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Comparative Study between the Effect of Magnesium Oxide Nanoparticles and Zinc Oxide Nanoparticles on Healing of Avulsion Wounds in Dogs

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ABSTRACT

The aim of present study was to investigate the efficacy of Magnesium oxide and zinc oxide nanoparticles on healing of avulsion wounds in dogs. Nine adult animals weighing (M±SD: 15 ± 5) Kg were utilized in this study. Four circular wounds (1.5 cm) including full skin thickness were induced in both sides on the dorsum of the dogs (two on each side. The wounds were separated into 4 groups: group treated with normal saline (control group), MgO group treated locally with 150 µg of Nano magnesium oxide; ZnO group treated locally with 150 µg Nano zinc oxide; and Mixed group treated locally by combination of Nano-magnesium oxide (150 µg) and Nano-zinc oxide (150 µg). The second dose of Nanomaterials repeated locally in all treated groups after 3rd day from induced injury. Wounds diameter was clinically monitored (0-day, 7th, 14th, 17th, 18th, and 21st days) post-operation. Wound samples collected at 7,14,21 days postoperatively. Results: Clinically 21-day post injury, more wound contraction with a narrow diameter was observed in MgO, ZnO, and mixed groups when compared with control group Histopathological results revealed a good healing process in MgO, ZnO, and mixed groups which are characterized by normal structure of epidermis layer and subcutaneous layer and papillary layer. In conclusion of this study, Nano Zinc Oxide and Magnesium Oxide nanoparticles accelerate wound healing process.

1. INTRUDUCTION

There are four stages in the complicated process of wound healing: inflammatory phase, phase of re-epithelialization, contraction and remodeling of wound. These phases are continuing without any demarcation between them (Ibrahim et al., 2025). The first aim of agents for wound healing is to accelerate the healing rate with minimal scar formation (Jasim et al., 2025). Blood flow, wound size, infection (Ibrahim et al., 2025), age, sex, and illnesses including diabetes (Mathews and Binnington, 2001) all have an impact on wound healing. Numerous scientific fields, including biomedical sciences, have used and introduced nanomaterial. These materials mimic natural tissues by offering the right extracellular environment for cells to grow and
survive. Utilizing nanoparticles appropriate for fixing significant tissue defects was crucial (Zhao-Gui et al., 2011). Due to its exceptional mechanical strength, good biocompatibility, and capacity to increase bone density, magnesium oxide (MgO) of various weight fractions has been widely utilized as a bioactive material for orthopedic implantation for many years (Kumar et al., 2020). Topical zinc and magnesium promote re-epithelialization, lowers inflammation and bacterial development, and speeds up the healing of minor and acute skin wounds (Soderberg et al., 2001; Agren, 1990). Zinc oxide nanoparticles (Nano-ZnOs) are widely used and possess great potential in agriculture and biomedicine (Baena et al., 2017).

Nano-ZnO show wide application in orthopaedics, and soft tissues, were demonstrated that the ZnO nanoparticles can enhance the proliferation, adhesion, and differentiation of fibroblasts and osteoblasts by affecting factors that facilitate the recruitment of mesenchymal stem cells which then start differentiating into specialized cells to build new tissue (Augustine et al., 2014). Nanoparticles are characterized by a wide range of biological activities like anti-inflammatory, antibacterial activity, and wound healing enhancement and acceleration with

minimal scar tissue formation (Sankar et al., 2015). MgO NPs can accelerate wound healing process when applied locally on the tissues (Hickey and Webster, 2015). Because of the major complications associated with wound healing process, we investigate the effect of MgO and ZnO NPs on wound healing and evaluate the positive role of these materials on regeneration of wound defects.

2. MATERIALS AND METHODS

2.1. Ethical Approval

According to the approval (Number 118/37) through the local committee of the animal care and use at the college of Veterinary Medicine, University of Basrah of Iraq.

2.2. Experimental animals

2.2.1. Animals

Nine adults apparently healthy dogs were included in this study. They were having body weight average (M±SD: 15 ± 5) Kg. They were kept in appropriate cages, having good conditions at the animal house/ College of Veterinary Medicine University of Basra. They were given food and provided with water and kept in their particular cages for 15 days before starting their studies.

All experimental animals were employed to remove 1.5 cm (induce Avulsion wound) of whole thickness of the skin at the back (dorsal) of the animals.

2.2.2. Experimental design

The animals will be divided randomly into four groups as follows:

Control Group (Group A)

Skin defect model (induce avulsion wound 1.5Cm) without treatment.

