Mitigating Cytotoxicity: Plant-Based Chitosan Nanoparticles And Their Stimulatory Effects

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I. Introduction

Cytotoxicity, in the context of chemical-based nanoparticles, explores the potential adverse effects of these nanomaterials on various biological systems. Understanding cytotoxicity is crucial for assessing the safety of nanoparticles in diverse environments. This encompasses plant cytotoxicity, examining the impact on plant cells and ecosystems; animal cytotoxicity, delving into the consequences for animal tissues and organisms; and microbial cytotoxicity, exploring how nanoparticles may affect microorganisms and their functions. Investigating these aspects is vital for responsible development and application of nanotechnology, ensuring its compatibility with different biological systems.

Plant cytotoxicity

Plant cytotoxicity assessment is a pivotal aspect in the evaluation of chemical-based nanoparticles, emphasizing their potential impact on plant cellular systems. As these nanoparticles interact with plant cells, understanding the intricate mechanisms involved becomes crucial for comprehending their environmental implications. Investigating plant cytotoxicity provides insights into the potential risks posed to the plant kingdom, prompting a meticulous examination of how these nanoparticles may influence plant growth, development, and overall ecological balance. This facet of cytotoxicity assessment plays a key role in shaping responsible and sustainable practices in the utilization of chemical-based nanoparticles within environmental contexts.

Animal cytotoxicity

Animal cytotoxicity evaluation is paramount in the scrutiny of chemical-based nanoparticles, aiming to discern potential adverse effects on diverse biological systems. Understanding how these nanoparticles interact with animal cells is pivotal for assessing their safety and potential implications for human health. The investigation of animal cytotoxicity delves into the intricate mechanisms that may influence physiological processes, organ functions, and overall well-being. This comprehensive assessment is indispensable in establishing a thorough understanding of the safety profile of chemical-based nanoparticles, guiding responsible applications to mitigate potential risks to animal and human populations alike.

Microbial cytotoxicity

Microbial cytotoxicity assessment is a critical component in the thorough examination of chemicalbased nanoparticles, shedding light on their potential impact on microbial communities and ecological processes. Understanding how these nanoparticles interact with microorganisms is essential for evaluating their safety and environmental consequences. Microbial cytotoxicity investigations delve into the intricate mechanisms that may influence the viability, growth, and functions of diverse microbial species. This aspect of cytotoxicity assessment contributes to our understanding of the potential repercussions on crucial ecological balances, emphasizing the need for responsible and sustainable utilization of chemical-based nanoparticles in order to safeguard microbial ecosystems and their pivotal role in environmental dynamics.

Dye exclusion

II. Cell Cytotoxicity Methods

Dye exclusion methods stand as fundamental tools in the assessment of cell cytotoxicity, offering a straightforward yet effective means to discern viable cells from non-viable ones. This approach involves utilizing vital dyes that selectively penetrate healthy cells, while being excluded by damaged or compromised cell membranes. The principle lies in the ability of viable cells to maintain membrane integrity, preventing the

entry of these dyes. By quantifying dye exclusion, researchers can gauge the viability of cell populations and infer potential cytotoxic effects induced by various agents. The simplicity and reliability of dye exclusion methods make them invaluable in cytotoxicity studies, providing a quick and accessible means to evaluate the impact of substances on cellular viability.

Onion root tip assay

The onion root tip assay serves as a unique and accessible method for evaluating cell cytotoxicity, particularly in the context of plant cells. Leveraging the regenerative capacity of onion root tips, this assay provides a dynamic platform to assess the impact of substances on cell division and growth. By exposing onion roots to test agents and observing subsequent changes in root tip morphology, researchers can infer potential cytotoxic effects. This method offers a practical and cost-effective means to study the consequences of various compounds on plant cell viability and division. The onion root tip assay, with its simplicity and reproducibility, emerges as a valuable tool in plant cytotoxicity assessments, contributing to our understanding of the potential effects of substances on plant cellular processes.

