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RESEARCH ARTICLE

Cytotoxic Effects of Green Synthesized Superparamagnetic Iron Oxide Nanoparticles (SPIONs) on Glioblastoma Multiform: An In Vitro Study

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Abstract: This study investigates the physicochemical characteristics and anticancer efficacy of superparamagnetic iron oxide nanoparticles (SPIONs) synthesized via a plant-mediated approach. FTIR spectroscopy revealed the presence of hydroxyl (-OH), carbonyl (C=O), and aliphatic hydrocarbon groups on the nanoparticle surface, confirming successful capping and functionalization that enhance stability and biocompatibility. Scanning electron microscopy (SEM) showed predominantly rod-shaped nanoparticles with a tendency to aggregate, likely mediated by interactions with plant-derived stabilizing biomolecules. The cytotoxic potential of SPIONs was assessed against SHSY5Y neuroblastoma cells using the MTT assay, which demonstrated dose-dependent inhibition of cell viability, with an IC_{50} of 18 ± 0.2 µg/mL. Microscopic examination revealed hallmark apoptotic changes, including cell shrinkage, membrane blebbing, and detachment, suggesting disruption of membrane integrity and cytoskeletal structure. Fluorescence staining further confirmed apoptosis through nuclear condensation and enhanced membrane permeability, indicating activation of programmed cell death pathways. These effects may involve ROS generation, mitochondrial depolarization, and caspase cascade activation, highlighting mechanistic pathways underlying SPION-induced cytotoxicity. Overall, the results demonstrate that these SPIONs combine structural stability, surface functionalization, and potent anticancer activity, offering a promising nanoplatform for targeted neuroblastoma therapy. Their biocompatibility, coupled with the ability to induce apoptosis selectively in cancer cells, underscores their potential as a therapeutic agent and encourages further exploration in preclinical cancer models and drug-delivery applications.

Keywords: Superparamagnetic iron oxide nanoparticles, FTIR spectroscopy, morphological characterization, cytotoxicity, apoptosis, neuroblastoma, fluorescence microscopy.

INTRODUCTION

Despite advancements in medical research, glioblastoma multiforme (GBM) is still considered to be one of the most aggressive [1]. Rapid growth, widespread infiltration into nearby brain tissue, and an exceptional capacity to become resistant to standard treatments including chemotherapy, radiation, and surgical resection are characteristics of this cancer [2]. By limiting the supply of therapeutic drugs to the tumor site. This emphasizes the urgent need for creative approaches to enhance medication delivery and therapeutic results [3]. Nanotechnology has become a game-changer in cancer in recent years, providing innovative approaches to imaging, diagnostics, and targeted medication administration. Because of their distinct magnetic characteristics, biocompatibility, and potential for surface functionalization, superparamagnetic iron oxide nanoparticles (SPIONs) have drawn a lot of attention among the many nanomaterials being studied [4]. By employing external magnetic fields to direct SPIONs to certain tumor locations, therapeutic payloads may be delivered precisely while reducing off-target effects. Furthermore, targeted ligands like peptides or antibodies can be added to their surface to increase their affinity for cancer cells, increasing the selectivity and effectiveness of therapy [5]. Concerns regarding environmental sustainability and biocompatibility are raised by the use of hazardous chemicals, high temperatures, and non-renewable resources in traditional SPION synthesis processes. Green synthesis techniques, on the other hand, create nanoparticles in an economical and environmentally beneficial way by using natural reducing and stabilizing agents such plant extracts, microbes, or biomolecules [6]. This process improves the stability and biocompatibility of the final nanoparticles, increasing their suitability for biomedical applications while simultaneously lessening the environmental impact of nanoparticle manufacturing. For example, it has been demonstrated that phytochemicals originating from plants, such as flavonoids and polyphenols, function as both capping and reducing agents, giving the nanoparticles additional medicinal qualities [7]. There is great potential for using green-synthesized SPIONs in GBM treatment. They are a perfect option for delivering chemotherapeutic drugs, nucleic acids, or other therapeutic compounds straight to tumor cells due to their ability to go across the barrier between the blood and the brain and their magnetic targeting properties [8]. Personalized medicine methods can be facilitated by the use of SPIONs in combination with imaging techniques like MRI [9].

