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Original Article

Potent Effects of Green Synthesized Zinc Nanoparticles Against some Cariogenic Bacteria (*Streptococcus mutans* and *Actinomyces viscosus*)

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ABSTRACT

Objectives: Tooth decay is an irreversible complication of calcified tooth tissues that is characterized by demineralization of the inorganic part and destruction of organic matter in the tooth and often leads to the formation of cavities. In the present study, we investigated the antibacterial effect of zinc nanoparticles (ZnNPs) green synthesized with *Lavandula vera* DC. by microwave method on *Streptococcus mutans* and *Actinomyces viscosus* compared with chlorhexidine.

Material and Methods: ZnNPs were green synthesized using *L. vera* extract using the microwave and were characterized by scanning electron microscope, X-ray diffraction technique, nano-sizer-zetacizer, and Fourier-transform infrared spectroscopy. Antibacterial effects were evaluated through determining the minimum inhibitory concentration (MIC) of ZnNPs in comparison with chlorhexidine on standard strains of *S. mutans* and *A. viscosus*, by microbroth dilution method according to Clinical and Laboratory Standards Institute guidelines.

Results: The absorption peak of ZnNPs was in the range of 230–330 nm. The size of ZnNPs varied from 30 to 80 nm, while most nanoparticles were between 50 and 60 nm. The best MIC related to ZnNPs + chlorhexidine was reported with 1.66 and 1.66 μg/ml for *S. mutans* and *A. viscosus*, respectively. Furthermore, the lowest MICs related to ZnNPs alone were obtained with 13.33 and 16.33 μg/ml for *S. mutans* and *A. viscosus*, respectively. ZnNPs + chlorhexidine in comparison with chlorhexidine had a significant ($P < 0.05$) antibacterial effect and inhibited the growth of both bacteria.

Conclusion: ZnNPs especially combined with chlorhexidine exhibited promising antibacterial effect in comparison with chlorhexidine alone. However, further studies are needed to clarify the accurate mechanisms and toxicity of ZnNPs.

Keywords: Green synthesis, Nanomedicine*, In vitro,* Antibacterial, *Lavandula vera*

INTRODUCTION

Tooth decay is an irreversible complication of calcified tooth tissues that is characterized by demineralization of the inorganic part and destruction of organic matter in the tooth and often leads to the formation of cavities.[1] It is a multipart and dynamic procedure that is influenced by many factors and causes the progression of the disease.[1] Tooth decay is still one of the main common diseases of people around the world that can affect people throughout their lives; approximately 36% of the world's population has caries on their permanent teeth.[2] The risk of caries depends on factors such as physical, biological, environmental, behavioral, and lifestyle conditions, and the most important factors predisposing to the onset of caries include the existence of bacterial species that are able to reduce salivary acidity to a critical level of 5.5, poor oral hygiene, diet, and tooth structure.[3]

Today, a Gram-positive bacteria called *Streptococcus mutans* are considered to play a key role in causing caries, which damages tooth enamel by fermenting sucrose and producing lactic acid; however, other microorganisms such as *Lactobacillus acidophilus* and *Actinomyces viscosus* are not ineffective.[4] Uncontrolled growth of bacteria leads to penetration into the dentin and infection of the soft tissue of the dental pulp, which causes severe complications and systemic infections.[5] The most important way to deal with tooth decay is to prevent it, and the most important ways to inhibit caries in conventional medicine include proper nutrition, oral hygiene, the use of groove blockers is fluoride therapy, and the use of non-cariogenic sweeteners such as xylitol, saccharin, and aspartame.[6] Treatment of dental caries imposes heavy costs on the government and society in all countries. Therefore, considering the priority of prevention over treatment, it is necessary to try to recognize and generalize methods of prevention of tooth decay and to attract the public to them.[7]

Nanomedicine is the use of nanoparticle technology in the medical applications, etc., prevention, diagnostic, and treatment of diseases.[8-10] In materials science, green synthesis is well-known as one of the reliable and environmentally friendly technique to synthesis various nanoparticles.[11-13] The microwave heating method is also one of the widely methods used in the synthesis of various nanomaterials.[13] This method is based on the properties and characteristics, etc., specific heat distribution, increasing response speed and accordingly, the decrease of time and energy in the synthesis of various nanomaterials.[13] In the present study, we investigated the antibacterial effect of zinc nanoparticles (ZnNPs) synthesized with *Lavandula vera* DC. by microwave method on *S. mutans* and *A. viscosus*.

