Evaluation of antidiabetic and anti-inflammatory action of selenium nanoparticles mediated through *aspalathus linearis* - An *in vitro* study

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ABSTRACT

Introduction: Selenium nanoparticles (SeNPs) have drawn a lot of interest among researchers because of their distinct impact on antioxidant activity, anti-inflammatory tests, antibacterial activity, and in the treatment of various diseases. A. linearis has shown great findings in biomedical applications because of its physio-chemical compounds such as Aspalathin, orientin, and isoorientin. The increasing demand for eco-friendly and sustainable nanomaterial synthesis has led to the development of green methods utilizing natural resources. The study's main objective is to synthesize green SeNPs using Aspalathus linearis and then test them for cytotoxic, anti-inflammatory, and anti-diabetic properties.

Materials and Methods: A UV-visible spectrophotometer and SEM were used to characterize the green synthesized SeNPs. The anti-inflammatory and anti-diabetic activities of green synthesized SeNPs were measured using the alpha-amylase inhibitory & beta-glucosidase enzyme inhibition assay and the egg albumin, bovine serum albumin, and membrane stabilization assays. A test for the mortality of brine shrimp was used to determine the cytotoxic impact of SeNPs.

Results: A. linearis powder was used for the green synthesis of selenium nanoparticles, which exhibited the highest peak at 440 nm when analyzed using a UV-visible spectrophotometer. The In vitro anti-inflammatory effect of synthesized SeNPs was maximally inhibited by 44-83% in the bovine serum albumin assay 54-79% in the egg albumin assay, and 54-86% in the membrane stabilization assay compared with standard. The inhibition percentage of antidiabetic activity was found to be 50-86% in the alpha-amylase assay and 49-85% in the beta-glucosidase assay when compared to standards at various concentrations. Furthermore, the cytotoxicity impact shows that 70% of brine shrimp were alive at the maximum fixation of 80 µg/mL. Conclusion: The SeNPs showed concentration-dependent anti-inflammatory and anti-diabetic action, and the green synthesized SeNPs demonstrated an excellent anti-inflammatory and anti-diabetic agent. The brine shrimp lethality assay confirmed the SeNPs' biocompatible nature even at high concentrations with less toxicity. Hence the study may enhance SeNPs in developing inflammation drugs and can also be utilized in diabetes management.

KEYWORDS:
Antidiabetic activity, Anti-inflammatory, Green synthesis, Selenite, Biomedical applications

INTRODUCTION

New advances in nanotechnology have prompted creativity in numerous fields, especially the biomedical field, where groundbreaking findings have produced nanoproducts with the potential to treat serious medical conditions. Concepts grounded in "nanotechnology" There is a lot of promise in using atoms and molecules to create useful structures.[1] Nanotechnology has enabled the creation of functional structures such as solid-liquid nanoparticles, dendrimers, liposomes, nanotubes, and nanocrystals.[2] Nanotechnology has the potential to be applied in many different medical fields, such as wound healing, cancer therapy, and diabetes mellitus management. Selenium is found in group 16 of the periodic table and is well known for its photoelectric and semiconductor properties. Additionally, it is used in biological processes, solar cells, rectifiers, and light exposure in photography.[3] Biomedical applications of green synthesized Selenium nanoparticles (SeNPs) include an anti-inflammatory test, antioxidant properties, antibacterial activity, antidiabetic activity, cytotoxic effect, etc.[4] A variety of plants and herbs were used in the synthesis of nanoparticles, which can serve as capping and reducing agents. As the human population grows, inflammatory disorders such as asthma, cardiovascular disease, rheumatoid arthritis, colitis, diabetes, psoriasis, autoimmune disease, lupus, vasculitis, cancer, celiac disease, and chronic obstructive pulmonary disease pose a threat to human health.[5] Previous studies have shown that the anti-inflammatory properties of herbal plants come from their bioactive components, including tannins, alkaloids, flavonoids, saponins, and phytosterols.

