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In vitro antioxidant and neuroprotective activity of green tea and papaya leaf formulation mediated silver nanoparticles

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ABSTRACT

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by oxidative stress, amyloid-beta (Aβ) aggregation, and cholinergic dysfunction. Plant-mediated synthesis of silver nanoparticles (AgNPs) offers a biocompatible approach to target these pathological features. This study investigates the antioxidant, acetylcholinesterase (AChE) inhibitory, and amyloid (Aβ1–42) aggregation inhibitory properties of AgNPs synthesized using green tea and papaya leaf extracts, with a focus on their potential neuroprotective effects.

Methodology: Silver nanoparticles were synthesized using green tea and papaya leaf extracts. The antioxidant activity of the AgNPs was assessed using DPPH and ABTS radical scavenging assays. The synthesized AgNPs were tested at varying concentrations (5–160 µg/mL) in all assays, and ascorbic acid was used as a standard antioxidant. The AChE inhibitory activity was evaluated using Ellman's method, while the anti-amyloid activity was measured by examining the inhibition of Aβ1–42 peptide aggregation.

Results: The AgNPs exhibited significant antioxidant activity, with DPPH inhibition reaching up to 89.85% and ABTS inhibition up to 85.43% at the highest concentration. The AChE inhibitory activity demonstrated concentration-dependent inhibition, with a maximum inhibition of 79.93% at 160 µg/mL. Furthermore, the AgNPs effectively inhibited Aβ1–42 aggregation, with only19.2% aggregation observed at 100µg/mL. The bioactivity of AgNPs is attributed to the polyphenolic compounds present in green tea and papaya leaf extracts, enhancing their neuroprotective properties.

Conclusion: The AgNPs synthesized from green tea and papaya leaf extracts demonstrated potent antioxidant, AChE inhibitory, and anti-amyloid aggregation activities, making them promising candidates for therapeutic applications in Alzheimer's disease. These findings support the potential of plant-mediated AgNPs as multifunctional agents in targeting key pathological mechanisms of AD, with future studies needed to explore their in vivo efficacy and mechanisms of action.

Keywords: Silver nanoparticles, Alzheimer's disease, antioxidant, acetylcholinesterase, amyloid

INTRODUCTION

Neurological diseases are a leading cause of disability and death worldwide, accounting for approximately 12% of all global fatalities. The rapidly increasing number of individuals affected by neurodegenerative diseases (NDDs), which now totals tens of millions annually, has been identified by the World Health Organization as one of the greatest health challenges of the 21st century [1]. Neurodegenerative disorders (NDs) refer to a group of chronic or hereditary conditions characterized by the progressive loss of specific types of neurons. Among the most impactful of these diseases in modern society are Parkinson's disease (PD) and Alzheimer's disease (AD). NDDs are marked by the deterioration of brain neurons, leading to a range of clinical and pathological symptoms, including impairments in speech, movement, and cognition [2]. One of the significant hurdles in treating neurodegenerative diseases is the blood-brain barrier (BBB)—a tightly regulated network of blood vessels and endothelial cells that prevents harmful substances from entering the brain. This protective

mechanism, while crucial for brain health, poses a considerable challenge in the development of effective therapeutics for NDDs [3].

Nanotechnology offers a ground-breaking and promising avenue, particularly due to the distinct properties of nanoparticles, which enable them to traverse the blood-brain barrier (BBB) and address various neurodegenerative disorders [4]. The high surface area of nano systems significantly enhances their potential for interaction with diagnostic biomarkers, therapeutic agents for drug delivery with prolonged retention, and damaged cells for regenerative therapies [5]. Owing to their adaptable and advanced physicochemical, morphological, and topological characteristics, nano systems have become crucial in targeted drug delivery, neuroimaging, neuroprotection, neurosurgery, and neuroregeneration strategies. These capabilities make them invaluable for treating neurodegenerative diseases, especially with their potential to cross the BBB [6].

