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Original Article

Antioxidant Assessment of *Albizia lebbeck* SLNs

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ABSTRACT

Aim: Nanotechnology is pivotal in the pharmaceutical industry, significantly augmenting drug properties and formulations. It enhances drug stability and controlled release, providing precise control over drug delivery systems, thereby maximizing therapeutic efficacy and minimizing adverse effects. Solid Lipid Nanoparticles (SLNs), composed of solid lipids, amalgamate lipid-based systems with nanotechnology to furnish controlled drug release, targeted delivery, and enhanced protection for encapsulated drugs. The integration of antioxidants within SLNs has been shown to enhance their efficacy in addressing diseases associated with oxidative stress. However, including the well-known antioxidant plant *Albizia lebbeck* leaf extract in the SLNs has been scarcely explored. **Materials and Methods:** The current study encapsulated the ethanolic extract from the leaves of *Albizia lebbeck* using stearic acid and polyvinyl alcohol. The encapsulated substance underwent microstructural, morphological, and chemical analysis using FE-SEM, FTIR, and BET techniques. A comprehensive study on antioxidant properties and MTT assay on C6 cells followed standard protocols. **Results:** The antioxidant properties of the drug were rigorously evaluated using various scavenging assays, including DPPH, superoxide, hydroxyl, nitric oxide, and hydrogen peroxide. The drug exhibited scavenging activity with an IC_{50} value of 03.07±0.04 µg/mL, 60.51±0.13 µg/mL, 65.57±0.21 µg/mL, 66.06±0.19 µg/mL, and 9.35±0.7 µg/mL, respectively, when compared with ascorbic acid. Although it was less effective than the strong antioxidant ascorbic acid in all assessments, it showed enhanced therapeutic potential, particularly in neurological diseases such as Alzheimer's. The MTT assay on C6 cells found that SLNA concentration from 12.5 to 200 mg/mL initiated apoptosis through cell disintegration, shrinkage, and reduced production of apoptotic bodies. However, it did not induce toxicity compared to the negative control, even at higher dosages. **Conclusion:** SLNA holds significant potential for advancing pharmaceutical formulations and therapies where controlled antioxidant release is required, particularly in managing neurological diseases.

Keywords: *Albizia lebbeck*, Anti-oxidant Release, Nanotechnology, Neurological Diseases, Pharmaceutical Formulations, Solid Lipid Nanoparticles.

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INTRODUCTION

The pharmaceutical industry continuously seeks advanced technologies to address health concerns, with nanotechnology emerging as a key focus.[1] Nanocarriers, for instance, can encapsulate active ingredients with low solubility, providing protection and controlled release, thus reducing the frequency of administration.[2] Among these nanocarriers, Solid Lipid Nanoparticles (SLNs) stand out. Composed of solid lipid materials, SLNs merge the benefits of lipid-based systems with nanotechnology, offering controlled drug release, targeted delivery, and enhanced protection of encapsulated drugs from degradation.[3]

The nanoscale size of SLN yields a large surface area and enables significant interface phase interactions, making them unique in the field.[4] These characteristics and their significant drug-loading capacity make SLNs very beneficial for enhancing pharmacological bioavailability and effectiveness.[5,6] SLNs have diverse applications, including delivery of both hydrophilic and lipophilic drugs provided that precise and targeted drug release,[7,8] Cosmetics,[9] Food Industry,[10] Vaccines,[11] Nutraceuticals,[12] clinical treatments,[13] and various research fields.[14,15] Additionally, SLNs are utilized for sterilization,[16] immobilization,[17] and incorporating biocompatible ingredients into pharmaceutical formulations. They improve drug stability,[18] enhance bioavailability[19] and enable controlled drug release. Overall, Enhancing therapeutic efficacy while reducing side effects highlights their potential to enhance pharmacological profiles across different

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pharmaceuticals, where SLN offers a viable approach.[20] Currently, SLNs are produced by high-pressure homogenization,[21] solvent emulsification-evaporation,[22] microemulsion-based techniques[23] and other methods.[24] Each method involves unique processes to form stable nanoparticles, ensuring the efficacy of the encapsulated compounds.

Current research accentuates the beneficial impact of Solid Lipid Nanoparticles (SLNs) in enhancing the solubility and bioavailability of drugs. Budiman *et al.*,[25] showcases how the solubility of glibenclamide significantly increased by creating a cocrystal with aspartame, leading to marked improvements in both solubility and rates of dissolution. SLNs with antioxidants like rosmarinic acid,[26] curcumin[27] and quercetin[28] show enhanced neuroprotection, making them crucial for Alzheimer's disease and other neurological disease therapy.[29,30] By encasing antioxidants[31] and improving their bioavailability,[32] stability, and activity,[33] SLNs provide a remedy to numerous problems that currently persist.

Using SLNs can facilitate the development of innovative antioxidant-based therapeutics with enhanced effectiveness and bioavailability as a viable antioxidant administration and therapy technique.[34] The importance of antioxidants in pharmaceuticals cannot be overstated.[35,36] Antioxidants inhibit the oxidation of other molecules, protecting against oxidative damage.[37,38] Antioxidants were significant in industrial applications, but their primary biological importance is protecting unsaturated fats from oxidation.[35] However, the therapeutic potential of antioxidants has been limited due to poor solubility, low permeability, instability during storage, and gastrointestinal degradation.[19] Rangaraj *et al.*,[39] revealed that nanoparticles made of Zirconia (ZrO_2) and Titania (TiO_2) display superior biocompatibility and antioxidant properties when compared to their micro-sized versions, emphasizing the crucial influence of particle size on the effectiveness of nanocarriers. ZnO nanoparticles produced with *Albizia lebbeck* exhibit significant antioxidant properties, demonstrated by their ability to scavenge free radicals like 1,1-Diphenyl-2-Picrylhydrazyl (DPPH).[11] The plant's phytochemicals, such as flavonoids and tannins, contribute to the antioxidant potential of these nanoparticles.[40,41]

