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Antimicrobial Activity of Piperine Based Zinc Oxide Nanoparticle against Dental Pathogens

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ABSTRACT

Background: The Piperaceae family, including black pepper, is known for its antibacterial properties. The oral cavity is a major source of microorganisms, including plaque biofilms, which cause infections. Advancements in nanotechnology have led to a growing interest in antibacterial therapies based on nanoparticles. Black pepper, a member of the Piperaceae family, contains polyphenolic substances used in traditional medicine for treating illnesses like melano derma and leprosy. In this study, Piperine incorporated with zinc oxide nanoparticles was tested for efficacy against dental pathogens.

Aim: To determine the antimicrobial activity of Piperine based zinc oxide nanoparticle against dental pathogens

Materials and methods: Zinc acetate dehydrate and NaOH were used as precursors in the direct precipitation technique to create zinc oxide nanoparticles, which were then examined under a scanning electron microscope. Using the 2, 2-diphenylpicrylhydrazyl (DPPH) experiment, antioxidants' capacity to scavenge radicals is assessed. 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) are the ABTS test used to quantify the interaction between an antioxidant and the cation of the pre-generated ABTS+ radical. The Zone of Inhibition test is used to assess a pathogen's sensitivity to antibacterial agents or resistance to them. The outcomes were assessed.

Results and discussion: In this study zinc oxide nanoparticles were doped with piperine and SEM was demonstrated. The SEM images revealed that the uniform distribution and almost spherically shaped piperine mediated ZnO NPs is observed. The DPPH assay & ABTS assay shows antioxidant activity of ascorbic acid, piperine & piperine doped ZnO NP against oral pathogens. In DPPH assay & ABTS assay, there was an increased inhibition rate in piperine mediated ZnO NP when compared ascorbic acid and piperine. Therefore results concluded that piperine mediated zinc oxide nanoparticles showed very good antimicrobial activity against all oral pathogens such as S. mutans, C. albicans and E. faecalis.

Conclusion: Nano-dentistry is a developing field with the potential to address new and improved applications in dentistry, and it has provided a new avenue for revolution in oral care. Our study conclusions suggested that PP-mediated ZnO NPs may provide a useful nanomaterial for the creation of drugs intended to treat oral infections. ZnO nanoparticles mediated by PP showed remarkably potent antibacterial activity against S. mutans, Candida albicans, and E. faecalis, among other oral pathogens. When using 28-nm PP ZnO NPs against E. faecalis, a greatest inhibition zone was seen.

Key words: Piperine mediated Zinc oxide nanoparticles, S.mutans, Candida albicans, E. faecalis, and Nano dentistry.

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INTRODUCTION

An abundance of bacteria is always present in the mouth cavity. Plaque biofilm is a complex community of bacteria or fungi that produces infection by shielding harmful germs from external pharmacological agents and evading the host defensive mechanisms. It is a primary cause of dental illnesses, including caries and periodontitis [1]. Despite the fact that many research have been conducted in an attempt to develop antimicrobial agents to address this problem, most of these efforts have not been able to provide the desired outcomes since antibacterial medications quickly break down and release, which causes low efficacy and safety concerns. With the growth in bacterial resistance, it is imperative to conduct long-term research on suitable alternatives to standard antibacterial treatments. The developments in

nanotechnology have led to a surge in interest in the dental field for antibacterial treatments based on nanoparticles because of their broad antibacterial spectrum, stability, and outstanding antibacterial properties at an inexpensive price [2]. And by overcoming the limitations of single therapy and boosting osteogenesis and remineralization based on antibacterial qualities, the application of multifunctional nanomaterials has considerably increased the long-term prevention and treatment of oral disorders [3].

Nanomaterials are being used more and more in items like semiconductors and antimicrobial surface coatings. Nanoparticles can differ significantly from micrometer-sized particles in terms of their hardness, active surface area, chemical reactivity, and biological activity. In fact, it has been proposed that the biocidal efficacy of metallic nanoparticles stems from their large size as well as their high surface-to-volume ratio [4]. They should be able to intimately interact with microbial membranes owing to these qualities, and the effect won't just come from the release of metal ions [5].Despite this, because of their large specific surface area and high charge density, nano-antibacterial agent's exhibit broad antibacterial range and persistent antibacterial efficacy. Therefore, Reactive Oxygen Species (ROS) are produced by catalytically active nanomaterials and can cause fatal oxidative stress. It also has a high drug loading capacity, can enter eukaryotic cells, and release ions [6].

