Egypt; Sodium hydroxide (NaOH) pellets; were purchased from Riedel-de Haen Seelze-Hannover, Germany. Kits for aminotransferases, alkaline phosphatase, protein profile, CRP and IL8 evaluations were purchased from Bio-diagnostic, Egypt.

Synthesis of Zinc oxide Nanoparticles: Zinc oxide nanoparticles were prepared using the Sol-Gel method. The preparation method can be summarized as follows: 1:1 molar ratio of an analytical grade of hexahydrate zinc nitrate Zn $(NO_3)_2.6H_2O$ and monohydrate citric acid $(C_6H_8O_7.H_2O)$ in double-distilled water using a magnetic stirrer. The reaction is carried out according to the following equation:

$$Zn\ (NO_3)_2 + 3C_6H_8O_7 + 2O_2 \rightarrow Zn\ O + N_2\uparrow + 6CO_2\uparrow + 10H_2O \quad (1)$$

After getting a clear solution, without any suspensions, the solution was kept at a temperature of 90 °C while stirring continued till the gel formed; The gel started to completely burn at 450 ±10 °C and time around 30 min to form a crust powder. The powder was manually milled using a gate grinder.

Zinc oxide Nanoparticles' Characterization: The structural characteristics of the synthesized powders were studied by X-ray diffraction (Shimadzu 6000, Japan) using a copper X-ray tube (40 kV, 30 mA, Kα1 line with the wavelength of 1.54056 Å). The scanning was carried out over the range $2\theta = 30°–70°$. The crystalline phases were identified using the JCPDS database. The obtained powders were characterized morphologically by a high-performance digital imaging transmission electron microscope (TEM) (JEM-2100F; JEOL, USA). TEM samples were prepared by placing a few drops of their aqueous dispersions on carbon coated copper grids and drying at room temperature[23]. The mean NP sizes were calculated from a random field of TEM images that showed the general morphology of the prepared NPs. Over 200 particles were analyzed, counted and measured, to determine the mean sizes and size distributions.

Irradiation of Zinc oxide Nanoparticles: All irradiations were performed using the cobalt-60 gamma chamber (dose rate (1.77 kGy/h), located at Cyclotron Project, Nuclear Research Center, Egyptian Atomic Energy Authority, Egypt.

Preparation of Zinc oxide and Zinc oxide Nanoparticles ointments: Zinc oxide or prepared nano-zinc oxide is put into the mortar and a few drops of liquid paraffin is placed on it. The mixture is well ground. The half of simple ointment is weighted in a porcelain dish and it is melted on the water bath. The hot melted simple ointment is added to the zinc oxide and it is mixed until smooth ointment is obtained. According to the designed treatment protocol, ointment was added as a thin layer on the wounds once a day, three times a week, for seven weeks.

Experimental groups: Animals were divided into 4 groups, 9 rats for each group. Group 1(G1); received no treatment, and served as control. Group 2 (G2); partial thickness burns were caused in normal rats and were topically treated with pure paraffin; Group 3 (G3); partial thickness burns were caused in normal rats and were topically treated with ordinary zinc oxide ointment. Group 4 (G4); partial thickness burns were caused and were topically treated with irradiated nano-zinc oxide ointment. The animals were accommodated individually to prevent contamination. Time duration for the experiment was seven weeks to see the healing effect in all groups.

Induction of partial thickness burns on Skin: Animals of each group except the control, anesthetized by a combination of Ketamine 50 mg/kg BWt, Xylazine 5 mg/kg BWt and Thiopental sodium 50 mg/kg BWt. The back regions of rats were shaved and cleaned; partial thickness burns were caused using 2N Na OH (2 mol/L); to be topically applied on skin using a Cotton probe for 15 seconds with no pressure. The burns were designed to have a diameter of 1 cm^2. 2N Na OH was selected to cause partial thickness burns in rats after carrying out a pilot test; comparing its ability to cause partial thickness burns versus different detergents.

Healing assessment of partial thickness burns. Time duration for the experiment was seven weeks to explore the healing process in all groups. Morphologically healing progress of the burns was observed.

Hematology test. Blood samples were collected in EDTA - treated tubes to investigate; Hemoglobin concentration, Red blood cells (RBCs), white blood count, Lymphocytes % and total Neutrophil count.

Biochemical test. Blood samples were collected in a clean dry centrifuge tube, then centrifuged at 3500 rpm for 15 minutes for serum preparation and stored at −20 °C for further biochemical test, Activities of serum aminotransferases, alkaline phosphatase, the concentrations of total protein (TP), albumin (Alb) and globulin and Inflammatory markers C-reactive protein (CRP) and Interleukin 8 (IL8) were determined following manufacturer's instructions.

