Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1438-1443 | ISSN:2251-6727

Evaluating the Anti Inflammatory Properties of Arrow Root Extract Silver Nanoparticles - An Invitro Study.

[1] Sai Krishna, [2] Rajprakash Bhaskaran, [3] Santhosh P Kumar

[1] Oral and maxillofacial surgery, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

[2, 3] Oral and maxillofacial surgery, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 24 July 2025)

Keywords

Anti Inflammatory, Properties, Arrow Root, Silver Nanoparticles

ABSTRACT:

Introduction: Silver nanoparticles are recognized for their antimicrobial and anti-inflammatory properties, making them valuable in biomedical applications. Green synthesis methods using plant extracts, such as arrowroot, provide an eco-friendly alternative for nanoparticle production. Arrowroot has traditionally been used for its medicinal benefits, and this study explores its potential in synthesizing silver nanoparticles with anti-inflammatory properties. This study aimed to evaluate the anti-inflammatory effects of silver nanoparticles (AgNPs) synthesized using arrowroot (Maranta arundinacea) extract in an in vitro model.

Materials and Methods: Arrow Root Extract Silver Nanoparticles (AR-AgNPs) were synthesized using arrow root extract and silver nitrate solution. The anti-inflammatory activity was evaluated using the Bovine Serum Albumin (BSA) Denaturation Assay, Egg Albumin Denaturation Assay, and Membrane Stabilization Assay. For BSA and egg albumin assays, protein denaturation was induced by heat (70°C for 10 min), and inhibition was measured spectrophotometrically at 660 nm. In the membrane stabilization assay, human red blood cells were exposed to hypotonic stress, and lysis was measured by recording absorbance at 560 nm. Different concentrations of AR-AgNPs (10-50 µg/mL) were tested in all assays.

Results: The synthesized Arrow Root Extract Silver Nanoparticles (AR-AgNPs) demonstrated significant anti-inflammatory activity in all three assays. In the BSA and egg albumin denaturation assays, AR-AgNPs showed a dose-dependent inhibition of protein denaturation, with the highest inhibition observed at 50 µg/mL. The membrane stabilization assay also showed a notable reduction in hemolysis, confirming the membrane-protective effect of AR-AgNPs. Across all assays, the nanoparticles exhibited a consistent anti-inflammatory response, with inhibition percentages comparable to standard anti-inflammatory drugs.

Conclusion: Arrowroot extract-synthesized silver nanoparticles showed promising anti-inflammatory properties in vitro, suggesting their potential use in treating inflammation-related conditions. Further research is necessary to evaluate their clinical application and ensure their safety for therapeutic use.

1. Introduction

Inflammation is a vital biological response that helps protect the body from infections, injuries, and harmful stimuli. While acute inflammation is essential for healing, chronic inflammation can lead to various diseases, including cardiovascular disorders, neurodegenerative diseases, autoimmune conditions, and cancer [1]. Current treatments for inflammation, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are effective but often cause side effects when used long-term [2]. Therefore, there is a growing need for safer, more sustainable alternatives. Nanotechnology, particularly silver nanoparticles (AgNPs), has emerged as a promising field in biomedical research. Silver nanoparticles are valued for their antibacterial, antifungal, and anti-inflammatory properties [3]. Their unique physical and chemical characteristics, such as a high surface area-to-volume ratio, make them ideal candidates for treating inflammation and related conditions [4]. However,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1438-1443 | ISSN:2251-6727

conventional synthesis methods for AgNPs often use toxic chemicals and energy-intensive processes, raising environmental and health concerns. To overcome these issues, green synthesis approaches that utilize biological materials like plant extracts have gained attention.

Green synthesis offers an eco-friendly and biocompatible alternative for producing nanoparticles. Plant-based extracts, rich in bioactive compounds, can serve as reducing and stabilizing agents in nanoparticle synthesis, leading to enhanced bioactivity in the final product [5]. Arrowroot (Maranta arundinacea), a tropical plant traditionally used for medicinal purposes, is a prime candidate for this process. Known for its anti-inflammatory, antioxidant, and antimicrobial properties, arrowroot contains bioactive compounds like flavonoids, polyphenols, and saponins, which may further enhance the therapeutic properties of silver nanoparticles [6]. In the green synthesis process, bioactive compounds in arrowroot extract reduce silver ions (Ag+) to silver nanoparticles (AgNPs), while also stabilizing them to prevent aggregation. This method creates arrowroot-silver nanoparticles (AR-AgNPs), which are expected to exhibit potent anti-inflammatory properties [7]. The combination of silver's inherent bioactivity with arrowroot's medicinal compounds suggests that AR-AgNPs could provide a safer, biocompatible alternative to conventional treatments.