Notwithstanding these developments, there are still issues with improving the stability, targeting effectiveness, and synthesis of green-synthesized SPIONs. To guarantee optimum performance in vivo, variables such particle size, surface charge, and functionalization techniques need to be properly regulated [10]. To guarantee their safety for clinical application, these nanoparticleslong-term biocompatibility and possible toxicity also need careful research. In order to create a sustainable and potent nanotherapeutic platform, this work focuses on the green manufacture of SPIONs and their usage in targeting GBM cells[11]. This study aims to solve the shortcomings of existing GBM therapies and promote precision medicine in cancer by fusing the special qualities of SPIONs with the concepts of green chemistry. The results of this work may lead to the creation of tailored, environmentally friendly nanotherapies that enhance patient outcomes while reducing their negative effects on the environment [12].

EXPERIMENTAL PROCEDURES AND MATERIALS

2.1 Synthesis of SPION'S

Superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized using a chemical co-precipitation method. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were dissolved in deionized water in a 2:1 molar ratio and mixed under constant magnetic stirring at room temperature. The pH of the solution was gradually adjusted to 10–11 by dropwise addition of 1 M sodium hydroxide, initiating the co-precipitation of iron oxide nanoparticles. The reaction mixture was maintained at 60–70°C under continuous stirring for 1–2 hours to ensure complete nucleation and growth of SPIONs, indicated by the appearance of a dark brown to black suspension. The nanoparticles were collected by centrifugation at 10,000 rpm for 15 minutes and washed repeatedly with deionized water and ethanol to remove unreacted salts and impurities. The purified SPIONs were dried at 50°C for 12 hours.

2.2 FT-IR Spectroscopy Analysis

Using Fourier transform infrared (FT-IR) spectroscopy, the chemical makeup and functional groups of the produced superparamagnetic iron oxide nanoparticles (SPIONs) were examined. For this, a Nicolet 5700 spectrometer (Thermo Scientific, USA) was used. By combining the SPIONs with potassium bromide (KBr) and compressing them into a thin, transparent disk, the samples were made utilizing the KBr pellet technique. The vibrational modes of the functional groups on the surface of the nanoparticle were revealed by the spectra, which were collected in the 500–4000 cm^{-1} range [13].

2.3 Surface and Morphology Examination

The SPION were spread out over a polycarbonate substrate and let to cure at room temperature in preparation for SEM examination. To eliminate any remaining moisture, the dried samples were further exposed to carbon dioxide for critical point drying. To improve conductivity, the samples were then sputter-coated with a thin coating of gold using a metallizer. High-resolution pictures of the nanoparticle morphology were obtained by imaging the coated samples at an accelerating voltage of 20 kV using JEOL JSM5600LV [14].

2.4 Preparation and Maintenance of Cell Lines

For this investigation, human neuroblastoma (SHSY5Y) cells were acquired from the National Center for Cell Sciences (NCCS, Pune, India). Dulbecco's Modified Eagle's Medium (DMEM) was used to cultivate the cells. It was supplemented with 0.1 mM non-essential amino acids, 1.5 g/L sodium bicarbonate, 2 mM L-glutamine, 1.5 g/L glucose, 1 mM sodium pyruvate, 10 mM HEPES buffer, and 10% fetal bovine serum (FBS, GIBCO, USA). Penicillin (100 IU/mL) and streptomycin (100 µg/mL) were added to the medium to stop bacterial contamination. The cells were kept at 37°C with a 5% CO_2 environment in a humidified incubator [15].

2.5 Assessment of Cellular Morphology

SHSY5Y colonies were planted onto sterile cover slips at a density of 1×10^5 cells per cover slip and left to adhere overnight in order to assess the morphological alterations brought about by the SPIONs. Following treatment with different doses of the SPIONs, the cells were incubated for a predetermined amount of time. Following treatment, the cells were placed onto glass slides and fixed using a 3:1 (v/v) ethanol:acetic acid solution. Using a bright-field inverted light microscope (Nikon, Japan) at 10× magnification, morphological changes were seen and recorded [16].