Histological test. Skin samples were fixed in 10% neutral formalin and then was prepared for Histological tests **[**16**]**, Sections were microscopically recorded and derived semi-quantitatively; epidermis was represented as an arrow and dermis was represented as a star; hair follicles were (h) & sebaceous glands were (s).

Statistical analysis. The obtained values are given as means ±standard deviation (S.D.) Contrasts between groups were carried out by one-way analysis of variance (ANOVA); The level of significance was set at $P \leq 0.05$.

Data availability. All data generated or analyzed during this study are included in this published article.

Ethics approval. Ethics approval and consent to participate this study was approved by the Research Ethics Committee of National Center for Radiation Research and Technology, Egypt.

3 Results and Discussion

I-List the results

Characterization of Zinc oxide nanoparticles

X-Ray Diffraction analysis. XRD analysis was used to check the phase structural of ZnO. The X-ray diffraction data were recorded by using Cu-K_α radiation (λ=1.5406 Å). Figure (1) shows the XRD pattern of the ZnO. The ZnO peaks are detected at 31.8°, 34.5°, 36.3°, 47.6°, 56.6°, 62.9°, 66.4°, 68.0°, 69.1° and 72.7° for the crystal planes (100) (002) (101) (102) (110) (103) (200) (112), (201) and (004), respectively and indicated a hexagonal crystal structure. No other phase or impurity were detected in the obtained spectrum and the entire diffraction peaks is in agreement with the standard JCPDS data (card **No 04-008-2750**) ZnO. The average grain size was calculated with the help of Scherrer equation using the FWHM of (101) peak according the equation:

$$D = 0.89\lambda/(\beta Cos\theta) \quad (2)$$

where D is the grain size, λ is the wavelength of x-ray radiation, β is the full width at the half maximum (FWHM) of the ZnO and θ is the diffraction angle. The crystallite size recorded 29 nm.

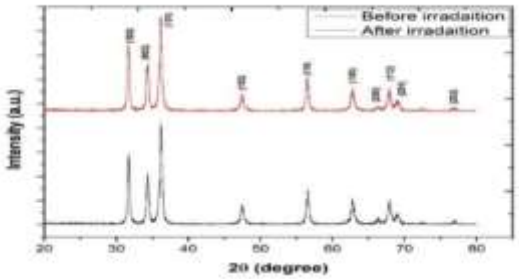


Fig.1:XRD pattern for ZnO nanoparticles before and after radiation.

Transmission Electron Microscopy (TEM). The ZnO nanoparticles' transmission electron microscopy (TEM) results are shown in Figure (2). Images of the samples show that the final product of ZnO nanoparticles have a semi-spherical form.

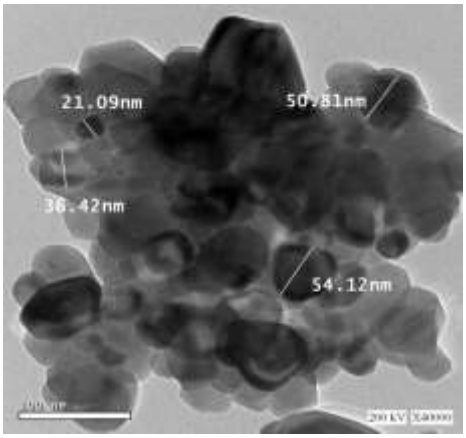


Fig.2: TEM image of ZnO nanoparticles.

Biochemical findings. Serum ALT, AST and ALP were significantly elevated ($p \leq 0.05$) in G2, G3 and G4; compared to control group. Serum total protein and Globulin concentrations showed significant variations ($p \leq 0.05$); although Albumin and A/G ratio showed insignificant change versus control group.

Serum inflammatory markers findings. Inflammatory markers were significantly elevated in G2 compared to control; while G3 and G4 showed a prolonged decrease in CRP and IL-8 ($p \leq 0.05$).

Hematology findings. Hb and RBCs showed mild decline in G2, G3 and G4 compared to control group; WBCs showed significant increase ($p \leq 0.05$) in G2 and G3 versus control group; but G4 showed a significant decline;

lymphocytes showed significant decrease in G2 and G3 versus control group; but group (4) showed an elevation.

