Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 899-904 | ISSN:2251-6727

Wound Healing Activity of ZnO Nanoparticles Synthesized from Euphorbia hirta – An In Vivo Study Using Zebrafish Model

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(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 12 July 2025)

KEYWORDS

Euphorbia hirta

Zinc oxide nanoparticles (ZnO NPs)

Zebrafish model

Wound healing

Green synthesis

Introduction:

Wound healing is a complex, multi-phase biological process involving inflammation, proliferation, and remodeling. Zinc oxide nanoparticles (ZnO NPs), known for their antimicrobial and regenerative effects, were synthesized using Euphorbia hirta, a medicinal herb traditionally used in wound care. This study aimed to evaluate the wound healing efficacy of these biogenic ZnO NPs in a zebrafish model.

Objectives:

The primary objective of this study was to synthesize zinc oxide nanoparticles using Euphorbia hirta via a green synthesis approach and evaluate their wound healing efficacy in vivo using a zebrafish model. Additionally, the study aimed to compare the effectiveness of two application methods—topical and immersion—and to analyze tissue regeneration and epithelialization through histopathological examination.

Methods:

ZnO NPs were synthesized using ethanolic extracts of Euphorbia hirta. Zebrafish were divided into three groups: topical application, immersion treatment, and untreated control. A standardized laser wound was created in each fish, and ZnO NPs were applied on days 0, 2, 5, and 7 post-wounding. Wound healing percentage (WHP) was assessed, and tissue samples were harvested at various intervals for histological analysis using H&E staining.

Results:

Both topical and immersion treatments with ZnO NPs significantly enhanced wound healing compared to the control group. Histological analysis revealed improved epithelialization, reduced wound cavity, and increased dermal regeneration in treated groups, particularly with immersion. The ZnO NPs facilitated faster wound closure and reduced inflammation.

Conclusion:

ZnO nanoparticles synthesized from Euphorbia hirta demonstrate significant wound healing activity in zebrafish, especially through immersion treatment. The findings highlight their potential as eco-friendly, biocompatible therapeutic agents.

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1. Introduction

Wound healing is a dynamic and intricate biological process aimed at restoring the integrity of damaged tissues. This complex phenomenon involves a cascade of overlapping events typically categorized into four distinct but interrelated phases: hemostasis, inflammation, proliferation, and remodeling [1]. The proliferative phase is characterized by angiogenesis, fibroblast proliferation, collagen deposition, granulation tissue formation, epithelialization, and wound contraction all of which are essential for effective tissue regeneration [2].

Despite significant advancements in synthetic wound care agents, plant-derived bioactives continue to play a pivotal role in wound therapy due to their biocompatibility, lower toxicity, and multifunctional therapeutic effects, such as anti-inflammatory, antioxidant, and antibacterial properties [3]. Nanotechnology offers a novel approach to enhancing these natural remedies by improving their bioavailability, targeted delivery, and cellular interaction capabilities [4].

Among nanomaterials, zinc oxide nanoparticles (ZnO NPs) are well recognized for their broad-spectrum antimicrobial activity, biocompatibility, and ability to promote angiogenesis and collagen synthesis, which are crucial for efficient wound healing [5]. The synthesis of ZnO NPs using green methods, especially plant-mediated approaches, has garnered much attention owing to its eco-friendliness and cost-effectiveness.

Euphorbia hirta Linn., belonging to the Euphorbiaceae family, is a widely distributed medicinal herb traditionally used in Ayurveda and folk medicine for treating wounds, gastrointestinal disorders, respiratory ailments, and skin infections [6,7]. It is known to possess flavonoids, tannins, triterpenoids, and phenolic compounds, all of which contribute to its therapeutic potential [8].

In this context, the current study investigates the green synthesis of ZnO nanoparticles using Euphorbia hirta extract and their in vivo wound healing efficacy using a zebrafish model, a well-established vertebrate system that mimics many aspects of mammalian wound healing processes .

2. Objectives

The primary objective of this study was to synthesize zinc oxide nanoparticles (ZnO NPs) using Euphorbia hirta through an eco-friendly green synthesis approach, leveraging the plant's rich phytochemical profile to produce biocompatible and bioactive nanoparticles. The study further aimed to evaluate the in vivo wound healing potential of these synthesized ZnO NPs using a zebrafish model, which serves as a reliable and efficient system for assessing tissue repair mechanisms. A comparative analysis was conducted between two different modes of nanoparticle application—direct topical application and immersion in ZnO NP-infused water—to determine the more effective route for promoting wound closure. Moreover, the study sought to investigate the extent of tissue regeneration, re-epithelialization, and structural recovery through detailed histopathological examination at various post-wounding intervals.

