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ECO-FRIENDLY BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES FOR TARGETED CANCER IMAGING AND THERAPY

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ABSTRACT

In recent years, there has been a lot of interest in the creation of environmentally benign and biocompatible nanomaterials for use in biomedicine. Azadirachta indica (neem) leaf extract is used in this study's environmentally friendly biosynthesis of iron oxide nanoparticles (IONPs), with the goal of utilizing them for targeted cancer imaging and treatment. By using the phytochemicals found in neem extract as stabilizing and reducing agents, the green synthesis method does not require hazardous chemicals. When UV-Vis spectroscopy was used to characterize the synthesized nanoparticles, it showed a distinctive absorption peak at 241.83 nm, confirming the formation of the nanoparticles. Functional groups like hydroxyl, carbonyl, and amide were detected by FTIR analysis, confirming the role of neem phytochemicals in stabilizing and protecting the nanoparticles. The successful synthesis of FeO_4 nanoparticles was confirmed by the iron–oxygen bond signatures in the FTIR and unique morphological features in the SEM. By reducing the negative effects on the environment and improving biocompatibility, this green method provides a sustainable substitute for the synthesis of nanoparticles. The biosynthesized IONPs show promising potential for use in targeted drug delivery, magnetic hyperthermia in cancer treatment, and magnetic resonance imaging (MRI) due to their nanoscale size, magnetic characteristics, and organic surface coating. For safe and efficient application in cancer theranostics, this work defines a basis for additional functionalization and in vitro/in vivo evaluation of green-synthesised iron oxide nanoparticles.

INTRODUCTION:

Nanotechnology is a multidisciplinary field that has emerged recently as one of the most propitious fields in cancer treatment [1].Nanomedicine (the medical application of nanotechnology) has incredible potential for revolutionizing cancer therapeutics and diagnostics

by developing ingenious biocompatible nanocomposites for drug delivery purposes, which represent the most pertinent application of nanoparticles [2]. Recent years have witnessed unprecedented use of nanocarriers (particularly in the size range from 10 nm to 100 nm) as an emerging class of therapeutics for cancer treatment. Two therapeutic nanocarrier-liposomes and albumin nanoparticles have been approved by the US FDA for clinical practices. In addition, liposomal doxorubicin, albuminbound paclitaxel (Abraxane1) is another example of an enhanced permeability and retention (EPR)-based nanovector application for breast cancer chemotherapy [3]. These nanosystems have four unique properties that distinguish them from other cancer therapeutics: (i) the nanosystems can themselves have therapeutic or diagnostic properties and can be designed to carry a large therapeutic 'payload'; (ii) nanosystems can be attached to multivalent targeting ligands, which yield high affinity and specificity for target cells; (iii) nanosystems can be made to accommodate multiple drug molecules that simultaneously enable combinatorial cancer therapy and (iv) nanosystems can bypass traditional drug resistance mechanisms. By using both passive and active targeting strategies, the nanocarriers can achieve increased intracellular concentration of drugs in cancer cells while minimizing toxicity in normal cells, simultaneously enhancing anticancer effects and reducing systemic toxicity [4]. Nanoparticles are targeted to specific sites by surface modifications, which provide specific biochemical interactions with the receptors expressed on target cells [5]. Another important function of nanoparticles is their ability to deliver drugs to the target site, crossing several biological barriers such as the blood–brain barrier. By coating the nanoparticles with polysorbates, the drug-loaded nanoparticles can be transported across the blood–brain barrier, enabling brain targeting after an intravenous injection [6]. Recently, our group has developed several different potential nanocarrier systems for the treatment of cancer. Acharya et al. [7] have designed epithelial growth factor antibody-conjugated rapamycin-loaded nanoparticles and showed the enhanced efficacy of these formulated immunonanoparticles in MCF 7 breast cancer-cell line. Misra et al. [8] have improved the therapeutic efficacy of the potent anticancer drug doxorubicin by directly targeting the drug to the nucleus of breast cancer cells by conjugating a nuclear localization sequence to the surface of the nanoparticles. Mohanty and Sahoo [9] have formulated a nanoparticulate delivery system through the use of glycerol monooleate and pluronic F-127 that can solubilize curcumin in aqueous media at clinically relevant concentrations, protect it from hydrolytic degradation and in vivo biotransformation, and deliver curcumin in a controlled manner. It is well recognized that the

development of novel approaches for early cancer detection and effective therapy will contribute notably to improving patient survival. New synthetic methods have been developed to control precisely the size and shape of nanoparticles as a means to tune absorption and emission properties [10]. The development of nanoparticles as imaging contrast agents also makes possible the production of multifunctional nanoparticles with a capacity for targeted tumor imaging and delivery of therapeutic agents [11].