In this study piperine incorporated with zinc oxide nanoparticles was used to assess the efficacy against dental pathogens. The Piperaceae family, which includes black pepper (*Piper nigrum L.*), is widely harvested for its fruit. The majority of the time, pepper is used in curry preparations and is used in Ayurveda and other traditional medicine cures [7]. Because of the polyphenolic chemicals including tannins, volatile compounds, and phenols, it is used in folk medicine to cure a variety of conditions including leprosy, rheumatoid arthritis, cough, melano-derma, and peripheral neuropathy. It is also used as an antiseptic & antimicrobial agent [8].One of the main reasons ZnO NPs are being studied as partners of antimicrobial drugs is because zinc is an essential trace element that is present in muscle, bone, skin, and the hard tissues of teeth [9].The "Trojan Horse Effect," a recent idea, explains this by claiming that an acidic lysosomal environment stimulates the breakdown of nanoparticles, which in turn causes the conversion of core metals into ions and the release of hazardous chemicals that subsequently stop cell division [10]. Therefore the aim of this study is to determine the antimicrobial activity of piperine based zinc oxide nanoparticles against dental pathogens.

MATERIALS AND METHODS

Characterization of Nanoparticle

Analyzing nanoparticles using a Scanning Electron Microscope (SEM) involves specific sample preparation methods to ensure proper imaging and accurate characterization. If the nanoparticles are part of a larger structure or matrix, consider using fixation methods to preserve the overall structure. If nanoparticles tend to agglomerate, employ methods to de-agglomerate them. This may involve sonication or other dispersion techniques. Attach the nanoparticles onto a clean SEM stub using a conductive adhesive, double-sided carbon tape, or another suitable mounting method. Ensure good electrical contact. If the nanoparticles are non-conductive, consider coating them with a thin layer of conductive material to enhance conductivity and reduce charging effects during imaging. Carefully load the sample stub into the SEM chamber using the designated sample holder. Ensure the sample is securely mounted. Calibrate the SEM instrument, including adjusting the electron beam energy, focus, and stigmation. Use calibration standards to ensure accurate imaging and measurement. Choose SE imaging mode for surface characterization of the nanoparticles. This mode provides detailed surface information. Set the working distance and magnification based on the size and features of the nanoparticles. Start with lower magnifications for an overview before moving to higher magnifications. Use image analysis software to measure particle size, shape, and distribution.

DPPH free radical scavenging assay

In the DPPH free radical scavenging assay, test samples are prepared at various concentrations, and equal volumes are mixed with a freshly prepared DPPH solution in ethanol. The reaction mixture is incubated in the dark at room temperature, allowing for the scavenging of DPPH radicals by antioxidants present in the test samples. After the incubation period, the absorbance of the reaction mixture is measured at around 517 nm using a spectrophotometer. The decrease in absorbance indicates the free radical scavenging activity of the test samples. A blank control without the test sample is included for background correction. The percentage of DPPH radical scavenging activity is calculated using the formula, and a dose-response curve is plotted to visualize the scavenging activity at different concentrations. The IC50, representing the concentration required to scavenge 50% of the DPPH radicals, can be determined, providing a quantitative measure of antioxidant efficacy. The assay is performed in triplicate or more, with appropriate controls and standards, to ensure accuracy and reliability of the results.

ABTS free radical scavenging assay

In the ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) free radical scavenging assay, a stock solution of ABTS is reacted with potassium persulfate to generate the ABTS radical cation (ABTS). The ABTS solution is then diluted to an absorbance of around 0.70 at a specific wavelength usually 734 nm. Test samples, such as antioxidants or plant extracts, are prepared at various concentrations and mixed with the diluted ABTS solution. The reduction of the ABTS radical by antioxidants leads to a decrease in absorbance, which is measured after a suitable incubation period using a spectrophotometer. The scavenging activity is calculated as the percentage inhibition of the ABTS radical compared to a control without the test sample. A dose-response curve is generated, and the IC50, representing the concentration at which 50% of the ABTS radicals are scavenged, can be determined. The assay is performed in triplicate or more, with appropriate controls, to ensure the accuracy and reliability of the results.

