RESEARCH PAPER

CHITOSAN NANOPARTICLES MITIGATE H_2O_2-MEDIATED TOXICITY IN SH-SY5Y CELLS

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Abstract

The neuroprotective potential of chitosan nanoparticles (CS NPs) were evaluated against hydrogen peroxide (H_2O_2) induced toxicity in SH-SY5Y cells. The ionic gelation was used for synthesizing the CS NPs and characterized using Dynamic light scattering, UV-Visible spectroscopy, X-ray diffraction, Scanning and high resolution electron microscopy. The characterization results confirmed the generation of spherical-shaped particles with a size, zeta potential, and polydispersity index of 116.9 nm, 17.4 mV and 0.260 respectively. The formation of CS NPs was further confirmed by UV-Vis spectroscopy and XRD. The toxic effects of H_2O_2 were mitigated by CS NPs through improvement in cell viability and membrane integrity through MTT and LDH assays. Further, the CS NPs treatment decreased ROS levels, indicating their ability to mitigate oxidative stress. These results support the possible use of chitosan-based nanoparticles as a promising neurotherapeutic carrier.

Keywords: Chitosan nanoparticles, Hydrogen peroxide, Cell viability, LDH, ROS

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Introduction

Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and other age-related neurological conditions are characterized by the gradual loss of structural integrity and dysfunction of neurons[1]. Oxidative stress (OS) is one of the major pathophysiological features underlying these neurological illnesses[2]. OS arises due to the surplus production of reactive oxygen species (ROS) and other free radicals. The surplus-generated radical species damages lipids, proteins, and nucleic acids, eventually resulting in impaired mitochondrial function, apoptosis, and death[3]. Particularly, the hydrogen peroxide (H_2O_2) is a reactive species known to cause OS and regarded as harmful to the cells[4]. Several findings have reported that H_2O_2 causes disruption in neuronal activity[5,6]. Therefore, the strategies which aim at reducing OS have attained considerable interest for the potential to delay neurodegeneration.

In the recent decade, researchers have shown tremendous interest in using natural polymers from marine sources due to their abundance and varied biological activity[7]. Chitosan (CS) is a bioactive molecule with multiple biomedical applications such as antioxidant, anti-diabetic, antimicrobial, anticancer, wound healing, etc[8]. CS also offers beneficial properties like non-toxicity, biodegradability, biocompatibility, and muco-adhesion[9]. CS is the second most prevalent natural biopolymer after cellulose. It is a cationic linear heteropolysaccharide made of N-acetyl-D-glucosamine and D-glucosamine bonded with β (1−4) glycosidic linkage[10,11]. Although CS has excellent potential for diverse biomedical applications, its use is limited due to low mechanical strength, less heat stability, and sensitivity to pH. However, the functional groups of CS can be altered to enhance its functionality through graft modification and crosslinking. The nanosized particles of CS can be easily created by cross-linking with anionic compounds like sodium tripolyphosphate (TPP), which increases its stability[12]. The chitosan nanoparticles (CS NPs) tend to have enhanced porosity, high surface area, and improved biological activity[13].

The CS NPs can be produced with various methods, including emulsion-based solvent evaporation, emulsification, micro-emulsion, polyelectrolyte complexation, solvent diffusion, and ionic gelation[14,15]. Despite the availability of several synthesis methods, ionic gelation is widely used as it offers excellent benefits, such as the use of non-hazardous solvents and an easy, convenient, and controllable process. Studies of Zhu et al.[16] reported the ability of chitosan oligomer to attenuate β-amyloid-mediated neurotoxicity and inflammation in a dose-dependent manner. Manigandan et al.[17] reports revealed the ROS scavenging and antioxidant activity of low molecular weight sulphonated chitosan, by reducing lipid peroxidation, alleviating oxidative stress, and reducing mitochondrial dysfunction.

Further, the studies reported by Ahlawat et al.[18] have revealed the potent neuroprotective ability of the bare CS NPs against rotenone-induced neurotoxicity in SH-SY5Y cells. However, studies on the neuroprotective effects of bare CS NPs against H_2O_2 toxicity in SH-SY5Y cells are very sparse. Therefore, our study focuses on synthesizing and characterizing CS NPs and investigating their neuroprotective potential against H_2O_2-induced toxicity in SH-SY5Y cells.

