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RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL, ANTIOXIDANT, AND CYTOTOXIC PROPERTIES OF GREEN SYNTHESISED ZINC OXIDE NANOPARTICLES FROM SANTALUM ALBUM: AN IN VITRO STUDY

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ABSTRACT

Background: Periodontal disease is a prevalent condition that requires effective treatments to manage inflammation and bacterial infections. Traditional therapies face challenges such as antibiotic resistance and limited efficacy. Green synthesised nanoparticles (NPs), particularly Zinc oxide nanoparticles (ZnO NPs), offer a promising alternative due to their unique biological activities. This study aims to green-synthesize ZnO NPs using *Santalum album (S. album)* leaf extract and evaluate their antibacterial, antioxidant, and cytotoxic properties, for application in the management of severe forms of periodontal disease

Materials and Methods: ZnO NPs were synthesized by mixing *S. album* leaf extract with Zinc acetate solution, followed by heating and centrifugation. The antibacterial activity was assessed using the Kirby-Bauer disk diffusion method against *Staphylococcus aureus (S. aureus)*, *Streptococcus mutans (S. mutans)*, *Escherichia coli (E. coli)*, and *Klebsiella pneumoniae (K. pneumoniae)*. Antioxidant activity was evaluated using DPPH and H_2O_2 scavenging assays. Cytotoxicity was determined through the zebrafish embryo model.

Results: The sZnO NPs demonstrated significant antimicrobial activity against *S. aureus, S. mutans, E. coli*, and *K. pneumoniae* with the highest activity against *S. mutans*. Their antioxidant activity was comparable to Vitamin C and Vitamin E, suggesting potential for further exploration. Zebrafish embryos treated with sZnO NPs showed proper development and comparable mortality rates with the control, indicating biocompatibility.

Conclusion: The green synthesised sZnO NPs have shown good antibacterial and antioxidant properties with low cytotoxic activity, allowing their further exploration for clinical application in the form of local targeted drug delivery agents for the treatment of severe forms of periodontal disease.

Keywords: Periodontal disease; nanoparticles; zinc oxide; green synthesis; antibacterial; antioxidant; cytotoxicity

1.INTRODUCTION

Periodontal disease has remained one of the major oral diseases affecting millions of individuals worldwide. According to the assessment of the status of the National Oral Health Policy in India in 2015, it is one of the two common oral diseases, with 89.2% of individuals affected by the disease in the age group of 35-44 years.[1] It is a chronic inflammatory disease affecting the supporting tissues of the teeth, that initiates in response to microbial plaque. The resultant host-microbial interaction leads to tissue destruction and the production of free radicles and reactive species, which further perpetuates the inflammatory process. Though various non-surgical treatment modalities have been explored, additional use of chemotherapeutics has been warranted in a few cases of hyper-responsive phenotypes. Oral and topical antimicrobials, local drug delivery, mouthwashes, and lozenges have been commonly used in the control of periopathogenic bacteria. With the emergence of super-resistant bacterial strains due to the imprudent use of systemic antibiotics and their associated side effects, alternative antibacterial agents are the need of the hour.[2] Moreover, the adjunctive use of antioxidants has also been shown to play a beneficial role in reinstating the tissues back to a state of health.

With the advances in biotechnology, nanodentistry has emerged as a promising solution for multiple facets of dental diagnosis and treatments. Nanomaterials, nanosensors, nanoimaging, and nanorobotics are aspects of nanotechnology that have been explored for application in the treatment of various periodontal diseases and conditions.[3-6] In particular, nanomaterials have been developed for application as local drug delivery agents, desensitizing solutions, mouthwashes, gum paints, etc[7]. Nanomaterials are particles ranging in size from 1-100 nm that are manipulated at the nanoscale for functionalization.[8] They have been further classified into organic and inorganic nanoparticles (NPs). These inorganic NPs express different biologic properties like antibacterial, antioxidant, anti-inflammatory, anti-cancer, and anti-viral.[9]

Zinc oxide nanoparticles (ZnO NPs) are tiny semiconducting particles known for their distinctive electrical and optical characteristics due to a substantial bandgap of 3.4 electron volts (eV).[10-12] Their unique properties trigger photochemical reactions by causing electrons to transition from the valence band to the conduction band, setting off a sequence of photochemical processes that generate reactive oxygen species. It is these reactive compounds that are accountable for the antibacterial effects of ZnO NPs.[10,13] They have also been found to be extremely biocompatible. Their increased surface area to volume ratio and improved tunability of the size, morphology, and surface properties are also advantageous for local drug delivery applications.

