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Research Article

Zein Coated Zinc Oxide Nanoparticles: Fabrication and Antimicrobial Evaluation as Dental Aid

[1]Ghada Hussein Naguib, [2]Faten Ebraheem Al-Hazmi, [3]Mallesh Kurakula, [4]Ayman Abdulaziz Al-dharrab, [5]Khaled Mohamed Hosny, [6]Hala Mohammed Alkhalidi, [4]Mohamed Tharwat Hamed, [7]Ali HabibAllah Hassan, [6]Ameen M Al-mohammadi, [8]Abeer Mohamed Alnowaiser and [9]David Henry Pashley

[1]Department of Conservative Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom Saudi Arabia
[2]Department of Physics, Faculty of Science, King Abdulaziz University, Kingdom Saudi Arabia
[3]Department of "Giacomo Ciamcian" Chemistry, Polymer Science Group, University of Bologna, Italy
[4]Department of Oral Rehabilitation, Faculty of Dentistry, King Abdulaziz University, Kingdom Saudi Arabia
[5]Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Kingdom Saudi Arabia
[6]Department of Clinical Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Kingdom Saudi Arabia
[7]Department of Orthodontics, Faculty of Dentistry, King Abdulaziz University, Kingdom Saudi Arabia
[8]Department of Oral basic and diagnostic science, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom Saudi Arabia
[9]Department of Oral Biology, The Dental College of Georgia, Augusta University, Georgia, USA

Abstract

Background and Objective: Bacterial strains resistance to conventional antibiotics has gained the attention of researchers to frame a materialistic approach and to identify an agent from physical source rather than biological. Zinc oxide (ZnO) has proven antimicrobial competence but had not explored as dental aid due to its solubility and agglomeration issues. This study aimed to utilize a newer technique to formulate ZnO nanoparticles and to refurbish with zein coating in order to optimize the desired pharmacological activity. **Materials and Methods:** Zein based ZnO nanoparticles were synthesized using hydrothermal technique at two different pH 1.24 and 11, respectively. Both zein coated and uncoated nanoparticles were characterized for particle size, surface charge, morphology, crystallinity and even metal oxide compatibility studies. *In vitro* dissolution studies were performed in order to determine the effect of zein over the solubility and release of ZnO nanoparticles. Antimicrobial assay for the zein coated ZnO nanoparticles were performed on *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans* using both Kirby-bauer and direct kill tests. **Results:** The uncoated ZnO nanoparticles were in 25 nm size but aggregate, tend to increase up to 280 nm after 1 h aqueous dispersion, oppositely zein coated were 40 nm with no aggregation. Scanning electron microscopy indicated agglomerate of uncoated ZnO nanoparticles while as distinct nanospheres when zein coated. Uncoated and zein coated nanoparticles exhibited a surface charge of 28 and -24 mV at pH 1.24 and -18 and -20 mV at pH 11, respectively. X-ray crystallography indicated crystalline nature and Fourier-transform infrared spectra with no possible interaction or formation of new product. Interestingly, at pH 1.4, zein coated ZnO nanoparticles release was just 20% at 12 h in comparison to uncoated indicating prolonged release. **Conclusion:** The synthesized zein coated ZnO nanoparticles were found to be highly crystalline with enhanced solubility and better compatibilities. Among the four microbial strains tested, zein coated ZnO nanoparticles exhibited higher pharmacological activity against *C. albicans* strains in comparison to the uncoated. These experimental outcomes can indicate the potential of ZnO nanoparticles in formulating an antimicrobial dental aid.

Key words: Zein, zinc oxide, nanoparticles, antimicrobial, dental aid

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Corresponding Author: Ghada H. Naguib, Department of Conservative Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom Saudi Arabia
Tel: 00966558190589

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fashionable materials of therapeutic importance have always been a great interest in nanotechnology world. Tailoring nanomaterials as per specific needs have outranged their use in wider backgrounds[1]. Biofunctional nanoparticles have emerged and gained huge importance due to their super special applications among which metallic based nanoparticles are the key players. Current biomedical and engineering demands have been answered with key innovations based on nanoparticles[2]. Apart from feasibility in altering their chemical properties for specific use, nanoparticle of metal or their oxides withstand rigorous processing conditions that makes them too attractive segment in nanotechnology research fields[3].

