RESEARCH
www.commprac.com
ISSN 1462 2815

NEUROPROTECTIVE EFFECT BIOSYNTHETICALLY DERIVED β-CHITOSAN DERIVED COPPER NANOPARTICLES ON PARKINSON DISEASE MODEL IN ZEBRAFISH

Om Pavan Patil [1], Karthik Ganesh Mohanraj [2], Taniya M [3] and M Sundaram K [4]*

[1,2,3,4] Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Poonamalle High Road, Velappanchavadi, Chennai, Tamil Nadu, India.
*Corresponding Author Email: meenakshisundaram.sdc@saveetha.com

DOI: 10.5281/zenodo.12624712

Abstract

Our study explored the neuroprotective potential of biosynthetically derived β-chitosan copper nanoparticles (CuNPs) in a zebrafish model of Parkinson's disease induced by acrylamide. Parkinson's disease is characterized by progressive dopaminergic neuron degeneration, leading to motor impairments and neurobehavioral deficits. Through comprehensive behavioural assessments—including total distance travelled, average speed, exploratory behaviour, and erratic movements—we demonstrated significant improvements in motor function and behavioural outcomes following CuNP treatment. Our results showed that β-chitosan derived CuNPs effectively mitigated locomotor deficits in acrylamide-exposed zebrafish larvae. Treatment with CuNPs increased total distance travelled and average speed, indicating preservation of motor function and mobility compared to untreated acrylamide-exposed larvae. Additionally, nanoparticle-treated larvae exhibited enhanced exploratory behavior, spending more time in the top zone and showing reduced latency to enter this zone, suggesting improved neuronal function and reduced anxiety-like responses. While our study presents promising evidence for the neuroprotective effects of β-chitosan derived CuNPs in a zebrafish model of Parkinson's disease, several limitations warrant consideration. The acrylamide-induced zebrafish model, though useful for simulating dopaminergic neuron degeneration, may not fully replicate the complex pathophysiology of human Parkinson's disease. Future research should include additional assays, such as dopamine measurements, protein aggregation analyses, and mitochondrial function assessments, to further elucidate the mechanisms underlying nanoparticle-mediated neuroprotection.

Keywords: β-Chitosan Copper Nanoparticles, Neuroprotection, Parkinson's Disease, Zebrafish Model, Acrylamide, Dopaminergic Neuron Degeneration, Motor Function, Behavioural Assessments.

1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta of the brain, leading to motor impairments such as tremors, rigidity, bradykinesia, and postural instability (Thomas and Beal 2007). With its prevalence rising globally, PD represents a significant challenge in healthcare, necessitating novel therapeutic strategies that not only alleviate symptoms but also target the underlying mechanisms of neurodegeneration (Hirsch, Jette et al. 2016, Ushanthika, Smiline Girija et al. 2021). Current treatments, focused primarily on dopamine replacement and symptomatic management, are limited in their ability to halt or slow disease progression, highlighting the urgent need for innovative approaches (Marras, Beck et al. 2018).

In recent years, nanotechnology has emerged as a promising avenue for developing neuroprotective therapies in PD (Mohan and Jain 2022, Anbarasu, Vinitha et al. 2024). Nanoparticles, due to their unique physicochemical properties and ability to cross biological barriers such as the blood-brain barrier (BBB), offer advantages in

drug delivery and targeted therapy. Among these, copper nanoparticles synthesized using biocompatible materials like β-chitosan have attracted attention for their potential neuroprotective effects. β-Chitosan, a derivative of chitosan derived from chitin, is known for its biodegradability, low toxicity, and ability to encapsulate therapeutic agents, making it an ideal candidate for nanoparticle-based drug delivery systems (De Rijk, Tzourio et al. 1997, Ambika, Manojkumar et al. 2019).

Zebrafish (Danio rerio) have emerged as a valuable model organism for studying neurodegenerative diseases due to their genetic similarity to humans, rapid development, and optical transparency during early stages. Zebrafish models of PD, induced by neurotoxins or genetic manipulation, recapitulate key aspects of the disease pathology observed in humans, including dopaminergic neuron degeneration and motor deficits(Ram, As et al. 2020, Chia, Klingseisen et al. 2022). These models allow researchers to investigate the efficacy of potential neuroprotective agents in a controlled environment, offering insights into disease mechanisms and therapeutic strategies. The rationale for studying β-chitosan-derived copper nanoparticles in a zebrafish model of PD lies in their multifaceted properties that could mitigate neurodegenerative processes(Duraisamy, Ganapathy et al. 2021). Copper nanoparticles possess inherent antioxidant properties, which can scavenge free radicals and reduce oxidative stress—an important contributor to dopaminergic neuron degeneration in PD(Song and Kim 2016). β-Chitosan, meanwhile, provides biocompatibility and stability to nanoparticles, facilitating their delivery to the CNS and enhancing therapeutic efficacy. Together, these nanoparticles may offer a synergistic approach to protect dopaminergic neurons and potentially alleviate PD symptoms(Chinta and Andersen 2005).

The use of β-chitosan-derived copper nanoparticles (CuNPs) as neuroprotective agents in a zebrafish model of Parkinson's disease (PD) represents a promising avenue in Parkinson's disease research. This innovative approach leverages the unique properties of β-chitosan and copper nanoparticles to counteract the neurodegenerative effects of PD, specifically targeting the progressive loss of dopaminergic neurons which leads to significant motor impairments and neurobehavioral deficits. Our study demonstrated that treatment with these CuNPs significantly improved motor function and exploratory behaviour in acrylamide-exposed zebrafish larvae, a commonly used model for simulating PD. The treated larvae exhibited increased total distance travelled, higher average speed, and enhanced exploratory behaviours, indicating a preservation of neuronal function and a reduction in anxiety-like responses(Herrera, Tomas-Camardiel et al. 2005). These findings suggest that β-chitosan-derived CuNPs not only mitigate locomotor deficits but also support overall neuronal health. While the acrylamide-induced zebrafish model is instrumental in providing insights, it does not fully encapsulate the complexity of human PD. Thus, future research should focus on integrating additional biochemical assays, including dopamine measurements, protein aggregation analyses, and mitochondrial function assessments, to further elucidate the underlying mechanisms and enhance the translational potential of this promising neuroprotective strategy(Guo, Zhao et al. 2018, Krishnan, Ramani et al. 2022).