However, diabetes is the major cause among the population, and phytochemicals like Polyphenols, terpenoids, coumarin,

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flavonoids, and other compounds that exhibit a decrease in blood glucose levels are responsible for the antidiabetic effect of medicinal plants.[6] Rooibos, or *Aspalathus linearis*, is a native South African plant that is highly valued for its therapeutic qualities. The herb has been used to treat a wide range of illnesses, including inflammation, diabetes, and cancer.[7,8] Numerous secondary metabolites, including flavonoids and polyphenols, are known to be present in Rooibos and have anti-inflammatory, antioxidant, and antidiabetic properties.[9,10] There are several ways to synthesize selenium nanoparticles, including chemical, biological, and physical processes. It has been demonstrated that biological techniques, such as the use of plant extracts as stabilizing and reducing agents, are more economical and environmentally beneficial than chemical and physical techniques.[11]

In this study, SeNPs were synthesized using *A. linearis*. Preliminary analyses were performed on the synthesized SeNPs using a UV-visible spectrophotometer in the range of 250 to 650 nm (nanometer). The anti-inflammatory activity of the synthesized SeNPs was evaluated using membrane stabilization, EA, and BSA assays. The antidiabetic effect was evaluated using alpha-amylase and beta-glucosidase enzyme inhibition assays. Moreover, cytotoxic effects were assessed using the brine shrimp lethality assay.

MATERIALS & METHODS

Plant Extract Preparation:
Powdered red tea (A. linearis) was purchased from a commercial supplier. A weight balance was used to weigh 1 g of A. linearis, which was combined with 100 mL of distilled water. Using a heating mantle, the combined solution was boiled for 15–20 min at 60 °C. A conical flask and glass funnel were used to filter the boiling solution through a Whatman No. 1 filter paper.

Synthesis of SeNPs:
Using a weighing balance, 20 mM sodium selenite was measured and mixed with 50 mL distilled water. The orbital shaker was filled with a mixture of 50 mL sodium selenite solution and 50 mL filtered *A. linearis* extract. After 36 h, the mixed solution was kept in an orbital shaker and analyzed using a UV-visible spectrophotometer. UV absorbance was measured at intervals of 12, 24, and 36-hour intervals. After 36 hours, the solution was centrifuged at 8000 rpm (rotation per minute), and the supernatant was discarded. Pellets were collected and stored for future studies. The steps involved in the green synthesis of SeNPs and their biomedical applications are shown in Figure 1.

Bovine Serum Albumin Denaturation Assay
According to previous studies, 12 bovine serum albumin (0.45 g) and various concentrations of green-synthesized selenium nanoparticles (10-50 µg/mL) were combined in 0.05 mL of the mixture. A pH of 6.3 was maintained. The mixture was incubated for 30 min at 55°C in a water bath for 10 min at room temperature. The standard group was diclofenac sodium, whereas the control group was dimethyl sulfoxide. The samples were examined by spectrophotometry at 660 nm. The following formula was used to obtain the denaturation percentage of the protein:

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

Egg Albumin Denaturation Assay
A gentle stir was given to a mixture containing 2.8 mL of phosphate buffer and 0.2 mL of fresh egg albumin. Various concentrations of *A. linearis*-mediated selenium nanoparticles were added to the reaction concentrations (10-50 µg/mL). A pH of 6.3 was maintained. After ten minutes at ambient temperature, the mixture was incubated for 30 min at 55°C in a water bath. The standard group used was diclofenac sodium, and the control group was dimethyl sulfoxide as stated in the study.[13] After that Spectroscopic analysis was performed at 660 nm using a UV visible spectrophotometer to examine the samples.

