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Original Article

An Albumin-Glutaraldehyde Nanoparticle Loaded with Berberine Inhibits Cell Growth and Induces Apoptosis in Colon Cancer HCT-116 Cells

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ABSTRACT

Background: The study aims to synthesize and characterize berberine-loaded albumin-glutaraldehyde nanoparticles and evaluate their anticancer activity against colon cancer HCT-116 cells. The research focuses on the mechanisms involved in oxidative stress-dependent apoptosis, crucial for inhibiting colon cancer growth and promoting overall health. **Materials and Methods:** The synthesized Berberine-Albumin NPs were characterized using several techniques, including UV-visible spectroscopy, X-ray Diffractometer (XRD), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Fourier Transform Infrared (FT-IR), Dynamic Light Scattering (DLS) and Photoluminescence (PL) studies. The antibacterial potentials of Berberine-Albumin NPs against various pathogens were examined using the well diffusion technique. The cytotoxicity of Berberine-Albumin NPs against HCT-116 cells was evaluated using the MTT assay. The study evaluated ROS production and apoptosis in HCT-116 cells treated with Berberine-Albumin NPs using fluorescence staining and oxidative stress marker levels using assay kits. **Results:** The characterization analyses revealed the presence of Berberine-Albumin NPs with a cuboidal shape and an average particle size of 155.80 nm. Several elements and functional groups in Berberine-Albumin NPs were also identified. The synthesized Berberine-Albumin NPs exhibited remarkable antimicrobial properties by efficiently suppressing the growth of several pathogens. The treatment with Berberine-Albumin NPs effectively suppressed the proliferation of HCT-116 cells. The findings of fluorescence staining experiments confirmed more ROS accumulation and apoptosis in HCT-116 cells treated with Berberine-Albumin NPs. The administration of Berberine-Albumin NPs resulted in an upregulation of oxidative stress in HCT-116 cells. **Conclusion:** In summary, this work revealed that synthesized Berberine-Albumin NPs had anticancer effects against colon cancer. Hence, the present findings indicate that Berberine-Albumin NPs hold promise as an effective candidate for colon cancer treatment. In future studies, we would like to evaluate clinical relevance, comparative analysis, long-term effects and biodistribution and pharmacokinetics of Berberine-Albumin NPs.

Keywords: Nanoparticles, Berberine, Albumin, Colon cancer, Oxidative stress, Apoptosis.

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INTRODUCTION

In 2020, Colorectal Cancer (CRC) was responsible for 10% of the total number of cancer cases globally and caused 9.4% of all cancer-related deaths. From an epidemiological perspective, the incidence of CRC has shown a consistent increase. In 2020, the number of new cases exceeded 1.9 million, making it the leading cause of cancer-associated mortality. It is anticipated that over 935,000 deaths were attributed to the CRC.[1] The International Agency for Research on Cancer (IARC) predicts that there will be approximately 1.6 million fatalities globally from the disease in 2040. The higher prevalence of CRC patients can be attributed to the growing elderly population, unhealthy diets, an increasing risk of smoking, obesity and less physical activity.[2] The increase in the occurrence of CRC can be primarily attributed to the change

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in lifestyle, which has led to greater exposure to environmental risk factors.

CRC typically progresses gradually over several years, initially manifesting as a harmless abnormal growth known as a polyp. Nevertheless, if not treated, these polyps have the potential to develop into cancer and metastasize to other regions of the body. CRC symptoms may encompass alterations in bowel patterns, like experiencing diarrhea or constipation, the presence of bloody stool, stomach pain and sudden weight loss. Nevertheless, certain individuals affected by CRC may remain asymptomatic, particularly throughout the initial phases of the disease.[3]

CRC arises due to mutations in oncogenes and tumor suppressor genes, which lead to the transformation of the typical colon epithelium into an adenoma. Presently, the available treatment options for CRC are determined by various aspects, such as the tumor's location, the disease stage and the patient's overall health condition.[4] Clinical practice commonly employs a combination of chemotherapy, surgery and radiation therapy to treat CRC. Nevertheless, chemotherapy for CRC has certain disadvantages, mainly the adverse effects of drugs following recurrent administrations. For chemotherapy to be successful, tumor cells must get sufficient amounts of a therapeutic substance while ensuring that the patient does not suffer from intolerable side effects.[5]

Nanomedicine focuses on the utilization of nano-delivery technologies as precise vehicles for drugs. Nanoparticles (NPs) have recently been employed in the treatment of several diseases, serving as chemotherapeutic agents, immunotherapeutic agents, biological agents, etc.,[6] NPs possess distinct electrical, chemical and physical characteristics that render them suitable for use in cancer diagnosis, drug transport and treatment. They serve as exceptional functional biometals.[7] NPs have a size range of 1 to 100 nm, which is comparable to the size of human proteins.[8] It has already been shown that NPs can influence crucial pathways involved in cancer, including apoptosis, oxidative stress and aberrant gene transcription.[9]

An emerging area of study focuses on the application of nanotechnology in conjunction with natural substances. Nanotechnology can be utilized to deliver drugs to treat cancer and other human diseases. NPs can enhance the bioavailability, targeting and controlled release of natural compounds.[10] Bovine Serum Albumin (BSA), a protein with high water solubility, can bind drug compounds and inorganic compounds through noncovalent interactions. Tian *et al.*[11] demonstrated that albumin may be synthesized in the form of microscopic particles and NPs by precipitating it in organic solvents and subsequently crosslinking it with glutaraldehyde. Berberine is a major bioactive compound derived from the *Coptis chinensis* plant that possesses potent pharmacological properties and has received more significant attention in recent times. Previous studies have shown that it possesses various pharmacological activities, including anti-diabetic encephalopathy,[12] cardioprotective, hepatoprotective, renoprotective,[13] neuroprotective,[14] anti-inflammatory,[15] antioxidant[16] and anti-atherogenic[17] properties. The present work was designed to synthesize and characterize berberine-loaded albumin-glutaraldehyde nanoparticles and evaluate their anticancer activity against colon cancer cells.