Among all the existing metal oxide nanoparticles, zinc oxide (ZnO) is a mineral zincite that is bio-safe, biocompatible with proven strong anti-bacterial properties, which can powerfully resist broad range of microorganisms due to their ability to generate reactive oxygen species (ROS) on the surface of oxides[4]. ZnO has wider applications in the fields of magnetic, gas sensing, optical and even in piezoelectric[5]. The zincite due to its, strong adsorption ability and large catalytic efficiency it is commonly used in the large scale manufacture of ceramics, pharmaceutical excipients, pigments, sunscreens lotions, in antifungal preparations and even in physical phenomenon of wastewater and rubber treatment as well[6-10]. ZnO and titanium dioxide (TiO_2) nanoparticles have indicated strong inhibitions against many resistant bacterial strains, hence forth employed in nanoscaffolds and even in medical devices[10-12]. But most of the research reported that metal oxide based nanoparticles have a drawback of agglomeration in dispersion due to their high surface energy. Therefore, there is a need to balance the surface charge sufficiently in order to obtain a stable redispersable nanoparticulate solution[13,14].

Hybrid materials in nanostructured form with unique properties are regarded as bionanocomposite. These are the end products obtained in the combination of life science, nanotechnology and material science[15]. Bionanocomposite is novel procreation from green technology and are well recognized for their biocompatibility, biodegradability, therefore extensively used in agriculture technologies, food packaging and pharmaceutical products[16,17]. Natural polymers used in bionanocomposite can be manufactured using a protein or mono/polysaccharide in combination. Starch from natural sources is low cost and is freely obtained, apart from bio safe it even exhibits easy film formation characteristics[18]. Zein polymer obtained from natural corn, is an alcohol-soluble protein contained in the endosperm tissue[19]. Zein is even reported as a promising material for film coating especially for preparation of micro or nanoparticles and nanowires of other metals such as magnesium oxide (MgO)[20]. Literature survey reported ZnO nanoparticles encapsulated in chitosan and polyvinyl alcohol based films and evaluated for their antibacterial activity especially on the species *S. aureus*[21]. ZnO based nanorods were evaluated for their potential as filler in starch-based films for its use as active packaging materials in the pharmaceutical and food industries[10]. Even green ZnO nanoparticles were synthesized, that exhibited inhibition against bacterial and fungal pathogens, therefore can to be used effectively in agricultural and food safety applications[22].

It is interesting to try and stabilize the metal oxides such as ZnO using natural polymers or polysaccharides or proteins and none of studies have been reported till date. Therefore, in the present study ZnO nanoparticles were synthesized and coat them using natural corn polymer zein. The prepared zein coated nanoparticles were characterized for physiochemical properties and evaluated for their role in stabilization, in-vitro release and percentage effective inhibition of bacterial and fungal strain in comparison to the uncoated ZnO nanoparticle formulation.

MATERIALS AND METHODS

This study was carried out in Department of Conservative Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia in 2016. Few characterizations were even performed in chemistry laboratory in University of Bologna, Italy. Organic solvents used were of HPLC grade, purchased from Sigma-Aldrich Co. All other chemicals and solvents were of analytical grade. Deionized and distilled water was used throughout the study.

Synthesis of ZnO nanoparticles: All chemicals used in the experiments were of analytical grade and were purchased from Sigma-Aldrich, USA. ZnO nanoparticles were prepared using conventional hydrothermal technique, that has been reported earlier[23].

Preparation of zein coated ZnO nanoparticles: The preparation involves in mixing both the zein and polyvinyl alcohol (PVA). The obtained mixture and ZnO nanoparticles were taken in the weight ratio of 2:1 and 4:1, respectively. Further, an earlier reported method without any modification was used in this study[24].