Histological findings. Photomicrograph of skin from G1 in figure (6-a); showed normal skin layers; normal epidermis, and dermis with normal skin appendages; hair follicles & sebaceous glands; as well as figure (6-b); showed higher magnification of figure (6-a); the normal epidermis and dermis with normal hair follicles were noticed. Figure (6-c) illustrated the Photomicrograph of skin from group (2) showing complete destruction of epidermal layers with severe dermal inflammatory reaction while Figure (6-d) represented the higher magnification of the dermal inflammatory reaction; note the diffuse mononuclear cells infiltration was noticed. Figure (6-e) demonstrated the photomicrograph of skin from group (3) showed good healing progress; note the formation of mature granulation tissue with leucocytic cells infiltration; and figure (6-f) illustrated the higher magnification of the mature. Figure (6-g) discussed the photomicrograph of skin from group (4) showing complete healing; normal skin layers; epidermis, dermis with normal skin appendages, hypodermis, and muscle. Figure (6-h) showed higher magnification of the complete healing with normal skin layers; normal epidermis and dermis with normal skin appendages were seen.

Table 1: Biochemical findings in G1, G2, G3 and G4

(mean ± SD). (*) means significantly different at (P<0.05)

Groups / Parameters	G1	G2	G3	G4
ALT	120±2.1	165.4±2*	140.2±5*	145±3.1*
AST	180.5±3.1	192.13±5*	189.5±0.96*	188.7±3.2*
ALP	105.3±1.8	130±3*	130.5±1.29*	131.25±2.2*
TP	6.35±0.2	5.9±0.4*	5.54±0.2*	5.06±0.3*
Albumin	3.076±0.4	2.47±0.1	2.79±0.3	2.67±0.4
Globulin	3.29±0.3	3.43±0.2*	2.75±0.4*	2.38±0.2*
A/G	0.93±0.2	0.72±0.4	1.02±0.2	1.13 ±0.4

Figure (3-a)

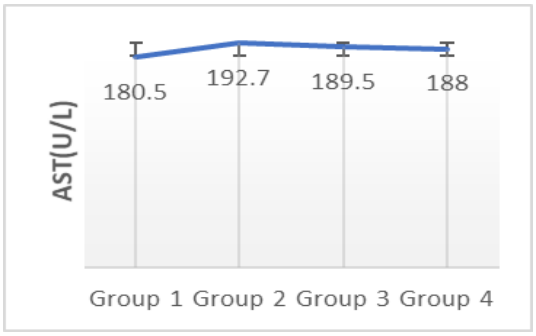


Figure (3-b)

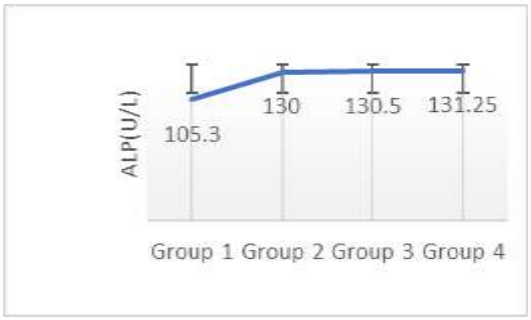


Figure (3-c)

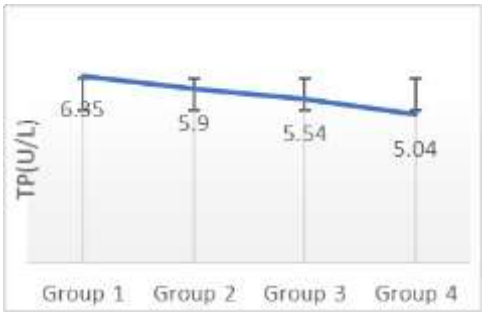


Figure (3-d)

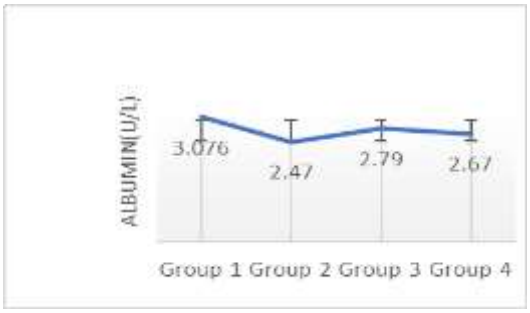


Figure (3-e)

Figure (3-f)

Figure (3-g)

Fig. 3: Biochemical findings of serum ALT, AST, ALP, Total protein, Albumin, Globulin and A/G ratio in G1, G2, G3 and G4.

Table 2. Inflammatory markers in G1, G2, G3 and G4 (mean ± SD). (*) means significantly different at (P<0.05).

