BOERHAVIA DIFFUSA MEDIATED SELENIUM NANOPARTICLES AND ITS ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

Running title : Effect of antioxidant and anti-inflammatory activities on *boerhavia diffusa* mediated selenium nanoparticles

ABSTRACT :

Introduction : *Boerhavia diffusa* belongs to species of flowering plant which is commonly called as *punarnava*. Its leaves are used as green leafy vegetables, with its high anti-inflammatory and expectorant properties selenium particles are synthesised. Selenium particles are used as nutritional supplements and in anti-microbial coating. Selenium (Se) was found to have anti-inflammatory properties, but its bioavailability and toxicity are limiting factors considerably.

Aim : To find the antioxidanmt and anti-inflammatory activities of *Boerhavia diffusa* mediated selenium nanoparticles

Materials and Methods : 1gm of *Boerhaavia diffusa*was added to 100 ml of distilled water and allowed to boil for 10-15 minutes at 70 degree celsius and filtered. 60 ml of 20 milli molar sodium selenite is prepared in 250 ml of conical flask, 40 ml of filtered plant extract was mixed to it. The synthesized nanoparticle was preliminarily analysed using UV visible spectroscopy. Prior to the final step the nanoparticle solution was centrifuged at 8000 rpm to prepare nanoparticle pellet powder, it was dried in a hot air oven at 80 degree celsius. The dried powder was sent for characterisation. The leftover nanoparticle solution extract was sent for evaluation of antioxidant activity through DPPH assay and anti-inflammatory activity through albumin denaturation assay.

Results : Selenium nanoparticles were achieved from boerhavia diffusa plant extract which was evident with its significant colour change in the cultivation medium. The final centrifuge was found to have potent antioxidant and anti-inflammatory activities.

Conclusion : The selenium nanoparticles synthesized from leaf extract of Boerhaavia diffusa were evaluated for antioxidant and anti-inflammatory activities. This study concludes that extract bound selenium nanoparticles had a potent antioxidant and anti-inflammatory activities. Therefore selenium nanoparticles may provide a great implication in the later studies for its potential activity.

Key words : Boerhavia diffusa, selenium nanoparticles, characterisation, antioxidant activity, anti-inflammatory activity, Innovative technology

INTRODUCTION :

Boerhavia diffusa belongs to species of flowering plant which is called as *punarnava*. Its leaves are used as green leafy vegetables, with its high anti-inflammatory and expectorant properties selenium particles are synthesized. Rural areas of many developing countries had a great awareness on usage of medicinal plants.*Boerhavia diffusa* is classified under the family Nyctaginaceae. It is a hairless, semi-prostrate, perennial herb of 60cm hight with a thick, fleshy rootstock that reproduces from seeds. The stem is slender, jointed, more or less fleshy and woody below. It is green or purple in colour, low branching, glabrous and not sticky from root hairs. The leaves are opposite, little fleshy, ovate shaped , 2.5-4cm long and 2-4cm wide, with petioles 1-3cm long, blunt-tipped and smooth(1). *Boerhavia diffusa* is grouped under the rasayana category according to ayurvedic claims. It is found to have anti-aging properties , disease prevention, and life strengthening activities which had much influence on disease burden and availability of healthcare in the world. Chemical analysis of *B.diffusa* provided a wide variety of chemical constituents namely -rotenoids, flavonoids, purine nucleoside, steroids etc.,. Various ethnopharmacological reports emphasize its role in disorders of reproductive system, gastrointestinal system, respiratory system, urinary system, hepatic system/jaundice, cardiovascular system, and cancer.(2), (3)