Pollen germination assay

Pollen germination assays constitute a pivotal method in the evaluation of cell cytotoxicity, particularly within the realm of plant reproductive biology. This technique involves exposing pollen grains to test substances and observing their subsequent germination and growth. The assay provides valuable insights into the impact of various agents on the crucial reproductive process of plants. By assessing the ability of pollen grains to germinate and produce pollen tubes, researchers gain a nuanced understanding of potential cytotoxic effects on plant reproductive cells. The pollen germination assay emerges as a specialized yet effective tool, offering a direct and sensitive means to investigate the influence of substances on plant cell viability and reproductive success.

Pollen cell viability asaay

The assessment of pollen cell viability stands as a pivotal method in unraveling the impact of various substances on plant reproductive cells. The pollen cell viability assay involves subjecting pollen to test agents and subsequently evaluating the integrity and vitality of pollen cells. By employing vital dyes or other indicators, researchers can discern viable from non-viable pollen cells, providing valuable insights into the cytotoxic effects of tested substances. This method offers a direct and specific approach to understanding the potential influence of agents on the reproductive success of plants. The pollen cell viability assay emerges as a valuable tool, contributing to our comprehension of the effects of substances on plant reproductive cell health and overall reproductive processes.

III. Plant Based Chitosan Nanoparticles Cytotoxicity Testing

In the pursuit of safer alternatives to chemical-based nanoparticles, plant-based nanoparticles, such as chitosan nanoparticles, have garnered attention for their potential mitigating effects on cytotoxicity. This study focuses on evaluating the cytotoxicity of plant-based chitosan nanoparticles, aiming to assess their safety profile and explore their potential as a less harmful alternative. By subjecting these nanoparticles to rigorous cytotoxicity testing, we aim to elucidate their impact on various biological systems and compare the findings with those observed with chemical-based counterparts. This research seeks to contribute valuable insights into the potential of plant-based nanoparticles, specifically chitosan nanoparticles, as a promising avenue to overcome the adverse effects associated with traditional chemical-based nanoparticles.

Onion root tip :

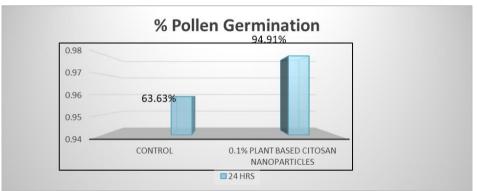
In the onion root tip assay, the evaluation of root length revealed notable differences between the control and the application of chitosan nanoparticles (0.1%). The control group exhibited a root length of 4.5 cm, while the introduction of chitosan nanoparticles led to a substantial increase, measuring 5.4 cm. This corresponds to a significant 22.22% rise in onion root length compared to the control on day 6. The observed augmentation in root length suggests a pronounced stimulatory impact of chitosan nanoparticles, emphasizing their role in promoting root development and suggesting a favourable aspect in mitigating adverse effects associated with chemical-based nanoparticles.

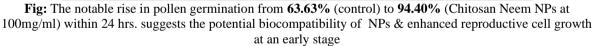


FIG: The above figure depicts different concertation of plant-based chitosan nanoparticles (0.01%,0.1%,1%, control, respectively). The left figure depicts microscopic image of mitosis in onion root tip with 0.1% of plant-based chitosan nanoparticles in 40X magnification.

Pollen germination

In the pollen germination assay, a significant increase in germination was observed, with chitosan neem nanoparticles at 100 mg/ml exhibiting a notable rise from 63.63% (control) to 94.40% within 24 hours. This substantial enhancement underscores the potential biocompatibility of the nanoparticles, indicating their favorable interaction with reproductive cells. The observed boost in pollen germination rates suggests an encouraging influence on early-stage reproductive cell growth. These results imply the potential of chitosan neem nanoparticles to enhance reproductive processes, highlighting their promise as a biocompatible alternative that may contribute to overcoming the adverse effects associated with chemical-based nanoparticles in the context of reproductive biology.





Pollen cell viability:

In the pollen cell viability assessment, a significant increase was observed, with pollen cell viability rising notably from 31.81% in the control group to 77.27% in the presence of chitosan nanoparticles at 100 mg/ml after 24 hours. This substantial enhancement suggests a potential lack of cytotoxicity associated with chitosan nanoparticles and indicates a possible beneficial impact on the survival of pollen cells under the experimental conditions. These results underscore the potential biocompatibility of chitosan nanoparticles, emphasizing their positive influence on the viability of reproductive cells and supporting their role as a promising alternative with reduced adverse effects compared to chemical-based nanoparticles.

