Eco. Env. & Cons. 29 (May Suppl. Issue) : 2023; pp. (S70-S74)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i03s.015

Investigation of the antibacterial efficacy of Zinc Oxide Nanoparticles (ZnO NPs) based Irrigant against *Enterococcus faecalis* Concerning Root Canal Disinfection

Jayant Pawar[1]*, G.R. Pathade[1] and E.A. Singh[2]

[1] KIMSDU's Krishna Institute of Allied Sciences, Karad 415 539, Maharashtra, India
[2]BVDU's Rajiv Gandhi Institute of IT and Biotechnology, Pune, Maharashtra, India

(Received 10 November, 2022; Accepted 20 January, 2023)

ABSTRACT

The present study is aimed to investigate the antibacterial efficacy of Zinc Oxide Nanoparticles (ZnO NPs) based irrigants for root canal disinfection. The ZnO NPs-based irrigant was tested *in-vitro* on *Enterococcus faecalis* (ATCC: 29212) with NaOCl as a positive control. In this direction, ZnO NPs are very important as they are biocompatible, non-cytotoxic, cheap and easy to produce. The synthesis of ZnO NPs was carried out by facile semi-solvo thermal route using zinc acetate and urea as precursors and a mixture of deionized water and ethylene glycol as solvent. The optical, structural and morphological analyses were carried out by UV-Vis spectroscopy, XRD, SEM and TEM. The qualitative and quantitative antimicrobial activity of ZnO NPs were tested against *Enterococcus faecalis* containing 1/*10^5 CFU/ml. The structural and morphological analysis of ZnO NPs reveals the formation of a hexagonal crystal structure having flower-like morphology where each flower is made up of petals with a thickness of 10-50 nm. The absorption edge was found to be shifted to the lower wavelength at 340 nm which can be due to the size quantization effect. The band gap value was calculated to be 3.65 eV. The inhibition zones were observed in all concentrations with positive control. The MIC and MBC of ZnO NPs were found to be 250 µg/ml and 1000 µg/ml, respectively. Overall, ZnO NPs synthesized by the semi-solvo thermal method were found to be an effective antibacterial agent against *E. faecalis*. Therefore, ZnO NPs can be used in irrigant solutions for better protection of the oral canal from *E. faecalis*.

***Key words**: ZnO NPs, Irrigants, NaOCl, Antibacterial efficacy, Enterococcus faecalis.*

Introduction

Dental caries and periodontal diseases are the major public oral health problems globally and the most often noncommunicable disease (NCD) affecting around 20-50 % of the population of world and the utmost cause of tooth loss (Haque *et al.*, 2019). Usually, endodontic therapy plays an important role to reduce microbial incursion, which consecutively promotes the normal healing process of the periapical tissues (Muliyar *et al.*, 2014; Ambulkar *et al.*, 2019).

Endodontic success depends on a range of treatments like negotiating the canal, disinfecting the canal by irrigation, intra-canal medication, debriding, and obturation of the canal space to prevent and treat apical periodontitis (Saoud *et al.*, 2016). However, the canal space is intricate and infected with a surfeit of microorganisms which may be inaccessible for conventional treatment methods.

Also, antibiotics are widely used in dental caries to treat odontogenic, nonodontogenic, local and focal infections and also for prophylactic reasons (Ahmadi *et al.,* 2021). But unfortunately, the overuse of antibiotics has rapidly developed antimicrobial resistance (Haque *et al.,* 2019). Due to this, the development of multidrug resistance in pathogenic bacteria has become a global challenge, especially in controlling of infectious diseases in endodontics.

Enterococcus faecalis (Lamont *et al.,* 1991) and other bacteria like *Streptococcus mutans* and *Streptococcus sanguis*, (Goudarzi *et al*., 2019) found in the oral cavity and found to be resistant to many commonly used antimicrobial agents (aminoglycosides, aztreonam and quinolones etc.) (Gonçalves *et al*., 2007) and irrigants like sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), and doxycycline (Doxy) have found effective antimicrobial agents but at higher concentrations (Carson *et al.,* 2005). So, there is a need to develop novel agents with broad-spectrum therapeutic potential to treat dental issues.