Berberine-loaded albumin-glutaraldehyde nanoparticles are a new formulation designed to improve the delivery and efficacy of berberine, a bioactive compound with known anticancer properties. Berberine, an isoquinoline alkaloid derived from plants, exhibits anti-inflammatory, antimicrobial and anticancer properties. Albumin, a carrier protein for drug delivery, enhances the solubility and stability of berberine, facilitating targeted delivery to cancer cells.[18] Glutaraldehyde used as a cross-linking agent, forms stable nanoparticles that encapsulate berberine. The nanoparticles are taken up by cancer cells through endocytosis, release berberine in a controlled manner and induce apoptosis by generating reactive oxygen species.[19] The advantages of this formulation include enhanced bioavailability, targeted delivery and controlled release. Potential applications include cancer therapy, particularly colon cancer and combination therapy with other anticancer agents. Challenges include toxicity, pharmacokinetics and scalability.

The objective of the current studies is a synthesize berberine-loaded albumin-glutaraldehyde nanoparticles to evaluate the efficacy of berberine-loaded albumin-glutaraldehyde nanoparticles in inhibiting the growth of colon cancer HCT-116 cells and investigate the mechanisms involved, specifically focusing on the induction of oxidative stress-dependent apoptosis.

MATERIALS AND METHODS

Chemicals

In this study, the chemicals and reagents, including albumin (A4737), berberine (633-65-8), glutaraldehyde (111-30-8), etc., were procured from Sigma-Aldrich, USA. The assay kits for the biochemical studies were purchased from Abcam, USA and Elabscience, USA, respectively.

Synthesis of Berberine-loaded Albumin NPs

To synthesize Berberine-Albumin NPs, 20 mg of berberine was initially mixed with a 4 mL ethanol solution. Subsequently, a solution containing 200 mg of albumin was dissolved in 1 mL of Milli-Q water. The albumin suspension was continuously stirred at 37°C, while the berberine solution was slowly added. After 2 hr, 30 µL of a solution containing 25% glutaraldehyde was mixed as a cross-linking agent. The solution was then agitated vigorously for 24 hr. Subsequently, the NPs were purified using centrifugation at 10,000 rpm for 15 min. The developed Berberine-Albumin NPs were subsequently used for further studies.

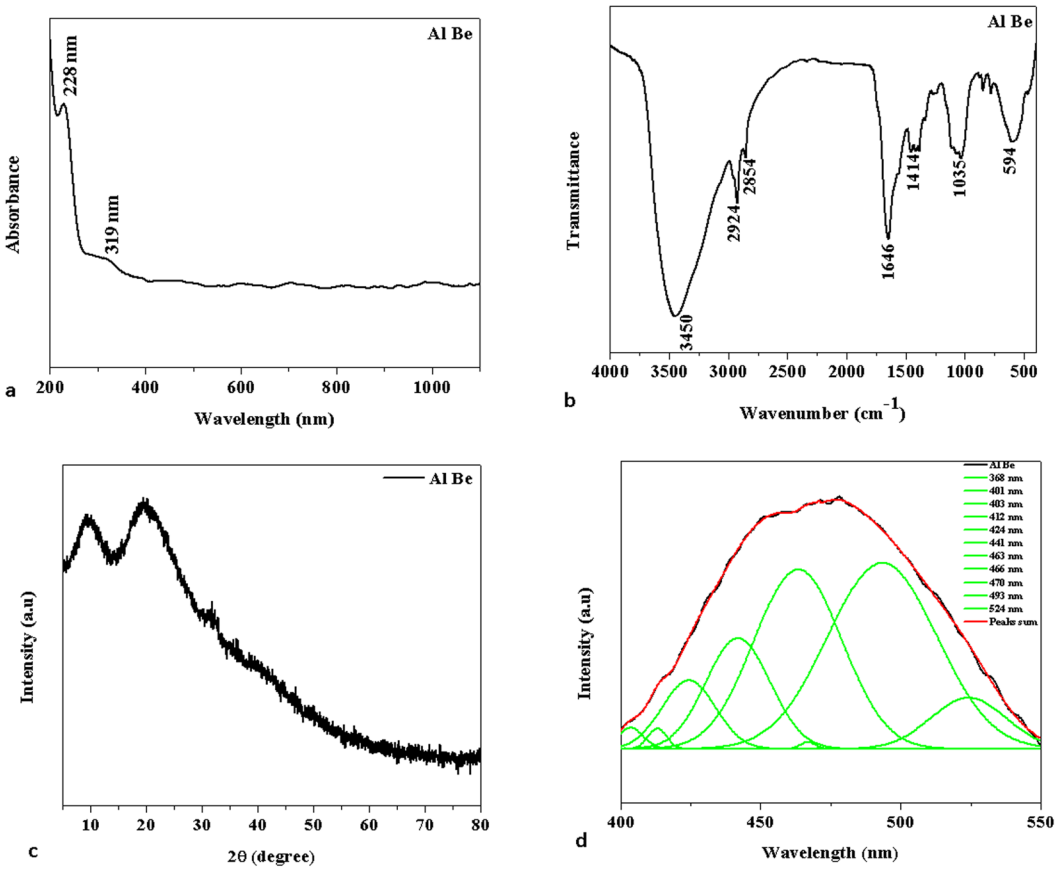


Figure 1: Spectral analysis of the synthesized Berberine-Albumin NPs. (A): UV-visible spectroscopic analysis; (B): Photoluminescence analysis; (C): X-ray diffraction analysis; (D): Fourier transform infrared analysis.