Compounds extracted from *Albizia lebbeck* have shown potential for treating cardiovascular diseases,[42] diabetes mellitus,[43,44] diarrhoea,[45] multiple sclerosis,[46] wounds[47] and embedding for drug targeting and controlled drug delivery,[48] Some nanostructured compounds can potentially treat neurological diseases[49] and have promising therapeutic applications in drug design.[50] On the other hand, *Albizia lebbeck* extracts also hold potential for other clinical applications,[50] such as treating chronic neurological diseases related to oxidative stress,[51] through direct clinical evidence remains limited. Since the plant's extracts, rich in polyphenols,[52] flavonoids[53] and other antioxidant compounds,[54] exhibit vigorous free radical scavenging activity, which is crucial in mitigating oxidative stress,[42] a key factor in the pathogenesis of neurological disorders like Alzheimer's disease,[55] Parkinson's disease[56] and multiple sclerosis.[29] By neutralizing Reactive Oxygen Species (ROS)[57] and reducing oxidative damage[58] to neurons, *Albizia lebbeck* extracts could theoretically protect against the progression of neurodegenerative diseases. Kumar *et al.*[42] examined the cardioprotective properties of *Albizia lebbeck*, noting its potential for wider therapeutic use due to its antioxidant and anti-inflammatory characteristics. Specifically, while the research primarily investigates its effects on myocardial infarction, the findings propose that *Albizia lebbeck* could also offer neuroprotective benefits, suggesting a broader application in medical treatments. Thus, it makes SLNs an ideal platform for utilizing the therapeutic potential of *Albizia lebbeck* in pharmaceutical industrial applications. However, still, no such efforts have been made by many researchers.

Overall, the research on SLNs shows promise in controlled drug release,[59-61] enhanced bioavailability, and adoption in various industrial applications. Moreover, adding nanocarriers and antioxidants to the SLNs enhances their treatment capacity. Still, more formulations need to be explored to improve effectiveness, expand the range of diseases that could benefit from SLNs, and use various assays to characterize antioxidant properties. Addressing these research gaps could significantly advance the application of nanotechnology in pharmaceuticals.

As per the author's knowledge from conducting a vast literature survey, very few studies have analyzed the antioxidant activity extensively on *Albizia lebbeck*-loaded SLN (SLNA). Thus, this study synthesized SLNA through the green synthesized method and characterized it using FE-SEM, FTIR, and BET techniques. A thorough analysis of the drug's antioxidant properties using DPPH, Superoxide, Hydroxyl, Nitric oxide, and Hydrogen peroxide scavenging assays was conducted, and the effects of shape, size, and chemical composition were also evaluated. Lastly, the toxicity of the drug's dosage was evaluated using the MTT assay on C6 cells.

MATERIALS AND METHODS

Materials

Albizia lebbeck plant leaves were obtained from a certified botanist at Central Ayurvedic Research Institute, Bengaluru. Other chemicals, such as Stearic acid**,** Acetone**,** Polyvinyl alcohol, and others, were purchased from Sigma Aldrich India.

Methods

Preparation of Albizia lebbeck Ethanolic Extract

10 grams of *Albizia lebbeck* plant material and 100 mL of ethanol were mixed to create the ethanolic extract. For a period of 3 hr, the mixture was constantly mixed while being heated to 40ºC. The combination was then filtered to extract the plant material's ethanolic extract.

Preparation of *Albizia lebbeck* encapsulated solid lipid Nanoparticles

The general procedure of the co-precipitation method employed in synthesizing is given in Figure 1 SLNA Preparation: 30 mg of Stearic Acid (SA) were dissolved in 10 mL of each of the acetone and ethanol solvent mixtures. This solution was heated to 60ºC in a water bath for 150-180 min. After adding 10 mg of the ethanolic extract, the mixture was shaken for 2 hr, ultrasonically agitated for 30 min, and acetone was removed by evaporating it while bringing it to room temperature. Several extracts were prepared and analyzed for their size and zeta potential. The final mixture was then mechanically stirred while being added to a cold solution of 1% polyvinyl alcohol (100 mL) that had been previously chilled in a refrigerator. After centrifuging the solidified nanoparticles at 1000 rpm, they were cleaned multiple times in deionized water. The completed SLNs were collected and kept for additional analysis.

Green synthesized SLNA-Structural and morphological characterization

The synthesized nanoparticles' morphology, size, and chemical composition were assessed using the following methods: scanning electron microscopy (SEM; *Jeol, Japan*); particle size and polydispersity index using the dynamic light scattering technique (Malvern Zeta sizer) and Fourier-Transform Infrared spectroscopy (FTIR; PerkinElmer Spectrum 1000). BEL: 2 SORP (Italy) was used for the analysis of the Braunauer-Emmett-Teller (BET) study of the SLNA.

Antioxidant evaluations

DPPH radical scavenging assay

For DPPH antioxidant evaluation,[62,63] a mixture of DPPH free radical scavenging assay was prepared by combining 2 mL of a 0.1 mM DPPH solution with 2 mL of methanol, and its absorbance was immediately measured at 517 nm to serve as a control. In the experimental procedure, an equal volume of the test extract replaced methanol and was shaken vigorously with the DPPH solution. After incubating for 30 min, which allowed antioxidants in the extract to reduce the DPPH radical to 1,1-Diphenyl-2-Picryl Hydrazine, the reduction in DPPH was measured by the decrease in absorbance at 517 nm, indicating the scavenging activity of the extract.

Hydroxyl radical scavenging assay

For the Hydroxyl antioxidant evaluation using its radical scavenging assay,[64] the reaction mixture (1.0 mL) was meticulously prepared as follows: 100 µL of 2-Deoxy-D-Ribose, 500 µL of the extract, and 200 µL of a 1.04 mM EDTA solution with $FeCl_3$ (200 mM) in a 1:1 ratio were sequentially added. Subsequently, 100 µL of 1.0 mM H_2O_2 and 100 µL of 1.0 mM ascorbic acid were added. The mixture was then incubated at 37ºC for 1 hr. After incubation, 1.0 mL of 1% TBA and 1.0 mL of 2.8% TCA were added, and the solution was subjected to a second incubation at 100ºC for 20 min.

Nitric oxide scavenging assay

In the Nitric oxide scavenging assay, a 10 mM sodium nitroprusside solution was mixed with a test or standard solution at different concentrations in phosphate-buffered saline (pH 7.4), and then incubated at 25ºC for 150 min. After incubation, the resulting mixture was combined with Griess' reagent, and the absorbance of the resulting chromophore was measured at 548 nm to determine the percentage of inhibition.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay combined the extract with phosphate-buffered saline and added hydrogen peroxide. The absorbance was recorded at 230 nm, and the scavenging activity was quantified based on the reduction in absorbance at 230 nm.

Superoxide free radical scavenging assay

A reaction mixture containing Tris-HCl buffer, NBT solution, NADH solution, sample solution, and PMS solution was incubated at 25ºC for 5 min to measure superoxide radicals. The absorbance was then measured at 560 nm, and the presence of antioxidants was indicated by a decrease in absorbance at 560 nm, suggesting the scavenging of superoxide anions.