Inflammation is mediated by complex signaling molecules such as cytokines, chemokines, and enzymes like cyclooxygenase (COX) and lipoxygenase (LOX). These molecules play critical roles in regulating the inflammatory response [8]. Excessive production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) can lead to tissue damage and the progression of chronic inflammatory diseases. The COX and LOX enzymes contribute to the production of inflammatory mediators like prostaglandins and leukotrienes. Therefore, inhibiting these enzymes and downregulating pro-inflammatory cytokines are key strategies for controlling inflammation [9]. Macrophages are a crucial component of the immune system and play a central role in the inflammatory response. Upon activation by stimuli such as LPS (a component of bacterial cell walls), macrophages release large amounts of pro-inflammatory cytokines and mediators, which contribute to inflammation [10]. Using LPS-stimulated macrophages as an in vitro model allows researchers to assess the anti-inflammatory effects of AR-AgNPs and determine their potential therapeutic benefits.

Recent studies suggest that silver nanoparticles can modulate the inflammatory response by inhibiting the production of pro-inflammatory cytokines and enzymes [6-8]. However, the specific anti-inflammatory potential of silver nanoparticles synthesized using arrowroot extract remains underexplored. This study aims to fill this gap by evaluating the in vitro anti-inflammatory properties of arrowroot-silver nanoparticles (AR-AgNPs).

2. Materials and Methods

Bovine serum albumin denaturation assay

In this method, 0.45mL of bovine serum albumin was mixed with 0.05 mL of different concentrations (10-50 µg/mL) of Arrow root-mediated silver nanoparticles. The pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm.

Percentage of protein denaturation was measured using the following equation, % inhibition = Absorbance of control- Absorbance of sample×100 Absorbance of control.

Egg Albumin Denaturation Assay

To perform the egg albumin denaturation assay, 0.2mL of fresh egg albumin was mixed with 2.8 mL of phosphate buffer. Different concentrations (10-50 µg/mL) of Arrow root-mediated silver nanoparticles were added to the reaction mixture. The pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm.

Percentage of protein denaturation was measured using the following equation, % inhibition= Absorbance of control- Absorbance of sample×100 Absorbance of control.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1438-1443 | ISSN:2251-6727

Membrane Stabilization Assay

The in vitro membrane stabilization assay is a widely used technique for evaluating the membrane stabilizing properties of natural and synthetic compounds. This assay measures the ability of a compound to stabilize the cell membrane by preventing its disruption and subsequent release of intracellular contents. The materials include Human red blood cells (RBCs), Phosphate-buffered saline (PBS), Tris-HCl buffer (50 mM, pH 7.4), Different concentrations of silver nanoparticles (10-50 µg/mL), Centrifuge tube, UV-Vis spectrophotometer.

Preparation of RBC suspension - Collect fresh human blood in a sterile tube containing anticoagulant. Centrifuge the blood at 1000 g for 10 minutes at room temperature to separate the RBCs from other blood components. Remove the supernatant and wash the RBCs three times with PBS. Resuspend the RBCs in Tris-HCl buffer to obtain a 10% (v/v) RBC suspension.

3. Results

Bovine Serum Assay (BSA)

The BSA was conducted to evaluate the percentage inhibition of Arrowroot (Ag/NPs) in comparison with a Standard across varying concentrations (10-50 µg/mL) as depicted in the Figure *1*. The results demonstrate a concentration-dependent increase in the percentage of inhibition for both samples. At the lowest concentration (10 µg/mL), Arrowroot (Ag/NPs) exhibited approximately 42% inhibition, whereas the Standard showed a slightly higher inhibition of around 50%. As the concentration increased to 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL, the inhibition for both samples steadily rose, with the Standard consistently outperforming the Arrowroot (Ag/NPs). At the highest concentration (50 µg/mL), the inhibition for Arrowroot (Ag/NPs) reached approximately 75%, while the Standard achieved around 80% inhibition. The error bars indicate low variability in the results, suggesting consistent inhibition across multiple trials.

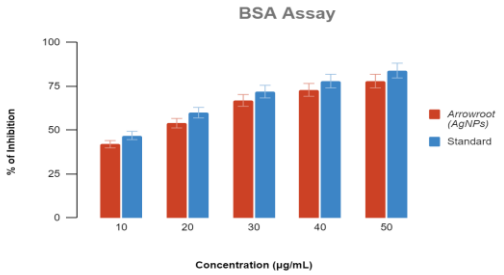

Figure 1. Depicting the percentage of inhibition of bovine serum assay at various concentrations.