Groups Parameters	G1		G2		G3		G4	
CRP	0.1466	5±0.3	0.462	190.67±5*	0.311	135.67±0.4*	0.239	93±3*
IL-8	0.107	8±0.4	0.399	450.33±2*	0.245	266.33±0.4*	0.169	167.67±2*

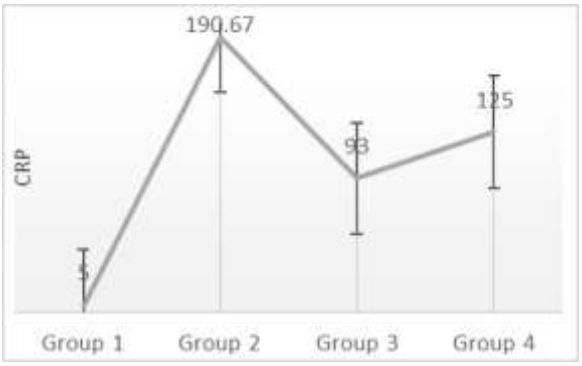


Figure (4-a)

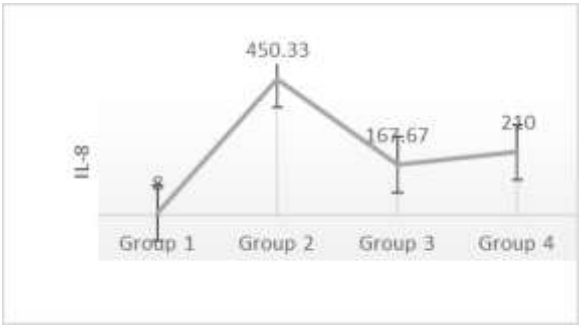


Figure (4-b)

Fig. 4: CRP (mg/dl) and IL-8 (pg/ml) in G1, G2, G3 and G4.

Table 3: Hematology findings. G1, G2, G3 and G4 (mean ± SD). (*) means significantly different at (P<0.05).

Groups Parameters	G1	G2	G3	G4
Hemoglobin	*15±0.2*	13.9±0.1*	14±0.2	14.2±0.4
RBCs	*5.4±0.1*	4.8±0.2*	4.9±0.1*	5.5 ±0.2
WBCs	*9.8±0.2*	18 ±0.3*	14.5±0.3*	9.8 ±0.1
Lymphocytes	*74.7±0.4*	73.5±0.8*	71.65±0.8*	74.5±1
Total Neutrophils	*20.2±0.2*	24±0.3*	21.3±0.2	21.16±0.1

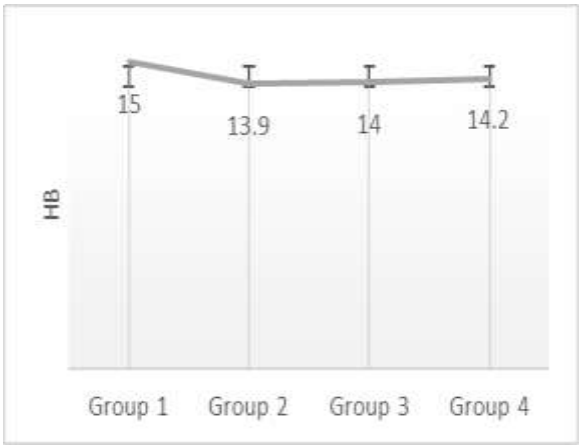


Figure (5-a)

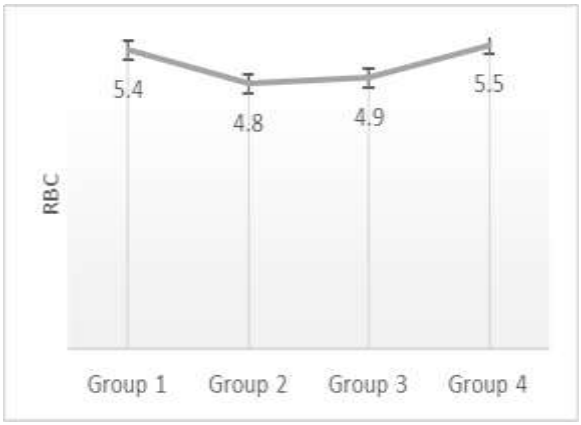


Figure (5-b)

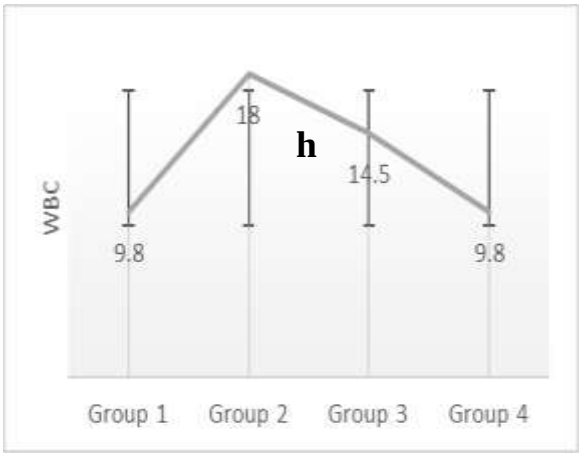


Figure (5-c)