Selenium particles are used as nutritional supplements and in anti-microbial coating. Selenium (Se) was found to have anti-inflammatory properties, but its bioavailability and toxicity are limiting factors considerably. Nanoparticles of Se were administered orally with a dosage of 2.55 mg/kg once before carrageenan administration in the first model and two times in the second model(4)),(5)The antioxidant activity of selenium nanoparticles was determined by production of reactive oxygen species using man's umbilical vein endothelial cells. Electron microscopic pictures show intracellular and extracellular deposits of selenium particles.These particles which are of<100 nm are used as preservatives due to antioxidant properties that are relevant to human health.(6)(7) . Selenium nanoparticles are related to health care and other concerned issues. Pectin, well known food grade polysaccharide is used as stabilizer to form better dispensed and stable SeNPs under a simple redox reaction , and also Se / PEC ratio affects the colour of suspension for easy identification(8), (9) Cytotoxic effect of the SeNPs and selenium dioxide (SeO2) on MCF-7 cell line was assessed by MTT assay. Transmission electron

micrograph (TEM) of the purified Se NPs showed individual and spherical nanostructures in a range of about 80–220 nm.(10), . In the previous studies , the biosynthesis of SeNPs that employ microorganisms, especially probiotics, had given a great attention and are considered as green process because of several advantages adding on safety, cost effectiveness, and an eco-friendly approach and is currently trending as an alternative to conventional methods which include, physical and chemical synthesis methods of SeNPs(11)(12)

The present study focussed on antioxidant and anti-inflammatory activity of boerhavia diffusa mediated selenium nanoparticles. Previous studies on various activities of selenium nanoparticles like antifungal , antimicrobial and cytotoxic activities and based on various sources of plants other than boerhavia diffusa had given a detailed explanation of all the activities. Our team has extensive knowledge and research experience that has translated into high quality publications(13–24).

MATERIALS AND METHODS :

In the present study, 1gm of boerhaavia diffusa was added in 100 ml of distilled water and boiled for 10-15 minutes at 70 degree celsius. After boiling, the plant extract was filtered by Whatman No 1 filter paper. 60 ml of 20 milli molar sodium selenite is prepared in 250 ml of conical flask, 40 ml of filtered plant extract was mixed to it and kept in a magnetic stirrer for nanoparticle synthesis. The synthesized nanoparticle was preliminarily analysed using UV visible spectroscopy. Prior to the final step the nanoparticle solution was centrifuged at 8000 rpm to prepare nanoparticle pellet powder, it was dried in a hot air oven at 80 degree celsius. The dried powder was sent for characterisation. Finally the left over solution was taken to calculate antioxidant and antiinflammatory activity. All the results were taken photographs and recorded in the excel sheets.

Figure 1: Preparation of nanoparticles

Antioxidant activity - DPPH method :

DPPH assay was used to test the antioxidant activity of biogenic synthesized selenium nanoparticles. Diverse concentrations (2-10 µg/ml) of boerhaavia diffusa leaf extract interceded selenium nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes.Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control. The percentage of inhibition was determined from the following equation,

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

Anti inflammatory activity - albumin denaturation assay :

The anti-inflammatory activity for selenium nanoparticles was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of Solanum torvum gel of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL 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 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.

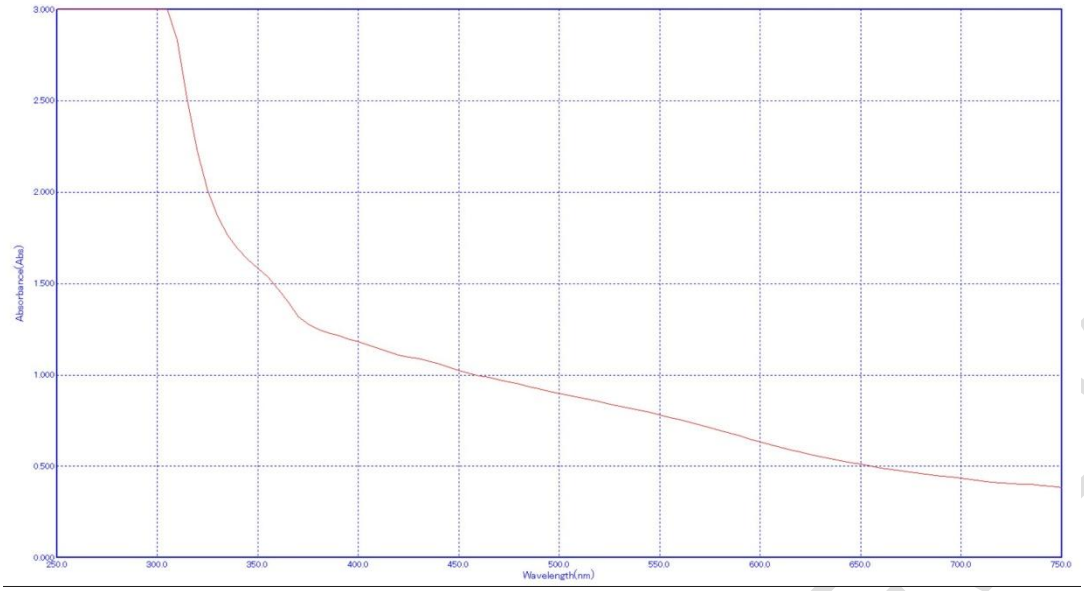
Percentage of protein denaturation was determined utilizing following equation,