Among metal oxide nanoparticles, silver nanoparticles (AgNPs) have garnered significant attention due to their outstanding antioxidant, anticancer, anti-Alzheimer'sand catalytic properties [7]. Regarding the central nervous system (CNS), AgNPs have demonstrated the ability to cross the BBB and accumulate in brain tissue following ingestion or inhalation, highlighting their potential for CNS-related applications [8-10]. Previous studies have demonstrated that AgNPs reduced brain inflammation and its associated toxicity besides attenuating spatial and recognition memory impairments in sporadic Alzheimer rat [11, 12]. However, studies have also reported detrimental impact caused by AgNPs on neuronal functions [13-15]. Given the background, the present study has aimed to investigate the antioxidant and neuroprotective effect of green tea and papaya leaf extract mediated AgNPs.

Materials and method

DPPH Free radical scavenging assay [16]

In DPPH radical scavenging assay, 10µL different concentrations of the synthesised AgNPs (5, 10, 20, 40, 80 & 160µg/ml) was added to 190µL of DPPH (150µM prepared in ethanol). The reaction mixture was shaken thoroughly and incubated in dark for 30 min at 37°C. After incubation, the absorbance was measured at 517 nm using Biotek synergy H4 hybrid microplate reader, USA. The reaction mixture without the nanoparticle was used as control and ascorbic acid was used as standard. The % inhibition of DPPH free radical formation was calculated as follows: [(Control - Test)/Control] *100

ABTS radical scavenging assay [17]

The ABTS (2,2'-azino-di [3-ethylbenzthiazoline sulfonate]) assay was performed as follows: 10µL different concentrations of the synthesised AgNPs (5, 10, 20, 40, 80 & 160µg/ml) was added to 10µL of metmyoglobin and 150µL of 2mM ABTS. The reaction was initiated by adding 40µL of H202 (441µM). The reaction mixture without the test drug was kept as control and ascorbic acid was used as standard. The absorbance was read at 690 nm using Biotek synergy H4 hybrid microplate reader, USA. The % inhibition of ABTS radical formation was calculated as follows: [(Control - Test)/Control] *100

In vitro acetylcholinesterase (AChE) inhibition assay [18]

Silver nanoparticles synthesised from papaya leaf and green tea were examined for its AChE inhibitory activities at different concentrations (10, 20, 40, 60, 80, 100µg/ml). The nanoparticles volume was made up to 200µl using 0.05M tris base. Briefly, in this method, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of nanoparticle solution at the different concentrations were mixed and incubated for 15 min at 30ºC. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200µl of AChE (0.3U/ml) solution were added to the initial mixture, to start the reaction and then the absorbance was determined.Control contained all components except the nanoparticles. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

IA (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100

All treatments were performed in triplicate with two replicates.

Assessment of Aβ (1–42) Concentration

Preparation of Aβ solution

The Aβ solution was prepared according to the method of Miyazaki et al., 2019. Briefly, synthetic β-Amyloid Peptide 1-42 **(**Aβ1-42**)** (PP69, Sigma Merck, USA) was dissolved in 0.1% ammonia solution at a final concentration of 250 µM and sonicated in ice-cold water for a total of 5 min (1 min × 5 times) to avoid pre-aggregation. For preparation of the Aβ solution, aliquots of Aβ were diluted to 25µM in 50mM phosphate buffer (pH 7.5) and 100 mM NaCl.

Thioflavin T fluorescence assay

The thioflavin T (ThT) fluorescence assay was performed as Miyazaki et al., 2019 [19]. Aβ solution (8µL) was mixed with the different concentrations of papaya leaf and green tea extract mediated Silver nanoparticles (10,

20, 40, 60, 80 & 100µg/ml) and the mixture then added to 1.6 mL of ThT solution containing 5µM ThT and 50mM NaOH- glycine-buffer (pH 8.5). The samples were incubated at 37°C and the fibrillogenesis rate was monitored by using ThT fluorescence assays. The samples ThT fluorescence levels were evaluated by using an Biotek Synergy H4 hybrid multi-mode reader (USA). The respective excitation and emission wavelengths were 446 nm and 490 nm.