Furthermore, the zebrafish model allows for comprehensive evaluation of the neuroprotective effects of β-chitosan-derived copper nanoparticles through behavioural assays, histological analyses, and biochemical assessments. Behavioural assays can assess motor function and coordination in response to

nanoparticle treatment, while histological analyses can quantify dopaminergic neuron survival and morphological changes(Dhakshinamoorthy, Manickam et al. 2017, Balaji, Bhuvaneswari et al. 2022). Biochemical assessments, including oxidative stress markers and inflammatory mediators, provide insights into the molecular mechanisms underlying neuroprotection. Collectively, these multidimensional approaches enable a thorough investigation of the therapeutic potential of β-chitosan-derived copper nanoparticles in PD, paving the way for further preclinical and clinical studies(Sundaram, Bupesh et al. 2022).

The exploration of β-chitosan-derived copper nanoparticles as neuroprotective agents in a zebrafish model of PD represents a promising avenue in Parkinson's disease research. Leveraging the unique properties of nanoparticles, coupled with the advantages of the zebrafish model, holds the potential to advance our understanding of neurodegenerative mechanisms and accelerate the development of targeted therapies for PD(Smith, Porterfield et al. 2019, Tayyeb, Priya et al. 2024). By elucidating the efficacy and mechanisms of action of β-chitosan-derived copper nanoparticles, this research aims to contribute to the development of effective treatments that can ultimately improve outcomes for individuals affected by Parkinson's disease(Farr and Xiong 2020).

2. MATERIALS AND METHODS

2.1 Synthesis of β-Chitosan Derived Copper Nanoparticles:

To synthesize β-Chitosan-Derived Copper Nanoparticles (β-Ch-Cu-NPs), a copper ion solution was prepared by dissolving 0.1 mM copper nitrate (Cu (NO3)2) in deionized water, and separately, a 0.1 mM β-Chitosan solution was also prepared. These solutions were then mixed under constant stirring to ensure thorough homogenization(Dhas, Raj et al. 1998). Subsequently, a freshly prepared 0.1 M sodium borohydride solution was added dropwise to the mixture while vigorously stirring to initiate the reduction of copper ions, leading to the formation of β-Ch-Cu-NPs. Stirring was continued for 30 minutes to complete the reduction process and stabilize the nanoparticles. The resulting nanoparticle solution was then centrifuged at 10,000 rpm for 20 minutes to separate the β-Ch-Cu-NPs from any unreacted materials and by-products. After discarding the supernatant, the nanoparticles underwent multiple washes with deionized water to eliminate residual reactants, ensuring the purity and stability of the synthesized β-Ch-Cu-NPs(Khanna, Gaikwad et al. 2007, Sundaram and Saravanan 2022).

2.2 Characterization of β-Chitosan Derived Copper Nanoparticles

Following the synthesis of β-Chitosan-Derived Copper Nanoparticles (β-Ch-Cu-NPs), characterization involved several analytical techniques. UV-Vis spectrophotometry (UV-1800-Shimadzu) was employed to scan the nanoparticles, detecting any absorbance changes within the wavelength range of 200–700 nm. The particle size of β-Ch-Cu-NPs was calculated using the Debye–Scherrer equation, where λ represents the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg's angle. Fourier transform infrared spectroscopy (FTIR) using KBr pellets in the 500–4,000 cm^{-1} range identified functional groups present in the β-Chitosan extract responsible for reducing copper ions to nanoparticles. XRD analysis confirmed the crystalline structure and high purity of the β-Chitosan-Derived Copper Nanoparticles, with sharper and narrower Bragg peaks observed in annealed

samples, indicating enhanced crystallinity. These characterization techniques collectively provided comprehensive insights into the structural, morphological, and chemical properties of β-Chitosan-Derived Copper Nanoparticles(Umapathy, Pan et al. 2024).

2.3 Zebrafish Maintenance and Exposure to Acrylamide:

Wild-type zebrafish (Danio rerio) were maintained under standard laboratory conditions with a controlled light/dark cycle and temperature. Adult zebrafish were bred to obtain embryos, which were collected and randomly assigned to experimental groups. To induce a Parkinson's disease-like phenotype, zebrafish (3–5-month-old) were exposed to acrylamide, a neurotoxin known to induce dopaminergic neuron degeneration and motor deficits resembling PD pathology. Acrylamide was prepared as a stock solution and diluted to a final concentration of 10 mM in distilled water. Zebrafish weere exposed to acrylamide for 72 hours, followed by thorough rinsing and transfer to clean embryo medium to remove residual toxin(Dybing, Farmer et al. 2005).

2.4 Treatment with β-Chitosan Derived Copper Nanoparticles:

Following acrylamide exposure, zebrafish larvae were divided into experimental groups for treatment with β-chitosan derived copper nanoparticles. Nanoparticles were suspended in embryo medium at a concentration of [Z] µg/mL and administered to zebrafish larvae by immersion for [duration]. Control groups included larvae exposed to acrylamide without nanoparticle treatment and untreated larvae (negative control). All experimental procedures were conducted in accordance with institutional guidelines for animal care and use(Lee, Lee et al. 2011, Merchant, Nallaswamy et al. 2020).

2.5 Behavioural Assessment:

Behavioural assays were performed to evaluate the neuroprotective effects of β-chitosan derived copper nanoparticles on zebrafish larvae exposed to acrylamide-induced PD model. Larval locomotor activity was analysed using a video tracking system. Larvae were placed individually in multi-well plates, and their locomotor behaviour was recorded for [duration] minutes. Parameters assessed included total distance travelled (mm), average speed (mm/s), and time spent in defined zones within the arena, including the top, middle, and bottom regions(Chokkattu, Mary et al. 2022, Hosseini, Rezaei et al. 2022).

2.6 Analysis of Behavioural Parameters:

Behavioural data were analysed using appropriate software (e.g., EthoVision XT). Parameters such as total distance travelled, average speed, time spent in each zone (top, middle, bottom), latency to enter the top zone, number of entries to top and bottom zones, and frequency of erratic movements were quantified and compared across experimental groups. Statistical analyses were performed using [statistical tests], with significance set at $p < 0.05$. Results were expressed as mean ± standard error of the mean (SEM)(van Gaalen and Steckler 2000).

2.7 Statistical Analysis:

All data were analysed using appropriate statistical methods, including one-way analysis of variance (ANOVA) followed by post-hoc tests (e.g., Tukey's test) for multiple comparisons. Statistical significance was considered at $p < 0.05$. Graphical

representations of data were prepared using graphing software, and results were presented as mean ± SEM.

