Research Article

Zein-Based Zinc Oxide Nanoparticles for Metformin Encapsulation: Synthesis, Characterization, and Biological Applications.

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ABSTRACT

Introduction and Aim: Type 2 diabetes mellitus (T2DM) remains a major global health challenge requiring improved drug delivery strategies. Zein, a maize-derived biopolymer, has attracted attention as a drug carrier because of its biocompatibility and controlled-release properties. This study aimed to synthesize zein-based zinc oxide nanoparticles (Zein-ZnONPs), characterize their physicochemical properties, evaluate their biological activities, and investigate their potential for metformin hydrochloride delivery.

Materials and Methods: Zein-ZnONPs were synthesized using a solution combustion approach and characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), dynamic light scattering (DLS), zeta potential analysis, and scanning electron microscopy (SEM). Metformin hydrochloride was encapsulated into the nanoparticles and evaluated by SEM and UV-Vis analysis. Antioxidant, anti-inflammatory, and antibacterial activities were assessed using DPPH radical scavenging, protein denaturation inhibition, and disc diffusion assays, respectively. Drug-release behavior was studied under simulated gastrointestinal conditions.

Results: Zein-ZnONPs exhibited particle sizes of 105-222 nm and a zeta potential of +4.5 mV. Characterization studies confirmed the formation of crystalline ZnO nanoparticles stabilized by zein. The nanoparticles showed concentration-dependent antioxidant activity with 95% DPPH inhibition at 100 µg mL^{-1} and anti-inflammatory activity with 90.7% inhibition of protein denaturation at 1000 µg mL^{-1}. Moderate antibacterial activity was observed against the tested microorganisms. Metformin-loaded Zein-ZnONPs exhibited a biphasic drug-release profile with an estimated half-life of 7.7 h.

Conclusion: Zein-ZnONPs demonstrated favorable physicochemical properties, biological activities, and sustained drug-release behavior, indicating their potential as a nanocarrier for metformin delivery. Further studies are required to evaluate their therapeutic applicability *in vivo.*

Keywords: *Zein-ZnONPs; Metformin; Antioxidant; Maize; Zinc oxide; Anti-inflammatory; Antibacterial*

1. INTRODUCTION

The enhancement in nanotechnology has completely evolved the field of biomedicine. From design to delivery, nanosystems have augmented the efficacy, target and reduced the side effects of several drugs. One of these nanocarriers is biopolymer-based nanoparticles. Their biodegradable and less toxic nature makes them an environmentally friendly alternative to synthetic polymers [1]. Zein is a prolamin-class protein which can be extracted from *Zea mays* i.e., maize. It is a polymer which is thermal-stable and hydrophobic in nature. It can be used to synthesize nanoparticles (NPs) or non-toxic films for food preservation. The coatings of these films can extend the durability of foods that are going to be perished [2]. It can also encapsulate many compounds which are bioactive starting with drugs (small-molecule) to peptides. NPs based on this natural polymer are reported to be highly efficient for encapsulation, pH responsive and controlled release of the drugs [3].

Zinc-oxide nanoparticles (ZnONPs) are known to possess excellent bioactivities. ZnONPs have been reported in several accounts for their strong antioxidant and anti-inflammatory activities. There have also been reports of showcasing certain antimicrobial and anticancer attributes. High surface area to volume ratio and regulatable surface charge constitutes the activity profile of ZnONPs. Zinc (Zn) is an element that is involved in the functioning of several enzymes and cellular pathways. ZnONPs can integrate with zein to provide a more stable (structure) and functional synergistic effects [4]. This integration of zein and ZnO nanocomposites is an example of hybrid technology development as well. As such the capacity of zein i.e., its activity as a drug carrier and the ZnO's stability and bioactivity are merged together [5]. This type of systems can become a carrier that has dual purpose. A zein-encapsulated ZnONPs can act as an agent for the reduction of oxidative stress while protecting against the antimicrobial activity. These bioactivities of the encapsulated zein-ZnONPs come into play in case of chronic conditions. For instance, in diabetes mellitus management, ZnONPs are reported to decrease the oxidative stress and inflammation. ZnONPs can also protect the pancreatic β-cells along with insulin sensitivity [6].

Type 2 Diabetes mellitus (DM2) poses a concern for the health condition worldwide. Inadequate insulin secretion coupled with insulin secretion (or without) results into chronic hyperglycemia leads to DM2. Such patients often show conditions including the likes of increasing in the levels of oxidative stress. This furthers into systemic inflammation and become susceptible to microbes as well [7]. These complications demand agents that can act in multiple ways. The available drug at present that is widely used for management is metformin hydrochloride (MH). It falls under biguanide-class and represents a first-line therapy for DM2. In this case, it suppresses gluconeogenesis to lower the blood glucose level. MH also reduces the risk of hypoglycemia through the activation of AMPK. It is safe and also benefits the cardiovascular health. In spite of its benefits, MH also faces challenges in case of renal clearance and bioavailability. It has also been reported to cause gastrointestinal complications [8]. This leads to the search for alternatives with better efficacy. Delivery systems based on nanoparticles have been researched to contemplate the limitations. The advantage of metformin encapsulated by nanocarriers based on biopolymer will enhance the efficacy. This hybrid matrix also enhances the retention of the drug and controlled release [3].