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

RESULTS :

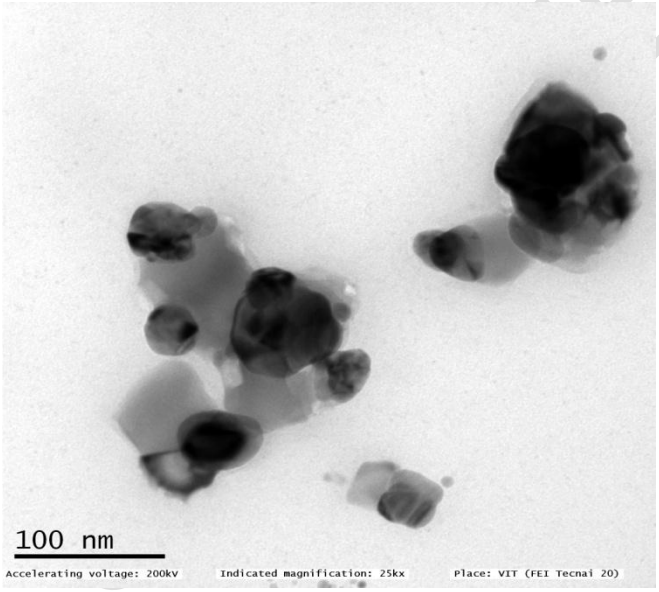
Selenium nanoparticles were achieved from Boerhavia diffusa plant extract which was evident with its significant colour change in the cultivation medium. The final centrifuge was found to have potent antioxidant and anti-inflammatory activities. All the parameters were recorded and represented. Fig 2 shows the UV light spectroscopy of selenium nanoparticles which showed decreased absorbance when compared with the serial wavelengths, electron microscopy picture showed that the nanoparticles are spherical and square and many undefined shapes are there may the binding of plant extract with nanoparticles (figure 3) later the antioxidant and anti-inflammatory bar graphs of selenium nanoparticles compared with standards showed a potent activity (figure 4 and 5)

Figure 2 : UV Spectroscopy



The above picture shows the UV light spectroscopy of selenium nanoparticles which showed decreased absorbance when compared with the serial wavelengths.

Figure 3 : Transmission electron microscopy



The figure 3 clearly shows that the nanoparticles are spherical and square and with many undefined shapes there may be the binding of plant extract with nanoparticles.