Dye exclusion

In the yeast cell viability assay utilizing dye exclusion, a notable increase in yeast cell viability was observed. The control group exhibited a baseline viability of 95.9%, while the introduction of chitosan nanoparticles at 0.1% led to a substantial rise to 97.91% after 24 hours. This significant enhancement suggests a potential lack of cytotoxicity associated with chitosan nanoparticles and indicates a possible beneficial impact on the survival of yeast cells under the experimental conditions. These findings support the notion of chitosan nanoparticles as biocompatible, highlighting their potential positive influence on yeast cell viability and signalling a promising avenue for further exploration as an alternative with reduced adverse effects compared to chemical-based nanoparticles.

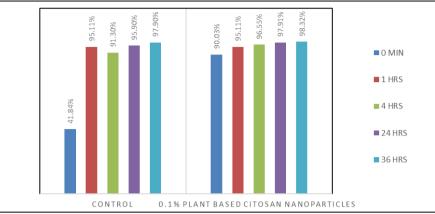


FIG – By dye exclusion method cell toxicity test of plant chitosan naoparticles by tryphan blue assay was performed on yeast cell where, there was significant increase in viability of cells in plant based chitosan nanoparticles with respect to each time interval and maximum 98.32 %viability was observed in plant based chitosan nanoparticles with 97.90% viability in control at 36 hours

IV. Conclusion:

The comprehensive findings from our research underscore the promising potential of chitosan nanoparticles as a biocompatible alternative to chemical-based nanoparticles. In the yeast cell viability assay, the substantial increase in viability from 95.9% to 97.91% suggests a lack of cytotoxicity and hints at a beneficial impact on yeast cell survival. Similarly, the pollen cell viability assessment demonstrates a remarkable rise from 31.81% to 77.27%, reinforcing the idea of chitosan nanoparticles as biocompatible and supportive of reproductive cell survival.

The pollen germination assay further highlights the positive influence of chitosan neem nanoparticles, with a significant increase from 63.63% to 94.40%, indicating their potential to enhance early-stage reproductive cell growth. In the onion root tip assay, the observed 22.22% rise in root length suggests a stimulatory effect of chitosan nanoparticles on onion root growth.

Collectively, these results support the notion that chitosan nanoparticles exhibit biocompatibility and beneficial effects on various cell types, making them a promising avenue for further exploration as an alternative with reduced adverse effects compared to chemical-based nanoparticles. This research contributes valuable insights into the potential applications of chitosan nanoparticles, particularly in addressing cytotoxicity concerns, and encourages further investigations for their safe and sustainable utilization.

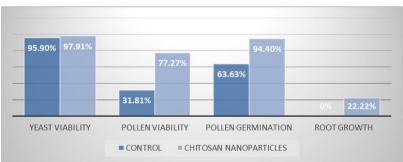


FIG: After Several Cell Toxicity Assays Performed, There Was Prominent Vialibility Observed In Pollen Germination With 94.40% Viability In Plant Based Chitosan Nanoparticles With Comparison To 63.63% In Control. The Pollen Viability Rose 77.27% In Plant Based Chitosan Nanoparticles Than 31.81% In Control. There Was Significant Increase In Root Length With 22.22% Growth, Also Notable Increase In Cell Viability From 95.90% To 97.91% Was Observed In Yeast Cells.