Type 2 diabetes mellitus is frequently associated with increased oxidative stress, chronic inflammation, and a higher susceptibility to infections, all of which contribute to disease progression and complications. Therefore, nanocarrier systems possessing antioxidant, anti-inflammatory, and antibacterial properties, in addition to drug delivery capability, may offer supplementary therapeutic advantages. Zein-ZnONPs may represent a promising multifunctional platform for metformin delivery because of the combined carrier properties of zein and the biological activities associated with ZnO nanoparticles. However, further experimental and in vivo investigations are required to validate these potential benefits under physiological conditions.

This study reports the synthesis of zein-ZnONPs to encapsulate MH. The potential of this encapsulation for controlled release delivery system has also been evaluated. Physico-chemical characterisations such as UV-Vis, DLS, FTIR, XRD, and SEM were performed. Their bioactivities were also assessed through DPPH radical scavenging assay, protein denaturation inhibition and disc diffusion tests. This research aims to provide a comprehensive evaluation of zein-ZnONPs and their therapeutic potential for managing DM2.

2. MATERIALS & METHODS

2.1 Extraction of zein protein

N-hexane was used to defat corn gluten meal (20 g) and dried at room temperature (RT). The powder achieved was mixed with 100 mL ethanol (70% v/v) and stirred for 2 h. The suspension underwent centrifugation (15 min, 12000 rpm) after solubilisation of zein. It assures to separate the residues that were insoluble. The

supernatant contains zein. It was further diluted with an equal volume of distilled water. This results into precipitation of the zein protein. The protein was further washed two times with ethanol (70%), air-dried and stored (desiccator) for future use [9].

2.2 Synthesis of zein-based zinc oxide nanoparticles (zein-ZnONPs)

A solution combustion approached was adopted to synthesize zein-ZnONPs [10]. 8 g Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) was stirred continuously with 10 mL distilled water. It forms a homogenous transparent solution. Zein protein solution (3 mL) was added drop by drop while magnetic-stirred for 30 min. The uniform mixture was transferred into a ceramic crucible. It was placed in a muffle furnace (400 ± 10 °C; preheat). The combustion started rapidly evidenced by the foamy and light-coloured combustion ash. This indicates preliminary formation of ZnONPs. They were calcined for 2h at 700 °C and resulted into a pale-yellow nanopowder. It was cooled off and ground into fine particles (mortar and pestle). The particles were accumulated in a air-tight container for further use.

2.3 Characterizations

Zein-ZnONPs underwent several characterization techniques. UV-Visible spectroscopy was employed to record the absorbance spectra at a range starting from 200 nm to 800 nm. This was followed by Dynamic Light Scattering (DLS) for particle size distribution and zeta potential analysis for colloidal stability and surface charge respectively. KBr pellet method was adopted for Fourier Transform Infrared Spectroscopy (FTIR). A range of 4000-400 cm^{-1} was scanned to identify the functional groups present in the formed NPs. X-ray Diffraction (XRD) analysis was improvised to check the crystallinity of zein-ZnONPs using Cu Kα radiation (2θ range of 10° to 80°). Zein-ZnONPs were gold-coated for imaging in high vacuum condition. Scanning Electron Microscopy (SEM) was used to determine the estimate particle size and surface morphology of zein-ZnONPs.

2.4 *Zein-ZnONPs* and Metformin encapsulation

Zein-ZnONPs (100 mg) was added with MH (50 mg) alongwith 1 mL ethanol (70%). It was vortexed and stirred gently ensuring to mix properly. This was added slowly to 10 mL of deionized water dropwise. In order to make certain of the precipitation and encapsulation, continuous magnetic stirring is extremely necessary. The dispersion, thus obtained, was incubated for 10 min at RT and centrifuged (30 min; 10000 rpm). The pellet washed with deionized water (twice) was transferred into a sterile petri dish. Parafilm was used to cover the sample. It was allowed to freeze for 24 h (-20 °C). Lyophilisation provided the dried powder i.e., Zein-ZnONPs loaded with metformin. It was kept at 4°C until further use. The drug loading was determined by UV-Vis spectrophotometry [11].

