Molecular Biology Reports (2024) 51:423
https://doi.org/10.1007/s11033-024-09407-7

ORIGINAL ARTICLE

Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells

Jehad Zuhair Tayyeb[1] · Madhu Priya[2] · Ajay Guru[2] · Meenakshi Sundaram Kishore Kumar[3] · Jayant Giri[4] · Akash Garg[5] · Rutvi Agrawal[5] · Khairiyah Binti Mat[6,7] · Jesu Arockiaraj[8]

Received: 23 January 2024 / Accepted: 29 February 2024

Abstract

Background Oral health remains a significant global concern with the prevalence of oral pathogens and the increasing incidence of oral cancer posing formidable challenges. Additionally, the emergence of antibiotic-resistant strains has complicated treatment strategies, emphasizing the urgent need for alternative therapeutic approaches. Recent research has explored the application of plant compounds mediated with nanotechnology in oral health, focusing on the antimicrobial and anticancer properties.

Methods In this study, curcumin (Cu)-mediated zinc oxide nanoparticles (ZnO NPs) were synthesized and characterized using SEM, EDAX, UV spectroscopy, FTIR, and XRD to validate their composition and structural features. The antioxidant and antimicrobial activity of ZnO-CU NPs was investigated through DPPH, ABTS, and zone of inhibition assays. Apoptotic assays and gene expression analysis were performed in KB oral squamous carcinoma cells to identify their anticancer activity.

Results ZnO-CU NPs showcased formidable antioxidant prowess in both DPPH and ABTS assays, signifying their potential as robust scavengers of free radicals. The determined minimal inhibitory concentration of 40 µg/mL against dental pathogens underscored the compelling antimicrobial attributes of ZnO-CU NPs. Furthermore, the interaction analysis revealed the superior binding affinity and intricate amino acid interactions of ZnO-CU NPs with receptors on dental pathogens. Moreover, in the realm of anticancer activity, ZnO-CU NPs exhibited a dose-dependent response against Human Oral Epidermal Carcinoma KB cells at concentrations of 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL. Unraveling the intricate mechanism of apoptotic activity, ZnO-CU NPs orchestrated the upregulation of pivotal genes, including BCL2, BAX, and P53, within the KB cells.

Conclusions This multifaceted approach, addressing both antimicrobial and anticancer activity, positions ZnO-CU NPs as a compelling avenue for advancing oral health, offering a comprehensive strategy for tackling both oral infections and cancer.

Keywords Curcumin · Zinc oxide nanoparticle · Dental Pathogen · Apoptosis

Introduction

Oral health is a pivotal aspect of daily life that often goes overlooked, despite its profound impact on overall well-being. The oral cavity harbors a diverse microbial community, and the delicate balance within this microbiome is crucial for maintaining oral health [1]. Several pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*, have been implicated in oral infections, posing a threat to both oral and systemic health [2]. The lifestyle choices we make play a significant role in creating an environment conducive to the proliferation of these pathogens. Poor oral hygiene practices, a high-sugar diet, and the use of tobacco and alcohol can create an imbalance in the oral microbiome, providing an opportunity for these pathogens to thrive [3]. *S. aureus*, for example, may exploit compromised oral tissues, while *S. mutans* and *E. faecalis* are notorious for their involvement in dental caries and root canal infections, respectively. *C.*

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Published online: 15 March 2024

albicans, a common fungal pathogen, can exacerbate oral infections in immunocompromised individuals [4–6].

Recent research has illuminated the intricate connection between oral pathogens and the development of oral cancer. Chronic infection and inflammation induced by these pathogens contribute to genetic mutations, epigenetic changes, and immune system dysregulation, all of which are known precursors to carcinogenesis [7]. *S. aureus* has been linked to the promotion of oncogenic pathways, while *S. mutans* and *E. faecalis* are associated with an increased risk of oral squamous cell carcinoma. *C. albicans*, in addition to its role in fungal infections, has been implicated in promoting inflammatory processes that may contribute to oral cancer progression [8–10]. In the realm of dentistry, the integration of innovative approaches is imperative to combat the formidable challenge posed by oral pathogens and their potential link to cancer. Curcumin (CU), a polyphenolic compound derived from the turmeric plant (*Curcuma longa*), has garnered significant attention for its multifaceted therapeutic properties. Notably recognized for its potent antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Its ability to modulate various signaling pathways makes it an attractive option for preventing and managing both oral infections and the progression of oral cancer [11, 12]. CU's antimicrobial can be used against a spectrum of oral pathogens. Beyond its antimicrobial properties, CU has demonstrated remarkable anticancer effects by inhibiting key pathways involved in tumor initiation and progression, making it an intriguing agent for oral cancer prevention and therapy. To enhance the bioavailability and targeted delivery of CU, the integration of zinc oxide nanoparticles (ZnO NPs), holds great promise. NPs can facilitate controlled release, improve stability, and enhance the penetration of curcumin into oral tissues. ZnO NPs, known for their biocompatibility and antimicrobial properties, complement curcumin's activity, creating a synergistic effect in combating oral pathogens [13]. The combination of CU and ZnO NPs presents a novel therapeutic approach that can revolutionize oral healthcare.

In this study, the integration of CU with ZnO NPs is a novel strategy aimed at synergistically enhancing the bioavailability and therapeutic efficacy of these compounds. The study systematically evaluates their antioxidant potential, highlighting their ability to neutralize oxidative stress. Furthermore, their antimicrobial activity is scrutinized against a spectrum of oral pathogens, emphasizing their potential to target specific biofilms and disrupt the receptors crucial for pathogen adherence. A critical facet of this research focuses on unraveling the anticancer potential of the synthesized ZnO-CU NPs in KB oral cancer cells. The study investigates the mechanism by which ZnO-CU NPs induce apoptosis, shedding light on the intricate signaling pathways involved in the suppression of oral cancer cells. This exploration not only underscores their potential as therapeutic agents but also provides valuable insights into the development of targeted strategies for combating oral cancer. By elucidating their mechanism of action and therapeutic potential, this research contributes to the evolving landscape of advanced interventions, paving the way for more effective and targeted approaches in oral healthcare.

Materials and methods

Synthesis of ZnO-CU NPs

A 10 mg/mL concentrated solution of CU was prepared by dissolving 50 mg of CU powder in 5 mL of ethanol. This solution was introduced into a ZnO precursor solution, by dissolving 486.4 mg of zinc acetate dihydrate in 30 mL of deionized water. Stirring at 5000 rpm was applied to ensure the dissolution of CU and the homogeneous dispersion of the ZnO precursor. The controlled addition of the CU solution to the ZnO precursor solution, followed by 30 min of stirring at room temperature, initiated the reduction of the Zn precursor, leading to the formation of ZnO-CU NPs. A subsequent 24 h incubation period facilitated the precipitation of NPs. The reaction mixture underwent centrifugation at 5000 rpm for 10 min to isolate the synthesized ZnO NPs. Following the removal of the supernatant, the NPs were subjected to three washes with deionized water to eliminate any residual chemicals. Subsequently, the purified ZnO NPs were dried in an oven at 60 °C for a duration of 6 h. Characterization techniques, including UV-visible spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR), were employed to assess the size, morphology, crystallinity, functional groups, and optical properties of the synthesized ZnO NPs [14].