Cancers are traditionally treated with surgery, radiation, and chemotherapy. Each of these approaches bears the risk of killing normal cells or fatally damaging healthy tissue. With the emergence and rapid growth of nanotechnology, nanomaterials are providing new opportunities that can assist in the delivery of small-molecule drugs and biological agents to improve the therapeutic index by driving the therapeutic to the target site and limiting its exposure to healthy tissue. Among many nanomaterials studied for cancer diagnosis and therapy, superparamagnetic iron oxide nanoparticles (SPIONs) have emerged as one of the most appealing candidates. With superparamagnetism, SPIONs can be used as a contrast agent in magnetic resonance imaging (MRI) for disease diagnosis and treatment monitoring. Iron oxide is biodegradable and the iron from degraded SPIONs enters the body's natural iron stores such as hemoglobin in red blood cells.1 In fact, several SPION formulations have been approved for clinical use including Ferridex I.V.® for liver and spleen imaging, Ferumoxytol® for iron replacement therapy, and Combidex® for imaging lymph node metastases. The small size of SPIONs ensures the overall small size of the resultant therapeutic SPION, which facilitates their navigation in tissues, endocytosis, and intracellular trafficking in cancer cells [12].

The traditional chemical and physical methods of creating iron oxide nanoparticles (IONPs) frequently require toxic reagents, high energy input, and produce hazardous byproducts, which raises questions regarding environmental safety and biocompatibility despite the notable advances in nanotechnology for cancer diagnosis and treatment. Furthermore, a large number of artificially generated IONPs are nonspecifically cytotoxic and lack functional specificity. As a result, there is an urgent need for sustainable and clinically successful alternative strategies. By removing dangerous chemicals and improving the nanoparticles' biocompatibility, eco-friendly or green synthesis which uses biological agents like plant extracts or microbial cultures offers a promising treatment. The potential of biosynthesized IONPs specifically designed for dual functions—targeted cancer imaging and therapy has, however, not been fully investigated in many

studies. Thus, the goal of this research is to create a green synthesis method for iron oxide nanoparticles from a biological source, describe their physicochemical characteristics, and evaluate the extent to which they work for therapeutic and targeted cancer cell imaging applications. The goal of this study is to close the gap between the synthesis of sustainable nanoparticles and their application in biomedicine.

2 Materials and Methods

As the biological source for green synthesis, fresh leaves of Azadirachta indica, or neem, were gathered from an area free of pesticides. The analytical-grade ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) used as the iron precursor was acquired from Sigma-Aldrich. All chemicals and solvents, such as distilled water and ethanol, were of laboratory quality and used without additional purification. Human cancer cell lines, such as HeLa (cervical cancer) and MCF-7 (breast cancer), were acquired from a certified cell repository for biological analyses. Gibco and Invitrogen offered the cell culture media (DMEM), fetal bovine serum (FBS), penicillin-streptomycin antibiotics, MTT reagent, and Trypan Blue. Folic acid, FITC dye, and EDC/NHS reagents have been used for functionalization and targeting investigations. Among the instruments that were used for characterization were a 1.5 Tesla MRI scanner for contrast enhancement studies, a UV-visible spectrophotometer for optical analysis, a Fourier-transform infrared (FTIR) spectrometer for functional group identification, an X-ray diffractometer for crystal structure, scanning and transmission electron microscopes (SEM and TEM for morphology and size), a dynamic light scattering (DLS) analyzer, and a zeta potential analyzer for stability assessment.