Green synthesis of NPs has received tremendous attention in recent years as an environmentally sustainable substitute for traditional chemical approaches that often use hazardous chemicals, produce toxic byproducts, increase environmental temperatures, and need costly complex equipment. The green synthesis of NPs involves the use of natural sources such as plants, bacteria, and fungi extracts which are great sources of biomolecules that can act as reducing and stabilizing agents in the synthesis of NPs. NPs synthesised using this method have shown many advantages like effectiveness, safety, scalability, and sustainability.[14]

Various herbal extracts have been explored for the synthesis of NPs.[15,16] *Santalum album (S. album)*, commonly known as Indian sandalwood, is a tree native to India, Indonesia, and several other countries in Southeast Asia. It is well known for its fragrant wood which is used in perfumes, incense, and traditional medicines. The essential oil extracted from the wood of *S. album* has been used for various medicinal purposes such as treating skin disorders, respiratory problems, and digestive issues. Studies have also reported potential antibacterial, anti-inflammatory, and anti-oxidant properties from santalum album extracts.[17] The aim of the present study was to green synthesise ZnO NPs using *S. album* extract (sZnO NPs) and to assess its antibacterial, antioxidant, and cytotoxicity properties.

MATERIALS AND METHODS

The study was conducted at the Department of Biomaterials, Saveetha Dental College, Chennai. Approval for conduction of the study was obtained from the Institutional Scientific Review Board.

ZnO NPs synthesis using *S. album (sZnO NPs)*: *S. album leaves* were freshly procured from Andhra Pradesh, India, from the latitude of 11,99674 and longitude of 92,9909. The leaves were carefully rinsed with running tap water to eliminate any dirt and dust particles adhering to them. Subsequently, they were allowed to air dry in the shade until completely moisture-free. They were then finely pulverized using an electric grinder. ZnO NPs were synthesised using the wet chemical process. The *S. album* leaf extract was prepared. Ten grams of the dried leaf powder was mixed with 100 ml of distilled water in a 500ml conical flask. The mixture was agitated at a constant speed of 130 rpm and 37 °C for 1 day to obtain a homogenous mixture. The leaf extract was acquired from the mixture by filtering it with the Whatman No 1 filter paper. Subsequently, 100 ml of the leaf extract was then added to 100 ml of 1M Zinc acetate solution while maintaining the Ph at 8. The flask with the mixture was

heated at 80 C for 3 hours, which lead to the precipitation of white material. The precipitate was washed with ethanol and subjected to centrifugation at 6000 rpm. The resultant pellet was collected and heated at 200 °C for 3 hours to obtain the pure sZnO NPs sample for further testing.

Antibacterial assay: The antibiotic susceptibility was carried out towards four common human oral bacteria namely *Staphylococcus aureus (S. aureus)* MTCC-740, *Streptococcus mutans (S. mutans)* MTCC-890, *Escherichia coli (E. coli)* MTCC-443, and *Klebsiella pneumoniae (K. pneumoniae)* MTCC-109. The nutrient broth was prepared and subjected to the sterilization process. The bacteria were individually inoculated and incubated at 37 °C for 8 hours to obtain bacterial colonies. The Kirby-Bauer disk diffusion test was performed. Each bacterial sample was evenly inoculated on the surface of Muller-Hinton agar plates. Four plates were prepared, one plate for each bacteria. In each plate 5mm discs were added in the plates. Disc A was loaded with antibiotic control (10µg/ml ceftazidime + 10 µg/ml erythromycin), disc B was loaded with a low concentration of sample (10 µg/ml) and Disc C was loaded with a high concentration (20 µg/ml). All the plates were incubated at 37 °C for 24 hours and the zone of inhibition was recorded and analyzed.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay: The antioxidant activity of sZnO NPs was assessed using the DPPH assay. Vitamin C was used as the control. Initially, a stock solution of DPPH was prepared by dissolving 22 mg of DPPH in 100 ml of methanol, resulting in a filtered solution with an absorbance of approximately 0.892 at 517 nm. Various ratios of DPPH and sZnO NPs were combined, with a standard ratio of 3 ml of DPPH solution in 100 µL of methanol. Following a 30-minute incubation period, the absorbance of the solutions was measured at 517 nm, and the percentage of antioxidants was then calculated and graphed.

Hydrogen peroxide (H_2O_2) assay: The H_2O_2 scavenging potential of sZnO NPs was investigated using the traditional UV method at 230 nm. To evaluate this activity, a colorimetric approach was employed. In this method, the reaction between H_2O_2, phenol, and 4-aminoantipyrine in the presence of horseradish peroxidase generates a pink quinoneimine dye. The alteration in color was used as an indicator of scavenging activity. This calorimetric technique was applied to both a standard antioxidant, ascorbic acid, and various concentrations of sZnO NPs (control, 40, 60, 80, 100, and 120 µg/mL). After a 30-minute incubation period at room temperature, the absorbance of the solutions was measured at 504 nm.

***In vitro* cytotoxicity assessment of sZnO NPs on zebrafish embryos:** The zebrafish model was used to analyse the *in-vitro* toxicity of the synthesised sZnO NPs. the test group were cultured with different concentrations of sZnO NPs while the control group were cultured with saline. The sZnO NPs were diluted at different ratios from 25-200 ug/ml. Twenty five numbers of zebrafish embryos were selected for the analysis. The zebrafish were well managed and assessed at different time periods. The number of dead zebrafish was estimated and differentiated with control samples. The zebrafish embryos were maintained at individual trails at a standard temperature for the generation of organs. The embryos were observed under a microscope at 40x magnification every 24 hours. At every four hour time period, the viable and dead embryos, and fish were identified. The dead embryos and fish were discarded at every inspection to minimise suspension contamination. The mortality ratio was calculated for every 24 hours.