Treated Group (Group B)

Skin defect model (induce avulsion wound 1.5Cm). They will be treated with Nano Magnesium oxide at dos (150 µg), would be treated with double dose local, the first dose applied immediately during operation and the second dose applied after 3rd days.

Treated Group (Group C)

Skin wound model (induce avulsion skin 1.5Cm) would be treated as local double dose of Nano Zinc Oxide at dose (150 µg). The first dose applied locally immediately during operation, and the second dose applied locally after 3rd days post-operation.

Treated Group (Mixed group) (Group D)

Skin wound model (induce avulsion skin 1.5Cm) would be treated as local double dose of Nano Zinc Oxide at dose (150 µg). The first dose applied locally immediately during operation, and the second dose applied locally after 3rd days post-operation.

2.2.3. Surgical preparation

The back (dorsal) was chosen in this study for all animals. After the area was clipped and shaved, it was thoroughly cleaned with distal water, scrubbed for two to three minutes with diluted liquid soap, treated with an antiseptic (70% ethanol alcohol), and then the incision site was treated with 2.5% tincture iodine. (Fig. 1).

2.2.4. Anesthetic Technique

Intramuscular injection of 2% xylazine hydrochloride at a dose of 5 mg/kg was used as the anesthetic method. After ten minutes, B.W. injects 15 mg/kg of 10% ketamine hydrochloride intramuscularly. B.W (Abduljaleel et al., 2025).

2.2.5. Surgical Operative Technique

Under general anesthesia and complete aseptic conditions, skin marks (1.5 cm diameter) were created in each side of the dorsal midline of the body in dogs (figure 1). Four full thickness of the marked skin was then cut carefully (figure 2, 3). These wounds were allocated into four groups, control group, Nano Zinc Oxide group, Nano Magnesium Oxide group and mixed group (combination of Nano Zinc Oxide and Nano Magnesium Oxide). All of the groups were represented in each dog (figure 1).

At zero, seven, fifteen, seventeen, eighteen, and twenty-one days following the injury, the wound diameter (in centimeters) was measured. Analyses of wound contraction data demonstrate the study of skin wound healing.

2.2.6. Post Operation Care

1. Daily monitoring of the animals for any abnormalities or complication
2. Daily systemic antibiotics (penicillin streptomycin) of a dosage 10000 International Unite/kg body weight for 3 days P.O.

Post-Operative Parameters for Results evaluation:

1. Wound dimensions' measure

Clinically, wound healing process was weekly evaluated by measuring wound size and contraction via using caliber and visual monitoring

2. Histopathological (microscopic) evaluation

The specimens were collected from all the animals in the study from the site of the wound after a period of 1st, 2nd, 3rd weeks post-operative (3 dogs for each subgroup in A, B, C and D groups). The skin samples have been fixed in a 10% buffered formalin for 48 hours (2 day) and then samples irrigate by distill water, then dehydrated with ethyl alcohol, washed with xylene first, later, embeded in paraffin wax. By microtome, transverse sections of 4–5 µm thickness has been prepared and then stain with hematoxylin and eosin (Liu and Lv, 2018; Mohsin et al., 2025).

2.2.7. Statistical analysis

The statistical test was done by SAS (2010) protocol for statistical comparison of different events in the research examination. The chi-square analysis for comparison of the percentage with the least significant difference; the LSD test (ANOVA) to study the significant comparison between means level of the present project, this level ($P \leq 0.05$) was considered significant.

3. RESULTS

3.1. Clinical finding

Clinical observations (3-7) day showed local inflammatory reaction, clot formation, slight to moderate swelling, and inflammatory exudate without signs of infection in the wound area in all groups (Fig. 2 and 3).

Macroscopical finding of the created wounds in all groups show same wound diameter immediately post-injury due to similarity of the lesion. While in the 3rd week post injury, wounds of MgO, ZnO, and mixed groups showed marked decrease with obvious wound contraction as compared with control group (Table 1) (Fig.1).

Fourteen-day post-operation, wounds were completely closed with minimum scar tissue formation in MgO, ZnO and mixed groups comparing with control group in which the wounds were nearly to be closed (figure 3 &4).

