Original Article

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Pharmacology Section

Green Synthesis of *Camellia sinensis*-mediated Selenium-doped Vitamin E and Chitosan Nanoparticles along with Evaluation of their Anti-inflammatory and Anticancer Activity: An Ex-vivo Study

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ABSTRACT

Introduction: Green synthesis is an eco-friendly and sustainable approach for developing bioactive compounds with enhanced therapeutic potential. The biomaterials used in the present study are known for their antioxidant, biocompatible and therapeutic properties. The green synthesis of *Camellia sinensis* extract-mediated selenium-doped vitamin E and chitosan may contribute to better therapeutic activity.

Aim: To synthesise *Camellia sinensis*-mediated selenium-doped vitamin E and chitosan nanoparticles and to leverage their synergistic anti-inflammatory and anticancer properties.

Materials and Methods: This ex-vivo study was conducted at AMR and Nanotherapeutics Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, from April 2024 to June 2024. The biosynthesis of *Camellia sinensis*-mediated Selenium-Doped Vitamin E and Chitosan (SeNPs-VitE/Chi) was performed and confirmed by Ultraviolet (UV)-visible spectroscopy. Further characterisation was conducted using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) analysis. Additionally, the anti-inflammatory activity was evaluated using the bovine serum albumin denaturation assay, followed by a cytotoxicity study on the lung cancer A549 cell line. The experiments were carried out in triplicate and the results were expressed as mean±Standard Deviation (SD). GraphPad Prism version 8 was utilised for statistical analysis. The Student's t-test was employed to determine statistical significance with a p-value of <0.05 for Control vs. Se/Vitamin E + chitosan nanoparticles in the experiments.

Results: The UV-Visible spectroscopy exhibited a characteristic peak at 310 nm for the Selenium Nanoparticles (SeNPs), accompanied by a visible colour change. SEM revealed rod-shaped structures with lengths ranging from 250 to 550 nm. Fourier Transform Infrared (FTIR) spectroscopy identified molecular bonds corresponding to C-I stretching, C-H, C-O and C=O groups in the SeNPs-VitE/Chi. The nanoparticles demonstrated a peak anti-inflammatory activity of 52.6% at a concentration of 100 µg/mL, although diclofenac, showed a higher activity of 65.1%. Cytotoxicity studies revealed an IC50 value of 121 µg/mL against the A549 lung cancer cell line, underscoring the potential biomedical applications of these nanoparticles.

Conclusion: The present study highlights the potential of green-synthesised SeNPs-VitE/Chi as a promising therapeutic agent with anti-inflammatory and anticancer properties, paving the way for future biomedical applications.

Keywords: Albumin denaturation assay, Biomaterials, Cell viability assay, Selenium nanoparticles

INTRODUCTION

Nanotechnology has revolutionised the field of biomedical sciences by providing innovative solutions for disease diagnosis, treatment and prevention [1]. Among the various types of nanomaterials, SeNPs have garnered significant attention due to their unique properties, including high biocompatibility [2]. However, conventional synthesis methods for nanoparticles often involve toxic chemicals, harsh conditions and high energy consumption, which limit their scalability and safety for biomedical applications. In this context, green synthesis has emerged as a sustainable and eco-friendly alternative for nanoparticle production [3,4].

Green synthesis employs natural resources such as plant extracts, microorganisms, or biomolecules to reduce and stabilise nanoparticles. This method is not only environmentally friendly but also imparts additional biological activity to the nanoparticles due to the presence of bioactive compounds from natural sources [5]. *Camellia sinensis* (*C. sinensis*), commonly known as green tea, is a widely studied plant for green synthesis due to its abundance of polyphenols, catechins, flavonoids and other phytochemicals. These compounds act as natural reducing and capping agents, enabling the synthesis of nanoparticles while enhancing their bioactivity. Green tea is well-documented for its antioxidant, anti-inflammatory and anticancer properties, making it an excellent candidate for developing biofunctional nanoparticles [6].

