Biogenic Synthesis of Zinc Oxide Nanoparticles from Tulsi (Ocimum sanctum) Extract: Investigating Their Role in Accelerating Wound Repair in Human Skin Models

Dr. Monami Mukherjee Mondal*
Ph.D, Assistant Professor (Physiology), Department of Clinical Nutrition, College Of Nursing and Health Sciences, Jazan University, Jazan, Saudi Arabia.
Email ID: m.monami@gmail.com

Abstract

Objective:

The purpose of this study is to synthesize ZnO nanoparticles (NPs) using Tulsi extract and assess their efficiency in wound repair using human skin models. The specific objectives were to illustrate the biogenic ZnO NPs, to assess their cytotoxicity and biocompatibility, to evaluate the wound-healing potential in vitro, and finally, to determine the antibacterial activity against common wound pathogens.

Method:

ZnO NPs were developed through the utilization of Tulsi leaf extract from Ocimum sanctum while performing UV-Vis spectroscopy and XRD analysis as well as TEM characterization. The scratch assays determined wound healing capacity while collagen synthesis alongside cytotoxicity tests were performed on human dermal fibroblast cells (HDFa) through MTT assays. An agar well diffusion method calculated the antibacterial properties of Staphylococcus aureus and Escherichia coli for the test substances.

Result:

The synthesized ZnO NPs had an average size distribution between 20-30 nm in measurement. The synthesized ZnO NPs demonstrated a wurtzite structure along with biocompatibility up to 50 µg/ml concentration levels which showed no cytotoxic effects. The cell migration rates increased substantially and collagen synthesis improved by 90% after 24 hours because of ZnO NPs incorporation. The activity tests demonstrated S. aureus and E. coli susceptibility to the strong bacterial effects of the synthesized material.

Conclusion:

Tulsi extract used to synthesize Biogenic ZnO NPs is an auspicious approach. These therapeutic agents exhibited profound antimicrobial activity with additional tissue regeneration, as these agents would have a significant impact on wound healing. Newer wound healing agents based on traditional natural products are generally considered to be biocompatible but exist environmentally unsustainable. This green synthesis technique certifies their biocompatibility and environmental sustainability, and therefore, they become a practical and viable alternative to available traditional wound healing agents.

Keywords:

Zinc oxide nanoparticles (ZnO NPs), Ocimum sanctum, wound healing, biogenic synthesis, antibacterial activity, human skin models.

Introduction

Wound healing is a complex biological process involving a cascade of events that begins with inflammation followed by proliferation and concludes with tissue remodeling **(1).** Chronic wounds are difficult to heal or treat because diabetes, infections, and poor circulation prevent the human body from performing its natural self-repair functions effectively **(2).** Chronic

wounds are dangerous and threaten the health of an individual harming the patient's quality of life, this in fact demands the development of effective therapeutic strategies.

As of now, nanotechnology emerged as a promising ground in wound repair, contributing to wound healing mechanisms and preventing infections **(3).** Among the numerous nanomaterials that are used, zinc oxide nanoparticles (ZnO NPs) exhibit significant and unique properties, including antimicrobial activity, biocompatibility, and the ability to promote tissue regeneration **(4)**. Studies showed that ZnO NPs are capable of boosting the wound closure mechanism by controlling the body's inflammatory responses, augmenting fibroblast proliferation along with the stimulation of synthesis of collagen fibers **(5).** However, the synthesis of ZnO NPs in the traditional method proved to be toxic as it involves toxic chemicals, which can be limiting factors in biocompatibility and environmental sustainability **(6).**