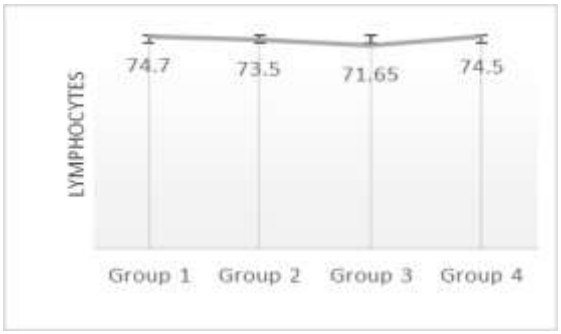


Figure (5-d)

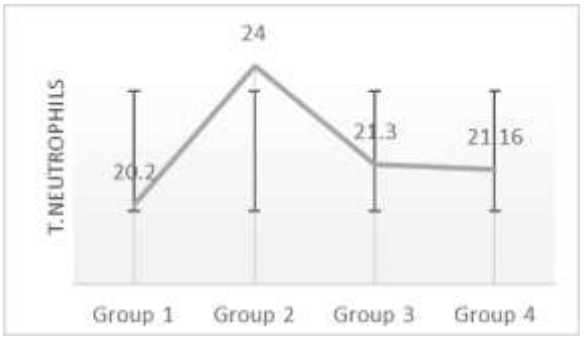
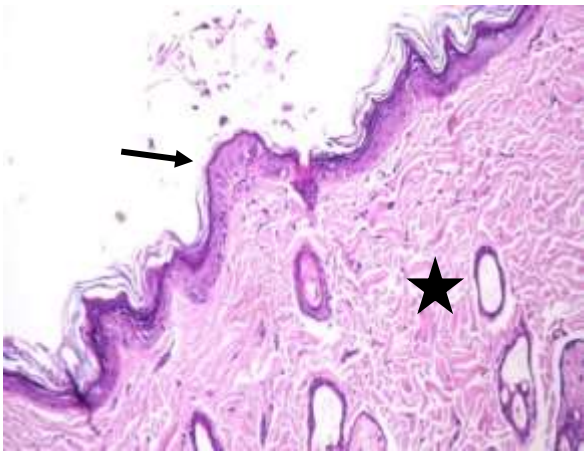


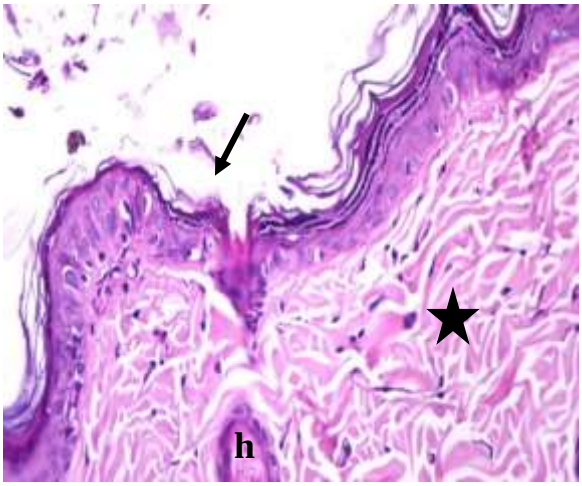
Figure (5-e)

Fig.5: Hb (g/dl), RBCs(x10^6/cmm) counts, WBCs (x10^3/cmm) counts, Lymphocytes % and total Neutrophil % in G1, G2. G3 and G4.



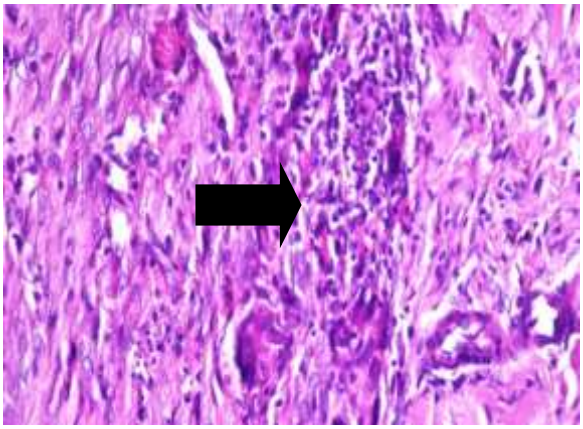
→	Epidermis
*	Dermis
h	Hair follicles
s	Sebaceous glands

Figure (6-a): Photomicrograph of skin from G1 showing normal skin layers; note the normal epidermis (arrow) and dermis (*) with normal skin appendages; hair follicles (h) & sebaceous glands (s), (H&E X200).