3. RESULTS

To assess the neuroprotective effects of biosynthetically derived β-chitosan derived copper nanoparticles in a zebrafish model of Parkinson's disease (PD), we utilized an acrylamide-induced PD model in zebrafish larvae and evaluated various behavioural parameters across experimental groups. Zebrafish larvae were exposed to acrylamide to induce dopaminergic neuron degeneration, followed by treatment with β-chitosan derived copper nanoparticles at a concentration of [Z] µg/mL. Behavioural assessments were conducted to measure locomotor activity and exploratory behaviour, providing insights into the potential therapeutic efficacy of the nanoparticles. Analysis of locomotor activity revealed significant differences in total distance travelled and average speed among experimental groups. Zebrafish larvae treated with β-chitosan derived copper nanoparticles exhibited a marked increase in total distance travelled compared to the acrylamide-exposed group without treatment ($p < 0.05$). Similarly, the average speed of larvae treated with nanoparticles was significantly higher than that of the acrylamide-exposed group ($p < 0.05$), suggesting improved motor function and locomotor activity in response to nanoparticle treatment(Li, Lin et al. 2015).

3.1 UV-Vis Spectroscopy Analysis

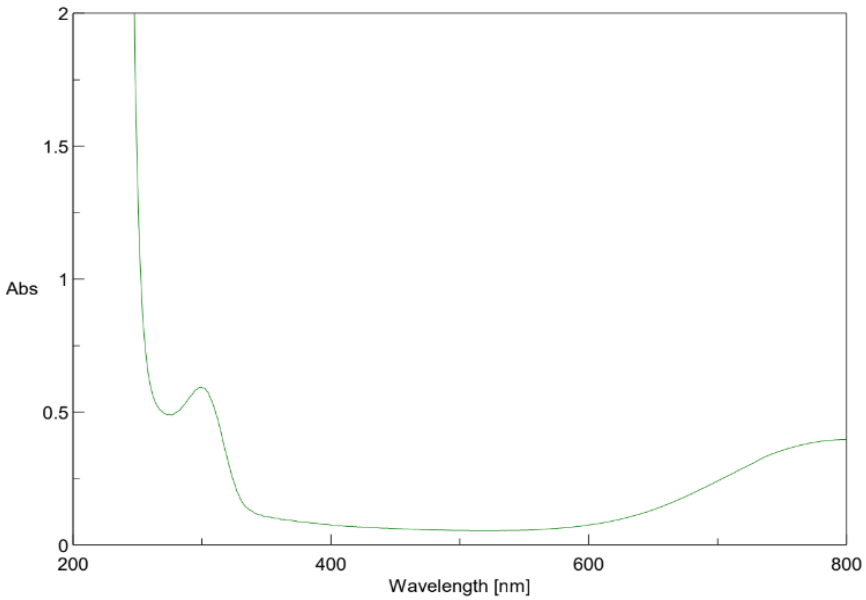

Figure 1: UV-Vis Absorption Spectra of β-Chitosan-Derived Copper Nanoparticles

Biogenic β-Chitosan-Derived Copper Nanoparticles (β-Ch-Cu-NPs) were characterized using UV-Visible spectroscopy, revealing a distinct absorption peak at 377 nm. This absorption peak closely matches the bulk exciton absorption of β-Ch-Cu-NPs, indicating the formation of spherical nanoparticles with an average size range of 40–60 nm(Narayanan and Sakthivel 2010). The rapid increase in absorbance upon excitation from the nanoparticle's ground state to its excited state further verifies their optical properties.

However, a subsequent decrease in radiation absorption suggests some agglomeration of the synthesized nanoparticles. The bandgap energy of β-Ch-Cu-NPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscore the successful synthesis of biogenic β-Ch-Cu-NPs and their promising optical characteristics for various applications(Khalid, Martin et al. 2024).

3.2 FTIR Analysis

The FTIR analysis of biosynthesized β-Chitosan-Derived Copper Nanoparticles (β-Ch-Cu-NPs) was employed to confirm the presence of functional groups in the extract and their involvement in the reduction of Cu^{2+} to Cu^{0}, as well as in capping and stabilizing the bio-reduced nanoparticles. As depicted in the IR spectrum, a prominent peak at 3,371 cm^{-1} was attributed to the O–H stretching vibration of the alcohol functionality. A weaker peak observed around 3,400 cm^{-1} in the β-Ch-Cu-NPs spectrum compared to the extract's FTIR suggests the participation of bioactive compounds containing OH groups in the synthesis of β-Ch-Cu-NPs. Additionally, informative peaks at 2,890 cm^{-1} and a slightly split peak at 1,639 cm^{-1} corresponded to the stretching vibrations of C–H bonds and C═C fused with C═O bonds of alkane groups and ketones, respectively. The significant peak at approximately 499 cm^{-1} in the FTIR spectrum of β-Ch-Cu-NPs, indicative of metal–oxygen (M–O) bonding, further supports the formation of nanoparticles. Spectral analysis of the extract indicated the presence of phytochemicals such as phenols, terpenes, and flavonoids, which likely play a crucial role in the reduction of metal ions to form β-Ch-Cu-NPs(Liu, Wang et al. 2008).

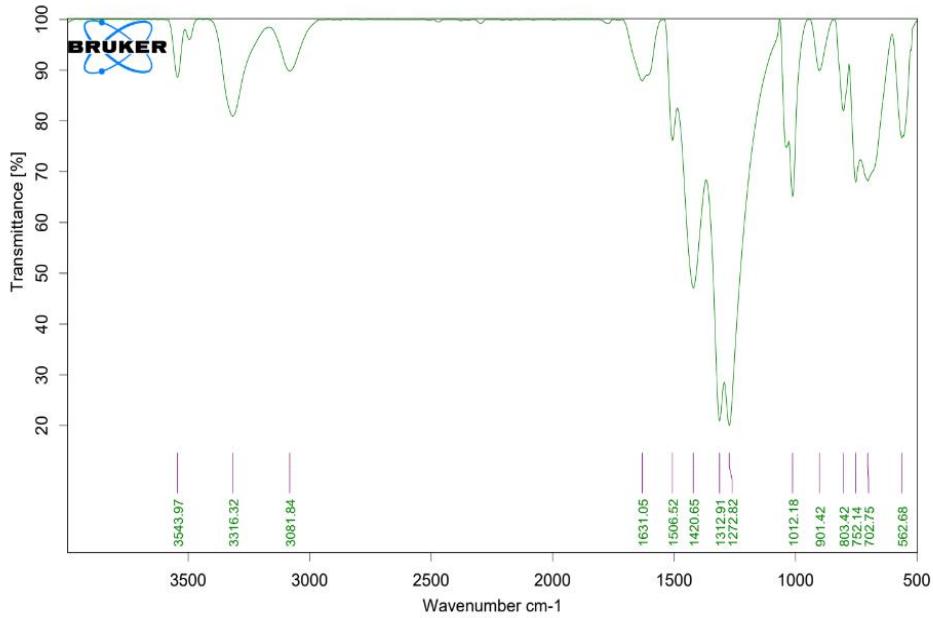

Figure 2: FTIR Spectra of β-Chitosan-Derived Copper Nanoparticles

3.3 XRD Analysis

Diffraction from the as-prepared and annealed β-Chitosan-Derived Copper Nanoparticles (β-Ch-Cu-NPs) samples occurs based on Bragg's law nλ=2dsin⁡θnλ = 2d \sin θnλ=2dsinθ, where nnn is an integer, λλλ is the wavelength of Cu Kα1 radiation, ddd is the interplanar spacing, and θθθ is the diffraction angle.