Characterization of the Berberine-Albumin NPs

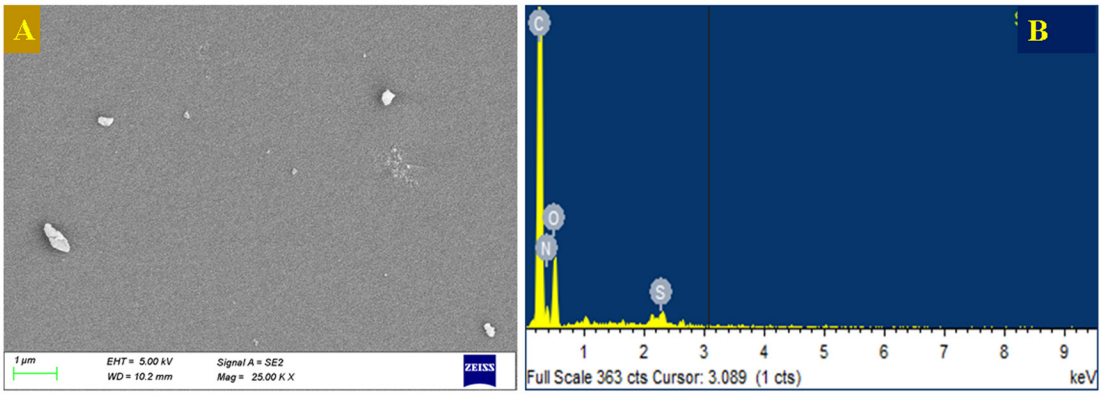
The UV-visible spectral analysis was done to assess the formation of Berberine-Albumin NPs. The quantification of the absorbance of NPs at various wavelengths ranging from 200 to 1000 nm was performed using a UV-visible spectrophotometer (Shimadzu, UV-3101PC, Japan). The crystalline structure of the Berberine-Albumin NPs was analyzed using a PANalytical X-ray diffractometer (X'Pert Pro, USA). The scanning range was configured to span from 2θ=10-80º. The synthesized Berberine-Albumin NPs were evaluated using a JEOL JSM-67001 Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray spectroscopy (EDAX). The TEM (TECNAI F30, USA) was employed to examine the average size and distribution of the Berberine-Albumin NPs. An electron beam was used to scan the NPs sample, which was placed on a copper grid, under electromagnetic radiation in a vacuum environment. The distribution patterns and particle size of the Berberine-Albumin NPs were assessed using Dynamic Light Scattering (DLS) analysis utilizing the Nano-Sight NS500 instrument (Malvern, UK). The Fourier Transform Infrared (FT-IR) technique was employed to analyze the functional groups, stretching and bonding of the Berberine-Albumin NPs. The Berberine-Albumin NPs were examined using a Shimadzu-8400S spectrometer. A spectrum was obtained using a KBr disc across the region of 4000-500 cm^{-1}. The Perkin Elmer-LS 14 spectrometer was used to record the Photoluminescence (PL) spectrum of the Berberine-Albumin NPs.

Antimicrobial activity

The well diffusion technique was employed to evaluate the antimicrobial properties of the Berberine-Albumin NPs. The various pathogens, such as *S. pneumoniae, B. subtilis, B. megatarium, K. pneumoniae, E. coli, V. cholerae* and *C. albicans* were cultivated on specific agar plates. Subsequently, 6 mm wells were developed on the surface of the agar plates. The wells were loaded with 1, 1.5 and 2 µg/mL of the Berberine-Albumin NPs, respectively and incubated for 24 hr. The amoxicillin antibiotic, with a concentration of 30 µg per well, was employed as a positive control. Subsequently, the inhibitory zones were assessed and the results were recorded.

Cell culture

The HCT-116 cells were purchased from ATCC, USA. Cells were grown on DMEM (D5648-10L) media supplemented with



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Element	Weight (%)	Atomic (%)
C K	55.84	61.69
N K	17.75	16.81
O K	25.42	21.08
S K	0.99	0.41
Totals	100.00	

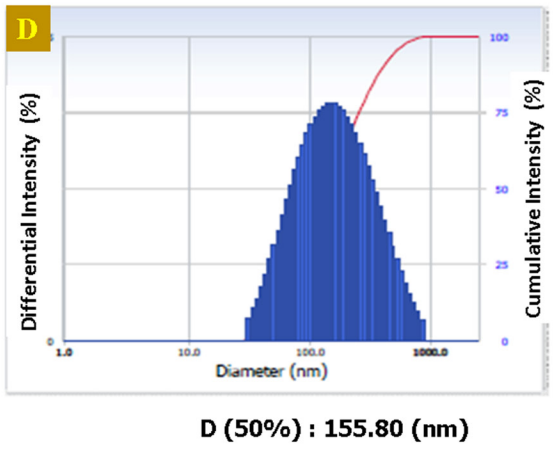

Figure 2: SEM, EDAX and DLS analyses of the synthesized Berberine-Albumin NPs. (A): Scanning electron microscope; (B): Energy dispersive X-ray analysis; (C): Percentage of elements; (D): Dynamic light scattering analysis.

10% fetal bovine serum (12103C-500ML) supplied from Sigma Aldrich (USA) and incubated in a 5% CO_2-supplied incubator. The buffer and other solvents used in this study were supplied from Sigma Aldrich (USA).

Cytotoxicity analysis

The cytotoxicity of Berberine-Albumin NPs was examined using an XTT colorimetric assay against HCT-116 and 3T3 cells. This test relies on the metabolic activity of cells and their capacity to convert Tetrazolium salt (XTT) into formazan. The 50 µL (8×10^3 cells/well) of cells was loaded in the 96-well plate and subsequently treated with the 2,16, 32, 64, 126 and 256 µg/mL of the Berberine-Albumin NPs, respectively, for 24 hr. Following 24 hr incubation, 24.70 µL of XTT reagent was mixed into each well for 4 hr. Finally, the absorbance was taken at 450 nm.

Dual staining

The apoptosis level was assessed by using dual staining on both the control and Berberine-Albumin NP-treated HCT-116 cells. The cells were cultivated on a 24-well plate and treated with 25 µg/mL of the Berberine-Albumin NPs and 2 µg of 5-FU (a standard drug) for 24 hr. Subsequently, the AO/EB dye mixture (100 µg/mL) was mixed into the wells for 5 min in a light-restricted environment. Afterward, the cells were studied using a fluorescent microscope to detect apoptosis.

DAPI staining

The DAPI staining method was employed to investigate the apoptotic levels in HCT-116 cells treated with Berberine-Albumin NPs. Following the introduction of HCT-116 cells into a 24-well plate, the cells were subjected to Berberine-Albumin NP treatment at concentrations of 24.70 µg/mL and/or 2 µg of 5-FU for 24 hr. After being fixed in paraformaldehyde (4%) for 30 min, the cells were stained using DAPI (200 µg/mL) for 15 min. The effect of Berberine-Albumin NPs on nuclear damage and apoptosis levels was further examined using a fluorescence microscope.

Analysis of oxidative stress markers

In this study, the assay kits were utilized to assess the levels of malondialdehyde (MDA: E-EL-0060), glutathione (GSH: E-BC-K030-M), superoxide dismutase (SOD: E-BC-K022-M) and catalase (CAT: E-BC-K031-M) in the cell lysates of both untreated and Berberine-Albumin NPs-treated HCT-116 cells. The assays were conducted in triplicates under the recommended protocols by the manufacturer (Elabscience, USA).