RESULTS

Effect of AgNPs on DPPH free radicals' formation

The antioxidant activity of green tea and papaya leaf extract-mediated silver nanoparticles (AgNPs) was evaluated using the DPPH free radical scavenging assay across a concentration range of 5-160µg/mL. The % inhibition of DPPH radical formation demonstrated a dose-dependent trend, starting with a slight negative inhibition at the lower concentrations (-0.03% and -9.35% at 5 and 10 µg/mL, respectively). However, as the concentration increased, a marked rise in DPPH inhibition was observed, with % inhibition reaching 89.85% at 160µg/mL. In comparison, the standard antioxidant, ascorbic acid, exhibited a higher and more consistent % inhibition across all concentrations, ranging from 0.56% at 5 µg/mL to 94.78% at 160µg/mL. The initial negative inhibition observed at the lower concentrations of AgNPs may indicate that, at lower doses, the nanoparticles either do not effectively scavenge DPPH radicals or may interact with the assay medium in a way that temporarily reduces free radical scavenging. However, at concentrations above 40µg/mL, the AgNPs showed a substantial increase in free radical scavenging, comparable to that of ascorbic acid at the highest concentrations tested.

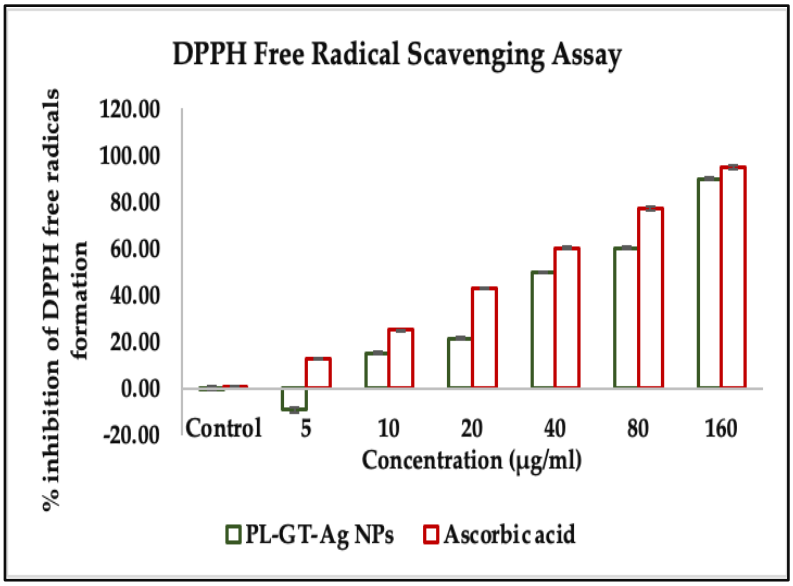

Figure 1: Effect of papaya leaf and green tea extracts mediated silver nanoparticles on DPPH free radical scavenging activity. Data are expressed as Mean±SEM (n=3).

Effect of AgNPs on ABTS free radicals' formation

The antioxidant potential of AgNPs was evaluated using the ABTS free radical scavenging assay across a concentration range of 5-160µg/mL. The results revealed a concentration-dependent increase in % inhibition of ABTS radicals, with inhibition values starting at 0% for the lowest concentration and rising to 85.43% at 160µg/mL. The standard antioxidant, ascorbic acid, also demonstrated a dose-dependent increase in % inhibition, ranging from 1.05% at 5 µg/mL to 92.76% at 160µg/mL. At lower concentrations (5-20 µg/mL), AgNPs displayed moderate radical scavenging activity, with inhibition levels slightly below those of ascorbic acid. However, as the concentration increased, the % inhibition of ABTS radicals by AgNPs improved substantially, reaching 85.43% at 160µg/mL, which is comparable to the 92.76% inhibition achieved by ascorbic acid at the same concentration.

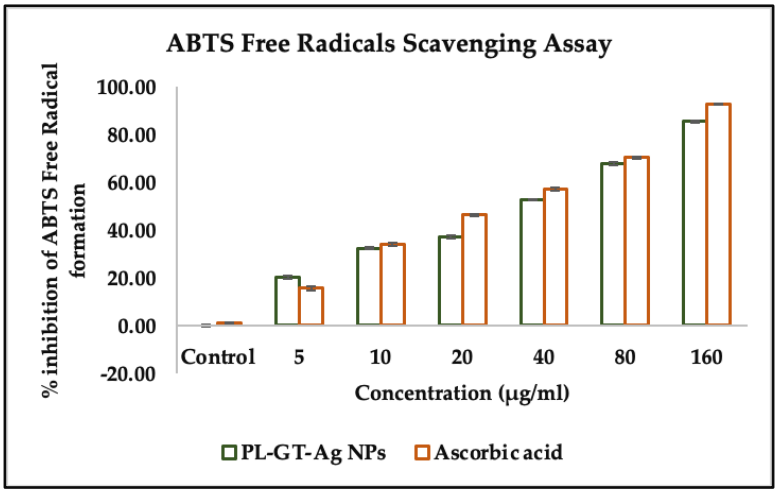

Figure 2: Effect of papaya leaf and green tea extracts mediated silver nanoparticles on ABTS free radical scavenging activity. Data are expressed as Mean±SEM (n=3).