Presently, metal oxide nanoparticles have been widely used for assorted applications in electronics, instrumentation, catalysis, nanosensors, food technology, biotechnology and medicine (Pawar *et al*., 2017; Viswanathan *et al.,* 2020; Aswini *et al*., 2022). The metal oxide nanoparticles which exhibit antimicrobial properties include magnesium oxide (MgO) (Jin *et al*., 2011), copper oxide (CuO) (Shirsat *et al.,* 2019), Tungsten Oxide (WO_3) (Harini *et al.,* 2021 and Qureshi *et al.,* 2021) and zinc oxide (ZnO) (Pisal *et al*., 2021). Perhaps, differences in the cell structure, physiology and metabolism, degree of contact of organisms with nanoparticles or rate of diffusion of nanoparticles, metal oxide nanoparticles would show different antibacterial efficacy (Azam *et al*., 2012). Among all other metal oxide nanoparticles, ZnO NPs has got major attention due to their biocompatibility and antimicrobial property (Pawar *et al*., 2019).

In the present study, Zinc Oxide nanoparticles (ZnO NPs) were synthesized by Semi-solvothermal methods and tested its antibacterial activity against *Enterococcus faecalis* (ATCC: 29212) by qualitative and quantitative antibacterial assays. The advantages of using the semi-solvothermal method are that it requires limited equipment and materials, a simple process compared to other modes of synthesis. This method may be useful for the students and researchers who do not have sufficient laboratory facilities to perform synthesis work.

The main focus of this study is to synthesise and characterization of ZnO NPs based irrigants and studied their antibacterial efficacy against *Enterococcus faecalis* (ATCC: 29212) to treat dental infections.

Materials and Methods

Chemicals and Solvents

All the bulk chemicals and solvents used in the study were of analytical reagent (AR) grade and procured from suppliers including SD fine-chemicals, Loba Chemie, Sisco Research Laboratories and Qualigenes, India.

Synthesis and Characterization of ZnO NPs

In the present study, the synthesis of ZnO NPs was carried out by a modified facile semi-solvothermal route (Pawar *et al*., 2017). Zinc acetate (0.1M) was dissolved in 50 ml DI (Deionized) water and Urea solution (0.6M) was prepared in 50 ml 40 % ethylene glycol. On constant stirring, both the solutions were mixed for 5 minutes to obtain a homogenous reaction mixture which was then transferred to a Teflon-lined stainless-steel autoclave. Further, the reaction mixture was heated at 160 °C for 24 hours in a muffle furnace followed by cooling at room temperature. The resultant precipitate was washed twice with DI water, once with absolute ethanol and again with DI water under centrifugation at 5000 rpm for 15 minutes to remove impurities. Subsequently, to remove all the volatile impurities and make the product friable, the final product was heated in a hot air oven at 400 °C for 8 hours.

The structural and morphological features of the resultant ZnO powder were characterized by UV-Vis spectroscopy, XRD, SEM and TEM for studying the various crystalline phases present in the powders as well as their morphological and optical properties.

Determination of antagonistic efficacy of ZnO NPs against *Enterococcus faecalis*

The antagonistic activities of ZnO were tested on *Enterococcus faecalis* (ATCC: 29212) found in the oral cavity. The antibacterial activity of ZnO was carried out by agar well diffusion assay (AWDA) (Pawar *et al.,* 2012; Jain *et al*., 2019). The Muller-Hilton (MH) agar plates were spread and inoculated with overnight-grown cultures of *Enterococcus faecalis* (ATCC: 29212). The synthesized ZnONPs were dispersed in sterile DI water by ultrasonication to make a colloidal solution of nanomaterials. On the surface of agar plates, wells of 5 mm in diameter and 18 µl in capacity were formed by using a sterile gel borer. The 15 µl of ZnO suspension was placed in each well and incubate all plates at 37 °C for 24 hours. The MIC and MBC of ZnO were performed by plate count method on MH agar plates (in the concentration range of 15 µg - 1000 µg/ml). The test cultures of final cell density of 1/*10^5 CFU/ ml were used for spread inoculation. The plates were incubated at 37 °C for 24 hours and subsequent growth inhibition of bacterial cultures was determined.

Results and Discussion

The absorption edge was found to be shifted to the lower wavelength at 340 nm which can be due to the size quantization effect (Figure 1(a)). The band gap value was calculated to be 3.65 eV. The crystal structure analysis of ZnO NPs by XRD revealed the formation of a hexagonal crystal structure of ZnO matching with JCPDS card number 36-1451. The peak broadening indicates the formation of nanoscale structures. The powder sample shows the crystalline nature with peaks corresponding to (100), (002), (101), (102), (110), (103), (200), (112), (004), (207) and (202) planes (Figure 1(b)). The FESEM images show the flower-like morphology where each flower is made up of petals with a thickness of 10-50 nm (Figure 2). TEM image shows a representative petal having a thickness of 50 nm (Figure 3).