Free radical scavenging assays

2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH free radical scavenging assay operated on the underlying principle of color alteration that transpires when DPPH radicals interact with substances exhibiting antioxidant properties. DPPH, being a stable free radical characterized by a violet color, undergoes a color shift from violet to yellow upon reduction by antioxidants. The assessment of the antioxidant potential of ZnO-CU NPs employed the DPPH assay. In accordance with the radical scavenging principle, a 0.1 mM solution of DPPH radicals was prepared. Various concentrations of ZnO-CU NPs were subsequently

combined with the DPPH solution, and the reduction of the DPPH radical was quantified by measuring absorbance at 517 nm following a 30 min incubation period. Trolox served as the positive control in this experiment [15].

DPPH Scavenging % = [(Absorbance of the control - Absorbance of the sample)÷Absorbance of the control] ×100.

2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS) acid assay

The ABTS radical cation (ABTS·+) was generated through the reaction between ABTS and an oxidizing agent. Upon introduction of antioxidants into the solution, electrons were donated, leading to the reduction of ABTS·+ and the formation of a non-radical form (ABTS-H), accompanied by a decolorization of the solution. The ABTS assay evaluated the capacity of ZnO-CU NPs to quench ABTS radical cations. Adhering to the principle of reduction, a solution of ABTS radical cations was prepared by incubating an ABTS solution with potassium persulfate. ZnO-CU NPs at varying concentrations were subsequently introduced, and the reduction in absorbance at 734 nm indicated their antioxidant potential. This assay yielded additional insights into the nanoparticles' capability to mitigate oxidative stress [16].

ABTS Scavenging % = [(Absorbance of the control - Absorbance of the sample)÷Absorbance of the control] ×100.

Minimal Inhibitory Concentration (MIC)

The MIC of ZnO-CU NPs against dental pathogens such as *S. aureus* (MTCC 1144), *S. mutans* (MTCC 497), *E. faecalis* (MTCC 2729), *C. albicans* (MTCC 183) was determined using a microplate assay. The MIC was defined as the lowest concentration inhibiting the visible growth of pathogens. Pathogen strains were sourced from Saveetha Dental College and Hospitals, and amoxicillin served as a positive control. The MIC determination involved incubating the microplate at 37 °C for 24–48 h, and growth was quantified by measuring absorbance at 600 nm [17].

Zone of inhibition

Antimicrobial activity was further evaluated using the Zone of Inhibition method. Clear zones around wells indicated the inhibition of microbial growth. ZnO-CU NP solutions (50 µL) were added to wells on agar plates, and after incubation at 37 °C for 24 h, the diameter of clear zones was measured in millimeters (mm) using a caliper. This method provided a visual representation and quantification of the nanoparticles' impact on microbial growth [18].

Autodock simulation

Molecular docking simulations using AutoDock Ver 1.5.6 provided insights into the interaction between Cu and specific receptors. The 3D structure of the dental pathogen receptor from the Protein Data Bank (https://www.rcsb.org/) and the CU ligand structure from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) were utilized. The simulation results were analyzed to identify the best-docked conformations based on binding affinity values on ligand-receptor interactions expressed in kcal/mol. The 3D and 2D structure of the amino acid interaction between the ligand and receptor was visualized in the Discovery Studio visualizer software [19].

Anticancer assay

The impact of ZnO-CU NPs on KB oral cancer cell viability was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. The KB cells with the passage number of 8 were procured from the National Centre for Cell Sciences, Pune. The cells were maintained in a humidified incubator at 37 °C with 5% CO_2. Seeding of KB cells into 96-well culture plates was carried out at a density of 5×10^4 cells /well. The cells were with various concentrations of ZnO-CU NPs (10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL). Control wells were kept untreated, while cyclophosphamide served as a positive control. The cells were incubated with ZnO-CU NPs for 24 h. At the end of the incubation period, the medium was aspirated, and 100 µL of MTT solution was added to each well. Subsequently, the cells were incubated for an additional 4 hrs to allow the formation of formazan crystals. Then 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals and the absorbance of the dissolved formazan was measured using a microplate reader at a wavelength of 570 nm [20].

Gene expression analysis

The molecular mechanisms underlying the effects of ZnO-CU NPs on KB cells were investigated through gene expression analysis focused on apoptotic genes (BCL2, BAX, P53). After the treatment of ZnO-CU NPs in KB cells the RNA was extracted using TAKARA RNAiso Plus. cDNA was synthesized using PrimeScript RT Reagent Kit (TaKaRa, Japan) and PCR was performed using TB Green Premix Ex Taq II (TaKaRa, Japan). Primers used in this study are listed in Table 1. RT-PCR was performed in the Roche LightCycler machine. The GAPDH was used as a housekeeping gene and the relative gene expression was calculated using $2^{-\Delta\Delta ct}$ [19].

Table 1 Primers used for gene expression

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Reference
GAPDH	GCCAAAAGGGTCATCATCTCTGC	GGTCACGAGTCCTTCCACGATAC	[21]
BCL-2	GACGACTTCTCCCGCCGCTAC	CGGTTCAGGTACTCAGTCATCCAC	[21]
BAX	AGGTCTTTTTCCGAGTGGCAG	GCGTCCCAAAGTAGGAGAGGAG	[21]
P53	ACATGACGGAGGTTGTGAGG	TGTGATGATGGTGAGGATGG	[22]

Statistical analysis

To determine the significance of differences between the various concentrations of ZnO-CU NPs and the control, a one-way analysis of variance (ANOVA) was performed, and then post-hoc multiple comparisons (Tukey's test) were done. At $p < 0.05$, the results were considered statistically significant. The data are shown as mean ± standard deviation, and each experiment has been carried out in triplicate.

Results

Synthesis and characterization of ZnO-CU NPs

Figure 1 shows the UV absorption spectra of ZnO-CU NPs. There is a strong absorption band peaked at 200–600 nm in the UV spectrum. The SEM analysis of ZnO-CU NPs exhibited a distinct spherical morphology, with particles falling within the range of 100–200 nm (Fig. 2). The uniform and well-defined structure observed in the SEM image suggests a controlled synthesis process, ensuring the reproducibility and stability of the NPs. The NPs size is noteworthy, as it falls within the nanoscale range, providing an increased surface area that could enhance interactions with various biological entities. These results establish the successful fabrication of ZnO-CU NPs with specific morphological characteristics for further investigation. The XRD pattern of the ZnO-CU NPs revealed multiple distinctive peaks at various 2θ values: 5.454°, 10.219°, 13.472°, 20.280°, 21.414°, 25.877°, 30.038°, 31.778°, 36.619°, 39.266°, 41.384°, and 56.588° (Fig. 3). These peaks indicate the crystalline nature

