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Anti-inflammatory and Antioxidant Activity of *Clitoria Ternatea* Extract Mediated Selenium Nanoparticles: An In-Vitro Study

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***Abstract*---**Selenium was used to hydrothermally synthesize selenium nanoparticles (SeNPs) using *Clitoria ternatea* extract, which is an important trace ingredient for human health. The green synthesis of nanoparticles has created a cost-effective, environmentally sustainable procedure for synthesis of non-materials, as well as raising healthy strategies. DPPH radical scavenging assay was used to investigate the antioxidant activity. At 50ml, the synthesized nanoparticles showed most antioxidant activity by scavenging 89.1% DPPH and excellent anti-inflammatory activity by dose-dependently reducing inflammation at inhibition percentage of 85.5%, which was found to be higher than the control. Selenium nanoparticles

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synthesized from *Clitoria ternatea* may be used as possible candidates for biomedical and environmental applications due to their eco-friendly synthesis, nontoxicity, and biocompatibility.

***Keywords*---**anti-inflammatory, antioxidant, clitoria ternatea, green synthesis, selenium.

Introduction

Nano materials are particles with a nanoscale dimension, whereas nanoparticles are very small particles with increased catalytic reactivity, thermal conductivity and chemical stability due to their high surface area to volume ratio (1). The nanoparticles used in various applications are gold, silver, silver oxide, copper, copper oxide, copper sulfide, zinc, zinc oxide, zinc sulfide, cadmium sulfide, titanium oxide, zirconium, cerium oxide, selenium among many others (2–4).

Different physical, chemical methods are used to synthesize nanoparticles such as electrochemical, laser deposition, wet chemical, electrode position (5,6). Nanoparticles have been incorporated into a variety of consumer industries, including industrial, health, oil, feed, room, chemical, and cosmetics, necessitating a green and environmentally sustainable approach to their synthesis (7), as the physical, chemical methods involve the use of costly equipment, high temperature, pressure, highly toxic chemicals detrimental to the environment (8,9). As a result, green methods for nanoparticle synthesis that are both eco-friendly and cost-effective are encouraged.

Selenium is an essential trace factor that the human body requires up to 40–300 g per day. It aids in the regulation of human body activity. It aids in the protection of cardiovascular wellbeing, the regulation of thyroid hormones and immune response, and the prevention of cancer progression (10). Due to its low cost, high impact, and environmentally friendly nature, the synthesis of selenium nanoparticles using green methods has sparked a lot of interest. These natural strains and plant extracts produce phytochemicals that serve as a reducing agent as well as a capping or stabilizing agent (11). Selenium Nanoparticles (SeNPs) have been studied in a variety of disease conditions due to their improved properties over Se. SeNPs have increased bioavailability with the added benefit of reduced toxicity. In a number of pathological disorders, the pro-oxidant and antioxidant results offer various opportunities for investigation (12). There are studies on biological methods for producing SeNPs from plant parts such as dried leaves, seeds, bulbs, and bark (13–16). *Clitoria ternatea,* commonly known as “blue pea” is a well-known Ayurvedic medication used for a variety of diseases that has been thoroughly studied scientifically for its antimicrobial, anti-stress, anxiolytic, antidepressant, anticonvulsant, tranquilizer and sedative activities (17). Studies have found that ethanolic extracts of *Clitoria ternaea* leaves and flowers have potent anti-diabetic, antioxidant, and anti-inflammatory properties (18,19). However, this study is a first of its kind in our knowledge, which aims to investigate the anti-inflammatory and antioxidant properties of *Clitoria ternatea* extract mediated selenium nanoparticles.

Materials and method

Study design: In-vitro
Preparation of *Clitoria ternatea* extract mediated selenium nanoparticles

Prior to use, dried *Clitoria ternatea* flowers were purchased from a specialized Ayurveda pharmacy and inspected for purity and phytochemical composition. The dried flowers were ground to a fine powder and 10 gm of *Clitoria ternatea* flower powder was boiled in 100 mL of double distilled water to make an aqueous extract. Following boiling, the solution was filtered using Whatman number 1 filter paper, and the filtrate was used for nanoparticle synthesis. 50 mL flower extract, 30 mM sodium selenium, and 50 mL purified water were combined. The solution was then put in an incubator with a cum shaker set to 250 rpm until a colour change indicated nanoparticle synthesis.

