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Anti-inflammatory, antioxidant activity, cytotoxic effect of Azadirachta indica mediated selenium nanoparticles

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Abstract

Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) have garnered interest due to their potential anti-inflammatory and antioxidant properties. This study aimed to evaluate the anti-inflammatory and antioxidant activities of A. indica (Se NPs) and their cytotoxic effects using various assays. A. indica (Se NPs) were synthesized and characterized. The anti-inflammatory activity was assessed using BSA, EA, and Membrane Stabilization assays, while antioxidant activity was evaluated using DPPH, H2O2, and FRAP assays. The cytotoxic effect was determined using a brine shrimp lethality assay. A. indica (Se NPs) exhibited dose-dependent anti-inflammatory effects in all assays, with comparable efficacy to diclofenac sodium. The antioxidant potential of A. indica (Se NPs) was demonstrated through scavenging of free radicals and protection against oxidative stress. The cytotoxicity study revealed a dose-dependent decrease in survival rates of brine shrimp nauplii with increasing concentrations of SeNPs. The findings suggest that A. indica (Se NPs) possess significant anti-inflammatory and antioxidant activities, making them potential candidates for therapeutic applications. The dose-dependent response observed in the assays indicates the importance of concentration in eliciting biological effects. The cytotoxicity results highlight the need for further investigation into the safety profile of SeNPs. In conclusion, A. indica mediated selenium nanoparticles exhibit promising anti-inflammatory and antioxidant properties, with a dose-dependent response observed in their biological activities. Further research is warranted to elucidate the mechanisms of action and potential clinical applications of A. indica (Se NPs) while ensuring their safety for therapeutic use.

Keywords: Azadirachta indica, Selenium nanoparticles, Green synthesis, Antioxidant agent, Anti-inflammatory agent

Introduction

Nanotechnology represents a transformative field that harnesses the unique properties of materials at the nanoscale (1-100 nanometers) for innovative applications across diverse sectors. In particular, selenium nanoparticles (SeNPs) have emerged as promising nanomaterials due to their distinctive characteristics and potential uses in various industries, including medicine, environmental science, and electronics.

Selenium, an essential trace element, exhibits intriguing properties at the nanoscale, distinct from its bulk form. Selenium nanoparticles possess exceptional antioxidant activity, making them valuable for combating oxidative stress-related diseases. Moreover, their demonstrated cytotoxic effects against cancer cells suggest potential applications in targeted cancer therapies. Additionally, SeNPs show antimicrobial properties, highlighting their potential in addressing antibiotic-resistant infections.

Green-synthesized selenium nanoparticles (SeNPs) signify a notable stride in nanotechnology, offering eco-friendly, cost-effective, and safe approaches to nanoparticle fabrication. These nanoparticles are produced using plant extracts, which not only serve as reducing agents but also aid in stabilizing the nanoparticles. This synthesis process yields SeNPs with diverse shapes and sizes, influenced by the specific plant parts utilized, including leaf extracts, fruits, seeds, fruit peels, and roots. Green synthesis stands out for its efficacy in removing heavy metals from polluted solutions and soils, showcasing promise for environmental remediation.

Azadirachta indica, commonly known as neem, is a versatile and highly valued tree native to the Indian subcontinent and widely cultivated across tropical and subtropical regions worldwide. Renowned for its medicinal, agricultural, and environmental applications, Azadirachta indica has been integral to traditional and modern practices owing to its diverse beneficial properties.

The neem tree is recognized for its rich array of bioactive compounds, including limonoids, triterpenoids, flavonoids, and azadirachtin, which contribute to its potent antimicrobial, insecticidal, and therapeutic attributes. In traditional medicine, various parts of the neem tree such as leaves, bark, seeds, and oil—are used to treat a range of ailments, including skin disorders, fevers, infections, and gastrointestinal issues.

Beyond its medicinal uses, Azadirachta indica plays a crucial role in agriculture as a natural pesticide and soil conditioner. Neem-based formulations are effective in pest management, promoting soil fertility, and enhancing crop productivity while minimizing environmental impact compared to synthetic chemicals.

In recent years, neem has gained prominence in scientific research and industrial applications, particularly in the development of eco-friendly products such as biopesticides, herbal cosmetics, and pharmaceuticals. Additionally, the utilization of neem extracts in green synthesis processes for nanoparticle production underscores its potential in nanotechnology for creating sustainable and biocompatible materials.

In this study, selenium nanoparticles were synthesized utilizing Azadirachta indica (neem) leaves extract and subsequently evaluated for their in vitro anti-inflammatory and antioxidant activities using various assays. Additionally, the biocompatibility of these selenium nanoparticles was assessed using a brine shrimp lethality assay.

Materials and methods

Preparation of *Azadirachta indica* extract:

In this study, *Azadirachta indica* extract is used as a reducing and capping agent to synthesize selenium nanoparticles. To prepare the extract, 1g of *Azadirachta indica* was measured and added to 100 mL distilled water. Then it was boiled using heating mantle at 60-70 ^{o}C for 20 minutes. The boiled extract was then filtered using Whatmann No:1 filter paper. The filtered extract was kept for selenium nanoparticle synthesis.

Green synthesis of selenium nanoparticles

20mM sodium selenite was measured and added to conical flask containing 60mL distilled water. To that, 40mL filtered *Azadirachta indica* extract was added. The reaction mixture was kept on a magnetic stirrer for about 700 rpm for 48h. The synthesized selenium nanoparticle solution was centrifuged at 8000 rpm for 10 minutes to separate pellet out of supernatant. The collected pellet was stored in an airtight Eppendorff tube for further characterization and biomedical activities.

