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Assessment of Antioxidant Activities of *Albizzia Lebbeck* Loaded Solid-Lipid Nanoparticles

Kavitha S. K.,[1] Dr. Mohammad Ali*[2]

[1]*Department of Pharmacology, Adichunchanagiri University, Faculty of Pharmacy, Sri Adichunchanagiri College of Pharmacy, B G Nagara, Karnataka-571418, India and Nitte College of Pharmaceutical Science, Bangalore -560064*

[2]*Professor, Department of Pharmacology, Faculty of Pharmacy, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University B G Nagara, Karnataka-571418, India*

Abstract

Background: Nanotechnology is vital role in therapeutic industry, SLNs combine lipid-based systems with nanotechnology to provide controlled drug release, & antioxidants in SLNs improves their efficacy in treating diseases to oxidative stress. Aim: To study the detail about Assessment of antioxidant activity of *Albizzia Lebbeck* loaded solid-lipid nanoparticles. Material & methods: However, very few studies have incorporated with antioxidant plant *Al* leaves extract into the SLNs. In the current study, ethanolic extract from the leaves of *Al* has been SLNs using stearic acid. The substance was further characterized by morphological, and chemical analysis using FE-SEM, FTIR, and BET techniques. The antioxidant properties of the drug were evaluated using various scavenging assays, including DPPH, superoxide, hydroxyl, nitric oxide, and the exhibited scavenging activity with an IC_{50} value of 34.69 µg/ml, 20.88 µg/ml, 60.00 µg/ml, 43.11 µg/ml, and 96.06 µg/ml, respectively, compared with ascorbic acid. In comparison to ascorbic acid, Results & Discussion: SLNs shows less effectiveness against certain radicals but exhibits stronger activity against superoxide. This SLNs could be effective in treating conditions primarily affected by these specific radicals. The MTT assay on C6 cells found that concentrations from 12.5 to 200 mg/L initiated apoptosis through cell collapse, reduced production of apoptotic. In summary, it is show that significant potential for pharmaceutical formulations & antioxidant stree release is required, it managing neurological diseases.

Keywords*: SLNs, AL, MTT, C6, DPPH, IC50 etc*

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 pharmaceuticals, where SLN offers a viable approach [20]. Currently, SLNs are produced 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 glipalamide significantly increased by creating a cocrystal with aspartame, leading to marked improvements in both solubility and rates of dissolution. SLNs with antioxidants like Rosmarinus 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]. Historically, antioxidants were significant in industrial applications, but their primary biological importance lies in 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 (ZrO2) and titania (TiO2) 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 *Albizzia 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]

Albizzia lebbeck offer the potential for controlled and site-specific drug delivery [42], making them promising for therapeutic applications in drug design. Specifically, nanoparticles of *Albizzia lebbeck* serve as a carrier for the bioactive compounds [43], enhancing drug stability, bioavailability, and targeted release [44]. Though direct clinical evidence remains limited, *Albizia lebbeck* extracts hold potential for clinical applications in treating chronic neurological diseases *[45]* related to oxidative stress. However, the plant's extracts, rich in polyphenols [46], flavonoids [47], and other antioxidant compounds [48], exhibit vigorous free radical scavenging activity, which is crucial in mitigating oxidative stress [49], a key factor in the pathogenesis of neurological disorders like Alzheimer's disease [50], Parkinson's disease [51], and multiple sclerosis [29]. By neutralizing reactive oxygen species (ROS)[52] and reducing oxidative damage[53] to neurons, *Albizia lebbeck* extracts could theoretically protect against the progression of neurodegenerative diseases. Kumar et al. [49] 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 *Albizzia lebbeck* in pharmaceutical industrial applications. However, still, no such efforts have been made by many researchers.

Overall, the research on Solid Lipid Nanoparticles (SLNs) shows promise in controlled drug release, enhanced bioavailability and employed in various industrial applications. Moreover, addition of nanocarriers and antioxidants into the SLNs enhance 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 property. Addressing these research gaps could significantly advance the application of nanotechnology in pharmaceuticals.

As per the authors knowledge in conducting vast literature survey, there are very few studies analysed 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: *Albizzia 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.

Preparation of *Albizzia Lebbeck* Ethanolic Extract: 10 grams of *Albizzia Lebbeck* plant material and 100 millilitres of ethanol were mixed to create the ethanolic extract. For a period of 3 hours, the mixture was constantly mixed while being heated to 40°C. The combination was then filtered to extract the plant material's ethanolic extract.