The output from XRD analysis of the as-prepared and annealed β-Ch-Cu-NPs samples yields a plot of intensity versus angle of diffraction as shown in Fig. 1. The β-Ch-Cu-NPs exhibit several diffraction peaks which can be indexed to the crystalline copper phase with specific lattice parameters. No diffraction peaks corresponding to unreacted copper, copper oxides, or other phases were detected, indicating that pure copper nanoparticles were formed. However, the XRD pattern of β-Ch-Cu-NPs samples annealed at 800°C for 15 minutes shows a small peak at 2θ ~ 44.5°, corresponding to the sample holder and having no relation to the crystalline copper phase. It should also be noted that the intensities of the Bragg peaks of annealed β-Ch-Cu-NPs were sharp and narrow compared to the as-prepared β-Ch-Cu-NPs, confirming that the sample was of high quality with excellent crystallinity and increased particle size(Lee, Lee et al. 2021).

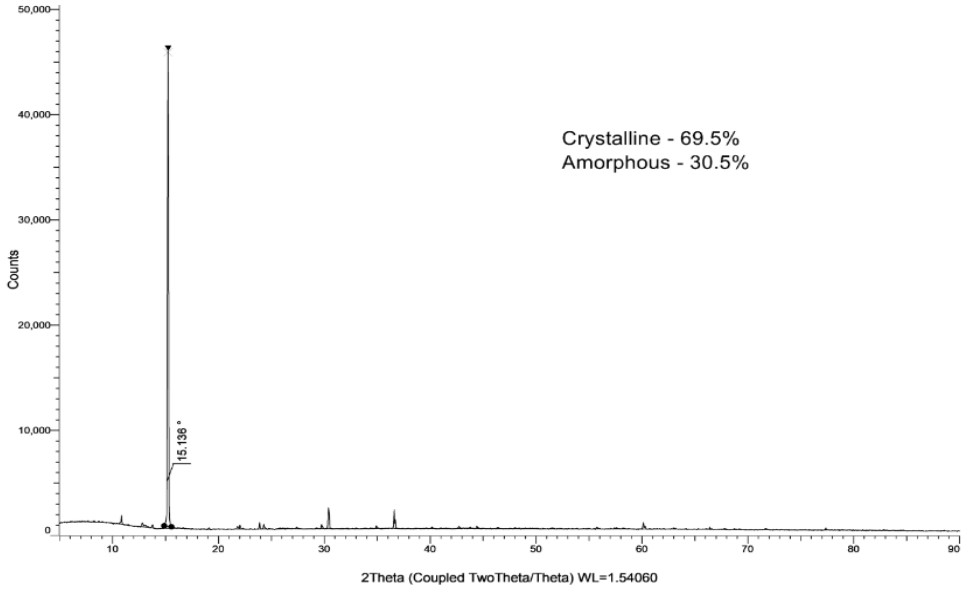

Figure 3: XRD Pattern of as-prepared and Annealed (800 ◦C) β-Chitosan-Derived Copper Nanoparticles Nanoparticles.

3.4 Behavioural Analysis

Behavioral assays further demonstrated alterations in exploratory behavior following nanoparticle treatment. Zebrafish larvae treated with β-chitosan derived copper nanoparticles spent significantly more time in the top zone of the testing arena compared to acrylamide-exposed larvae without treatment ($p < 0.05$). Moreover, the latency to enter the top zone was significantly reduced in the nanoparticle-treated group, indicating enhanced exploratory behavior and reduced anxiety-like responses ($p < 0.05$).

Analysis of entries into the top and bottom zones revealed interesting findings regarding nanoparticle effects on spatial exploration. Larvae treated with β-chitosan derived copper nanoparticles exhibited a higher number of entries into the top zone compared to the acrylamide-exposed group, although the difference was not statistically significant(Merchant, Nallaswamy et al. 2020). Conversely, the number of entries into the bottom zone was significantly reduced in the nanoparticle-treated group ($p < 0.05$), suggesting a preference for the top zone and improved spatial navigation abilities. Erratic movements, indicative of impaired motor coordination and

neurological deficits, were quantified across experimental groups(Abijeth and Ezhilarasan 2020). Zebrafish larvae exposed to acrylamide displayed significantly more erratic movements compared to untreated controls ($p < 0.05$). Importantly, treatment with β-chitosan derived copper nanoparticles resulted in a significant reduction in erratic movements compared to the acrylamide-exposed group ($p < 0.05$), demonstrating the potential neuroprotective effects of the nanoparticles in mitigating motor deficits associated with PD. Overall, our results indicate that biosynthetically derived β-chitosan derived copper nanoparticles exert significant neuroprotective effects in a zebrafish model of Parkinson's disease induced by acrylamide. The nanoparticles enhanced locomotor activity, improved exploratory behavior, and reduced erratic movements, suggesting preservation of dopaminergic neurons and functional improvements in motor coordination. These findings support further investigation into the therapeutic potential of β-chitosan derived copper nanoparticles as a novel treatment strategy for Parkinson's disease and other neurodegenerative disorders.