Antioxidant activity:

DPPH assay

The in vitro DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is commonly employed to assess the antioxidant properties of various compounds, including plant extracts. This method relies

on the compound's capacity to neutralize this free radicals, stable dark-coloured crystalline compounds. The reduction of DPPH radicals into DPPH-H, a colourless or light-yellow compound, serves as an indicator of the antioxidant's ability to neutralize free radicals, evaluating its free radical scavenging activity (RSA).

To create the stock solution, 24 milligrams of DPPH were dissolved in 100 mL of methanol, resulting in a filtrated mixture with an absorbance of approximately 0.973 at 517 nm. Different concentrations of Azadirachta *indica* mediated selenium nanoparticles (10µg/mL-50 µg/mL) were then combined with 3 mL of this DPPH solution, and the mixture was incubated in complete darkness for 30 minutes. Subsequently, absorbance was measured at 517 nm, and the percentage of antioxidant activity was calculated using the formula:

% of antioxidant activity = [(Ac−As) ÷ Ac] × 100,

where Ac represents the control reaction absorbance, and As is the testing specimen absorbance.

H2O2 assay

In the in vitro hydrogen peroxide radical scavenging assay using *Azadirachta indica* mediated selenium nanoparticles as the test substance, the following steps were followed. Hydrogen peroxide (H2O2) was prepared as a stock solution at a concentration of 3% (w/v). Horse radish peroxidase (HRP) was used as the peroxidase enzyme, with a stock solution prepared at a concentration of 1 mg/mL in phosphate buffer (pH 7.4). The substrate solution, containing 4-aminoantipyrine (4-APA) that changes color upon reduction, and phosphate buffer (pH 7.4) as the buffer solution were also prepared. Control solutions without the test substance were included for comparison.

For the test solution, *Azadirachta indica* mediated selenium nanoparticles was dissolved in an appropriate solvent (Distilled water) to achieve the desired concentration (10µg/mL- 50 µg/mL). The assay mixture, comprising hydrogen peroxide, HRP, substrate solution, and the test solution, was prepared in a 96-well plate. The uniform final volume in each well was ensured. The assay mixture was then incubated at 37°C for 30 minutes to facilitate the reduction of hydrogen peroxide by the enzyme and the scavenging of hydrogen peroxide.

After incubation, the absorbance of each well was measured at 504 nm using a spectrophotometer, corresponding to the color change of the substrate solution. Data analysis involved comparing the absorbance values of the test solution wells with the control wells. The percentage of inhibition of the color change was calculated using the formula

[Percentage of Inhibition = (Absorbance of Control - Absorbance of Test Solution) / Absorbance of Control) × 100].

The percentage of inhibition obtained serves as an indicator of the scavenging efficiency. A higher percentage suggests superior antioxidant activity, signifying the ability of the test substance to effectively neutralize or reduce the activity of hydrogen peroxide.

FRAP assay

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely utilized technique for assessing the total antioxidant capacity of biological specimens. In this method, a FRAP reagent is prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3.6H2O solution in a 10:1:1 ratio.

To conduct the FRAP assay, varying concentrations (10µg/mL- 50 µg/mL) of *Azadirachta indica* mediated selenium nanoparticles and standards are dispensed into a 96-well plate in triplicate, approximately 20 µL each. Subsequently, 200 µL of the prepared FRAP reagent is added to each well, and the plate is incubated at 37°C for 30 minutes. The absorbance at 593 nm is then measured for each well using a microplate reader.

The FRAP value for each sample or standard is determined by comparing the absorbance to a standard curve generated using known concentrations of a standard antioxidant, such as trolox. The FRAP assay assesses the capacity of antioxidants in the samples to convert Fe3+ to Fe2+ within the FRAP reagent, resulting in a color change directly proportional to the antioxidant capacity of the sample. This method offers a quantitative measure of the antioxidant activity present in biological specimens, making it a valuable tool for evaluating the overall antioxidant potential of green synthesized nanoparticles, nanocomposites and other substances.

Anti-inflammatory activity:

Anti-inflammatory activity

The green synthesized selenium nanoparticles was comparatively tested for its anti-inflammatory activity using three assays such as Bovine serum albumin denaturation assay, Egg albumin denaturation assay, Membrane stabilization assay

Bovine serum albumin denaturation assay

A solution containing 0.45 mL of bovine serum albumin was prepared by mixing it with 0.05 mL of green synthesized selenium nanoparticles which were present in various concentrations ranging from 10 to 50 µg/mL. Subsequently, the pH of the solution was adjusted to 6.3. The mixture was then incubated at room temperature for a duration of 10 minutes. Following this, it was subjected to a 30-minute incubation period in a water bath at 55°C. For comparison purposes, diclofenac sodium was utilized as the standard group, while dimethyl sulphoxide served as the control. Finally, the samples were analyzed spectrophotometrically at a wavelength of 660 nm.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Egg Albumin denaturation assay

For the egg albumin denaturation assay, a reaction mixture was prepared by mixing 0.2 mL of fresh egg albumin with 2.8 mL of phosphate buffer. To this mixture, Azadirachta indica mediated selenium nanoparticles were added in varying concentrations, ranging from 10 to 50 µg/mL. The pH of the solution was then adjusted to 6.3. The mixture was subsequently incubated at room temperature for a period of 10 minutes. Following this, it was subjected to a 30-minute incubation period in a water bath at 55°C. For comparison, diclofenac sodium was employed as the standard group, whereas dimethyl sulphoxide was utilized as the control. Finally, the samples were analyzed spectrophotometrically at a wavelength of 660 nm.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Membrane stabilization assay

The in vitro membrane stabilization assay evaluated the membrane-stabilizing properties of the compounds. This assay assessed the potential of the Azadirachta indica mediated selenium nanoparticles (10-50 µg/mL) to prevent the disruption of cell membranes and the subsequent release of intracellular contents. The assay utilized tris-HCl buffer, human red blood cells (RBCs), phosphate-buffered saline (PBS), centrifuge tubes, and a UV-visible spectrophotometer.