2.6 Evaluation of Cell Viability

The inhibitory effect of that combination on SHSY5Y cells was evaluated using the MTT assay. In 96-well plates, cells have been planted at a density of 1×10^4/well and cultivated to 80% confluency. Cells were cultured for 48 hours after the media was changed to a serially diluted test chemical. After removing the medium, 100 µL of MTT solution (0.5 mg/mL in PBS) was added, and the plates were set aside for four hours at 37°C to cause the production of formed crystals of formazan. Cell viability was calculated using the formula:

2.7 Analysis of Programmed Cell Death

Apoptotic cell death was evaluated using a dual-staining technique with acridine orange (AO) and ethidium bromide (EtBr). Briefly, 1×10^5 cells/mL were treated with the SPIONs, harvested, and washed with PBS (pH 7.2). The cell suspension was mixed with 1 µL of a dye mixture containing 100 µg/mL AO and 100 µg/mL EtBr. After incubation for 2 minutes, the cells were washed twice with PBS and visualized under a fluorescence microscope (Nikon Eclipse, Japan) at 10× magnification. AO stains live cells green, while EtBr stains apoptotic

and necrotic cells red, allowing for the differentiation of cell viability and death [17].

RESULTS AND INTERPRETATION

3.1 Infrared Spectroscopy Findings

The FTIR spectrum is shown in **Figure 1**, which displays transmittance (%) as a function of wavenumber (cm^{-1}). Certain functional groups present in the sample are represented by the observed absorption bands. The O–H stretching vibrations, suggestive of hydroxyl (-OH) groups, are responsible for the large peak at 3703 cm^{-1}. C–H stretching, commonly linked to aliphatic hydrocarbons, is represented by the absorption at 2987 cm^{-1}. Carbonyl (C=O) stretching vibrations, potentially from ketones, esters, or carboxyl groups, are suggested by the band at 1936 cm^{-1}. Furthermore, C-O stretching, reminiscent of the apex at 1066 cm^{-1}, is likely connected to alcohol or ether functionalities. The presence of out-of-plane bending vibrations, possibly associated with aromatic rings or other structural components, is suggested by the absorption at 708 cm^{-1}. These spectral characteristics help characterize the material's structure by revealing information about the functional groups present.

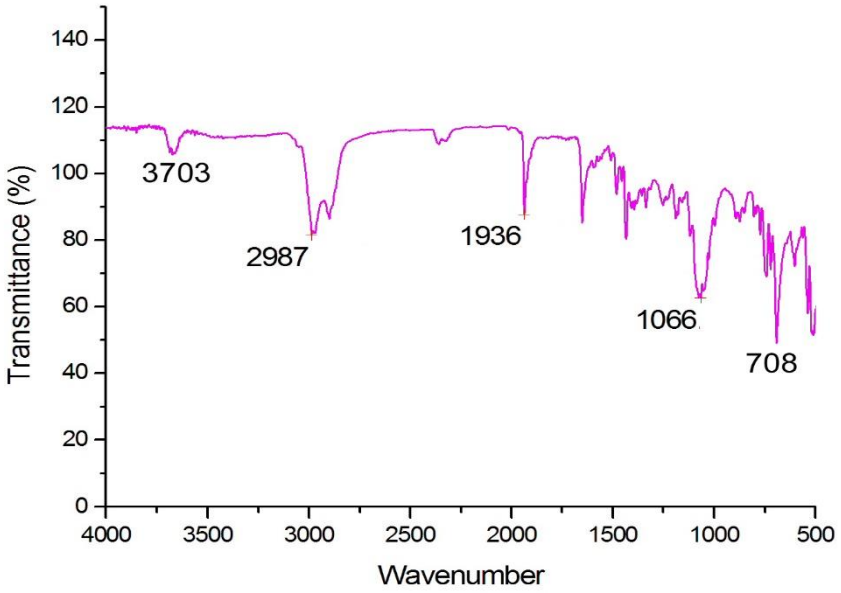

Figure 1. FTIR spectrum of SPIONs showing characteristic peaks of functional groups.

3.2 Morphological and Structural Characterization

Utilizing scanning electron microscopy (SEM), the quantity, arrangement, and dispersion of the synthesized SPIONs were examined. **Figure 2** presents SEM images showing a predominance of rod-shaped nanoparticles, which exhibited a tendency to form aggregates. This aggregation phenomenon suggests that the reduction process and nucleation of metal ions were influenced by the presence of secondary metabolites in the plant extract. These metabolites may have facilitated the binding of metal ions, leading to the formation of larger clusters. Despite the aggregation, the nanoparticles exhibited a relatively uniform dispersion, indicating effective stabilization by the capping agents. The SEM results highlight the role of plant-derived compounds in modulating the morphology and structural properties of the SPIONs.