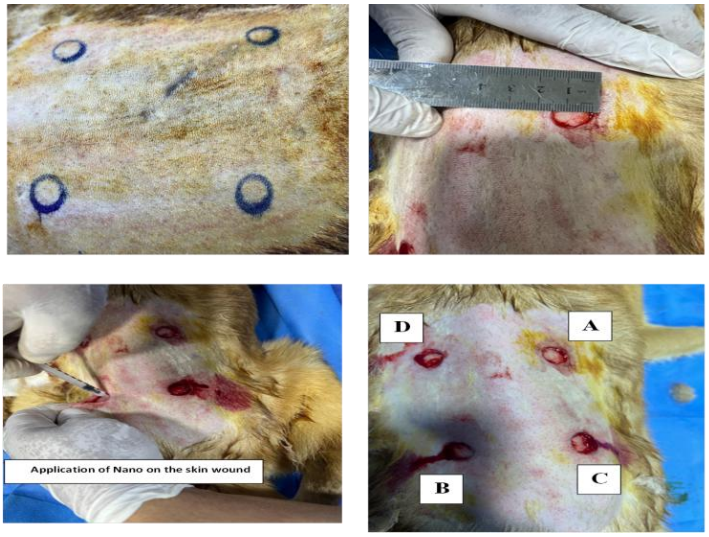

Fig. (1): Photographic appearance of wound immediately post-operation (A) Control group. (B) ZnO group. (C) MgO group. (D) Mixed group.

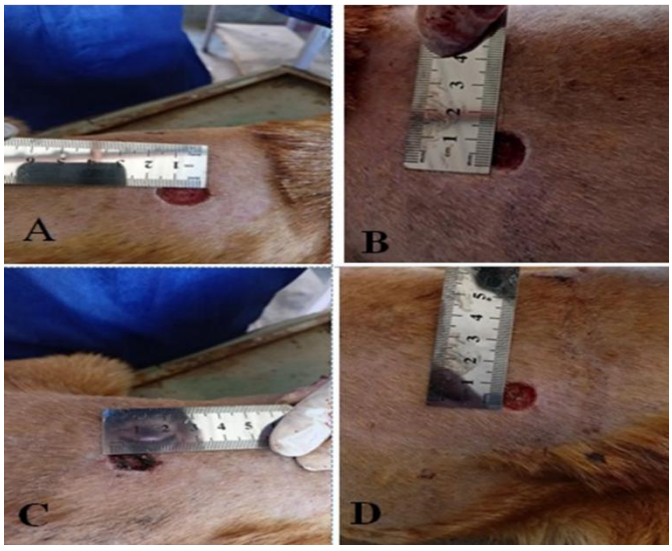

Fig. (2): Photographic appearance of wound in 7th days post-operation (A)Control group. (B)ZnO group. (C)MgO group. (D)Mixed group.

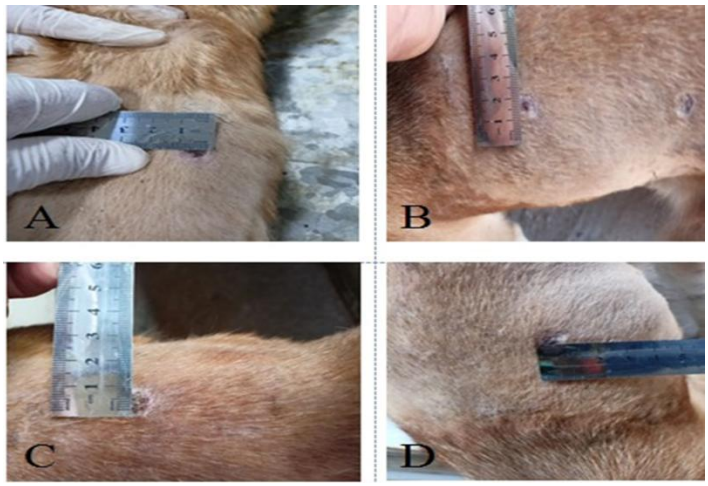

Fig. (3): Photographic pictures of wound in 14th days post-operation (A)Control group. (B)ZnO group. (C)MgO group. (D)Mixed group.

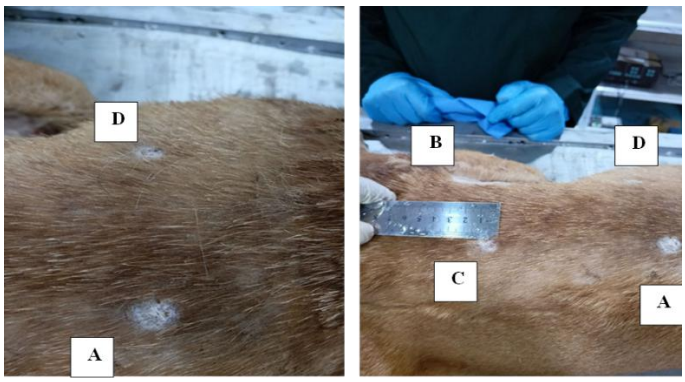

Fig. (4): Photographic pictures of wound in 21th days post-operation (A)Control group. (B)ZnO group. (C)MgO group. (D)Mixed group.