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

Membrane Stabilization Assay
A popular method for assessing the ability of a compound to stabilize membranes in a controlled setting is the in vitro membrane stabilization assay. This assay tests the ability of a substance to maintain the integrity of the cell membrane by preventing the disintegration of the membrane and subsequent release of intracellular contents. PBS, centrifuge tubes, Tris-HCl buffer (50 mM, pH 7.4), human red blood cells, different quantities of selenium nanoparticles (10-50 µg/mL), and a UV-Vis spectrophotometer were used in this assay.

An RBC suspension was prepared by collecting human blood and placing it in an anticoagulant-filled sterile tube. Centrifuge the blood at 3000 rpm (rotations per minute) for 10 minutes to separate the red blood cells from other blood constituents. RBCs were washed with (phosphate-buffered saline PBS (Phosphate buffer solution) and the supernatant was discarded. To achieve an RBC suspension of 10% (v/v), RBCs were resuspended in Tris-HCl buffer. Each centrifuge tube was filled with 1 mL of RBC suspension using a pipette. Next, varying quantities of SeNPs were introduced into each tube. After gentle mixing, the tubes were incubated at 37°C. The tubes for ten minutes at room temperature at 2500 rpm (rotation per minute). A UV-visible spectrophotometer at 540 nm was used to determine the absorbance of the supernatant.

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

where the absorbance of the RBC suspension without the test chemical(s) is referred to as the OD control, and the absorbance of the RBC suspension with the test compound present is referred to as the OD sample.[14]

In-vitro Antidiabetic Assay
Alpha-amylase and alpha-glucosidase enzyme inhibition assays were the two methods used to carry out the in vitro anti-diabetic assay.

Alpha-Amylase Inhibitory Assay:
To determine whether alpha-amylase inhibition was present, the amount of maltose released during the experiment was assessed. Various quantities of selenium nanoparticles (10, 20, 30, 40, and 50 µg/mL) were pre-incubated in a 100 µg/mL solution of -amylase (1 U/mL) for the first 30 min. The mixture was allowed to settle at room temperature for five minutes, and then 100 µg/mL of a 1% w/v starch solution was added. The mixture was heated in a water bath for five minutes before adding 100 µL of 96 mM (3, 5-dinitrosalicylic acid solution (DNSA) reagent to stop the reaction. When the same volume of sodium phosphate buffer was substituted, the control was maintained. A steady pH of 6.9 is maintained. The samples were analyzed using a multi-beam spectrophotometer set up at 540 nm and the readings were recorded; acarbose was used as a control, as described in previous studies.[15]

% of inhibition = C-T/C x 100, where C is the control and T is the test sample.

Beta-Glucosidase Enzyme Inhibition
Following mixing with the starch substrate solution (2% w/v maltose or sucrose), the SeNPs solutions at concentrations of 10, 20, 30, 40, and 50 µg/ml were incubated for 5 minutes at 37° C in the presence of 0.2 M pH 8.0 Tris buffer. Beta-glucosidase enzyme (1 µg/ml) was added, and the mixture was incubated for 40 min at 35 °C. The process ended when two milliliters of 6 N HCl was added and acarbose was used as a control.[16]

% of inhibition = C-T/C x 100, where C is the control and T is the test sample.

Brine Shrimp Lethality Assay
200 milliliters of distilled water were used to dissolve two grams of iodine-free salt. Ten to twelve milliliters of saline water were added to each of the six-well ELISA plates. Gradually, 10 nauplii were added into each well at varying concentrations (5 10, 20, 40, and 80 µL) were added to each well. The plates were incubated for an entire day. The ELISA plates were inspected after a 24-hour interval to count the number of live nauplii and calculate the percentage of quantity using the following procedure as mentioned in previous studies.[17] The total number of dead nauplii was equal to the sum of the live nauplii and 100.

$$\text{Percentage of live nauplii} = \frac{\text{No of live nauplii} - \text{No of dead nauplii}}{\text{No of dead nauplii}} \times 100$$

Statistical Analysis
All experiments in this study were performed in triplicate to guarantee the accuracy of the findings. The standard error (SE) was used in the statistical analysis of the measured anti-inflammatory and antidiabetic activity data. A measure of the sample means' variability is provided by the standard error, which enables the evaluation of the accuracy and significance of the results.