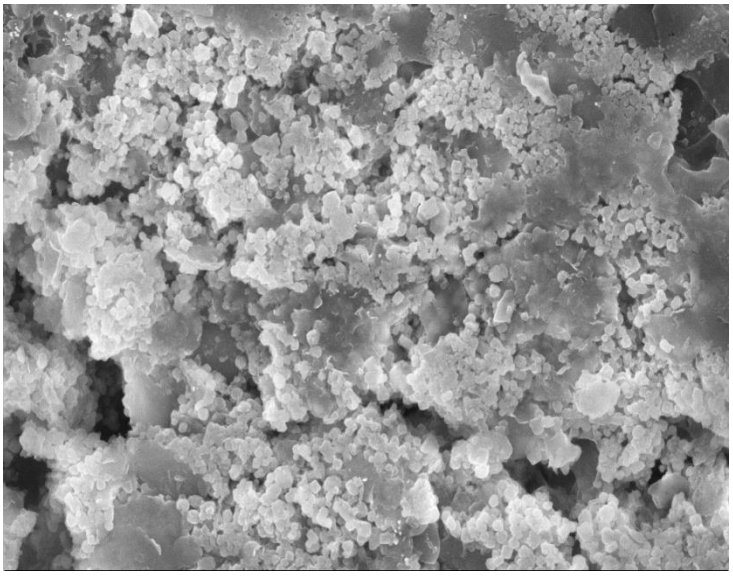

Figure 2. SEM images of SPIONs revealing rod-shaped morphology and aggregation.

3.3 Cytotoxicity Assessment

The cytotoxic potential of SPIONs was evaluated using the MTT assay on human neuroblastoma (SHSY5Y) cells. **Figure 3** demonstrates a dose-dependent inhibition of cell proliferation, with the SPIONs exhibiting significant anticancer activity. The half-maximal inhibitory concentration (IC50) was determined to be **18 ± 0.2 µg/mL**, indicating potent cytotoxicity at relatively low concentrations. This finding underscores the potential of SPIONs as a therapeutic agent for targeting cancer cells, requiring minimal dosage for effective inhibition.

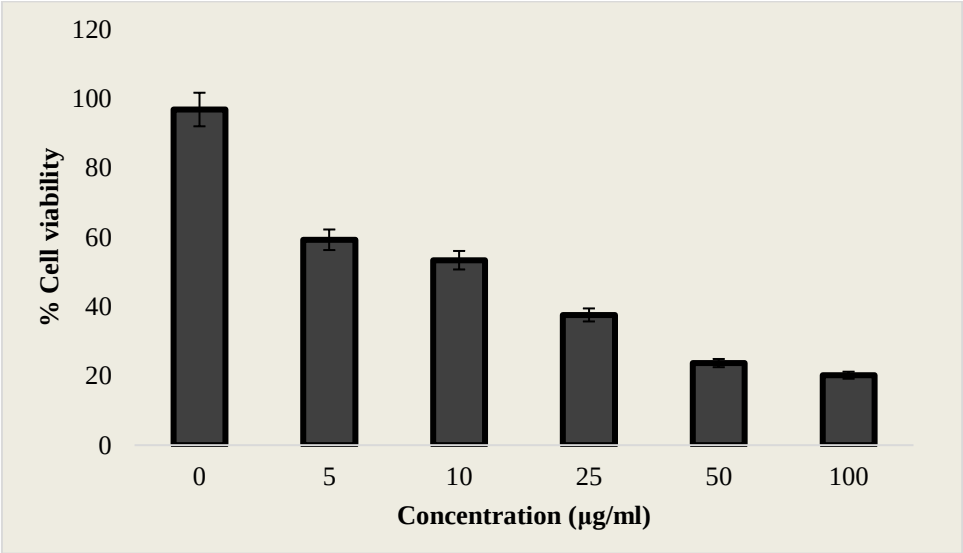

Figure 3. MTT assay results showing dose-dependent cytotoxicity of SPIONs (IC50 = 18 ± 0.2 µg/mL).

3.4 Morphological Changes in Cancer Cells

The impact of SPIONs on the morphology of SHSY5Y cells was assessed using bright-field microscopy. **Figure 4A** and **Figure 4B** illustrate that untreated control cells maintained their typical structure, while cells exposed to SPIONs exhibited pronounced morphological alterations, including **cell shrinkage, membrane blebbing, and detachment from the substrate.** These changes are characteristic of apoptotic cell death, suggesting that the SPIONs induce cytotoxicity through mechanisms that disrupt cellular integrity and function. The observed dose-dependent effects further validate the antiproliferative potential of the nanoparticles.