These findings suggest that both Arrowroot (Ag/NPs) and the Standard demonstrate effective inhibition, with the Standard consistently producing higher inhibition percentages at all concentrations tested. This pattern highlights the potential of Arrowroot (Ag/NPs) for significant inhibition, albeit slightly less effective than the Standard at similar concentrations.

Egg Albumin Denaturation Assay (EADA)

The EADA was performed to measure the percentage inhibition of Arrowroot (Ag/NPs) compared to a Standard across concentrations ranging from 10 µg/mL to 50 µg/mL. The results depicted in the Figure *2* reveal a dose-dependent increase in the inhibition for both the Arrowroot (Ag/NPs) and the Standard. At 10 µg/mL, Arrowroot (Ag/NPs) demonstrated approximately 50% inhibition, which was slightly lower than the Standard, which achieved about 55%. As the concentration increased, both Arrowroot (Ag/NPs) and the Standard showed a consistent increase in the percentage of inhibition. At 20 µg/mL and 30 µg/mL, inhibition for both the Arrowroot and the Standard was around 65% and 70%, respectively, with the Standard maintaining a slight advantage. At the highest concentration of 50 µg/mL, Arrowroot (Ag/NPs) achieved around 80% inhibition, while the Standard exhibited slightly higher inhibition at about 85%. The error bars reflect low variability in the results, indicating that the inhibition values are consistent and reproducible across trials.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1438-1443 | ISSN:2251-6727

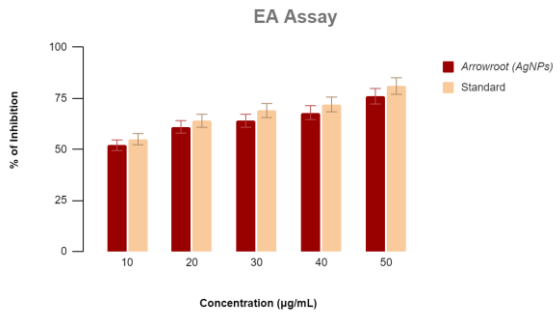

Figure 2. Depicting the egg albumin serum assay at various concentrations.

These findings suggest that both Arrowroot (Ag/NPs) and the Standard possess strong inhibitory effects, with the Standard marginally outperforming Arrowroot (Ag/NPs) at all tested concentrations. However, the Arrowroot (Ag/NPs) still demonstrates potent inhibition, indicating its potential effectiveness in this assay.

Membrane Stabilization Assay:

The bar graph in the Figure *3* depicts the membrane-stabilizing effects of Arrowroot AgNPs with a Standard across a range of concentrations (10 to 50 µg/mL). The y-axis shows the percentage of inhibition, which correlates with the ability to stabilize the membrane. Both Arrowroot (AgNPs) and the Standard exhibit a concentration-dependent increase in membrane stabilization. As the concentration increases, the percentage of inhibition also rises for - At lower concentrations (10-20 µg/mL), Arrowroot AgNPs show slightly lower membrane stabilization compared to the Standard. At higher concentrations (30-50 µg/mL), the difference between Arrowroot and Standard diminishes, with Arrowroot AgNPs displaying inhibition levels almost equal to or slightly exceeding the Standard.

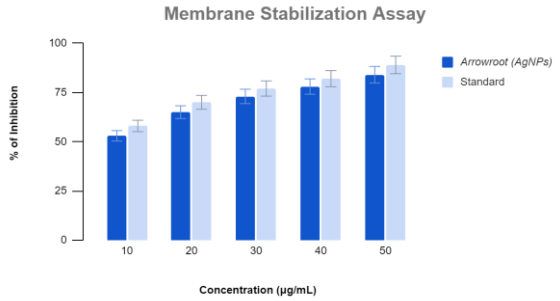

Figure 3. Depiction the Membrane stabilisation assay

4. Discussion

This study aimed to investigate the anti-inflammatory properties of Arrow Root Extract Silver Nanoparticles (AR-AgNPs) using different in vitro models, including the Bovine Serum Albumin (BSA) Denaturation Assay, Egg Albumin Denaturation Assay, and Membrane Stabilization Assay. These assays are commonly utilized to screen potential anti-inflammatory agents, as they replicate processes involved in inflammation, particularly protein denaturation and membrane stabilization.