SeNPs: Selenium nanoparticles; *A. linearis*: Aspalathus linearis
A) BSA assay
B) EA assay
C) Membrane stabilization assay

Error bars in the graph represent standard error. Two-way ANOVA was used to evaluate the significance. The P value ($P<0.0005$) was statistically significant.

RESULTS

Synthesis of Selenium Nanoparticles and Its Characterization Analysis
SeNPs were synthesized using *A. linearis* extract, and the color change was preliminarily confirmed. After 24 h of incubation, a color change from light orange to dark brown was noted, indicating the reducing and capping ability of the *A. linearis* extract. The color change is shown in Figures 2A and 2B. Three milliliters of the solution were sampled at 12-, 24-, and 36-hour intervals and the decrease in Se ions was then measured using a UV-vis spectrophotometer. Spectral analysis was performed to determine the maximum absorption by measuring the wavelength between 250 and 650 nm. The green synthesized selenium nanoparticles using *A. linearis* exhibited a maximum peak at 440 nm, and a visual representation is provided in Figure 2C. The synthetic SeNPs were spherical with a consistent size distribution, as confirmed by SEM imaging Figure 2D. The nanoparticles were evenly distributed throughout the substrate, with no evidence of aggregation or clustering. Smooth and spherical particles with no visible defects or abnormalities are depicted in the high-resolution SEM images, which offer comprehensive insights into the surface morphology of SeNPs. Given that the nanoparticles were spherical, the synthesis process likely produced monodisperse SeNPs. The homogeneous size distribution of the SEM images suggests that the synthesis process is repeatable.

Anti Inflammatory Assay
Bovine Serum Albumin Denaturation Assay
The anti-inflammatory properties of the synthesized SeNPs were evaluated and compared with those of the reference drug using the bovine serum albumin assay. The concentration-dependent anti-inflammatory effects of SeNPs showed an increasing percentage of inhibition. When the SeNPs were concentrated to a maximum of 50 µg/mL, they had 79% anti-inflammatory activity; at 10 µg/mL, the inhibition percentage was 44%. In contrast, the BSA test revealed that the standard had an anti-inflammatory effect of 47% at a concentration of 10 µg/ml and 84% at the highest concentration of 50 µg/ml. Table IA displays the dose-dependent inhibition of BSA using SeNPs.

EA Assay
An assay for denaturing egg albumin was used to evaluate the anti-inflammatory capabilities of the green-synthesized SeNPs. The results showed that SeNPs had anti-inflammatory action, with 54% inhibition at 10 µg/mL, 65% inhibition at 30 µg/mL, and 79% inhibition at the maximal dose of 50 µg/mL. In contrast, 55% of the anti-inflammatory activity of

Table I: Anti-inflammatory activity of A. linearis mediated selenium nanoparticles using three different In vitro assays

A) BSA assay

Concentration (µg/mL)	10	20	30	40	50
Standard (%)	47.34	60.45	72.56	78.89	84.24
SeNPs (%)	44.24	56.46	69.67	76.45	83.21

B) EA assay

Concentration (µg/mL)	10	20	30	40	50
Standard (%)	55.45	64.34	69.76	72.56	81.34
SeNPs (%)	54.56	62.34	65.72	68.76	79.21

C) MSA assay

Concentration (µg/mL)	10	20	30	40	50
Standard (%)	58.23	70.34	77.21	82.56	89.09
SeNPs (%)	54.67	67.24	74.3	78.54	86.87

Table II: Graphical representation of inhibition percentage of *A. linearis* mediated selenium nanoparticles using A) Alpha-amylase inhibition Assay and B) Beta-glucosidase enzyme inhibition

A)