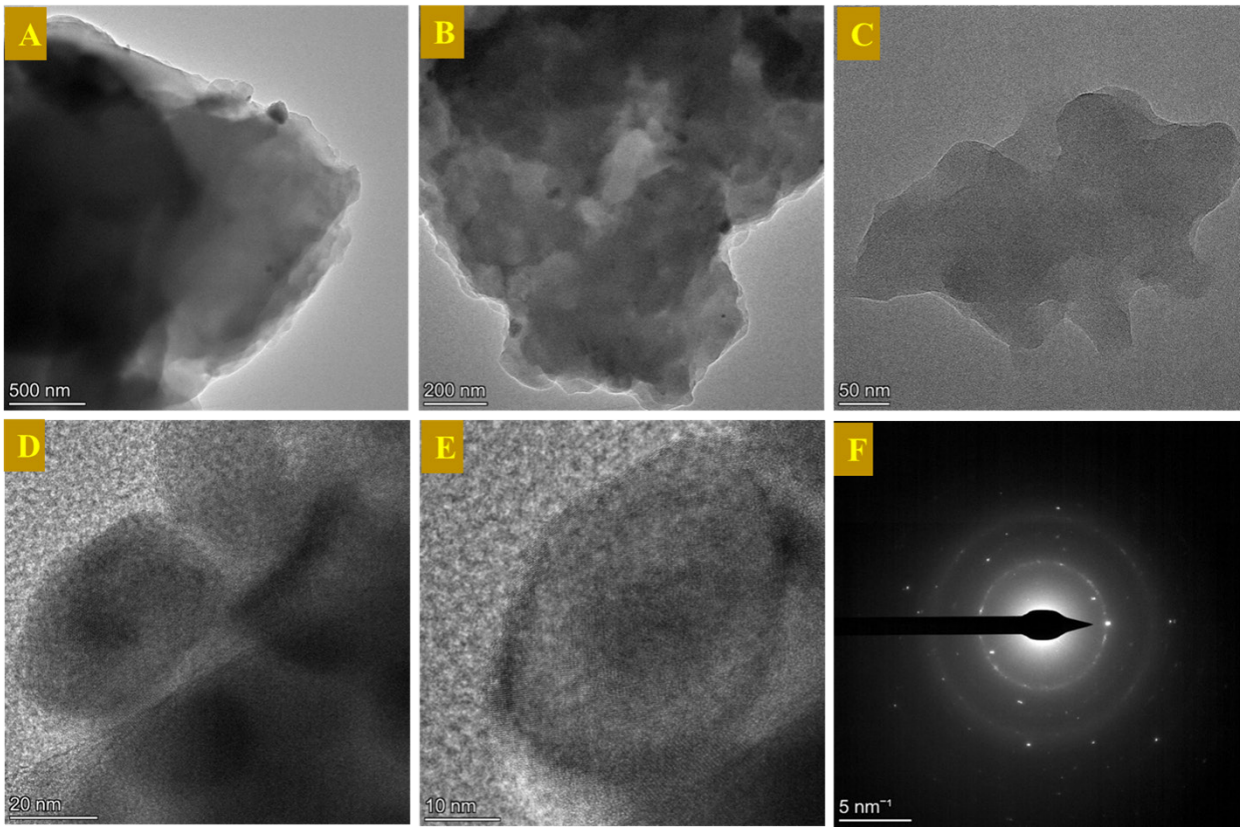

Figure 3: TEM analysis of the synthesized Berberine-Albumin NPs. (A): Transmission electron microscope; (B): SAED pattern of the Berberine-Albumin NPs.

Statistical analysis

The data set was statistically analyzed using SPSS software. The results are reported as the mean±SD of triplicates (n=3). The values are analyzed by ANOVA and Dunnett's multiple range test to ascertain the statistical significance (p<0.05).

RESULTS

Characterization of the synthesized Berberine-Albumin NPs

The UV-visible spectrum analysis results of the Berberine-Albumin NPs are shown in Figure 1(a). The absorbance of the Berberine-Albumin NPs was measured at wavelengths from 200 to 1000 nm. The production of Berberine-Albumin NPs was confirmed by observing their maximal absorbance peaks at wavelengths of 228 and 319 nm (Figure 1a).

Figure 1(b) presents the findings of a PL analysis conducted on the Berberine-Albumin NPs. The Berberine-Albumin NPs displayed absorptions at specific wavelengths of 368, 401, 403, 412, 424, 441, 463, 466, 470, 493 and 524 nm. The PL spectrum offers information on the crystal structure, surface properties and structural imperfections of the Berberine-Albumin NPs. The presence of peaks at wavelengths 368, 401 and 403 nm indicates the existence of free exciton recombination. The presence of interstitial oxygen vacancies can be attributed to the green-blue emissions observed at the peaks of 412, 424, 441, 463, 466 and 470 nm. Figure 1(b) demonstrates that the Berberine-Albumin NPs exhibit emissions at wavelengths of 493 and 524 nm, indicating the occurrence of a singly ionized oxygen vacancy.

The purity and crystalline characteristics of the Berberine-Albumin NPs were evaluated by XRD analysis (Figure 1c). The peaks observed in Figure 1(c) represent the unique crystallographic orientations of the Berberine-Albumin NPs in their crystalline state, exhibiting multiple different peaks.

The FTIR analysis was used to detect the specific functional groups responsible for the bioreduction, capping and stability of the synthesized NPs (Figure 1d). The FT-IR spectra of the Berberine-Albumin NPs exhibit a prominent peak at 3450 cm^{-1}, indicating the Hydroxyl (OH) groups stretching that are absorbed onto the surface of the Berberine-Albumin NPs. The peak noted at 2924 cm^{-1} and 2854 cm^{-1} corresponds to the asymmetric stretching of the Al-O bond. Moreover, the detection of the O-Al-O bond at 1646 cm^{-1} denotes the development of crystallized Berberine-Albumin NPs. The peaks corresponding to the elongation of the C-O bond were detected at a wavelength of 1414 cm^{-1}. The peaks at 1035 cm^{-1} and 594 cm^{-1} were corresponding to the stretching of the C-O and C-C bonds (Figure 1d).