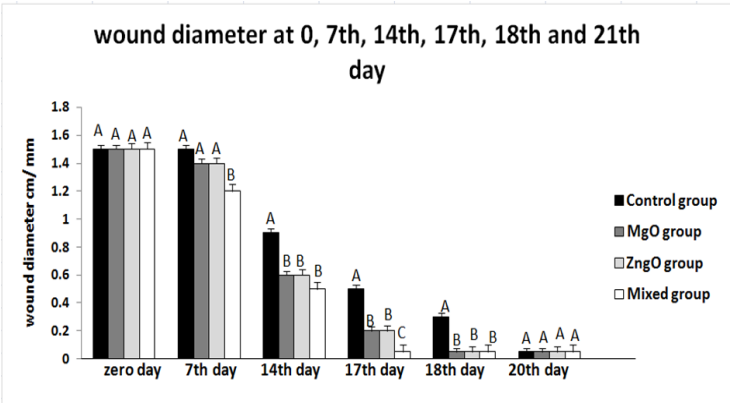

Figure 5: Wound contraction study, diameter of the wounds for control, MgO, ZnO, mixed groups post wounding (0, 7th, 14th, 17th, 18th 21 day). ABC Different letters within group indicates significant differences ($P < 0.05$)

Table 1: Wound contraction study, diameter of the wounds for control, MgO, ZnO, mixed groups post wounding at (0, 7th,

14th, 17th, 18th 21 day). post wounding, in different times post wounding (means and standard errors).

Types of group / Wound-diameter according The times post wounding / day	Control group	MgO group	ZnO group	Mixed Group
Wound diameter at 0 day	1.5±0.00^{A}	1.5±0.00^{A}	1.5±0.00^{A}	1.5±0.00^{A}
Wound diameter at 7th day	1.5±0.03^{A}	1.4±0.03^{A}	1.4±0.04^{A}	1.2±0.05^{B}
Wound diameter at 14th day	0.9±0.03^{A}	0.6±0.04^{B}	0.6±0.04^{B}	0.5±0.03^{B}
Wound diameter at 17th day	0.5±0.31^{A}	0.2±0.31^{B}	0.2±0.31^{B}	0.00±0.00^{C}
Wound diameter at 18th day	0.3±0.01^{A}	0.00±0.00^{B}	0.00±0.00^{B}	0.00±0.00^{B}
Wound diameter at 21th day	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	10.00±0.00^{A}

3.2. Histopathological examination

The results of histopathological investigation revealed in 7th day post-operation in control group, showed abnormal structure of papillary layer and structure of dermis, also alteration of papillary layer. In Zinc group and MgO group, showed showed incomplete of structure of epidermis layer, but in Mixed group, show the normal structure of dermis, abnormal structure of papillary layer, and incomplete of subcutaneous layer (Fig. 6). In 15th days post-operation, Control group showed semi healing of wounds, abnormal structure of papillary layer. Zinc group showed semi healing of wound, normal structure of epidermis layer, and papillary layer. (C)MgO group, showed normal structure of epidermis layer, and semi wound healing. (D)Mixed group, show the normal structure of dermis (a), normal structure of subcutaneous layer (Fig.7).

In 21st days post-operation in Control group, show abnormal structure of papillary layer and semi normal structure papillary layer of dermis, also alteration of papillary layer. Zinc group showed semi normal structure of epidermis layer, and semi healing of wound. MgO group, showed normal of structure of epidermis layer, and normal of papillary layer. Mixed group, show the normal structure of dermis (a), normal structure of papillary layer, and normal of layer (Fig. 8).