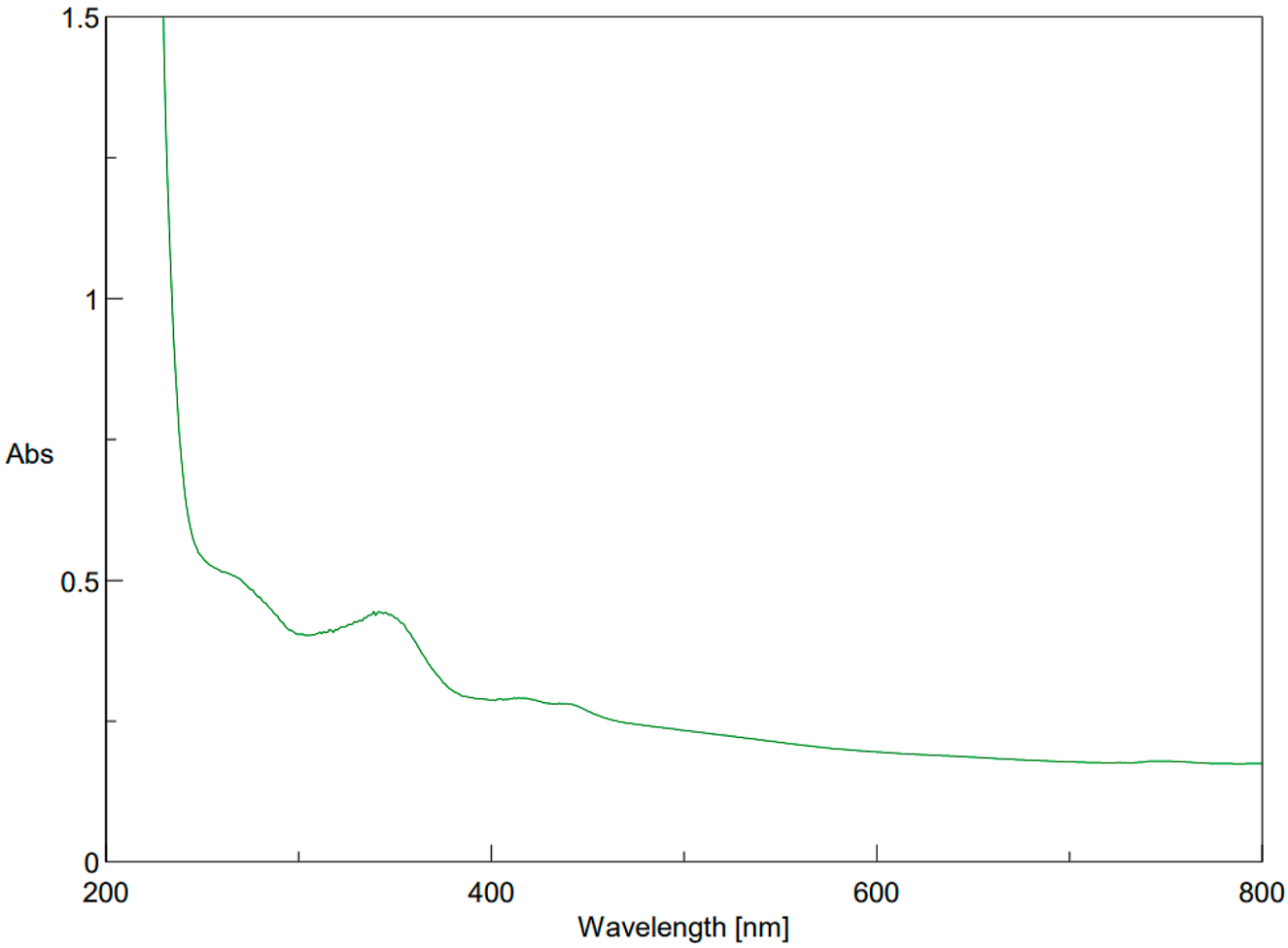

Fig. 1 UV absorption spectra of synthesized ZnO-CU NPs

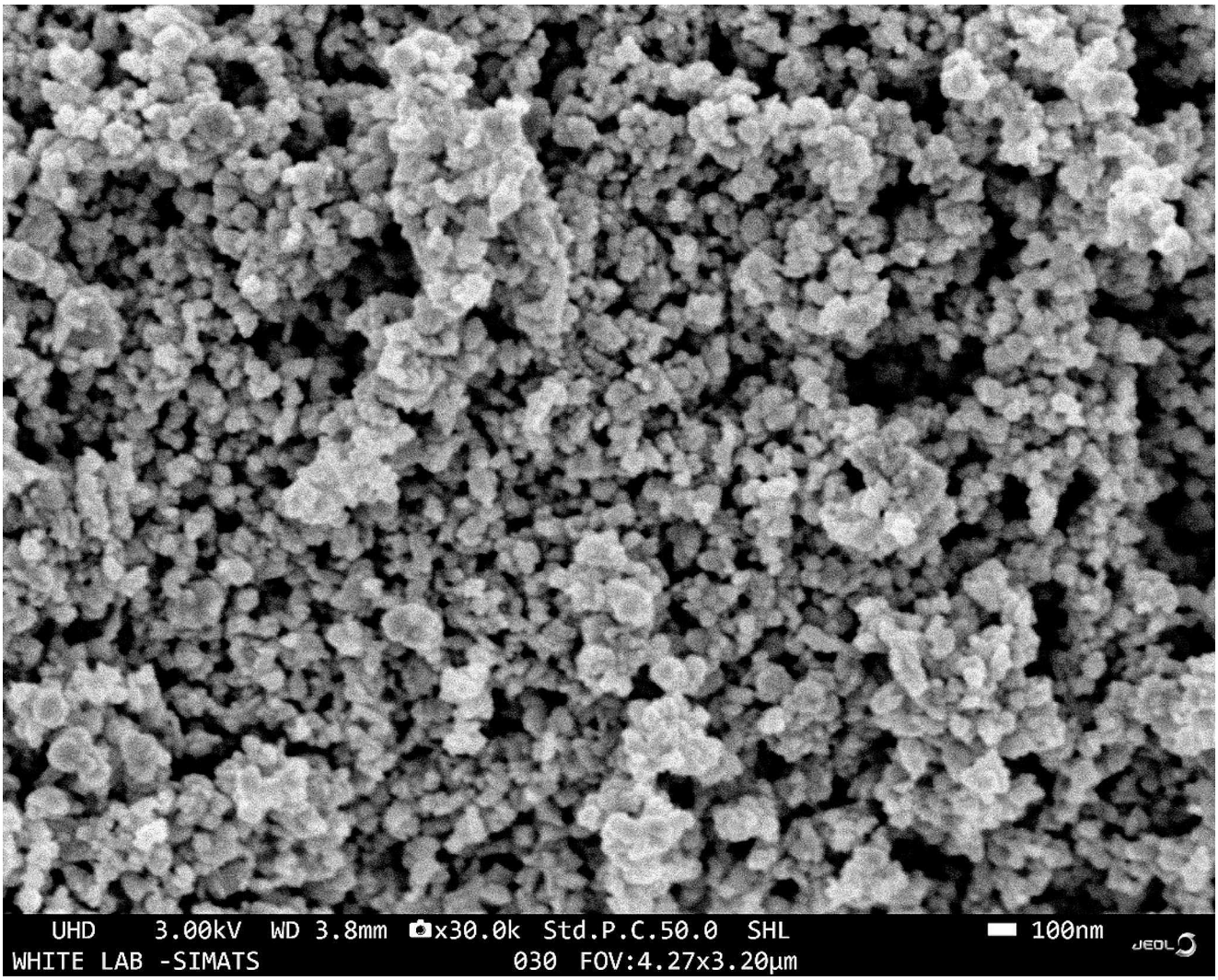

Fig. 2 SEM images of ZnO-CU NPs showed the spherical-like shapes

of the synthesized NPs, providing crucial information about their structural characteristics. Additionally, the observed peaks at 2θ values of 30.038°, 31.778°, 36.619°, 39.266°, 41.384°, and 56.588° may signify the presence of other crystalline phases or the incorporation of CU in the composite, potentially enhancing the anticancer and antimicrobial capabilities. The FTIR peaks observed in the ZnO-CU NPs composite hold significance for their proposed applications in anticancer and antimicrobial contexts (Fig. 4). The peak at 1548.99 cm^{-1} corresponds to C = C stretching vibrations, likely associated with CU, a compound known for its anticancer properties. Peaks at 1337.82 cm^{-1} and 1241.69 cm^{-1} suggest the presence of phenolic groups and C-O stretching vibrations in CU, contributing to its antioxidant and anticancer capabilities. Additionally, the peaks at 1130.22 cm^{-1} and 1081.35 cm^{-1}, associated with Zn-O stretching vibrations in ZnO, align with its antimicrobial activity. The various peaks in the fingerprint region (996.26 cm^{-1}, 941.97 cm^{-1}, 821.92 cm^{-1}, 717.22 cm^{-1}, and 563.77 cm^{-1}) further confirm the presence of essential functional groups crucial for both anticancer and antimicrobial applications.