Cytotoxicity Assessments of SLNA

To conduct the cytotoxicity study, the standard protocol is followed established by various studies.[65-67] C6 cell suspensions at a density of 20,000 cells per well in a 96-well plate seeded, using 200 µL of suspension per well, allowed the cells to grow for 24 hr without any test agents. Doxorubicin was used as a positive control in the cytotoxicity assessment. Following the initial growth period, the test agents were prepared at specified culture media concentrations and added to the plate wells. Then, we incubated the plate at 37ºC in a 5% CO2 atmosphere for 24 hr. Post-incubation, media spent media was removed from each well and the MTT reagent to a final 0.5 mg/mL concentration. The plate was wrapped in aluminum foil to protect the reaction from light and returned to the incubator for 3 hr. Subsequently, the MTT reagent was carefully removed, and 100 µL of DMSO was added to each well to dissolve the formazan crystals formed, occasionally requiring gentle agitation or pipetting to ensure complete dissolution in dense cultures. The absorbance of each well was then measured using a spectrophotometer or an ELISA reader at a wavelength of 570 nm. The percentage of cell viability was calculated by comparing the mean absorbance of treated cells to that of untreated cells, using equation (1) to accurately assess the test agents' cytotoxic effects.[68]

$$\%\ \text{cell viability} = \frac{\text{Mean abs of treated cells}}{\text{Mean abs of Untreated cells}} \times 100$$

RESULTS

Structural and Morphological Characterization

The *Albizia lebbeck* loaded SLNA FTIR spectra are shown in Figure 2a and revealed distinct vibrational frequencies at 548.7 cm^{-1}, 725.11 cm^{-1}, 1296.5 cm^{-1}, 1701.4 cm^{-1}, and 2971.2 cm^{-1}. Potentially indicating alkyl halides, aromatics, aliphaticiodo compounds, C-I stretch and C-H bending vibrations of organic compounds, C-N stretching vibrations of amine groups, C=O stretching vibrations in carbonyl groups, C-H stretching vibrations in lipid-based nanoparticles.[69-72] In the UV-visible spectroscopic analysis of SLNA, a prominent peak was observed at 215.64 nm, shown in Figure 2b. This peak is indicative of strong absorbance within the UV region, which is commonly associated with π-π* transitions of aromatic compounds or n-π* transitions of non-bonding electrons to π-antibonding orbitals in functional groups present within the compounds.[73] BET analysis of SLNA revealed a specific surface area (S_BET) of 45.54 m^2/g, accompanied by a Type IV isotherm, as shown in Figure 2c. The surface morphology of SLNA was evaluated through Scanning Electron Microscopy (SEM). SLNA showed agglomeration of nanoparticles with a non-uniform size distribution, as shown in Figure 3 (a and b). The agglomeration observed in the synthesized SLNA is due to the polarity and electrostatic attraction. The analysis of Particle size found that 68.13 nm of size. To further establish the synthesized particles' elemental properties, EDX analysis was performed, wherein peaks for C and O elements were observed in Figure 3c. The insert table in Figure 3 (d and f) shows the weight and atomic percentages of Carbon 67.81% and oxygen 32.19%, indicating that the synthesized SLNA is majorly constituted of C and O.

Antioxidant Activity

Figure 4 illustrates the assessment of antioxidant activity through a series of distinct assays, which collectively provide a comprehensive understanding of the antioxidant potential of SLNA compared to ascorbic acid. Figure 4a evaluates SLNA's ability to donate an electron or a hydrogen atom to neutralize the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, which indicates scavenging activity. This assay, widely employed for its simplicity and rapidity, offers a swift overview of antioxidant potential. Here, the comparison with ascorbic acid shows that SLNA exhibited less potency at each dosage but followed a similar trend. In Figure 4b, the Hydroxyl Radical Scavenging Assay evaluates SLNA's antioxidant capability, revealing that at 60 μg/mL, SLNA's antioxidant capacity closely aligns with that of ascorbic acid. The Nitric Oxide Scavenging Assay, depicted in Figure 4c, demonstrates that SLNA exhibits the same antioxidant potential as ascorbic acid at a 20 μg/mL concentration. However, its efficacy decreases as the concentration increases. Interestingly, at a significantly higher concentration (100 μg/mL), SLNA exhibits a much closer antioxidant potential to ascorbic acid. Figure 4d presents the Hydrogen Peroxide Scavenging Assay, where SLNA's antioxidant potential exhibits a consistent trend comparable to ascorbic acid, particularly at dosages of 20, 40, 80 and 100 μg/mL. However, a slightly larger variance was observed at the 60 μg/mL dosages indicating that SLNA's antioxidant potential closely aligns with ascorbic acid at certain dosages but diverges at the