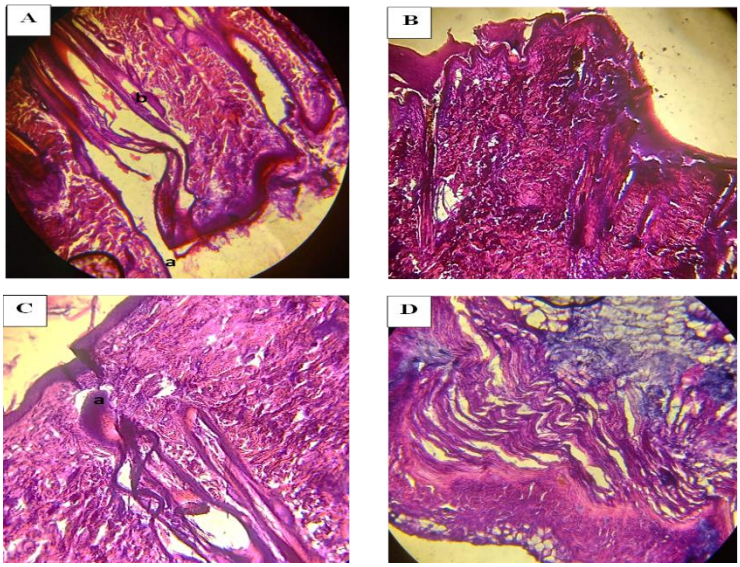

Fig. (6): Histopathological sections of Skin at period 1st week post-operation. (A) Control group, show abnormal structure of papillary layer and structure of dermis, also alteration of papillary layer. (B)Zinc group, showed semi normal structure of epidermis layer, and semi healing of wound. (C)MgO group, showed incomplete structure of epidermis layer, and semi normal of papillary layer. (D)Mixed group, show the normal structure of dermis (a), abnormal structure of papillary layer, and incomplete of subcutaneous layer.

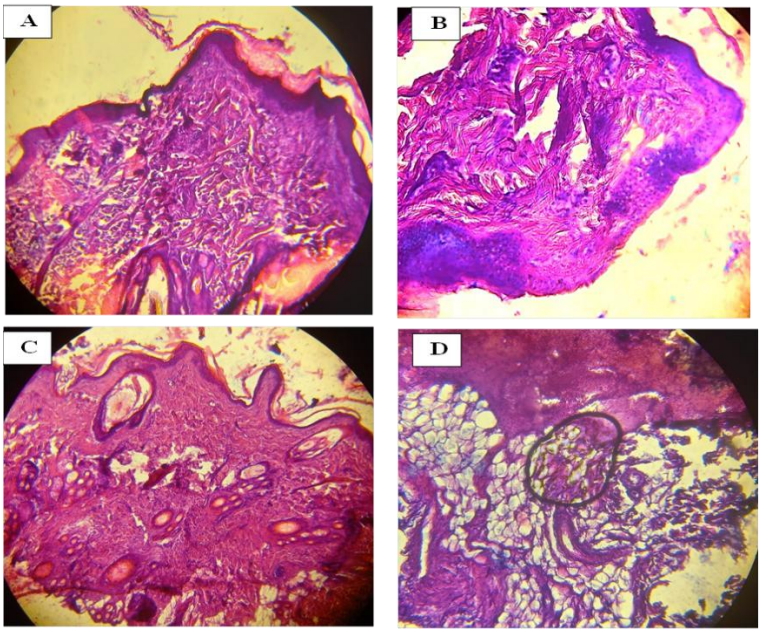

Fig. (7): Histopathological sections of Skin at period 2nd week post-operation. (A) Control group showed semi healing of wounds, abnormal structure of papillary layer (a). (B) Zinc group showed semi healing of wounds, normal structure of epidermis layer, and papillary layer. (C) MgO group, showed normal of structure of epidermis layer, and semi wound healing. (D)Mixed group, show the normal structure of dermis (a), normal structure of subcutaneous layer.