Berberine-Albumin NPs were analyzed using SEM and EDX techniques to analyze the composition and structural

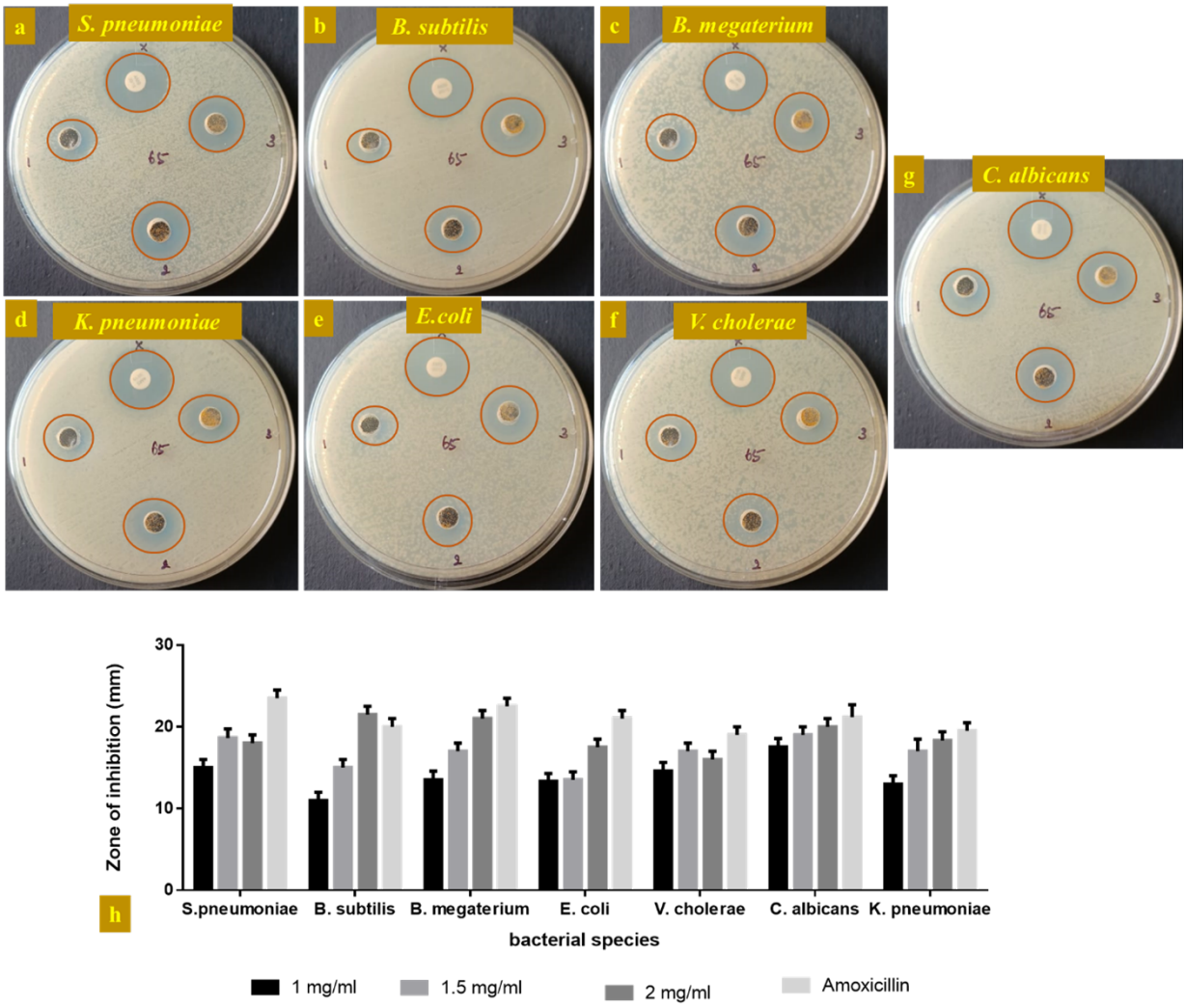

Figure 4: Antimicrobial activity of the synthesized Berberine-Albumin NPs

arrangement of the NPs. The findings of these analyses are depicted in Figures 2 (a and b). The SEM images indicated that the Berberine-Albumin NPs exhibited cuboidal and randomly distributed particles across the observed region (Figure 2a). The EDX study of the Berberine-Albumin NPs (Figure 2b) revealed the existence of many elements, including Carbon (C), Oxygen (O), Nitrogen (N) and Sulfur (S).

Figure 2(c) presents the results of the DLS analysis, which was used to examine the distribution and average size of the Berberine-Albumin NPs. The analysis revealed distinct peaks, indicating an average particle size of 155.80 nm.

The sizes and morphology of the Berberine-Albumin NPs were analyzed using TEM. SAED is a technique that is commonly used in combination with TEM to examine the crystalline properties of NPs (Figure 3a-e). The TEM images revealed the presence of nanostructures composed of Berberine-Albumin NPs, which exhibited cubic-like structures. The selected area of the SAED pattern (Figure 3f) of the synthesized Berberine-Albumin NPs likewise confirmed their crystalline structure.

Antimicrobial activity of the synthesized Berberine-Albumin NPs

The antibacterial activities of Berberine-Albumin NPs against various pathogens, such as *S. pneumoniae, B. subtilis, B. megatarium, K. pneumoniae, E. coli, V. cholerae* and *C. albicans*, were assessed using the well diffusion technique (Figure 4). The treatment of different doses of Berberine-Albumin NPs has demonstrated a significant effect on inhibiting the growth of all tested strains. Berberine-Albumin NPs treatment efficiently inhibited the growth of pathogens, more effectively against *S. pneumoniae, B. subtilis* and *B. megaterium* (Figure 5). The observed inhibition zones surrounding the wells treated with Berberine-Albumin NPs provide evidence of the growth inhibition of these pathogens.

The treatment of different doses of Berberine-Albumin NPs has demonstrated significant effectiveness in inhibiting the growth of all tested strains. More sensitivity was shown by *S. pneumoniae (a), B. subtilis (b), B. megaterium (c), K. pneumoniae (d), E. coli (e), V. cholerae(f)* and *C. albicans (g)*. Antimicrobial activity (Zone

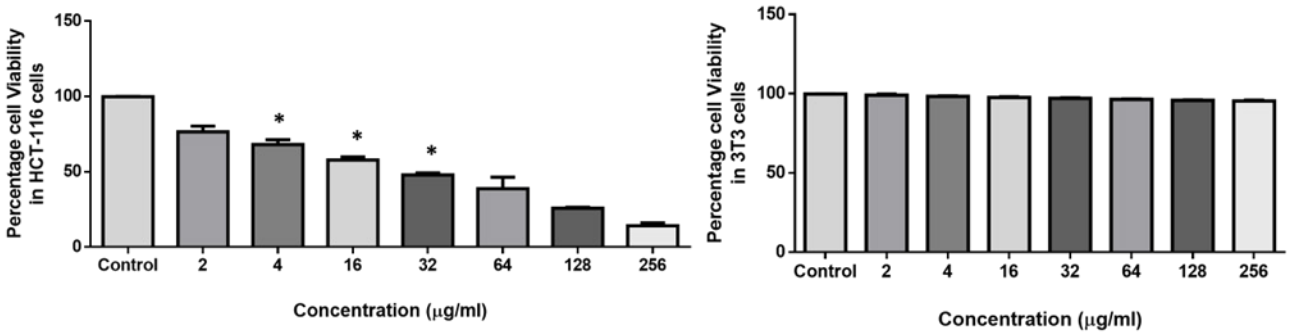

Figure 5: Effect of Berberine-Albumin NPs on the viability of HCT-116 and non-malignant 3T3 cells