The BSA denaturation assay relies on the concept that inflammation leads to protein denaturation, a key event in the inflammatory process [11]. Heat-induced denaturation of proteins is a major pathway for inflammation, and BSA is often used as a model protein to measure the anti-inflammatory activity of compounds based on their ability to prevent denaturation [12]. In this study, AR-AgNPs demonstrated a dose-dependent reduction in BSA denaturation. This stabilization effect may be due to interactions between the silver nanoparticles and BSA, possibly forming a protective barrier around the protein molecules. These findings suggest that AR-AgNPs exhibit significant anti-inflammatory properties by preventing protein denaturation, a central feature of inflammation.

The egg albumin denaturation assay, similar to the BSA assay, evaluates the inhibition of protein denaturation. Inflammation-like conditions cause denaturation of egg albumin proteins when exposed to heat or stress. Anti-inflammatory agents are expected to prevent this process [13]. The results of this study show that AR-AgNPs effectively inhibited egg albumin denaturation in a dose-dependent manner. This finding confirms that the nanoparticles possess the ability to stabilize proteins against denaturation, reinforcing their anti-inflammatory potential. The substantial inhibition of egg albumin denaturation further supports the claim that AR-AgNPs can protect proteins from inflammation-induced damage. The membrane stabilization assay assesses the ability of a compound to prevent red blood cell (RBC) lysis under stress conditions [14]. Inflammation often triggers the release of lysosomal enzymes and other pro-inflammatory mediators that destabilize cell membranes, leading to cell lysis and tissue injury [15]. Compounds

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1438-1443 | ISSN:2251-6727

that prevent cell membrane disruption and stabilize the membrane are considered anti-inflammatory agents.

In this study, AR-AgNPs were found to stabilize RBC membranes against hypotonic-induced lysis in a dose-dependent manner. This membrane-protective effect suggests that the nanoparticles can help reduce tissue damage by preventing the release of inflammatory mediators [16]. The membrane stabilization observed in this assay is comparable to that of standard NSAIDs, highlighting the potential of AR-AgNPs to protect cells during inflammation. All three assays consistently demonstrated that AR-AgNPs have strong anti-inflammatory activity. The nanoparticles effectively inhibited protein denaturation and stabilized cellular membranes, both of which are key processes in inflammation. The results from the BSA and egg albumin assays highlight the role of AR-AgNPs in maintaining protein integrity, while the membrane stabilization assay indicates their potential to protect cell membranes from inflammation-induced damage.

However, a study done by Chi NT et al., using AgNPs with Azadirachta indica kernel aqueous extract, showed an anti-inflammatory potential of 69.77% against the standard with potency of 81.15% [17]. The dose-dependent inhibition observed in these assays suggests that the anti-inflammatory activity of AR-AgNPs is closely related to their concentration and their interaction with biological macromolecules. The silver nanoparticles likely interact with proteins and membranes at a molecular level, blocking the pathways that trigger inflammation [18]. Similar types of results were also noted in the study conducted by the Dharman et al., where turmeric silver nanoparticles were used to evaluate the anti-inflammatory properties [19]. Tharani et al., in their study has shown the significant activity of silver nanoparticles in reducing the inflammation [20]. Roy et al., in their study has shown potent anti-inflammatory effects of neem and aloe vera extract silver nanoparticles [21].

Given their ability to inhibit protein denaturation and stabilize cell membranes, AR-AgNPs hold promise for therapeutic applications in treating inflammatory conditions that involve protein degradation and cell lysis. They may serve as an alternative or complementary therapy to conventional anti-inflammatory drugs, especially in cases where synthetic drugs are less effective or cause adverse effects.

5. Conclusion

In conclusion, this study demonstrates that Arrow Root Extract Silver Nanoparticles (AR-AgNPs) possess significant anti-inflammatory activity, as evidenced by their performance in the BSA denaturation, egg albumin denaturation, and membrane stabilization assays. These findings offer a strong foundation for the therapeutic potential of AR-AgNPs in managing inflammation-related disorders. Further research, including in vivo studies and deeper investigations into the underlying mechanisms, is necessary to fully explore the clinical applications of AR-AgNPs as an anti-inflammatory treatment.