Materials and methods

Chemicals

CS (low molecular weight) and sodium tripolyphosphate (TPP) were obtained from Sigma Aldrich, USA. Dulbecco's modified Eagle's medium (DMEM), F12 medium, Fetal bovine serum (FBS), Antibiotic-antimycotic solution, Dichloro difluorescein diacetate (DCFDA), 3(4, 5-Dimethylthiazol-2)-2, 5-Diphenyl tetrazolium bromide (MTT), were procured from Hi-media, Mumbai. All other general chemicals were purchased from Sisco Research Laboratories (SRL), Mumbai.

Methods

Preparation of CS NPs

The ionic gelation process was utilized for the CS NPs preparation following our earlier protocol with slight change[19]. The acetic acid (1%) was used to dissolve CS (1 mg/mL) under constant stirring (600 RPM) for 12 h. Then 2 mL of TPP (0.5 mg/mL) dissolved in deionized water was added dropwise into the CS solution (5 mL) under the stirrer. Then the suspension was centrifuged (16000 RPM, 45 min) to obtain CS NPs in the pellet, which was washed with deionized water, lyophilized and stored at 4 °C.

Characterization of CS NPs

Dynamic light scattering (DLS)

The particle size (PS), polydispersity index (PDI) and zeta potential (ZP) of CS NPs were analyzed using DLS technique (Malvern zetasizer, USA).

UV- Visible (UV-Vis) spectroscopy

The UV−vis spectrum of CS NPs were obtained in the range of 200-800 nm using a multimode reader (Synergy LX, Agilent Technologies).

X-ray diffraction (XRD)

A powder X-ray diffractometer (X-pert PRO, PANalytical) was used to detect the physical nature of these NPs at a scanning range of 10-80°.

Scanning electron microscopy

The surface morphological features, like shape, size, and smoothness, were observed using the SEM (Apreo 2, Thermo Scientific).

High-resolution transmission electron microscopy (HR-TEM)

The HR-TEM images were attained using the TecnaiG2 20 instrument to further validate the shape and size of the CS NPs.

In Vitro Studies

Cell viability (MTT) assay

MTT assay was accomplished following the protocol of Mossman[20]. SH-SY5Y cells (1×10^4 cells/well) were seeded in a 96-well plate (24 h) in a DMEM and F12 (1:1 ratio), supplemented with 10% FBS, and 1% antimycotic and antibiotic solution. Followed by pre-treatment with varying concentrations of CS NPs 1 h before the exposure of 135 µM H_2O_2 and further incubated (24 h). Then, the addition of MTT (5 mg/mL) in the dark (37°C, 4 h). Subsequently, the addition of DMSO (100 µL) and measuring the absorbance at 570 nm using multimode reader (Synergy LX, Agilent Technologies).

Lactate Dehydrogenase (LDH) Release Assay

The LDH assay kit (DIATEK diagnostics) was used for evaluating the LDH release following the manufacturer's protocol. The LDH amount released in the culture medium was used for monitoring the cell injury. In a 96-well plate, 1×10^5 cells/well were plated (24 h) and then treated with 135 µM H_2O_2 (24 h, 37°C) with or without pretreatment of CS NPs 1 h prior. Then, the culture medium (10 µL) was mixed with catalyst solution (100 µL, 30min, 37°C) and the absorbance was measured at 340 nm.

Estimation of reactive oxygen species (ROS)

The dichlorofluorescein diacetate (DCFDA) dye method was used for estimating ROS production via the Periyakaruppan et al.[21] protocol. A black bottom plate was used for growing the cells (5×10^4 cells/well) for 24 h, followed by pretreatment with CS NPs 1 h prior to 135 µM H_2O_2 exposure followed by incubating with DCFDA (100 µM) for 30 min after a mild PBS wash. The multimode reader equipped with a green fluorescent filter (Synergy LX, Agilent Technologies) was used for detecting the fluorescence intensity (excitation:488 nm and emission:525 nm).