Size and zeta potential of zein coated ZnO nanoparticles: The particle size distribution of Zein coated ZnO nanoparticles were measured using dynamic light scattering (Nano-ZS, Malvern Instruments, UK). From the measured distributions, Z-average diameters of the nanoparticles were calculated. A micro-electrophoresis device (Nano-ZS, Malvern Instruments, UK) was used in order to determine the surface charge of the Zein coated ZnO nanoparticles. For both the measurements, the samples were diluted with the water (same pH) or buffer solution in order to avoid or reduce the multiple scattering effects.

Surface morphology of the zein coated ZnO nanoparticles: In order to record the surface morphology of the prepared Zein coated ZnO nanoparticles, the scanning electron microscopy (SEM) was performed. (Carl Zeiss Meditec AG, Jena, Germany) The nanoparticles were evenly mounted on to the metallic stub and further sputter gold coated. All the sample images were recorded at an acceleration voltage of 15-18 kV. The data was recorded and saved.

X-ray diffraction (XRD) of the Zein coated ZnO nanoparticles: The extent of crystallinity of the prepared Zein coated ZnO nanoparticles were examined using Shimadzu XRD-6000 X-ray powder diffractometer. The measurements were performed CuKα radiation (0.15500 nm) at 16 kV and 31 mA for the X-ray tube. The phase type and content was recorded keeping the scanning rate was 5°C min^{-1} as constant. A complete 2θ scan was made between 10 and 80°C for individual measurement. The data was recorded and saved.

Differential Scanning Calorimetry (DSC) of the Zein coated ZnO nanoparticles: Thermogravimetric analysis (TGA) of Zein coated ZnO nanoparticles was executed by an non-isothermal condition using Shimadzu differential scanning calorimeter DSC-60 with an accuracy of 61.1 K under air flow at a flow rate of 50 mL min^{-1}. Dried Zein coated ZnO nanoparticles (approx. 2–3 mg) were sealed in clean aluminum pans. Empty sealed aluminum pan has served as control. The samples were heated from temperature of 25-800°C at a scan rate of 10°C min^{-1}. The data was recorded and saved.

Fourier Transform Infrared spectroscopy (FTIR) of the nanoparticles: Fourier Transform Infrared (FTIR) spectroscopy for the prepared nanoparticle samples were performed using a Nicolet DTGS TEC detector spectrophotometer from 400-4000 cm^{-1} using the KBr pellet method.

Antimicrobial assay: Antimicrobial activity of the ZnO zein nanoparticles were examined using Kirby-Bauer agar diffusion test[25] and also by modified direct contact test[26].

Preparation of the materials and bacterial cultures: The antimicrobial efficiency of the ZnO nanoparticles was determined by measuring the zone of inhibition diameter against the strains of bacteria and fungi stricken over the blood and sabouraud agar plates. *Streptococcus mutans* (ATCC 25175, gram-positive coccus), *Staphylococcus aureus* (ATCC 6538, gram-positive coccus), *Enterococcus faecalis* (ATCC 29212 gram positive coccus) and *Candida albicans* (ATCC10231 fungus) were used to study the antibacterial activity of zein based ZnO nanoparticles. All the strains used in the study were purchased from the American Type Culture Collection (ATCC).

Determination of antimicrobial activity of zein coated ZnO nanoparticles

By kirby-bauer test: Fresh bacterial inoculum was prepared and cultured on sterile agar plates. Paper disc of 6 mm diameter was prepared and treated by placing them in test solution (100 µL). After the treatment the discs were placed on the plates. Further the plates were incubated at 37°C for 24 h. Each plate had 4 treated discs and zone of inhibitions were measured around each disc. The experiments were performed in triplicate and standard deviation was calculated.

By direct kill test

Nanoparticles preparation: Both ZnO nanoparticles and Zein coated ZnO nanoparticles solutions were prepared in the concentration range of 0.3, 0.5, 1 and 2%, respectively, using distilled water. All the prepared samples were sonicated at 30 kHz using probe sonicator (Sonics vibra cell, VCX 750, Connecticut) for about 3 min.