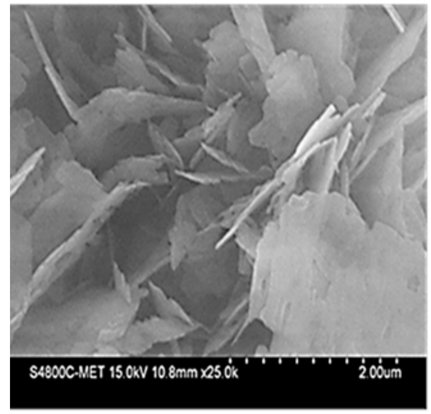

Fig. 2. FESEM Images of ZnO NPs revealed the flower-like morphology where each flower is made up of petals with a thickness of 10-50 nm.

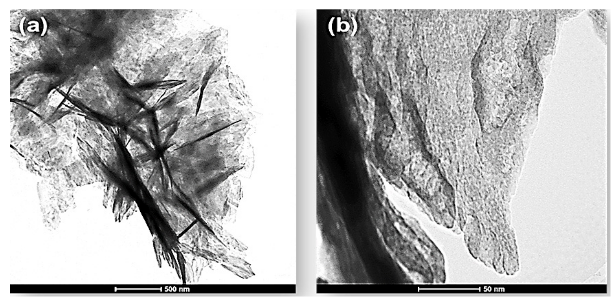

Fig. 3. TEM Images of ZnO NPs at (a) low, (b) high magnification. TEM image shows a representative petal having a thickness of50 nm.

The antagonistic activity of ZnO NPs suspension of 1000 µg/ml concentration was tested compared with NaOCl (a positive control) against *E. faecalis* and found to have antibacterial activity (Figure 4). The MIC and MBC of ZnO NPs were found to be 250 µg/ml and 1000 µg/ml for *E. faecalis* (Table 1).

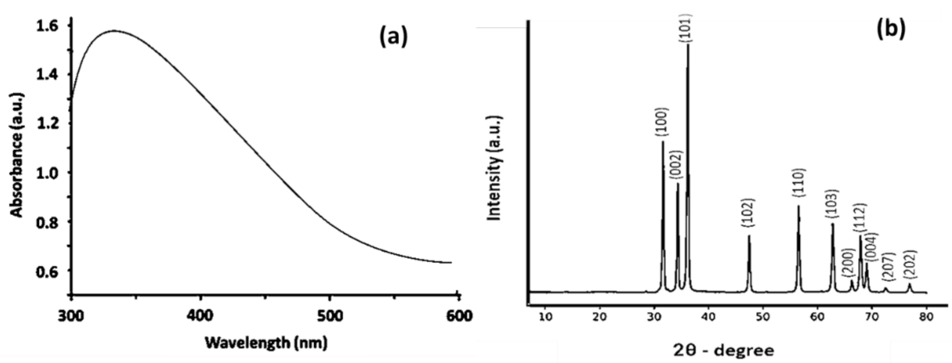

Fig. 1. UV-Vis Spectroscopy and XRD of ZnO NPs: (a) The maximum absorption (λmax) was found to be at 340 nm and (b) The X-ray diffractogram of powder reveals the formation of the hexagonal crystal structure of ZnO NPs.

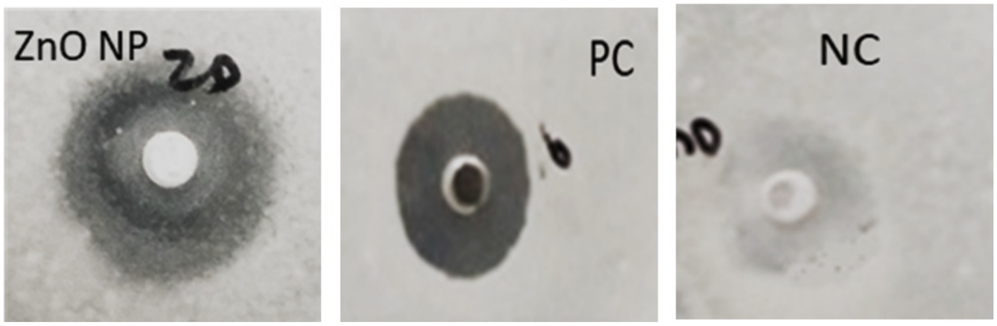

Fig. 4. Testing of antagonistic activity of ZnO NPs by AWDA method against *E. faecalis*showed a zone of inhibitions in both ZnO NPs (1000 μg/ml) and 2 % NaOCl (PC) indicating that the bacterial culture is sensitive to the tested materials.

The researchers reported that the antibacterial activity of ZnO NPs might be due to the oxygen species released on the surface of ZnO NPs, which causes fatal damage to microorganisms by penetrating the cell membrane by H_2O_2 produced after reacting the oxygen with hydrogen ions (Hamouda *et al*., 2020).

