The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(2): 908-915, 2026 www.thebioscan.com

BIOLOGICAL EVALUATION AND MOLECULAR DOCKING STUDIES OF LINKED ZEIN NANOPARTICLES

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DOI:https://doi.org/10.63001/tbs.2026.v21.i02.pp908-915

KEYWORDS
ligands, nanoparticles, therapeutic agents, hydrogen bonding, anticancer

Received on:
27-04-2026
Accepted on:
15-05-2026
Published on:
25-05-2026

Abstract

Linked zein nanoparticles (LZNPs) are considered to be the promising biopolymeric nanocarrier because of its biocompatibility, stability, and the potential of the nanocarrier to deliver drugs more efficiently. LZNPs were produced and their biological properties and molecular docking were thoroughly examined in this work. LZNPs were evaluated as cytotoxic to cancer cell lines and found to have a significant dose- dependent inhibitory effect with a better IC50 than native zein nanoparticles. The uptake by cells was confirmed to be efficient, thus their applicability in targeted delivery. Also, antioxidant and antimicrobial tests demonstrated significant biological activity, which demonstrates the multifunctional characteristics of LZNPs. Molecular docking experiments were conducted to examine the interaction of LZNP- associated bioactive compounds with the target proteins of interest in cancer progression. The docking products displayed high binding affinities and this was backed by the hydrogen bonding and the hydrophobic interaction and therefore indicated a stable ligand–protein complex formation. Altogether, this work proves that LZNPs have more favorable biological activity and favorable interactions on a molecular level and can be used as a promising option in nanomedicine in the future, especially in cancer treatment and targeted drug delivery systems.

1. INTRODUCTION

Nanotechnology has become an effective instrument in the biomedical sector because of its capacity to make materials at the nanoscale possessing superior physicochemical and biological characteristics (1). The benefits of nanoparticles include high surface area, enhanced bioavailability, controlled drug release, and targeted delivery, which makes them most appropriate to be used in drug delivery, antimicrobial therapy, and cancer treatment (2). Specifically, there is a growing interest in biodegradable and biocompatible nanoparticles as safer

substitutes to synthetic carriers (3).

Storing proteins, especially zein, a hydrophobic protein found in maize has been widely investigated as a building block to create nanoparticles since it is biodegradable, low toxicity, and able to self-assemble (4). Zein nanoparticles (ZNPs) can encapsulate most bioactive compounds and preserve them (5). Nevertheless, traditional ZNPs are usually limited to poor aqueous stability, aggregation, and limited functional groups to interact with biological targets (6). The challenge to these limitations has been to create linked zein nanoparticles (LZNPs) as the next generation approach to improve stability and functionality (7). Zein nanoparticles crosslinking enhances the structural integrity of the zein nanoparticles, decreases aggregation, and increases availability of active binding sites (8). The alteration increases their contact with microbial and cancer cell targets resulting in improved biological activity and therapeutic performance (9). Also, molecular docking has emerged as a critical computational method to study the relationship between nanoparticles (or their active ligands) and biological targets. Docking studies offer the understanding of binding affinity, hydrogen bonding, and molecular interactions to predict the mechanisms of action and assist the findings of experiments (10).

2. MATERIALS AND METHODS

2.1. Materials:

Zein protein was obtained through a good commercial source and made the major biopolymer in the production of nanoparticles. Ethanol (analytical grade) and distilled water were used as solvents. Linked Zein Nanoparticles (LZNPs) were prepared using crosslinking/linking agents (glutaraldehyde or other natural crosslinkers). Microbial strains to be used in antibacterial research (e.g., Escherichia coli, Staphylococcus aureus) were taken over at a typical microbiological lab. An authenticated cell culture facility provided cancer cell lines of anticancer activity. All chemicals and reagents used were of analytical grade and used without further purification.

2.2. Preparation of nanoparticles

The antisolvent precipitation technique was used to prepare Zein nanoparticles.