Effect of AgNPs on in vitro acetylcholinesterase activity

The inhibition of acetylcholinesterase (AChE) activity by silver nanoparticles (AgNPs) was assessed across a concentration range of 10-100µg/mL. The results demonstrated a concentration-dependent inhibition, with % inhibition increasing from 0.21% at 10 µg/mL to 79.93% at the highest concentration of 100µg/mL. At lower concentrations, AgNPs exhibited minimal AChE inhibitory activity, but as the concentration increased, there was a marked rise in inhibition, with 46.79% inhibition observed at 80µg/mL and a substantial inhibition of 79.93% at 100 µg/mL. These findings indicate that AgNPs exhibit strong inhibitory effects on AChE activity at higher concentrations. The dose-dependent increase in inhibition suggests that AgNPs have the potential to modulate acetylcholinesterase activity, which could be explored further for applications related to neurological conditions where AChE inhibition is beneficial, such as Alzheimer's disease.

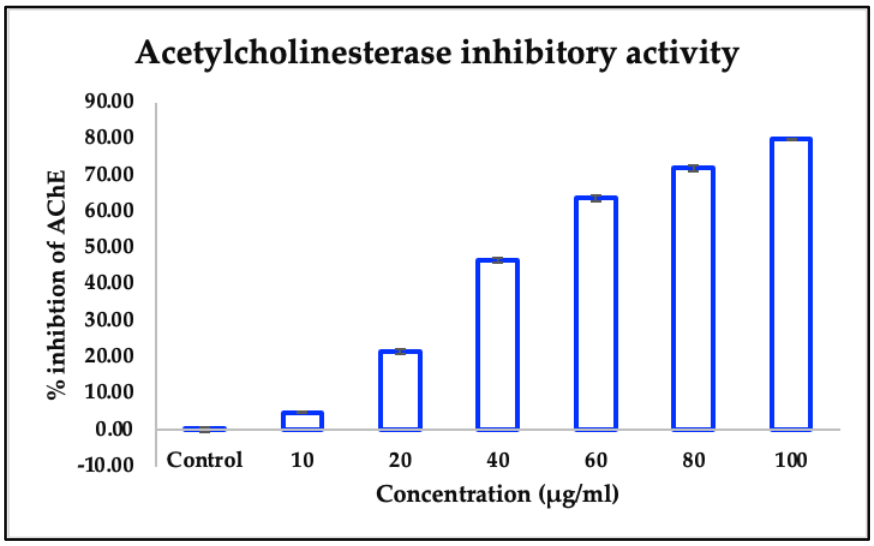

Figure 3: Effect of papaya leaf and green tea extracts mediated silver nanoparticles on acetylcholinesterase activity. Data are expressed as Mean±SEM (n=3).

Effect of AgNPs on in vitro amyloid (Aβ 1-42) peptide aggregation

The inhibition of amyloid (Aβ1–42) peptide aggregation by silver nanoparticles (AgNPs) was assessed across concentrations ranging from 10 to 100µg/mL. The results showed a concentration-dependent decrease in the amyloid aggregation. At the lowest concentration of 10µg/mL, the AgNPs exhibited a % aggregation of 99.85%. As the concentration increased, the inhibitory effect progressively increased, with % aggregation values of 86.55% at 20µg/mL, 64.66% at 80µg/mL, and 23.72% at the highest concentration of 100µg/mL. These findings suggest that AgNPs are highly effective in inhibiting Aβ1–42 aggregation at almost all the tested concentrations.

The strong inhibitory effect against amyloid aggregation highlights the potential of AgNPs as a therapeutic agent for conditions like Alzheimer's disease, where amyloid aggregation plays a key pathological role.