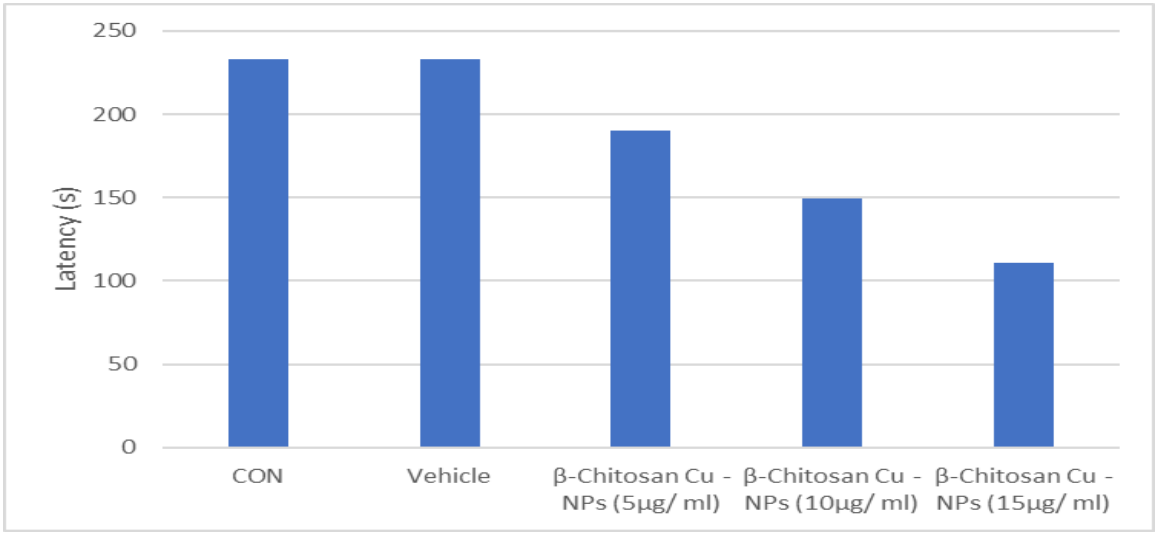

Figure 4: Latency to Enter the Top (s)

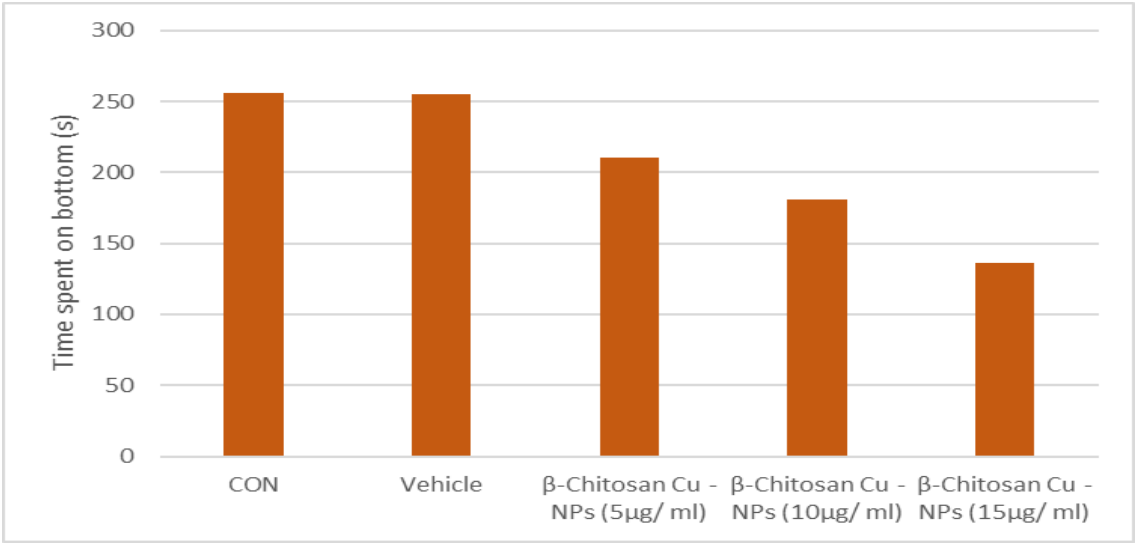

Figure 5: Time Spends on Bottom (s)