Preparation of RBC Suspension:

Fresh human blood was collected in a sterile tube with anticoagulants. After centrifuging the blood at 1000 g for 10 minutes at room temperature, the RBCs were separated. The RBCs were washed three times with PBS and resuspended in tris-HCl buffer to create a 10% (v/v) RBC suspension.

Assay Procedure:

1 mL of the RBC suspension was placed into each centrifuge tube, followed by the addition of different concentrations of Azadirachta indica mediated selenium nanoparticles. The tubes were gently mixed and incubated at 37°C for 30 minutes. After centrifuging the tubes at 1000 rpm for 10 minutes at room temperature, the absorbance of the supernatant was measured at 540 nm using a UV-visible spectrophotometer. The percentage inhibition of hemolysis was calculated as follows:

% inhibition = (OD control - OD sample) / OD control × 100

Here, OD control is the absorbance of the RBC suspension without the test compound(s), and OD sample is the absorbance of the RBC suspension with the test compound.

Cytotoxic effect:

Brine shrimp lethality assay

A quantity of 2 grams of iodine-free salt was measured and dissolved within 200 mL of distilled water. Following this, 6 well ELISA plates were utilized, and each well was filled with approximately 10 to 12 mL of saline water. Subsequently, 10 nauplii were introduced gradually into every individual well, each containing varying concentrations (5µg, 10µg, 20µg, 40µg, 80µg) of the green synthesized SeNPs. The plates were then placed under incubation for a duration of 24 hours. Once the 24-hour incubation period had elapsed, the ELISA plates were examined and the count of live nauplii was recorded. The calculation was performed using the subsequent formula:

number of dead nauplii / [number of dead nauplii + number of live nauplii] × 100.

Result

Anti-inflammatory activity

BSA assay

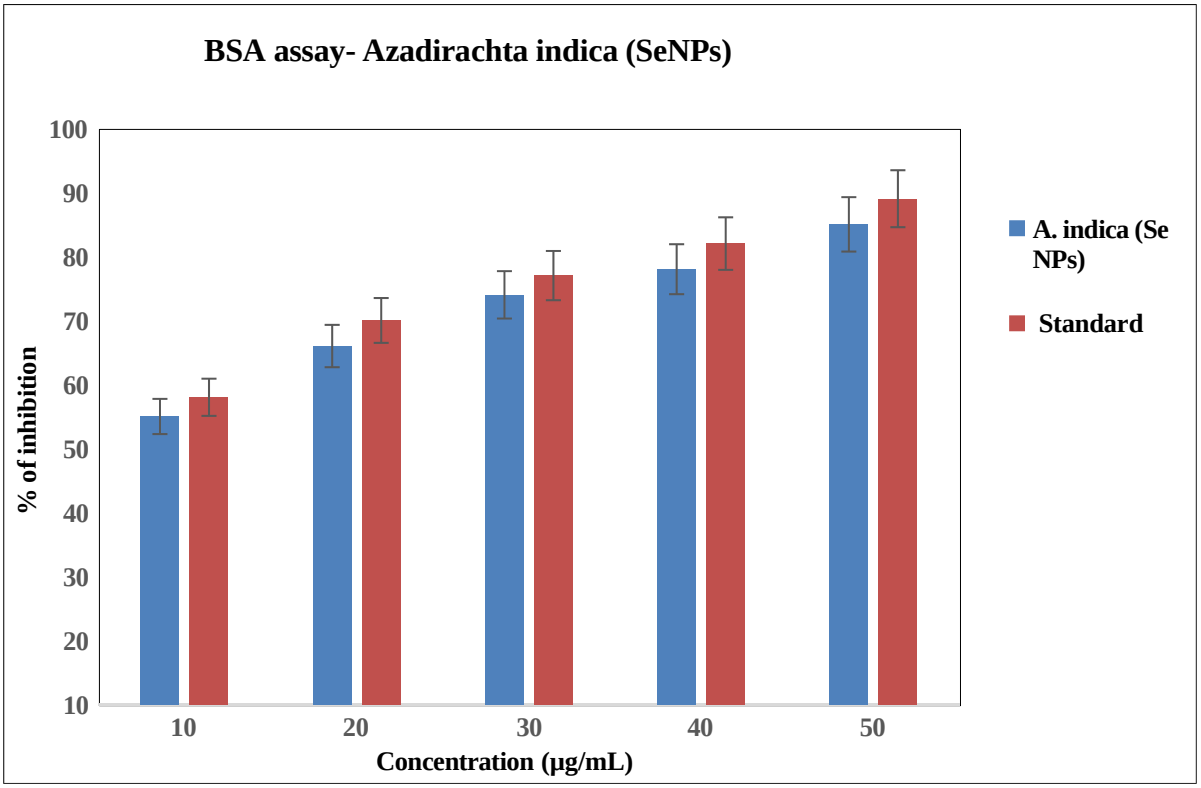

Figure 1: Anti-inflammatory activity of Azadirachta indica mediated selenium nanoparticles using BSA assay

The anti-inflammatory activity of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) was assessed using the Bovine Serum Albumin (BSA) assay and compared with diclofenac sodium as a standard reference drug.