Antioxidant activity of ZnO-CU NPs

The DPPH assay results (Fig. 5A) illustrate a dose-dependent scavenging effect of ZnO-CU NPs on DPPH free radicals. At concentrations of 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL, the percentage of DPPH radical scavenging activity increased, showing values of 17%, 32%, 61%, and 81%, respectively. Notably, the antioxidant capacity of ZnO-CU NPs at concentrations of 40 µg/mL and 80 µg/mL demonstrated comparable activity to the positive control, with no significant difference.

The ABTS assay results (Fig. 5B) reveal a concentration-dependent scavenging effect of ZnO-CU NPs on ABTS radicals. The conversion of ABTS to its radical

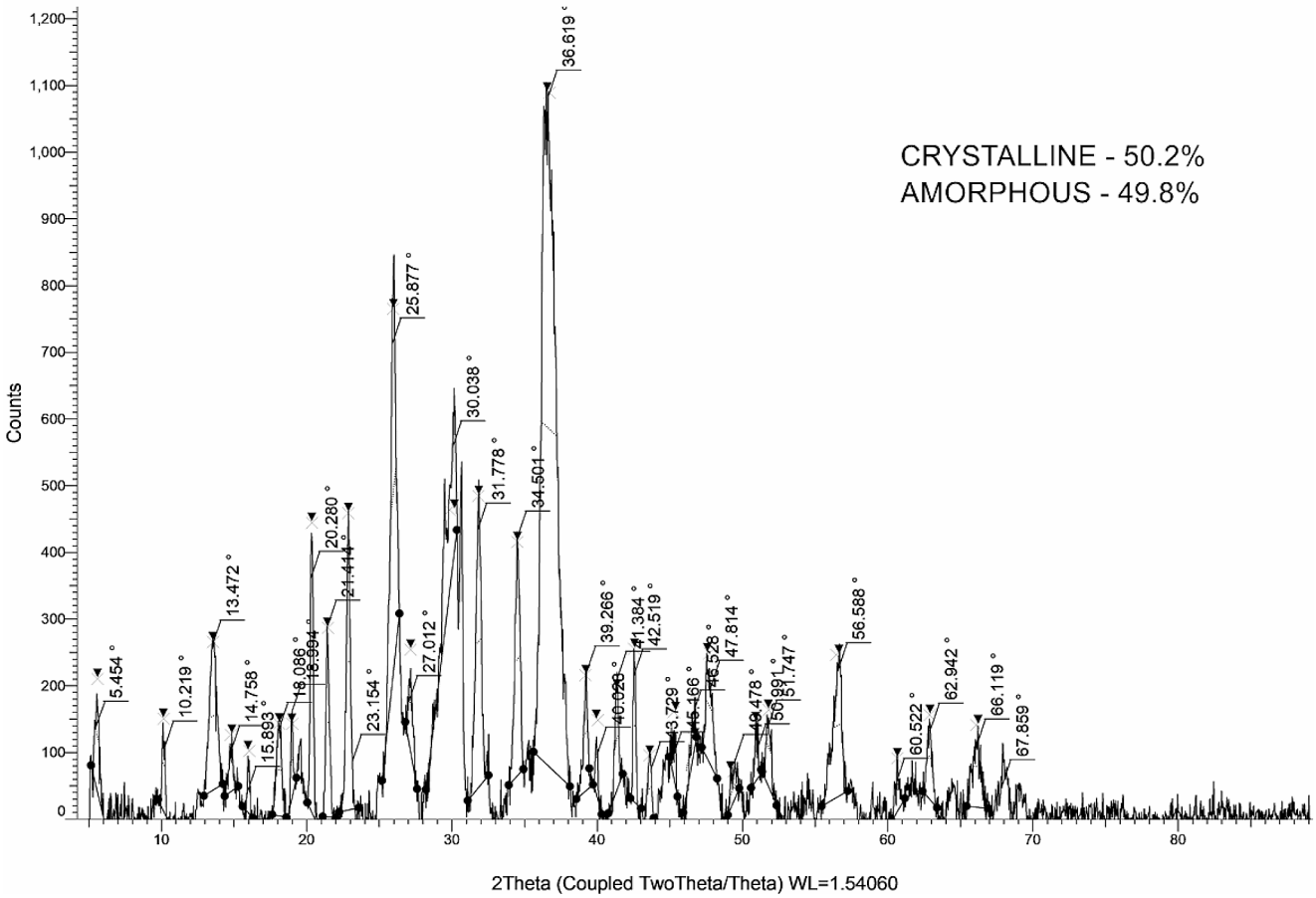

Fig. 3 XRD analysis of ZnO-CU NPs

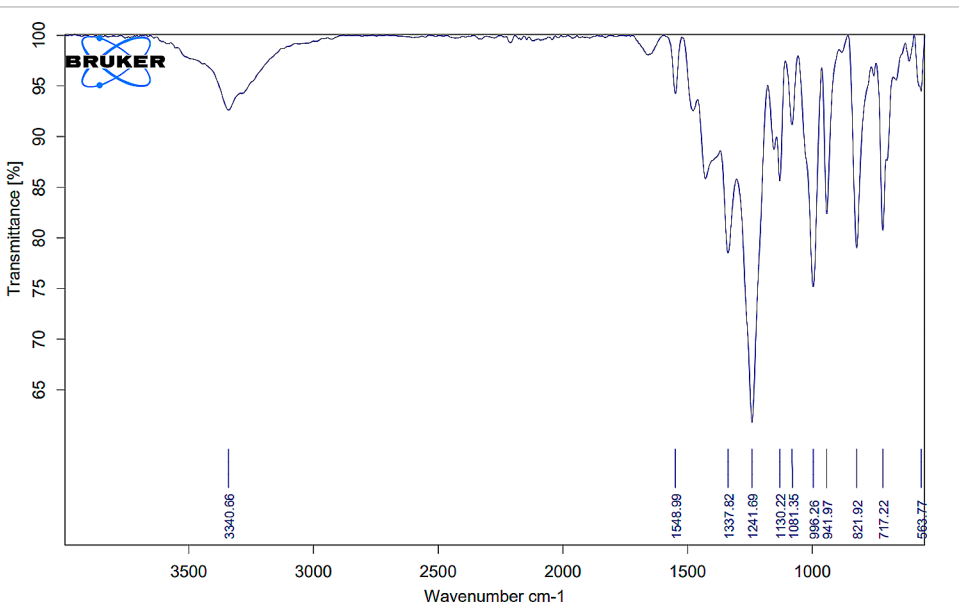

Fig. 4 FTIR characterization of ZnO-CU NPs

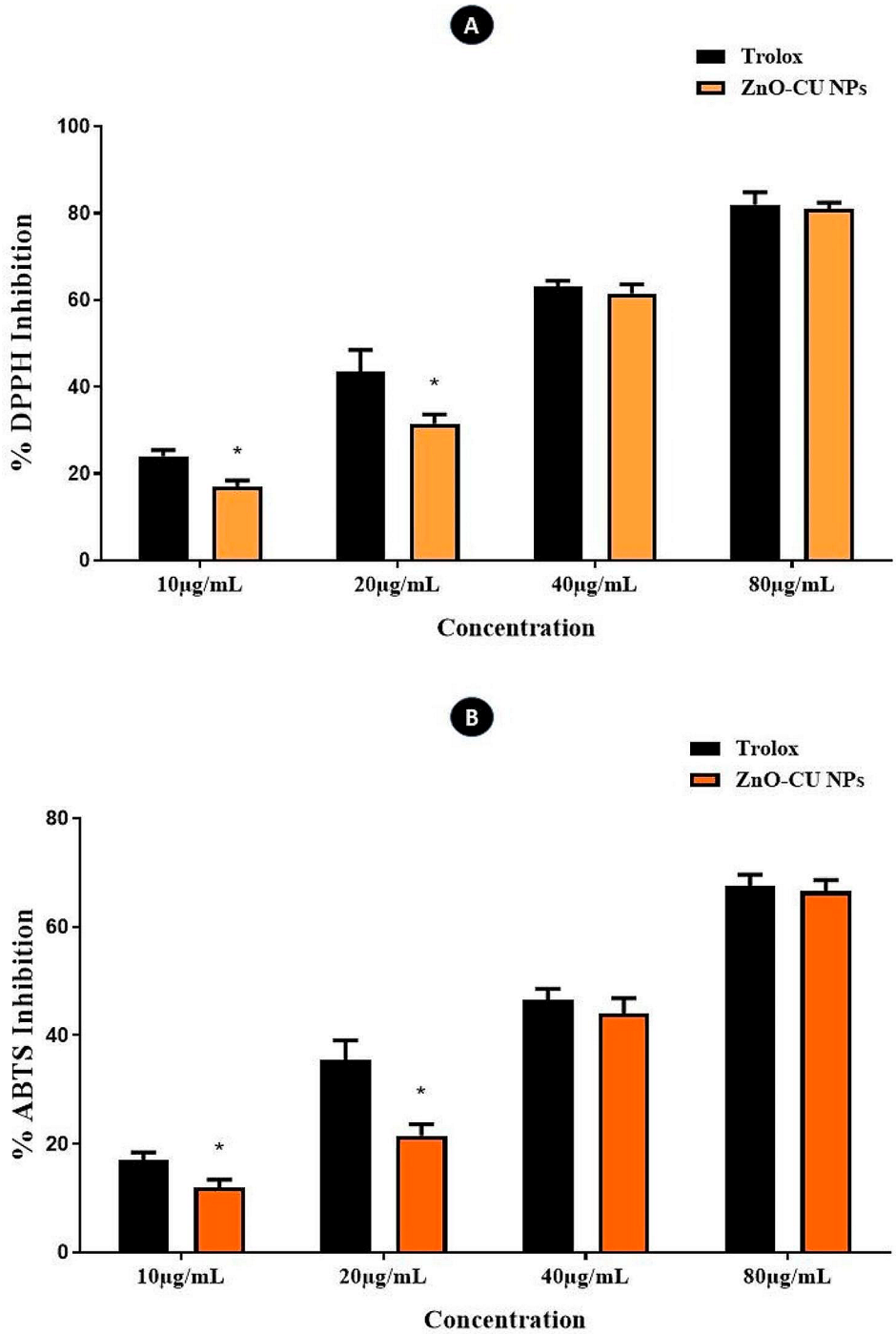

Fig. 5 Free radical scavenging activity of ZnO-CU NPs (10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL) on (**A**) DPPH and (**B**) ABTS. Trolox was used as a positive control. The * represented the level of significance ($p < 0.05$) when the results were compared to the control. Data were presented as mean ± SD of three independent experiments

cation (ABTS•+) and subsequent reduction by ZnO-CU NPs resulted in a decrease in absorbance, indicating the scavenging of ABTS radicals. As the concentration of ZnO-CU NPs increased (10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL), the percentage of ABTS radical scavenging also increased, with values of 12%, 21%, 44%, and 67%, respectively. Similarly, the ABTS radicals scavenging by ZnO-CU NPs at concentrations of 40 µg/mL and 80 µg/mL demonstrated comparable activity to the positive control, with no significant difference.

Antimicrobial activity of ZnO-CU NPs against dental pathogens

ZnO-CU NPs demonstrated consistent Minimum Inhibitory Concentration (MIC) values against the tested dental