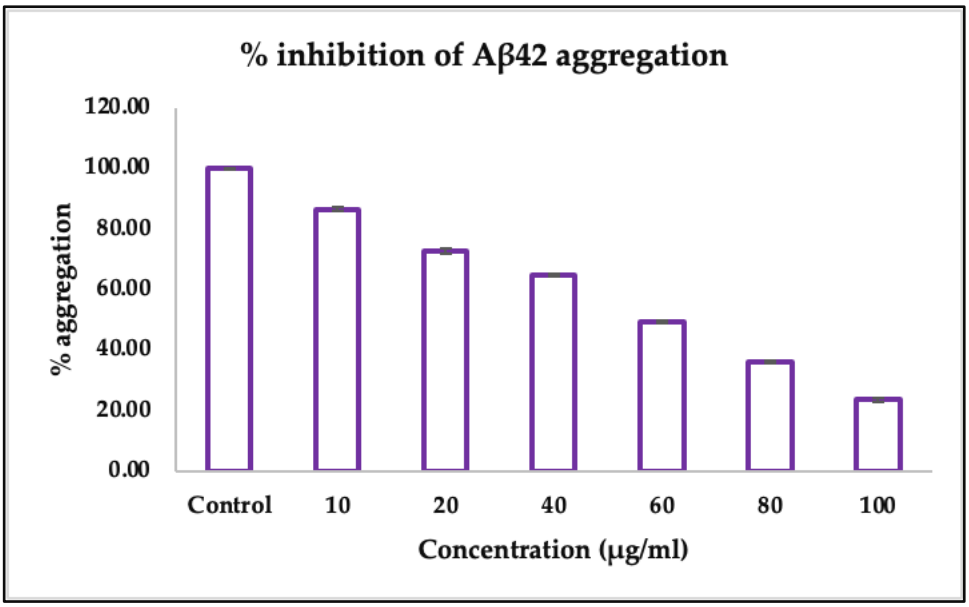

Figure 4: Effect of papaya leaf and green tea extracts mediated silver nanoparticles on in vitro Aβ42 aggregation. Data are expressed as Mean±SEM (n=3).

Discussion

The present study investigated the antioxidant, acetylcholinesterase (AChE) inhibitory, and amyloid (Aβ1–42) aggregation inhibitory activities of silver nanoparticles (AgNPs) synthesized using green tea and papaya leaf extracts. The findings reveal significant potential for these AgNPs in modulating oxidative stress and neurodegenerative processes, which are critical factors in conditions like Alzheimer's disease (AD).

The antioxidant activity of the synthesized AgNPs, as demonstrated by their ability to inhibit DPPH and ABTS free radicals, was concentration-dependent, with higher inhibition percentages observed at increasing concentrations. This aligns with previous studies indicating the potent antioxidant properties of silver nanoparticles synthesized from natural plant extracts [20, 21]. The high antioxidant activity can be attributed to the polyphenolic content of green tea and papaya leaf extracts, which are known to enhance the bioactivity of AgNPs [22, 23]. Green tea catechins, in particular, have been shown to possess strong free radical scavenging abilities, potentially mitigating oxidative stress, a major contributor to neurodegeneration [24, 25].

In addition to their antioxidant potential, the AgNPs also demonstrated significant AChE inhibitory activity, with inhibition increasing as the concentration of AgNPs rose. The inhibition of AChE, a key enzyme involved in the breakdown of acetylcholine, is crucial for the management of Alzheimer's disease [20]. Previous research supports the idea that plant-based AgNPs, particularly those synthesized from green sources like Kickxiaelatine and pawpaw leaf extract, exhibit AChE inhibitory properties [26]. The alkaloids and phenolic compounds present in papaya leaf extracts, coupled with the bioactive compounds in green tea, likely contribute to the enhanced AChE inhibition observed in our study. This supports the potential therapeutic role of these AgNPs in managing AD symptoms by improving cholinergic transmission.