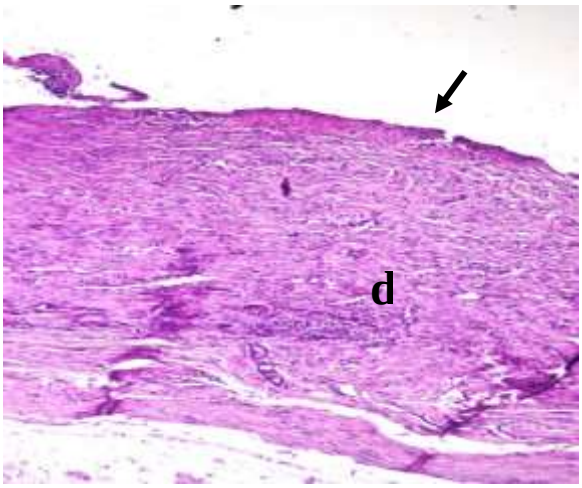
→	Epidermis
*	Dermis
h	Hair follicles

Figure (6-b): Photomicrograph of skin from G1 showing higher magnification of figure 1; note the normal epidermis (arrow) and dermis (*) with normal hair follicles (h), (H&E X400).



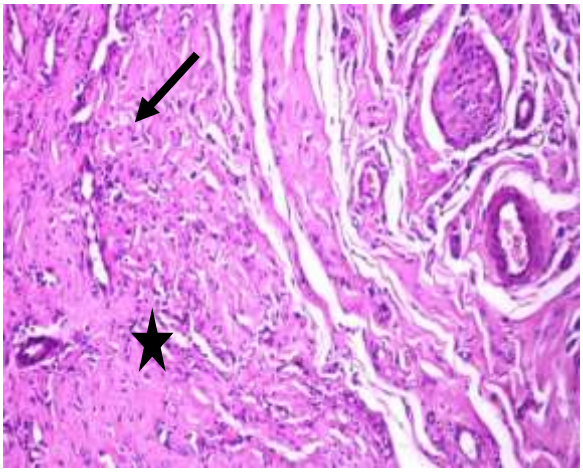
➡	diffuse mononuclear cells infiltration

Figure (6-d): Photomicrograph of skin from G2 showing higher magnification of the dermal inflammatory reaction; note the diffuse mononuclear cells infiltration (arrow head), (H&E X400).



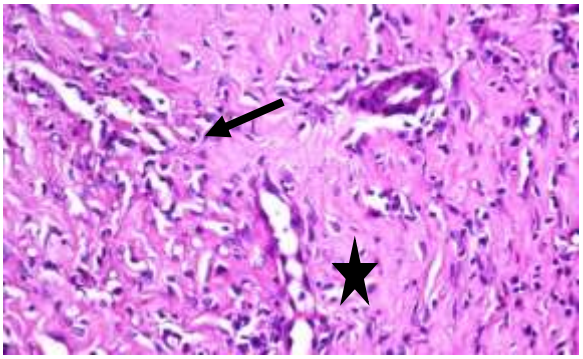
→	Leucocytic cells infiltration
*	Mature granulation tissue

Figure(6-c): Photomicrograph of skin from G2 showing complete destruction of epidermal layers (arrow) with severe dermal inflammatory reaction (d), (H&E X200).



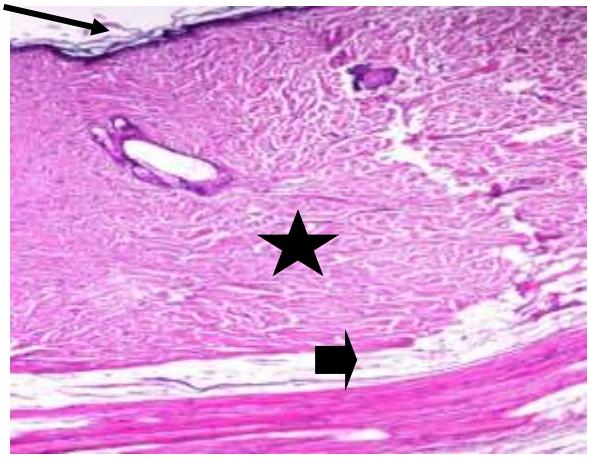
→	Leucocytic cells infiltration
*	Mature granulation tissue

Figure(6-e): Photomicrograph of skin from G3 showing good healing progress; note the formation of mature granulation tissue (*) with leucocytic cells infiltration (arrow), (H&E X200).