2.5 Biological Activity Assays

2.5.1 Antioxidant activity (DPPH)

Zein-ZnONPs were checked for their antioxidant properties through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [12]. The Zein-ZnONPs were prepared for concentrations varying from 20, 40, 60, 80 to 100 μg mL^{-1} in distilled water. 1 mL of each NPs' concentrations was added with 1 mL of 0.1 mM DPPH solution (in 75% ethanol) in a test tube. The mixtures were vortexed (30 s) and incubated in the dark (30 min) at RT. The absorbance was recorded at 517 nm. Ascorbic acid was the positive control and ethanol (75%), the blank. The antioxidant activity calculated using the formulae:

$$\%\ Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ represents absorbance of the control i.e., DPPH and ethanol,

A_{sample} represents absorbance of the test sample i.e., DPPH and Zein-ZnONPs was expressed in the form of percentage inhibition.

2.5.2 Anti-inflammatory activity

Protein denaturation Inhibition

Egg albumin was the source of protein used for protein denaturation inhibition assay [13]. 0.2 mL of egg albumin was added with phosphate buffer saline (2.8 mL, pH 6.4 PBS). Different

concentrations of zein-ZnONPs (250, 500, 750, and 1000 μg mL^{-1}) were prepared in ethanol. 0.6 mL of each was mixed with to the mixture of egg albumin and PBS. Pre-incubation [10], (10 min; 37 °C) and heating (20 min; 70 °C) of samples in a water bath is a must for inducing protein denaturation. The mixtures were allowed to cool to RT and absorbance was recorded at 660 nm. Samples without the nanoparticles acted as negative control. The positive control was diclofenac sodium. The percentage inhibition was determined from the following formula.

$$\% \, Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ denotes the absorbance of the controls,

A_{sample} denotes the absorbance of the test sample.

2.5.3 Antibacterial activity

Disc diffusion technique was used to assess the zein-ZnONPs' antibacterial activity [14]. *Escherichia coli* and *Salmonella abony* were selected for the Gram-negative isolates and *Staphylococcus aureus* as Gram-positive isolates. Prior to testing, they were sub-cultured from the cultures in the nutrient agar slants maintained at 4 °C. 0.5 McFarland standard (~ 1.5×10^8 CFU mL^{-1}) population was upkept while preparing the Mueller-Hinton agar plates. 6 mm paper discs with Zein-ZnONPs' different concentrations (0.25, 0.5, 1.0 mg mL^{-1}) were placed to the inoculated agar plates. The positive control was tetracycline and sterile distilled water, the neagtive control. This was followed by incubation for 24 h (37 °C) under aerobic environment. The zones of inhibition were measured (in mm) for the antibacterial efficacy. All tests were conducted in triplicates.

2.6 *In-vitro* Drug Release Study

A gastrointestinal environment of simulated gastric fluid (SGF, pH 1.2) and phosphate buffer (pH 6.8) was used as a mimic. Zein-ZnONPs loaded with metformin (≡ 1 mg drug) was added to 10 mL of respective dissolution medium. It was placed in a beaker (37 °C) with constant stirring at 100 rpm. 1 mL aliquots were taken from the release medium at time intervals of 0, 1, 2, 4, 6, and 24 h. It was immediately substituted with fresh buffer every time to maintain sink conditions. The amount of metformin released from the collected and filtered samples were estimated by reading the absorbance at 280 nm. A metformin standard calibration curve was used to determine the drug concentration of each sample. The cumulative release data was fitted to mathematical models like zero-order, first-order, Higuchi, and Korsmeyer-Peppas to analyze the kinetics of drug release.

3. RESULTS & DISCUSSION

3.1 Extraction of zein protein

Zein protein was successfully extracted from corn gluten meal using 70% ethanol in combination with alkali and reducing agents. The extracted protein appeared as a yellowish-orange powder, consistent with previous reports on zein obtained using alcohol-based solvents [9].

3.2 Synthesis of zein-ZnO nanoparticles

Zein-ZnONPs were successfully synthesized using the solution combustion method, with zein serving as a capping and stabilizing agent. The reaction of zinc precursor in the aqueous zein medium produced a foamy ash during combustion at elevated temperature. The resulting material was calcined at 700 °C to improve ZnO crystallinity and remove residual organic matter. A pale yellow powder was obtained after calcination, confirming the formation of ZnO nanoparticles. Zein contributed to nanoparticle stabilization by promoting dispersion and limiting agglomeration through the formation of a protein-based matrix around the ZnO core. The method provided a simple, reproducible and greenroute for producing zein-ZnONPs suitable for drug loading and subsequent biological evaluation.

3.3 Characterizations

3.3.1 UV-Visible spectroscopy

UV-Visible spectrophotometry was employed to confirm the formation of zein-ZnONPs and assess their optical properties. The absorption spectrum revealed a distinct absorption peak at ~367 nm (Fig. 1a). This is characteristic of ZnO nanoparticles and corresponds to the intrinsic band-gap absorption arising from electronic transitions from the valence band to the conduction band [15]. Wali *et al.*, reported a similar peak at 360 nm, supporting the formation of nanooscale ZnO [16]. The slight blue shift from the typical bulk ZnO peak (~370-380 nm)

indicates particle size redeuction to the nanoscale through quantum confinement. Zein may also mix into the matrix and alter the surface electrons, affecting the sharpness and position of the peak [17]. Enhanced absorption between 200 and 300 nm was also observed, which may arise from zein protein and its interaction with ZnO.