MATERIAL AND METHODS

Preparation and characterization of ZnNP

Zn sulfate solution with a concentration of 1 mM was prepared with deionized water and different amounts of prepared plant extracts (*L. vera*) were added to it separately. The mixture was exposed to microwaves of different strengths and times in the microwave. Based on the color change of the mixture to dark gray, which is the color characteristic of ZnNPs production, and drawing the Uv-Vis spectrum of the mixture using a spectrophotometer and observing the peak of ZnNPs at 266 nm, nanoparticle formation was investigated qualitatively.[14] The characterization of the synthesized nanoparticles was determined using scanning electron microscope, X-ray diffraction (XRD) technique, nano-sizer-zetacizer, and Fourier-transform infrared spectroscopy (FTIR).

Bacteria

S. mutans (ATCC 35668) and *A. viscosus* (PTCC 1202) were grown in tryptic soy broth, blood agar and mitis salivarius agar at 37°C in an atmosphere containing 5% CO_2.

Preparation of standard McFarland 0.5 solution

At first, 0.5 ml of $BaCl_2$ (0.048 mol/l) ($2H_2O$ *W*/*V* $BaCl_{20}$ 1/175%) was mixed to 99.5 ml of sulfuric acid (0.18 mol/l) (*V*/*V* 1%). The optical density of mixture was studied by a spectrophotometer at 625 nm.[15]

Determining the minimum inhibitory concentration (MIC)

To determine the MIC of ZnNPs on standard strains of *S. mutans* and *A. viscosus*, broth micro-dilution method was used in 96 well sterile plates according to Clinical and Laboratory Standards Institute guidelines.[16] Normal saline and chlorhexidine are used for negative and positive control, respectively. In addition, the lowest concentration of the NPs with no bacteria survived was reported as the minimum bactericidal concentrations of NPs.

Statistical analysis

We performed data analysis using SPSS software version 25.0. $P < 0.05$ will be considered as a significant level.

RESULTS

Characterization of ZnNPs

By UV-VIS spectral analysis, the absorption peak in the range of 230–330 nm. Furthermore, EDX analysis of ZnNP showed that Zn adsorption peaks including $ZnL\alpha_1$, $ZnK\alpha_1$, and $ZnK\beta_1$ were 1.01, 8.64, and 9.57 kg, respectively. The results of XRD pattern indicated that 101, 201, 202, 203, and 300 were refractive peaks at 19.6, 36.9, 39, 43.9, and 54.8° [Figure 1a]. By FTIR analysis, ZnNPs had peaks at 1401, 1262, 1064, and 580 cm, which can be linked to CC, CN, CN, and C- (F, Cl or BR). The highest adsorption at 1627 cm, 3418 cm, and 2923 cm can be caused by the polypeptide amide Bond-1, OH stretching [Figure 1b].

By SEM, the synthesized ZnNPs show a spherical shape with some grains of different lengths [Figure 2a]. The size of ZnNPs varied from 30 to 80 nm, while most nanoparticles were between 50 and 60 nm [Figure 2b].