pathogens, with *S. aureus*, *S. mutans*, *E. faecalis*, and *C. albicans* exhibiting susceptibility at 40 µg/mL. This implies that concentrations equal to or above 40 µg/mL effectively inhibited the growth of these dental pathogens (Fig. 6). Additionally, the zone of inhibition assay results revealed ZnO-CU NPs' antimicrobial activity against all tested dental pathogens. At 40 µg/mL, the zones of inhibition ranged from 11, 16, 9, and 14 mm, while at 80 µg/mL, the zones increased to 16, 16, 12, and 14 mm for dental pathogens of *S. aureus*, *S. mutans*, *E. faecalis*, and *C. albicans* (E-Supplementary Fig. 1). The comparison with the positive control, amoxicillin (50 µg/mL), showed that ZnO-CU NPs had slightly lower zones of inhibition. However, at 80 µg/mL of ZnO-CU NPs, the zones of inhibition for all pathogens significantly improved, indicating better antimicrobial activity compared to amoxicillin.

Amino acid interaction between CU and dental pathogen biofilm receptor

The molecular docking analysis revealed promising interactions between CU and the receptor proteins of four dental pathogens: *S. aureus, S. mutans, E. faecalis, and C. albicans* (Fig. 7). The binding affinity values for CU indicated higher binding affinity with the pathogen receptors, underscoring its potential as a therapeutic agent against dental infections (E-Supplementary Table 1). In the docking result with *S. aureus* surface protein G (PDB ID: 7SMH), CU exhibited a binding affinity of -6.77 kcal/mol. The identified amino acids involved in the interaction, including THR, suggest potential disruption of the crucial surface protein, hindering the pathogenicity of *S. aureus*. For *S. mutans*, the docking analysis with Antigen I/II carboxy-terminus (PDB ID: 3QE5) resulted in a binding affinity of -5.6 kcal/mol. Interactions with TYR, LYS, PRO, and GLY suggest that CU could disrupt the function of the Antigen I/II carboxy-terminus. Meanwhile, with Enterococcal surface protein (PDB ID: 6ORI), CU demonstrated a binding affinity of -7.4 kcal/mol with the amino acid interaction of ALA, TYR, TYR, and THR. By interfering with this protein, CU may hinder biofilm formation and attenuate the pathogenicity of *E. faecalis* infections. Finally, in the docking analysis with *C. albicans* ALS3 (PDB ID: 4LEE), CU displayed a binding affinity of -7.2 kcal/mol. Interactions with TYR, GLU, and PHE suggest that CU might interfere with the function of this essential protein involved in adherence to host surfaces, a critical step in biofilm formation.