3.3.2 XRD analysis

XRD analysis was performed to determine the crystalline nature and phase purity of zein-ZnONPs. The diffraction pattern (Fig. 1b) displayed prominent peaks at 2θ values of approximately 31°, 34°, 36°, 47°, 56°, 62°, and 68°, corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes of the hexagonal wurtzite structure of ZnO. The peak positions are consistent with JCPDS card No. 36-1451 and previously reported data from Shefali *et al.*, [18]. The well-defined peaks confirm the crystalline nature of the ZnO component. No additional peaks corresponding to crystalline impurities were detected, indicating the absence of detectable secondary crystalline phases.

3.3.3 FTIR analysis

FTIR spectroscopy was performed to identify the functional groups of zein-ZnONPs and assess interactions between zein and ZnO (Fig. 1c-d). FTIR spectra of zein-ZnONPs showed characteristic bands at 3327 cm^{-1} (corresponding to O-H and N-H stretching vibrations), 2973-2926 cm^{-1} (associated with aliphatic C-H stretching), 1650-1540 cm^{-1} (corresponding to the amide I and amide II bands of zein) and 540-516 cm^{-1}, assigned to Zn-O stretching vibrations. The amide I band, mainly associated with C=O stretching, reflects the protein backbone conformation and secondary structural elements, including α-helices, β-sheets, and β-turns [19]. Shifts in the O-H and N-H stretching region suggest hydrogen bonding and electrostatic interactions between zein and ZnO.

The FTIR spectrum of zein protein revealed peaks observed at 2961 and 2871 cm^{-1}, corresponding to C-H stretching vibrations, and at 2372 and 2337 cm^{-1}, which are attributable to adsorbed atmospheric CO_2. Bands at 982-872 cm^{-1} were associated with C-O and C-H bending vibrations. Signals at 703, 579, 556, 525, and 506 cm^{-1} were assigned to Zn-O stretching vibrations. Similar amide I, amide II, and Zn-O bands have been reported by Schmitz *et al.*, [20]. The observed protein- and ZnO-associated bands therefore support the formation of the zein-ZnO nanocomposite and indicate interactions between the biopolymer and metal oxide.

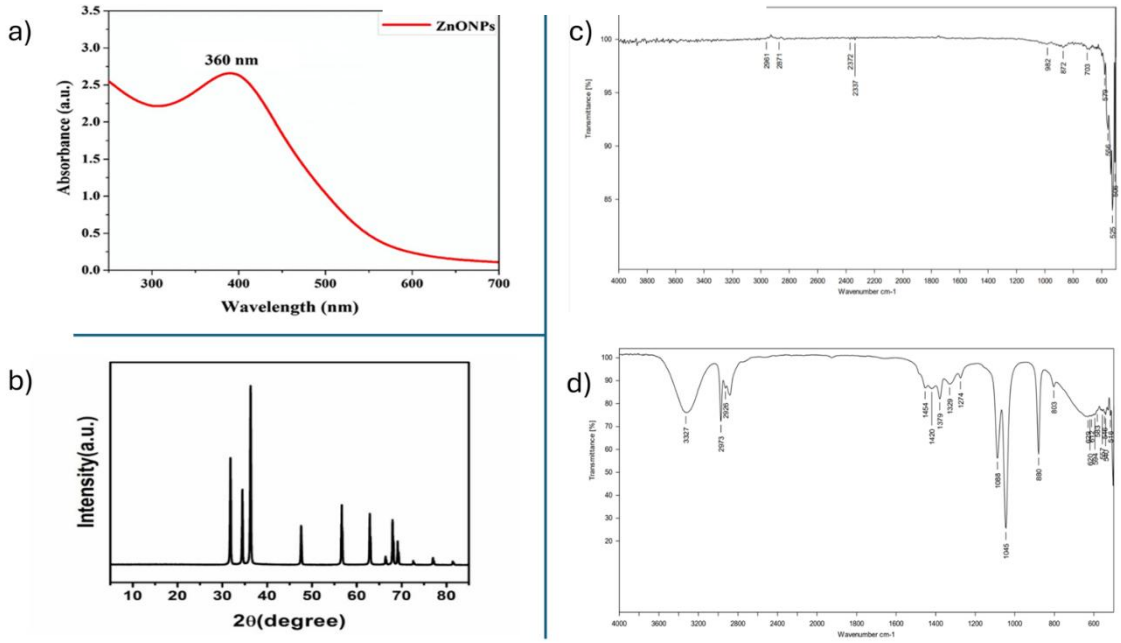


Fig. 1 Physicochemical characterization of Zein-ZnONPs showing (a) UV-visible absorption spectrum confirming nanoparticle formation, (b) X-ray diffraction (XRD) pattern indicating the crystalline nature of ZnO nanoparticles, (c) FTIR spectrum of pure zein protein, and (d) FTIR spectrum of Zein-ZnONPs demonstrating the interaction between zein and ZnO nanoparticles.