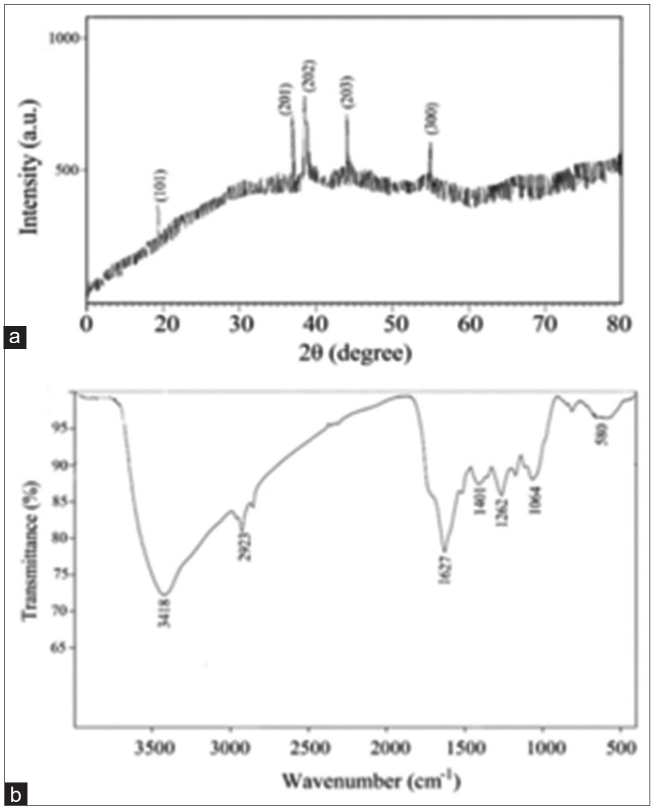

Figure 1: X-ray diffraction patterns (a) and Fourier-transform infrared spectroscopy (b) of zinc nanoparticles using extraction of lavender extract based on microwave method.

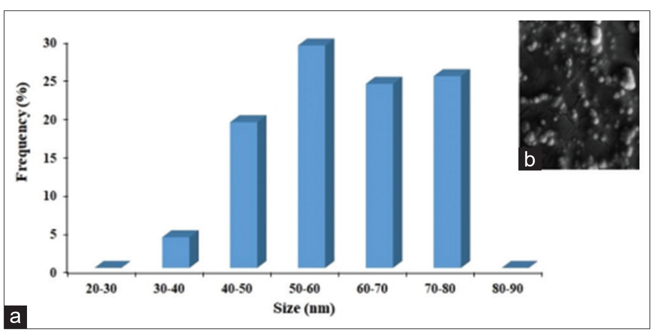

Figure 2: Particle size distribution (a) and scanning electron microscope (b) of zinc nanoparticles using extraction of lavender extract based on microwave method.

Antibacterial effects on *S. mutans* and *A. viscosus*

The MIC after three replications for ZnNPs, chlorhexidine, and the combination of ZnNPs + chlorhexidine on both bacterial species is given in [Table 1]. The highest MICs related to ZnNPs + chlorhexidine were reported with 1.66 and 1.66 µg/ml for *S. mutans* and *A. viscosus*, respectively. Furthermore, the lowest MICs related to ZnNPs alone were obtained with 13.33 and 16.33 µg/ml for *S. mutans* and *A. viscosus*, respectively. ZnNPs + chlorhexidine in comparison with chlorhexidine had a significant ($P < 0.05$) antibacterial effect and inhibited the growth of both bacteria.

DISCUSSION

Oral disease can limit a person's activities at school, at work or at home resulting in the loss of millions of hours of work and study worldwide; in the meantime, tooth decay is one of the most common diseases in the world.[17] Observance of oral hygiene is considered a determining factor in reducing or eliminating tooth decay, so that carries and tooth loss are considered as indicators of the health of the community; but more than 99% of people in human society are still infected and only a few people have not been infected in their lifetime.[18] Various studies have identified several factors involved in predicting the risk of tooth decay, including previous caries experience, patient health habits, socioeconomic factors, diet, and oral microbial flora. Environmental and personal factors such as diet, fluoride exposure, oral hygiene, saliva flow, and safety factors can affect the distribution of oral microflora and the development of dental caries.[19]

The colonization of microorganisms in the mouth begins immediately after birth, and after the teeth erupt, a more complex flora is formed in the mouth. Germs exert their effect on teeth and periodontium through dental plaque, so that the accumulation of some microorganisms on the adjacent dental and gingival surfaces in the form of dental plaque causes caries and periodontal disease and subsequent causes tooth loss.[20] The majority of people in the community cannot effectively remove microbial plaque from their dental surfaces by mechanical means; therefore, the use of chemical methods such as the use of mouthwashes, if they can improve home and daily care, will be an effective way to eliminate or control the formation of microbial plaque and its complications.[21]

Chlorhexidine is a diguanhexidine with specific antiseptic properties that have been accepted to date as the best substance in the treatment of gingivitis. Topical and reversible side effects of this substance include browning of teeth, tongue and silicate and resin restorations, and transient disturbance in the sense of taste.[22] Chlorhexidine mouthwash contains 12% alcohol, which is a concern for clinicians and patients; because regular use of alcohol increases the risk of oral and pharyngeal cancers.[22]

In recent years, nanotechnology has been able to make profound changes in the field of research and production of various products.[9] This technology uses nanometer-sized materials to produce new products; because the material in nanoscale shows a different behavior compared to the same material as a mass.[10] One of the products of nanotechnology is ZnNPs; the effect of ZnNPs has long been known, so

Table 1: Determination of antibacterial effect of ZnNPs by the determining the MIC.