Anticancer activity of ZnO-CU NPs

As shown in E-Supplementary Fig. 2, the results revealed significant alterations in KB cell viability with increasing concentrations of ZnO-CU NPs. At a lower concentration of 10 µg/mL, there was only a minimal effect on cell viability after 24 h of exposure. In contrast, the Cyclophosphamide group exhibited a substantial reduction in cell viability, reaching 27%. However, at higher concentrations of 80 µg/mL, a remarkable decline in KB cell viability (31%) was observed, resembling the cell viability in the positive control group. These findings suggest a concentration-dependent anticancer effect of ZnO-CU NPs on KB cells, indicating potential implications for oral cancer.

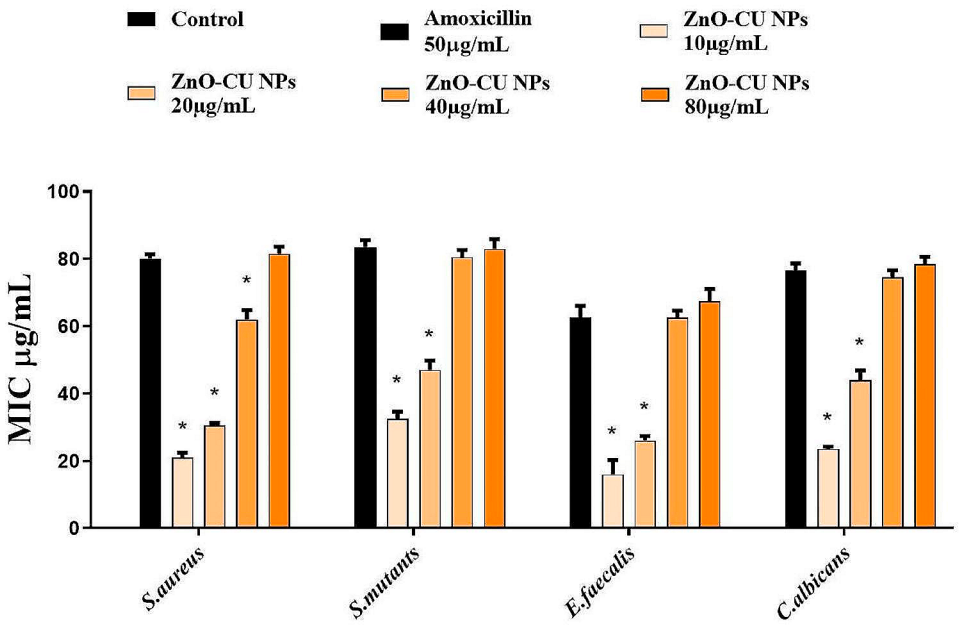

Fig. 6 MIC of ZnO-CU NPs at different concentrations against dental pathogens of *S. aureus*, *S. mutans*, *E. faecalis*, and *C. albicans*. Amoxicillin was used as a positive control. The * represented the level of significance ($p < 0.05$) when the results were compared to the control. Data were presented as mean ± SD of three independent experiments

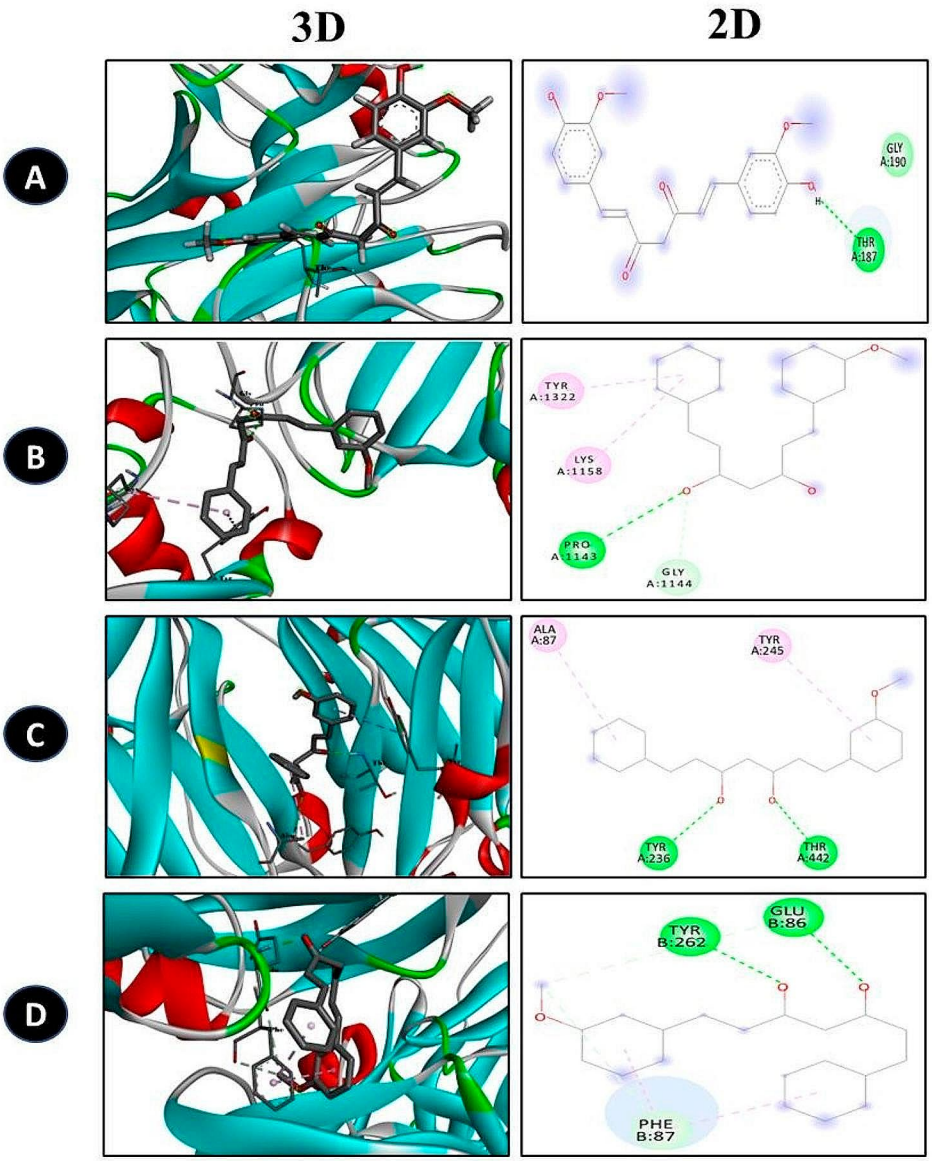

Fig. 7 3D and 2D representative images of CU and dental pathogen receptor interactions. (**A**) *Staphylococcus aureus* surface protein, G (**B**) Antigen I/II carboxy-terminus, (**C**) Enterococcal surface protein, (**D**) ALS3. The interaction is observed with amino acids such as PRO (Proline), GLY (Glycine), GLU (Glutamic acid), ALA (Alanine), Lysine (Lysine), PHE (Phenylalanine), THR (Threonine), and TRY (Tryptophan)

Targeting apoptotic specific pathway

The investigation of ZnO-CU NPs at a concentration of 80 µg/mL revealed superior antioxidant, antimicrobial, and antiapoptotic activity compared to other concentrations. This concentration was identified as optimal and subsequently utilized in gene expression studies. The effective anticancer activity involves the downregulation of BCL2 and the upregulation of BAX and P53 gene expression levels. In accordance with these findings, our study demonstrated that KB cells treated with ZnO-CU NPs at 80 µg/mL exhibited a significant decrease ($p < 0.05$) in BCL2 expression (0.5 fold) and concurrent upregulation of BAX (3.3 fold) and P53 (2.1 fold) levels compared to the control group (Fig. 8).