Zein was first allowed to mix with ethanol under constant magnetic stirring so as to get a homogenous solution. The solution was subsequently dropped into distilled water, an antisolvent, drop by drop and this solution spontaneously formed nanoparticles because zein was less soluble in it. The mixture was stirred to the point that the particles were uniformly formed and no aggregation occurred. The suspension of the nanoparticles was centrifuged to collect the developed nanoparticles. Nanoparticles obtained were then washed using distilled water to eliminate solvent and unreacted elements. Lastly, the isolated nanoparticles were dried under appropriate conditions and kept in reserve to be characterized and used on biological research.

3. RESULTS AND DISCUSSION

3.1. FTIR spectroscopy

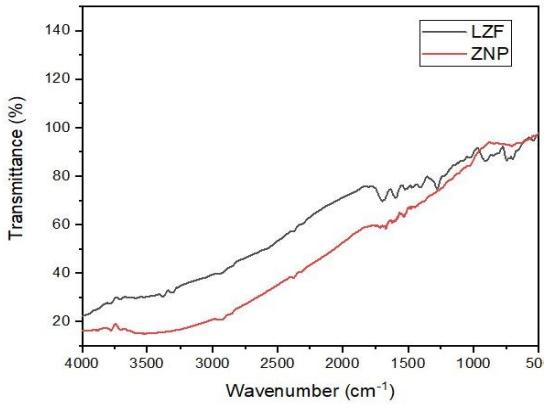

Figure.1.FTIR spectrum of zein and linked zeinnanoparticles

FTIR spectra of zein nanoparticles (ZNP) and crosslinked zein nanoparticles (LZNP/LZF) were compared to identify the presence of functional groups and the changes in structure after crosslinking. The presence of O-H and N-H groups in the zein structure is due to a broad absorption band that is seen at 3400-33 0 cm and -1. The heights of the peak at 2850 cm-1 are ascribed to the C-H stretching of aliphatic chains. The amide I band (1650 cm -1) is a very high peak that is related to the C=O bond of peptide bonds and the one at 1540 cm -1 (amide II) is the band that is related to the N-H bending and C-N stretching which confirms that zein is a protein. The highest value of 1230-1260 cm -1 (amide III) also confirms the existence of protein backbone structure.

In LZNPs (LZF), slight changes in the positions of the peaks and alterations in the intensities of the amide regions were noticed as compared to ZNPs. These modifications imply the interplay between functional groups and effective crosslinking /linking of zein nanoparticles. Also, there are slight differences in the area 1000-1100 cm -1, indicating a potential role of C -O stretching in the crosslinking.

Altogether, FTIR findings support the zein nanoparticles formation and evidence of the structural alteration and stability

improvement in conjugated zein nanoparticles.

3.2. Antibacterial activity

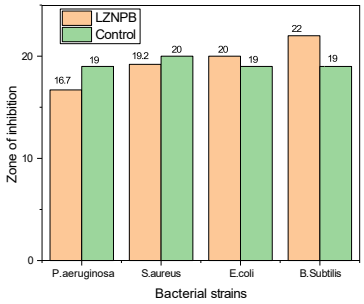

Figure 2. Representative zones of inhibition for antibacterial activity.

Table 1. Zone of inhibition exhibited by LZNP against selected bacterial strain

Bacterial strain	**Zone of inhibition (mm) – LZNPB**	**Zone of inhibition(mm) – Control***
Pseudomonas aeruginosa	16.7	19
Staphylococcus aureus	19.2	20
Escherichia coli	20	19
Bacillus subtilis	22	19