Selenium is an essential trace element involved in various physiological processes, including the regulation of oxidative stress, immune response and thyroid hormone metabolism. Selenium compounds have shown potential in preventing and treating cancer, inflammation and other chronic diseases [7,8]. Selenium nanoparticles (SeNPs), in particular, offer a controlled release of selenium ions and reduced toxicity compared to bulk selenium or selenium salts [9]. However, the therapeutic efficacy of SeNPs can be further enhanced by functionalising them with bioactive molecules or polymers.

Vitamin E, a lipid-soluble antioxidant, is well-known for its ability to neutralise free radicals and protect cellular membranes from oxidative damage [10]. Incorporating vitamin E into SeNPs can improve their stability and antioxidant potential, offering a synergistic effect against oxidative stress and inflammation. Similarly, chitosan, a natural polysaccharide derived from chitin, is widely recognised

for its biocompatibility, biodegradability and antimicrobial properties. Functionalising SeNPs with chitosan can enhance their stability, biocompatibility and targeted delivery to diseased tissues [10,11].

The present study addresses the need for eco-friendly and biocompatible therapeutic agents by utilising green synthesis to develop *Camellia sinensis* mediated selenium-doped vitamin E and chitosan nanoparticles. The novelty lies in the synergistic combination of these biomaterials, enhancing stability, bioavailability and therapeutic efficacy against inflammation and cancer, offering a sustainable alternative to conventional treatments. The synthesised nanoparticles were systematically characterised to evaluate their physicochemical properties, stability and bioactivity. Furthermore, their therapeutic efficacy was assessed through anti-inflammatory and anticancer studies, aiming to explore their potential in addressing critical health challenges [12,13]. By integrating green chemistry principles with the therapeutic benefits of *Camellia sinensis*, selenium, vitamin E and chitosan, this research aims to contribute to the development of sustainable and effective nanomedicine [14].

The present study highlights the potential of green-synthesised SeNPs-VitE/Chi using *Camellia sinensis* as a promising therapeutic agent with anti-inflammatory and anticancer properties, paving the way for future biomedical applications.

MATERIALS AND METHODS

The present ex-vivo study was conducted at the AMR and Nanotherapeutics Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, from April 2024 to June 2024, after obtaining approval from the Scientific Review Board (SDC/RO8/2024).

Study Procedure

Chemicals: Selenium dioxide (SeO_2), sodium selenite (Na_2SeO_2), vitamin E (CAS No. 10191-41-0), chitosan (CAS No. 9012-76-4), Bovine Serum Albumin (BSA) and diclofenac sodium were obtained from SRL Laboratories in Mumbai, India. The National Centre for Cell Science (NCCS), located in Pune, India, supplied the lung cancer A549 cell line.

Extraction of the plant sample: Ten grams of commercially available powder of *C. sinensis* was added to 200 mL of sterile distilled water. The mixture was subjected to autoclaving using the autoclave-assisted method and then filtered with Whatman No. 1 filter paper.

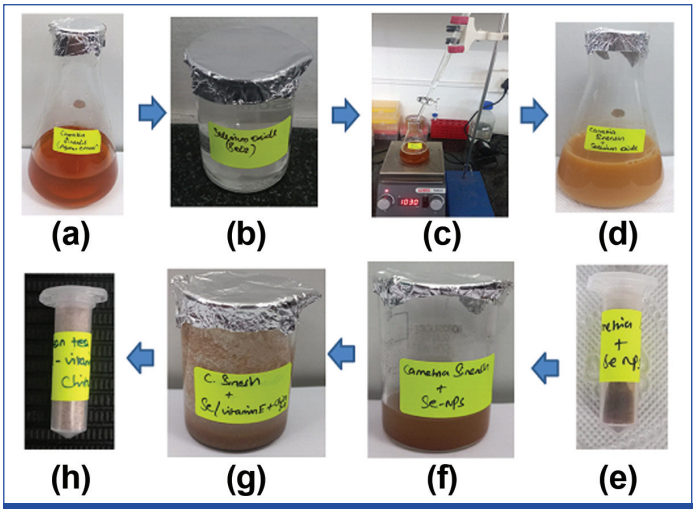
Biosynthesis of SeNPs doped vitamin E, chitosan:

Biosynthesis of SeNPs: The prepared aqueous extract of *C. sinensis* was placed in a conical flask. A 25 millimolar (25 mM) of SeO_2 was taken in a burette. Using the titration method, SeO_2 was added dropwise to the extract and the pH of the solution was maintained between 7 and 8. The colour of the mixture changed from red to brown during overnight incubation at 180 rpm in an orbital shaker. The final solution was subjected to lyophilisation and the produced SeNPs were collected in powder form.