Discussion

In the context of anticancer applications, the combination of ZnO and CU in NPs form holds great promise. ZnO have demonstrated cytotoxic effects on cancer cells, and CU is known for its anti-inflammatory and anticancer properties [23]. The synergistic effects of these components within the NPs structure may lead to enhanced anticancer activity. The controlled size and spherical morphology observed in the SEM analysis are crucial for effective cellular uptake and targeted interactions with cancer cells and dental pathogen receptors. Meanwhile, the identification and confirmation of these characteristic peaks of ZnO and CU in NPs lay the foundation for understanding the chemical composition and structure of the synthesized ZnO-CU NPs. The XRD

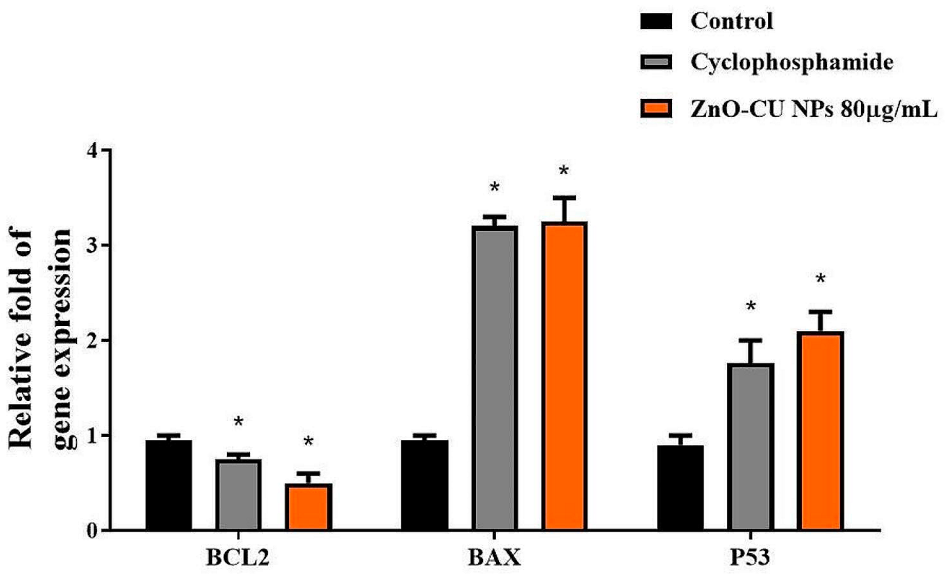

Fig. 8 Effect of ZnO-CU NPs treatment group on the mRNA expression level of BCL2, BAX, and P53. Data were expressed as mean + SD of three independent experiments. *$p < 0.05$ as compared to the control

pattern showed the presence of characteristic peaks associated with the hexagonal wurtzite phase of ZnO suggesting the retention of its crystalline structure, known for its cytotoxic effects against cancer cells and antimicrobial properties [14].

The antioxidant properties of plant compounds with NPs play a pivotal role in both anticancer and antimicrobial activities. In the context of anticancer effects, these NPs, enriched with antioxidants, scavenge reactive oxygen species (ROS), mitigating oxidative stress and protecting DNA from damage, thereby reducing the risk of mutations and cancer initiation [24, 25]. Concurrently, the antioxidant-rich plant NPs demonstrate effectiveness in inhibiting biofilm formation. By disrupting microbial biofilms, they prevent the persistence of microbial communities on surfaces, crucial in the context of infections [26, 27]. The ability to hinder biofilm formation contributes to antimicrobial activity, reducing the resistance of microbes to treatment. This dual functionality underscores the versatile nature of plant NPs, making them promising candidates for integrated therapeutic approaches, addressing both cancer prevention and microbial infection control [28, 29]. In this study, the DPPH and ABTS assay outcomes highlight the concentration-dependent antioxidant activity of ZnO-CU NPs, indicating their potential in anti-oral cancer and antimicrobial applications against dental pathogens. The increased percentage of DPPH and ABTS radical scavenging with rising NPs concentrations underscores their efficacy in neutralizing radicals linked to oxidative damage, a crucial factor in cancer development and microbial infections. The antioxidant behavior observed aligns with the known properties of CU and the potential synergistic effects when combined with ZnO. The scavenging effect on DPPH and ABTS radicals further emphasizes the ZnO-CU NPs role as promising multifunctional agents for oral health interventions, supporting their exploration for potential therapeutic applications.

The antimicrobial activity of zinc oxide nanoparticles (ZnO NPs) and curcumin against dental pathogens is attributed to their unique properties and modes of action, combining to create a synergistic effect. ZnO NPs can release Zn^{2+} ions, which have antimicrobial effects. The Zn^{2+} ions can interfere with microbial cell membranes, disrupting their structural integrity and permeability [30, 31]. This disruption can lead to the leakage of cellular contents and eventually cell death. Also, it can inhibit the activity of microbial enzymes essential for various cellular processes. This interference with enzymatic function disrupts vital metabolic pathways, contributing to the overall antimicrobial effect [32, 33]. Dental pathogens often form biofilms on oral surfaces, contributing to the development of oral infections [34]. CU has been shown to inhibit biofilm formation by interfering with the adhesion of microbial cells to surfaces. It has been reported to disrupt microbial cell membranes [35]. This interference can compromise the structural integrity of the cell membrane, leading to increased permeability and ultimately causing cell death [36]. In this study, the consistent MIC values and significant zones of inhibition demonstrated by ZnO-CU NPs against dental pathogens underscore their potential as antimicrobial agents. Surface proteins in bacteria often play a crucial role in adhesion to host tissues or abiotic surfaces, a crucial step in biofilm formation [37]. From the result of docking studies, it showed that CU interferes with the binding site of *S. aureus* surface protein G, so that it can disrupt the initial adhesion of bacteria, inhibiting biofilm formation. The Antigen I/II proteins in *S. mutans* are known to play a crucial role in bacterial

adhesion and biofilm formation [38]. These proteins facilitate the binding of bacteria to tooth surfaces and promote the formation of dental plaque, which is a precursor to biofilm development. The carboxy-terminus of Antigen I/II is particularly important for its function in adhesion [39]. This CU interference could potentially prevent the efficient adhesion of *S. mutans* to tooth surfaces, inhibiting the initial steps of biofilm formation. Surface proteins in bacteria, including *E. faecalis*, play a crucial role in adhesion to host tissues or abiotic surfaces [40]. CU interacts with the binding sites of the Enterococcal surface protein, so it can interfere with the initial adhesion of *E. faecalis* to surfaces. This disruption could prevent the bacteria from attaching to dental tissues and forming the foundation of a biofilm. Candida albicans ALS3 protein is known for mediating adhesion to host tissues [41]. Since CU interacts with the binding sites of ALS3, it can disrupt the initial adhesion of Candida albicans to surfaces. This interference could prevent the yeast cells from attaching to dental tissues and forming the foundation of a biofilm.