RESULTS

Antibacterial assay: On analysis of the antibacterial activity observed with sZnO NPs, the zone of inhibition increased in proportion with the concentration of the sZnO NPs while the zone of inhibition noted with the antibiotic standard was consistently lower than that noted with the low concentration of the sZnO NPs, for all the bacterial strains. The results are depicted in Fig 1, Fig 2, and Table 1. A statistically significant difference in the antibacterial activity of sZnO NPs in comparison to the antibiotic standard was observed for both *S.aureus* and *S.mutans*, with the most antibacterial activity by sZnO NPs noted against s.mutans at both low and high concentrations. However, a variability in response was noted between gram-positive and gram-negative strains highlighting the importance of cell wall composition in determining bacterial resistance or susceptibility to nanoparticles.

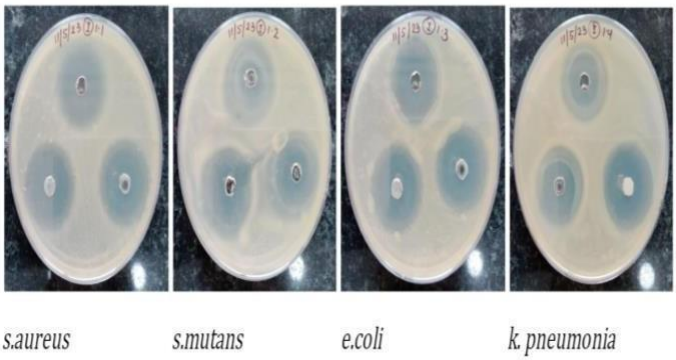


Figure 1. Antibacterial assay performed using sZnO NPs

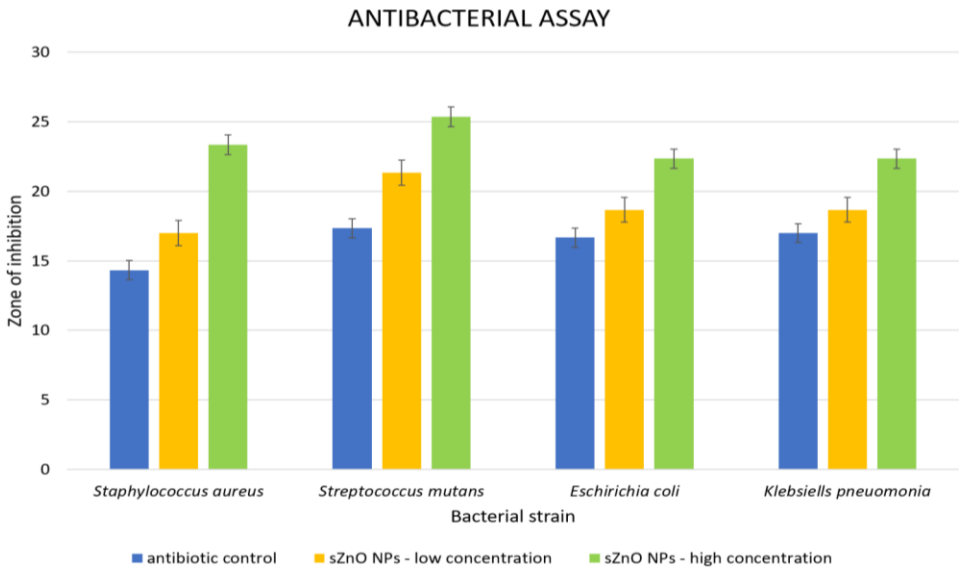


Figure 2. Comparison of the antibacterial activity of sZnO NPs with the antibiotic control

Table 1. Comparison of antibacterial activity of sZnO NPs and antibiotic control - one-way ANOVA

Strain	Concentration	N	Mean (Zone of Inhibition)	Standard Deviation	P Value
Staphylococcus Aureus	Antibiotic Standard	3	14.33	0.577	0.031*
	sZnO NPs - Low Concentration	3	17	1.000	0.001*
	sZnO NPs - High Concentration	3	23.33	1.154	0.000*
Streptococcus Mutans	Antibiotic Standard	3	17.33	1.527	0.042*
	sZnO NPs - Low Concentration	3	21.33	1.527	0.042*
	sZnO NPs - High Concentration	3	25.33	1.628	0.002*
Escherichia coli	Antibiotic Standard	3	16.66	1.423	0.315
	sZnO NPs - Low Concentration	3	18.66	1.652	0.059
	sZnO NPs -	3	22.33	1.715	0.009*

	High Concentration				
Klebsiella pneumoniae	Antibiotic Standard	3	17.00	2.000	0.647
	sZnO NPs - Low Concentration	3	18.66	2.081	0.186
	sZnO NPs - High Concentration	3	22.33	2.516	0.058

DPPH assay: The results of the DPPH assay, as depicted in Fig 3 and Table 2, demonstrate the antioxidant activity of sZnO NPs across varying concentrations, compared to Vitamin C (Vit C) as a standard. While both sZnO NPs and Vit C exhibited a concentration-dependent increase in DPPH radical scavenging activity with Vit C consistently showing higher activity at all tested concentrations, no statistically significant difference in the antioxidant activity was observed between the two samples. The results suggest that the antioxidant activity of sZnO NPs is comparable to Vit C. The observed trend highlights sZnO NPs potential as an antioxidant, albeit not as effective as the standard, Vit C. These findings could support the exploration of sZnO NPs as a complementary antioxidant agent for clinical applications.