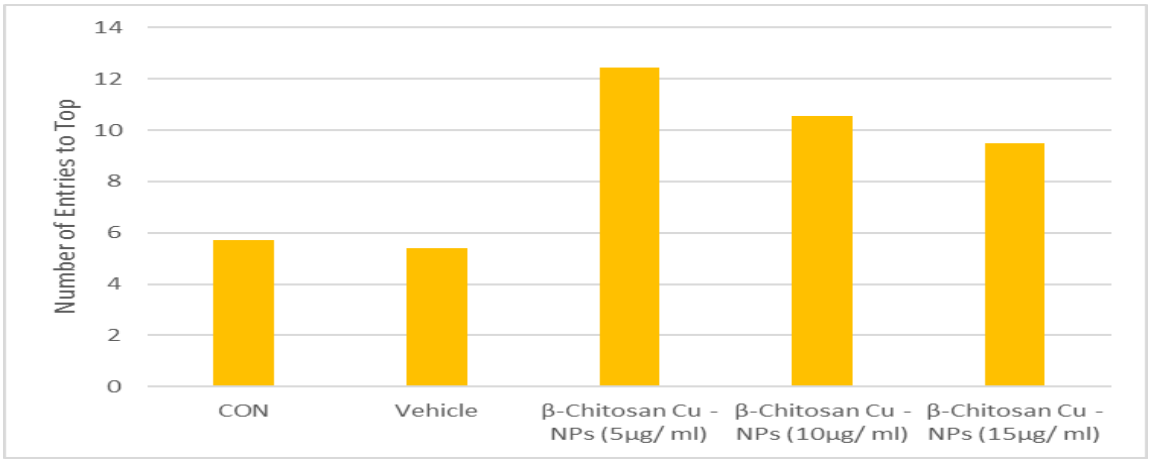

Figure 6: Number of Entries to Top

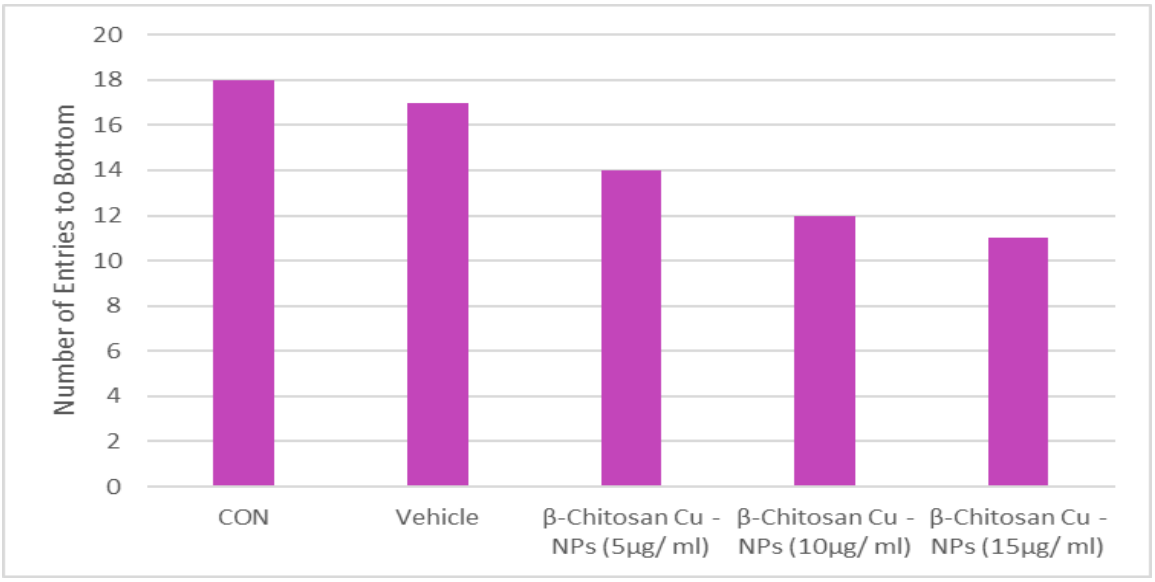

Figure 7: Number of Entries to Bottom

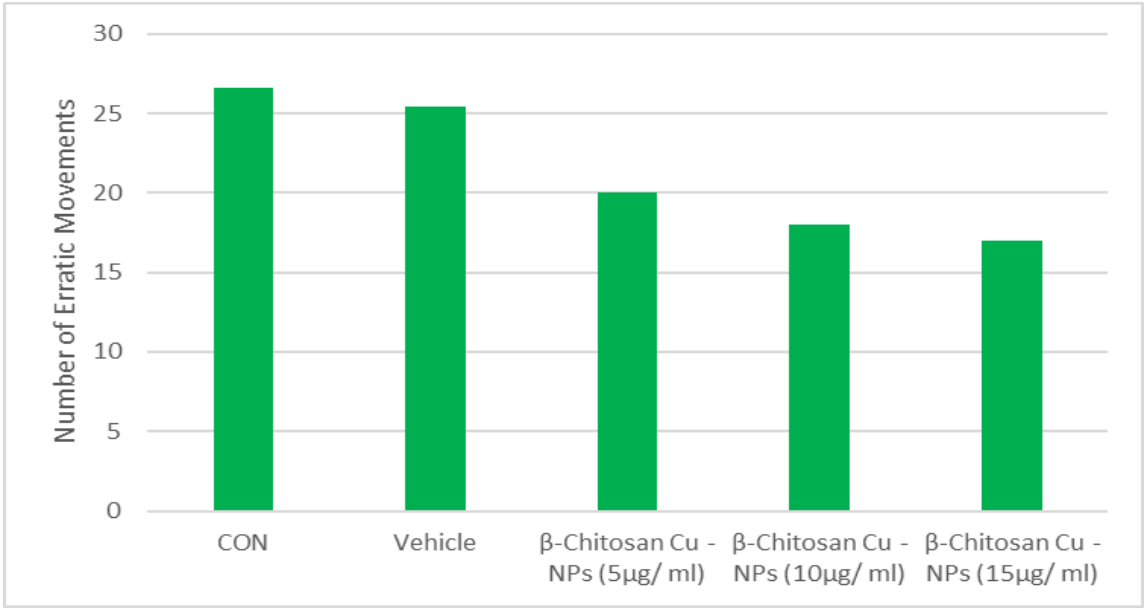

Figure 8: Number of Erratic Movements

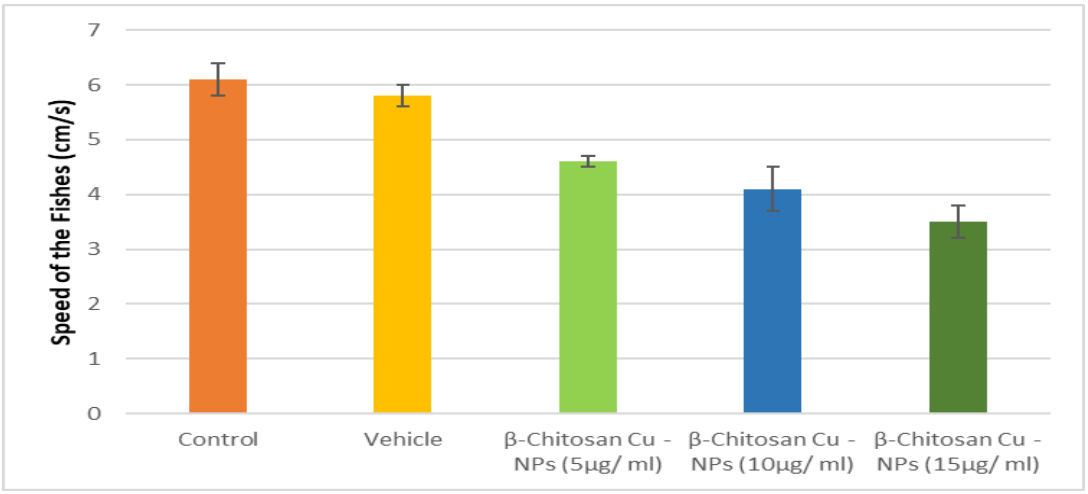

Figure 9: Speed of the Fish (cm/s)