Preparation and stabilisation of SeNPs with chitosan: One gram of chitosan was dissolved in 100 mL of 1% (v/v) acetic acid solution and stirred at room temperature for 12 hours until a homogeneous mixture was obtained. Any undissolved particles were removed by filtration. The prepared chitosan was added to the SeNPs suspension in a 1:1 ratio. The mixture was stirred at room temperature for two hours to allow proper coating of SeNPs with chitosan.

Doping with vitamin E: Vitamin E was dissolved in ethanol to obtain a final concentration of 1 mM and was added dropwise to the chitosan-stabilised SeNPs under gentle stirring. The solution was then subjected to lyophilisation and the produced SeNPs were collected in powder form, as shown in [Table/Fig-1].

Characterisation techniques: The maximum absorbance of green synthesised SeNPs-VitE/Chi was measured using UV-visible



[Table/Fig-1]: Overview of biosynthesis of SeNPs doped vitamin E, chitosan using *C. sinensis*: a) Aqueous extract of *C. sinensis*; b) Selenium dioxide; c) Titration process; d) Synthesised SeNPs; e) Power form of SeNPs; f, g) SeNPs doped vitamin E, chitosan; h) Powder form of SeNPs-VitE/Chi.

spectroscopic analysis (Jasco V-730) over a wavelength range of 200-600 nm [15]. Fourier Transform Infrared (FTIR) spectroscopy (Bruker) was used to identify the functional groups and chemical bonds present in the SeNPs-VitE/Chi. To prepare the sample, it was mixed with Potassium Bromide (KBr) and then pressed into pellets [16]. The spectra were recorded in the range of 4000-500 cm^{-1}. Phase expansion and crystalline nature of SeNPs-VitE/Chi were analysed by observing Cu-Kα radiation (λ=1.5406 Å) using X-ray Diffraction (XRD) with a Bruker D8 Advanced diffractometer operating at 40 kV and 30 mA. Diffraction patterns over a 2θ range of 10° to 80° were obtained at a scanning speed of 2°/min [17]. The size and morphology of the synthesised SeNPs-VitE/Chi were analysed using Scanning Electron Microscopy (SEM). To improve conductivity, the samples were sputter-coated with a thin layer of gold after being mounted on conductive tape. A 3 kV accelerating voltage was used for imaging and the scale bars ranged from 200 nm to 1 µm. Acquiring X-ray spectra and mapping images at different magnifications and analytical sites allowed for an analysis of the elemental composition and distribution in the sample. The quantitative elemental analysis of the data was carried out using EDX software.

Albumin denaturation assay: A 96-well microtiter plate was used for the albumin denaturation assay to evaluate the anti-inflammatory properties. A 1% BSA solution was used to treat each well. One well was designated as a blank, devoid of samples, while the others received SeNPs-VitE/Chi at doses ranging from 20 to 100 µg/mL. A standard row contained diclofenac sodium and 1% BSA. After 15 minutes at room temperature, the plate was incubated for 20 minutes at 55°C. Following incubation, Absorbance (Abs) at 660 nm was measured using a microplate reader. The following formula was used to determine the percentage of BSA denaturation inhibition:

Percentage (%) of inhibition=Abs of control-Abs of sample/Abs of control×100

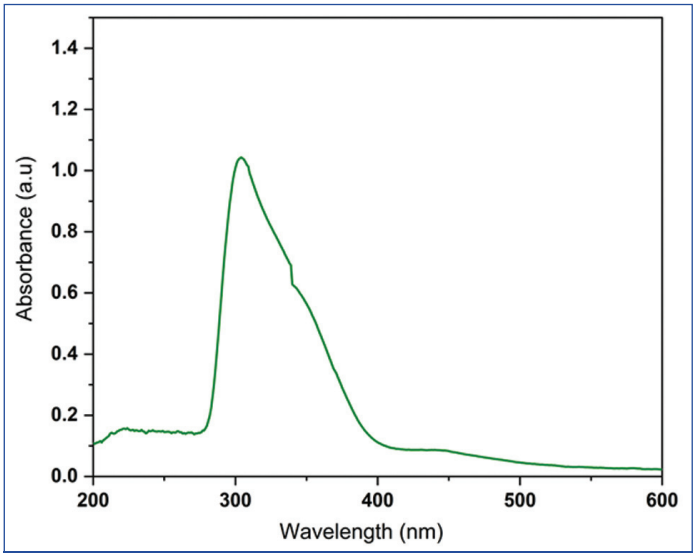
Cytotoxicity activity: To achieve 70% confluency, lung cancer cells were plated at a density of 6000 cells per well in a 96-well plate and incubated in a CO_2 environment for 24 hours. Various concentrations of SeNPs-VitE/Chi were subsequently introduced into the cells. Following the 24-hour treatment period, MTT solution was added and the cells were then incubated in the dark for an additional three hours. After incubation, the supernatant was carefully discarded and the resulting crystals were solubilised by adding 100 µL of Dimethyl Sulfoxide (DMSO). Measurements of absorbance were recorded at 490 nm using a microplate reader. Changes in the morphology of the cells, whether treated or untreated with SeNPs-VitE/Chi, were examined using an inverted light microscope (Euromex, Arnhem, Netherlands).

STATISTICAL ANALYSIS

The results were expressed as mean±standard deviation and each experiment was carried out in triplicate. GraphPad Prism version 8 was utilised for the analysis. The Student's t-test was employed to determine statistical significance, with $p<0.05$ for control vs. Se/Vitamin E+chitosan nanoparticle.

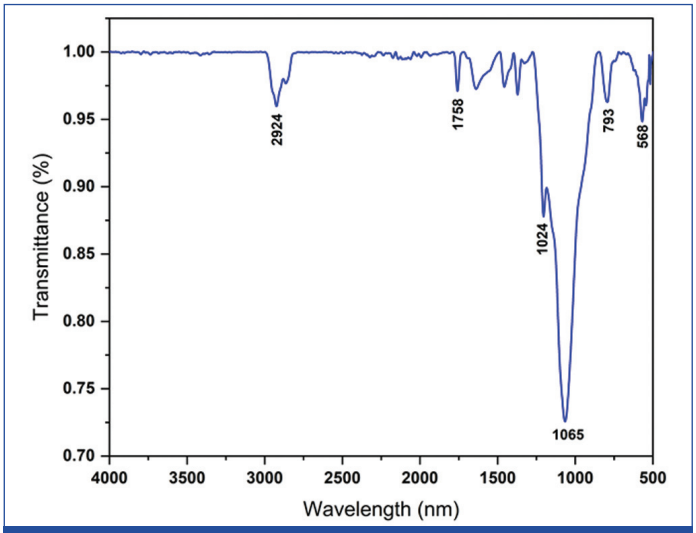
RESULTS

UV-Visible spectra analysis: UV-Visible spectroscopy is frequently used to verify the formation and assess the optical properties of synthesised nanoparticles. The characteristic absorption peaks for SeNPs typically occur around 310 nm due to the excitation of surface plasmon vibrations in the nanoparticles [Table/Fig-2]. These absorption peaks in the UV-Vis spectrum are a clear indication of SeNPs formation through the biosynthesis process.