Table 2. Comparison of antioxidant activity of sZnO NPs and vitamin C using the DPPH assay - unpaired t-test

Concentration	Sample	N	Mean (% Of Antioxidant Activity	Standard Deviation	P Value
2µg/ml	sZnO NPs	3	21.66	3.511	0.632
	Vit C	3	26.66	2.516	
4µg/ml	sZnO NPs	3	27	2.000	0.801
	Vit C	3	29.66	2.081	
6µg/ml	sZnO NPs	3	32.66	3.511	0.305
	Vit C	3	39.33	1.527	
8µg/ml	sZnO NPs	3	39.33	2.309	0.328
	Vit C	3	43.33	1.527	
10µg/ml	sZnO NPs	3	43.33	1.635	0.519
	Vit C	3	50.33	2.092	

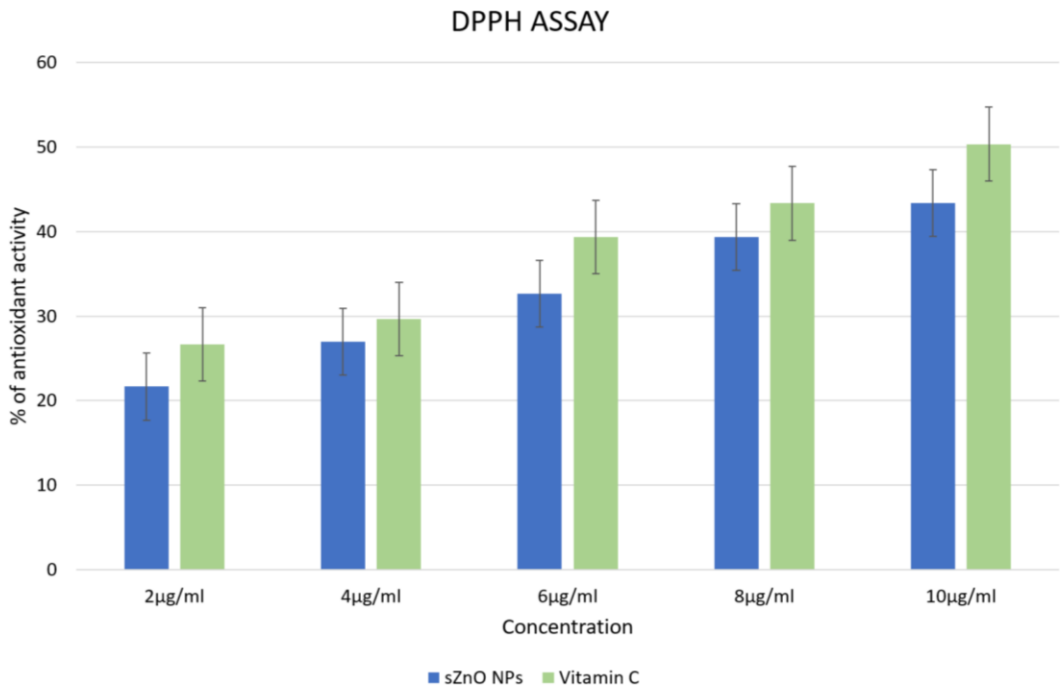


Figure 3. Antioxidant analysis of sZnO NPs using the DPPH assay

H_2O_2 assay: The results as depicted in Fig 4 and Table 3, indicate the antioxidant activity of sZnO NPs in comparison to Vitamin E (Vit E) across various concentrations. Both sZnO NPs and Vit E demonstrate a concentration-dependent increase in activity, as evidenced by the rising mean percentages of scavenging activity. However, Vit E consistently exhibits slightly higher activity than sZnO NPs at all tested concentrations, though there was no statistically significant difference between them at all time points. The standard deviations for both sZnO NPs and Vit E remain relatively low, suggesting consistency in the results. Overall, sZnO NPs demonstrate promising antioxidant activity, comparable to that of Vit E. These findings highlight the potential of sZnO NPs as an alternative or complementary antioxidant agent, warranting further exploration.