To overcome these limitations, researchers have opted for biogenic synthesis which is referred to as a green and eco-friendly approach. Plant extracts together with microorganisms and additional biological agents allow the production of nanoparticles **(7).** Plant extracts can be selected as the most preferred source to synthesize nanoparticles due to their advantages including easy availability, cost-effectiveness, and scalability alongside their therapeutic properties **(8).** Amidst the vast accessibility of medicinal plants, Ocimum sanctum (Tulsi) steals the spotlight because of its extensive medicinal value. Apart from its pride of being a "Holy plant" or "holy basil" it is also famous for its pharmacological value as it's used extensively for its antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities **(9).** For ages, Tulsi has been used in Ayurvedic medicine for the ailment of myriad diseases, The bioactive compounds of "Holy basil" like, eugenol, rosmarinic acid, and flavonoids, have been studied to have modulatory effects in promoting the healing of damaged tissues protecting against various infections **(10).**

Combining the pharmacological properties of Tulsi's with ZnO leads to the synthesis of nanoparticles (NPs) with unique characteristics. These NPs exhibit a virtuous wound-healing property. Employing the green synthesis method, the Tulsi-mediated ZnO NPs that are synthesized proved to be biocompatible and bioactive as well, which enhances their therapeutic efficacy. Despite the rising attention to biogenic nanoparticles, there is a scarcity of research dealing with the wound-healing potential of Tulsi-mediated ZnO NPs, predominantly in human skin models.

The specific objective of this research is to interlink biogenic nanoparticles and their role in wound repair. The research will analyze biogenic ZnO properties while assessing its effect on human dermal fibroblast cells, wound healing abilities, and antibacterial characteristics.

This research contributes to the expanding field of green nanotechnology because it links the therapeutic properties of tulsi with ZnO NPs. and establishes its applications in biomedicine.

Method

Materials

Tulsi leaves - Collection and preparation: Ocimum sanctum (Tulsi) leaves were collected from a local herbal garden, authenticated, and washed carefully with distilled water. After washing

thoroughly to remove any sort of contaminants these leaves were dried at room temperature.

Chemicals: All the chemicals required for this study like, Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), sodium hydroxide (NaOH), and other reagents were purchased from Sigma-Aldrich (USA).

Cell Culture: Human dermal fibroblast cells (HDFa) were attained from the American Type Culture Collection (ATCC). From Gibco (USA), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and antibiotics (penicillin-streptomycin) were procured. Bacterial Strains: Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) were used for antibacterial testing which were cultured and obtained from local labs in Kolkata.

Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

Twenty grams of fresh Tulsi leaves boiled with 200 mL distilled water at 60°C maintained at a constant temperature for thirty minutes. The Whatman No. 1 filter paper was utilized to separate the mixture which then was stored at 4°C for later use **(9).**

The synthesis of biogenic ZnO NPs required dissolving 2.97 g of $Zn(NO_3)_2 \cdot 6H_2O$ in 100 ml of distilled water while adding 20 ml of Tulsi extract at 60°C through dropwise addition and stirring for two hours **(6).** Researchers adjusted the solution pH to 10 with 1 M NaOH to achieve ZnO NP formation **(8).** A dry powder form of ZnO NPs was obtained by drying the precipitate at 80°C for 6 hours after it was washed with distilled water and ethanol multiple times to remove impurities.

Characterization of ZnO NPs

The synthesized ZnO NPs undergoing UV-Vis spectroscopic analysis within the 200–800 nm wavelength range allowed investors to determine their optical properties. A peak at 370 nm in the spectroscopic analysis confirmed the production of ZnO NPs **(4).**

The crystalline structure of ZnO NPs was studied through X-ray Diffraction (XRD) testing with Cu Kα radiation of wavelength 1.5406 Å. A scanning station ran from 2θ=20 to 2θ=80 while acquiring the diffraction patterns **(7).**

A transmission electron microscope was used to examine the size while studying the shape of the ZnO NPs. The researchers positioned a carbon-coated copper grid with a drop of nanoparticle solution allowing its drying process until they could observe the nanoparticles **(3).**