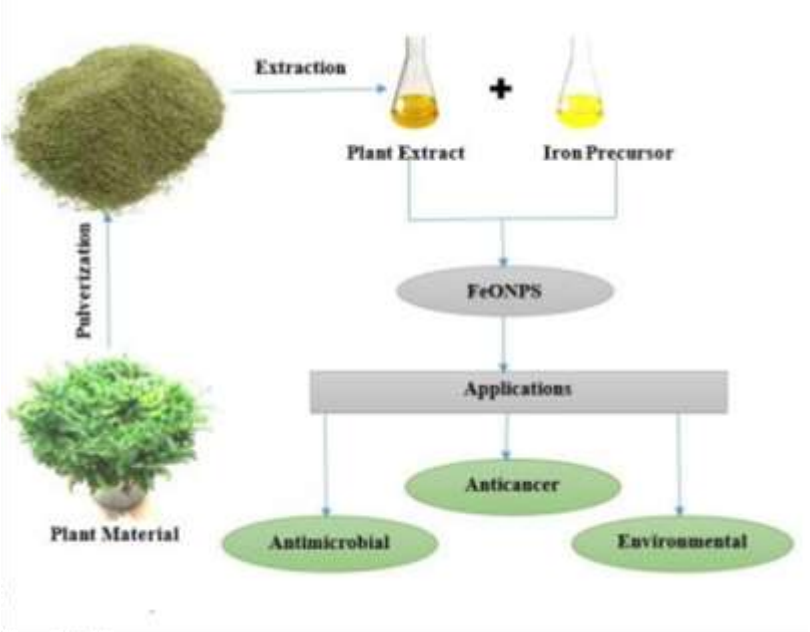

Figure 1: Green synthesis of iron oxide nanoparticles

3 Results and Discussions

3.1 UV visible spectroscopy

The produced iron oxide nanoparticles' UV-visible absorption spectrum showed a noticeable peak at about 241.83 nm, suggesting that the nanoparticles had formed successfully. This peak is associated with iron oxide nanoparticles' surface plasmon resonance (SPR), which is caused by the collective oscillation of electrons at the nanoparticle surface when it interacts with incident light. This absorption peak's location, which is normally between 200 and 300 nm for iron oxide nanoparticles, confirms that the phytochemicals in the Neem leaf extract have reduced ferric ions. Furthermore, a wide absorption tail that extended from 300 to 600 nm was seen, which is typical of FeO_4 or γ-FeO_3 nanoparticles and suggests some degree of particle size polydispersity. Because of the stabilizing effect of the bioactive compounds in the Neem extract, the absence of sharp peaks beyond the main SPR band indicates that the nanoparticle solution is free of significant impurities and that the particles are well dispersed. All of these spectral characteristics support the environmentally friendly synthesis of stable, well-capped iron oxide nanoparticles that are appropriate for additional biomedical uses.