At a BSA concentration of 10 µg/mL, A. indica (Se NPs) demonstrated a 43% inhibition of inflammation, whereas diclofenac sodium exhibited a slightly higher inhibition of 47%. As the BSA concentration increased to 20 µg/mL and 30 µg/mL, both A. indica (Se NPs) and diclofenac sodium showed a dose-dependent increase in anti-inflammatory activity, with A. indica (Se NPs) exhibiting 57% and 68% inhibition, respectively, and diclofenac sodium showing 60% and 72% inhibition at the corresponding concentrations.

Further escalation of the BSA concentration to 40 µg/mL and 50 µg/mL resulted in enhanced anti-inflammatory effects for both A. indica (Se NPs) and diclofenac sodium. At 40 µg/mL BSA, A. indica (Se NPs) achieved 74% inhibition, whereas diclofenac sodium reached 78% inhibition. Similarly, at the highest BSA concentration tested (50 µg/mL), A. indica (Se NPs) exhibited 77% inhibition, and diclofenac sodium displayed 84% inhibition, indicating a notable increase in the anti-inflammatory activity for both compounds.

These findings demonstrate a dose-dependent response of A. indica (Se NPs) and diclofenac sodium in inhibiting inflammation, with diclofenac sodium consistently exhibiting slightly greater potency across all tested concentrations of BSA.

EA assay

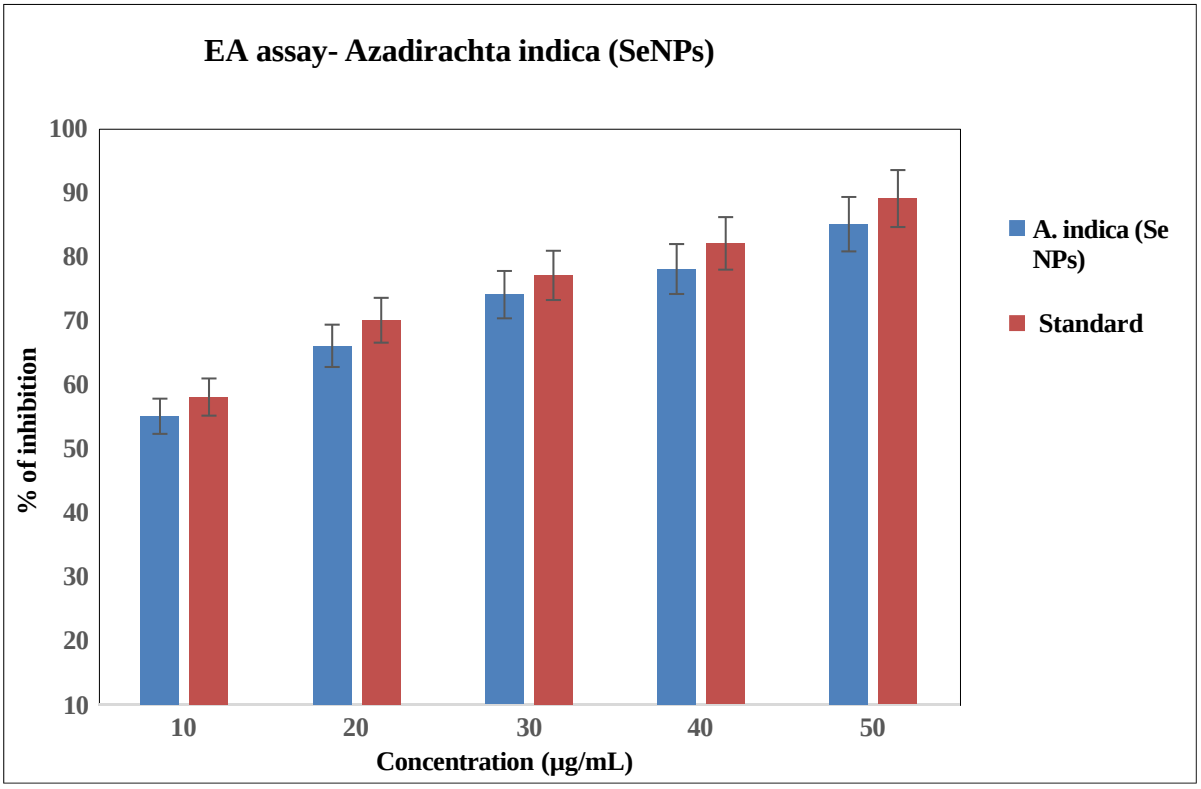

Figure 2: Anti-inflammatory activity of Azadirachta indica mediated selenium nanoparticles using EA assay

The anti-inflammatory activity of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) was investigated using an Egg Albumin (EA) denaturation assay and compared with a standard compound (diclofenac sodium) across various concentrations of EA.

The results demonstrate the percentage inhibition of egg albumin denaturation by A. indica Se NPs and the standard compound across increasing concentrations of EA. As the EA concentration increased from 10 µg/mL to 50 µg/mL, both A. indica Se NPs and the standard compound exhibited dose-dependent anti-inflammatory effects.