3.3.4 Dynamic light scattering and zeta potential

DLS was used to determine the hydrodynamic diameter and size distribution of the zein-ZnONPs in suspension. The analysis showed an average hydrodynamic diameter of 105-222 nm, with a relatively low polydispersity index (PDI), indicating a comparatively narrow and uniform size distribution (Fig. 2a). The hydrodynamic diameter measured by DLS includes the nanoparticle core and the surrounding hydrated layer, including surface-associated biomolecules, and therefore may exceed the physical particle diameter observed by electron microscopy [21]. Comparatively, nanoparticles synthesized using shilajit extract showed an average size of 348 nm, which was higher than that observed for the present formulation [22]. The measured hydrodynamic diameter confirms the nanoscale nature of the zein-ZnONPs. Zeta potential analysis was performed to evaluate the surface charge and colloidal stability of the nanoparticle suspension. Zein-ZnONPs exhibited a zeta potential value of approximately +4.5 mV (Fig. 2b). This slight positive surface charge may be associated with protonated amino groups ($-NH_3^+$) present in zein. Zeta potential values between −30 and +30 mV generally indicate limited electrostatic stabilization, whereas higher absolute values are associated with greater electrostatic repulsion and colloidal stability [23]. A zeta potential of −7.45 mV has previously been reported for biosynthesized ZnO nanoparticles, indicating differences in the surface chemistry of the capping biomolecules [24]. The relatively low positive charge observed in the present formulation suggests that steric stabilization by the zein matrix may contribute to colloidal stability in addition to electrostatic effects. The DLS and zeta potential results therefore support the formation of a dispersed Zein-ZnONP system in aqueous suspension.

3.3.5 Scanning electron microscopy

SEM was conducted to study the surface morphology and structural features of zein-ZnONPs. The micrographs showed predominantly spherical to slightly irregular particles arranged in loosely aggregated clusters (Fig. 2c). The average particle size estimated from the SEM micrographs was approximately 140 nm, which was within the hydrodynamic size range obtained by DLS. The particle surfaces appeared rough and textured, consistent with the presence of the zein matrix. Similar spherical morphology and aggregation patterns have been reported for biopolymer-associated ZnO nanoparticles [25]. Upon encapsulation of metformin, SEM images revealed denser and slightly larger structures, indicating the successful incorporation of the drug into the nanoparticle matrix. This morphological change supports the findings from UV-Vis and FTIR analyses and confirms the suitability of the nanoparticles as effective drug carriers.

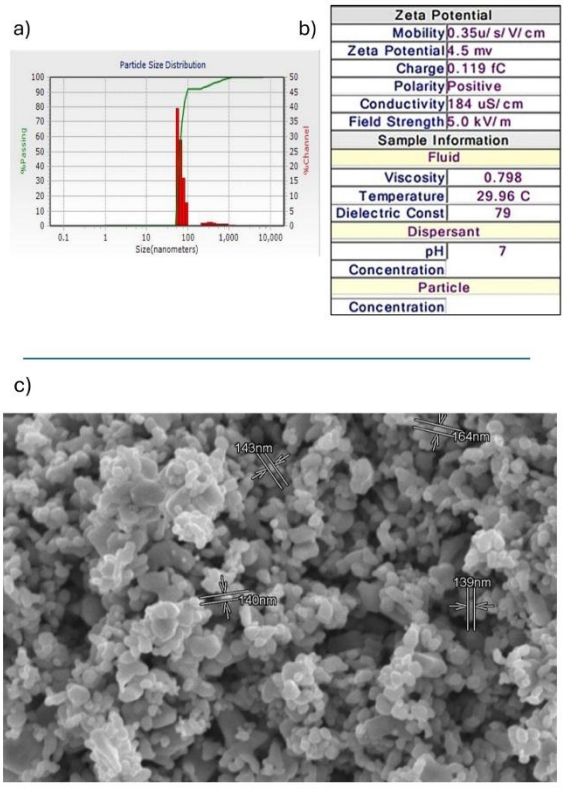


Fig. 2 Particle characterization of Zein-ZnONPs showing (a) particle size distribution by DLS analysis, (b) zeta potential analysis revealing the colloidal stability and surface charge characteristics, and (c) SEM micrograph illustrating the morphology and nanoscale dimensions of the synthesized Zein-ZnONPs.