Moreover, the AgNPs synthesized in our study were also effective in inhibiting the aggregation of amyloid (Aβ1–42) peptides, which is a hallmark of Alzheimer's disease pathology. Our findings demonstrated a significant inhibition of amyloid aggregation at all tested lower concentrations of AgNPs. This is consistent with previous reports that silver nanoparticles, particularly those synthesized from green sources, can interfere with amyloid fibril formation [11]. The ability of AgNPs to inhibit amyloid aggregation suggests that they could play a preventative role in the development of AD, as amyloid plaques are a key factor in the neurotoxicity and cognitive decline associated with the disease [22].

Additionally, the neuroprotective properties of AgNPs may extend beyond amyloid inhibition. Recent in vivo studies have shown that AgNPs can reduce brain inflammation and oxidative stress through the induction of H2S-synthesizing enzymes, which further contributes to their neuroprotective role [11]. The combination of antioxidant, AChE inhibitory, and anti-amyloid aggregation properties make AgNPs synthesized from green tea and papaya leaf extracts a promising candidate for further exploration in neurodegenerative disease treatment strategies.

Conclusion

In conclusion, the AgNPs synthesized from green tea and papaya leaf extracts demonstrated multifaceted bioactivities, including strong antioxidant effects, AChE inhibition, and amyloid aggregation inhibition. These findings align with previous studies on the neuroprotective and anti-inflammatory potential of AgNPs (Nam et al., 2021). Future research should focus on in vivo models to further elucidate the mechanisms behind these effects and their potential therapeutic applications in the treatment of Alzheimer's disease and other neurodegenerative disorders.

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Conflict of Interest

The authors declared no conflict of interest pertaining to the study.

References

1. Holbrook JA, Jarosz-Griffiths HH, Caseley E, Lara-Reyna S, Poulter JA, Williams-Gray CH, Peckham D, McDermott MF. Neurodegenerative disease and the NLRP3 inflammasome. Frontiers in pharmacology. 2021 Mar 10;12:643254.
2. Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, Bohr VA. Ageing as a risk factor for neurodegenerative disease. Nature Reviews Neurology. 2019 Oct;15(10):565-81.
3. Thukral P, Chowdhury R, Sable H, Kaushik A, Chaudhary V. Sustainable green synthesized nanoparticles for neurodegenerative diseases diagnosis and treatment. Materials Today: Proceedings. 2023 Jan 1;73:323-8.
4. Naz S, Beach J, Heckert B, Tummala T, Pashchenko O, Banerjee T, Santra S. Cerium oxide nanoparticles: a 'radical'approach to neurodegenerative disease treatment. Nanomedicine. 2017 Mar 1;12(5):545-53.
5. Chen J, Zhou Z, Luo S, Liu G, Xiang J, Tian Z. Progress of advanced nanomaterials in diagnosis of neurodegenerative diseases. Biosensors and Bioelectronics. 2022 Dec 1;217:114717.
6. Tiwari S, Atluri V, Kaushik A, Yndart A, Nair M. Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. International journal of nanomedicine. 2019 Jul 19:5541-54.
7. Huda NU, Ghneim HK, Fozia F, Ahmed M, Mushtaq N, Sher N, Khan RA, Ahmad I, Al-Sheikh YA, Giesy JP, Aboul-Soud MA. Green synthesis of Kickxiaelatine-induced silver nanoparticles and their role as anti-acetylcholinesterase in the treatment of Alzheimer's disease. Green Processing and Synthesis. 2023 Jun 7;12(1):20230060.
8. Tang J, Xiong L, Zhou G, Wang S, Wang J, Liu L, Li J, Yuan F, Lu S, Wan Z, Chou L. Silver nanoparticles crossing through and distribution in the blood-brain barrier in vitro. Journal of nanoscience and nanotechnology. 2010 Oct 1;10(10):6313-7.
9. Loeschner K, Hadrup N, Qvortrup K, Larsen A, Gao X, Vogel U, Mortensen A, Lam HR, Larsen EH. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Particle and fibre toxicology. 2011 Dec;8:1-4.
10. Ji JH, Jung JH, Kim SS, Yoon JU, Park JD, Choi BS, Chung YH, Kwon IH, Jeong J, Han BS, Shin JH. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhalation toxicology. 2007 Jan 1;19(10):857-71.
11. Gonzalez-Carter DA, Leo BF, Ruenraroengsak P, Chen S, Goode AE, Theodorou IG, Chung KF, Carzaniga R, Shaffer MS, Dexter DT, Ryan MP. Silver nanoparticles reduce brain inflammation and related neurotoxicity through induction of H2S-synthesizing enzymes. Scientific reports. 2017 Mar 2;7(1):42871.
12. Zhang X, Li Y, Hu Y. Green synthesis of silver nanoparticles and their preventive effect in deficits in recognition and spatial memory in sporadic Alzheimer's rat model. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2020 Nov 20;605:125288.
13. Greish K, Alqahtani AA, Alotaibi AF, Abdulla AM, Bukelly AT, Alsobyani FM, Alharbi GH, Alkiyumi IS, Aldawish MM, Alshahrani TF, Pittalà V. The effect of silver nanoparticles on learning, memory and social interaction in BALB/C mice. International journal of environmental research and public health. 2019 Jan;16(1):148.
14. Węsierska M, Dziendzikowska K, Gromadzka-Ostrowska J, Dudek J, Polkowska-Motrenko H, Audinot JN, Gutleb AC, Lankoff A, Kruszewski M. Silver ions are responsible for memory impairment induced by oral administration of silver nanoparticles. Toxicology letters. 2018 Jun 15;290:133-44.
15. Kruszewski M, Grzelak A. Nanoparticle toxicity and reactive species: An overview. Toxicology. 2021 Jan 1:11-21.
16. Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Analysis, 2002; 13: 8-17.