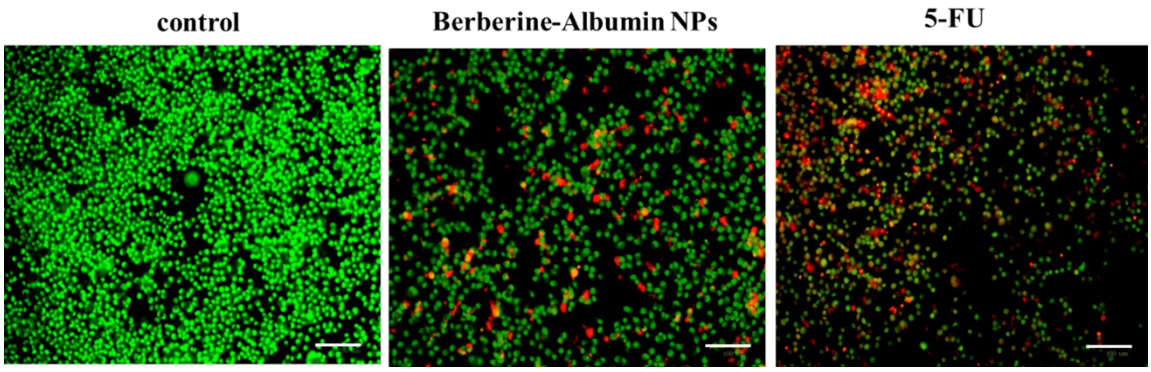

Figure 6: Effect of Berberine-Albumin NPs on the apoptosis in colon cancer HCT-116 cells.

of inhibition) of the synthesized Berberine-Albumin NPs (h) and the results are reported as the mean±SD of triplicates (n=3). The data were subjected to one-way ANOVA and Dunnett's multiple-range tests. '*' reveals the significance at p<0.05 from the control group.

Effect of Berberine-Albumin NPs on the viability of HCT-116 and non-malignant 3T3 cells

Figure 5 presents the findings of the XTT cytotoxicity assay, illustrating the effect of Berberine-Albumin NPs on HCT-116 and 3T3 cell viability. Berberine-Albumin NPs treatment significantly decreased the viability of HCT-116 cells at concentrations of 1-64 µg/mL. Berberine-Albumin NPs treatment at doses ranging from 1 to 64 µg/mL did not have any cytotoxicity on the viability of 3T3 cells. The higher concentration of Berberine-Albumin NPs had less effect on 3T3 cell growth, as seen in Figure 6. The Berberine-Albumin NP's IC_{50} concentration was set at 24.70 µg/mL for HCT-116 cells and this dosage was selected for subsequent assays.

The results are reported as the mean±SD of triplicates (n=3). The data were subjected to one-way ANOVA and Dunnett's multiple-range tests. '*' reveals the significance at p<0.05 from the control group.

Effect of Berberine-Albumin NPs on the apoptosis in HCT-116 cells

The dual staining procedure was performed to study apoptosis in both the control and Berberine-Albumin NP-treated HCT-116 cells. The findings of this study are given in Figure 6 After being treated with 24.70 µg/mL of Berberine-Albumin NPs; the HCT-116 cells exhibited increased levels of yellow and orange fluorescence, providing evidence of the occurrence of apoptotic cell deaths. The 5-FU treatment findings also demonstrated an increased number of cells exhibiting more yellow/orange fluorescence, which proves the occurrence of apoptosis (Figure 6). The observed increases in apoptotic cell death provide evidence of the elevated production of endogenous ROS in the HCT-116 cells treated with Berberine-Albumin NPs. The 5-FU treatment also showed increased green fluorescence, indicating an increase in the accumulation of endogenous ROS.

The fluorescent microscope was employed to measure the intensity of the developed fluorescence. The presence of more apoptotic cell death was observed in the HCT-116 cells following treatment with Berberine-Albumin NPs shown by increased yellow/red fluorescence. The standard drug 5-FU at a concentration of 5 µM/mL as a positive control. A representative image of the triplicate experiment at 20X magnification is shown here.

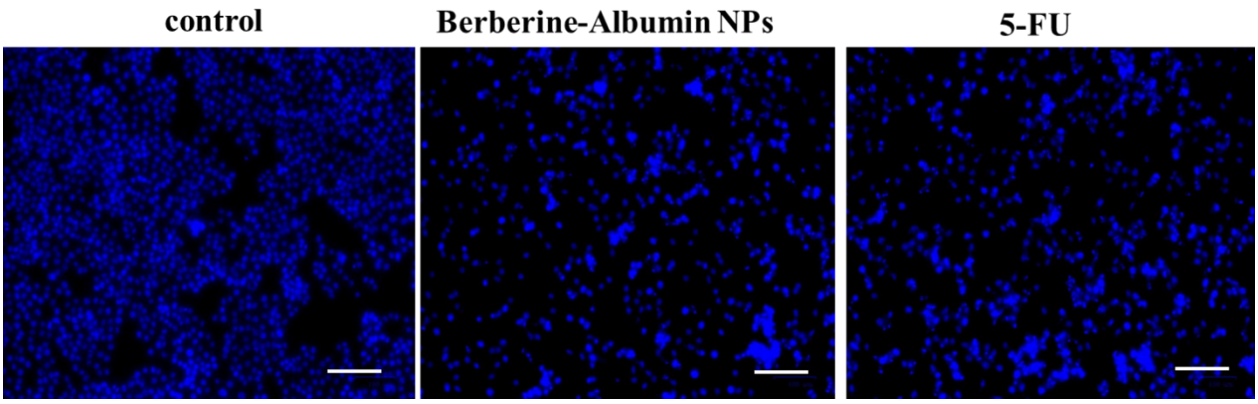

Figure 7: Apoptosis in HCT-116 cells was determined using DAPI nuclear staining.