Table 1. MIC and MBC of ZnO NPs against *E. faecalis*

Conc. of ZnO NPs	*Enterococcus faecalis* (CFU)
Culture Control	$2.21 X10^6$
125 μg/ml	$1.50 X10^5$
250 μg/ml	$2.05 X10^3$
500 μg/ml	$1.00 X10^2$
1000 μg/ml	Nil

Guan *et al*., (2021) proposed that the adherence and penetration of ZnO NPs into the bacterial cell wall led to the loss of integrity of bacterial cell membrane and cell wall permeability. Nisar *et al.* (2019) reported the release of zinc ions can interact with the thiol groups of many vital enzymes and inactivate the bacterial cells. However, the exact mechanism of ZnO NPs engaged to cause antibacterial effect is not known completely and needs to explore.

The present study suggests exploring the use of ZnO NPs solutions as an alternative to root canal irrigating solutions not only just due to their bactericidal potential but also for their biocompatibility, particularly in low concentrations.

Conclusion

The structural and morphological analysis of ZnO NPs reveals the formation of a hexagonal crystal structure having flower-like morphology where each flower is made up of petals with a thickness of 10-50 nm. The absorption edge was found to be shifted to the lower wavelength at 340 nm which can be due to the size quantization effect. The band gap value was calculated to be 3.65 eV. The inhibition zones were observed in all concentrations with positive control. The MIC and MBC of ZnO NPs were found to be 250 μg/ml and 1000 μg/ml, respectively. Overall, ZnO NPs synthesized by the semi-solvo thermal method were found to be an effective antibacterial agent against *E. faecalis*. Therefore, ZnO NPs can be used in irrigant solutions for root canal disinfection from *E. faecalis*.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

Ahmadi, H., Ebrahimi, A. and Ahmadi, F. 2021. Antibiotic therapy in dentistry. *International Journal of Dentistry*. *2021*.

Ambulkar, S., Tale, V., Khilari, S. and Pawar, J. 2019. Antibacterial and Antibiofilm Activity of Quercus infectoria Galls on Rothiadentocariosa isolated from Dental Caries. *Asian Journal of Pharmaceutical and Clinical Research.* 12 (10).

Aswini, A., Vijayapradeep, S., Kumara Swamy D., Prakash V., Rabinder Henry and Jayant Pawar, 2022. Determination of Antagonistic Effect of CuO NPs against Bacterial Cultures. *Asian Journal of Convergence in Technology.* 8(1) : 138-143.

Azam, A., Ahmed, A.S., Oves, M., Khan, M.S., Habib, S.S. and Memic, A. 2012. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *International Journal of Nanomedicine*. 7: 6003.

Carson, K.R., Goodell, G.G. and McClanahan, S.B. 2005. Comparison of the antimicrobial activity of six irrigants on primary endodontic pathogens. *Journal of Endodontics.* 31(6) : 471-473.

Gonçalves, M.O., Coutinho Filho, W.P., Pimenta, F.P., Pereira, G.A., Pereira, J.A.A., Mattos Guaraldi, A.L. and Hirata Jr, R. 2007. Periodontal disease as reservoir for multi resistant and hydrolytic enterobacterial species. *Letters in Applied Microbiology.* 44(5) : 488-494.

Goudarzi, M., Mehdipour, M., Hajikhani, B., Sadeghinejad, S. and Sadeghi-Nejad, B. 2019. Antibacterial properties of citrus limon and pineapple extracts on oral pathogenic bacteria (*Streptococcus mutans* and *Streptococcus sanguis*). *Int J Enteric Pathog.* 7(3): 99-103.

Guan, G., Zhang, L., Zhu, J., Wu, H., Li, W. and Sun, Q. 2021. Antibacterial properties and mechanism of biopolymer-based films functionalized by CuO/ZnO nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. *Journal of Hazardous Materials*. 402: 123542.

Hamouda, R.A., Yousuf, W.E., Mohammed, A.A., Darwish, D.B. and Abdeen, E.E. 2020. Comparative Study Between Zinc Oxide Nanoparticles Synthesized by Chemical and Biological Methods in View of Characteristics, Antibacterial Activity and Loading on Antibiotics *In vitro*. *Digest Journal of Nanomaterials & Biostructures (DJNB)*. 15(1).

Haque, M., Sartelli, M. and Haque, S.Z. 2019. Dental infection and resistance-global health consequences. *Dentistry Journal.* 7(1): 22.

