Type of study: Original study

EVALUATION OF ANTIOXIDANT AND CYTOTOXIC EFFECT OF SELENIUM NANOPARTICLES SYNTHESISED USING *CAPPARIS DECIDUA*

Running Title: Evaluation of antioxidant and cytotoxic effect of Selenium Nanoparticles using *Capparis decidua*

Abstract:

Among the nanoparticles, selenium nanoparticles (SeNP) are one of the most extensively studied as Se has zero oxidation state, non toxic and biologically inert material. This is the reason why Selenium is considered as a major nanoparticulate. In this study SeNPs were extracted from the fruit of *Capparis decidua* which is a xerophytic small herb. The aim of the present study is to evaluate the cytotoxic effect and antioxidant capacity of selenium nanoparticles. The cytotoxic effect of SeNPs was evaluated using Brine Shrimp assay and the antioxidant activity was determined using DPPH assay considering ascorbic acid as the standard. From the study of this assay the shrimps introduced into the well were almost alive in different concentrations and this indicates that there is no cytotoxicity in selenium nanoparticles. The percentage inhibition of Selenium nanoparticles in 10 µl was 15.4±0.1, 20 µl was 38.36±0.15, 30 µl was 45.3±0.1, 40 µl was 59.6±0.15 and 50 µl was 65.6± 0.1. It can be inferred that percentage inhibition increases with increase in concentration but it was less when compared to the percentage inhibition of the standard. The selenium nanoparticles extracted from *Capparis decidua*do not have any cytotoxic effect on shrimps. The SeNPs possessed significant antioxidant activity in increasing concentrations compared to the standard used. Thus SeNPs are biologically useful and can be used as eco-friendly, cost effective and efficient biomedical agents and therapeutics.

Keywords: Selenium nanoparticles, *Capparis decidua,* cytotoxicity, antioxidant, non toxic, brine shrimps, DPPH radical.

1.Introduction:

Selenium (Se) plays a vital role in the antioxidant defense mechanism of the liver and thus protecting against oxidative stress [1]. Also Selenium prevents the accumulation of free radical species, and reduces the cellular damage which makes the researchers choose Selenium for study [2–4]. As it is familiar that Selenium is one of the essential trace elements with zero oxidation state which enhances bioavailability compared to other forms of Selenium [5,6]. But there

also exists a limitation of dose which could be toxic when exceeded [7]. Yet another limitation would be that Selenium is a thermostable and biologically inert element and thus it is restricted to be food intake [8,9]. Selenium was designed as a nano-vehicle using polysaccharides , proteins, etc., as stabilisers [10,11]. Cytotoxic refers to a substance or process that results in cell damage or cell death. Even our own immune systems have cells that are considered to be cytotoxic, such as T cells which kill bacteria, viruses, and also cancer cells [12]. These cytotoxic drugs work by interrupting the cells in their growth cycle [13]. The term antioxidant itself infers that it inhibits oxidation. Oxidation being a chain reaction, can produce free radicals that may damage the cells of organisms. Certain vitamins like beta carotene, Vitamin A and Vitamin E being a dietary supplement for antioxidant activities has no positive effect on mortality rate [14]. In case of Selenium, it is considered as a better antioxidant supplement though not a dietary intake but it was limited that selenium had no positive impact on cardiovascular disease [15]. The most interesting factor is that antioxidants being reducing agents, can act as pro oxidants but the relative importance of pro oxidants is still a matter of discussion [16]. *Capparis decidua,* commonly known as Karira which is used as folk medicine and herbalism grown in drought resistant areas [17]. From the prior studies, it was evident that C.decidua possesses sterols, fatty acids, flavones and alkaloids [18–20]. Thus the plant *Capparis decidua* was chosen to enable better results.

In previous studies, involving the antioxidant capacity of SeNPs it establishes low cytotoxicity and enhanced antioxidant capacity. This study by Chitosan also speaks on the ability of considered nanoparticles to penetrate cells or tissue effectively [21]. The present study is the extension of prior studies undertaken with *Capparis decidua* as it had been acknowledged that this plant is a potent source of various bio chemicals and therapeutics. Similarly, with consideration of selenium it possesses a better antioxidant activity. Thus determining the antioxidant and cytotoxic effect of Selenium nanoparticles with the extract of C.decidua would enhance the pharmacological development of drugs in future. The study on the combination of both antioxidant and cytotoxic effects of Selenium nanoparticles as a subject is the uniqueness of our study. Our recent research portfolio slides numerous articles in reputed journals [22–30]. Based on this experience we planned to pursue the cytotoxicity and antioxidant property of Selenium nanoparticles. Thus the aim of the study is to evaluate antioxidant and cytotoxic effects of selenium nanoparticles using *Capparis decidua.*

Comment [KayodeBab1]: statement source

2. Materials And Methods:

The current study has been approved by the Scientific review board, Saveetha Dental College, Chennai.