Bacteria preparation: 0.5 Mc Farland in broth solutions were prepared using the bacterial strains of *Streptococcus mutans* ATCC 25175, *Staphylococcus aureus* (ATCC 6538, gram-positive coccus), *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231. Both the bacterial and individual nanoparticle solutions were taken in a ratio of 1:1 and incubated at 37°C at different intervals of 2, 24 h, 3, 4, 5, 6 and 7 days. At each incubation interval the concentration of the ZnO nanoparticles alone and the ZnO nanoparticles coated with zein were tested for bacteria/fungi growth by streaking agar plates, incubating them 37°C for 24 h and evaluating for bacterial or fungal growth by colony forming units (CFU). The number of colonies formed were plotted and graphed for individual strains.

***In vitro* dissolution studies of zein coated ZnO nanoparticles:** *In vitro* dissolution studies were performed in order to ascertain the role of zein over altering the solubility or release of ZnO nanoparticles. Two formulations i.e. uncoated ZnO, zein coated ZnO nanoparticles prepared at pH 1.2 and 11 were considered for the release test. The dissolution studies were performed using earlier reported method[20].

RESULTS

Size and charge of zein coated ZnO nanoparticles: The uncoated ZnO nanoparticles were determined having an average size of 25 nm but tend to aggregate closely on exposing in aqueous dispersion for 1 h gradually increases the size up to 280 nm. Interestingly, the zein coated ZnO nanoparticles were found to have an average size of 40 nm. There was no further size increase observed for exposure up to 12 h in the aqueous medium.

Surface morphology of zein coated ZnO nanoparticles: The SEM images of pure uncoated ZnO nanoparticles revealed size about 50-60 nm wide irregularly shaped in appearance in the state of aggregation (Fig. 1a). After coating with the 1% zein polymer, the ZnO nanoparticles were in spherical form and very distinct as nanospheres without any aggregation (Fig. 1b).

X-ray diffraction Zein coated ZnO nanoparticles: The x-ray diffractometry spectra of zein pure polymer have not indicated any sharp peaks proving the amorphous nature. The spectra of zein coated ZnO nanoparticles indicated sharp and strong peaks located at the 2θ value of 28.8, 33.7 and 36.4°C relating to (111), (200) and (220) planes respectively indicating the high crystalline state in comparison to the uncoated ZnO nanoparticles having peaks at 30.1, 34.3 and 37.2°C. Further, no additional diffraction peaks were observed, reflecting that the zein coated ZnO nanoparticles were pure in nature (Fig. 2).

Differential scanning calorimetry (DSC) of zein coated ZnO nanoparticles: DSC spectra of uncoated ZnO nanoparticles showed a single endothermic peak at 350.25°C indicating that the sample is pure. In case of zein coated ZnO nanoparticles the peak has shifted to 375.3°C, this transition takes place at a much higher temperature. The pure zein polymer showed an endothermic peak at 381.5°C (Fig. 3).

Fourier transform infrared spectroscopy (FTIR) zein coated ZnO nanoparticles: FTIR spectra of uncoated ZnO nanoparticles showed that IR absorption bands at 2750 and 3200 cm^{-1}, these corresponds at O-H stretching mode of hydroxyl groups present due to moisture over the superficial layer (Fig. 4). The peaks at 3200 cm^{-1} is majorly due to the