Statistical Analysis

The statistical analysis was accomplished using GraphPad Prism and the data represented as mean ± SE. One-way analysis of variance (ANOVA) followed by Tukey's test were carried out to compute statistically significant probability values ($p < 0.05$).

Results and discussion

DLS

The particle size (PS), polydispersity index (PDI), and zeta potential (ZP) of the nanoparticles are commonly determined by the DLS technique[18]. The PS and PDI of CS NPs synthesized through ionic gelation were found to be 116.9 nm and 0.260, as shown in Fig. 1. The PS is crucial in cellular uptake, drug release, permeation, endocytosis across brain capillary endothelial cells, and biodistribution. Particularly, the NPs with < 200 nm are considered the optimum size for brain targeting[22]. While the PDI values greater than 0.7 generally indicate a broad size distribution, whereas values < 0.2 indicate monodisperse particles, indicating the homogeneity of colloidal dispersions[23]. The PDI value of CS NPs obtained in our study conforms well within the accepted

range, suggesting a homogeneous size distribution. The ZP of CS NPs is depicted in Fig.1B, which is +17.4 mV, indicative of moderate stability, signifying greater electrostatic repulsion between particles, which helps prevent their aggregation. The physicochemical characteristics like size, surface charge, stability, and homogeneity are essential for biodistribution, localization, cellular uptake, and retention of NPs, which are prerequisites for their therapeutic applications[24].

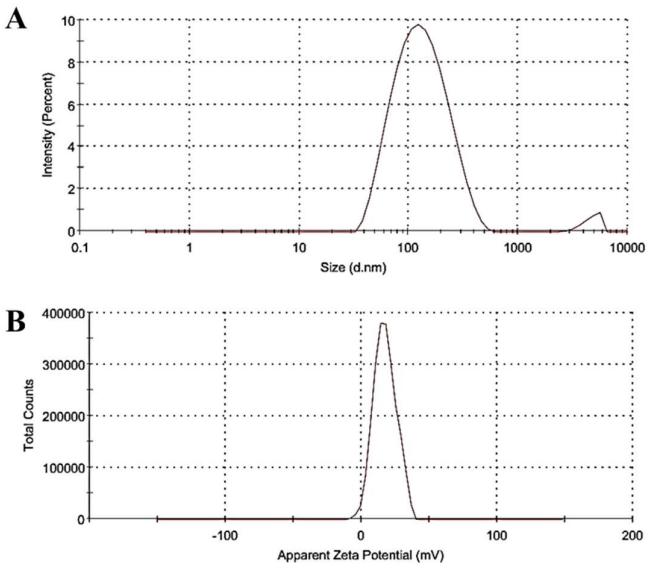

Fig. 1. (A) Particle size distribution and (B) zeta potential of CS NPs.

UV-Vis spectroscopy

The optical properties of CS NPs were analyzed by UV-Visible spectroscopy. As shown in Fig.2A, CS NPs showed the absorbance peak at 264 nm, that can be attributed to their pi-pi transition. Similar UV-Vis spectroscopic results were also obtained by the studies of Thamilarasan et al.[25].

XRD

As shown in Fig. 2B, the XRD pattern of the CS NPs reveals no sharp intensity peaks indicating its amorphous nature. The obtained results align with the investigations of Qi et al.[26] and Shirkhani et al.[27].

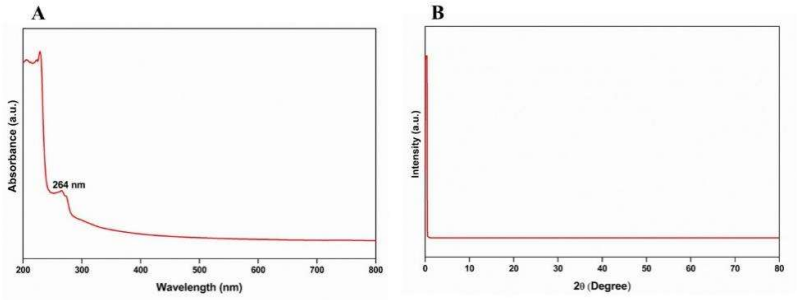

Fig. 2. (A) UV-Visible spectra, and (B) XRD pattern of CS NPs.