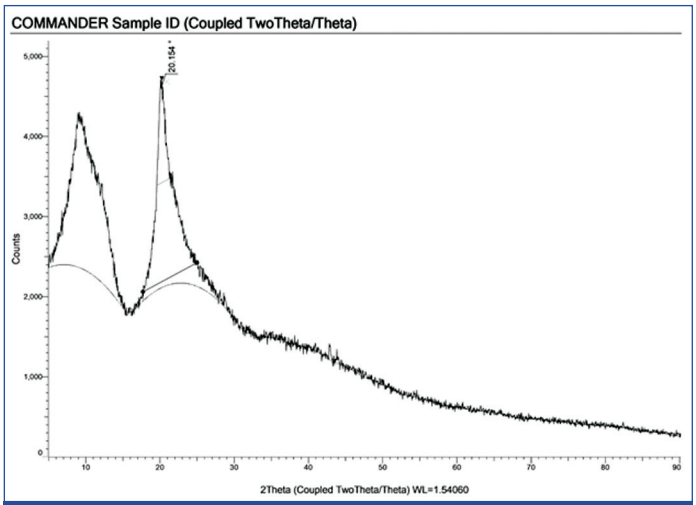
[Table/Fig-2]: UV-Visible spectra of synthesised SeNPs-VitE/Chi.

Fourier transform infrared spectroscopy: FTIR is a useful method for analysing the chemical composition and interactions of synthesised nanoparticles. A wide spectrum of infrared light is passed through the sample during the analysis and certain wavelengths of this light are absorbed by various chemical bonds, causing them to vibrate. In the synthesised SeNPs-VitE/Chi [Table/Fig-3], the spectrum revealed distinct functional groups, with peaks observed at 568, 793, 1065, 1758 and 2924 cm^{-1}. These peaks correspond to functional groups such as C-I stretching, C-H, C-O and C=O, respectively. These characteristic peaks confirm the successful functionalisation of the SeNPs-VitE/Chi and demonstrate the chemical interactions within the nanoparticle structure.



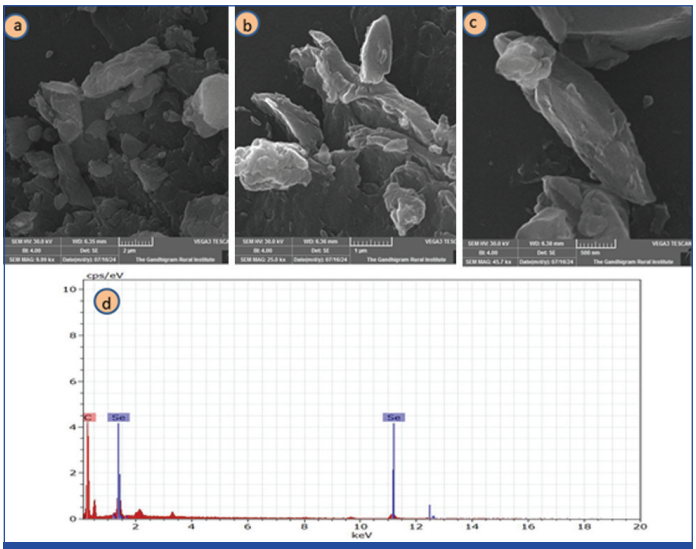
[Table/Fig-3]: Showing FTIR absorbance spectrum of green synthesised SeNPs-VitE/Chi.

X-Ray Diffraction (XRD) analysis: The XRD spectrum shows distinct peaks at specific 2θ values, which indicate the crystalline nature of the sample. Sharp and intense peaks, like those seen around 10° and 20.15° 2θ, suggest that the material has clear crystalline phases. Broader peaks or regions of low intensity between the sharp peaks indicate some degree of amorphous content or disorder within the structure. The most intense peak around 20.15° 2θ is likely associated with a dominant crystallographic plane in the sample. This peak can be indexed using standard databases such as the Joint Committee on Powder Diffraction Standards (JCPDS) to identify the crystalline phase present, as shown in [Table/Fig-4].



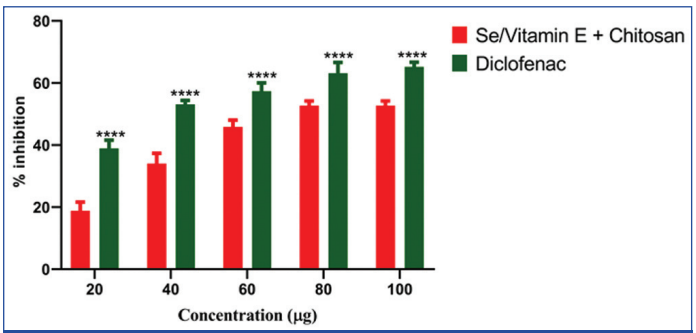
[Table/Fig-4]: XRD analysis of synthesised SeNPs-VitE/Chi.