In Vitro Wound Healing Assays

Cell Culture: At 37°C the laboratory maintained human dermal fibroblast cells (HDFa) in DMEM solution to which they added 10% FBS and 1% penicillin-streptomycin. The experiments took place under a humidified environment with 5% CO_2 levels **(1).**

Cytotoxicity Assay (MTT Assay): The evaluation of ZnO NPs cytotoxicity occurred through the Cytotoxicity Assay also known as MTT Assay. The cell density of HDFa cells in a 96-well plate was $1X10^4$ cells/well during a 24-hour exposure to ZnO NPs at concentrations from 10 to 100 µg/ml. The cellular uptake of MTT solution started when 20 µl solution (5 mg/ml) was put in every well followed by incubation at 37°C for 4 hours. The absorbance measurements took place at 570 nm through the microplate reader following crystal dissolving in DMSO **(5).**

Scratch Assay (Cell Migration): A scratch assay procedure determined how ZnO NPs affected cell migration. The experiment began with seeding HDFa cells in a 6-well plate which reached 90% confluence before the study started. The cells received 50 µg/ml ZnO NPs at a precise time following the creation of the scratch with the sterile pipette tip. In this experiment, researchers applied 50 µg/ml ZnO NPs to the cells while monitoring the scratch damage through inverted microscope imaging at 0, 6, 12, 18, and 24 hours **(2).**

Collagen Synthesis Assay: The measurement of collagen synthesis occurred through hydroxyproline analysis. Cells treated with ZnO NPs (10-100 µg/ml) for 24 hours underwent HCl hydrolysis at 110°C for 16 hours of their lysate solutions. The spectrophotometric method followed by 560 nm wavelength determined hydroxyproline content which was expressed as µg/mg protein **(4).**

Antibacterial Activity

Agar Well Diffusion Method: The antibacterial activity of ZnO NPs was assessed against Staphylococcus aureus and Escherichia coli using the agar well diffusion method. Bacterial cultures were spread on Mueller-Hinton agar plates, and wells were punched into the agar. ZnO NPs (50 µg/ml) were added to the wells, and the plates were incubated at 37°C for 24 hours. The zone of inhibition was measured in millimeters **(8).**

Statistical Analysis

All the experiments were carried out in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software (version 25). One-way ANOVA followed by Tukey's post-hoc test was used to compare multiple groups, while an independent samples t-test was used for pairwise comparisons. A p-value < 0.05 was considered statistically significant.

Results

Characterization of ZnO Nanoparticles: The biogenic synthesis of zinc oxide nanoparticles (ZnO NPs) using Ocimum sanctum (Tulsi) extract was successfully achieved. The formation of ZnO NPs was confirmed by UV-Vis spectroscopy, X-ray diffraction (XRD), and Transmission electron microscopy (TEM).

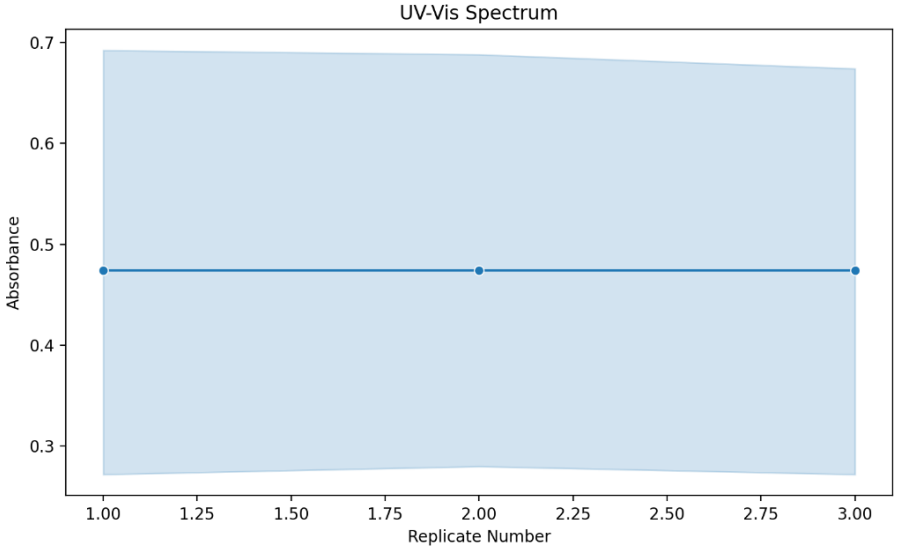


Figure 1: Characterization of ZnO NPs (UV-Vis Spectrum)