Preparation of *Albizzia Lebbeck* encapsulated solid lipid Nanoparticles:

The general procedure of synthesizing is given in 30 milligrams of stearic acid (SA) were dissolved in 10 millilitres of each of the acetone and ethanol solvent mixtures. In a water bath, this solution was heated to 60°C. After adding 10 milligrams of the ethanolic extract, the mixture was shaken for two hours and ultrasonically agitated for thirty minutes. Several extracts were prepared and analysed 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 millilitres) 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.

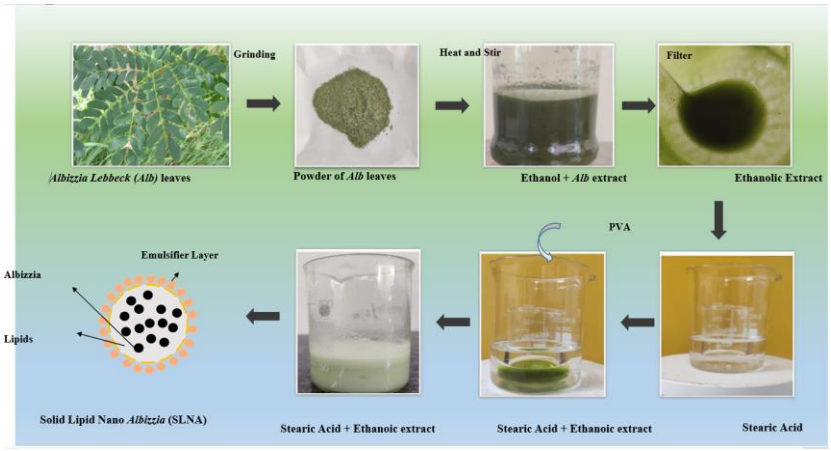
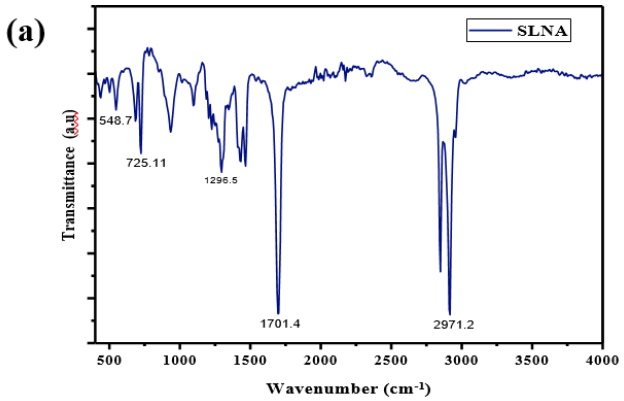


Fig. 1. SLNA Preparation

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; HITACHI, USA -10 KV); energy-dispersive X-ray spectroscopy (EDS; Zeiss Supra 55VP, Japan); particle size and polydispersity index using the dynamic light scattering technique (Malvern Zeta sizer). X-ray diffraction (XRD; Rigaku Smart Lab using Cuka radiation); 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.