Anti-inflammatory activity

The anti-inflammatory efficacy of *Clitoria ternatea* extract-mediated selenium particles was assessed using the Muzushima and Kabayashi convention, with some modifications (20). 0.05 mL of varying amounts of Cl ternatea-SeNP of various concentrations (10µL, 20 µL,30 µL,40 µL and 50 µL) was added to 0.45 ml of bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 minutes before being heated in a water bath for 30 minutes at 55 degrees Celsius. After cooling the samples, the absorbance was measured spectro-photometrically at 660 nm. As a control, diclofenac sodium was used. The monitoring substance is DMSO.

Antioxidant activity

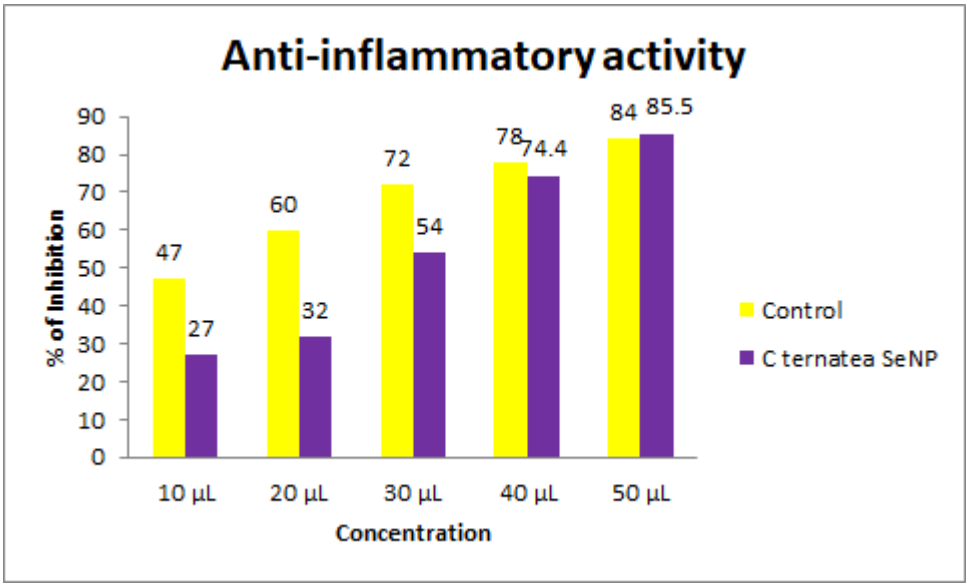
1 ml of 0.1 mM 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) in methanol solution and 450 l of 50 mM Tris-HCl buffer were combined with various concentrations (10 µL, 20 µL, 30 µL, 40 µL 50 µL,) of nanoparticles at pH 7.4. The reduction in the number of DPPH free radicals was calculated based on the absorbance at 517 nm after incubation. Ascorbic acid was used as the standard control, and the percent (%) inhibition was calculated as [(Absorbance of control – Absorbance of test sample)/Absorbance of control] ×100.