Concentration (µg/mL)	10	20	30	40	50
Standard (%)	52.23	64.45	76.67	80.12	88.65
SeNPs (%)	50.45	61.34	73.78	77.54	86.12

B)

Concentration (µg/mL)	10	20	30	40	50
Standard (%)	52.56	64.87	76.45	80.12	88.23
SeNPs (%)	49.67	62.34	74.56	76.12	85.67

Table III: Cytotoxic effect of green synthesized SeNPs using brine shrimp lethality assay

Concentration (µg/mL)	Control	% of live nauplii
5	100	90
10	100	80
20	100	80
40	100	70
80	100	60

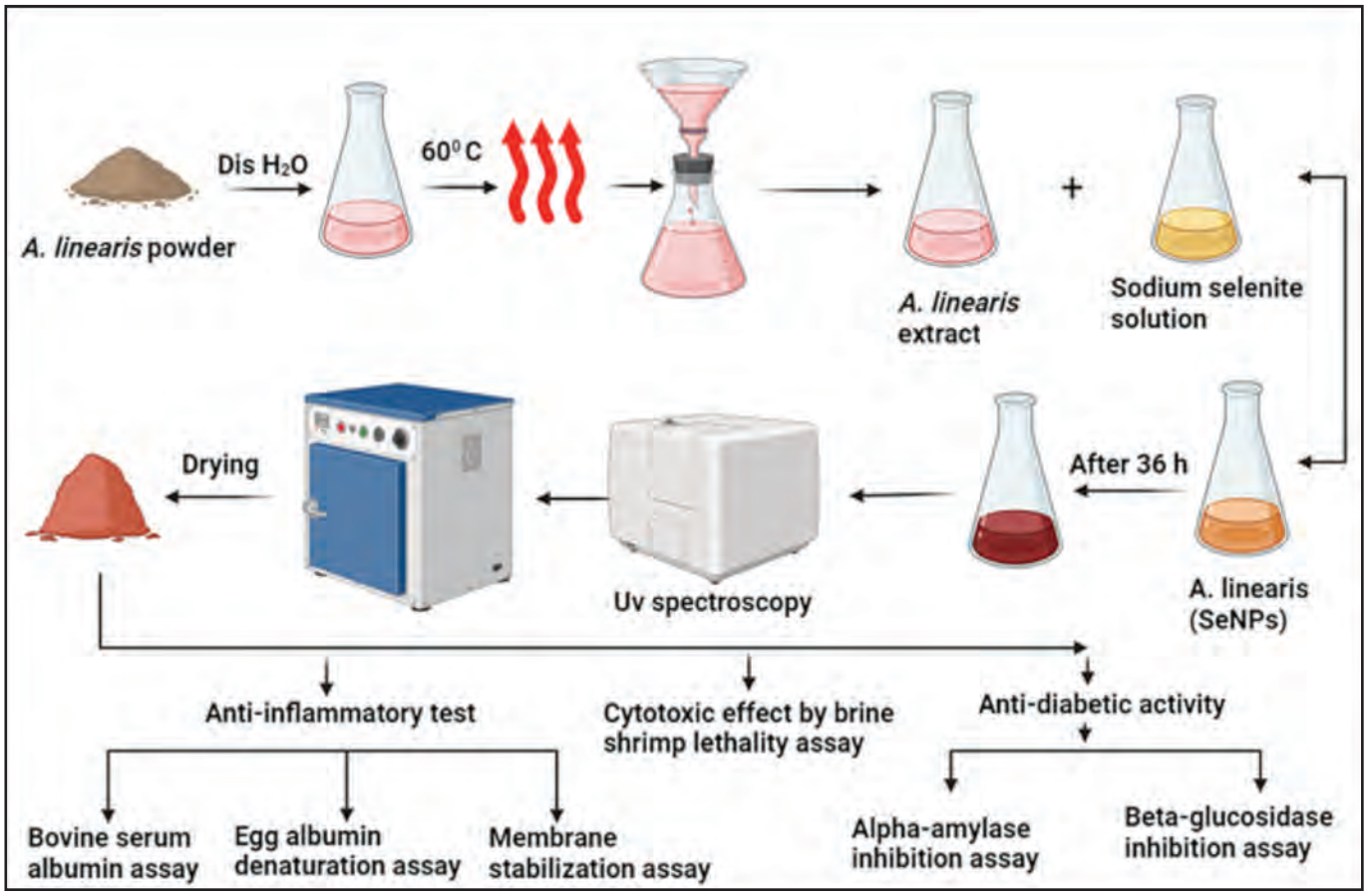

Fig. 1: Graphical illustration of the overall synthesis of selenium nanoparticles using *A. linearis* Anti-inflammatory assay

the standard was demonstrated by the BSA test at 10 µg/mL, 69% at 30 µg/mL, and 84% at the highest dosage of 50 µg/mL. The readings are listed in Table IB.

Membrane Stabilization Assay

Table IIIC shows a visual representation of the inhibition % of green-synthesized SeNPs as determined by a membrane stabilization experiment. *A. linearis*-mediated SeNPs' anti-inflammatory potential was contrasted with that of a common medication (diclofenac sodium). The produced nanoparticles were evaluated at 10, 20, 30, 40, and 50 µg/mL and compared to the standards. The results indicated that the percentage inhibition of SeNPs was 55% at 10 µg/mL, 74% at 30 µg/mL, and 86% at 50 µg/mL. In contrast, the standard indicated that the percentage of inhibition was 58% at 10 µg/mL, 77% at 30 µg/mL, and 89% at the highest concentration of 50 µg/mL. The overall inhibition percentage of SeNPs is notable, and the percentage of inhibition is shown in Table IC.

In vitro Antidiabetic Assay

Alpha-Amylase Assay