Effect of Berberine-Albumin NPs on the apoptosis in the HCT-116 cells

The apoptotic cells in both control and Berberine-Albumin NP-treated HCT-116 cells were examined using DAPI staining and the findings are given in Figure 7. The HCT-116 cells treated with Berberine-Albumin NPs at concentrations of 24.70 µg/mL showed increased occurrences of apoptosis, characterized by nuclear condensation and damage, membrane damage and apoptotic body development. The Berberine-Albumin NPs treatment significantly enhanced apoptosis and resulted in a reduction in cell counts in the HCT-116 cells, which is similar to the 5-FU treatment results.

Al Be nanoparticles were treated with IC_{50} doses of standard drug 5-FU at a concentration of 5 µM/mL compared to control for 24 hr (control cells, cells treated with IC_{50} and cells treated with 5-FU). This is a representative image of the triplicate experiment.

Effect of Berberine-Albumin NPs on the oxidative stress markers in the HCT-116 cells

Figure 8 displays the MDA, GSH, CAT and SOD levels in HCT-116 cells that were exposed to Berberine-Albumin NPs, as well as in the control group. The control cells exhibited increased levels of GSH, CAT and SOD, while a decrease in MDA levels was observed. Meanwhile, when HCT-116 cells were treated with 25 µg/mL of Berberine-Albumin NPs, there was an increase in MDA levels and a decrease in GSH, CAT and SOD levels compared to the control (Figure 8). The treatment of 5-FU also resulted in an increase in the MDA level and a decrease in the SOD, CAT and GSH levels in the HCT-116 cells.

The results are reported as the mean±SD of triplicates (n=3). The data were subjected to one-way ANOVA and Dunnett's multiple-range tests. '*' reveals the significance at $p<0.05$ from the control group.

DISCUSSION

Colorectal Cancer (CRC) is characterized by the unregulated growth of cells, resulting in the development of malignancies through the modification of intracellular pathways and the manipulation of apoptosis. Therefore, it is essential to block the uncontrolled growth of tumor cells to treat Colorectal cancer.[20] The present findings proved that Berberine-Albumin NPs treatment effectively inhibited HCT-116 cell growth in a concentration-dependent manner. A CRC malignant tumor develops when there is a disruption in the regulation of cellular processes that control normal cell growth and division, including apoptosis.[21] To develop medicines that can effectively combat CRC, it is essential to accurately detect the precise regulatory systems within tumor cells. A significant obstacle to CRC treatment is the inadequate targeting of drugs, which leads to substantial damage to healthy tissues. In response to this problem, nanoscale drug delivery systems have been developed as a viable solution to improve the therapeutic efficacy of different drugs and bioactive substances. This is achieved by utilizing the Enhanced Permeability and Retention (EPR) effect.[22]

Developments in the use of phytocomponents conjugated with nanoparticles have made it possible to enhance the treatment of colon cancer using a wide range of NPs.[23] Currently, this conjugated nanoparticle approach has emerged as a potent treatment strategy used in this study and has combined NPs with other nanometals or pharmaceuticals to treat colon cancer. Chemotherapy is a highly successful approach to treating various forms of Colorectal cancer.[24] Chemotherapeutic medicines induce genotoxicity. They have some drawbacks, including limited specificity in their interactions, an insufficient rate of distribution throughout the body, significant adverse effects and the emergence of drug resistance.[25] Hence, the process of producing NPs from natural bioactive substances offers novel combinations that can reduce the toxic effects on normal cells. Therefore, in this work, we report the synthesis and characterization of berberine-loaded albumin-glutaraldehyde NPs and their anticancer activity against colon cancer HCT-116 cells.

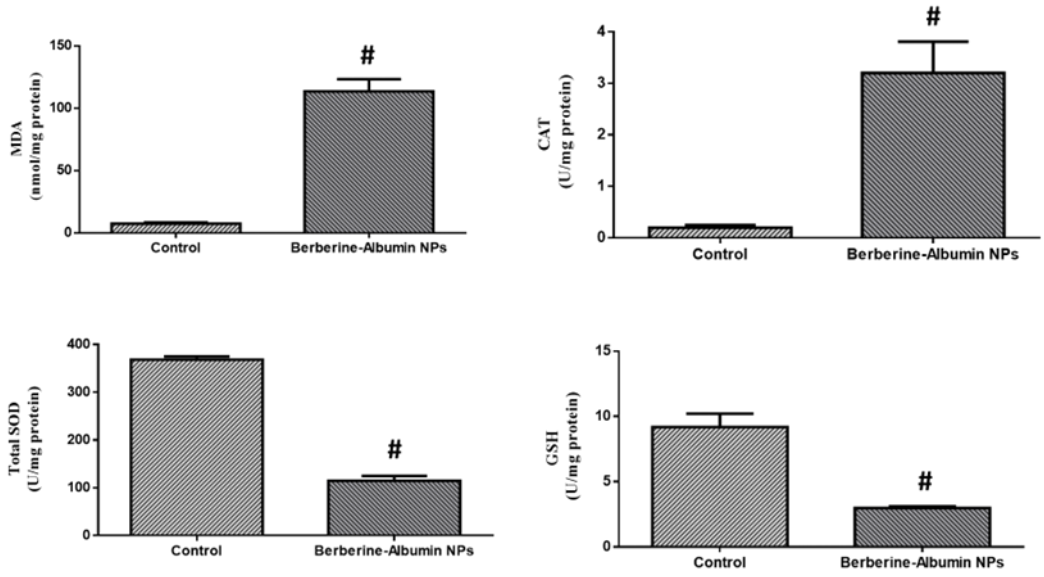

Figure 8: Effect of Berberine-Albumin NPs on the oxidative stress markers in the HCT-116 cells

In regards to the antimicrobial activity of Berberine-Albumin NPs, the synthesis of conjugative nanoparticles provides several opportunities for modifying the physico-chemical properties of drugs, thereby improving novel biological applications. Many types of NPs have been found to effectively combat different diseases, including infections that are resistant to antibiotics. The antibacterial capabilities of NPs can be altered by many processes, including changes in their size, surface area, shape and physicochemical properties. As the size of the NPs decreases, the ratio of their surface area to volume increases.[26] The more surface areas of NPs promote increased contact with bacteria, thereby impacting their antimicrobial activities. The findings of this study proved that the Berberine-Albumin NPs treatment efficiently inhibited the growth of pathogenic bacteria and fungi.