2.1 Preparation Of Plant Extract:

The well dried *Capparis decidua* fruits were collected and made into a powder using mortar and pestle. 1 grams of C.decidua powder was dissolved in distilled water and boiled for 10 mins at a controlled temperature of 60 degree in a hot mantle. The solution was filtered.

2.2 Preparation Of Nanoparticle Solution:

0.01 milligram of Sodium selenite was dissolved in 8 ml of distilled water. 40 ml of plant extract was added with 60 ml of prepared metal solution and were made into 100 ml solution. This solution was kept in a shaker and readings were taken.

2.3 UV Characterization:

Synthesised nanoparticle solution is characterised using UV spectroscopy in the range of 250-650 nm. These results were recorded for graphical analysis.

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2.4 Preparation Of Nanoparticle Powder:

Selenium nanoparticle solution was centrifuged using refrigerated centrifuge at 8000 rpm for 3 minutes and pellet was collected and washed with distilled water twice. The purified pellet was collected and dried for 2-3 days in a hot air oven. Finally, the nanoparticle powder was collected and stored in an airtight Eppendorf tube.

2.5 Determination Of Cytotoxic Effect:

The cytotoxic effect was determined by carrying out brine shrimp assay method. Distilled water was taken in different concentrations such as 10 microliter, 20 microliter and till 50 microliter. The shrimps were introduced into each well in the count of exactly 10 involving the control. Then the extract was added according to the concentration of distilled water in each well excluding the control. This setup was undisturbed and then observed after 24 hours. Now the alive shrimps were counted.

2.6 Determination Of Antioxidant Activity:

Antioxidant activity of Se nanoparticle was determined on the basis of radical scavenging mechanism in DPPH assay (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) which is a free radical method based on electron transfer. The value of RSC% (Radical Scavenging Capacity) was calculated using the following formula:

Radical scavenging activity, RSC% = A0-A1/A0 multiplied by 100

where A0 is the absorbance of the control and A1 is the absorbance of the mixed solution of the antioxidant and free radical agent. This assay was carried out based on the work of Xu B J et.al., 2007[31]. 0.2 mL of nanoparticle powder was mixed vigorously with 3.8 mL of DPPH radical ethanol solution with DPPH concentration as 0.1 mmol/L , and then maintained at room temperature in the dark for 30 minutes. The absorbance was measured at 517 nm with a UV spectrophotometer.

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3.Results And Discussion:

The cytotoxic activity of Selenium nanoparticles shows that all the introduced shrimps were alive in the control whereas in the well of 10 µl , 9 shrimps were alive, in 20 µl nanoparticle well 9 shrimps were alive, in the well of 30 µl and 40 µl nanoparticles also 9 shrimps were alive. However, in the well with a concentration of 50 µl of nanoparticles only 8 shrimps were alive after 24 hours. It is presented in a bar graph (Figure 4). Thus it is evident that there is no significant cytotoxicity in Selenium nanoparticles. For the evaluation of antioxidant

Comment [KayodeBab4]: From the graphical presentation, 10 shrimps each were alive both for the control and the 10ul well but your statement said 9 shrimps were alive for 10ul. There is a conflict statement with your graphical presentation, please make the necessary correction.

capacity, the percentage inhibition of 10 µl was 15.4±0.1, 20 µl was 38.36±0.15, 30 µl was 45.3±0.1, 40 µl was 59.6±0.15 and 50 µl was 65.6± 0.1 which were presented in the form of a graph in comparison with the percentage inhibition of the standard (Ascorbic acid) used (Figure 3). This can be concluded that with increase in concentration, the percentage inhibition of Selenium nanoparticles is increased but it remains less than the percentage inhibition of standard ascorbic acid. Hence Selenium nanoparticles possess better antioxidant capacity but it is less effective when compared to the antioxidant property of the standard used (Table 1). .

The synonymous study carried by R. S. Das et.al., performed the similar DPPH assay on SeNPs with ascorbic acid as standard and established the same result which adds evidence to our study and exists as a supporting study [32]. Another supporting study by Mughal Quayam et.al., also performed DPPH assay for antioxidant determination also total phenolic content was estimated ensuring the significant antioxidant capacity in C.decidua and thus adds an evidence to our present study [33]. Yet another similar study by Tapiero H et.al., establishes that selenium compounds like selenite induce cytotoxicity causing apoptosis but Selenium has no cytotoxic effect which is synonymous with our study and thus adds evidence to the present study [1]. The study by Mojdeh Safari et.al., involving SeNPs, cytotoxicity was determined by MTT assay which showed low toxicity toward investigated cell lines for 24 hrs which contradicts our findings and thus remains as an opposite finding to our study [34].