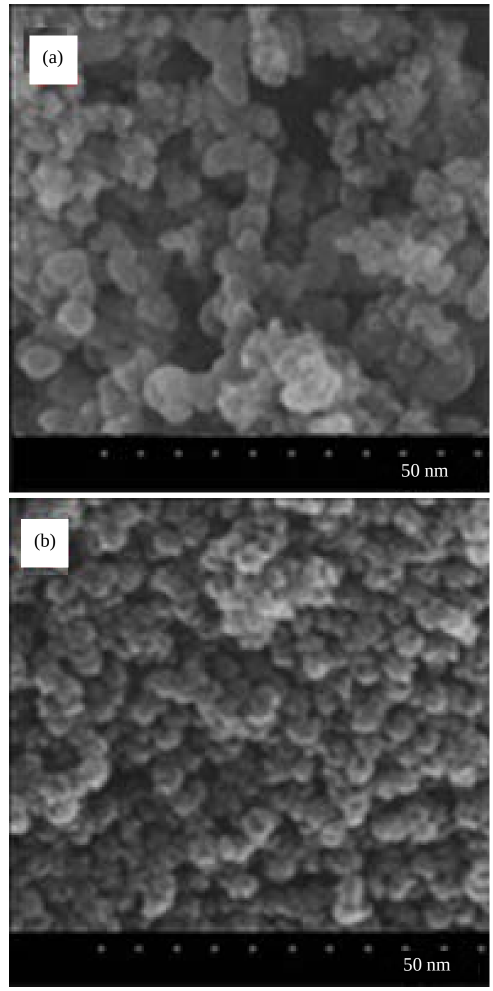

Fig. 1(a-b): SEM images (a) Uncoated ZnO nanoparticles (b) Zein coated ZnO nanoparticles

C-H bond resembling in the spectra of pure zein (Fig. 5) and in zein coated ZnO nanoparticles. A symmetric stretching band at 1400-1450 cm^{-1} is observed in both pure zein and zein coated ZnO nanoparticles due to the CH2 group present in zein polymer[27]. A sharp intense peak is observed at 3400 cm^{-1} in zein coated ZnO nanoparticles (Fig. 6) indicating the H-bonding between ZnO and OH of zein. No other peaks were observed indicating formation of new compounds.

***In vitro* dissolution release of nanoparticles:** The uncoated ZnO nanoparticles released 90% of ZnO in 12 h indicating high solubility of the ZnO with respect to zein stabilized nanoparticles with 18%. Both coated ZnO nanoparticles formulations at different pH indicated interesting release pattern. At 12 h, pH 1.2 stabilized ZnO nanoparticles indicated a sustained release of about 40% in comparison to 70% release of ZnO stabilized at pH 11 (Fig. 7).

Evaluation of Antibacterial activity of ZnO nanoparticles

By kirby-bauer test: Zein coated ZnO nanoparticles in 0.3, 0.5, 1 and 2% concentrations showed zones of inhibition against *C. albicans* (18, 19, 17 and 19 mm, respectively). While non-coated ZnO nanoparticles and zein polymer did not show any zones of inhibition (0 mm).

By direct kill test: All the different concentrations of zein coated ZnO nanoparticles (ZnOZ) and of ZnO alone showed same growth of bacteria (*S. mutans*, *S. aureus*, *E. faecalis*) and the fungi *C. albicans* after 2 h, 1, 2, 3, 4, 5, 6 and 7 days incubation at 37°C (10^6 CFU). While at day 4 All the concentrations of ZnO-zein showed decrease in *C. albicans* count (10^4 CFU) then in day 7 (10^3 CFU). While at day 4 all the concentrations of ZnOZ showed decrease in *C. albicans* count (10^4 CFU) then in day 7 (10^3 CFU). While different concentration of ZnO alone showed a decrease in *C. albicans* count by day 6 (Fig. 8).

In case of *S. aureus*, all the different concentrations of zein coated ZnO nanoparticles (0.3, 0.5, 1 and 2%) showed a decrease in bacteria count by day 6 (10^4 CFU) then day 7 (10^2 CFU). While all the different concentrations of ZnO nanoparticles alone (0.3, 0.5, 1 and 2%) showed a decrease in bacteria count by day 6 (10^4 CFU) then no growth by day 7 (0 CFU) (Fig. 9).

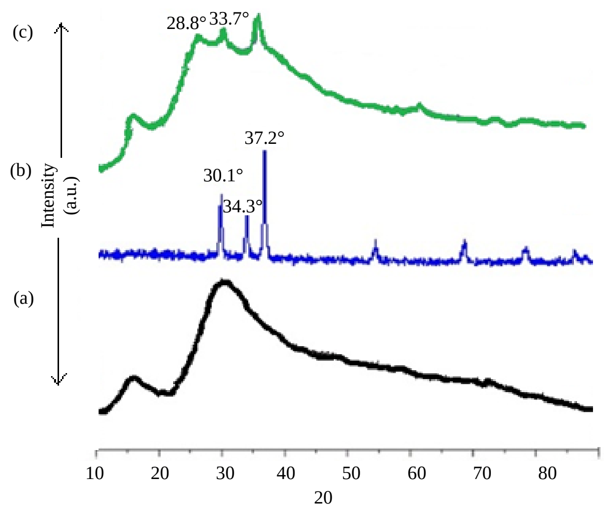

Fig. 2(a-c): XRD pattern of (a) Pure zein polymer, (b) Uncoated ZnO nanoparticles, (c) Zein coated ZnO nanoparticles