Table 2A illustrates the concentration-dependent suppression of A. linearis-mediated SeNPs, with percentages of 50, 61, 73, 77, and 86% at concentrations of 10, 20, 30, 40, and 50 µL, respectively. In contrast, at equivalent doses, conventional acarbose demonstrated a percentage of inhibition ranging from 52% to 88%, which illustrates the potential of SeNPs-mediated *A. linearis* in modifying α-amylase activity, as demonstrated by these studies.

Beta-Glucosidase Inhibition Assay

SeNPs exhibited concentration-dependent inhibition in the β-glucosidase enzyme assay at different concentrations (10, 20, 30, 40, and 50 µL), with corresponding inhibition percentages of 49, 62, 74, 76, and 85%, respectively. Similar concentration-dependent inhibitory patterns, with percentages ranging from 52 to 88%, are shown in the standard reference. These results highlight the antidiabetic potential of SeNPs, which may explain their observed β-glucosidase-inhibitory actions. Compared to the standard drug acarbose, the β-glucosidase-inhibitory activities of SeNPs synthesized using *A. linearis* were enhanced in a dose-dependent manner, as shown in Table IIB.

Cytotoxic Effect

The cytotoxic effect was assessed using a brine shrimp lethality assay, and the results showed that 100% live nauplii were present in each well on day 1. On day 2, 100% of live nauplii was noted at a concentration of 5 µg/mL, 90% at 10µg/mL and 20 µg/mL, 80% at 40 µg/mL, and 70% of live nauplii were present at a maximum concentration of 80 µg/mL, where the nauplii with salt water was used as a control. Overall, the SeNPs synthesized using *A. linearis* showed lower toxicity, as shown in Table III.