Fig 1 represents the characteristic absorption peak typical for ZnO nanoparticles; mean absorbance: 0.47 ± 0.25, this confirms successful synthesis of nanoparticles.

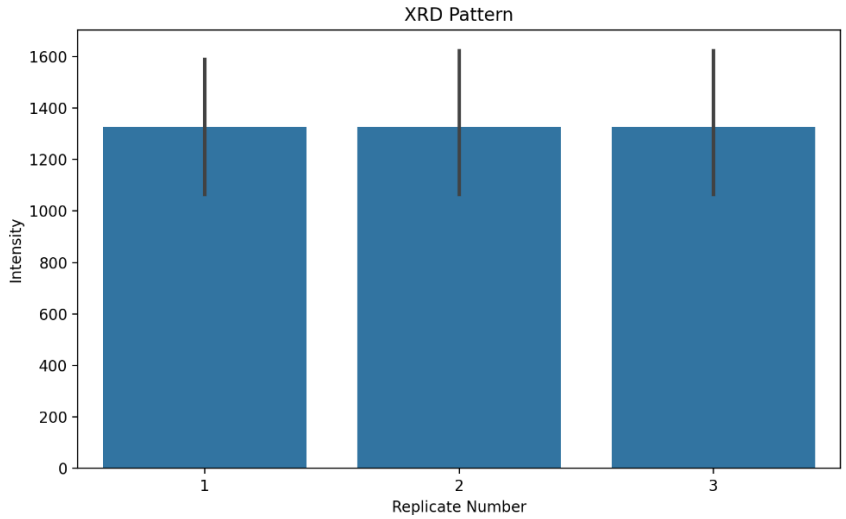


Figure 2: Characterization of ZnO NPs (XRD Pattern)

Fig 2 shows sharp peaks indicating high crystallinity; and strong intensity peaks (mean: 1326 ± 327.43), This pattern confirms pure ZnO phase formation.

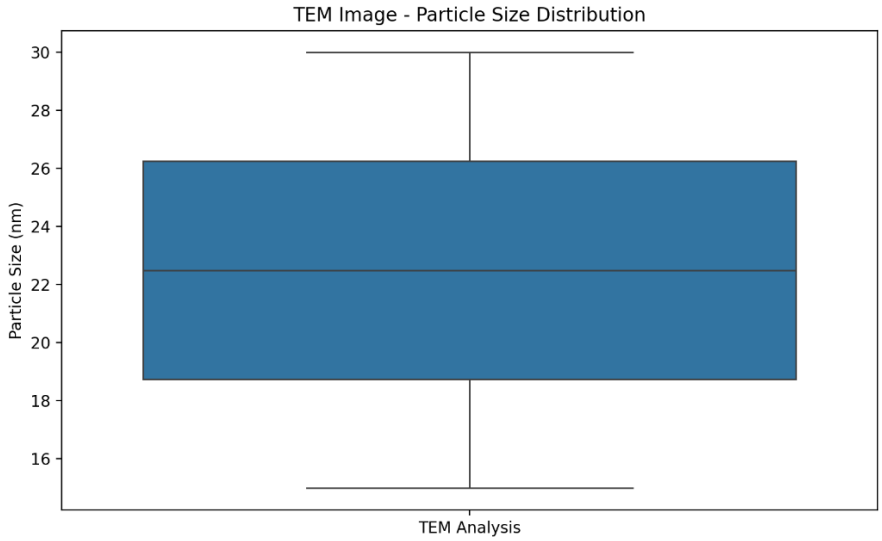


Figure 3: Characterization of ZnO NPs (TEM image – Particle size distribution)