References:

- Ricardo Carneiro Borra(A) Mônica Andrade Lotufo(A) Sonia Maria Gagioti(A) Fabiana De Mesquita Barros(B) Priscila Maria Andrade(C), 2009 : A Simple Method To Measure Cell Viability In Proliferation And Cytotoxicity Assays, Braz Oral Res 2009;23(3):255-62.
- [2] H.A. Abdelgadir A, S.D. Johnson B, J. Van Staden A,*2011,Pollen Viability, Pollen Germination And Pollen Tube Growth In The Biofuel Seed Crop Jatropha Curcas (Euphorbiaceae);South African Journal Of Botany 79 (2012) 132–139.
- [3] Te-Hsiu Ma A,,, Zhidong Xu A, Chengen Xu A, Heike Mcconnell A, Eugenia Valtierra Rabago B, Gemma Adriana Arreola B, Hongen Zhang C. The Improved Allium/Vicia Root Tip Micronucleus Assay For Clastogenicity Of Environmental Pollutants Mutation Research 334 (1995) 185-195, 1994.

- [4] Terry Riss, Phd, 1 Andrew Niles, Ms,1 Rich Moravec, Bs,1 Natashia Karassina, Ms,1 And Jolanta Vidugiriene, Phd1, 2019; Cytotoxicity Assays: In Vitro Methods To Measure Dead Cells, Us- National Library Of Medicine: Https://Www.Ncbi.Nlm.Nih.Gov/Books/.
- [5] A. Hensten-Pettersen Niom, Scandinavian Institute Of Dental Materials, Haslum, Norway Comparison Of the Methods Available For Assessing Cytotoxicity International Endodontic Journal {1988} 21, 89-9.
- [6] Y. Faqir, J. Ma, Y. Chai, Chitosan In Modern Agriculture Production, Plant Soil Environ. 67 (12) (2021) 679–699.
- [7] R. Vani, S.A. Stanley, Studies On The Extraction Of Chitin And Chitosan From Different Aquatic Organisms, Adv. Biotech. 12 (12) (2013) 12–15.
- [8] A.M. Papineau, D.G. Hoover, D. Knorr, D.F. Farkas, Antimicrobial Effect Of Water- Soluble Chitosans With High Hydrostatic Pressure, Food Biotechnol. 5 (1) (1991) 45–57.
- M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial Properties Of Chitosan And Mode Of Action: A State Of The Art Review, Int. J. Food Microbiol. 144 (1) (2010) 51–63.
- [10] E.V. Campos, J.L. Oliveira, C.M. Da Silva, M. Pascoli, T. Pasquoto, R. Lima, P.C. Abhilash, F.L. Fernandes, Polymeric And Solid Lipid Nanoparticles For Sustained Release Of Carbendazim And Tebuconazole In Agricultural Applications, Sci. Rep. 5 (1) (2015) 1–4.
- [11] C.N. Hernandez-Tellez, F.J. Rodríguez-Cordova, E.C. Rosas-Burgos, M.O. Cortez- Rocha, A. Burgos-Hernandez, J. Lizardi-Mendoza, W. Torres-Arreola, A. Martínez- Higuera, M. Plascencia-Jatomea, Activity Of Chitosan–Lysozyme Nanoparticles On The Growth, Membrane Integrity, And B-1, 3-Glucanase Production By Aspergillus Parasiticus, 3 Biotech 7 (5) (2016) 1–3.
- [12] P.L. Kashyap, X. Xiang, P. Heiden, Chitosan Nanoparticle-Based Delivery Systems For Sustainable Agriculture, Int. J. Biol. Macromol. 77 (2015) 36–51.
- [13] A.F. Sahab, A.I. Waly, M.M. Sabbour, L.S. Nawar, Synthesis, Antifungal And Insecticidal Potential Of Chitosan (Cs)-G-Poly (Acrylic Acid) (Paa) Nanoparticles Against Some Seed Borne Fungi And Insects Of Soybean, Int. J. Chemtech Res. 8 (2) (2015) 589–598.
- [14] F.N. Maluin, M.Z. Hussein, Chitosan-Based Agronanochemicals As A Sustainable Alternative In Crop Protection, Molecules 25 (7) (2020) 1611.
- [15] P.D. Van, B.D. Du, T.H. Van, N.Q. Hien, Preparation And Foliar Application Of Oligochitosan-Nanosilica On The Enhancement Of Soybean Seed Yield, Int. J. Environ. Agric. Biotechnol. 2 (1) (2017), 238688.
- [16] R. Li, J. He, H. Xie, W. Wang, S.K. Bose, Y. Sun, J. Hu, H. Yin, Effects Of Chitosan Nanoparticles On Seed Germination And Seedling Growth Of Wheat (Triticum Aestivum L.), Int. J. Biol. Macromol. 126 (2019) 91–100.
- [17] A.B. Muley, P.R. Shingote, A.P. Patil, S.G. Dalvi, P. Suprasanna, Gamma Radiation Degradation Of Chitosan For Application In Growth Promotion And Induction Of Stress Tolerance In Potato (Solanum Tuberosum L.), Carbohydr. Polym. 210 (2019) 289–301.
- [18] B.R. Rizeq, N.N. Younes, K. Rasool, G.K. Nasrallah, Synthesis, Bioapplications, And Toxicity Evaluation Of Chitosan-Based Nanoparticles, Int. J. Mol. Sci. 16 (2018) 101–112.