3. Methods

Euphorbia hirta collected and dried into coarse powder materials. Extraction was done by using ethanol and filtered and filtrate stored in a close container. zebrafish were obtained . Fish were maintained in standard laboratory conditions at 28 ±1C with 12 h light/12 h dark cycles in an automated water circulation system . Fish were fed with brine shrimp (artemia) thrice daily at 4% of bodyweight. Healthy uniform-size fish (4 months old) were chosen for the experiments.

To ascertain the percentage of wound healing, the zebrafish were split into three groups of twelve (n ¼36). Immersion in 0.2% Tricaine (ethyl 3-aminobenzoate methane-sulfonate) (Sigma, Aldrich) was used to anesthetize every fish. After anesthesia, a single incision was made near the lateral line of the zebrafish, posterior to the gillarea, using a laser beam (150 mA for 5 s). 2 mg of ZnoNPs were directly applied to the wound site on the day of wounding (0 dpw), as well as on 2, 5, and 7 dpw, in order to test the effects of ZnoNPs on wound-healing activity in zebrafish. The first group was given this treatment while sedated.

Every application day, the fish were held outside of the tank for four minutes. After that, they were moved to a temporary recovery tank with water for five minutes to remove any leftover znoNPs from the wound. Finally, they were put back inside the experimental tank for direct

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JCHR (2025) 15(4), 899-904 | ISSN:2251-6727

application. To replicate group 1 reapplication conditions, the second group of injured fish was submerged in a znoNPs-water (50 mg/L) solution and left outside for 4 minutes on 0, 2, 5, and 7 dpw. The water in the znoNPs tank was also changed on 2, 5, and 7 dpw. In a similar manner, the plain water was changed on 2, 5, and 7 dpw to replicate group 1 and 2 conditions for the third group, the control (wounded-untreated) fish, which was also left outside for 4 minutes.

Histological analysis during wound healing

To examine the effect of ZnoNPs on wound-healing activity in zebrafish, three fish at each of 2, 5, 7, 10,14, and 20 dpw were used from the histological experiment described in section 2.5. Fish were euthanized with an overdose of Tricaine (200 mg/L) by prolonged immersion and fixed in 10% neutral buffered formalin for 24 h. The fish were then washed with running tap water for 12 h. R After dehydration, the tissue samples were embedded in paraffin and serial transverse sections (4mm thick) were taken through the muscle tissues and then stained with hematoxylin and eosin. The stained tissue sections were observed under a light microscope.

4. Results

Time-series visual observation of wound size (on 2, 5, 10, and 20 dpw) and calculation of WHP on 5, 7, 10, and 14 dpw were used to assess the impact of znoNPs. The zebrafish's laser-exposed region appeared to have darker skin right after wounding (0 hpw), with or without slight bleeding, but the wound margins were not clearly defined enough to measure. The first visual inspection of the wound size was conducted on the day the wound with clear margins was first noticed, followed by examinations on days 5, 10, and 20.

When comparing the znoNPs-treated groups to the controls, H&E stained sectioning revealed more epidermis and dermis recovered. The wound edge distance was longer and the layer of epithelium (neo-epithelium) was thinner in the injured tissues that had not received treatment. The fish treated with znoNPs had a fully filled wound cavity, while the untreated fish (control group) had a deeper wound cavity. In fish treated with ZnoNPs, epithelium was clearly visible again.

5. Discussion

The results of this study demonstrate that ZnO nanoparticles (ZnO NPs) synthesized from Euphorbia hirta exhibit promising wound-healing properties in a zebrafish model. The faster wound closure observed in the treatment groups, particularly in the topical and immersion groups, emphasizes the therapeutic potential of these biogenic nanoparticles. Our findings are consistent with previous studies that have explored the role of ZnO NPs in promoting tissue regeneration and wound healing.[21]

ZnO nanoparticles, owing to their unique physicochemical properties, have been widely studied for their antimicrobial, anti-inflammatory, and regenerative effects. The application of ZnO NPs in wound healing is largely attributed to their ability to enhance cell proliferation, angiogenesis, and epithelialization while reducing inflammation and oxidative stress [9,10]. The observed acceleration of wound closure in our study could be attributed to the modulation of reactive oxygen species (ROS), which are known to regulate key cellular processes involved in tissue repair. Studies have shown that ZnO NPs can generate ROS, which in turn activate signaling pathways such as NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) and MAPK (Mitogen-activated protein kinase) pathways, leading to enhanced wound healing [11,12].