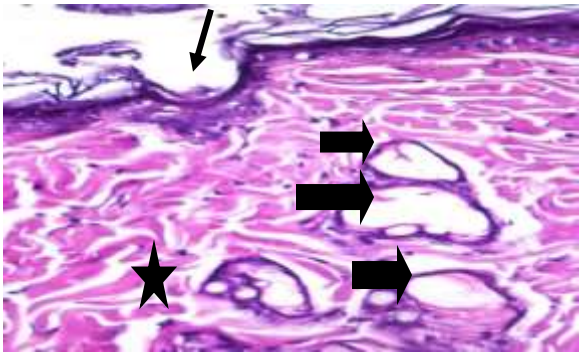
→	Leucocytic cells infiltration
*	Mature granulation tissue

Figure (6-f): Photomicrograph of skin from G3 showing higher magnification of the mature granulation tissue (*) with leucocytic cells infiltration (arrow), (H&E X400).



→	Epidermis
*	Dermis
➡	Hypodermis
m	Muscle

Figure (6-g): Photomicrograph of skin from G4 showing complete healing; note the normal skin layers; epidermis (arrow), dermis (*) with normal skin appendages, hypodermis (arrow head), and muscle (m), (H&E X200).



→	Epidermis
*	Dermis
➡	Normal skin appendages

Figure (6-h): Photomicrograph of skin from G4 showing higher magnification of the complete healing with normal skin layers; note the normal epidermis (arrow) and dermis (*) with normal skin appendages (arrow head), (H&E X400).

Fig. 6: Histopathological assessment for G1, G2, G3 and G4.

The present obtained results can be explained as the following:

Burns are a multi-organ pathophysiological condition that affects numerous organ systems mainly the skin **[**1**]**. Data from the present work, showed that chemical partial thickness burn induced complete destruction of epidermal layers and severe dermal inflammatory reaction. Dermal inflammatory reaction was characterized by diffuse mononuclear cell infiltration as shown in figure (6-c) and figure (6-d). These results are in accordance with other biochemical and hematology findings in which there were significant increases in WBC count in the peripheral blood, total neutrophil count, CRP, IL-8. CRP is an acute phase reactant that is released from the liver in response to inflammatory stimuli upon the surge of IL-6 IL-8 is a chemokine that modulate the migration of inflammatory cell to the inflamed site. Hepatic integrity findings showed significant increase of serum AST, ALT, ALP enzyme activities and significant decrease of total proteins, albumin, and A/G ratio as shown in table (1) and figures (3-a), (3-b), (3-c), (3-d), (3-e), (3-f) & (3-g). This was in line with tissue damage induced by burn and exudation of plasma at the burn site.

Data from animal skin treated non-nano zinc oxide showed moderate healing progress; the formation of mature granulation tissue with mild leucocytic cell infiltration in comparison to animal receiving paraffin. Zinc is an important trace element in the body and its role in health and disease is well recognized **[**17**].** The role of zinc in wound healing has been well demonstrated. Zinc acts as a cofactor in a number of transcription factors and enzymes such as zinc-dependent matrix metalloproteinases that promote auto-debridement and keratinocyte migration during wound repair **[**18**]**. It also confers resistance to epithelial apoptosis through cell resistance to reactive oxygen species and bacterial toxins perhaps through antioxidant action of the cysteine-rich metallothionein [19]. Zinc insufficiency can lead to pathological changes and delayed wound repair. Oral zinc supplementation has been used in treating zinc-deficient patients with leg ulcer patients [19]. In accordance with the US Food and Drug

Administration (FDA), zinc oxide is safe medication **[**9**]**. The detailed mechanism of zinc oxide-induced healing has not been explored in the present study. Its ability to promote the growth of damaged skin and hair, boosts the antibacterial properties of the coating in a dose-dependent manner, speeds up the healing process, and reduces inflammation have been previously demonstrated **[**20**]**. Zinc oxide produces reactive oxygen species (ROS) - a safe and powerful
antibacterial agent -, which harm pathogens' cell membranes. To release deadly Zn2+ ions in water that are adsorbed on microbial cells and damage their cell walls [21]. One possible mechanism of action of zinc oxide in the present study is its ability to down-regulate the inflammatory reaction at the burn site. This is evidenced by the decline in TWBs count, CRP and IL-8. Although inflammatory responses to burn induction contribute the healing process, exaggerated inflammatory response induced by burn may contribute to tissue damage and scarring [22].