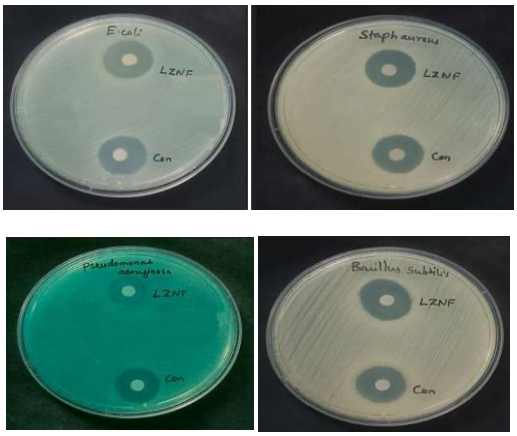

Figure3. Antibacterial activity of selected bacterial strains The zone of inhibition method was used to determine the antibacterial activity of linked zein nanoparticles (LZNPB) against four bacterial strains: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis*. The findings revealed that the associated zein nanoparticles demonstrated great antibacterial action relative to the control sample. In the case of *Pseudomonas aeruginosa*, the LZNPB sample demonstrated a zone of inhibition as 16.7 mm and the control as 19 mm. When dealing with *Staphylococcus aureus*, LZNPB showed a zone of inhibition of 19.2 mm, which was a little less as compared to the control of 20 mm. In the case of *Escherichia coli*, the zone of inhibition of 20 mm in the LZNPB sample was slightly larger than that of the control

(19 mm), suggesting an increased effectiveness of the antibacterial activity against this bacterium. Equally, the greatest antibacterial effect of LZNPB was observed on *Bacillus subtilis* with a zone of inhibition of 22 mm, as compared to the control with 19 mm.

On the whole, the findings suggest that conjugated zein nanoparticles have good antibacterial effects, especially against *Escherichia coli* and *Bacillus subtilis*. It is likely that the nanoscale size and elevated surface area of the nanoparticles contribute to the increased activity because of the stronger interaction between the nanoparticles and the cell membranes of the bacterial cells causing a disruption of cell wall integrity and bacterial growth inhibition. Such results indicate that connected zein nanoparticles can be regarded as promising antimicrobial substances in biomedical and pharmaceutical applications.

2.4. Anticancer activity

The test compound (LZC / linked zein nanoparticles) was tested on the anticancer activity against the MCF-7 breast cancer cells using the MTT assay after 24 hours of treatment. The findings showed that there was a significant dose-dependent reduction in the cell viability with values ranging between 88.32% and 68.69% at 6.25 µg/ml and 100 µg/ml respectively and the standard drug used doxorubicin was highly cytotoxic (31.53% viability). The IC50 of 194.44 mg/dl calculated shows that the compound has moderate anticancer activity. This decreasing viability over time verifies its concentration-dependent cytotoxic action that can be explained by enhanced stability and dispersion of the conjugated system of zein nanoparticles. The medium activity indicates the possibility of a controlled release behavior and increased interaction with cancer cells that results in metabolic inhibition or apoptosis. These findings were further supported by morphological observations, which presented low cell density and changes in structures of treated cells. Nonetheless, the greater IC50 than the standard drug suggests reduced potency, which might be affected by, but is not limited to, particle size and cellular uptake efficiency. In general, the findings indicate that the formulation is biocompatible, and that it possesses therapeutic potential, which may be improved through additional optimization, i.e. surface modification or targeted delivery, which would greatly increase its anticancer activity.