SEM and HR-TEM

The surface morphological features and size of the synthesized CS NPs were analyzed using SEM and HRTEM (Fig. 3). SEM images revealed that the CS NPs exhibited relatively smooth and spherical morphology. Further, the HRTEM analysis confirmed the nanoscale size of the synthesized CS NPs with well-defined spherical morphology. Moreover, the particles within 200 nm are suitable for neuroprotective applications with enhanced permeability, retention, and ability to cross the BBB[28] .

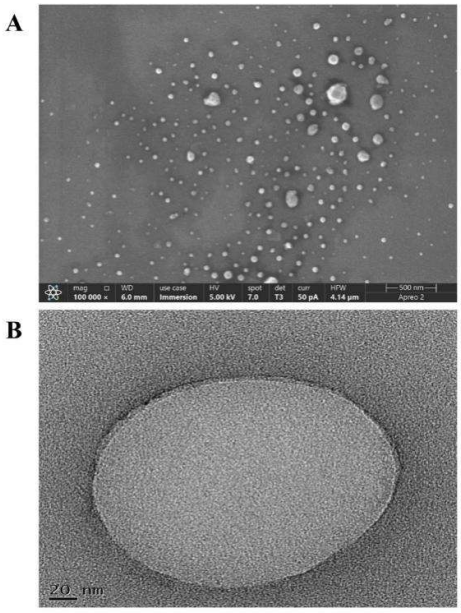

Fig. 3. (A) SEM, and (B) HR-TEM images of CS NPs.

MTT Assay

The cytocompatibility and neuroprotective potential of the CS NPs was evaluated by MTT assay. Initially, different doses of CS NPs were treated with SH-SY5Y cells to determine the non-toxic concentration. As apparent in Fig. 4, CS NPs up to 30 µg/mL showed relatively less cytotoxicity, maintaining over 80% viability. Thus, the non-toxic concentrations (7.5, 15 and 30 µg/mL) were selected to determine their neuroprotective potential upon H_2O_2-induced toxicity in SH-SY5Y cells. The IC_{50} value for H_2O_2 toxicity in SH-SY5Y cells was found to be 135 µM as per the previously published studies[29]. Therefore 135 µM was used to induce the neurotoxicity in all the further experiments. Subsequently, the pretreatment with non-toxic doses of CS NPs upon H_2O_2 toxicity showed prominent neuroprotective effect at 15 and 30 µg/mL. The phase-contrast microscopic images further supported the MTT findings. As depicted in Fig. 5, the control group shows a dense, healthy network of cells. Contrastingly, 135 µM H_2O_2 treated cells show a substantial decrease in cell density and disrupted morphology, indicating cytotoxicity. However, the cells pretreated with 15 and 30 µg/mL CS NPs upon H_2O_2 induction displayed improved cell morphology and density, suggesting that the CS NPs confer dose-dependent neuroprotection. The observed protective effect may be attributed to chitosan's antioxidant, anti-inflammatory, and membrane-stabilizing properties[30]. Similar to our results, Chen et al.[31] reported the neuroprotective activity of CS NPs against BV-2 cells induced with hydrogen peroxide (H_2O_2) toxicity.

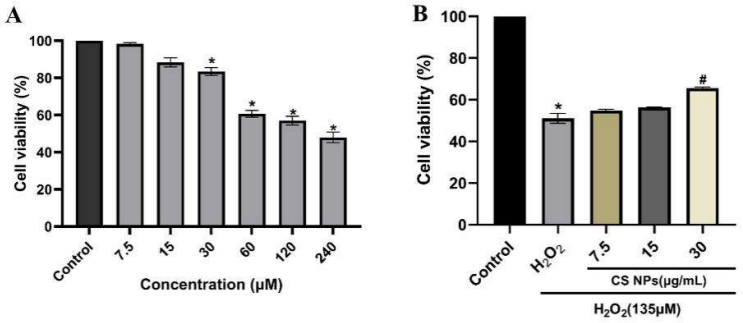

Fig. 4. (A) Effect of CS NPs on SH-SY5Y cell viability, and (B) Neuroprotective activity of CS NPs on H_2O_2 induced toxicity in SH-SY5Y cells. The data is expressed as mean ± SE (n = 3) and analyzed by one-way ANOVA followed by Tukey's test. *p < 0.05 indicates a significant difference compared to control and #p < 0.05 indicates a significant difference compared to the H_2O_2 treated group.