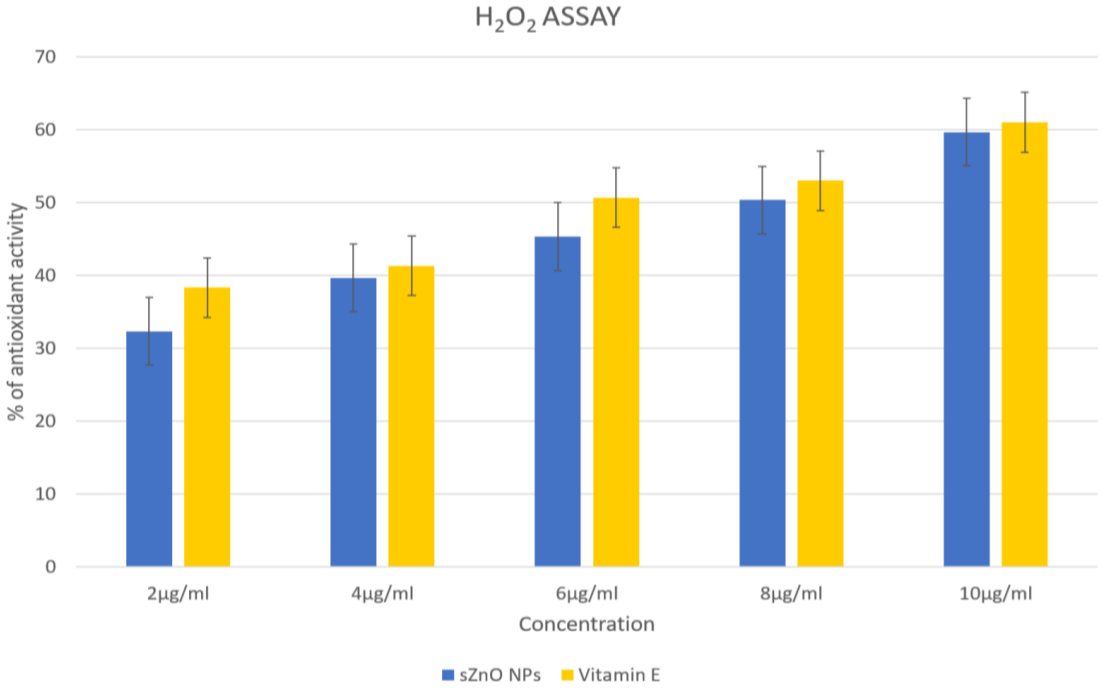


Figure 4. Antioxidant analysis of sZnO NPs using the H_2O_2 assay

Table 3. Comparison of antioxidant activity of sZnO NPs and vitamin E using the H_2O_2 assay - unpaired t test

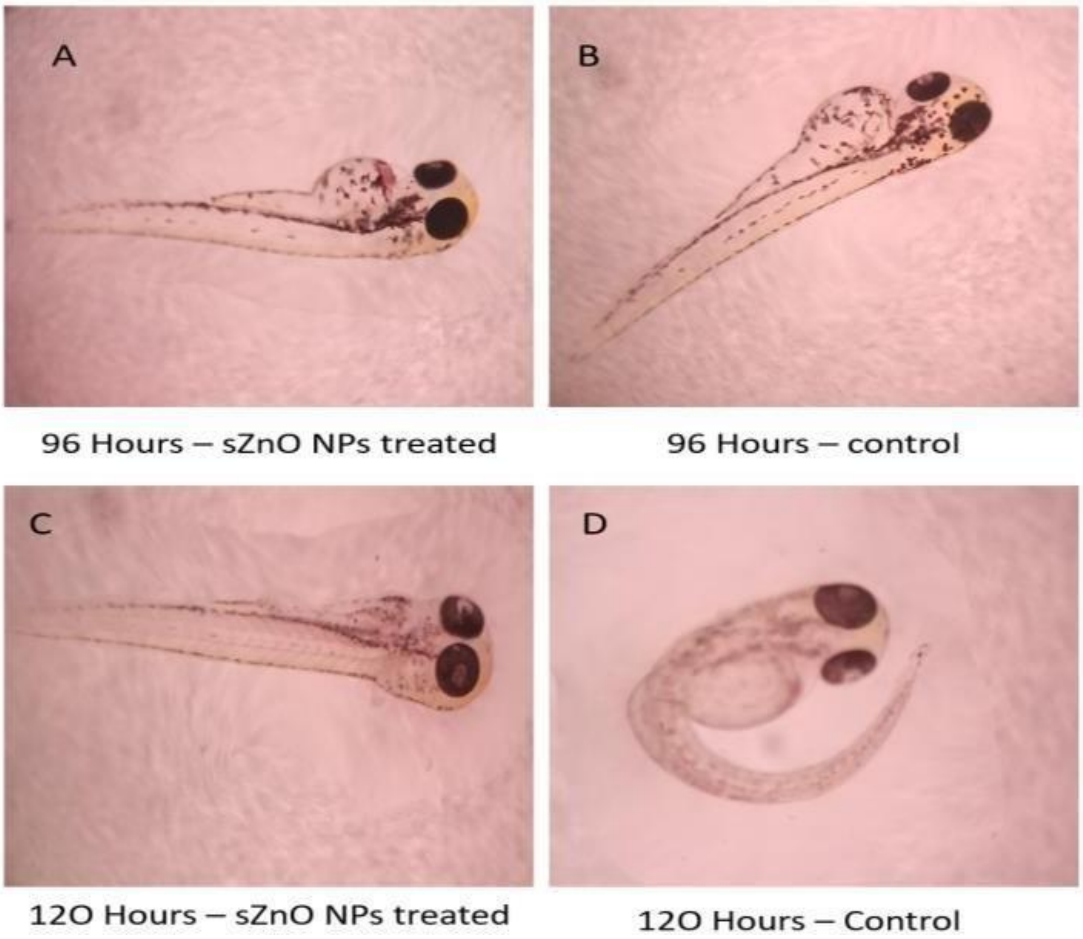
Concentration	Sample	N	Mean (% Of Antioxidant Activity)	Standard Deviation	P Value
2µg/Ml	sZnO NPs	3	32.33	4.041	0.655
	Vit E	3	38.33	3.055	
4µg/mL	sZnO NPs	3	39.66	2.081	0.812
	Vit E	3	41.33	2.516	
6µg/mL	sZnO NPs	3	45.33	1.527	0.519
	Vit E	3	50.66	2.081	
8µg/mL	sZnO NPs	3	50.33	0.577	0.057
	Vit E	3	53	2.645	
10µg/mL	sZnO NPs	3	59.66	2.081	0.801
	Vit E	3	61	2.000	