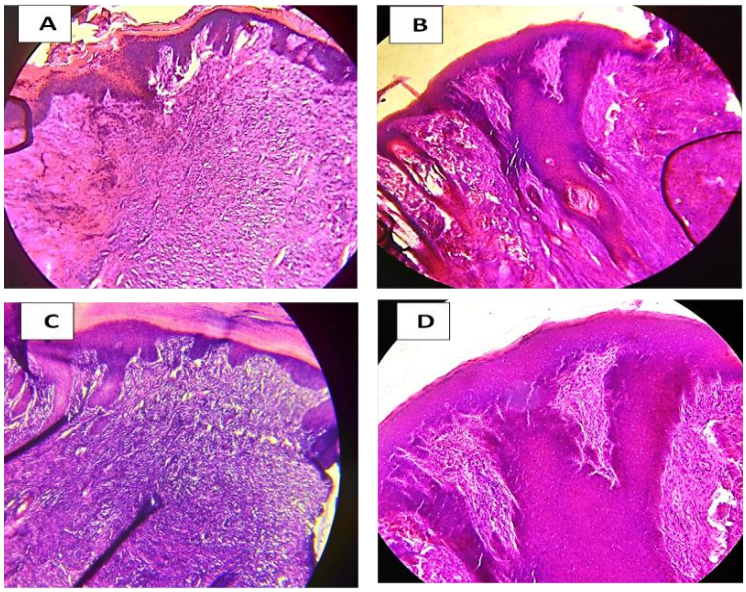

Fig. (8): Histopathological sections of Skin at period 3rd week post-operation. (A) Control group, show abnormal structure of papillary layer and semi normal structure papillary layer of dermis, also alteration of papillary layer. (B)Zinc group, showed semi normal structure of epidermis layer, and semi healing of wound. (C)MgO group, showed normal of structure of epidermis layer, and normal of papillary layer. (D)Mixed group, show the normal structure of dermis(a), normal structure of papillary layer, and normal of layer.

4. DISCUSSION

Dog was used in the current study as a model because it can be suitable for many surgical treatments like wound healing (Landén and Stahle, 2016), Achilles tendon injury repair (Asmaa et al., 2019), and esophageal anastomosis (Al-Maseeh and Eesa, 2009).Clinical observation of the present study revealed that MgO and ZnO nanoparticles display a positive effect on wound healing by increasing wound contraction and facilitate wound closure. This result agrees with Hickey and Webster, (2015) who proven that nanoparticles have ability to accelerate wound healing when it used locally on the wound. Also, Lansdown et al. (2007) and Kogan et al. (2017) have reported that zinc can improve wound healing process and this process become worse with zinc deficiency. The current results are in com with other results reported by Al-Zubaedi, (2017) who show that using of nanoparticles in a specific dose via local rout directly on the wound resulted in a good healing process. A significant wound contraction and new reepithelialization is clear when ZnO NPs applied in treatment of wound in rats (Ekta et al., 2018).

Histopathological signs of the current study showed the beneficial value of MgO NPs and ZnO NPs on wound healing process due to their role as anti-inflammatory, antibacterial, and antioxidant properties (Jiajia et al., 2020; Mingyue et al., 2021). MgO NPs have ability of stimulate the endothelial cells and pro-angiogenic activity by increase the expression of vascular endothelial growth factor (VEGF) which is primary cytokine that increase and promote new blood vessels formation and ultimately enhance wound healing (Lili et al., 2015; Ge et al., 2011). Chronic and acute wound healing can be accelerated by using ZnO NPs as a treatment model due to their epithelialization and bacteriostatic properties (Aksoy et al., 2010). Naraginti et al. (2016) reported appearance of fibroblasts, neovascularization, complete re-epithelialization and fewer inflammatory cells werer observed when ZnO NPs used for treatment of tissues. Al-Zubaedi et al. (2020) found that using of Zinc oxide Nanoparticles for treatment of wound healing in goats suffer from zinc deficiency will affect positively wound healing and regeneration of epidermal tissue.

5. Conclusion

Magnesium and zinc oxide nanoparticles gives a promising result for treatment of wound healing through their ability to accelerate wound healing and regeneration. The synergistic effect of Nano-Zinc Oxide and Nano-Magnesium Oxide on wound healing showed the best effect rather than using MgO NPs or ZnO NPs a lone.

6. Recommendations:

Future studies should evaluate the clinical applicability of Hibiscus sabdariffa nano-phytosome in patients with early-stage diabetic nephropathy to confirm the renoprotective effects observed in experimental models. Long-term investigations are required to assess its safety and sustained efficacy on renal function. Further research should also elucidate the molecular mechanisms underlying its nephroprotective activity, particularly its role in oxidative stress, inflammation, and apoptosis pathways. In addition, optimization of dosing strategies, delivery systems, and potential combination therapy with established antidiabetic agents such as metformin or SGLT-2 inhibitors is recommended. The use of renal biomarkers and advanced imaging techniques may further improve the monitoring of therapeutic outcomes and early detection of diabetic kidney injury.

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Authors' declarations

Publication consent

The authors have demonstrated their consent for the publication of the current manuscript.

Data and material availability

All data is available on request

Conflict of interests

The author stated that absence of any conflicts of interest.

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Authors' contributions

All authors contributed to the design and conception of the study. Ammar Maatoq Hashim and Montaser Mohamad Helal: prepared the materials, collected, and revised the manuscript. Jumma Qusai Saleh; wrote the first draft of the manuscript, and all authors commented on earlier versions. All authors read and approved the final manuscript.

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