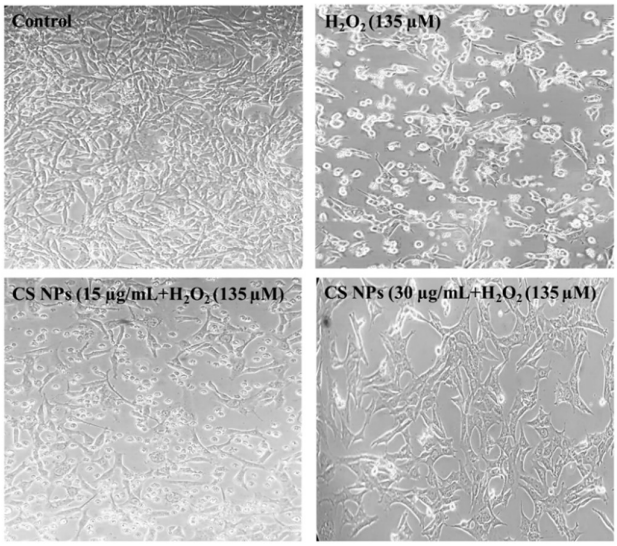

Fig. 5. Phase contrast microscopic images depicting the morphological changes in SH-SY5Y cells (Magnification:20X).

LDH release assay

The LDH assay was implemented to validate the neuroprotective ability of CS NPs on 135 µM H_2O_2 induced toxicity. LDH, a cytosolic enzyme, leaks into the extracellular space when the membrane integrity is compromised during stress conditions[32]. As shown in Fig. 6A, SH-SY5Y cells upon treatment with H_2O_2 notably elevating the LDH levels compared to the control. However, the LDH levels were markedly lowered with the pretreatment of CS NPs. Similarly, Cho et al.[33] showed a considerable reduction in LDH leakage upon CS NPs treatment in PC12 cells induced with acrolein toxicity.

Estimation of intracellular ROS generation

The effect of CS NPs upon intracellular ROS was evaluated to further validate its protective ability against H_2O_2 induced oxidative damage. Fig. 6B represents the increased ROS generation upon treatment with H_2O_2 over the control cells. Whereas, the pretreatment with CS NPs considerably reduced the ROS generation in a dose-dependent pattern indicating the CS NPs ability to scavenge ROS, which results in mitigation of OS.

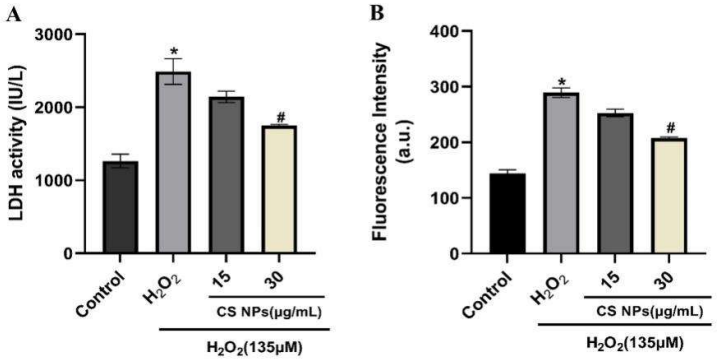

Fig. 6. Effect of CS NPs on A) LDH leakage and (B) ROS generation in H_2O_2 induced toxicity in SH-SY5Y cells. The data is expressed as mean ± SE (n = 3) and analyzed by one-way ANOVA followed by Tukey's test. *p < 0.05 indicates a significant difference compared to control and #p < 0.05 indicates a significant difference compared to the H_2O_2 treated group.