- [19] S.F. Hosseini, M. Rezaei, M. Zandi, F. Farahmandghavi, Fabrication Of Bio- Nanocomposite Films Based On Fish Gelatin Reinforced With Chitosan Nanoparticles, Food Hydrocolloids 44 (2015) 172–182.
- [20] N.K. Bari, M. Fazil, M.Q. Hassan, M.R. Haider, B. Gaba, J.K. Narang, S. Baboota, J. Ali, Brain Delivery Of Buspirone Hydrochloride Chitosan Nanoparticles For The Treatment Of General Anxiety Disorder, Int. J. Biol. Macromol. 81 (2015) 49–59.
- [21] C. Xu, Y. Zeng, X. Zheng, R.T. Wang, Surface-Fluorinated And Ph-Sensitive Carboxymethyl Chitosan Nanoparticles To Overcome Biological Barriers For Improved Drug Delivery In Vivo, Carbohydr. Polym. 208 (2019) 59–69.
- [22] M. Sathiyabama, R. Parthasarathy, Biological Preparation Of Chitosan Nanoparticles And Its In Vitro Antifungal Efficacy Against Some Phytopathogenic Fungi, Carbohydr. Polym. 151 (2016) 321–325.
- [23] A. Anitha, V.G. Deepagan, V.V. Divya, R.D. Menon, S.V. Nair, R. Jayakumar, Preparation, Characterization, In Vitro Drug Release And Biological Studies Of Curcumin Loaded Dextran Sulphate–Chitosan Nanoparticles, Carbohydr. Polym. 84 (3) (2011) 1158– 1164.
- [24] S.S. Mukhopadhyay, Nanotechnology In Agriculture: Prospects And Constraints, Nanotechnol. Sci. Appl. 7 (2014) 63–71.
- [25] L. Orzali, B. Corsi, C. Forni, L. Riccioni, Chitosan In Agriculture: A New Challenge For Managing Plant Diseases, In: E. Shalaby (Ed.), Biological Activities And Application Of Marine Polysaccharides, Intech, 2017, Pp. 17–36.
- [26] F. Behboudi, T.Z. Sarvestani, M.Z. Kassaee, S.A.M.M. Sanavi, A. Sorooshzadeh, S.B. Ahmadi, Evaluation Of Chitosan Nanoparticles Effects On Yield And Yield Components Of Barley (Hordeum Vulgare L.) Under Late Season Drought Stress, J. Water Environ. Nanotechnol. 3 (2018) 22–39.
- [27] F. Behboudi, Z. Tahmasebi-Sarvestani, M.Z. Kassaee, S.A.M. Modarres-Sanavy, Sorooshzadeh, A. Mokhtassi-Bidgoli, Evaluation Of Chitosan Nanoparticles Effects With Two Application Methods On Wheat Under Drought Stress, J. Plant Nutr. 42 (13) (2019) 1439.
- [28] H.M. Abdel-Aziz, M.N. Hasaneen, A.M. Omer, Nano Chitosan-Npk Fertilizer Enhances The Growth And Productivity Of Wheat Plants Grown In Sandy Soil, Spanish J. Agric. Res. 14 (2016), 0902.
- [29] R.C. Choudhary, R.V. Kumaraswamy, S. Kumari, A. Pal, R. Raliya, P. Biswas, V. Saharan, Synthesis, Characterization, And Application Of Chitosan Nanomaterials Loaded With Zinc And Copper For Plant Growth And Protection, In: R. Prasad, M. Kumar, V. Kumar (Eds.), Nanotechnology, Springer, Singapore, 2017a, Pp. 227–247.
- [30] R.C. Choudhary, R.V. Kumaraswamy, S. Kumari, S.S. Sharma, A. Pal, R. Raliya, P. Biswas, V. Saharan, Cu-Chitosan Nanoparticle Boost Defence Responses And Plant Growth In Maize (Zea Mays L.), Sci. Rep. 7 (9754) (2017b) 1–11.
- [31] C.N. Siddaiah, K.V.H. Prasanth, N.R. Satyanarayana, Et Al., Chitosan Nanoparticles Having Higher Degree Of Acetylation Induce Resistance Against Pearl Millet Downy Mildew Through Nitric Oxide Generation, Sci. Rep. 8 (2018) 2485.
- [32] J.T. Buchman, W.H. Elmer, C. Ma, K.M. Landy, J.C. White, C.L. Haynes, Chitosan- Coated Mesoporous Silica Nanoparticle Treatment Of Citrullus Lanatus (Watermelon): Enhanced Fungal Disease Suppression And Modulated Expression Of Stress-Related Genes, Acs Sustain. Chem. Eng. 7 (2019) 19649–19659.
- [33] N. Chookhongkha, T. Sopondilok, S. Photchanachai, Effect Of Chitosan And Chitosan Nanoparticles On Fungal Growth And Chilli Seed Quality, Acta Hortic. 973 (2013) 231–237.
- [34] S. Chandra, N. Chakraborty, A. Dasgupta, J. Sarkar, K. Panda, K. Acharya, Chitosan Nanoparticles: A Positive Modulator Of Innate Immune Responses In Plants, Sci. Rep. 5 (2015), 15195.
- [35] S. Chandra, N. Chakraborty, K. Panda, K. Acharya, Chitosan-Induced Immunity In Camellia Sinensis (L.) O. Kuntze Against Blister Blight Disease Is Mediated By Nitric- Oxide, Plant Physiol. Biochem. 115 (2017) 298–307.
- [36] P. Khati, P. Chaudhary, S. Gangola, P. Bhatt, A. Sharma, Nanochitosan Supports Growth Of Zea Mays And Also Maintains Soil Health Following Growth, 3 Biotech 7 (1) (2017) 81.