Figure 4 : Bar graph showing antioxidant activity of SeNps

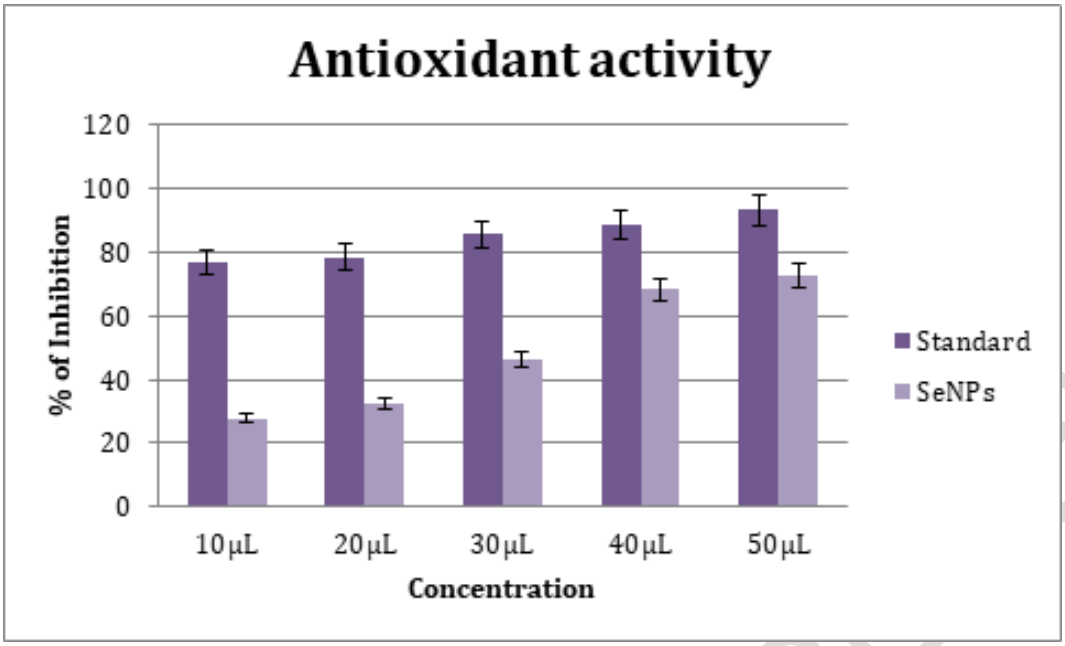


Figure 4 : The bar graph above denotes the antioxidant activity of selenium nanoparticles derived from the plant extract of boerhaavia diffusa. X-axis denotes the concentration of plant extract with selenium nanoparticles and Y-axis denotes the percentage of inhibition towards the oxidant activity. It shows the increase in percentage of inhibition with increase in concentrations but decreased activity when compared with the standard