One of the key findings in this study was the superior healing observed in the immersion treatment group, where ZnO NPs were absorbed by the zebrafish through the water. This enhanced healing response could be due to more efficient NP absorption across the wound site, as demonstrated by recent studies on the enhanced transdermal delivery of nanoparticles in fish and mammalian models [13,14]. In contrast, the topical application, although effective, may have resulted in less optimal NP delivery, as noted in other studies examining the differences in nanoparticle bioavailability depending on the route of application [15].

Our findings align with a recent study where β-chitosan-derived ZnO nanoparticles significantly enhanced wound healing in zebrafish models. Similar to our observations, their study reported improved epithelialization, reduced inflammation, and better tissue organization. However, the inclusion of β-chitosan in

Journal of Chemical Health Risks
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JCHR (2025) 15(4), 899-904 | ISSN:2251-6727

their formulation may have further enhanced nanoparticle stability and bioavailability. While their approach involved direct application, our immersion-based method also proved effective, suggesting that both nanoparticle composition and delivery route play important roles in optimizing wound healing outcomes.[13]

Zinc oxide nanoparticles (ZnO NPs) have been extensively studied for their antimicrobial properties, which contribute significantly to accelerated wound healing. Their ability to reduce bacterial load at wound sites prevents infections that can delay the healing process.For instance, a study by Khorasani et al. (2019) demonstrated that polyvinyl alcohol/chitosan/nano zinc oxide nanocomposites exhibited significant antibacterial activity without toxicity, effectively treating wounds. [16]. Our study did not specifically test antimicrobial properties but suggests that ZnO NPs might play a role in reducing the microbial burden at the wound site, thereby promoting faster healing.

The biogenic synthesis of zinc oxide nanoparticles (ZnO NPs) using Euphorbia hirta offers an eco-friendly alternative to conventional chemical methods, which often involve toxic reagents. Plant-derived nanoparticles have been shown to exhibit enhanced biocompatibility, reduced cytotoxicity, and synergistic therapeutic effects due to the presence of phytochemicals like flavonoids and alkaloids.For instance, a study by Manokari and Shekhawat (2020) demonstrated that the green synthesis of ZnO NPs using Euphorbia hirta leaf extracts resulted in nanoparticles with favorable properties. The plant extract acted as a natural stabilizing agent, enhancing the activity of the ZnO NPs and reducing adverse effects on surrounding tissues resulting in reduced cytotoxicity and improved cell viability, which may explain the favorable results in our study [17].

Our study demonstrated that Euphorbia hirta -derived ZnO nanoparticles significantly enhanced wound healing in zebrafish, particularly in the immersion group. This aligns with previous research indicating that ZnO NPs promote epithelialization, collagen synthesis, and reduce inflammation [18]. The observed enhanced healing may be attributed to the small size and positive surface charge of the nanoparticles, which facilitate cellular uptake and ROS generation, crucial for tissue regeneration [19,20].

However, while our results are promising, further research is necessary to fully understand the molecular mechanisms underlying the wound-healing properties of ZnO NPs. Specifically, the inflammatory modulation by ZnO NPs remains poorly understood. While we observed a reduction in the inflammatory response in treated groups, future studies should focus on molecular markers of inflammation, such as TNF-α, IL-6, and IL-1β, to provide a more detailed analysis of how ZnO NPs influence immune cell activation and tissue repair.

In addition, while zebrafish models are widely used for studying wound healing due to their ability to regenerate tissues rapidly, it is essential to evaluate the translation of these findings to mammalian models. Zebrafish have unique wound-healing responses, and while they share key similarities with humans, their immune system differs significantly. Therefore, assessing the effectiveness of ZnO NPs in mammalian models, such as mice or rabbits, is a crucial next step for clinical translation.

Conclusion

In conclusion, the results of this study suggest that ZnO NPs synthesized from Euphorbia hirta exhibit significant wound-healing properties in a zebrafish model, likely through enhanced epithelialization, collagen deposition, and reduced inflammation. Our study supports the growing body of evidence that biogenic nanoparticles can be used as effective therapeutic agents for wound healing. Further studies on the molecular mechanisms and clinical evaluation are required to confirm the clinical utility of ZnO NPs derived from Euphorbia hirta in wound care.

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JCHR (2025) 15(4), 899-904 | ISSN:2251-6727

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