During the past two decades, nanotechnology has rapidly progressed, opening up alternatives for the treatment of several disorders. Metal oxides, with at least one dimension of nanoscale structure is considered nanotherapeutics [23**]**. Zinc oxide nanoparticles are utilized as a UV filter in topical treatments, they are superior and safe preservative, they have antimicrobial action as well as they do not produce acute cutaneous toxicity, sensitization, nor irritation [24; 25; 26; 27 and 28]. Metal nanotherapeutics have a number of benefits, including the ability to: (1) augment drug level at application sites, which minimizes drug dose with lesser toxic effects; (2) overcome bacterial resistance, enhance medication diffusion across tissue barriers and (3) increase the stability of nano particles.

Animals treated with zinc oxide nanoparticles showed completely healed epidermis, dermis, normal skin appendages, hypodermis, and muscle layer. A more or less full recovery to normal skin appearance was observed in animals treated irradiated zinc oxide nano particles. Metal oxide, metal, and other nanotherapeutics have been used extensively to treat burn wounds because of the benefits listed above. ZnO, shows broad-spectrum anti-bacterial action by dissolving bacterial biofilms, eradicating bacterial DNA, or producing ROS to thwart bacterial development [29].

Data from the present study showed the irradiated zinc oxide has negligent effect on the hepatic function. Due to the positive charge of the polymer, certain cationic polymeric nanoparticles, like chitosan, exhibit bactericidal and bacteriological capabilities. They stick to bacterial surfaces and cause damage to the membrane wall, which inhibits microbial growth [30].

Data from the present study showed that, topical application of zinc oxide nanoparticle was superior to topical administration of non-nano form of zinc oxide in promoting healing of burn. Morphologically a moderate healing progress was observed on the burns of G3 after 45 days, while G4 showed full recovery process after 39 days only. According to biochemical findings, G3 and G4 showed a relative decline in serum (ALT), (AST) and (ALP) activities compared to G2; as well as a significant decline in the levels of CRP and IL-8; which indicated that topical treatments in G3 and G4 had a positive effect on the inflammation, and on the healing progress for both groups Proper delivery of zinc oxide through nanoparticulation adds to its ability to act as an anti-pholgestic. It also minimizes necrotic material via the enhancement of local defense systems and breakdown of collagen [31], Nano-particulation supports the sustained release of zinc ions with expected stimulation of epithelialization of wounds.

Compared to bigger particles, zinc oxide nanoparticles are more effective in attacking both gram-positive and gram-negative bacteria because of their high surface-to-volume ratio. Zinc nanoparticles activated by gamma-radiation, increase the creation of H_2O_2, which penetrates microbial cell membranes and kills bacteria such as *S. aureus*, *E. coli*, *C. albicans*, and *P. aeruginosa.* [24; 32; and 33]. Irradiation strengthens the antibacterial preservative properties of zinc oxide nanoparticles against pathogenic organisms in topical treatments **[**34-37**]** without altering drug kinetics. It is unlikely that zinc transport through the skin from applied formulations seems has a systemic effect. The present study adds to the few studies conducted on topical zinc oxide nanoparticle treatment and supports evidence of its role in acceleration of wound healing.

In conclusion, histological and biochemical findings from the present work demonstrate that irradiated nano-zinc oxide ointment has more advantage on partial thickness burns compared to ordinary zinc oxide ointment in term of the quality of healing and the duration needed for full recovery. Further study is needed to explore the possible mechanism by which zinc oxide nano-particles offer a better modality to treat chemical burn than non-nano zinc oxide.

4 Conclusions

In our study, histological and biochemical findings proved that irradiated nano-zinc oxide ointment has advantage in healing partial thickness burns compared to ordinary zinc oxide ointment. Irradiated nano-zinc oxide ointment can prevent bacterial contamination and sepsis, and being readily available and less expensive. Further investigations are needed to clarify the mode of action of irradiated nano-zinc oxide.

Abbreviations: KGy: Kilo-gray; XRD:X-ray powder diffraction; TEM: Transmission electron microscopy; ALT: Alanine aminotransferase; AST Aspartate

aminotransferase; ALP: Alkaline Phosphatase; WBC: White blood **cells;** CRP: A/G: Albumin /Globulin ratio; RBC: Red blood cells; CRP: C-reactive protein; IL-8: Interleukin-8; γ- irradiated nano-zinc oxide: gamma-irradiated nano-zinc oxide.

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Declarations

Competing interests. The authors declare no competing interests.

Consent for publication. All authors agreed to publish this article.

Data availability. The authors confirm that all data support their published claims and comply with field standards.

Animal Guidelines and Ethical approval. The research protocol with (Serial Number 10 A/23) for the purpose of over-seeing and monitoring experimental animals was approved by the National Centre for Radiation Research and Technology's Research Ethics Committee.

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