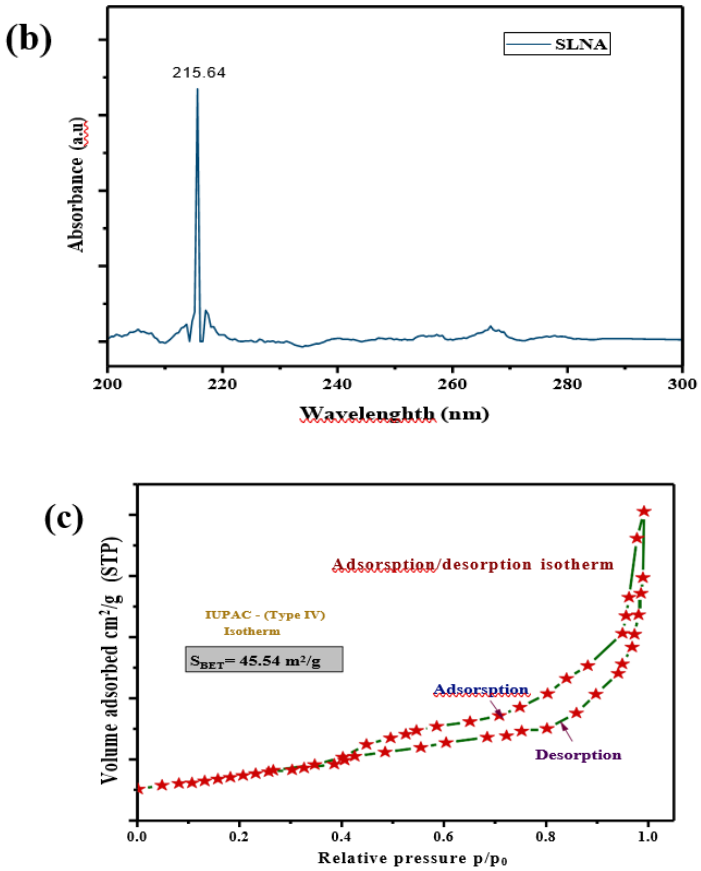
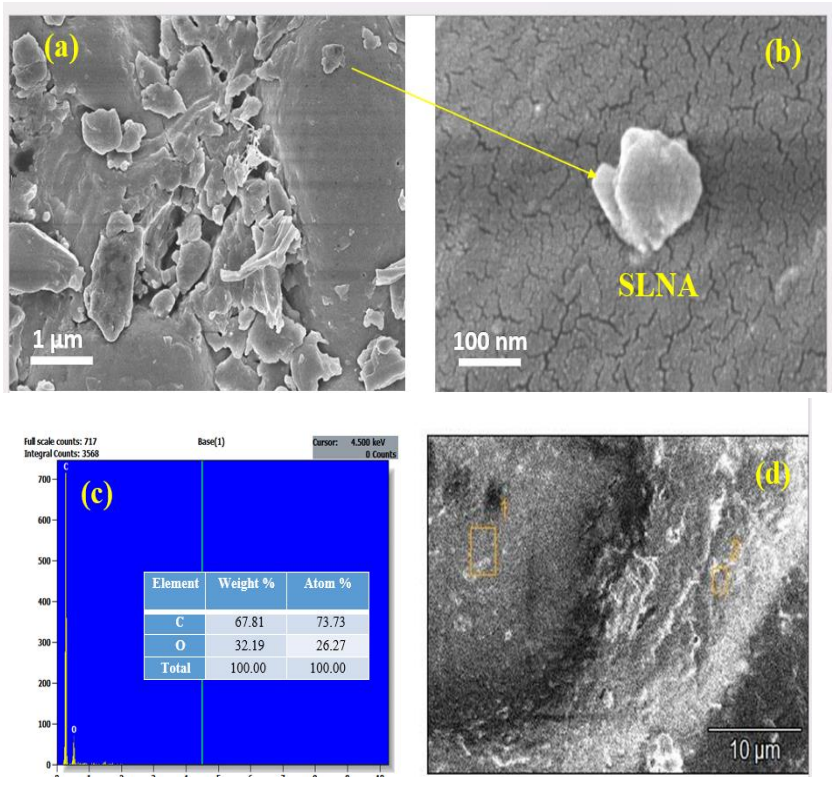


Fig. 2. (a) FTIR Spectral analysis (b) UV Spectra and (c) BET Surface area analysis of SLNA.



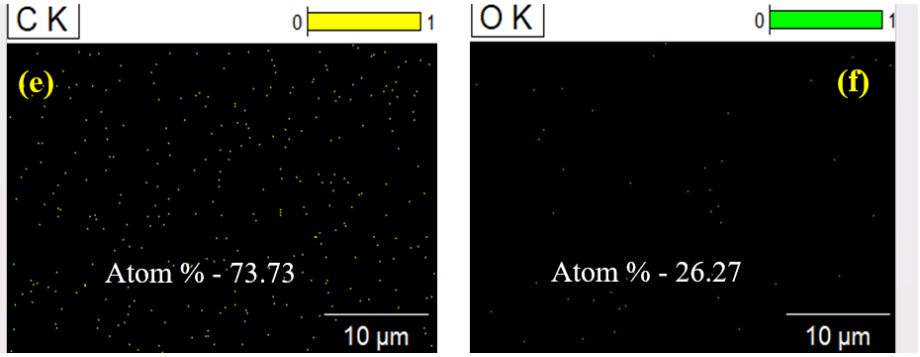


Fig. 3. (a and b) SEM analysis of SLN nanoparticles resolution of 1um and 100 nm, (c)EDAX elemental Composition and (d-f) Elemental mapping SLNA particles.