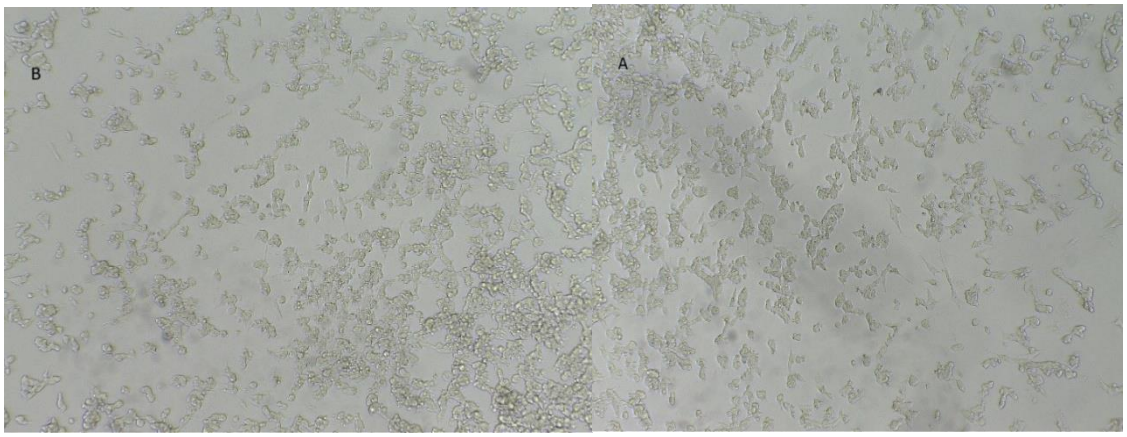

Figure 4. (**A)** Bright-field microscopy of SHSY5Y cells; SPION-treated cells show shrinkage and blebbing. **(B)** Higher magnification image highlighting membrane blebbing and cell detachment

3.5 Apoptosis Induction Analysis

The apoptotic effect of SPIONs on SHSY5Y cells was examined using fluorescence microscopy. **Figures 5A and 5B** display fluorescence microscopy images of untreated and SPION-treated cancer cells. Untreated control cells displayed **green fluorescence**, indicative of viable cells with intact membranes. In contrast, cells treated with SPIONs exhibited a shift to **orange/red fluorescence**, signifying membrane permeabilization and nuclear condensation hallmarks of apoptotic cell death. These findings confirm that the SPIONs trigger programmed cell death in cancer cells, further supporting their potential as a therapeutic agent.

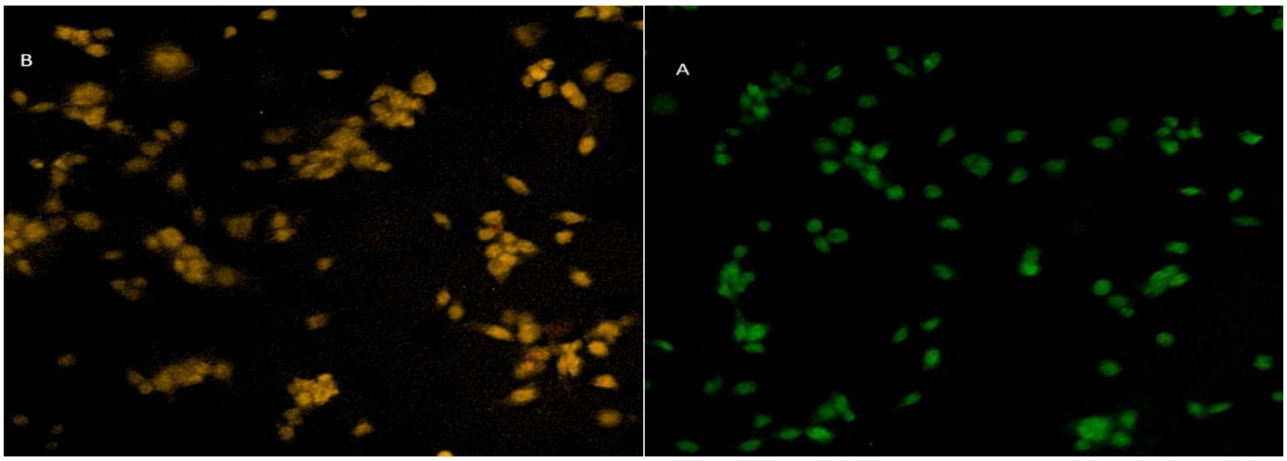

Figure 5. (A) Fluorescence microscopy of control cells showing green fluorescence (viable cells) **(B)** Fluorescence microscopy of SPION-treated cells showing orange/red fluorescence (apoptosis).