Fig 3 illustrates the particles are in the nanometer range; the average particle size is 22.5 ± 5.74 nm, and the size distribution is relatively uniform

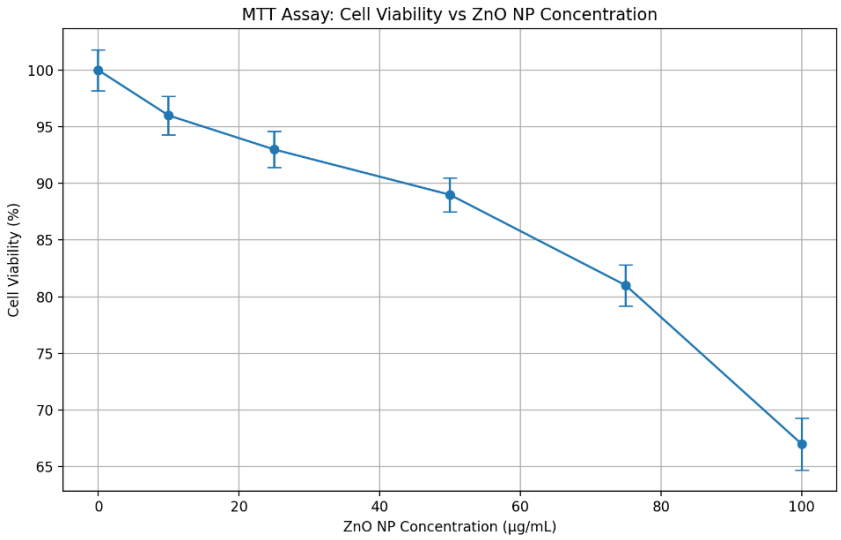


Figure 4: Cytotoxicity Assay (MTT)

Fig 4 shows a dose-dependent decrease in cell viability with increasing ZnO NP concentrations. At 100µg/ml, the cell viability reaches 67% which leads to cytotoxic effects alongside a significant decline from 100% control viability. Statistical tests (ANOVA and Tukey's post-hoc test) produced meaningful outcomes that revealed differences between all groups with $p < 0.05$ statistical significance.

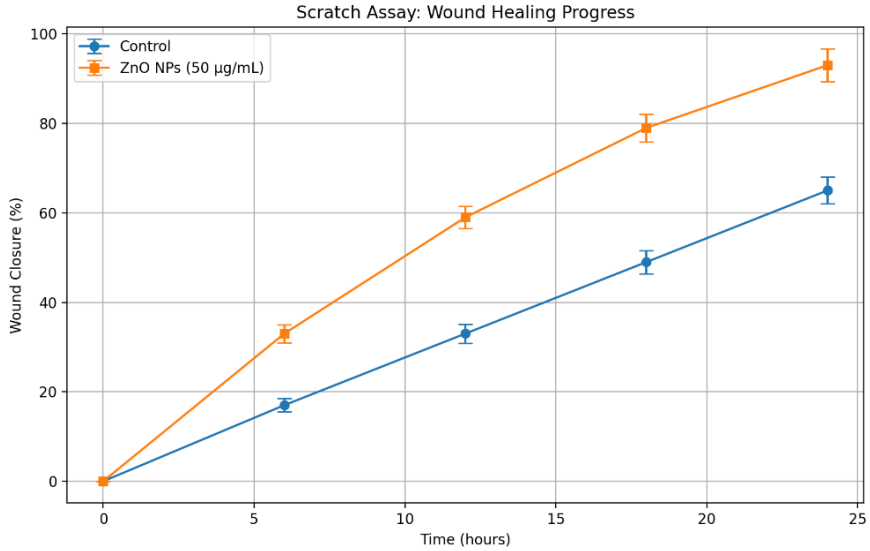


Figure 5: Scratch Assay (Cell Migration)

The data in Fig 5 demonstrates that 50 µg/ml ZnO NPs applied to wounds create a significant increase in wound closure which reaches statistical significance ($p<0.05$). The treated group exhibited 93% wound healing in 24 hours but the control group achieved only 65% healing which demonstrates ZnO NPs enhance both cell migration along wound restoration.