Antioxidant evaluations

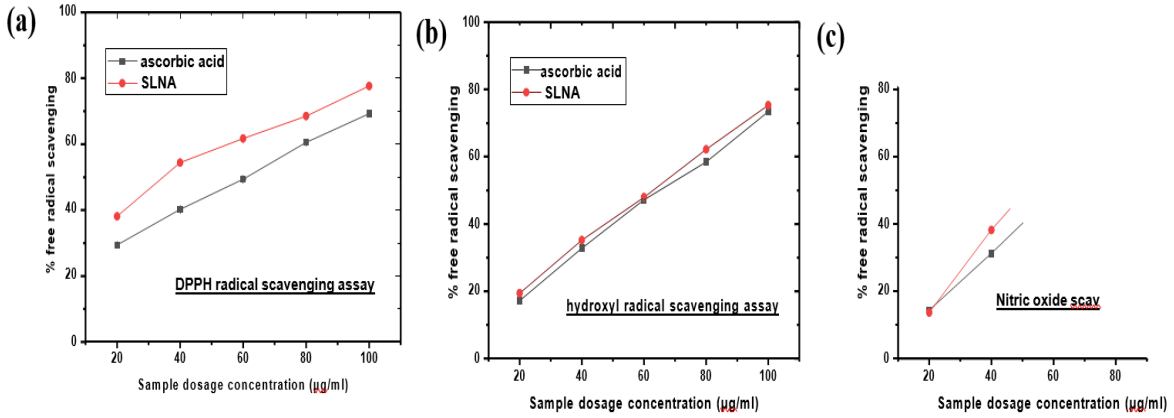
For DPPH antioxidant evaluation [54-55], a mixture of DPPH free radical scavenging assay was prepared by combining 2 mL of a 0.1 mM DPPH solution with 2 mL of methanol, and its absorbance was immediately measured at 517 nm to serve as a control. In the experimental procedure, an equal volume of the test extract replaced methanol and was shaken vigorously with the DPPH solution. After incubating for 30 minutes, 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.

For the Hydroxyl antioxidant evaluation using its radical scavenging assay [56], 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 FeCl3 (200 mM) in a 1:1 ratio was sequentially added. Subsequently, 100 µL of 1.0 mM H2O2 and 100 µL of 1.0 mM ascorbic acid were added. The mixture was then incubated at 37°C for 1 hour. 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 100°C for 20 minutes.

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 25°C for 150 minutes. 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 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.

A reaction mixture containing Tris-HCl buffer, NBT solution, NADH solution, sample solution, and PMS solution was incubated at 25°C for 5 minutes 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.