Oral cancer, including malignancies affecting the tongue, lips, and oral cavity, poses a significant health concern worldwide [42]. The unique challenges associated with oral cancer treatment include the need for effective therapies that selectively target cancer cells while minimizing adverse effects on healthy tissues. Recent studies have already reported that ZnO-CU NPs exhibited the best balance between showing the least cytotoxic effect against healthy human embryonic kidney cells and demonstrating good anticancer activity on rhabdomyosarcoma cells [43]. The promising anticancer effects observed in KB cells following exposure to ZnO-CU NPs at higher concentrations indicate their potential as agents for addressing oral cancer. Oral cancer is characterized by dysregulation in apoptosis, a process crucial for maintaining tissue homeostasis. BCL2, an antiapoptotic gene, often displays elevated expression in cancer cells, contributing to their survival and resistance to cell death [44]. Conversely, BAX and P53 are proapoptotic genes involved in initiating and regulating apoptosis [45]. The observed downregulation of BCL2 in KB cells treated with ZnO-CU NPs at 80 µg/mL indicates a potential shift towards an apoptotic phenotype. BCL2 suppression is associated with a reduction in antiapoptotic signals, making cancer cells more susceptible to programmed cell death. This effect aligns with the notion that an effective anticancer agent should target and reduce the expression of antiapoptotic genes [46]. Concomitantly, the significant upregulation of BAX and P53 is indicative of an enhanced proapoptotic response. BAX promotes apoptosis by facilitating the release of mitochondrial cytochrome c and caspase-9, while P53 acts as a crucial regulator of cell cycle arrest and apoptosis [47]. The observed increase in BAX and P53 expression levels suggests activation of apoptotic pathways, reinforcing the potential of ZnO-CU NPs to induce programmed cell death in KB cells. The modulation of apoptosis-related gene expression by ZnO-CU NPs at 80 µg/mL holds promise for the development of targeted therapies in oral cancer treatment. The shift towards a proapoptotic phenotype in cancer cells could contribute to limiting tumor growth and enhancing the efficacy of anticancer strategies. In brief, our research introduces the synthesis of curcumin-loaded zinc oxide nanoparticles (ZnO-CU NPs) and investigates their dual functionality against dental pathogens and oral cancer cells (Fig. 9). These nanoparticles

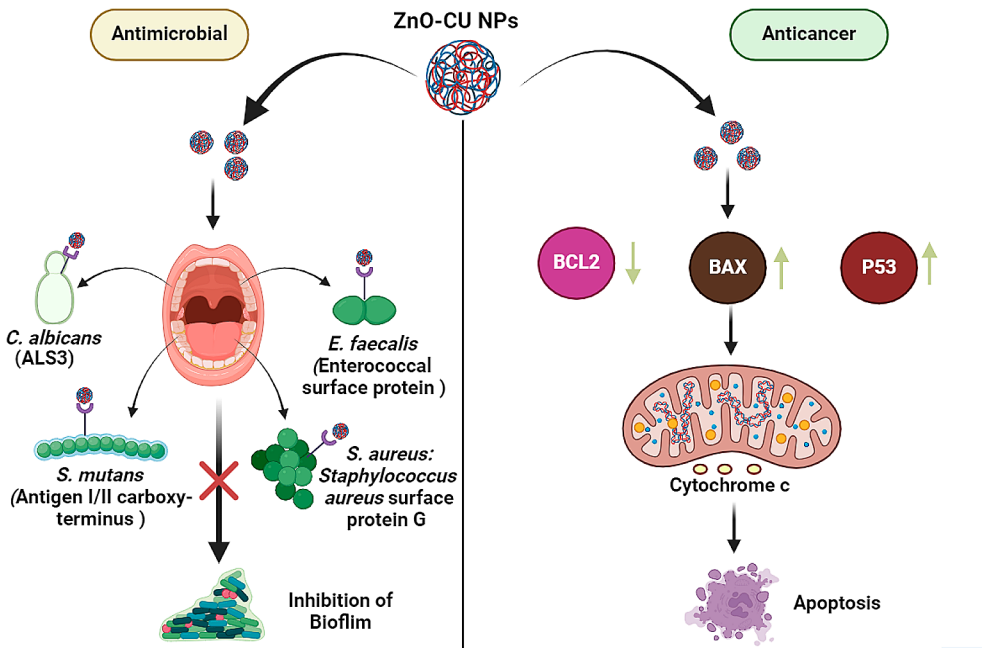

Fig. 9 ZnO-CU NPs were synthesized for dual action against dental pathogens and oral cancer cells. ZnO-CU NPs bound to biofilm receptors, combating dental infections. Additionally, they modulated apoptotic pathways, inducing apoptosis, and inhibiting proliferation in oral cancer cells

exhibit enhanced antimicrobial activity by targeting biofilm receptors, disrupting biofilm formation, and destabilizing existing biofilms, presenting a promising strategy for combating dental infections. Additionally, ZnO-CU NPs demonstrate potent anticancer activity against oral cancer cells by modulating apoptotic-specific pathways, inducing apoptosis, and inhibiting proliferation. During synthesis and treatment, no difficulties were encountered, yielding excellent nanoparticles. However, limitations include the need to develop ZnO-CU NPs into suitable materials or drugs for clinical use, requiring optimization and rigorous testing for safety, efficacy, and biocompatibility. Future research will focus on addressing these limitations and advancing ZnO-CU NPs towards clinical applications in oral healthcare.

Conclusion

The multifunctional capabilities of ZnO-CU NPs, encompassing antioxidant, antimicrobial, and anticancer activities, position them as promising candidates for integrated therapeutic approaches in oral health interventions. However, further in vivo studies and clinical trials are essential to validate their safety, efficacy, and translational potential in addressing oral cancer and microbial infections. The findings from this study provide a robust foundation for the continued exploration and development of ZnO-CU NPs for potential therapeutic applications in oral healthcare.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-024-09407-7.

Acknowledgements This research was supported under a postdoctoral scheme publication under University Malaysia Kelantan.

Author contributions Conceptualization, Data curation, Investigation: J.Z.T, M.P, J.G. Formal analysis: M.S.K.K, R.A. Writing—review and editing: A.G, K.B.M, and J.A. All authors have read and agreed to the published version of the manuscript.

Funding Not applicable.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Jehad Zuhair Tayyeb[1] · Madhu Priya[2] · Ajay Guru[2] · Meenakshi Sundaram Kishore Kumar[3] · Jayant Giri[4] · Akash Garg[5] · Rutvi Agrawal[5] · Khairiyah Binti Mat[6,7] · Jesu Arockiaraj[8]

✉ Ajay Guru
ajayguru.sdc@saveetha.com

✉ Khairiyah Binti Mat
khairiyah@umk.edu.my

✉ Jesu Arockiaraj
jesuaroa@srmist.edu.in

[1] Department of Clinical Biochemistry, College of Medicine, University of Jeddah, Jeddah 23890, Saudi Arabia

[2] Department of Cariology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

[3] Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Department of Anatomy, Saveetha Dental College, Chennai 600 077, Tamil Nadu, India

[4] Department of Mechanical Engineering, Yeshwantrao Chavan College of Engineering, Nagpur, India

[5] Rajiv Academy for Pharmacy, Mathura 281001, Uttar Pradesh, India

[6] Department of Agricultural Sciences, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Jeli 17600, Malaysia

[7] Advanced Livestock and Aquaculture Research Group, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Jeli 17600, Malaysia

[8] Toxicology and Pharmacology Laboratory, Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Kattankulathur603203, Chengalpattu District, Tamil Nadu, India