Figure 5 : Bar graph showing anti-inflammatory activity of SeNps

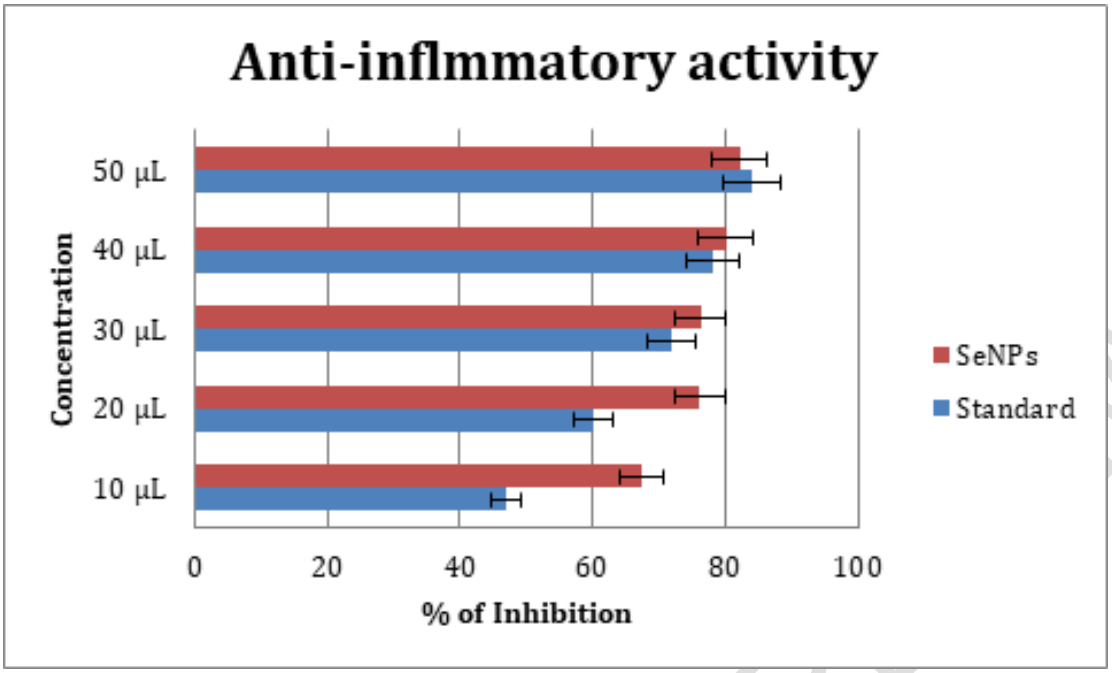


Figure 5 : The bar chart above shows the anti-inflammatory activity of selenium nanoparticles derived from the boerhavia diffusa plant extract. X-axis denotes the percentage of inhibition and Y-axis denotes the concentration of plant extract containing selenium nanoparticles. It shows the increase in percentage of inhibition for inflammatory activity with increase in concentrations. At 50 µl concentration the activity of sel;enium nanoparticles is more compared to the standard whereas the activity is less than standard activity at the rest of the concentrations.

DISCUSSION :

Green tea extract was used as the Na2SeO3-reducing agent at room temperature and the LBP acted as the coating agent to prevent SeNPs agglomeration in dispersion, finally enhancing the stability and biological activity under physiological conditions. Results have shown that SeNPs have ability to utilize as food materials in green synthesis of selenium nanoparticles and also in applications in biomedical, cosmetic and pharmaceutical products, as main antioxidant supplement and neuroprotective agent.(25), (26), (27)FTIR results show that SeNPs were combined to the hydroxyl groups of GA,The hydroxyl radical scavenging ability and DPPH scavenging ability of GA-SeNPs were higher than those of AGHA-SeNPs and could reach 85.3 ± 2.6%, 85.3 ± 1.9% at a concentration of 4 mg/ml, respectively.(28) SeNPs show an excellent antioxidant performance by the 2,2′-azino-bis (3-ethylbenzothiazoline -6- sulphonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) methods.(29) The biogenic biomolecules capped-SeNPs possess low cytotoxicity, and significant antioxidant and anticancer activities. The current study suggests that probiotics could provide a better alternative to synthesize biogenic SeNPs with potential applications as anticancer and antioxidant agents.(30,31)

SeNP at a concentration of 250 µg/kg b.w. acted as potent anti-inflammatory agent and significantly reduced ($p < 0.05$) arthritis induced parameters. The enzymatic antioxidant levels in liver, kidney, and spleen were regained significantly ($p < 0.05$) at 500 µg/kg b.w. while CRP was regained to normal at concentration of 100 µg/kg b.w. determining SeNP at 500 µg/kg b.w. can be a potential antiarthritic drug supplement.(32) The synthesized nanoparticles were assessed for the anti-inflammatory and antioxidant properties by albumin denaturation assay and α, α-diphenyl-β-picryl hydrazyl free radical scavenging assay. From the synthesized selenium nanoparticles, both the anti-inflammatory and antioxidant activities showed a good percentage of inhibition.(32,33)(34,35)

LIMITATIONS : Only antioxidant and anti-inflammatory activities are evaluated.

FUTURE SCOPE : We expect further studies on characterisation of selenium nanoparticles and other activities derived from different studies.

CONCLUSION :

The selenium nanoparticles synthesized from leaf extract of boerhaavia diffusa were evaluated for antioxidant and anti-inflammatory activities. This study concludes that selenium nanoparticles had a potent antioxidant and anti-inflammatory activities. With these properties it can be used in healthcare centers and camps. Therefore selenium nanoparticles may provide a great impact in later studies for the potential activity.

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