Results

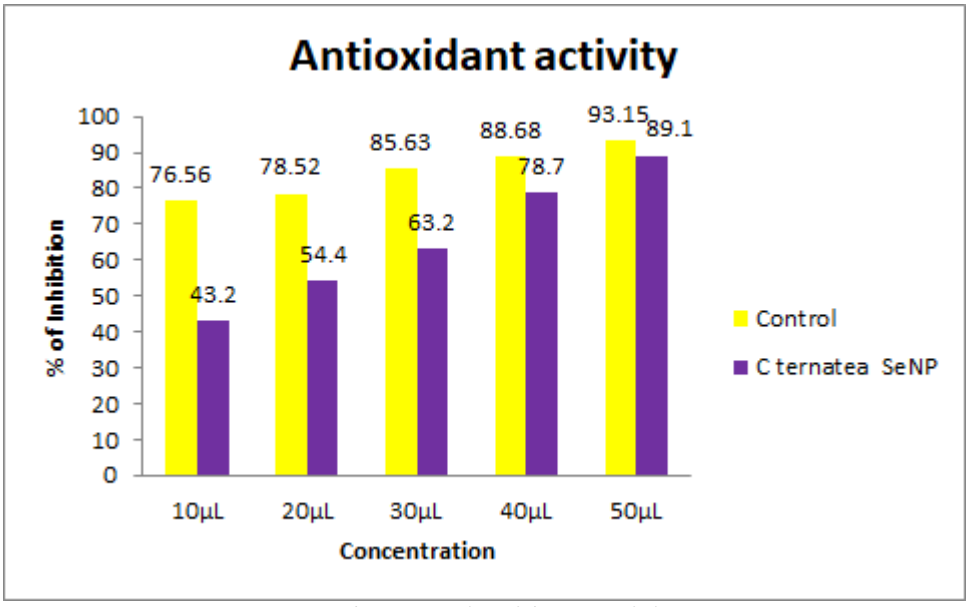
At 10ml, there was 27% inhibition of Cl. ternatea-SeNP at 20 µL, we observed a 32%, at 30 µL 54%, at 40 µL 74.4% and at 50 µL 85.5% inhibition rate. Therefore, at 50 µL, the Cl. ternatea SeNP had a better anti-inflammatory action as compared to the control. (graph 1)

Figure 5 shows the antioxidant activity of *Clitoria ternatea* extract mediated selenium nanoparticles. At 10 µL, 43.2% inhibition was obtained as compared to 76.5% of the control, at 20 µL 54.4% inhibition was obtained to 78.5% of the control. With every increased concentration, an increased anti oxidising property

was reported. At 50 µL, the highest inhibition was seen at 89.1%, however the control was at 93.1% (graph 2)



Graph 1 : Anti-inflammatory activity



Graph 2 : Anti-oxidant activity

Discussion

Due to its high bioavailability and lower toxicity than inorganic and organic sources, where inorganic compounds are more toxic than organic compounds, the nano form of selenium has gotten much more interest (21–23). Selenium nanoparticles (SeNPs) have different biological properties depending on their size: bigger particles have more activity. The cellular consumption of NPs is affected by particle size; in vitro absorption of 0.1 m particles was observed to be 2.5 and 6 times greater than that of 1 and 10 m particles, respectively (24,25).

The visual appearance, UV-Vis spectra, and antioxidant activity of SeNPs obtained under ideal conditions were analyzed to determine their stability, in this study. The visual color change was observed in the current study after 72 hours, which confirmed the synthesis of the nanoparticles. The colour is generated by the mutual oscillations of free conduction electrons in nanoparticles (26). Increased nanoparticle synthesis was observed when the colour strength was increased when the incubation time was extended, as has been observed and proved in other related studies too (27–30).

In the current study, DPPH assay was used to assess the free radical scavenging in order to investigate the antioxidant properties of the synthesized nanoparticles. The experiment was performed in triplicate and average was taken for determination of percentage inhibition. It was observed that the biologically synthesized Cl. ternatea-SeNPs have 89.1% maximum inhibition activity which although compared less to the control, but was an excellent antioxidant activity. Earlier studies have reported Cl.ternatea- MgONPs to have 65 % maximum inhibition activity (31), effective antioxidant activity of *Cl.ternatea* with silver and gold nanoparticles (32). The enhanced antioxidant properties could be due to the bioactive components present in the plant extract.

An increased anti-inflammation inhibitory activity was also observed at 85.5% in this study. Animal studies have also reported increased anti-inflammatory activity of Cl.ternatea due to presence of a high concentration of triterpenoids including taraxerol and taraxerone. Inflammation is also caused by secondary enzymes such as phosphlolipase A2, cycloxygenase, lipo-xygenase, and nitric oxide synthase and different parts of the plant are known to reduce these enzymes (33). Ethanolic extract of *Clitoria ternatea* also possesses good anti-inflammatory activity, as reported by Suganya G et al (18). To the best of our knowledge, this was one of the first of the studies which assessed the anti-inflammatory activity of Cl.ternatea-SeNP, therefore further parallels could not be drawn.

Conclusion

The findings of the study conclude, a novel green method for synthesis of selenium nanoparticles was developed using *Clitoria ternatea* extract as reducing and stabilizing agents. The synthesized nanoparticles possess promising anti-inflammatory and antioxidant properties.

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