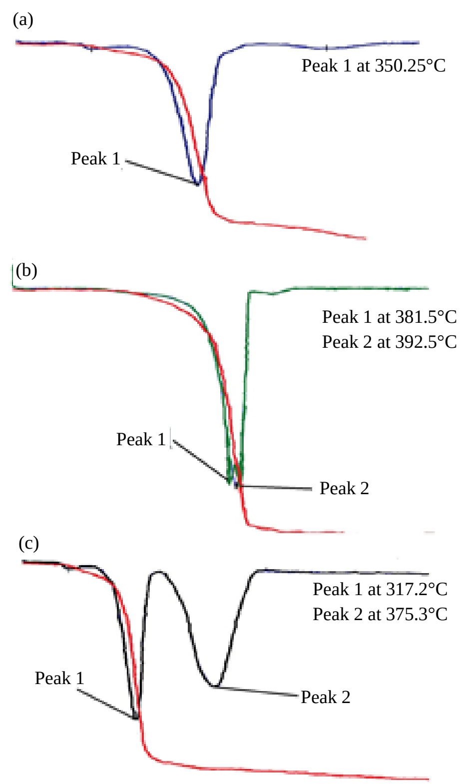

Fig. 3(a-c): DSC spectra of (a) Uncoated ZnO nanoparticles (b) Pure zein polymer, (c) Zein coated ZnO nanoparticles