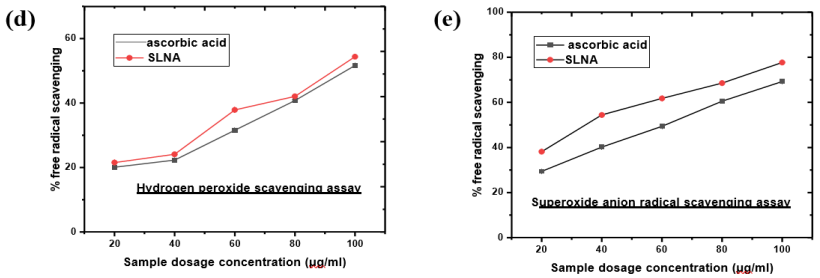
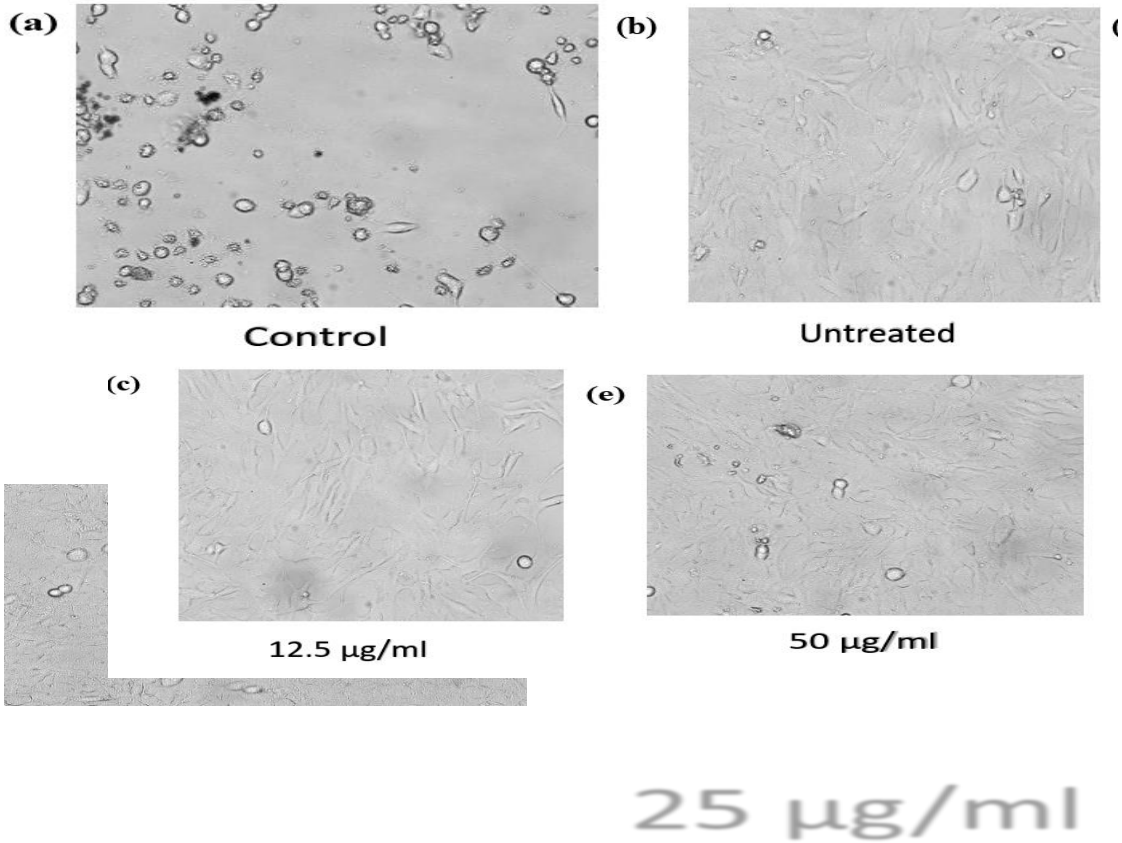


Fig. 4. Antioxidant assessment using (a) DPPH radicle scavenging assay, (b) Hydroxyl radicle scavenging assay, (c) Nitric oxide scavenging assay, (d) Hydrogen peroxide scavenging assay and (e) Superoxide anion radicle scavenging assay

Cytotoxicity assessments of SLNA

To conduct the cytotoxicity study, standard protocol is followed established by various studies [57]–[59]. C6 cells suspensions at a density of 20,000 cells per well in a 96-well plate seeded, using 200µl of suspension per well, and allowed the cells to grow for 24 hours without any test agents. Following this initial growth period, the test agents ere prepared at specified concentrations in the culture media and added these to the plate wells. Then, we incubated the plate at 37°C in a 5% CO2 atmosphere for 24 hours. Post-incubation, media spent media was removed from each well and the MTT reagent to a final 0.5mg/mL concentration. To protect the reaction from light, the plate was wrapped in aluminium foil and returned to the incubator for a further 3 hours. Subsequently, the MTT reagent was carefully removed, and 100µl 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 570nm. 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 [60].