At the lowest EA concentration (10 µg/mL), A. indica (Se NPs) showed 53% inhibition of protein denaturation, while the standard compound demonstrated 55% inhibition. This trend continued with increasing EA concentrations, where A. indica (Se NPs) consistently

displayed substantial inhibition of protein denaturation, reaching 76% at 50 µg/mL EA. Similarly, the standard compound showed increased inhibition, reaching 81% at the highest EA concentration tested.

Overall, the data suggest that Azadirachta indica mediated selenium nanoparticles possess notable anti-inflammatory activity as evidenced by their ability to inhibit egg albumin denaturation.

Membrane stabilization assay

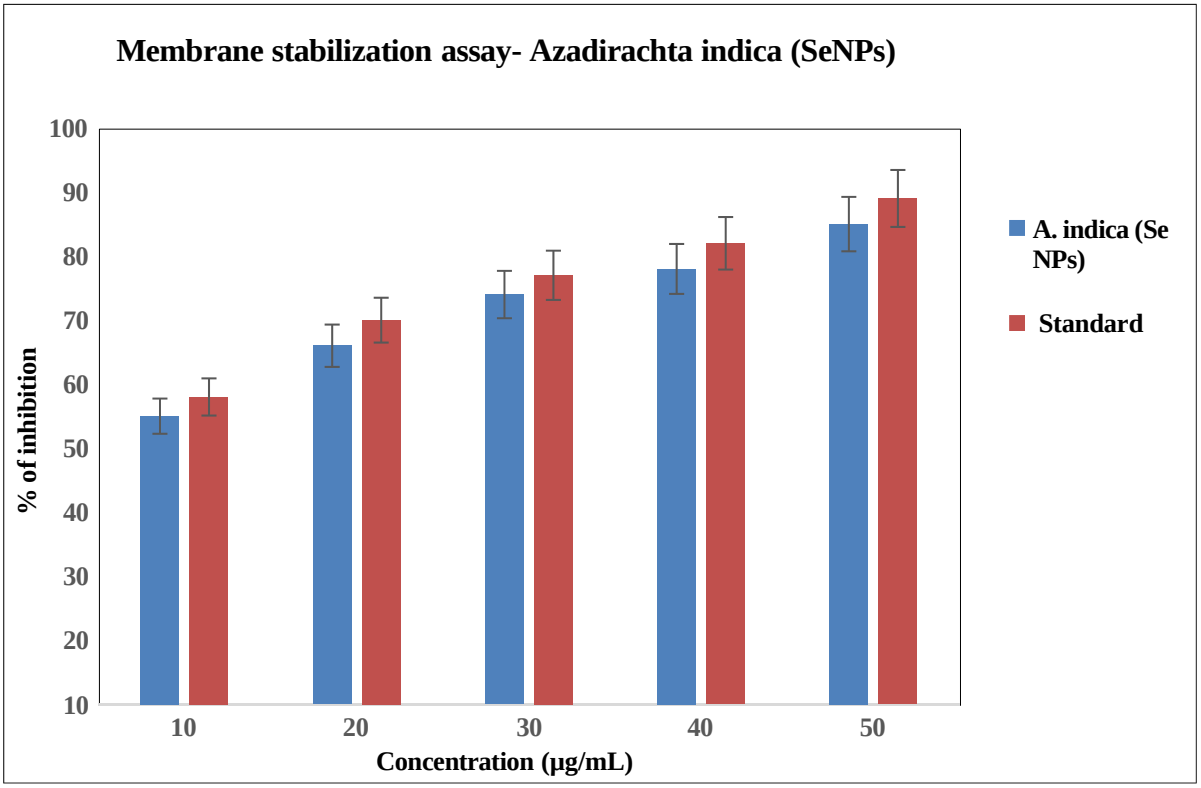

Figure 3: Anti-inflammatory activity of Azadirachta indica mediated selenium nanoparticles using Membrane stabilization assay

The anti-inflammatory activity of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) was evaluated using a Membrane Stabilization Assay (MSA) and compared with diclofenac sodium as a standard compound across various concentrations.

At the lowest concentration (10 µg/mL), A. indica (Se NPs) exhibited 55% inhibition of membrane stabilization, while diclofenac sodium showed 58% inhibition. As the concentration of the assay component increased up to 50 µg/mL, both A. indica (Se NPs) and diclofenac sodium displayed dose-dependent increases in anti-inflammatory activity, with A.

indica (Se NPs) achieving 85% inhibition and diclofenac sodium reaching 89% inhibition at the highest concentration tested.

These results highlight the significant anti-inflammatory activity of A. indica mediated selenium nanoparticles, with efficacy comparable to diclofenac sodium, a widely used anti-inflammatory drug. The dose-dependent response observed suggests the potential therapeutic utility of A. indica (Se NPs) in inflammation-related conditions, supporting further investigation into their mechanisms of action and safety profile for clinical applications.