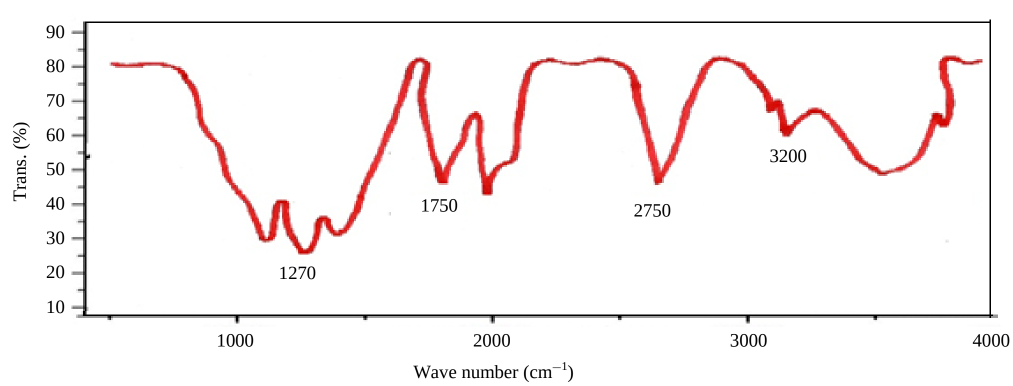

Fig. 4: FTIR spectra of uncoated ZnO nanoparticles

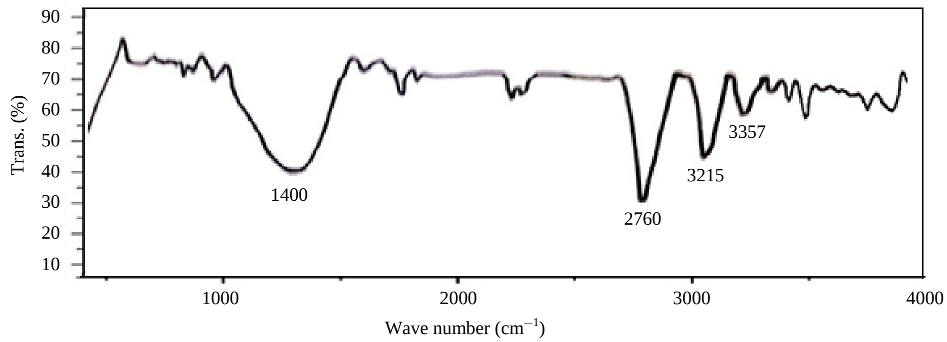

Fig. 5: FTIR spectra of pure zein polymer

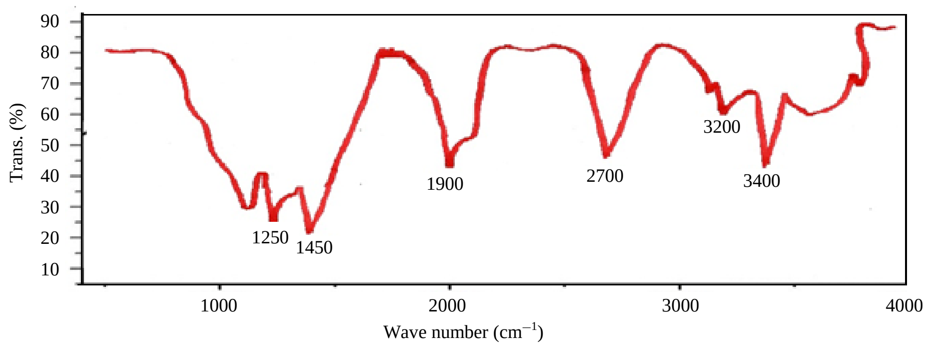

Fig. 6: FTIR spectra of zein coated ZnO nanoparticles

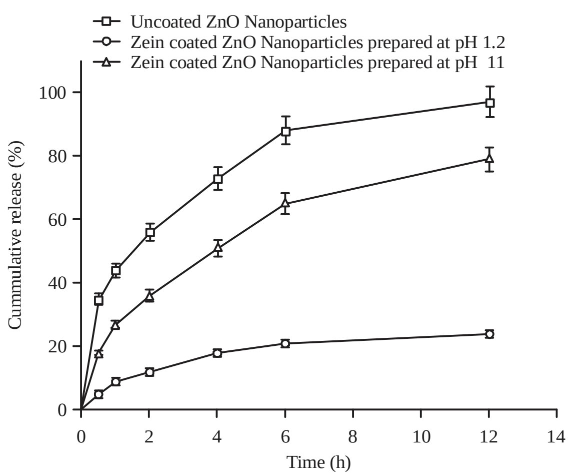

Fig. 7: Comparative In-vitro dissolution release of uncoated ZnO nanoparticles and zein coated ZnO nanoparticles prepared at pH 1.2 and pH 11, respectively

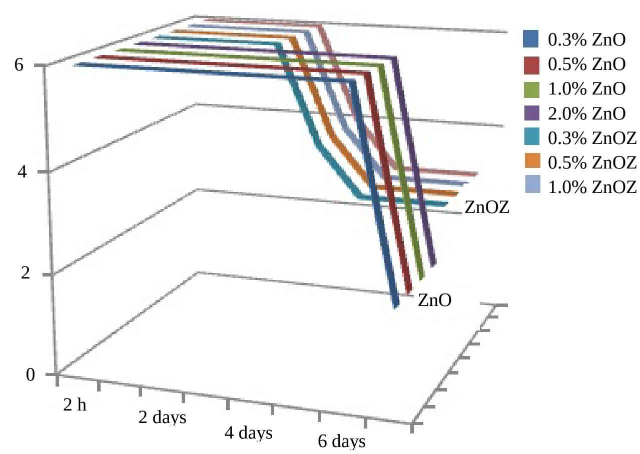

Fig. 8: Line Graph showing the fungi count (CFU) of *C. albicans* after direct contact with different solutions from 2 h till 7 days

DISCUSSION

In the present study, the zein coated ZnO nanoparticles have been successfully synthesized having an average size of 40 nm in comparison to uncoated. The surface charge of the nanoparticles prepared at pH 1.2 changed from 22 to -24 mV. This is due to the fact that ZnO exhibits a positive charge below its iso-electric point (pH 8.4). Both +ve/-ve charges between ZnO and zein establishes an electrostatic stabilization allowing efficient coating in comparison to the