Colorectal cancer therapy faces resistance due to cancer cells' ability to fight apoptosis, disrupting intracellular pathways, causing growth and spread and promoting tumor growth.[27] In this study we aim to enhance apoptosis for cancer management, minimizing damage to normal cells and supporting clinical management to combat therapy resistance caused by cancer cells' ability to fight apoptosis using Berberine-Albumin NPs. Commercially available drugs for CRC that induce the programmed cell death of tumor cells are a highly effective technique for cancer treatment and multiple pathways contribute to this complex process.[28] As previously reported several antitumor agents demonstrate their antitumor activities by triggering apoptosis and apoptotic cell death plays a crucial role in the regulation of cellular balance and suppressing the growth of malignancy.[29]

Our results demonstrated that Berberine-Albumin NPs induce apoptotic cell death and exhibit distinct features, including nuclear fragmentation, cell shrinkage, RNA degradation and DNA fragmentation. Apoptosis prevents the occurrence of unregulated cellular proliferation. Dysregulation of apoptosis is a major contributing element to the advancement of Colorectal cancer.[30] Hence, the exploration of innovative therapeutic strategies that particularly target the apoptotic pathway in cancer treatment exhibits substantial promise. A previous study has shown that non-encapsulated chemotherapeutic medicines specifically cause cell death in tumor cells without disturbing non-malignant cells.[31]

In this work, the results of fluorescent staining analyses showed that the treatment of Berberine-Albumin NPs effectively promoted apoptotic cell death in HCT-116 cells. Therefore, it was clear that Berberine-Albumin NPs could inhibit the development of colon cancer by promoting colon cancer cells. ROS disrupts the equilibrium inside cells between oxidative stress and anti-oxidant processes, resulting in persistent cellular damage.[32,33] Generally, CRC tumor cells rely on a high quantity of ROS to carry out essential biological processes, including differentiation and development, as well as to regulate metabolism. Furthermore, ROS at the necessary threshold also contributes to the advancement of tumors, metastasis and the formation of new blood vessels. Nevertheless, the concentration of ROS surpasses the threshold, resulting in oxidative stress, particularly in tumor cells.[34]

Mitochondrial dysfunction in tumor cells increases susceptibility to oxidative stress, leading to ROS generation, toxicity and cell death when exogenous agents are used to elevate ROS levels.[35,36] The present findings demonstrated that Berberine-Albumin NPs treatment appreciably elevated ROS accumulation in HCT-116 cells. Hence, it can be reported that Berberine-Albumin NPs may trigger apoptosis in colon cancer cells via increasing oxidative stress. All chemotherapeutic drugs cause apoptosis in tumor cells due to oxidative stress, which can be reduced by antioxidants. Cancer cells accumulate more drugs, indicating enhanced

defense mechanisms against ROS. Therefore, redox-modifying techniques are crucial to counteract resistance and improve cancer therapy efficacy.[37] The assay results showed a notable reduction in antioxidant levels and a simultaneous increase in oxidative stress in HCT-116 cells exposed to NPs. This could potentially lead to cell death caused by oxidative stress.

In summary, the study explores the use of Berberine-Albumin Nanoparticles (NPs) in treating colon cancer by inhibiting the growth of HCT-116 cells in a concentration-dependent manner. NPs improve drug targeting and reduce damage to healthy tissues. Combining NPs with other nanometals or pharmaceuticals is an emerging treatment strategy. Despite challenges like limited specificity and drug resistance, NPs from natural bioactive substances can reduce toxicity to normal cells. The study also highlights the potential of NPs in targeting apoptosis, reducing Reactive Oxygen Species (ROS) levels and managing therapy resistance.

CONCLUSION

As a result of this study, it was determined that synthesized Berberine-Albumin NPs were anticancer in their effects against colon cancer. In HCT-116 cells, berberine-albumin NPs inhibit growth and increase oxidative stress markers, thereby promoting apoptosis. HCT-116 cells treated with Berberine-Albumin nanoparticles showed significantly reduced viability, enhanced ROS production, elevated oxidative stress markers and ultimately apoptosis. The present findings demonstrate that Berberine-Albumin nanoparticles are effective candidates for treating colon cancer. Furthermore, further research should be conducted on the specific therapeutic functions of Berberine-Albumin NPs in combating colon cancer. The study on berberine-loaded nanoparticles for cancer treatment is limited by *in vitro* nature, lack of long-term studies and toxicity and safety studies. It primarily focuses on oxidative stress-dependent apoptosis but may not explore other pathways. Prospects include *in vivo*, animal models, clinical trials and regulatory approval.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

pH: Potential of hydrogen; **M:** Molar; **mg**: Milligram; **µg:** Micro gram; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **CAT:** Catalase; **mL:** Microliter; **IC_{50}:** Concentration of a compound with half-maximal cell viability; **DMSO:** Dimethyl sulfoxide; **MMP:** Mitochondrial Membrane Potential (ΔΨm); **ROS:** Reactive Oxygen Species; **AO:** Acridine Orange; **XTT:** 2;3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetr azolium-5-carboxanilide; **EtBr:** Ethidium Bromide; **CO_2:** Carbon dioxide; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **BAX:** BCL-2 associated X proteins; **BCL-2:** B cell lymphoma 2; **CRC:** Colorectal cancer; **ER:** Endoplasmic reticulum; **KRAS:** Kirsten rat sarcoma viral oncogene homolog; **5FU:** Fluorouracil; **DAPI:** 4';6-diamidino-2-phenylindole; **cm^2:** Square Centimeter; **PI:** Propidium Iodide; **PBS:** Phosphate buffer saline.

SUMMARY

Recent advances in Nanoparticle (NP) technology have enabled the use of various NPs in colon cancer treatment. This study aimed to synthesize and characterize berberine-loaded albumin-glutaraldehyde nanoparticles (Berberine-Albumin NPs) and evaluate their anticancer activity against colon cancer HCT-116 cells. The synthesized nanoparticles were analyzed using UV-visible spectroscopy, X-ray diffraction, electron microscopy and other techniques, revealing cuboidal particles with an average size of 155.80 nm and the presence of various elements and functional groups. Berberine-Albumin NPs showed significant antimicrobial activity against multiple pathogens. In cytotoxicity tests, these nanoparticles effectively inhibited the proliferation of HCT-116 cells. Fluorescence staining assays indicated increased ROS production and apoptosis in treated HCT-116 cells, alongside elevated oxidative stress markers. These findings suggest that Berberine-Albumin NPs exhibit strong anticancer effects, making them a promising candidate for colon cancer treatment.

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