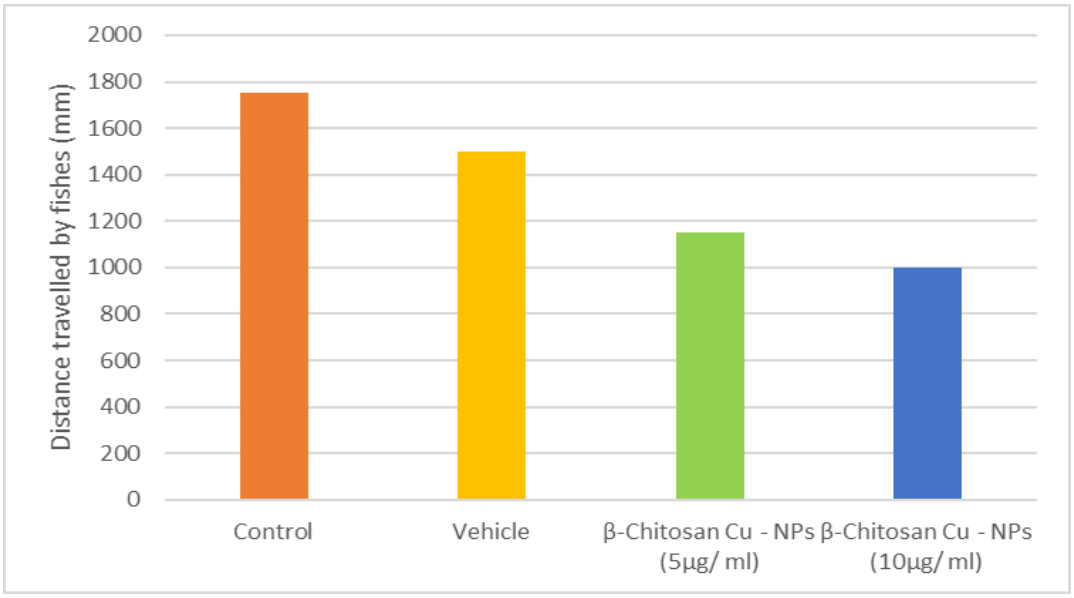

Figure 10: Distance Travelled by Fish

4. DISCUSSION

The present study investigated the potential neuroprotective effects of biosynthetically derived β-chitosan derived copper nanoparticles in a zebrafish model of Parkinson's disease (PD) induced by acrylamide(Haleagrahara, Siew et al. 2011). Parkinson's disease is characterized by the progressive loss of dopaminergic neurons, leading to motor deficits and neurobehavioral abnormalities. Our findings demonstrate that treatment with β-chitosan derived copper nanoparticles effectively mitigated behavioural impairments associated with PD in zebrafish larvae exposed to acrylamide. Behavioral assessments revealed significant improvements in locomotor activity and exploratory behavior following nanoparticle treatment(Pinho, Reis et al. 2016). Zebrafish larvae treated with β-chitosan derived copper nanoparticles exhibited increased total distance traveled and average speed compared to the acrylamide-exposed group without treatment. These findings suggest that nanoparticle treatment preserved motor function and mobility, potentially by protecting dopaminergic neurons from degeneration induced by acrylamide.

Exploratory behavior, assessed through time spent in defined zones within the testing arena and latency to enter the top zone, further supported the neuroprotective effects of β-chitosan derived copper nanoparticles(Thayumanavan, Jeyabalan et al. 2022). Larvae treated with nanoparticles spent more time in the top zone and showed reduced latency to enter this zone compared to acrylamide-exposed larvae without treatment. These results indicate enhanced exploratory behavior and reduced anxiety-like responses in nanoparticle-treated larvae, reflecting improved neuronal function and behavioural outcomes. The number of entries into the top and bottom zones provided additional insights into spatial exploration and navigation abilities. Although the difference in the number of entries into the top zone was not statistically significant between groups, nanoparticle-treated larvae showed a trend towards increased entries into the top zone compared to the acrylamide-exposed group. Moreover, a significant reduction in entries into the bottom zone was observed in the nanoparticle-treated group, indicating a preference for the top zone and improved spatial awareness and navigation abilities following treatment(Duraisamy, Ganapathy et al. 2021).

Erratic movements, a measure of motor coordination and neurological deficits, were significantly reduced in zebrafish larvae treated with β-chitosan derived copper nanoparticles compared to those exposed to acrylamide alone. This finding underscores the potential neuroprotective effects of nanoparticles in preserving motor coordination and reducing the severity of movement impairments associated with PD. The observed neuroprotective effects of β-chitosan derived copper nanoparticles may be attributed to several underlying mechanisms. Copper nanoparticles possess potent antioxidant properties, which can scavenge free radicals and mitigate oxidative stress—a key contributor to dopaminergic neuron degeneration in PD. β-Chitosan, as a biocompatible and biodegradable polymer, enhances the stability and delivery of copper nanoparticles to the central nervous system, facilitating their therapeutic efficacy in protecting neurons from toxic insults such as acrylamide. The use of the zebrafish model in this study offers several advantages for evaluating nanoparticle-based therapies in PD. Zebrafish larvae are genetically tractable, allowing for the manipulation of disease-relevant genes and pathways to simulate PD pathology. The optical transparency of zebrafish larvae during early developmental stages enables real-time visualization and quantification of neuronal structure and function, facilitating detailed morphological and behavioural analyses following experimental interventions. Despite the promising findings, several limitations should be considered when interpreting the results of this study. The acrylamide-induced zebrafish model, while useful for studying dopaminergic neuron degeneration, may not fully recapitulate the complex pathophysiology of Parkinson's disease observed in humans. Future studies could benefit from incorporating additional PD-related assays, such as dopamine measurements, protein aggregation assays, and mitochondrial function analyses, to provide a more comprehensive understanding of nanoparticle-mediated neuroprotection. Our study provides compelling evidence for the neuroprotective effects of biosynthetically derived β-chitosan derived copper nanoparticles in a zebrafish model of Parkinson's disease induced by acrylamide. The nanoparticles improved locomotor activity, enhanced exploratory behavior, and reduced erratic movements, suggesting preservation of dopaminergic neurons and functional improvements in motor coordination. These findings support further exploration of β-chitosan derived copper nanoparticles as a promising therapeutic strategy for Parkinson's disease and other neurodegenerative disorders, highlighting

their potential to alleviate disease burden and improve patient outcomes in clinical settings.

5. CONCLUSION

In summary, our findings support further investigation of biosynthetically derived β-chitosan derived copper nanoparticles as a promising therapeutic approach for Parkinson's disease and other neurodegenerative disorders. By enhancing motor function, improving behavioural outcomes, and reducing neuronal damage in a zebrafish model, these nanoparticles hold potential for clinical translation, aiming to alleviate disease progression and improve quality of life for patients affected by Parkinson's disease.

References

1) Abijeth, B. and D. Ezhilarasan (2020). "Syringic acid induces apoptosis in human oral squamous carcinoma cells through mitochondrial pathway." Journal of Oral and Maxillofacial Pathology **24**(1): 40-45.

2) Ambika, S., et al. (2019). "Biomolecular interaction, anti-cancer and anti-angiogenic properties of cobalt (III) Schiff base complexes." Scientific reports **9**(1): 2721.

3) Anbarasu, M., et al. (2024). "Depolymerization of PET Wastes Catalysed by Tin and Silver doped Zinc oxide Nanoparticles and Evaluation of Embryonic Toxicity Using Zebrafish." Water, Air, & Soil Pollution **235**(6): 433.

4) Balaji, A., et al. (2022). "A review on the potential species of the zingiberaceae family with anti-viral efficacy towards enveloped viruses." J Pure Appl Microbiol **16**(2): 796-813.

5) Chia, K., et al. (2022). "Zebrafish as a model organism for neurodegenerative disease." Frontiers in molecular neuroscience **15**: 940484.

6) Chinta, S. J. and J. K. Andersen (2005). "Dopaminergic neurons." The international journal of biochemistry & cell biology **37**(5): 942-946.