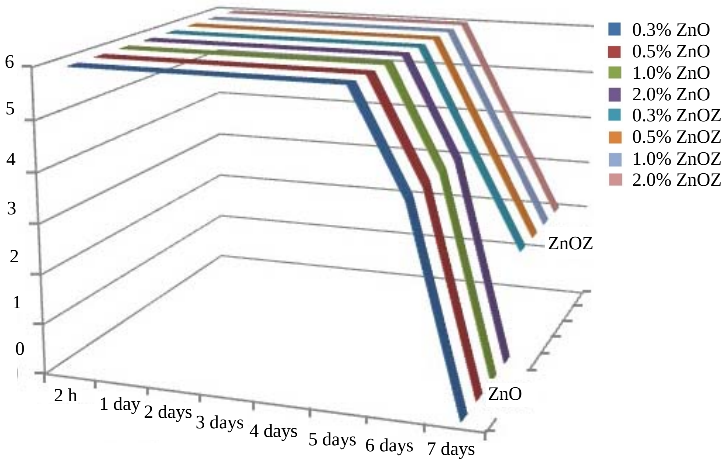

Fig. 9: Line graph showing the bacteria count (CFU) of *S. aureus* after direct contact with different solutions from 2 h till 7 days

formulation at pH of 11. Scanning electron microscope (SEM) images indicated that ZnO resembling as nanospheres, crystalline form similar to the earlier reported magnesium oxide nanoparticles[20] with great stability. The x-ray diffraction pattern (Fig. 2) for the zein coated nanoparticles indicates that the powder is highly crystalline and pure[28,29]. DSC test usually corresponds to the melting point and purity of the analyze, if any. In case of ZnO nanoparticles, ZnO melted at 381°C and shifted to 345°C when coated with zein indicating the decrease in degree of crystallinity of ZnO, possible due to interaction between the polymer and nanoparticles[18]. Well-defined absorption bands were observed in the FTIR spectrum with no possible interactions or no formation of new compounds (Fig. 4). The band at 3310 cm^{-1}, corresponds to the hydrogen bonded hydroxyl group of zein and ZnO indicating that they blend well. This hydrogen bonding aids in ZnO nanospheres stability as reported in previous research[12]. The *in vitro* dissolution was even found to be sustained, cumulative release was approx. 20% for about 12 h for the formulation prepared at pH 1.2. This is majorly due to the fact that at pH 1.2, zein was efficiently coated over ZnO nanoparticles and swells by absorbing moisture. Therefore, a stable formulation with respect to uncoated and prepared at pH 11[20]. This feature can be beneficial in designing a dental aid for prolonged antimicrobial activity. The zein coated ZnO nanoparticles[30,31] exhibited better antimicrobial activity with clear zones of inhibition over *C. albicans* in the Kirby bauer test[32] (Fig. 6) in comparison to other three microbial strains. Also, coating with zein polymer usually increases the ability of ZnO to form more uniform pastes as zein polymer decreases the roughness in the surface of ZnO particles and make the surface more smooth which is essential in preparation of ZnO based dental pastes[33]. This acquired property may play an important role in formulating a sustained release dental aid of specifically having antifungal property[34].

CONCLUSION

Zein based ZnO nanoparticles was formulated and pharmacologically evaluated in the current study. Coating of ZnO using zein in the fabrication of nanoparticles has proven to enhance both the physiochemical and release properties in comparison to ZnO nanoparticles. These zein based anti-microbial materials are safer and can be used both as dental aid and redesigning an antibacterial delivery system.

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SIGNIFICANCE STATEMENT

This study suggests a novel technique to optimize the activity of ZnO as dental aid, as ZnO is biocompatible, non-toxic agent but has solubility and agglomeration issues, therefore is not explored as complete antimicrobial agent or aid. Refurbishing ZnO nanoparticles by coating with zein as stabilizing agent outcomes a dental aid with superior pharmacological and antibacterial activity. Furthermore, it will help researchers to uncover an important topic utilizing and exploring coating of other metallic oxide antimicrobial agents with zein polymer.

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