3.4 Encapsulation of the metformin hydrochloride

Metformin hydrochloride was successfully encapsulated into zein-ZnONPs using a solvent diffusion-assisted nanoprecipitation method. Lyophilization produced a dry, free-flowing

powder. SEM analysis further showed denser and slightly larger structures (105-222 nm) compared with the unloaded zein-ZnONP (Fig. 3a). Increased surface roughness and particle density were also observed. UV-Visible analysis showed an absorption peak at 357 nm for the metformin-loaded formulation compared with approximately 360 nm for the unloaded ZnO nanoparticles (Fig. 3b). The observed changes in optical and morphological characteristics provide qualitative evidence of metformin incorporation into the Zein-ZnONP system. However, drug-loading content and encapsulation efficiency were not quantitatively determined in the present study.

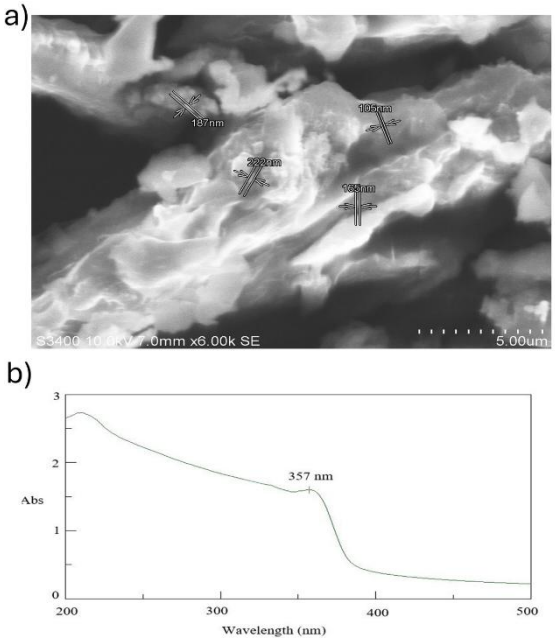


Fig. 3 Encapsulation of metformin hydrochloride: (a) SEM image showing the morphology of metformin-loaded zein-ZnONPs, and (b) UV-visible spectrum confirming drug encapsulation.

3.5 *In-vitro* drug release profile

The *in-vitro* release of metformin hydrochloride from zein-ZnONPs was evaluated under simulated gastrointestinal conditions. The metformin release profile showed a biphasic pattern, consisting of an initial rapid release during the first 2 h. followed by a slower release phase over the subsequent 22 h. (Fig. 4). The initial release may be associated with the diffusion of surface-associated drug, whereas the subsequent phase may result from gradual diffusion of metformin through the zein matrix.

The release data showed a good fit to the first-order kinetic model. The calculated release rate constant (k) was 0.0039 h^{-1}, corresponding to a half-life of approximately 7.7 h., calculated using:

$$t_{\frac{1}{2}} = \frac{0.693}{k}$$

The prolonged release observed for the nanoparticle formulation compared with free metformin suggests that incorporation within the zein-ZnO matrix modified the release kinetics of the drug. Ezzat et al. similarly reported an initial rapid release followed by sustained release from ZnO-MET folic acid nanoparticles over 72 h. [26].

The relatively shorter release duration in the present formulation may be related to differences in nanoparticle composition and the absence of additional components such as folic acid. Previous work has also reported enhanced therapeutic effects of ZnO-metformin nanocomposites compared with metformin alone in an experimental model of CCl_4-induced liver injury [27]. This demonstrates that the combination of ZnO with metformin not only modulates drug release but also enhances pharmacological activity, offering a dual advantage in drug delivery applications. The present release findings indicate that zein-ZnONPs can modulate the release of metformin and may therefore serve as a potential carrier for controlled drug delivery. The suitability of Zein-ZnONPs as an oral bio-nanocarrier is supported by their nanoscale particle size, successful metformin encapsulation, and sustained drug-release behavior observed under simulated gastrointestinal conditions. These properties suggest their potential to improve drug retention and provide controlled release of metformin. However, in vivo pharmacokinetic and antidiabetic studies are required to establish their effectiveness for oral diabetes management.

Zein contributes to metformin delivery by acting as a biocompatible polymeric matrix capable of encapsulating the drug and regulating its release. The hydrophobic nature of zein facilitates the formation of stable nanoparticles, while the incorporation of ZnO nanoparticles provides additional structural stability to the hybrid

system. The sustained release profile observed in the present study suggests that Zein-ZnONPs may prolong drug availability compared with free metformin and support their application as a controlled-release nanocarrier for metformin delivery.

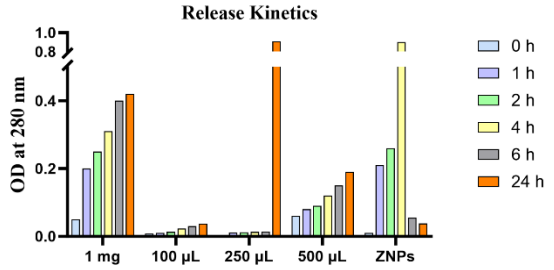


Fig. 4 In vitro drug release profile of metformin hydrochloride from Zein-ZnONPs, showing the release kinetics over time.