References:

1. Chen L, Deng H, Cui H, et al.: Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018, 9:7204-7218. 10.18632%2Foncotarget.23208
2. Bindu S, Mazumder S, Bandyopadhyay U: Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochem Pharmacol. 2020, 180:114147. 10.1016/j.bcp.2020.114147
3. Bruna T, Maldonado-Bravo F, Jara P, Caro N: Silver Nanoparticles and Their Antibacterial Applications. Int J Mol Sci. 2021, 22:7202. 10.3390/ijms22137202
4. Abbasi R, Shineh G, Mobaraki M, Doughty S, Tayebi L: Structural parameters of nanoparticles affecting their toxicity for biomedical applications: a review. J Nanopart Res. 2023, 25:43. 10.1007/s11051-023-05690-w
5. Hano C, Abbasi BH: Plant-Based Green Synthesis of Nanoparticles: Production, Characterization and Applications. Biomolecules. 2021, 12:31. 10.3390/biom12010031
6. Pyasi R: Potential health benefits and industrial scope of arrowroot. Int. J. Adv. Biochem. Res. 2024, 8:282-284. 10.33545/26174693.2024.v8.i3Sd.722
7. Liaqat N, Jahan N, Khalil-Ur-Rahman, Anwar T, Qureshi H: Green synthesized silver nanoparticles: Optimization, characterization, antimicrobial activity, and cytotoxicity study by hemolysis assay. Front Chem. 2022, 10:952006. 10.3389/fchem.2022.952006

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1438-1443 | ISSN:2251-6727

8. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MNM: The crucial roles of inflammatory mediators in inflammation: A review. Vet World. 2018, 11:627-635. 10.14202%2Fvetworld.2018.627-635
9. Ricciotti E, FitzGerald GA: Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011, 31:986-1000. 10.1161/ATVBAHA.110. 207449
10. Arango Duque G, Descoteaux A: Macrophage cytokines: involvement in immunity and infectious diseases. Front Immunol. 2014, 5:491. 10.3389/ fimmu.2014.00491
11. M A, I MA, Ramalingam K, S R: Evaluation of the Anti-inflammatory, Antimicrobial, Antioxidant, and Cytotoxic Effects of Chitosan Thiocolchicoside-Lauric Acid Nanogel. Cureus. 2023, 15:46003. 10.7759%2Fcureus.46003
12. Mendez-Encinas MA, Valencia D, Ortega-García J, et al.: Anti-Inflammatory Potential of Seasonal Sonoran Propolis Extracts and Some of Their Main Constituents. Molecules. 2023, 28:4496. 10.3390/ molecules28114496
13. Derbel H, Elleuch J, Mahfoudh W, Michaud P, Fendri I, Abdelkafi S: In vitro antioxidant and anti-inflammatory activities of bioactive proteins and peptides from Rhodomonas sp. Appl Sci. 2023, 13:3202. 10.3390/app13053202
14. Yesmin S, Paul A, Naz T, et al.: Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clin Phytoscience. 2020, 6:1. 10.1186/s40816-020-00207-7
15. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB: Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014, 20:1126-1167. 10.1089/ars.2012.5149
16. Brusini R, Varna M, Couvreur P: Advanced nanomedicines for the treatment of inflammatory diseases. Advanced drug delivery reviews. 20201, 157:161-78. 10.1016/j.addr.2020.07.010
17. Chi NT, Narayanan M, Chinnathambi A, et al.: Fabrication, characterization, anti-inflammatory, and anti-diabetic activity of silver nanoparticles synthesized from Azadirachta indica kernel aqueous extract. Environmental Research. 2022, 15:112684. 10.1016/j.envres.2022.112684
18. Mikhailova EO: Silver Nanoparticles: Mechanism of Action and Probable Bio-Application. J Funct Biomater. 2020, 11:84. 10.3390/jfb11040084
19. Dharman S, Maragathavalli G, Shanmugam R, Shanmugasundaram K: Biosynthesis of Turmeric Silver Nanoparticles: Its Characterization and Evaluation of Antioxidant, Anti inflammatory, Antimicrobial Potential Against Oral Pathogens an In vitro Study. Journal of Indian Academy of Oral Medicine and Radiology. 2023, 1:299-305. 10.4103/jiaomr.jiaomr_309_22
20. Tharani M, Rajeshkumar S, Al-Ghanim KA, Nicoletti M, Sachivkina N, Govindarajan M: Terminalia chebula-assisted silver nanoparticles: Biological potential, synthesis, characterization, and ecotoxicity. Biomedicines. 2023, 18:1472. 10.3390/biomedicines11051472
21. ROY A, RAJESHKUMAR S, THANGAVELU L: Anti-Inflammatory Activity of Silver Nanoparticles Synthesised Using Hing Oil: An In Vitro Study. International Journal of Pharmaceutical Research (09752366.2020, 2:10.31838/ijpr/2020.SP1.422