7) Chokkattu, J. J., et al. (2022). "Embryonic toxicology evaluation of ginger-and clove-mediated titanium oxide nanoparticles-based dental varnish with zebrafish." The Journal of Contemporary Dental Practice **23**(11): 1158.

8) De Rijk, M. d., et al. (1997). "Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease." Journal of Neurology, Neurosurgery & Psychiatry **62**(1): 10-15.

9) Dhakshinamoorthy, V., et al. (2017). "Neurobehavioural toxicity of iron oxide nanoparticles in mice." Neurotoxicity research **32**: 187-203.

10) Dhas, N. A., et al. (1998). "Synthesis, characterization, and properties of metallic copper nanoparticles." Chemistry of materials **10**(5): 1446-1452.

11) Duraisamy, R., et al. (2021). "Nanocomposites Used In Prosthodontics And Implantology-A Review." I nternational journal of dentistry and oral science **8**(9): 4380-4387.

12) Dybing, E., et al. (2005). "Human exposure and internal dose assessments of acrylamide in food." Food and Chemical Toxicology **43**(3): 365-410.

13) Farr, A. C. and M. P. Xiong (2020). "Challenges and opportunities of deferoxamine delivery for treatment of Alzheimer's disease, Parkinson's disease, and intracerebral hemorrhage." Molecular pharmaceutics **18**(2): 593-609.

14) Guo, J. D., et al. (2018). "Damage to dopaminergic neurons by oxidative stress in Parkinson's disease." International journal of molecular medicine **41**(4): 1817-1825.

15) Haleagrahara, N., et al. (2011). "Neuroprotective effect of bioflavonoid quercetin in 6-hydroxydopamine-induced oxidative stress biomarkers in the rat striatum." Neuroscience letters **500**(2): 139-143.

16) Herrera, A., et al. (2005). "Inflammatory process as a determinant factor for the degeneration of substantia nigra dopaminergic neurons." Journal of neural transmission **112**: 111-119.

17) Hirsch, L., et al. (2016). "The incidence of Parkinson's disease: a systematic review and meta-analysis." Neuroepidemiology **46**(4): 292-300.

18) Hosseini, S. F., et al. (2022). "Bioactive functional ingredients from aquatic origin: A review of recent progress in marine-derived nutraceuticals." Critical Reviews in Food Science and Nutrition **62**(5): 1242-1269.

19) Khalid, J. P., et al. (2024). "Exploring Tumor-Promoting Qualities of Cancer-Associated Fibroblasts and Innovative Drug Discovery Strategies With Emphasis on Thymoquinone." Cureus **16**(2).

20) Khanna, P., et al. (2007). "Synthesis and characterization of copper nanoparticles." Materials Letters **61**(25): 4711-4714.

21) Krishnan, R. P., et al. (2022). "Microscopic appearances of commonly implanted food particles." Journal of Oral and Maxillofacial Pathology **26**(3): 352-355.

22) Lee, H.-J., et al. (2011). "Biological synthesis of copper nanoparticles using plant extract." Nanotechnology **1**(1): 371-374.

23) Lee, S.-M., et al. (2021). "Analysis of activation process of carbon black based on structural parameters obtained by XRD analysis." Crystals **11**(2): 153.

24) Li, Q., et al. (2015). "Differential behavioral responses of zebrafish larvae to yohimbine treatment." Psychopharmacology **232**: 197-208.

25) Liu, Q., et al. (2008). "Mechanism study of wood lignin pyrolysis by using TG–FTIR analysis." Journal of analytical and applied pyrolysis **82**(1): 170-177.

26) Marras, C., et al. (2018). "Prevalence of Parkinson's disease across North America." NPJ Parkinson's disease **4**(1): 21.

27) Merchant, A., et al. (2020). "Radiographic Evaluation of Marginal Accuracy of Metal Coping in Sectioned and Unsectioned 3D Printed Models and Gypsum Models." World **11**(5): 386-391.

28) Mohan, R. and R. K. Jain (2022). "An in vitro comparative evaluation of surface roughness characteristics of different orthodontic archwires: An atomic force microscopy study." Journal of Dental Research, Dental Clinics, Dental Prospects **16**(2): 91.

29) Narayanan, K. B. and N. Sakthivel (2010). "Biological synthesis of metal nanoparticles by microbes." Advances in colloid and interface science **156**(1-2): 1-13.

30) Pinho, B. R., et al. (2016). "Pharmacological modulation of HDAC1 and HDAC6 in vivo in a zebrafish model: therapeutic implications for Parkinson's disease." Pharmacological Research **103**: 328-339.

31) Ram, A. J., et al. (2020). "Overexpression of BASP1 indicates a poor prognosis in head and neck squamous cell carcinoma." Asian Pacific journal of cancer prevention: APJCP **21**(11): 3435.

32) Smith, E. S., et al. (2019). "Leveraging the interplay of nanotechnology and neuroscience: Designing new avenues for treating central nervous system disorders." Advanced drug delivery reviews **148**: 181-203.

33) Song, J. and J. Kim (2016). "Degeneration of dopaminergic neurons due to metabolic alterations and Parkinson's disease." Frontiers in aging neuroscience **8**: 65.

34) Sundaram, K. K. M., et al. (2022). "Instrumentals behind embryo and cancer: a platform for prospective future in cancer research." AIMS Molecular Science **9**(1): 25-45.

35) Sundaram, K. K. M. and K. M. Saravanan (2022). "Deciphering Role of Chameleon Fragments in Folding of Amyloidogenesis."

36) Tayyeb, J. Z., et al. (2024). "Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells." Molecular Biology Reports **51**(1): 423.

37) Thayumanavan, G., et al. (2022). "Silibinin and Naringenin against Bisphenol A-Induced neurotoxicity in zebrafish model—Potential flavonoid molecules for new drug design, development, and therapy for neurological disorders." Molecules **27**(8): 2572.

38) Thomas, B. and M. F. Beal (2007). "Parkinson's disease." Human molecular genetics **16**(R2): R183-R194.

39) Umapathy, S., et al. (2024). "Selenium Nanoparticles as Neuroprotective Agents: Insights into Molecular Mechanisms for Parkinson's Disease Treatment." Molecular Neurobiology: 1-28.

40) Ushanthika, T., et al. (2021). "An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine." Natural product research **35**(11): 1893-1898.

41) van Gaalen, M. M. and T. Steckler (2000). "Behavioural analysis of four mouse strains in an anxiety test battery." Behavioural brain research **115**(1): 95-106.