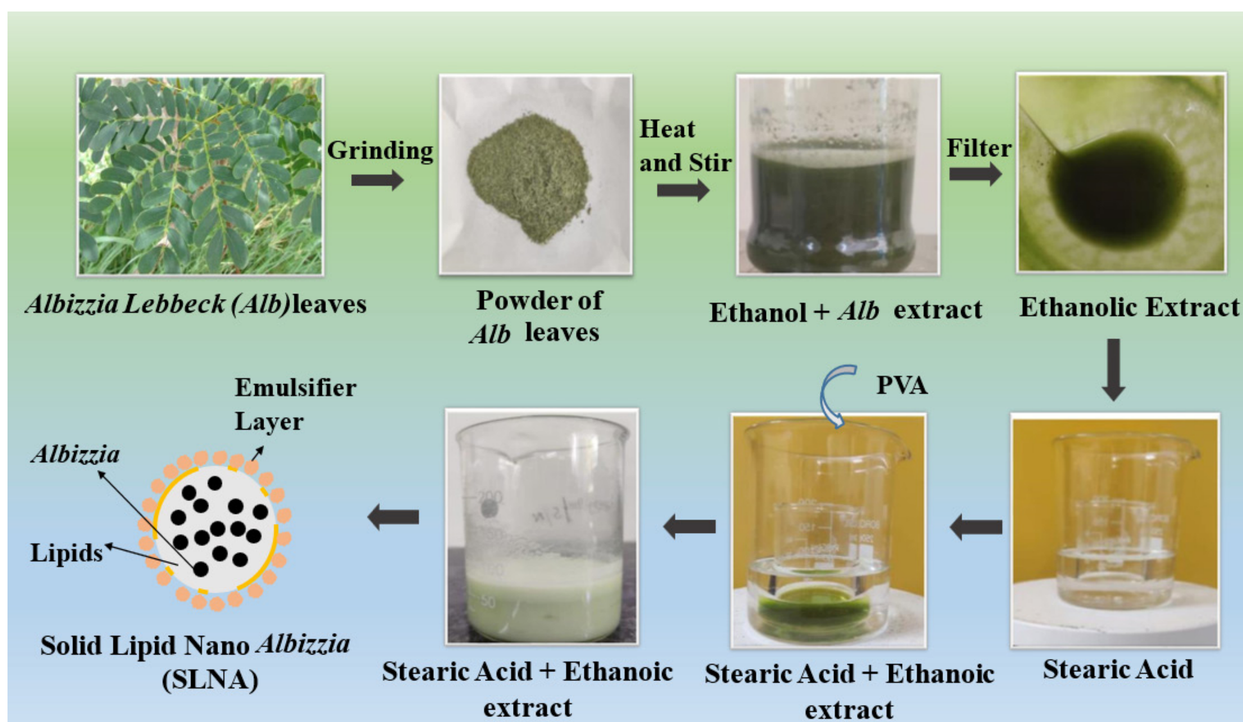

Figure 1: Synthesis methodology for *Albizia lebbeck* encapsulated Solid Lipid nanoparticle by Green synthesis route.

60 µg/mL dosage. Lastly, Figure 4e, illustrating the Superoxide Anion Radical Scavenging Assay, indicates that although SLNA exhibits a similar trend to that of ascorbic acid, it shows a lower potential. Notably, at a dosage of 40 µg/mL, a larger variance is observed compared to other dosages, suggesting a potential dosage-dependent effect on the antioxidant activity of SLNA. The data provided in the Table 1 shows that ascorbic acid has stronger effects in the DPPH and hydroxyl radical scavenging assays, highlighting its capability to counteract these radicals successfully. Conversely, SLNA is more effective in the superoxide and nitric oxide scavenging assays, suggesting its potential in addressing these particular radicals.

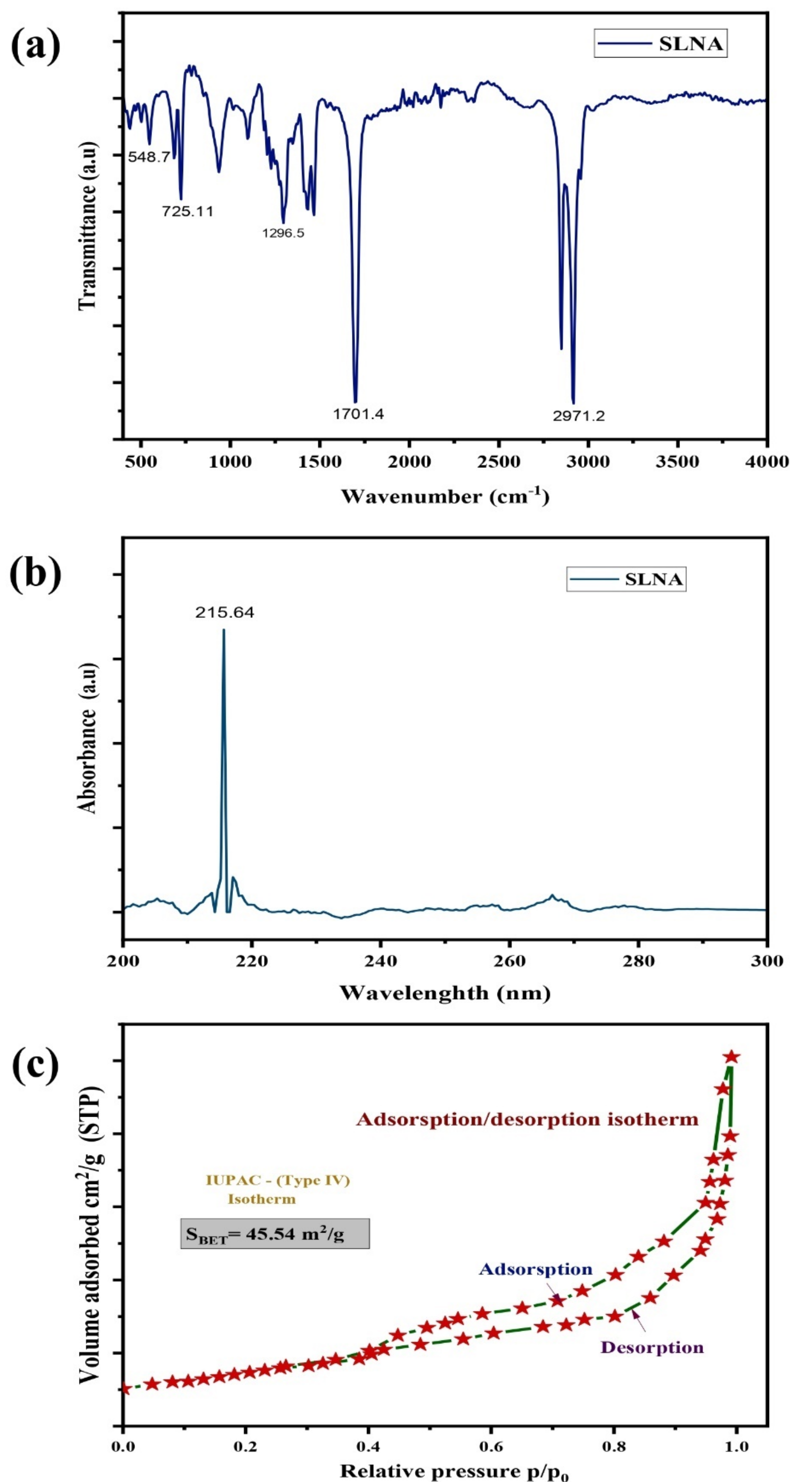

Figure 2: (a) FTIR spectral analysis (b) UV spectra and (c) BET surface area analysis of SLNA.

Cytotoxicity

Figure 5 examines how the SLNA water extract impacts C6 cells at various doses, demonstrating its cytotoxic effects. Subsections Figure 5 (a-g) illustrates how the cells react to differing levels of the SLNA extract, specifically showing changes in their structure that suggest apoptosis is occurring. Figure 5h presents a chart that illustrates how cell survival rates vary with different concentrations of an extract. When cells were exposed to SLNA concentrations ranging from 12.5 to 200 mg/L for 24 hr, lower concentrations showed minimal impact on cellular interactions, indicating low toxicity. This chart clearly demonstrates that the toxicity of the extract is related to its concentration, underscoring the importance of precisely controlling the dosage to find the right balance between its therapeutic benefits and potential harmful effects. This evidence supports the observation that cytotoxicity depends on concentration, highlighting the need for careful dosage adjustment to optimize therapeutic effectiveness while minimizing adverse effects.