17. Gonzalez-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Tellez-Tellez M, Gupta VK, Díaz-Godínez G, Soriano-Santos J. Evaluation of the Antioxidant Activity of Aqueous and Methanol Extracts of Pleurotusostreatusin Different Growth Stages. Front Microbiol. 2016 Jul 12;7:1099.
18. Ellman GL, Courtney D, Andres V, Featherston RM 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. BiochemPharmacol 7: 88-95.
19. Miyazaki H, Okamoto Y, Motoi A, Watanabe T, Katayama S, Kawahara SI, Makabe H, Fujii H, Yonekura S. Adzuki bean (Vigna angularis) extract reduces amyloid-β aggregation and delays cognitive impairment in Drosophila models of Alzheimer's disease. Nutr Res Pract. 2019 Feb;13(1):64-69.
20. Fu X, Rehman U, Wei L, Chen ZS, Abourehab MA, Kesharwani P, Cheng ZH. Silver-dendrimer nanocomposite as emerging therapeutics in anti-bacteria and beyond. Drug Resistance Updates. 2023 May 1;68:100935.
21. Huda NU, Ghneim HK, Fozia F, Ahmed M, Mushtaq N, Sher N, Khan RA, Ahmad I, Al-Sheikh YA, Giesy JP, Aboul-Soud MA. Green synthesis of Kickxiaelatine-induced silver nanoparticles and their role as anti-acetylcholinesterase in the treatment of Alzheimer's disease. Green Processing and Synthesis. 2023 Jun 7;12(1):20230060.
22. Zhang X, Li Y, Hu Y. Green synthesis of silver nanoparticles and their preventive effect in deficits in recognition and spatial memory in sporadic Alzheimer's rat model. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2020 Nov 20;605:125288.
23. Padmapriya A, Preetha S, Selvaraj J, Sridevi G. Effect of Carica papaya seed extract on IL-6 and TNF-α in human lung cancer cell lines-an In vitro study. Research Journal of Pharmacy and Technology. 2022;15(12):5478-82.
24. Janani M, Viswanathan D, Pandiaraj S, Govindasamy R, Gomathi T, Vijayakumar S. Review on phyto-extract methodologies for procuring ZnO NPs and its pharmacological functionalities. Process Biochemistry. 2024 Aug 25.
25. Afzal O, Dalhat MH, Altamimi AS, Rasool R, Alzarea SI, Almalki WH, Murtaza BN, Iftikhar S, Nadeem S, Nadeem MS, Kazmi I. Green tea catechins attenuate neurodegenerative diseases and cognitive deficits. Molecules. 2022 Nov 6;27(21):7604.
26. Adedayo BC, Oyeleye SI, Okeke BM, Oboh G. Anti-cholinesterase and antioxidant properties of alkaloid and phenolic-rich extracts from pawpaw (Carica papaya) leaf: A comparative study. Flavour and Fragrance Journal. 2021 Jan;36(1):47-54.