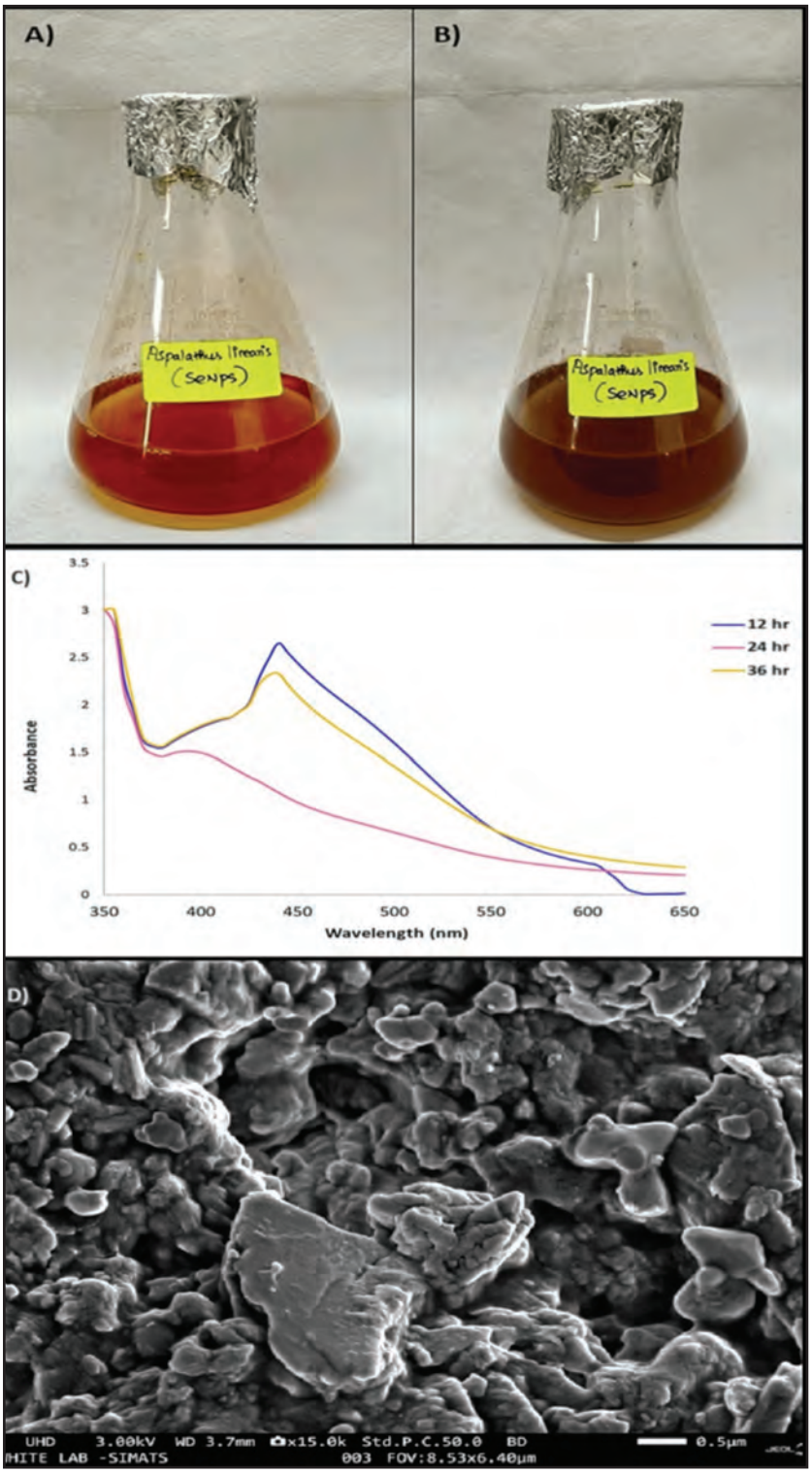

Fig. 2: A) Synthesis of SeNPs using A. linearis before incubation B) After incubation of 24 h C) UV absorbance of *A. linearis* mediated SeNPs D) SEM analysis of SeNPs