Antibacterial activity

The Zone of Inhibition assay is employed to evaluate the antimicrobial activity of substances. Initially, a microbial culture is evenly spread on a solid agar medium. Sterile paper discs or wells are then impregnated with the test substance, such as antibiotics or plant extracts, and placed onto the inoculated agar surface. Following an appropriate incubation period at the microorganism's optimal growth temperature, the plates are examined for the presence of clear zones surrounding the discs or wells, indicating inhibition of microbial growth. The diameter of these zones is measured as the Zone of Inhibition, providing a quantitative measure of the antimicrobial efficacy of the test substance. The assay is performed in triplicate or more for statistical reliability, and appropriate controls, including a blank disc or well, are included to account for any non-specific effects. The Zone of Inhibition assay serves as a valuable tool for screening and comparing the antimicrobial potential of different agents.

Statistics

The data presented in this study are the mean of three replicates and their respective Standard Deviation (SD). Also, the data were subjected to one-way ANOVA and post-ANOVA using Graph Pad Prism (version 5.0). Results were evaluated.

RESULTS

In this study zinc oxide nanoparticles were doped with piperine and SEM was demonstrated. The SEM images revealed that the uniform distribution and almost spherically shaped piperine mediated nO NPs is observed in the SEM image in [Figure 1].

Some piperine mediated ZnO NPs are present with a hexagonal shape and the average particle size is around 25-30 nm. Then DPPH Assay & ABTS assay were accessed, The DPPH assay & ABTS assay shows antioxidant activity of ascorbic acid, piperine & piperine doped ZnO NP against oral pathogens [figures 2 & 3].

In DPPH assay & ABTS assay, there was an increased inhibition rate in piperine mediated ZnO NP when compared ascorbic acid and piperine. And also the zone of inhibition against *Candida albicans*, *streptococcus mutans*, and *Enterococcus faecalis* were tabulated below [figure 4].

The largest inhibition zone of PP ZnO NPs against E. faecalis were observed with 28-nm and while the largest inhibition zones against *S. mutans* and *C. albicans* were observed with 26-nm & 23-nm. Therefore results concluded that piperine mediated zinc oxide nanoparticles showed very good antimicrobial activity against all oral pathogens such as *S. mutans, C. albicans and E. faecalis*. In that the piperine mediated zinc oxide nanoparticles show increased activity when compared with standard drugs.

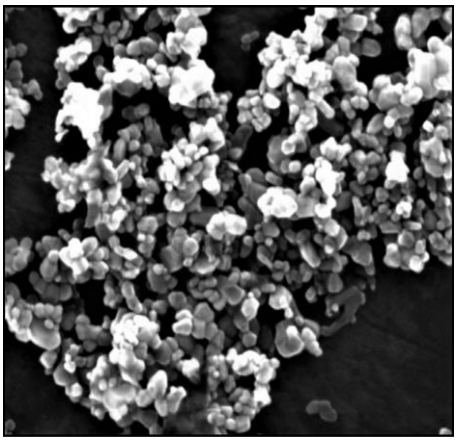

Figure 1: Piperine mediated Zinc oxide SEM image.

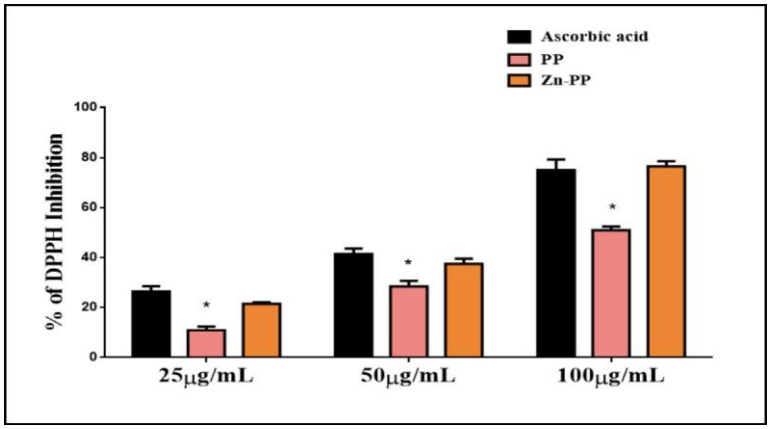

Figure 2: DPPH Assay.