Morphological and elemental composition of synthesised SeNPs-VitE/Chi: Scanning Electron Microscopy (SEM) is a valuable tool for analysing the surface morphology and structural features of nanoparticles. It provides high-resolution images of the surface, detailing the size, shape and distribution of the nanoparticles. The synthesised SeNPs-VitE/Chi displayed rod-shaped structures with lengths ranging from 250 to 550 nm [Table/Fig-5a-c]. Energy Dispersive X-ray (EDX) spectroscopy analysis showed that high-energy electrons or X-rays excite the sample, causing it to emit characteristic X-rays corresponding to the elements present. The nanoparticles helped in identifying and quantifying key elements, ensuring that the composition aligns with the intended material properties. The results confirmed the presence of SeNPs, along with peaks indicating the presence of other elements associated with carbon in the synthesised SeNPs-VitE/Chi, as illustrated in [Table/Fig-5d].



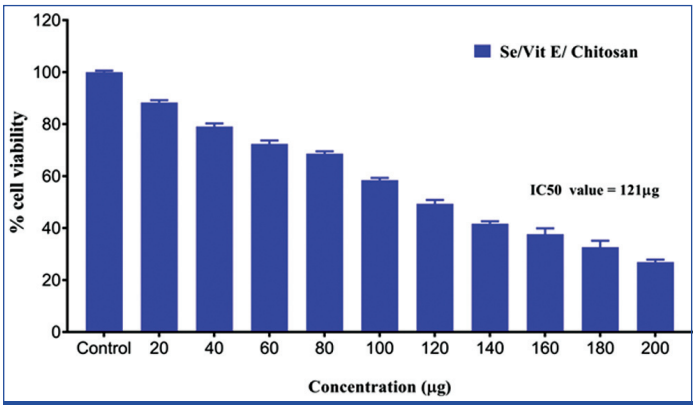
[Table/Fig-5]: Morphology of green synthesis of SeNPs-VitE/Chi using *C. sinensis* was analysed by SEM at different scale bars and elemental composition.

Albumin denaturation inhibitory assay: In [Table/Fig-6], the percentage of heat-induced albumin denaturation inhibition of SeNPs-VitE/Chi is represented. Compared to 65.1% for diclofenac, the maximum anti-inflammatory activity observed was 52.6% at a concentration of 100 µg/mL. The nanoparticles have an IC50 value of 64 µg.



[Table/Fig-6]: Anti-inflammatory activity of SeNPs-VitE/Chi: in comparison with diclofenac by BSA denaturation inhibitory assay. The data represents mean±SD of triplicate experiments Statistical significance analysis by the student t-test method shows **** which denotes p≤0.0001 for standard vs. Se/Vitamin E + chitosan.

Anticancer activity: This research focused on examining the anticancer properties of green-synthesised SeNPs-VitE/Chi in relation to the A549 lung cancer cell line. The evaluation of the cytotoxic effects of SeNPs-VitE/Chi was conducted using the MTT assay, demonstrating that exposure to the sample led to a concentration-dependent decrease in cell viability. Notable cytotoxic effects were observed at elevated concentrations, indicating the potential anticancer properties of the nanoparticles [Table/Fig-7]. The IC50 value for SeNPs-VitE/Chi against A549 cells, following 24 hours of treatment, was found to be 121 µg/mL.



[Table/Fig-7]: Anticancer activity of SeNPs-VitE/Chi using C. sinensis against lung cancer A549 cell line assessed by MTT assay. Data expressed as mean±SD of triplicate experiments and the IC50 value was 121 µg.