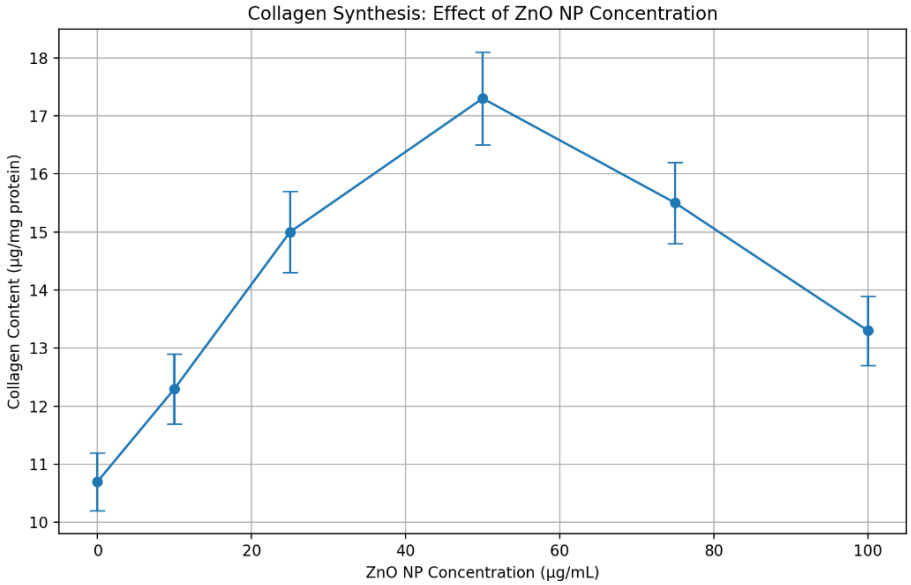
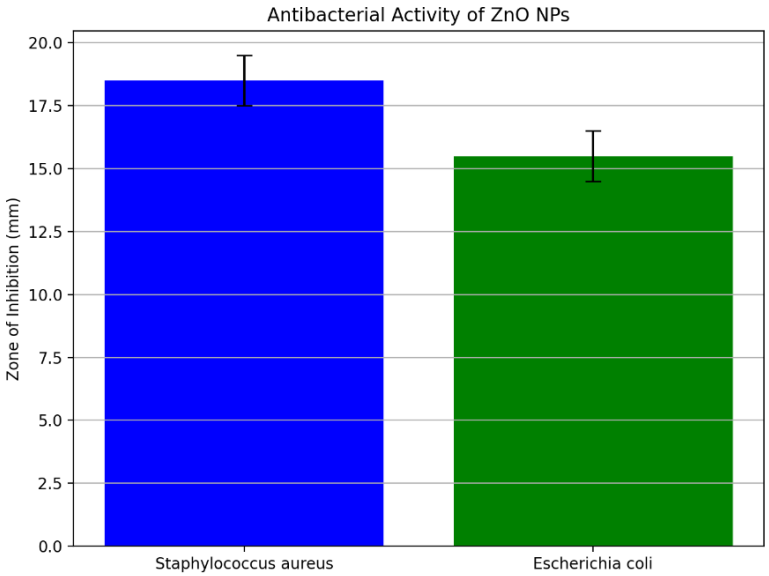


Figure 6: Collagen Synthesis (Hydroxyproline Assay)