Drug	MIC (μg/ml)		MBC (μg/ml)	
	Streptococcus mutans	*Actinomyces viscosus*	*Streptococcus mutans*	*Actinomyces viscosus*
ZnNPs	13.33±2.4	16.33±1.15	16.33±1.15	16.33±1.15
Chlorhexidine	4.66±1.15	5.33±2.4	5.33±2.4	5.33±2.4
ZnNPs+chlorhexidine	1.66±1.15*	1.66±1.15*	1.66±1.15*	1.66±1.15*

MBC: Minimum bactericidal concentrations, MIC: Minimum inhibitory concentration, ZnNPs: Zinc nanoparticles, * $p < 0.05$ difference was statistically significant

that the ability to deliver zinc through the structure of nanoparticles has dramatically increased its biological and antimicrobial value; so that a very small amount of nanoparticles of Zn is needed to have an antimicrobial effect similar to Zn in mass.[23]

Due to the weaknesses of chlorhexidine mouthwash and the widespread antimicrobial properties of zinc and ZnNPs, in the present study, the antimicrobial effect of ZnNPs alone and in combination with chlorhexidine mouthwash on two dental plaque microorganisms, *S. mutans* and *A. viscosus,* was investigated. The laboratory method used was the sequential Broth-microdilution, which is used as a standard method in laboratories and is more accurate and reliable compared to other approaches such as the diffusion method in the well and the results are easier to interpret than other. In the present survey, the highest MIC of ZnNPs + chlorhexidine was reported 1.66 μg/ml for *S. mutans* and *A. viscosus*; based on this finding, both bacteria showed the same sensitivity to this substance. Furthermore, the lowest MIC values for ZnNPs alone were 13.33 and 16.33 μg/ml for *S. mutans* and *A. viscosus*, respectively. Based on the results, the combination of ZnNPs + chlorhexidine in comparison with chlorhexidine showed a significant antibacterial effect and inhibited the growth of both bacteria. In other words, the combination of ZnNPs + chlorhexidine, in comparison with ZnNPs alone and chlorhexidine alone, at lower concentrations exerted their inhibitory effect on the microorganisms. This means that ZnNPs have a synergistic effect on the antibacterial properties of chlorhexidine.

Similar studies have been performed to evaluate the antibacterial effects of ZnNPs; Shakibaie *et al.* (2019) showed the antibacterial and anti-biofilm effects of ZnNPs and $ZnSO_4$ against *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Proteus mirabilis* with MICs values more than 2560 μg/ml.[24] In another study, Mahdavi *et al.* (2019) have studied the synthesis of Zn NPs by *Ziziphora clinopodioides* Lam and evaluated its *in vitro* and *in vivo* antibacterial (against *Escherichia coli*, *P. aeruginosa* and *Salmonella typhimurium*), antifungal (against *Candida crocus*, and *C. glabrata*), cytotoxicity, antioxidant and wound healing effects. The results of this study showed that ZnNPs have no toxicity *in vivo* on rats and have significant antimicrobial properties on the studied bacteria and fungi.[25] The results of the previous research suggested that ZnNPs may exhibit their antimicrobial effects through disrupting cell permeability, inhibition of cell growth, and induction of apoptosis; producing oxidative stress through the production of H_2O_2 and the release of zinc ions into the environment, which penetrate them through the cell wall and exert their toxic effects.[26] The results of all studies on the antibacterial effect of ZnNPs so far show that this substance inhibits the growth and death of microorganisms in low concentrations. Because eukaryotic cells are much larger than prokaryotes and have more complex structural and functional growths, higher concentrations of zinc ions are required to produce a cytotoxic effect in eukaryotic cells.[26] Therefore, it seems unlikely that the use of ZnNPs in low concentrations that are effective against microorganisms; has toxic effects on eukaryotic cells, of course, this issue needs further investigation.

CONCLUSION

ZnNPs especially combined with chlorhexidine exhibited promising antibacterial effect in comparison with chlorhexidine alone. However, further studies are needed to clarify the accurate mechanisms and toxicity of ZnNPs.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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