DISCUSSION

Using *Albizia lebbeck* extracts in nanoparticle synthesis reflects a growing shift towards green chemistry and environmentally friendly production methods. The study investigates the detailed analysis of the *Albizia lebbeck* embedded in SLN, now onwards referred to as SLNA. Analysis including chemical and morphological characterization. Additionally, the antioxidant

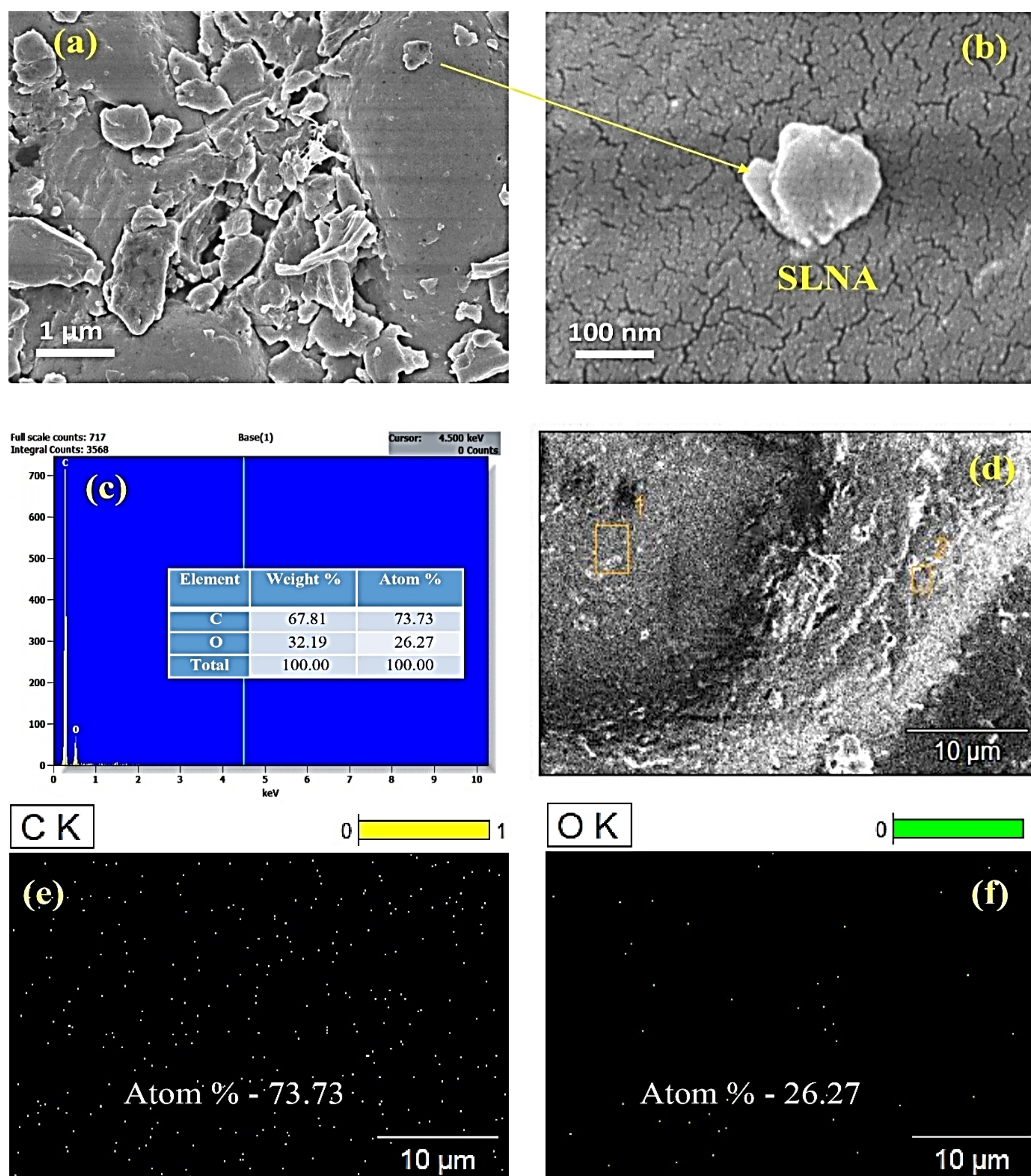

Figure 3: (a and b) SEM analysis of SLN nanoparticles resolution of 1µmand100nm, (c) EDAX elemental composition and (d-f) Elemental mapping SLNA particles.

performance and biocompatibility functionality of SLNA are discussed, highlighting the impact of SLNA in enhancing the efficacy and biocompatibility of the extracts for potential neurological applications.

Structural and Morphological Evaluation

FTIR spectral range of each corresponding to specific molecular interactions,[74] chemical groups[75] and the Active Pharmaceutical Ingredients (API) encapsulated in matrix,[76] analyzing the spectrum for specific absorption bands is crucial for identifying the functional groups within the lipid matrix. Figure 2a, corresponding to C=O stretching near 1700 cm^{-1}, indicates the presence of ester groups, which are common in lipid materials.[77] The methylene stretching vibrations observed at approximately 2850 cm^{-1} (symmetric) and 2920 cm^{-1} (asymmetric) provide information on the structural order of the lipid chains within the nanoparticles.[78] Bobby *et al.*,[71] analysed powdered leaves and the evaporated ethanolic extract of *Albizia lebbeck* the spectra of FTIR profiled amides, alkynes, alkanes, carboxylic acids, alkenes, aromatics, aliphatic amines, and alkyl halides, with significant peaks at 3654.12, 3307.55, 2918.44, 2849.92, 1643.73, 1454.46, 1054.13, and 510.34 cm^{-1} respectively. Similarly, the FTIR analysis of the dry ethanolic extracts indicated the presence of alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones, and alkyl halides, with major peaks observed at 3370.19, 2955.65, 2925.68, 2853.40, 1739.72, 1463.02, and 506.57 cm^{-1}.[71] Variations in these bands or their intensities may reflect changes in lipid matrix packing, potentially influencing the release characteristics of the encapsulated compounds.