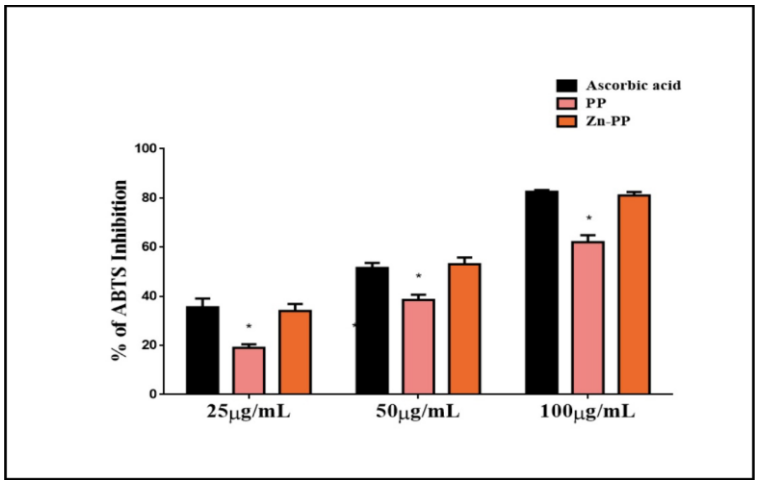

Figure 3: ABTS Assay.

Treatment	Average diameter of Zone of inhibition (mm)		
	Candida albicans	*Streptococcus mutans*	*Enterococcus faecalis*
Amoxicillin	24	27	26
Piperine	14	18	18
Zinc oxide-Piperine Nanoparticle	23	26	28

Figure 4: zone of inhibition rate.

DISCUSSION

Antimicrobial nanomaterials were incorporated into dental materials as a promising substitute for conventional antibacterial medications, leading to numerous advancements in treatment approaches [11]. Nanomaterial-based system advancements can give unique solutions in the prevention and treatment of dental illnesses, particularly in the control of oral pathogenic microorganisms [12].

Zinc oxide nanoparticles can bind to bacterial cells and subsequently enter them, producing structural alterations in the cell membrane such as increased permeability and cell death [13]. This study examined the antimicrobial activity of Piperine mediated zinc oxide nanoparticles against *S. mutans*, *Candida albicans*, and *Enterococcus faecalis*, three oral pathogens. For each of the three pathogens, the PP-mediated ZnO NPs displayed a zone of inhibition. Compared to the other two, the zone of inhibition for E. faecalis was larger, indicating a stronger effect of PP-mediated ZnO nanoparticles against the same. The highest inhibition zone of PP ZnO NPs against *E. faecalis* was seen in the current investigation at 28 nm, which was consistent with prior studies revealed that lower concentrations of nZnO eliminated *E. faecalis* in contrast to other microbes [14].The generation of Active Oxygen Species (AOS), which cling to the cell surface or collect in the cytoplasm of the cell, is responsible for the significant antibacterial action of nZnO on *E. faecalis* [15].

Additionally, Yousef and Danial examined the antimicrobial activity of ZnO and nZnO against a variety of pathogen strains, such as *Aspergillus Niger*, *E. coli*, and *Candida albicans*. They also reported the minimum inhibitory concentration (MIC) of nZnO against *Candida albicans*, which was found to have the lowest antimicrobial effect. This finding is consistent with our current study, which resulted in lower inhibition rate for *Candida albicans* when compared to other pathogens.

Thus, the findings of this investigation indicated that PP-mediated ZnO NPs may be an effective nanomaterial for the development of medications to treat oral infections. Because of their large specific surface area and high charge density, nano-antibacterial agents exhibit sustained antibacterial activity and a broad antibacterial range. Moreover, nanoparticles have a high drug loading capacity, may enter eukaryotic cells, and release ions. The ability of certain bactericidal pathways to prevent antibiotic resistance processes and more successfully combat antibacterial agents presents a chance to address the problem of antibiotic resistance in public health and safety [16].

CONCLUSION

Based on our findings, we conclude that, in comparison to the usual medicine, PP ZnO nanoparticles showed extremely strong antibacterial efficacy against all oral infections, including *S. mutans*, *C. albicans*, and *E. faecalis*. The biological technique appears to be a highly economical substitute for the traditional physical and chemical processes for synthesizing PP Zno nanoparticles, and it would be appropriate for creating a biological process for large-scale manufacturing. To further explore the antifungal effects, more research on high doses of nZnO is recommended. It is also important to investigate the impact of various nZnO solution production techniques on antibacterial activity.

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