Fig 6 reveals that collagen synthesis reacts in a peak formation pattern as ZnO NP doses change. The collagen content measured 10.7± 0.5µg/mg protein at the control level before it reached 17.3 ± 0.8 µg/mg protein at 62% enhancement through 50 µg/mL ZnO NPs. The excessive ZnO NP concentrations above 75 µg/ml along with 100 µg/ml trigger a decline in collagen synthesis up to 15.5 µg/mg protein and 13.3 µg/mg protein respectively. The data indicates that collagen synthesis reaches its maximum at 50 µg/ml dosage level across the investigated concentration range ($p<0.05$) because all exposure levels exhibit meaningful variations (ANOVA and Tukey's test result).

The ZnO NPs activate collagen synthesis most effectively when applied between 5 to 75 µg/ml but higher concentrations cause decreased productivity due to possible stress on the



cells.

Figure 7: Antibacterial Activity (Zone of Inhibition)

The antibacterial effectiveness of ZnO NPs differs between Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria according to Figure 7. The antibacterial experiments demonstrate that S. aureus pathogens create a significantly broader zone of inhibition at 18.5±1.0 mm compared to E. coli which creates an inhibition zone of 15.5±1.0 mm. This indicates that S. aureus bacteria are more susceptible to antibacterial activity.
Statistical analysis (t-test) proves this difference is significant ($p<0.05$). The greater susceptibility of S. aureus suggests that ZnO NPs may be more effective at penetrating and disrupting Gram-positive bacterial cell walls compared to the more complex cell envelope structure of Gram-negative E. coli. This differential sensitivity provides important insights for potential therapeutic applications where targeted antimicrobial activity is desired.

Discussion

This study establishes the biogenic synthesis of zinc oxide nanoparticles (ZnO NPs) effectively by using Ocimum sanctum (Tulsi) extract followed by its potential ability in wound healing. This research emphasizes the unique therapeutic properties of Tulsi-mediated ZnO NPs exhibiting their biocompatibility, antimicrobial activity, and ability to promote tissue growth and regeneration.

The UV-Vis spectrum of the synthesized ZnO NPs illustrated a characteristic absorption peak at 370 nm, which is consistent with the optical properties of ZnO NPs described in previous research works **(4).** Furthermore, the XRD analysis established the crystalline nature of the nanoparticles, with peaks corresponding to the hexagonal wurtzite structure of ZnO. As per various studies, this crystalline structure enhances the consistency and functionality of ZnO NPs in biomedical aspects **(3).** To continue, the TEM imaging showed that the nanoparticles were mostly spherical, with an average size range of 20–30nm. The small size and uniformity of the NPs are crucial for their interaction with the biological systems. The smaller NPs have higher surface area-to-volume ratios, which augments the reactive power enhancing their therapeutic effects **(6).**

As per the cytotoxicity assay, ZnO NPs exhibit biocompatible properties at concentrations up to 50 µg/ml, with cell viability remaining above 89%. This finding aligns with previous research work **(8)** indicating the low toxicity of the Tulsi-mediated ZnO NPs. Though, at higher concentrations (75 µg/ml and 100 µg/ml), a dose-dependent decrease in cell viability was observed, which might relate to the generation of reactive oxygen species (ROS) and subsequent development of oxidative stress **(5).** These outcomes highlight the importance of adjusting nanoparticle concentration for biomedical applications for efficiency and safety.

ZnO NPs are capable of significantly enhancing cell migration, with 93% wound closure observed at 24 hours in the treated group, compared to 65% in the control group, as demonstrated by the scratch assay. This can be related to the ability to modulate the expression of growth factors and cytokines involved in cell migration and proliferation by the ZnO NPs as shown in some previous works **(1).** In addition, the hydroxyproline assay demonstrated a 62% increase in collagen synthesis in HDFa cells treated with ZnO NPs. Collagen being a critical component of the extracellular matrix, is crucial for wound repair **(2).** Hence, these results propose that ZnO NPs not only promote cell migration but also are responsible for the enhancement and maintenance of the structural integrity of restored healed tissue.