3.6 Antioxidant assay

The Zein-ZnONPs exhibited concentration-dependent DPPH radical-scavenging activity. The percentage inhibition increased from 27% at 20 µg mL^{-1} to 80% at 80 µg mL^{-1} and reached 95% at 100 µg mL^{-1} (Fig. 5). The IC_{50} was estimated to be 50-60 µg mL^{-1}, indicating appreciable radical-scavenging activity. Similar concentration-dependent DPPH scavenging has been reported for *Tectona grandis* leaf extract-stabilized ZnO nanoparticles [28]. In contrast, *Punica granatum* peel extract-mediated ZnO nanoparticles reported an IC_{50} value of 240 µg mL^{-1}, indicating lower radical-scavenging efficiency than the present Zein-ZnO formulation [29]. The observed activity may be associated with the combined contribution of the zein matrix and ZnO surface. Similar antioxidant effects have been reported for chitosan-ZnO and gelatin-ZnO nanocomposites, suggesting that biopolymer-ZnO interactions can influence radical-scavenging activity [30]. The results indicate substantial antioxidant activity of the Zein-ZnO nanoparticles under the experimental conditions used. The antioxidant activity of Zein-ZnONPs may be attributed to the ability of ZnO nanoparticles to participate in electron-transfer and hydrogen-donating reactions, thereby scavenging free radicals. In addition, the zein matrix may improve nanoparticle dispersion and stability, increasing the accessibility of reactive sites involved in free radical neutralization. The observed concentration-dependent DPPH scavenging activity supports the antioxidant potential of the synthesized nanocomposite.

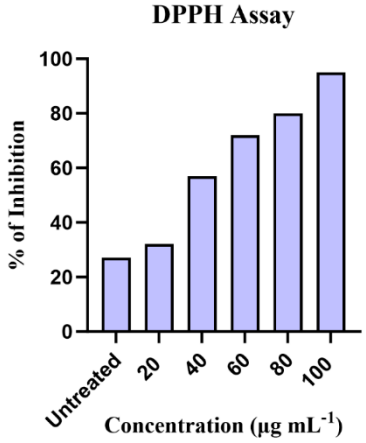


Fig. 5 DPPH free radical scavenging activity of Zein-ZnONPs, demonstrating their antioxidant potential.

3.7 Anti-inflammatory assay

Zein-ZnONPs exhibited a concentration-dependent inhibition of protein denaturation (Fig. 6). The inhibition increased from 46.4% at 250 µg mL^{-1} to 71.4% at 500 µg mL^{-1} and 87.0% at 750 µg mL^{-1}, reaching 90.7% at 1000 µg mL^{-1}. The activity was comparable to that of the standard anti-inflammatory drug used in the assay. ZnO nanoparticles prepared using *Senecio chrysanthemoides* extract have previously demonstrated anti-inflammatory activity, with 73.01% inhibition reported at 100 µg mL^{-1} [31]. Another study reported that ZnO nanoparticles prepared using *Cassia sieberiana* demonstrated notable anti-inflammatory effects via inhibition of protein denaturation and suppression of inflammatory cytokines [32]. Differences in particle size, synthesis conditions, surface chemistry, and formulation composition may contribute to variations in activity between studies. The observed inhibition may result from interactions between the ZnO surface and protein molecules that reduce protein denaturation. Zein may also contribute through its protein-stabilizing properties and interactions with the nanoparticle surface [33]. These findings indicate that Zein-ZnONPs possess notable in vitro anti-inflammatory activity. The anti-inflammatory effect observed in the protein denaturation assay may be associated with the ability of ZnO nanoparticles to stabilize protein structures and reduce denaturation under stress

conditions. Previous studies have suggested that ZnO nanoparticles can modulate inflammatory responses through regulation of reactive oxygen species and inflammatory mediators [32]. The presence of zein may further contribute to nanoparticle stability and enhance the overall bioactivity of the formulation.

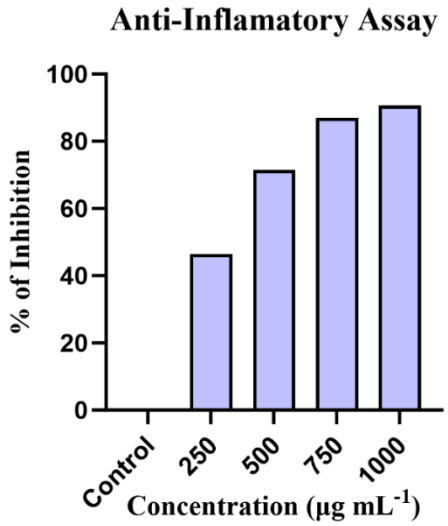


Fig. 6 In vitro anti-inflammatory activity of zein-ZnONPs, showing their effectiveness in reducing protein denaturation compared to control.