Conclusion

Our study highlights the neuroprotective potential of CS NPs against the SH-SY5Y cells induced with H_2O_2 toxicity. Upon H_2O_2 exposure, these cells exhibited decreased viability, increased LDH leakage, and increased ROS levels. However, pretreatment with CS NPs considerably lowered these effects and displayed improved cell viability, reduced LDH leakage and ROS levels, confirming the mitigation of the H_2O_2-induced toxicity. Hence, we can conclude that chitosan-based nanoparticles have the potential to reduce OS associated neuronal disorders.

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Conflict of Interest
The authors declare that they have no competing interests.

Bibliographic References:

1. Ricci C. Neurodegenerative disease: from molecular basis to therapy. International Journal of Molecular Sciences.2024;25(2):967.
2. Houldsworth A. Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants. Brain Communications. 2024; 6(1):fcad356.
3. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology. 2023;97(10):2499-2574.
4. Jacquemot N, Wersinger E, Brabet P, Cia D. Hydrogen Peroxide Affects the Electroretinogram of Isolated Perfused Rat Retina. Current Eye Research.2023;48(12):1179-1188.
5. Ostrowski TD, Hasser EM, Heesch CM, Kline DD. H_2O_2 induces delayed hyperexcitability in nucleus tractus solitarii neurons. Neuroscience. 2014;262:53–69. doi: 10.1016/j.neuroscience.2013.12.055.
6. Ohashi M, Hirano T, Watanabe K, Shoji H, Ohashi N, Baba H, Endo N, Kohno T. Hydrogen peroxide modulates neuronal excitability and membrane properties in ventral horn neurons of the rat spinal cord. Neuroscience. 2016;331:206–220.
7. Satchanska G, Davidova S, Petrov PD. Natural and synthetic polymers for biomedical and environmental applications. Polymers. 2024;16(8):1159.
8. Harugade A, Sherje AP, Pethe A. Chitosan: A review on properties, biological activities and recent progress in biomedical applications. Reactive and Functional Polymers. 2023;191:105634.
9. Zivari-Ghader T, Rashidi MR, Mehrali M. Biological macromolecule-based hydrogels with antibacterial and antioxidant activities for wound dressing: A review. International Journal of Biological Macromolecules. 2024:134578.
10. Tabassum Z, Girdhar M, Kumar A, Malik T, Mohan A. ZnO nanoparticles-reinforced chitosan–xanthan gum blend novel film with enhanced properties and degradability for application in food packaging. ACS Omega. 2023;8(34):31318-32.
11. Wijesekara T, Xu B. New insights into sources, bioavailability, health-promoting effects, and applications of chitin and chitosan. Journal of Agricultural and Food Chemistry. 2024;72(31):17138-52.
12. Alehosseini E, Shahiri Tabarestani H, Kharazmi MS, Jafari SM. Physicochemical, thermal, and morphological properties of chitosan nanoparticles produced by ionic gelation. Foods. 2022;11(23):3841.
13. Zhang Y, Zhao M, Cheng Q, Wang C, Li H, Han X, Fan Z, Su G, Pan D, Li Z. Research progress of adsorption and removal of heavy metals by chitosan and its derivatives: A review. Chemosphere. 2021;279:130927.
14. Wathoni N, Herdiana Y, Suhandi C, Mohammed AF, El-Rayyes A, Narsa AC. Chitosan/alginate-based nanoparticles for antibacterial agents delivery. International Journal of Nanomedicine. 2024:5021-44.
15. Jha R, Mayanovic RA. A review of the preparation, characterization, and applications of chitosan nanoparticles in nanomedicine. Nanomaterials. 2023;13(8):1302.
16. Zhu L, Li R, Jiao S, Wei J, Yan Y, Wang ZA, Li J, Du Y. Blood-brain barrier permeable chitosan oligosaccharides interfere with β-amyloid aggregation and alleviate β-amyloid protein mediated neurotoxicity and neuroinflammation in a dose-and degree of polymerization-dependent manner. Marine Drugs. 2020;18(10):488.
17. Manigandan V, Nataraj J, Karthik R, Manivasagam T, Saravanan R, Thenmozhi AJ, Essa MM, Guillemin GJ. Low molecular weight sulfated chitosan: neuroprotective effect on rotenone-induced in vitro Parkinson's disease. Neurotoxicity Research. 2019;35:505-15.
18. Ahlawat J, Deemer EM, Narayan M. Chitosan nanoparticles rescue rotenone-mediated cell death. Materials. 2019;12(7):1176.
19. Sultana S, Shwetha HJ, Jayakumar MN, Lakshmeesha TR, Raichur AM, Ravikiran T. Chitosan-assisted delivery of Decalepis hamiltonii extract for improved anticancer efficacy in HeLa cell lines. International Journal of Biological Macromolecules. 2025;319:145359.
20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods.1983;65(1-2):55-63.
21. Periyakaruppan A, Kumar F, Sarkar S, Sharma CS, Ramesh GT. Uranium induces oxidative stress in lung epithelial cells. Archives of Toxicology. 2007;81(6):389-95.
22. Rajamanickam G, Manju SL. Formulation and characterization of chitosan nanoparticles loaded with neuroprotective flavonoid from