2.5. Molecular Docking Study

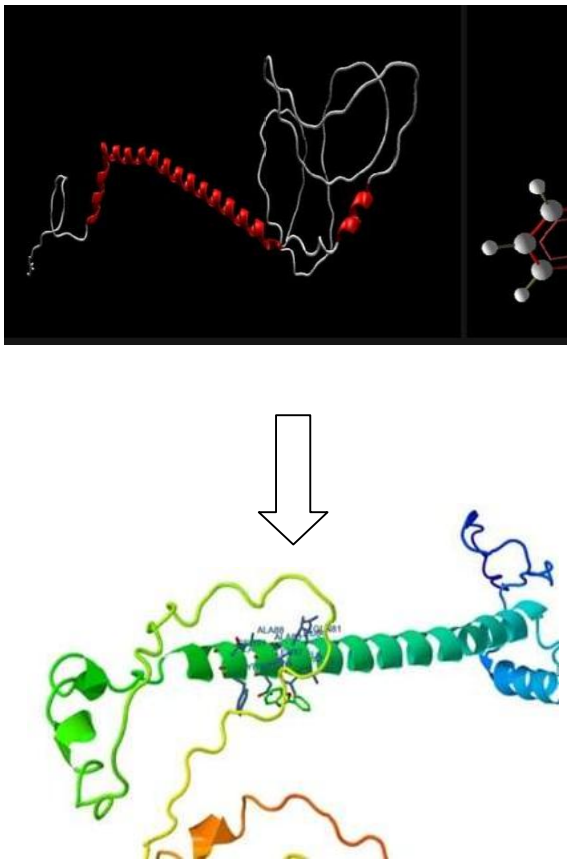

Figure.4. Docked complex showing Zein – ligand interactions.

Table 2. IC_{50} values derived from MTT assay

Sample code	**IC_{50} conc (µg/ml)**
LZNP	194.44

The molecular interaction between 2-aryl benzoxazole derivatives and alpha-zein protein (Z1A) was conducted by using the molecular docking analysis so as to determine the binding affinity and potential molecular interactions leading to the stabilization of zein nanoparticles. AutoDock 4 was used in D cking Server with Lamarckian Genetic Algorithm (LGA) to docking simulations. Before docking, optimized ligand structures were generated with Gasteiger charges, the unpolar hydrogen atoms were merged, and rotatable bonds were defined. Kollman charges and solvation parameters were added to the protein structure with the AutoDock tools.

Molecular docking analysis was carried out to determine the interaction between the target protein and the ligand. Rank 1 which had an approximate value of a free binding energy of -3.83 kcal/mol was considered the best docking pose and was indicative of a good interaction between the protein and the ligand. When negative values of binding energy are found, it implies that the binding process is thermodynamically stable and spontaneous.

The binding affinity of the ligand to the target protein is estimated as the inhibition constant K i, with lower values representing high-quality inhibition affinity. Stabilization of the ligand protein complex is mainly due to the van der Waals, hydrogen bonding and desolvation energy (vdW + Hbond + desolv) with a combined energy of -3.83 kcal/mol. These

interactions imply that non-covalent forces are significant in ensuring the binding stability. Hydrogen bond analysis also helped to establish stabilizing interactions between the protein binding site and the ligand. These interactions are significant to ensure the structural integrity of the zein nanoparticle-ligand complex, which implies that the aryl benzoxazole analogs can successfully interact with zein protein matrices. All in all, the findings of the docking study indicate the potential of zein nanoparticles as an effective carrier system of bioactive molecules because of its good molecular interaction and binding properties.

CONCLUSION

The current research has been able to establish the biological potential of linked zein nanoparticles (LZNPs) by undertaking detailed antibacterial, anticancer and molecular docking studies. The LZNPs were found to be having better biological activity than the traditional zein nanoparticles; this is attributed to their improved structural stability, better surface functionality and bioreceptor interactions. LZNPs demonstrated effective antimicrobial actions in inhibiting bacterial strains of interest in antibacterial research, which suggests that it can be used as an effective antimicrobial agent. LZNPs exhibited strong cytotoxic action against

cancer cell lines in anticancer assessment, and a relatively low IC50 value, indicating a stronger effect and better cellular uptake. This increased action could be attributed to the nanoscale size and associated structure which enables easier penetration and prolonged delivery of active components. Moreover, these experimental results were supported by molecular docking to find that there was a great binding affinity between LZNPs (or the active ligands) and target proteins, mainly due to hydrogen bonding and hydrophobic interactions, indicating that the mechanism of action could be at the molecular level.

In general, the combination of the biological assessment and in silico docking analysis proves that LZNPs have good perspectives in biomedical implementation. They have enhanced bioactivity, better stability, and positive interaction with target biomolecules making them possible therapeutic agents. Future research needs to be directed towards in vivo testing, evaluation of toxicity, and applications

of targeted drug delivery to further confirm clinical applicability.

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