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



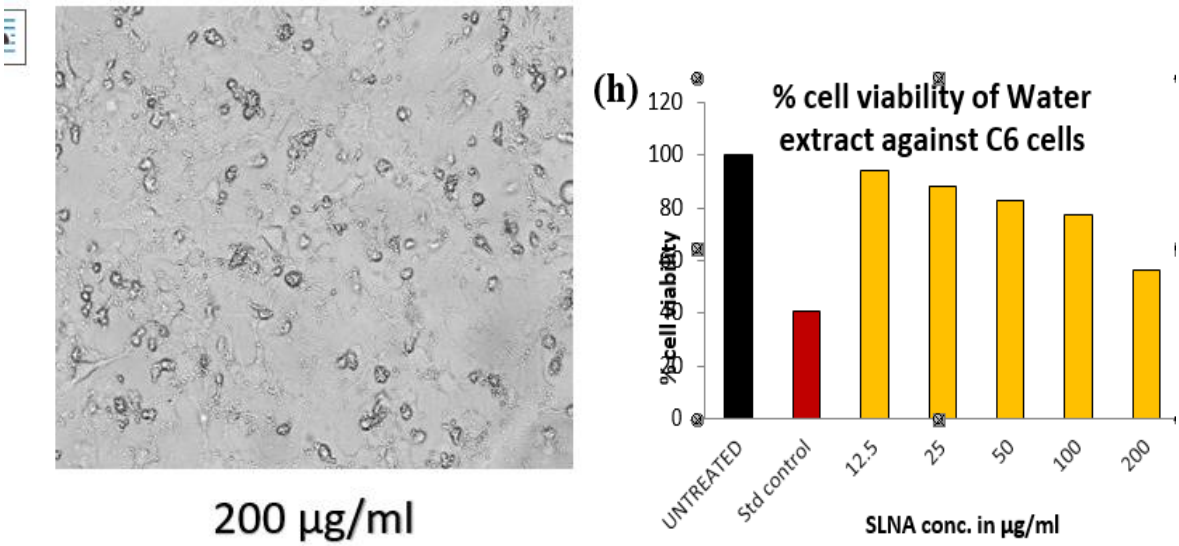


Figure 5: (a-g) Cytotoxic ability of the SLNA water extract upon different dosages for treatment against C6 cells, and (h) percentage cell viability of the water extract against C6 cells.

Results And Discussion

The use of *Albizzia 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 Albizzia lebbeck embedded in SLN now onwards referred as SLNA. Analysis, including chemical and morphological characterization. Additionally, antioxidant performance, and biocompatibility functionality of SLNA, is discussed, which is highlighting the impact of SLNA in enhancing the efficacy and biocompatibility of the extracts for potential neurological applications.

Green synthesized slna- structural and morphological characterization.

The *Albizzia lebbeck* loaded SLNA FTIR spectra are shown in **Fig 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, aliphatic Odo compounds, C-I stretch and C-H bending vibrations of organic compound, and C-N stretching vibrations of amine groups, C=O stretching vibrations in carbonyl groups, C-H stretching vibrations in lipid-based nanoparticles [61]– [64]. FTIR spectral range of each corresponding to specific molecular interactions [65], chemical groups [66] and the active pharmaceutical ingredients (API) encapsulated in matrix [67], analyzing the spectrum for specific absorption bands is crucial for identifying the functional groups within the lipid matrix. For example, a peak corresponding to C=O stretching near 1700 cm^{-1} indicates the presence of ester groups, which are common in lipid materials [68]. 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 [69]. Bobby et al. [63] 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 major peaks at 3654.12, 3307.55, 2918.44, 2849.92, 1643.73, 1454.46, 1054.13, and 510.34 cm^{-1}. 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}[63]. Variations in these bands or their intensities may reflect changes in lipid matrix packing, potentially influencing the release characteristics of the encapsulated compounds.

In the UV-visible spectroscopic analysis of SLNA, a prominent peak was observed at 215.64 nm shown in **Fig 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 [70]. Considering *Albizzia Lebbeck*, which is rich in bioactive

compounds, including in polyphenols [46], flavonoids [47], saponins [71], and alkaloids [63], the observed peak may be attributed to such phytochemicals embedded within the solid lipid matrix of the SLNAs. 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 *Albizzia Lebbeck* extracts. This could potentially improve the stability, bioavailability, and targeted delivery of the encapsulated phytochemicals.

BET analysis of SLNA revealed a specific surface area (S_BET) of 45.54 m^2/g, accompanied by a Type IV isotherm are shown in **Fig 2c**. This specific surface area indicates a moderately high level of porosity for the nanoparticles, suggesting that the encapsulation of *Albizzia Lebbeck* has contributed to creating an intricate internal structure within the lipid matrix [72]. 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 the pores within these nanoparticles are not only of uniform size but also indicate the presence of slit-like or cylindrical pores. 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. Additionally, the mesoporous structure of these *Albizzia Lebbeck* loaded Solid Lipid Nanoparticles could enhance the bioavailability of the loaded compounds, making them more effective in industrial applications [73], [74]. 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. Further investigations into the pore volume and pore size distribution would provide a more comprehensive understanding of their potential applications. This article is restricted for understanding the preliminary synthesis of SLNA.