Antioxidant activity:

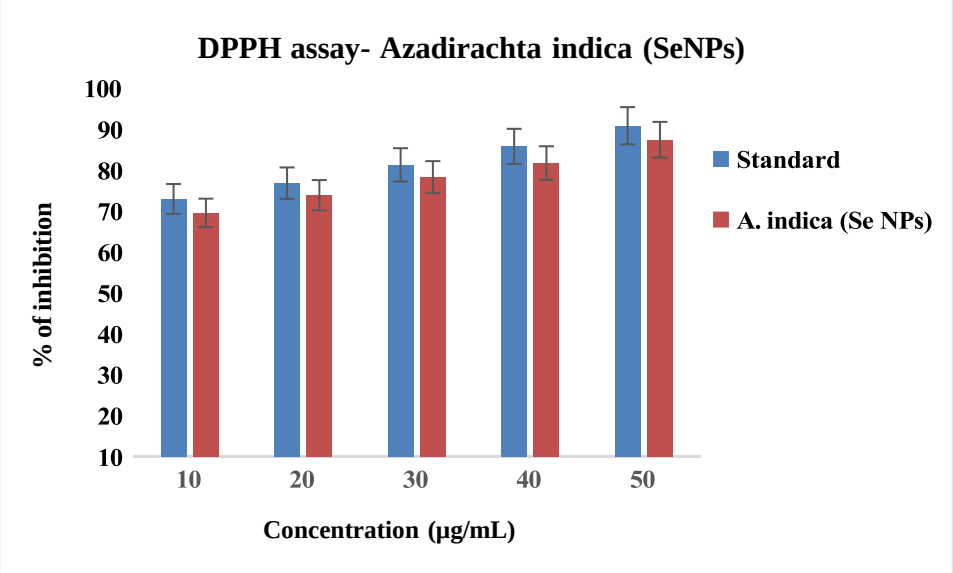

Figure 4: Antioxidant activity of Azadirachta indica mediated selenium nanoparticles using DPPH assay

The antioxidant activity of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and compared with a standard compound (Ascorbic acid) across different concentrations.

Figure 4 presents the percentage inhibition of DPPH radical by Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) and the standard compound across increasing concentrations of DPPH.

At a DPPH concentration of 10 µg/mL, A. indica (Se NPs) exhibited 63.79% inhibition of the DPPH radical, while the standard compound demonstrated 66.25% inhibition. As the

concentration of DPPH increased up to 50 µg/mL, both A. indica (Se NPs) and the standard compound showed dose-dependent increases in antioxidant activity. A. indica (Se NPs) achieved 90.44% inhibition at 50 µg/mL DPPH, whereas the standard compound reached 93.15% inhibition.

These results demonstrate the antioxidant potential of Azadirachta indica mediated selenium nanoparticles, with efficacy comparable to the standard compound (not specified) across the tested concentrations. The dose-dependent response suggests the ability of A. indica (Se NPs) to scavenge free radicals and inhibit oxidative stress, supporting its potential application as a natural antioxidant agent.

H202 assay

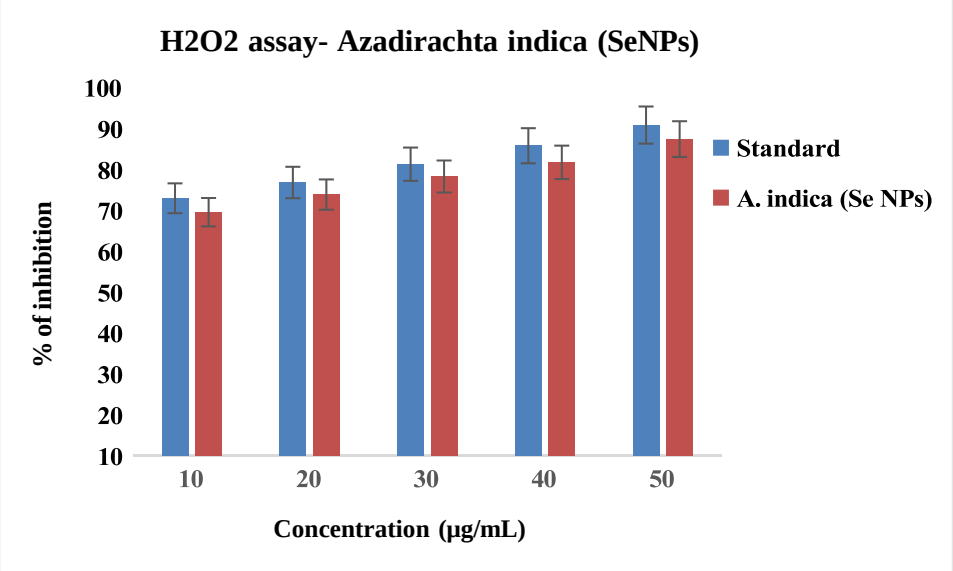

Figure 5: Antioxidant activity of Azadirachta indica mediated selenium nanoparticles using H2O2 assay

The antioxidant activity of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) was evaluated using the H2O2 assay and compared with ascorbic acid as the standard compound across different concentrations of hydrogen peroxide (H2O2).

Figure 5 presents the percentage inhibition of hydrogen peroxide (H2O2) by Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) and ascorbic acid across increasing concentrations of H2O2.

At an H2O2 concentration of 10 µg/mL, A. indica (Se NPs) exhibited 49.6% inhibition of H2O2, while ascorbic acid demonstrated 51.1% inhibition. As the concentration of H2O2 increased up to 50 µg/mL, both A. indica (Se NPs) and ascorbic acid showed dose-dependent increases in antioxidant activity. A. indica (Se NPs) achieved 85.2% inhibition at 50 µg/mL H2O2, whereas ascorbic acid reached 89.9% inhibition.

These results indicate the antioxidant potential of Azadirachta indica mediated selenium nanoparticles, with efficacy comparable to ascorbic acid across the tested concentrations of hydrogen peroxide. The dose-dependent response suggests the ability of A. indica (Se NPs) to scavenge hydrogen peroxide radicals and protect against oxidative stress, supporting its potential application as a natural antioxidant agent.