Cytotoxicity assay

The zebrafish embryos treated with sZnO NPs, as depicted in Fig 5, showed proper development of head, tail, eye, and internal organs at the correct time intervals, comparable to that of the control group, with several hatching and displaying various growth stages at 92 and 120 hours. The mortality trends observed in this study, as depicted in Fig 6 and Table 4, show that both the sZnO NPs and control groups show a consistent increase in mortality percentages, peaking at 120 hours, with sZnO NPs at 84.33% and the control at 85.66%.

This indicates a time- dependent factor influencing mortality, potentially linked to environmental conditions, experimental setup, or toxic progression. The close mortality rates at 120 hours suggest that sZnO NPs treatment does not significantly alter long- term outcomes compared to the control.

Furthermore, at all time points no statistically significant difference in mortality rate between the two groups was observed. This lack of significance implies that sZnO NPs does not introduce notable toxicity relative to the control, which proves that sZnO NPs is as biocompatible as the control.



Figur 5. Development of zebrafish embryos treated with sZnO NPs and control at various time points

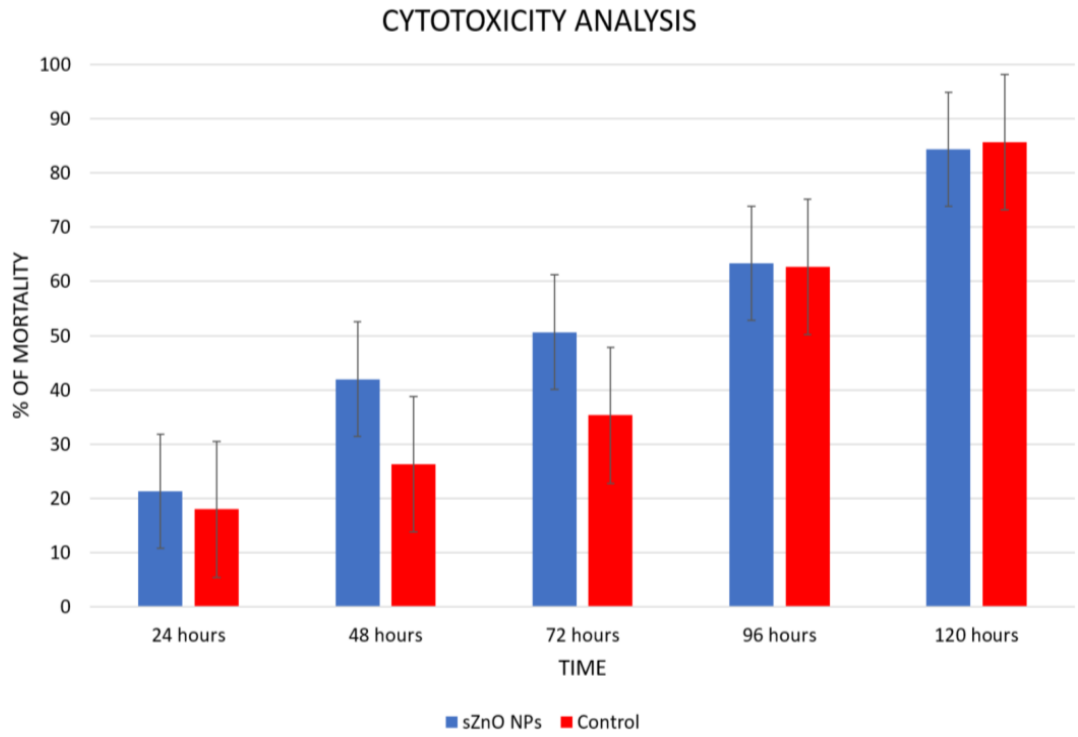


Figure 6. Cytotoxicity analysis of sZnO NPs observed in zebrafish embryos

Table 4. Comparison of cytotoxicity of sZnO NPs and control - unpaired t-test

Time	Sample	N	Mean (% Of Mortality)	Standard Deviation	P Value
24 HRS	sZnO NP	3	21.33	3.511	0.425
	Control	3	18.00	2.000	
48 HRS	sZnO NP	3	42.00	3.605	0.751
	Control	3	26.33	4.163	
72 HRS	sZnO NP	3	50.66	3.055	0.875
	Control	3	35.33	3.511	
96 HRS	sZnO NP	3	63.33	3.214	0.539
	Control	3	62.66	2.516	
120 HRS	sZnO NP	3	84.33	4.163	0.667
	Control	3	85.66	3.511	

DISCUSSION

Over the recent years, with the increase in life expectancy of the global population, there has been a greater emphasis on achieving enhanced quality of life. Periodontally healthy tissues improve the masticatory function, nutritional status, and esthetics of an individual. Though various mechanical therapies aim to remove the primary etiologic factors like plaque and calculus, complete eradication of the microbes from the intra-epithelial sources is not possible. They act as a source of reinfection, perpetually maintaining the tissues in a state of disease. In order to reduce the microbial load in the soft tissues, adjuvant antimicrobial therapy is often employed in the form of systemic or topical antimicrobials. However, due to the injudicious use of systemic antimicrobials, super-resistant bacterial strains have emerged causing aggressive forms of periodontal disease. Moreover, the inflammation-mediated free radical and reactive species generation has made the adjuvant use of antioxidants desirable. Hence multiple natural and synthetic sources are being explored to discover components with antimicrobial and antioxidant properties of which NPs have gained attention.

The antibacterial assay revealed that sZnO NPs exhibit a concentration-dependent antimicrobial effect, with higher concentrations consistently showing greater efficacy against the tested bacterial strains. This trend indicates that the sZnO NPs inhibitory activity likely increases with dosage, possibly due to enhanced interaction with bacterial cells or improved reactive oxygen species (ROS) generation at higher concentrations. For *S. aureus* and *S. mutans*, the antibacterial activity of sZnO NPs at both low and high concentrations significantly surpasses the antibiotic control, with statistically significant