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 **Fig. 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**.13nm of size. To further establish the synthesized particles' elemental properties, EDX analysis was performed**,** wherein peaks for C and O elements were observed **Fig. 3 c**. The insert table in **Fig. 3 d- 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.

Assessment of Antioxidant Activity

The assessment of antioxidant activity in various studies reveals the importance of natural compounds in combating oxidative stress and its related diseases [75]. SLNA were studied for their antioxidant activity using a variety of assays, such as DPPH radical scavenging, superoxide anion radical scavenging, hydroxyl radical scavenging, nitric oxide scavenging, and hydrogen peroxide scavenging assays.

Table 1. Percentage of different scavenging activities

Method	**IC50 values for ascorbic acid**	**IC50 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

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.

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 [54], [76]. The assay revealed that Solid Lipid Nanoparticles (SLNA) exhibit significant antioxidant activity, with an IC_{50} value of 34.69 µg/ml, though it is less potent than ascorbic acid (IC_{50} = 28.21 µg/ml). 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 [38-77].

The *Albizia lebbeck* extract, rich in polyphenols [46], and flavonoids [47], showed DPPH radical scavenging activity, indicating high antioxidant capacity [78], [79]. Another study compared the antioxidant activity of *Albizia lebbeck* with Acacia nilotica and found that *Albizia lebbeck* extracts showed considerable free radical scavenging activity, although slightly less potent than Acacia nilotica in specific assays [79]. 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 [78]. Recent studies on antioxidants in Ficus religiosa using DPPH radical scavenging activity, which measures antioxidant potential by evaluating free radical scavenging capabilities [78]. Another reseach on antioxidants in ashitaba herb using a DPPH radical scavenging assay, showing moderate activity with an IC50 value of 129.40 ± 7.36 ppm compared to ascorbic acid [79]. 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, yet also 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 *Albizzia 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. The lower efficacy of SLNA could be attributed to its active components' slower release or reduced bioavailability. 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 proximities 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) exhibited moderate scavenging activity in this assay with an IC_{50} of 96.06 µg/ml. Similarly, ascorbic acid showed same effect, with an IC_{50} of 96.95 µg/ml. While SLNA's antioxidant

efficacy may helpful in contexts requiring a milder antioxidant effect or as part of a broader antioxidant strategy, potentially due to lower toxicity or synergistic interactions with other compounds.

Superoxide free radical scavenging assay

The Superoxide anion radical scavenging assay is a crucial method for evaluating antioxidant activity, explicitly targeting superoxide anion radicals (O_2^-), which are significant contributors to cellular damage if not neutralized. In this study, Solid Lipid Nanoparticles of *Albizzia lebbeck* (SLNA) exhibited scavenging activity, with an IC_{50} value of 20.88 µg/ml, indicating a strong activity compared to ascorbic acid (IC_{50} = 29.64 µg/ml). The moderate scavenging capacity of SLNA in this assay aligns with broader antioxidant research, emphasizing the importance of reliable assays in screening antioxidant properties.

Cytotoxicity assessments of SLNA (Water extract) through *C6* cells via MTT assay

Cytotoxicity assays are crucial in evaluating the potential harmful effects of synthesized nanomaterials on living organisms [80]. 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. Upon exposure to various concentrations of SLNA (ranging from 12.5 to 200 mg/L) over a 24-hour period, it was observed that lower concentrations did not significantly affect cellular interactions or binding activity, indicating minimal cytotoxicity at these levels. However, as the SLNA concentration increased from 12.5 to 200 mg/L, 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 **(Fig 5)**. This concentration-dependent response underscores the importance of carefully regulating SLNA dosage to mitigate cytotoxic effects while optimizing therapeutic efficacy. The results indicate the potential cytotoxic effects of SLNA on C6 cells, providing insights into its safety and efficacy for further applications. These assays are integral in determining the safest and most effective dosage levels for therapeutic applications [57]. These findings emphasize the critical role that cytotoxicity assays, like the MTT assay, play in determining the most appropriate dosage levels for therapeutic interventions [81], ensuring both the safety and effectiveness of such treatments.

Using *Albizzia lebbeck* extracts in nanoparticle synthesis reflects a 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.

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