FRAP assay

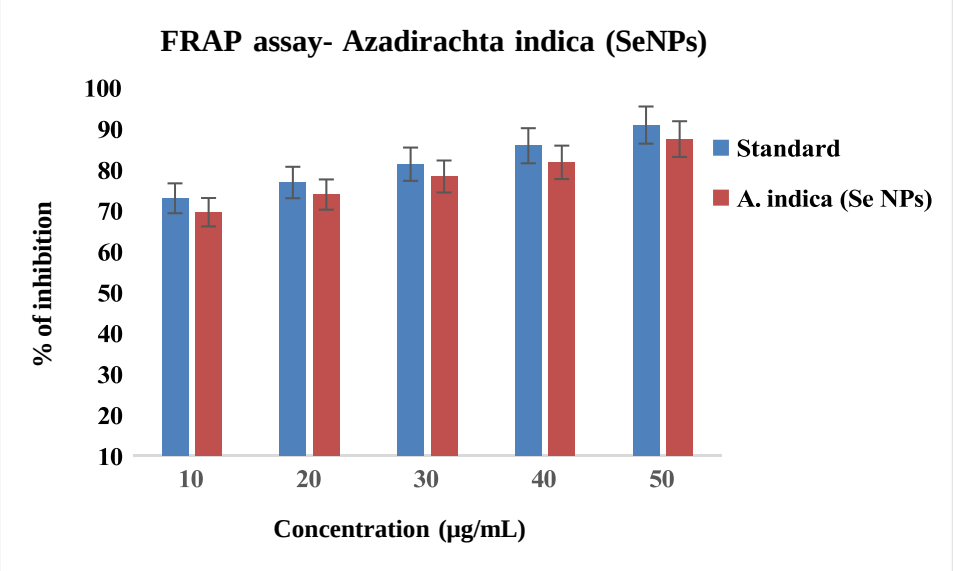

Figure 6: Antioxidant activity of Azadirachta indica mediated selenium nanoparticles using FRAP assay

The antioxidant activity of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) was evaluated using the FRAP (Ferric Reducing Antioxidant Power) assay and compared with ascorbic acid as the standard compound across different concentrations.

Figure 6 presents the percentage inhibition of oxidative stress by Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)) and ascorbic acid across increasing concentrations in the FRAP assay.

At a concentration of 10 µg/mL, A. indica (Se NPs) exhibited 30.42% inhibition of oxidative stress, while ascorbic acid showed 27.21% inhibition. As the concentration increased up to 50 µg/mL equivalents, both A. indica (Se NPs) and ascorbic acid displayed varying degrees of inhibition, with A. indica (Se NPs) showing decreased inhibition compared to ascorbic acid at higher concentrations.

These results demonstrate the antioxidant potential of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)), although the efficacy in inhibiting oxidative stress appears to be lower compared to ascorbic acid across the tested concentrations in the FRAP assay. The varying degrees of inhibition observed highlight the importance of dose-dependency in antioxidant activity and suggest the potential application of A. indica (Se NPs) as a natural antioxidant agent, albeit with different efficacy compared to the standard compound.

Cytotoxic effect

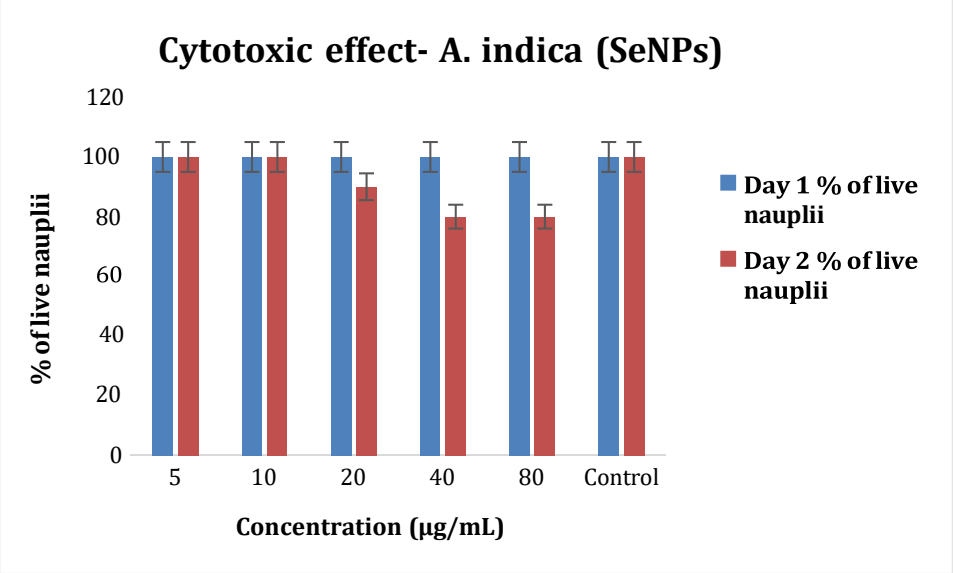

Figure 7: Cytotoxic effect of green synthesized SeNPs using Brine shrimp lethality assay

The cytotoxic effect of green-synthesized selenium nanoparticles (SeNPs) was evaluated using a brine shrimp lethality assay over a two-day period.

On the first day of exposure, all concentrations of SeNPs (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL) showed 100% survival of the brine shrimp nauplii, similar to the control

group (saline water only). This suggests that SeNPs at these concentrations did not cause immediate mortality or adverse effects on the nauplii within the initial 24-hour period.