P-values ($P < 0.05$). In the case of *E. coli*, significant improvement is observed only at the high concentration of sZnO NPs, while for *K. pneumoniae*, the observed increase in inhibition zones at higher concentrations lacks statistical significance. These findings suggest that sZnO NPs are particularly effective against certain bacterial strains, such as *S. aureus* and *S. mutans*, and their efficacy increases with higher concentrations. However, differences in sensitivity across strains could be attributed to variations in cell wall structure or bacterial resistance mechanisms.

The gram-positive bacteria in this study, showed significant zones of inhibition for both low and high concentrations of sZnO NPs, exceeding the antibiotic control in both cases. This heightened susceptibility can be attributed to the simpler cell wall structure of gram-positive bacteria, which primarily consists of a thick peptidoglycan layer. The lack of an outer membrane may have enabled easier NPs penetration and interaction with cellular components, disrupting metabolic processes or inducing oxidative stress. In contrast, the gram-negative bacteria demonstrated relatively lower sensitivity to sZnO NPs. While *E. coli* showed significant inhibition at high concentrations, *K. pneumoniae* did not achieve statistically significant differences at either concentration. The reduced susceptibility of gram-negative bacteria can be linked to their more complex cell wall architecture, which includes an outer membrane rich in lipopolysaccharides, which may have acted as a protective barrier, limiting the penetration of nanoparticles and reducing their efficacy. These results highlight the potential of sZnO NPs as a broad-spectrum antimicrobial agent, though optimization may be necessary to enhance effectiveness against less responsive strains.

Studies have postulated various mechanisms leading to the antimicrobial activity of ZnO NPs.[18-21] It has been proposed that the positive charges from ZnO NPs are attracted to the negative charges in the peptidoglycan and lipoteichoic acid present in the cell membrane. This causes electron gradient development, along with a chelation reaction between the lipoteichoic acid and zinc ions. This destabilises the membrane potential, causing damage to the cell surface, cell morphology and the deoxyribonucleic acid (DNA) ultimately leading to cell rupture.[18,20,21] Its photocatalytic activity and strong oxidizing nature also lead to the formation of ROS which impairs protein synthesis and DNA replication.[22]

A recent study synthesised ZnO NPs using four different plant extracts and analysed their antibacterial and antifungal activity.[23] They observed that all samples exhibited antibacterial activity against the chosen bacteria except *Beta vulgaris-based* ZnO NPs that were ineffective against *S. aureus*. They concluded that green synthesised ZnO NPs were cost-effective and environment-friendly and must be further explored for their antimicrobial activity. Another study stated that ZnO NPs prepared using leaf aqueous extract of *Azadirachta indica (A. indica)* depicted increased antimicrobial activity in accordance with the increase in the concentration of the NPs.[24] This is similar to the findings of our study. They also stated that the ZnO NPs showed good antimicrobial activity which may be attributed to the increase in H_2O_2 concentration released from the surface of the NPs. ZnO NPs synthesized using *Aloe vera (A. vera)* were assessed for antimicrobial activity against *Staphylococcus epidermidis (S. epidermidis), K. pneumoniae, E. coli,* and fungi[25].