Harini, S., Aswini, A., Kale, S., Narawane, J., Pawar, J., Masurkar, S. and Ruikar, S. 2021. Microwave-Assisted Solvothermal Synthesis of Tungsten Oxide (WO. *Int J Cur Res Rev.* 13(02) : 76.

Jain, N., Bhosale, P., Tale, V., Henry, R. and Pawar, J. 2019. Hydrothermal assisted biological synthesis of silver nanoparticles by using honey and gomutra (Cow Urine) for qualitative determination of its antibacterial efficacy against *Pseudomonas* sp. isolated from contact lenses. *EurAsian Journal of BioSciences.* 13(1): 27-33.

Jin, T. and He, Y. 2011. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. *J Nanopart Res.* 13: 6877-6885.

Kavithayeni, V., Prabhu, A., Prakash, V., Henry, R. and Pawar, J. 2020. Evaluation of Photocatalytic Dye Degradation Efficacy of ZnO Nanoparticles Synthesized by Sol-Gel Method at Different Calcination Temperatures. *Pollution Research.* 39 (4) : 1082-1088.

Lamont, R.J., Demuth, D.R., Davis, C.A., Malamud, D. and Rosan, B. 1991. Salivary-agglutinin-mediated adherence of *Streptococcus mutans* to early plaque bacteria. *Infection and Immunity.* 59(10) : 3446-3450.

Muliyar, S., Shameem, K.A., Thankachan, R.P., Francis, P.G., Jayapalan, C.S. and Hafiz, K.A. 2014. Microleakage in endodontics. *Journal of International oral Health: JIOH. 6*(6) : 99.

Nisar, P., Ali, N., Rahman, L., Ali, M. and Shinwari, Z.K. 2019. Antimicrobial activities of biologically synthesized metal nanoparticles: an insight into the mechanism of action. *JBIC Journal of Biological Inorganic Chemistry*. 24(7) : 929-941.

Pawar, J., Bipinraj, N.K., Singh, E.A. and Nikam, N. 2012. Determination and Partial Characterization of Antimicrobial Material of *Pseudomonas aeruginosa* Isolated from Milk. *Preservation.* 10: 13.

Pawar, J., Henry, R., Viswanathan, P., Patwardhan, A. and Singh, E.A. 2019. Testing of antibacterial efficacy of CuO nanoparticles by methylene blue reduction test against *Bacillus cereus* responsible for food spoilage and poisoning. *Indian Chemical Engineer*. 61(3): 248-253.

Pawar, J., Prakash, V., Vinchurkar, P., Shinde, M., Henry, R., Mandke, Y., Patwardhan, A. and Singh, E.A. 2017. Application of Semi-Solvo Thermally Synthesized Zinc Oxide (ZnO) Nanoparticles in Food Technology and Its Characterization. *Int. J. Nanotechnol.* 11(1) : 75-80.

Pawar, J., Shinde, M., Chaudhari, R. and Singh, E.A. 2017. Semi-solvo thermal synthesis and characterization of zinc oxide nanostructures against food borne pathogens. *Int J Pharm Bio Sci.* 8(2): 311-315.

Pisal, S., Pawar, J., Kale, S.C., Nanaware, J., Henry, R., Ruikar, S. and Masurkar, S. 2021. Antibacterial Effects of ZnO NPs Synthesized by Solvothermal Method against *Pseudomonas* sp. Extracted from Saliva Ejector Tubing. *Int J Cur Res Rev.* 13(03): 60.

Qureshi, N., Lee, S., Chaudhari, R., Mane, P., Pawar, J., Chaudhari, B., Shinde, M., Rane, S., Kim, T. and Amalnerkar, D. 2021. Hydrothermal Generation of 3-Dimensional WO Nanocubes, Nanobars and Nanobricks, Their Antimicrobial and Anticancer Properties. *J Nanosci Nanotechnol*. 1; 21(10) : 5337-5343.

Saoud, T.M.A., Ricucci, D., Lin, L.M. and Gaengler, P. 2016. Regeneration and repair in endodontics-a special issue of the regenerative endodontics-a new era in clinical endodontics. *Dentistry Journal.* 4(1):3.

Shirsat, S., Pawar, D., Jain, N., Pawar, J., Tale, V.S. and Henry, R. 2019. Synthesis of Copper Oxide Nanoparticles by Chemical Precipitation Method for the Determination of Antibacterial Efficacy against *Streptococcus* Sp. and *Staphylococcus* Sp. *Synthesis.* 12(5).

Viswanathan, P., Patel, A.K., Pawar, J., Patwardhan, A. and Henry, R. 2020. Fabrication of Tin Oxide Nanoparticles for CO_2 Gas Sensing Layer. *IETE Journal of Research*. 66(4): 460-465.