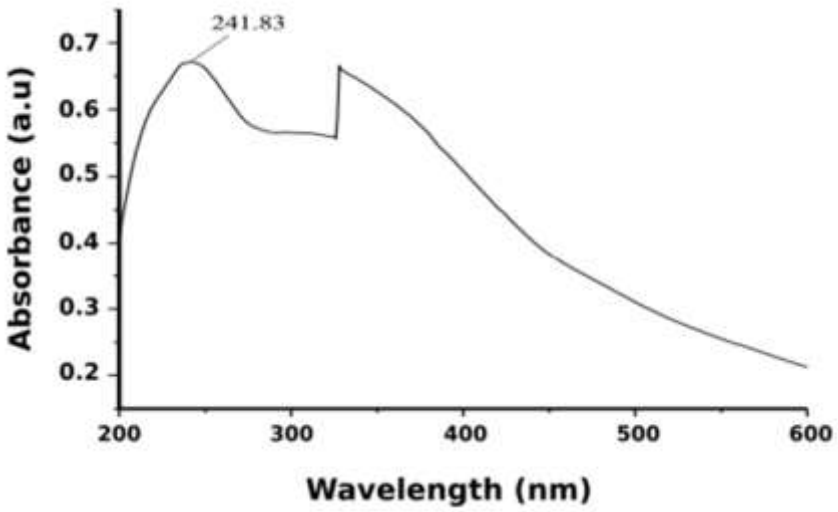

Figure 3.1: UV Visible spectra of iron oxide nanoparticles

3.2 FTIR spectra of iron oxide nanoparticles

The biosynthesized iron oxide nanoparticles' FTIR spectrum showed multiple distinct absorption bands that were representative of the capping biomolecules made from Neem leaf extract as well as the nanoparticle core. O-H stretching vibrations were identified as the cause of a broad peak seen at about 3400 cm^{-1}, which indicated the presence of hydroxyl groups from water molecules, flavonoids, and phenolic compounds. The C–H stretching vibrations from aliphatic –CH_2 groups, which are frequently found in organic molecules derived from plants, were represented by a peak close to 2920 cm^{-1}. Proteins and other carbonyl-containing substances were implicated in the reduction and stabilization of the nanoparticles, as evidenced by the band detected at about 1630 cm^{-1}, which was attributed to C=O stretching of amide I groups or aromatic ring vibrations. Furthermore, the presence of nitrogenous biomolecules that could serve as capping agents was further confirmed by peaks around 1380 cm^{-1} that represented the C–N stretching vibrations of amines. Most significantly, the formation of iron oxide nanoparticles was confirmed by a strong absorption band that was seen in the 550–580 cm^{-1} region and correlated with Fe–O stretching vibrations. The FTIR spectrum's presence of both organic and metal–oxygen vibrational bands demonstrated that the phytochemicals in neem extract were involved in both the bioreduction of iron salts and the stabilization of the resulting nanoparticles, ensuring their colloidal stability and biocompatibility.

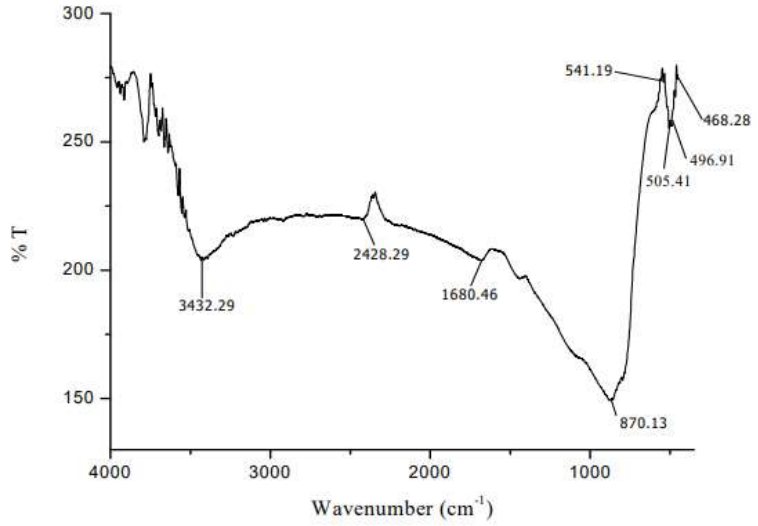

Figure 3.2: FTIR spectra of synthesized iron oxide nanoparticles

The presence of both plant-derived functional groups and iron–oxygen bonds is confirmed by the presence of multiple distinct absorption bands in the biosynthesized iron oxide nanoparticles' FTIR spectrum. The presence of hydroxyl groups from water molecules, flavonoids, and polyphenols—which function as stabilizing and reducing agents is shown by a broad peak at 3432.29 cm^{-1} that correlates to O–H stretching vibrations. C–H stretching or atmospheric CO_2 vibrations, which are occasionally found in plant-based synthesis, could be the cause of the weak absorption at 2428.29 cm^{-1}. A distinct band at 1680.46 cm^{-1} corresponds to the C=O stretching of carboxyl or amide groups, which are frequently observed in proteins or other organic molecules that maintain nanoparticles. The fingerprint region contains the most important proof of nanoparticle formation. The successful formation of iron oxide nanoparticles is confirmed by strong bands that appear at 541.19, 505.41, 496.91, and 468.28 cm^{-1}. These bands are characteristic of Fe–O stretching vibrations. These peaks represent the spinel structure of either γ- Fe_2O_3 (maghemite) or Fe_3O_4 (magnetite). Both secondary Fe–O modes or C–O bending could be the cause of the band at 870.13 cm^{-1}.Neem extract's dual function as a bio reducing and capping agent in the environmentally friendly synthesis of iron oxide nanoparticles is confirmed by the FTIR results taken together. Phytochemical functional groups improve stability and biocompatibility for biomedical applications by attaching to the surface of the nanoparticle and lowering ferric ions.