- [37] S. Shah, M.S. Hashmi, Chitosan-Aloe Vera Gel Coating Delays Postharvest Decay Of Mango Fruit, Hortic. Environ. Biotechnol. 61 (2020) 279–289.
- [38] V. Saharan, G. Sharma, M. Yadav, M.K. Choudhary, S. Sharma, A. Pal, R. Raliya, P. Biswas, Synthesis And In Vitro Antifungal Efficacy Of Cu-Chitosan Nanoparticles Against Pathogenic Fungi Of Tomato, Int. J. Biol. Macromol. 75 (2015) 346–353.
- [39] V. Saharan, R. Kumaraswamy, R.C. Choudhary, S. Kumari, A. Pal, R. Raliya, P. Biswas, Cu-Chitosan Nanoparticle Mediated Sustainable Approach To Enhance Seedling Growth In Maize By Mobilizing Reserved Food, J. Agric. Food Chem. 64 (2016) 6148–6155.
- [40] H. Hernandez-Hernandez, S. Gonzalez-Morales, A. Benavides-Mendoza, H. Ortega- Ortiz, G. Cadenas-Pliego, A. Juarez-Maldonado, Effects Of Chitosan-Pva And Cu Nanoparticles On The Growth And Antioxidant Capacity Of Tomato Under Saline Stress, Molecules 23 (1) (2018) 178.
- [41] R. Li, J. He, H. Xie, W. Wang, S.K. Bose, Y. Sun, J. Hu, H. Yin, Effects Of Chitosan Nanoparticles On Seed Germination And Seedling Growth Of Wheat (Triticum Aestivum L.), Int. J. Biol. Macro