DISCUSSION

The characteristic absorption peaks for SeNPs typically occur around 310 nm, due to the excitation of surface plasmon vibrations in the nanoparticles. Similarly, a few studies have reported the synthesis of SeNPs using different plants [18,19]. The calculated energy band gap for *C. sinensis*-mediated SeNPs was found to be 6.41 eV. FTIR is a useful method for analysing the chemical composition and interactions of synthesised nanoparticles. In the synthesised SeNPs-VitE/Chi, the spectrum revealed distinct functional groups, with peaks observed at 568, 793, 1065, 1758 and 2924 cm^{-1}. These peaks correspond to functional groups such as C-I stretching, C-H, C-O and C=O, respectively. This observation is similar to the recent report by Vasanthakumar et al., (2024), which described the synthesis of SeNPs [20]. In another study, when SeNPs reduced by *Cassia javanica* flowers were analysed by FTIR, distinctive peaks were observed at 3232 cm^{-1} for O-H stretching vibrations, 1588 cm^{-1} for tertiary amides, 1423 cm^{-1} for COO groups and 725 cm^{-1} for C-H stretching vibrations [21]. FTIR analysis results for synthesised SeNPs from *Cymbopogon citratus* and *Syzygium aromaticum* were similar [22].

The XRD spectrum showed distinct peaks at specific 2θ values, indicating the crystalline nature of the sample. The most intense peak around 20.15° 2θ was likely associated with a dominant crystallographic plane in the sample. A previous report by Soliman MK et al., (2024) revealed that the XRD pattern exhibited sharp peaks, validating that the synthesised SeNPs were crystalline in structure [21]. Similar crystalline bands of SeNPs have been reported previously [23]. The synthesised SeNPs-VitE/Chi exhibited rod-shaped structures with lengths ranging from 250 to 550 nm. The results confirmed the presence of SeNPs, along with peaks indicating the presence of other elements associated with carbon in the synthesised SeNPs-VitE. According to another study, the SEM image of the synthesised SeNPs from the aqueous extract of *Moringa oleifera* leaves exhibited highly uniform grain structures that were well distributed within the matrix [23]. In a previous study by Alipour S et al., (2021), SeNPs prepared using *Cyanobacterium spirulina* were found to be spherical in shape, with a particle size of around 100 nm. The elemental analysis showed a selenium signal along with peaks for carbon and oxygen groups, confirming the presence of selenium at 44.99% (wt.) in the sample. Additionally, carbon (29.9%) and oxygen (25.1%) signals were detected in the analysis [24].

The percentage of heat-induced albumin denaturation inhibition by SeNPs-VitE/Chi was 52.6% at a concentration of 100 µg/mL, compared to 65.1% for diclofenac. The nanoparticles demonstrated an IC50 value of 64 µg. The present study, when compared to work by Singh S et al., (2023), showed significant anti-inflammatory activities of *Camellia sinensis*-mediated nanoparticles [25]. The anticancer properties of green-synthesised SeNPs-VitE/Chi against the A549 lung cancer cell line were also assessed, revealing an IC50 value of 121 µg/mL. One previous study also reported the cytotoxic activity of SeNPs incorporated into nano chitosan [26].

Limitation(s)

While the present study demonstrates the successful green synthesis of selenium-doped vitamin E and chitosan nanoparticles using *Camellia sinensis*, several limitations must be acknowledged. The biological evaluations were primarily conducted in-vitro, limiting the understanding of their effectiveness in complex biological systems. Furthermore, the exact molecular mechanisms underlying their anti-inflammatory and anticancer activities remain unclear. The study also lacks comprehensive toxicity assessments and long-term stability analyses, which are crucial for clinical translation. Additionally, challenges related to scalability and reproducibility in the synthesis process must be addressed for potential large-scale production and biomedical applications.

CONCLUSION(S)

The present study successfully synthesised SeNPs doped with vitamin E and stabilised with chitosan using *Camellia sinensis* extract. The nanoparticles exhibited enhanced stability, biocompatibility and significant anti-inflammatory and anticancer effects. They selectively targeted cancer cells while sparing normal cells, demonstrating their potential as a safer therapeutic alternative. These findings highlight the promise of SeNPs-VitE/Chi as a sustainable and effective nanotherapeutic option for inflammation and cancer treatment. Future research should focus on understanding the molecular mechanisms, conducting in-vivo studies and optimising large-scale production for clinical applications.

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- iThenticate Software: Jun 12, 2025 (10%)

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- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

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