It was observed that the NPs were effective against *E. coli* and *Aspergillus niger* (*A. niger)* while the efficacy against other tested bacteria and fungi was less in comparison to the commercial antibiotic control. They postulated that the efficacy against gram-positive bacteria may have been less due to the presence of thick peptidoglycan layers of the cell membrane. This is in contrast to the results observed in our study.

The antioxidant analysis highlights the ability of sZnO NPs to exhibit antioxidant activity comparable to established standards like Vit C and Vit E across varying concentrations. Despite consistently showing slightly lower activity than both standards, the absence of statistically significant differences underscores the promise of sZnO NPs as viable alternatives or complementary agents in antioxidant applications. The results of this study are in accordance with another study that synthesized ZnO NPs mediated by *Coccinia abyssinica (C. abyssinica)* and showed slightly lower radical scavenging capacity than standard ascorbic acid at all concentrations, with an IC50 value of 127.74 µg/ml[26]. A recent study that analysed the antioxidant activity of ZnO NPs synthesised using *Pelargonium odoratissimum (P. odoratissiumum*) aqueous leaf extract, showed scavenging effect with an IC50 value of 28.11 ± 0.01 µg mL−1 compared to L-ascorbic acid. They stated that the comparable antioxidant activity to Vit C may be attributed to the bioactive constituents and higher phenolics and flavonoid content in *P. odoratissimum*.[27] Similar results of comparable antioxidant properties of green synthesised ZnO NPs to that of Vit C have been reported by recent research.[28-32]

The antioxidant potential exhibited by ZnO NPs can be explained through multiple mechanisms, leveraging their unique nanoscale properties and composition. They effectively scavenge free radicals, such as ROS, by donating electrons or hydrogen atoms to stabilize these harmful molecules, thus protecting cellular components from oxidative stress.[33] Additionally, ZnO NPs have been shown to enhance the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are critical for neutralizing ROS.[34] The zinc ions released by ZnO NPs also play a role in their antioxidant function, as these ions protect sulfhydryl groups in proteins and compete with redox-active metals, thereby reducing ROS generation.[35] Moreover, the small size and high surface area of ZnO NPs amplify their reactivity and improve their interaction with biological systems, enhancing their overall antioxidant capacity.[36] These mechanisms collectively highlight the potential of ZnO NPs in mitigating oxidative stress and their possible periodontal applications.

In relation to the cytotoxicity analysis of sZnO NPs on the zebrafish, it can be inferred that sZnO NP does not significantly alter mortality compared to the control under the given experimental conditions. The increasing trend in mortality for both groups might be influenced by external factors such as environmental conditions, depletion of resources, or inherent progression in the setup. These outcomes suggest that while sZnO NP does not appear to introduce significant toxicity compared to the control. Hence, we can conclude that sZnONPs are safe to be employed as antibacterial and antioxidant agents for periodontal applications. Similar results were observed in other studies that assessed the cytotoxicity of ZnO NPs developed through green synthesis.[37,38]

In summary, the green synthesised sZnO NPs exhibited good antibacterial activity against the chosen bacterial strains with greater zones of inhibition noted with gram-positive bacteria. The antioxidant activity of sZnO NPs increased proportional to the concentration of the NPs and was comparable to that of Vit C and Vit E. The cytotoxicity analysis showed a comparable mortality rate to that of the control, suggesting that the developed NPs did not induce cytotoxic activity on their own, other than that which occurred due to other experimental and environmental conditions. Hence, sZnO NPs warrant further exploration for their applicability as a local drug delivery agent with synergistic antibacterial and antioxidant activity. It's combined effect would allow for a more targeted effect that would also be more acceptable to the patient. The limitations of this study were that the synthesised sZnO NPs were not subjected to characterisation. Also, the biocompatibility could have been assessed with cell lines. Further, *in vitro* and *in vivo* studies can be conducted to study the antifungal, antiviral, and anti-inflammatory properties of the NPs and explore their clinical applicability in the management of severe forms of periodontal disease.

CONCLUSION

The study reveals that ZnO nanoparticles green synthesised from *S. album* exhibit good antibacterial and antioxidant activity. Moreover, they are safe for biomedical application which was proven by the comparable cytotoxicity observed with the control. These NPs are environmentally friendly and cost-effective, reducing the use of harsh chemicals and toxic byproducts. The findings suggest that further exploration of ZnO NPs antibacterial and antioxidant properties could lead to the development of new targeted local drug delivery applications for combating severe forms of periodontal diseases.

DECLARATIONS

Acknowledgments

None

Conflicts of interest and financial disclosures

The authors declare no conflict of interest and there was no external source of funding

Ethical approval: Approval for the conduction of the study was obtained from the Institutional Scientific Review Board.

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