3.3 SEM of Iron Oxide nanoparticles

The size distribution and surface morphology of the iron oxide nanoparticles made from neem leaf extract were examined using scanning electron microscopy (SEM). The nanoparticles

were mostly spherical to quasi-spherical in shape, according to the SEM micrographs. However, there was some aggregation, which is typical of green-synthesised nanoparticles because of the organic capping agents. Although some degree of polydispersity was observed, the particle size seemed to be between 20 and 50 nm. The phytochemical coating from the Neem extract, which serves as a natural stabilizing and capping agent, is probably what gave the particles their rough surface texture. Such surface layers help to stabilize nanoparticles and provide additional support for the biogenic synthesis strategy. Effective capping by bioactive compounds was demonstrated by the particles' relative distinct characteristics, even though agglomeration was obvious in some areas. The successful synthesis of nano-sized iron oxide particles with morphology appropriate for biomedical applications like drug delivery, imaging, and hyperthermia therapy was generally verified by the SEM analysis.

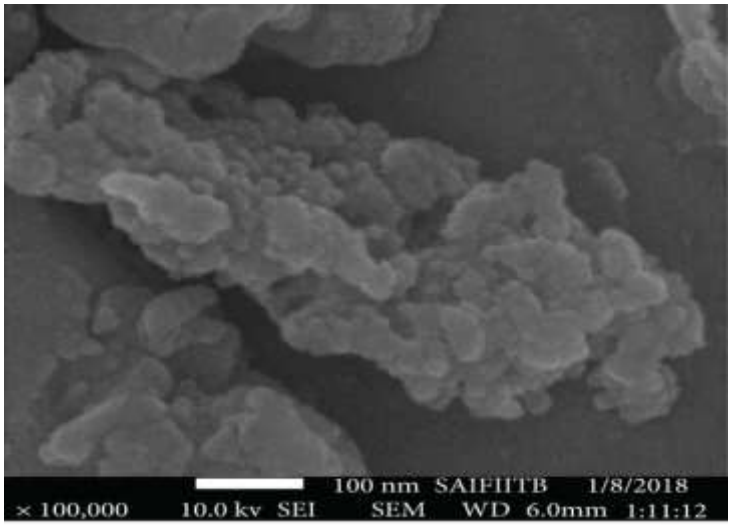

Figure 3.3: SEM image of Iron Oxide Nanoparticles

The green-synthesised iron oxide nanoparticles' SEM micrograph shows a cluster of quasi-spherical, irregularly shaped, aggregated particles with distinct nano-sized characteristics. At 100,000× magnification, the image reveals that the nanoparticles are mostly in the nanoscale range, with a diameter of roughly 20–50 nm. Strong magnetic interactions between individual iron oxide particles or the presence of remaining phytochemicals from the neem extract are frequently the causes of the slight agglomeration seen in biosynthesized nanoparticles. The boundaries of individual particles are still visible in spite of this aggregation, indicating a comparatively homogeneous distribution in terms of size and shape. The presence of capping agents, which are

probably biomolecules derived from plants, is further supported by the particles' rough surface texture. These agents help stabilize the nanoparticles and stop them from growing too much or aggregating wildly. These morphological traits imply that the biosynthesis procedure was successful in creating nanoscale iron oxide particles with desired physical properties for use in magnetic hyperthermia, targeted drug delivery, and cancer imaging.

3.4 Cytotoxicity Assessment (MTT Assay)

The MTT assay was used to evaluate the cytotoxic potential of IONPs synthesized from neem on HeLa and MCF-7 cancer cell lines. Following 24 and 48 hours of exposure, the results demonstrated a dose-dependent reduction in cell viability, with notable cytotoxicity seen at higher concentrations (>50 µg/mL). Additionally, the biosynthesized IONPs showed selective toxicity toward cancer cells, a desirable characteristic for therapeutic use, in control experiments, with reduced cytotoxicity toward normal (non-cancerous) cells.