By the second day, a dose-dependent cytotoxic effect of SeNPs was observed. At 20 µg/mL SeNPs, the survival rate of the nauplii decreased to 90%. At 40 µg/mL and 80 µg/mL SeNPs, the survival rate further decreased to 80%.

These results indicate that higher concentrations of green-synthesized SeNPs have a significant impact on the survival of brine shrimp nauplii over a 48-hour period compared to lower concentrations and the control group. The decrease in survival rates observed with increasing concentrations of SeNPs suggests a potential dose-dependent cytotoxicity of SeNPs towards brine shrimp nauplii.

Discussion:

The results presented in this study demonstrate the anti-inflammatory and antioxidant properties and cytotoxic effect of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)), as evaluated through a series of in vitro assays. These findings suggest the potential therapeutic applications of A. indica (Se NPs) in inflammatory disorders and oxidative stress-related conditions.

The anti-inflammatory effects of A. indica (Se NPs) were assessed using three different assays: Bovine Serum Albumin (BSA) assay, Egg Albumin (EA) denaturation assay, and Membrane Stabilization Assay (MSA). The results indicate that A. indica (Se NPs) exhibit dose-dependent inhibition of inflammatory processes, as evidenced by their ability to reduce protein denaturation and stabilize cellular membranes.

The mechanisms underlying the anti-inflammatory activity of A. indica (Se NPs) could involve inhibition of key mediators of inflammation, such as prostaglandins and cytokines, or modulation of oxidative stress pathways. The observed effects are comparable to diclofenac sodium, a commonly used non-steroidal anti-inflammatory drug (NSAID), suggesting that A. indica (Se NPs) may act through similar pathways to mitigate inflammation.

The significant anti-inflammatory activity of A. indica (Se NPs) highlights their potential as a natural alternative or adjunct to conventional anti-inflammatory therapies. The ability to

inhibit multiple inflammatory pathways, as demonstrated in different assays, underscores the broad-spectrum nature of A. indica (Se NPs) in combating inflammation-associated disorders.

A. indica (Se NPs) exhibited potent antioxidant activity in the DPPH, H2O2, and FRAP assays, indicating their capacity to scavenge free radicals and mitigate oxidative stress. The DPPH assay measures the ability of antioxidants to neutralize stable radicals, while the H2O2 and FRAP assays assess the reduction of hydrogen peroxide and the ferric ion, respectively.

The antioxidant efficacy of A. indica (Se NPs) compares favorably with ascorbic acid, a well-known antioxidant, across different concentrations. This suggests that A. indica (Se NPs) may act as an effective natural antioxidant agent capable of protecting cells from oxidative damage[-26].

Oxidative stress is implicated in the pathogenesis of various diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer. The antioxidant properties of A. indica (Se NPs) hold promise for mitigating oxidative stress-related damage and supporting overall health and wellness.

The cytotoxic effects observed in the brine shrimp lethality assay highlight the importance of dosage optimization and safety assessments for A. indica (Se NPs). While lower concentrations showed no immediate toxicity, higher concentrations resulted in reduced survival rates of brine shrimp nauplii[27-32].

These findings underscore the need for further studies to delineate the therapeutic window of A. indica (Se NPs) and establish safe and effective dosing regimens for clinical applications. Understanding the mechanisms underlying cytotoxicity is crucial for ensuring the safety profile of A. indica (Se NPs) as a potential therapeutic agent.

Limitations and Future Directions

Further investigations are needed to elucidate the precise mechanisms by which A. indica mediated selenium nanoparticles exert their anti-inflammatory and antioxidant effects. This includes identifying specific molecular targets and signaling pathways involved in mediating the observed activities.

Preclinical studies, such as animal models of inflammation and oxidative stress, are essential to validate the efficacy and safety of A. indica (Se NPs) in vivo. Subsequently, clinical trials can assess the translational potential of A. indica (Se NPs) as a novel therapeutic intervention for inflammatory and oxidative stress-related disorders in humans.

Optimization of nanoparticle formulations, such as encapsulation or surface modification of A. indica (Se NPs), may enhance their bioavailability, stability, and therapeutic efficacy. Formulation strategies should be tailored to maximize the delivery and targeting of A. indica (Se NPs) to specific tissues or organs affected by inflammation or oxidative stress.

Conclusion

The study investigated the potential therapeutic properties of Azadirachta indica mediated selenium nanoparticles (A. indica (Se NPs)). A. indica (Se NPs) demonstrated significant anti-inflammatory and antioxidant activities across multiple in vitro assays, including Bovine Serum Albumin (BSA) assay, Egg Albumin (EA) denaturation assay, Membrane Stabilization Assay (MSA), DPPH assay, H2O2 assay, and FRAP assay.

The anti-inflammatory effects of A. indica (Se NPs) were comparable to diclofenac sodium, a standard anti-inflammatory drug, indicating their potential as a natural alternative for inflammation management. Moreover, the potent antioxidant properties of A. indica (Se NPs) suggest their ability to scavenge free radicals and protect against oxidative stress-related damage. However, cytotoxic effects observed in the brine shrimp lethality assay highlight the importance of dose optimization and safety assessments for A. indica (Se NPs) in future studies. Mechanistic investigations are warranted to elucidate the underlying pathways involved in mediating the observed pharmacological effects. Overall, the findings support the therapeutic potential of A. indica mediated selenium nanoparticles as novel candidates for inflammation and oxidative stress-related disorders, paving the way for further preclinical and clinical studies to validate their efficacy and safety in diverse disease contexts.

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