Considering *Albizia lebbeck*, which is rich in bioactive compounds, including polyphenols,[52] flavonoids,[53] saponins[79] and alkaloids,[71] the observed peak may be attributed to such phytochemicals embedded within the solid lipid matrix of the SLNAs. Figure 2b, show the presence of this peak at 215.64 nm in the UV-visible spectrum suggests that the encapsulation process into the SLNAs might have preserved the integrity of these bioactive compounds, maintaining their characteristic absorbance properties. This finding is significant because it suggests that Solid Lipid Nanoparticles (SLNAs) could serve as an efficient carrier system for *Albizia lebbeck* extracts. This could potentially improve the stability, bioavailability, and targeted delivery of the encapsulated phytochemicals.

Specific surface area indicates a moderately high level of porosity for the nanoparticles, suggesting that the encapsulation of *Albizia lebbeck* has contributed to creating an intricate internal structure within the lipid matrix.[80] Figure 2c, indicates the Type IV isotherm is characteristic of mesoporous materials, often observed in the adsorption-desorption processes involving capillary condensation within the pores. The presence of a hysteresis loop in the Type IV isotherm further suggests that

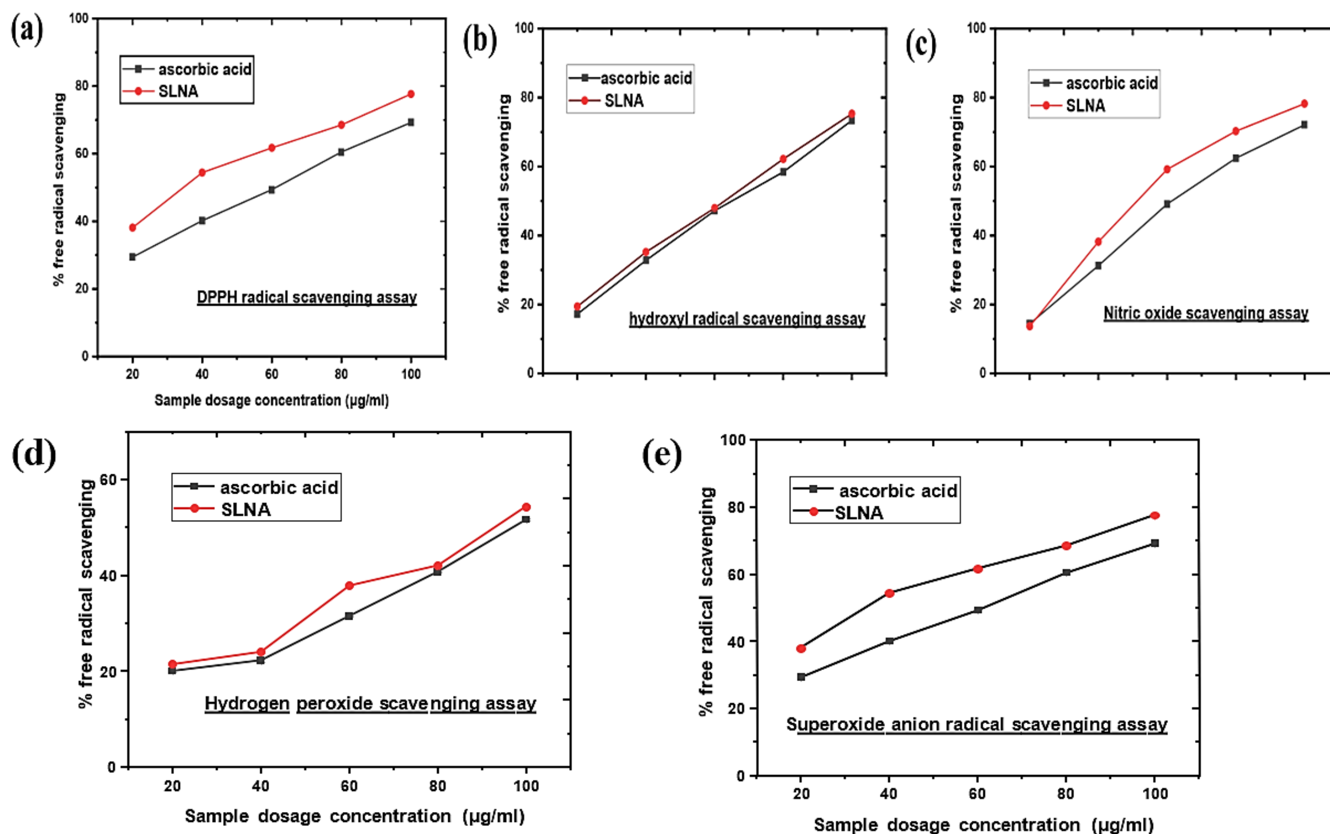

Figure 4: Antioxidant assessment using (a) DPPH radicle scavenging assay, (b) Hydroxyl radicle scavenging assay, (c) Nitric oxide scavenging assay, (d) Hydrogen peroxide scavenging assay and (e) Superoxide anion radicle scavenging assay.

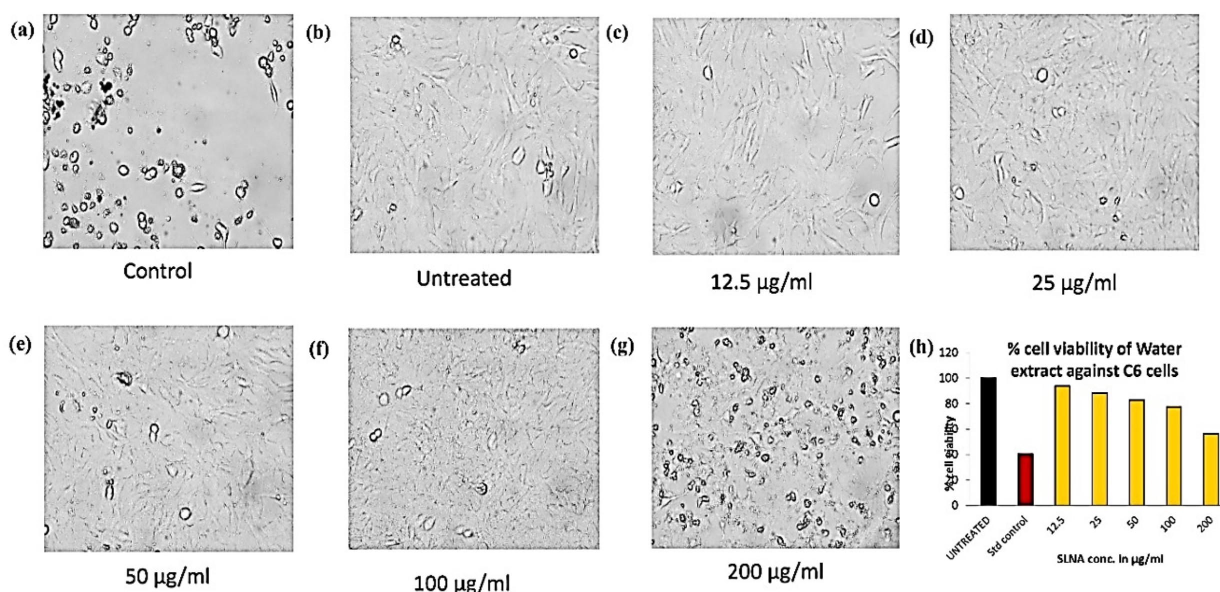

Figure 5: (a-g) Cytotoxic ability of the SLNA water extract upon different dosages for treatment against C6 Cells and (h) % Cell viability of water extract against C6 cells.