Phyllanthus niruri Linn. Macromolecular Research. 2023;31(1):13-24.
23. Hoseini B, Jaafari MR, Golabpour A, Momtazi-Borojeni AA, Karimi M, Eslami S. Application of ensemble machine learning approach to assess the factors affecting size and polydispersity index of liposomal nanoparticles. Scientific Reports. 2023;13(1):18012.
24. Dadashi H, Vandghanooni S, Karamnejad-Faragheh S, Karimian-Shaddel A, Eskandani M, Jahanban-Esfahlan R. A rapid protocol for synthesis of chitosan nanoparticles with ideal physicochemical features. Heliyon. 2024;10(11).
25. Thamilarasan, V., Sethuraman, V., Gopinath, K., Balalakshmi, C., Govindarajan, M., Mothana, R.A., Siddiqui, N.A., Khaled, J.M. and Benelli, G., Single step fabrication of chitosan nanocrystals using Penaeus semisulcatus: potential as new insecticides, antimicrobials and plant growth promoters. Journal of Cluster Science. 2018;29(2):375-384.
26. Qi L, Xu Z, Jiang X, Hu C, Zou X. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrate research. 2004;339(16):2693-2700.
27. Shirkhani Z, Chehregani Rad A, Mohsenzadeh F. Improving Cd-phytoremediation ability of Datura stramonium L. by Chitosan and Chitosan nanoparticles. Biologia. 2021;76(8):2161-2171.
28. Xue Y, Wang N, Zeng Z, Huang J, Xiang Z, Guan YQ. Neuroprotective effect of chitosan nanoparticle gene delivery system grafted with acteoside (ACT) in Parkinson's disease models. Journal of Materials Science & Technology. 2020;43:197-207.
29. Mamatha MG, Ansari MA, Begum MY, Prasad BD, Al Fatease A, Hani U, Alomary MN, Sultana S, Punekar SM, MB N, Lakshmeesha TR, Ravikiran T. Green synthesis of cerium oxide nanoparticles, characterization, and their neuroprotective effect on hydrogen peroxide-induced oxidative injury in human neuroblastoma (SH-SY5Y) cell line. ACS Omega. 2024;9(2):2639-2649.
30. Abdel-Ghani MA, El-Bab AFF, Elhawari SF, Abdel-Raheem SM, El-Sharawy MES, Naiel MA. Chitosan-stabilized selenium nanoparticles stimulate performance, feed utilization, health biomarkers, and histological integrity in Litopenaeus vannamei. Aquaculture International. 2025; 33(6):516.
31. Chen B, Li J, Borgens RB. Neuroprotection by chitosan nanoparticles in oxidative stress-mediated injury. BMC Research Notes. 2018;11(1):49.
32. Zhou Y, Qi M, Yang M. Current status and future perspectives of lactate dehydrogenase detection and medical implications: a review. Biosensors. 2022;12(12):1145.
33. Cho Y, Shi R, Ben Borgens R. Chitosan nanoparticle-based neuronal membrane sealing and neuroprotection following acrolein-induced cell injury. Journal of Biological Engineering. 2010;4(1):2.