DISCUSSION

In the present study, *A. linearis* was used to synthesize selenium nanoparticles in an environmentally friendly manner. The maximum peak of the synthesized SeNPs was analyzed at 440 nm using a UV-visible spectrophotometer, which was preliminarily confirmed. The synthesized *A. linearis*-mediated selenium nanoparticles showed 79% anti-inflammatory effects at a concentration of 50µg/mL and 42% anti-inflammatory effects at a concentration of 10µg/mL using the bovine serum albumin test. The maximal percentage of inhibition in the egg albumin assay was 53% at 10µg/mL and 78% at 50µg/mL. Compared to standards,

the maximum absorbance in the membrane stabilization assay was 86%. Furthermore, at 50 µg/mL, the Alpha-Amylase Assay showed 86% anti-diabetic activity. By contrast, the standard displayed approximately 88% inhibition. In the beta-glucosidase assay, the percentage of inhibition was 85% at the maximum concentration (50 µg/mL). These results demonstrate the effectiveness of the herbal plant in enhancing its antidiabetic properties in addition to its dose-dependent behavior. The above information points to a possible direction for developing cutting-edge diabetic treatment plans utilizing SeNPs. These results demonstrate the inhibitory potential of the green-synthesized SeNPs. In addition, using the brine shrimp lethality assay, the cytotoxic impact revealed that 70% of the nauplii remained alive at the highest dose of 80 µg/mL, which is less toxic.

An herbal combination of *C. sativus, C. macroptera*, and glycerol extract was prepared for prior investigation; its anti-inflammatory and antioxidant qualities were comparable to those of the standards. Comparatively, SeNPs showed comparable activity when tested for three criteria of the standard.[18] SeNPs made with arrowroot were examined for their cytotoxic and anti-inflammatory properties. Evidence suggests that an increase in concentration may enhance anti-inflammatory activities. The cytotoxic impact results indicated that at a concentration of 30 µg/mL, only one nauplii remained alive. Similarly, SeNPs revealed the presence of 7 live nauplii at an 80 µg/mL concentration.[19]

The anti-diabetic effects of SeNPs biosynthesized by Fagonia cretica were studied. SeNPs demonstrated dose-dependent inhibition of α-glucosidase and α-amylase at concentrations ranging from to 62-1000 µg mL-1, with IC50 values of 92 and 100 µg mL–1, respectively, using α-amylase inhibition and beta-amylase inhibition assays.[20] In vitro and in vivo studies were conducted to investigate the effects of dextrin-stabilized Se nanoparticles (SeNPs), which have a size of 64 ±0.158, as a strong antioxidant with lower toxicity. SeNPs showed strong anti-inflammatory effects and significantly ($p<0.05$) decreased the markers associated with arthritis at a dose of 250 µg/kg body weight. At 500 µg/kg b.w., the enzymatic antioxidant levels in the liver, kidney, and spleen were significantly ($p<0.05$) restored, while CRP returned to normal at a dose of 100 µg/kg b.w. SeNPs were used to identify concentration-dependent inhibition of the α-glucosidase enzyme. At a dose of 1000 µg/mL, the highest relative inhibition observed in this study was 19.26%. By contrast, the acarbose-treated control group showed a significant drop-in enzyme activity, with a concentration of 100 µg/mL.[21,22]

CONCLUSION

The synthesized *A. linearis*-mediated selenium nanoparticles exhibited a very similar percentage of inhibition of anti-inflammatory activity when compared with the standard drug. The effect of selenium nanoparticles synthesized using *A. linearis* should be further studied and utilized as a potential component of anti-inflammatory drugs. The anti-diabetic activity shows that the synthesized SeNPs have a huge impact and may be used in the treatment of diabetes as well as other biological applications. However, the cytotoxicity assay performed using brine shrimp lethality assay showed less toxicity. Overall, SeNPs synthesized using *A. linearis* have great potential for future biomedical applications.

CONFLICT OF INTEREST

The authors declare no conflicts of interest that would prejudice the impartiality of this scientific work.

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