the pores within these nanoparticles are not only of uniform size but also indicate the presence of slit-like or cylindrical pores.[80,81] This is valuable for applications requiring controlled release, as the porosity and surface area directly impact the rate at which the encapsulated compounds are released into the target environment.[80] Additionally, the mesoporous structure of these *Albizia lebbeck* loaded Solid Lipid Nanoparticles could enhance the bioavailability of the loaded compounds, making them more effective in industrial application.[82,83] Based on the BET analysis and Type IV isotherm characterization, these nanoparticles have a specific surface area and pore structure that make them ideal for targeted drug delivery applications, especially those that call for controlled or sustained release.[84,85] Further investigations into the pore volume and pore size distribution would provide a more comprehensive understanding of their potential applications. This article restricts for understanding the preliminary synthesis of SLNA.

Assessment of Antioxidant Activity

The assessment of antioxidant activity emphasizes the importance of natural compounds in combating oxidative stress and related diseases. Various assays, including DPPH, superoxide anion, hydroxyl radical, nitric oxide, and hydrogen peroxide scavenging, have been utilized to investigate the antioxidant capabilities of SLNA. These assays measure an antioxidant's ability to neutralize reactive radicals and reduce harmful nitric oxide levels, potentially protecting against oxidative damage and inflammation. This underscores the critical role of antioxidants in maintaining cellular integrity against oxidative damage.

DPPH radical scavenging assay

The DPPH radical scavenging assay is a standard method for assessing antioxidant activity, measuring a compound's ability to neutralize the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical.[62,86] The assay conducted on Solid Lipid Nanoparticles (SLNAs) revealed that they possess notable antioxidant activity, which is quantified by an IC_{50} value of 34.69 µg/mL. This indicates the concentration of SLNAs required to inhibit 50% of free radicals in the test system. While the antioxidant capacity of SLNAs is significant, it is important to note that their potency is lower than that of ascorbic acid, which has an IC_{50} value of 28.21 µg/mL. This comparison highlights that ascorbic acid is more effective at scavenging free radicals than SLNAs. The findings underscore the potential of SLNAs as antioxidant agents; however, they also suggest that ascorbic acid remains a more powerful option for applications requiring strong antioxidant properties. Further research may be necessary to explore the mechanisms by which SLNAs exert their effects and to optimize their formulations for enhanced efficacy. Despite its lower potency, SLNA's antioxidant capacity remains valuable, potentially offering benefits in applications where moderate efficacy is preferred due to its unique chemical structure and reduced side effects.[38,87]

The *Albizia lebbeck* extract, rich in polyphenols,[52] and flavonoids,[53] showed DPPH radical scavenging activity, indicating high antioxidant capacity.[88,89] Recent research has investigated the antioxidant properties of two plant species: *Albizia lebbeck* and Acacia nilotica. The findings indicate that extracts derived from *Albizia lebbeck* exhibit significant free radical scavenging activity, which is a critical measure of a substance's potential to counteract oxidative stress in biological systems, it is found that *Albizia lebbeck* extracts showed considerable free radical

Table 1: Comparison of scavenging activities.

Method	IC_{50} values for ascorbic acid	IC_{50} values for SLNA
DPPH	28.21 µM	34.69 µg/m
Hydroxyl radical scavenging assay.	45.66 µg/mL	60.00 µg/mL
Superoxide anion radical scavenging (SO) assay.	29.64 µg/mL	20.88 µg/mL
Nitric oxide scavenging assay.	45.00 µg/mL	43.11 µg/mL
Hydrogen peroxide Scavenging assay.	96.95 µg/mL	96.06 µg/mL

scavenging activity, although slightly less potent than Acacia nilotica in specific assays.[90,91] Additionally, the seed extracts of *Albizia lebbeck* were reported to possess low antioxidant activity compared to standards like Propyl gallate, highlighting a variation in activity based on the plant part and extraction method.[92] Baliyan *et al.*[93] assess antioxidants in Ficus religiosa using DPPH radical scavenging activity, which measures antioxidant potential by evaluating free radical scavenging capabilities. Muliasari *et al.*[94] assess antioxidants in *Ashitaba* herb using a DPPH radical scavenging assay, showing moderate activity with an IC_{50} value of 129.40±7.36 ppm compared to ascorbic acid. The significant yet variable antioxidant potential of *Albizia lebbeck* underscores the importance of the extraction solvent and the specific part of the plant used. This comparison highlights ascorbic acid's superior efficacy in antioxidant assays y*et al*so positions SLNA as a viable option with meaningful, albeit slightly lower, free radical neutralizing properties.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay is an essential technique for evaluating the antioxidant potential of compounds, including Solid Lipid Nanoparticles (SLNs) such as those derived from *Albizia lebbeck* (SLNA). SLNs are widely utilized in cosmetics, pharmaceuticals, and the food industry due to their biocompatibility and biodegradability. In this study, SLNA exhibited moderate hydroxyl radical scavenging activity, with an IC_{50} value of 60.00 µg/mL, indicating it requires a higher concentration to achieve 50% inhibition compared to ascorbic acid, which had an IC_{50} 45.66 µg/mL. Although less potent than ascorbic acid, SLNA's antioxidant activity suggests its potential as a supplementary antioxidant in therapeutic applications, warranting further research to improve its efficacy.