3.8 Antibacterial activity

The antibacterial activity of zein-ZnONPs was evaluated by the disc diffusion method against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* and *Salmonella abony*. Nanoparticle concentrations of 0.25, 0.5, and 1.0 mg mL^{-1} were tested, with tetracycline (15 µg mL^{-1}) and sterile water used as positive and negative controls, respectively. The nanoparticles exhibited concentration-dependent antibacterial activity, with larger inhibition zones observed at higher concentrations. At 1.0 mg/mL, inhibition zones of 12, 9, and 10 mm were observed against *S. aureus*, *S. abony*, and *E. coli* respectively (Fig. 7; Table 1). The activity was lower than that of the tetracycline control but demonstrated measurable antibacterial effects against all three test organisms. Similar concentration-dependent activity has been reported for biosynthesized ZnO nanoparticles against Gram-positive and Gram-negative bacteria [34].

The antibacterial activity of ZnO nanoparticles has been associated with several mechanisms, including reactive oxygen species generation, Zn^{2+} release, membrane damage, and disruption of intracellular components. The nanoscale dimensions and high specific surface area of ZnO can facilitate interactions with bacterial cells. The zein matrix may additionally influence nanoparticle dispersion and Zn^{2+} release. Similar antibacterial effects of ZnO nanoparticles have been reported against a broad range of bacterial species [35]. The antibacterial effect observed in the present study is consistent with these mechanisms. The nanoscale size of Zein-ZnONPs facilitates close interaction with bacterial cell surfaces, while the zein coating may improve nanoparticle dispersion and availability. Together, these effects contribute to the measurable antibacterial activity observed against both Gram-positive and Gram-negative bacterial strains. The present findings therefore demonstrate moderate in vitro antibacterial activity of the Zein-ZnO nanoparticles against the tested bacterial strains.

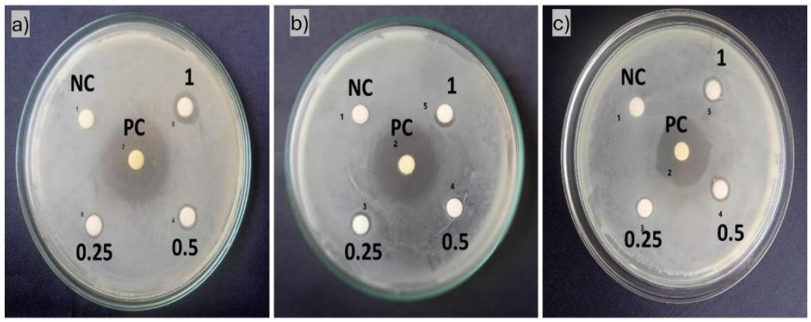


Fig. 7 Antibacterial activity of Zein-ZnONPs against pathogenic strains determined by the disc diffusion method using 6 mm sterile paper discs: (A) *Staphylococcus aureus*, (B) *Salmonella abony*, and (C) *Escherichia coli*. Labels: PC, positive control (tetracycline); NC, negative control (water); discs labeled 0.25, 0.5, and 1 correspond to Zein-ZnONPs concentrations of 0.25, 0.5, and 1.0 mg mL^{-1}, respectively.

Table 1. Antibacterial activity of Zein-ZnONPs determined by the disc diffusion method

Bacterial strain	Zone of inhibition (Diameter in mm) Zein-ZnONPs			
	Zein-ZnONPs			
	0.25 mg mL^{-1}	0.5 mg mL^{-1}	1.0 mg mL^{-1}	Tetracycline
Staphylococcus aureus	7	9	12	22
Salmonella abony	8	7	9	21
Escherichia coli	7	9	10	18

Values are expressed as total zone of inhibition diameters (mm) measured using a standard antibiotic zone measuring scale and including the 6 mm sterile paper disc diameter. Tetracycline (15 µg mL^{-1}) was used as the positive control.

4. CONCLUSION

This study demonstrated the synthesis, characterization, and biological evaluation of zein-based zinc oxide nanoparticles (Zein-ZnONPs) as a potential drug delivery system for metformin. Zein served as a biopolymer matrix for the stabilization of ZnO nanoparticles and incorporation of the drug. Physicochemical characterization confirmed the nanoscale dimensions, crystalline ZnO structure, and zein-associated surface functional groups of the formulation. The Zein-ZnONPs also exhibited concentration-dependent antioxidant and anti-inflammatory activities, together with moderate antibacterial activity against the tested bacterial strains. In vitro release studies showed a biphasic metformin release profile, with an initial rapid phase followed by sustained release over the subsequent period. The release kinetics indicated slower drug release from the nanoparticle formulation compared with free metformin. Overall, these findings demonstrate the potential of Zein-ZnO nanoparticles as a multifunctional carrier for controlled metformin delivery. Further studies involving drug-loading efficiency, cytotoxicity, in vivo pharmacokinetics, biodistribution, and long-term stability are required to establish their suitability for therapeutic applications.

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CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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ETHICAL INFORMATION

Authors have to clearly mention if any ethical information is involved in the research work.

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