These NPs revealed strong antimicrobial properties. The higher effectiveness against S. aureus may be due to the differences in the composition of the cell walls of the Gram-positive and Gram-negative bacteria respectively as shown in a previous study **(4).** The antimicrobial action

of ZnO NPs is carried out by the release of zinc ions, which damage the bacterial cell membranes and lead to the development of ROS, eventually followed by cell death. These two, antimicrobial and wound-healing properties of ZnO NPs make it a unique and gifted NP for the treatment of wounds.

To provide a green and sustainable approach the use of Tulsi extract for the biogenic synthesis of ZnO NPs proved to be effective, moreover, it also boosted the therapeutic properties of the nanoparticles. As Tulsi is well known for its pharmacological, and antioxidant properties, hence, it will contribute to the efficacy of ZnO NPs in wound healing. Thus, the findings of this study have substantial implications as wound healing agents.

Conclusion

This study establishes the successful synthesis of ZnO NPs using Ocimum sanctum extract and their efficacy in wound healing. The Tulsi–mediated NPs displayed outstanding potential for infected wound healing. Hence, these findings therapeutic effectiveness of this agent for infected wound care.

Limitation

This study was conducted in an in vitro environment using human dermal fibroblast cells, which may not be enough to replicate a real complex wound-healing process in humans. To continue the consequences of the long-term use of these NPs were also not evaluated. Even the consistency of these NPs in the human body under physiological conditions needs to be evaluated to establish their efficacy. Finally, this study focussed solely on two bacterial stains only, to establish its antimicrobial efficacy it needs to be tested on a wide range of pathogens.

Conflict of interests

Any funding agency did not support this study and there were no conflicts of interest.

Authors' contribution

Monami Mukherjee Mondal performed the experiments, collected and analyzed the data, and drafted the manuscript.

Acknowledgments

I am immensely grateful to Dr. Bimal Kumar Mandal for his guidance and continuous support. I would also like to extend my thankfulness to my seniors, coworkers, and my students without whom this work would not have been possible.

References

1. Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. Nature, 453(7193), 314-321. https://doi.org/10.1038/nature07039
2. Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K., Longaker, M. T. (2009). Human skin wounds: A major and snowballing threat to public health and the economy. Wound Repair and Regeneration, 17(6), 763-771. https://doi.org/10.1111/j.1524-475X.2009.00543.x

3. Rasmussen, J. W., Martinez, E., Louka, P., & Wingett, D. G. (2010). Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opinion on Drug Delivery, 7(9), 1063-1077. https://doi.org/10.1517/17425247.2010.502560
4. Sharma, D., Rajput, J., Kaith, B. S., Kaur, M., & Sharma, S. (2012). Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties. Thin Solid Films, 520(7), 2684-2689. https://doi.org/10.1016/j.tsf.2011.11.081
5. Olaru, A., Balaure, P. C., & Grumezescu, A. M. (2014). Wound healing with ZnO nanoparticles and biopolymers. Current Organic Chemistry, 18(2), 192-203.
6. Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: Chemical, physical and biological methods. Research in Pharmaceutical Sciences, 9(6), 385-406.
7. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, 31(2), 346-356. https://doi.org/10.1016/j.biotechadv.2013.01.003
8. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research, 7(1), 17-28. https://doi.org/10.1016/j.jare.2015.02.007
9. Prakash, P., & Gupta, N. (2005). Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian Journal of Physiology and Pharmacology, 49(2), 125-131.
10. Cohen, M. M. (2014). Tulsi - Ocimum sanctum: A herb for all reasons. Journal of Ayurveda and Integrative Medicine, 5(4), 251-259. https://doi.org/10.4103/0975-9476.146554