Nitric oxide scavenging assay

The nitric oxide scavenging assay is critical for assessing antioxidant capacity, with ascorbic acid demonstrating superior efficacy, consistent with its established antioxidant properties. Ascorbic acid effectively reduces superoxide and prevents peroxynitrite formation, thereby mitigating oxidative stress. It also contributes to non-enzymatic nitric oxide production in low pH environments, such as the stomach, and enhances the activity of key antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and catalase, amplifying its free radical scavenging ability. In the assay, Solid Lipid Nanoparticles (SLNA) showed moderate nitric oxide scavenging with an IC_{50} of 43.11 µg/mL, compared to ascorbic acid's much lower IC_{50} of 45.00 µg/mL indicating SLNA is more potent. Despite lower efficacy, SLNA may be valuable in situations requiring a milder antioxidant effect to minimize toxicity at higher doses. Ascorbic acid and its derivatives, like 2-O-β-d-glucopyranosyl-l-ascorbic acid (AA-2βG), offer enhanced protection against oxidative stress-induced cell damage. Natural antioxidants from medicinal plants such as *Pueraria montana* var. lobata and *Oryza sativa* also modulate nitric oxide and oxidative stress, with ascorbic acid often used as a benchmark. The involvement of nitric oxide pathways in therapeutic effects, like lycopene's role in reducing seizures and improving memory, further highlights the importance of nitric oxide scavenging in health. These findings emphasize ascorbic acid's potent antioxidant properties and its potential as a therapeutic agent in managing oxidative stress.

Hydrogen peroxide scavenging assay

The Hydrogen Peroxide (H_2O_2) scavenging assay is commonly employed to evaluate the antioxidant capacity of compounds by their ability to neutralize H_2O_2, a reactive oxygen species linked to oxidative stress and cellular damage. Solid Lipid Nanoparticles (SLNA) was evaluated for their antioxidant activity using a scavenging assay, and the results indicated moderate efficacy. Specifically, the half-maximal Inhibitory Concentration (IC_{50}) was determined to be 96.06 µg/mL, suggesting that at this concentration, SLNA can inhibit 50% of free radical activity in the tested system. In comparison, ascorbic acid, a well-known antioxidant, displayed similar scavenging activity with an IC_{50} of 96.95 µg/mL.

The observed antioxidant efficacy of SLNA may provide advantages in various applications requiring a gentler antioxidant effect. This characteristic can be particularly valuable in formulations intended for sensitive populations or conditions where higher levels of antioxidative compounds may induce toxicity. Additionally, SLNA's moderate activity could contribute to a synergistic approach when combined with other antioxidants, potentially enhancing the overall antioxidant strategy. This synergy might arise from complementary mechanisms of action or improved bioavailability of active compounds due to the lipid-based delivery system. Further studies are warranted to explore these interactions and optimize the use of SLNA in antioxidant formulations.

Superoxide free radical scavenging assay

The Superoxide Anion Radical Scavenging Assay serves as a vital technique for assessing the antioxidant properties of various substances. This assay specifically targets superoxide anion radicals (O_2^-), which are reactive oxygen species that play a significant role in causing oxidative stress and cellular damage if not effectively neutralized. SLNA were evaluated for their ability to scavenge these harmful superoxide anion radicals.

The findings revealed that SLNA exhibited notable scavenging activity, with an IC_{50} value of 20.88 µg/mL. The IC_{50} value indicates the concentration of a substance required to inhibit 50% of the radicals' activity, and a lower IC_{50} value signifies a stronger antioxidant effect. In comparison, ascorbic acid-which is widely recognized for its antioxidant properties-showed an IC_{50} value of 29.64 µg/mL. This comparison demonstrates that the scavenging activity of SLNA is significantly stronger than that of ascorbic acid. The moderately robust scavenging capacity of SLNA observed in this assay aligns well with existing literature on antioxidant research. These results underscore the importance of utilizing dependable assays, such as the Superoxide Anion Radical Scavenging Assay, when screening various compounds for their antioxidant capabilities.

Cytotoxicity Examination

Cytotoxicity assays are crucial in evaluating the potentially harmful effects of synthesized nanomaterials on living organisms.[95] The cytotoxicity assessments of SLNA, explicitly utilizing a water extract, on C6 cells-a rat glioma cell line-were meticulously conducted through the MTT assay. This assay is a vital tool for measuring cell viability by detecting the reduction of MTT dye to its formazan by metabolically active cells. SLNA concentration increased from 12.5 to 200 mg/L, and there was a noted increase in apoptosis, characterized by cell disintegration, shrinkage, and a decrease in the formation of apoptotic bodies compared to the control group (Figure 5). This concentration-dependent response underscores the importance of carefully regulating SLNA dosage to mitigate cytotoxic effects while optimizing therapeutic efficacy. These assays are integral in determining the safest and most effective dosage levels for therapeutic applications.[65] These findings emphasize the critical role that cytotoxicity assays, like the MTT assay, play in determining the most appropriate dosage levels for therapeutic interventions,[96] ensuring safe treatments.

Using *Albizia lebbeck* extracts in nanoparticle synthesis reflects a growing shift towards green chemistry and environmentally friendly production methods. These approaches make nanoparticle production more sustainable and improve the biocompatibility and effectiveness of nanoparticles for applications such as drug delivery and other biomedical uses.

CONCLUSION

The research findings indicate that *Albizia lebbeck* encapsulated in Solid Lipid Nanoparticles has significant antioxidant capabilities and a satisfactory safety record. FTIR, UV-Vis, and BET analysis were among the characterization methods that verified the nanoparticles' stable structural integrity and effective encapsulation. SLNA showed a strong potential for antioxidants, with an IC_{50} value that suggested a promising level of effectiveness. Even at larger doses, SLNA did not cause cytotoxicity in the MTT experiment on C6 cells, indicating that it is safe for future applications. These findings underscore SLNA's potential in enhancing pharmaceutical formulations, especially in therapeutic areas requiring controlled antioxidant release, such as neurological diseases. Future research should focus on optimizing SLNA formulations to enhance antioxidant efficacy and explore broader therapeutic applications.

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CONFLICT OF INTEREST

The authors declare that they do not have any known competing financial interests or personal relationships that could have influenced the work reported in this paper.

ABBREVIATIONS

SLN: Solid-lipid nanoparticles; **AL:** *Albizia lebbeck*; **SLNA:** *Albizia lebbeck* loaded Solid Lipid Nanoparticles; **SA:** Stearic Acid; **ROS:** Reactive oxygen species; **DPPH:** 1,1-diphenyl-2-picrylhydrazyl; **FTIR:** Fourier-transform infrared spectroscopy; **BET:** Braunauer-Emmett-Teller.

AUTHOR CONTRIBUTION

Regarding the CRediT taxonomy, the author's contributions were: **Kavitha S K:** Conceptualization, visualization, validation, methodology, investigation, data curation, formal analysis, writing-original draft. **Ali Mohammad:** Supervision, project administration, writing - review & editing.

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