DISCUSSION

Particularly in the context of glioblastoma multiforme (GBM), the green manufacture and characterisation of superparamagnetic iron oxide nanoparticles (SPIONs) has shown great promise for targeted cancer therapy. The existence of functional groupings was validated by the FT-IR analysis and secondary metabolites derived from the aqueous extract, which played a crucial role in the reduction and stabilization of the nanoparticles. These findings align with previous studies highlighting the efficacy of plant-based compounds in nanoparticle synthesis, offering a sustainable and biocompatible alternative to conventional chemical methods [18]. The distinct absorption peaks observed in the FT-IR spectra provide evidence of the successful integration of bioactive compounds, ensuring the stability and functionality of the SPIONs. The morphological analysis using SEM revealed a predominance of rod-shaped nanoparticles with a tendency to form aggregates [19]. This aggregation can be attributed to the presence of secondary metabolites in the plant extract, which may have facilitated the binding of metal ions and influenced the nucleation process. Similar observations have been reported in other studies, where plant-derived compounds were found to modulate the size and shape of nanoparticles, impacting their biological activity [20]. Despite the aggregation, the uniform dispersion of the nanoparticles suggests effective stabilization, which is critical for their application in biomedical settings. The method known as MTT was used to gauge the cytotoxic potential of the SPIONs, and the results showed a dose-specific inhibition of the growth of human

neuroblastoma (SHSY5Y) cells. The IC50 value of 18 ± 0.2 µg/mL indicates potent anticancer activity at relatively low concentrations. This result is in line with other studies on iron oxide nanoparticles cytotoxic effects, which have been demonstrated to trigger apoptosis [21]. And inhibit cancer cell growth through mechanisms such as oxidative stress and mitochondrial dysfunction [22]. The ability of SPIONs to achieve significant cytotoxicity at low doses underscores their potential as a targeted therapeutic agent for GBM. Morphological changes observed in SHSY5Y cells following treatment with SPIONs, including membrane blistering and diminishing cells are signs of apoptotic dying of cells [23]. These alterations were further confirmed by fluorescence microscopy using AO/EtBr staining, which revealed nuclear condensation and membrane permeabilization in treated cells. The induction of apoptosis is a key mechanism by which SPIONs exert their anticancer effects, as evidenced by the shift from green to orange/red fluorescence in treated cells. Similar results have been reported in studies investigating the apoptotic effects of nanoparticles on cancer cells, highlighting their potential for targeted therapy [24]. But the application of green synthesis techniques offers distinct advantages, including enhanced biocompatibility, reduced environmental impact, and the potential for additional therapeutic properties conferred by plant-derived compounds. These advantages make green-synthesized SPIONs a promising candidate for further development and clinical translation [25].

CONCLUSION

In conclusion, this study successfully demonstrated the green synthesis, characterization, and biological evaluation of SPIONs for targeting glioblastoma multiforme cells. The FT-IR and SEM analyses confirmed the successful integration of plant-derived compounds, ensuring the stability and functionality of the nanoparticles. The SPIONs exhibited potent cytotoxic effects on human neuroblastoma cells, inducing apoptosis and disrupting cellular morphology at low concentrations. These results demonstrate how green-synthesized SPIONs may be used as a targeted medical remedy for GBM, providing a viable and efficient substitute for traditional therapies. Future research should focus on optimizing the synthesis process to minimize aggregation and enhance targeting efficiency. Additionally, in vivo studies are needed to evaluate the biocompatibility, biodistribution, and therapeutic efficacy of SPIONs in animal models. The integration of SPIONs with imaging techniques, such as MRI, could further enhance their application in theranostics, allowing for the continuous surveillance of tumor growth and response to therapy. Overall, this study contributes to the advancement of nanotechnology in oncology, clearing the path for the creation of efficient and environmentally sustainable nanotherapies for glioblastoma multiforme.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Not required.

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