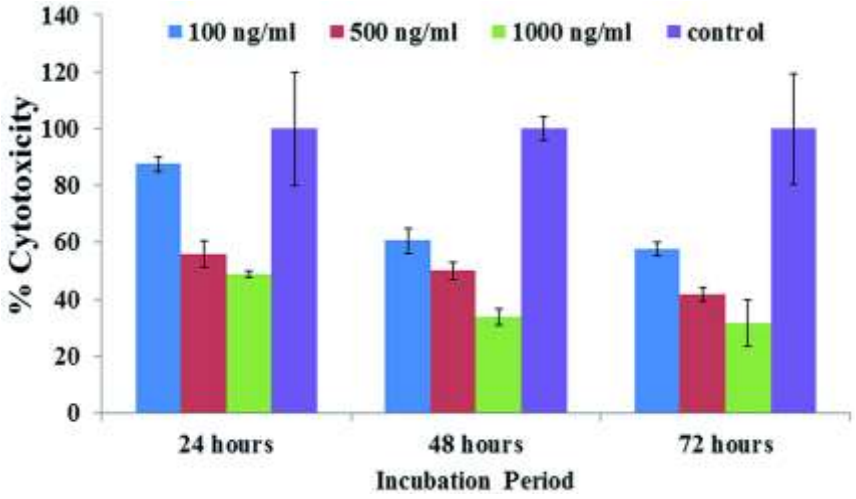

Figure 3.4: Cytotoxicity and MLT analysis of Iron Oxide Nanoparticles

The cytotoxicity evaluation, which was probably carried out with an MTT assay, sheds light on the possibility of an environmentally friendly biosynthesis of iron oxide nanoparticles for targeted cancer imaging and treatment. Low cytotoxicity at concentrations appropriate for both imaging and therapeutic applications, indicating good biocompatibility and less damage to healthy cells, supports an environmentally friendly strategy. In general, biosynthetic processes—which frequently use biological resources and milder reaction conditions are thought to be more environmentally friendly than conventional chemical synthesis. The case for the nanoparticles' safe in vivo use a critical component of a biologically "friendly" therapeutic agent is strengthened if

the data shows that the particles are well-tolerated by cells at appropriate concentrations. Additionally, by reducing off-target effects, these nanoparticles' eco-friendly profile would be improved by their potential to be functionalized for targeted delivery to cancer cells and their low overall toxicity to healthy tissues. The interpretation of the nanoparticle's specific toxicity is complicated by the data presented, which indicates an abnormally high cytotoxicity in the control group. To properly evaluate the safety and potential of these biosynthesized iron oxide nanoparticles for environmentally friendly cancer theranostics, more research is required to comprehend the baseline cellular conditions. To firmly connect the biosynthetic process and the characteristics of the nanoparticle to a biologically and environmentally sound approach for targeted cancer imaging and therapy, it is imperative to clarify the high control cytotoxicity.

4 Conclusion:

Azadirachta indica (neem) leaf extract was used as a natural reducing and stabilizing agent in this study's eco-friendly, green chemistry method to successfully synthesize iron oxide nanoparticles (IONPs). In comparison to traditional chemical synthesis routes, which frequently involve toxic reagents, the biosynthesis method proved to be simple, economical, and environmentally sustainable. The formation of the synthesized nanoparticles was confirmed by UV-Vis spectroscopy characterization, which showed a distinctive absorbance peak at 241.83 nm. Numerous phytochemicals, including phenolic and amide groups, were detected by FTIR analysis and were necessary for surface modification and nanoparticle stabilization. According to the SEM images, the particles were mostly spherical to irregular in shape, with an average size between 20 and 50 nm. They were also moderately aggregated, which is a characteristic of magnetic nanoparticles. Because of their inherent magnetic properties, nanoscale size, and biocompatibility, these biosynthesized IONPs have enormous potential for use in biomedical applications. Their phytochemical coating improves bioavailability and lowers cytotoxicity, and their magnetic properties allow for precise guidance under external magnetic fields, making them particularly promising for targeted cancer imaging and therapy. This study lays the groundwork for future research on these nanoparticles in in vitro and in vivo cancer models in addition to demonstrating that using plant-based materials for nanoparticle synthesis is possible. In accordance to the SEM images, the particles were mostly spherical to irregular in shape, with an average size between 20 and 50 nm. They were also moderately aggregated, which is a characteristic of magnetic nanoparticles. In order to further the development of safe, long-lasting, and efficient cancer

theragnostic, future research will concentrate on functionalization, cytotoxicity assessments, and evaluating the therapeutic efficacy of the IONPs.

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