Comment [KayodeBab5]: Who is the first author. Gunti L. or Das R.S? please kindly compare with your reference.

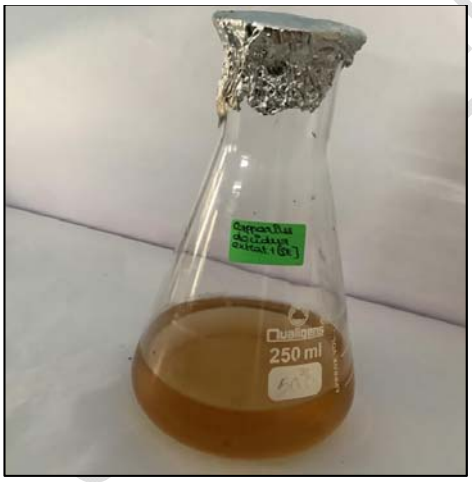

Figure: 1 shows the preparation of selenium nanoparticle

Figure : 2 shows the DPPH assay

Concentration (µl)	**% inhibition of DPPH (Selenium nanoparticle)**	**% inhibition of DPPH (Std)**
10 µl	15.4 ± 0.1	24.69 ± 0.59
20 µl	38.36 ±0.15	51.34 ± 1.46
30 µl	45.3 ±0.1	65.54 ± 1.05
40 µl	59.6 ±0.15	78.42 ± 0.73

50 µl	65.6 ± 0.1	91.53 ± 1.51

Table : 1 represents the percentage inhibition of selenium nanoparticles and the standard ascorbic acid for corresponding concentration in DPPH assay.

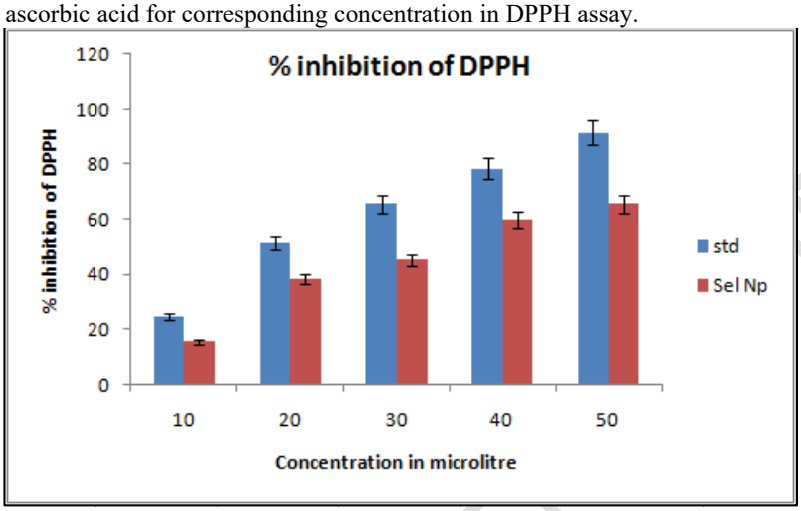

Figure : 3 shows the percentage inhibition of selenium nanoparticles and the standard used with respect to concentration. This graph represents that with increase in concentration, the percentage inhibition of Selenium nanoparticles is increased but it remains less than the percentage inhibition of standard ascorbic acid.

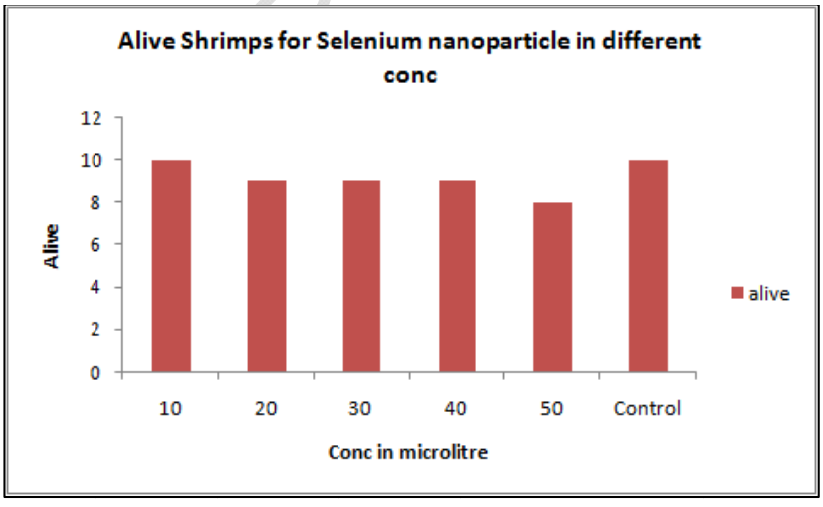

Figure 4: shows the count of alive shrimps after 24 hours in Brine shrimp assay.

4.Conclusion:

From the above study it is evident that SeNPs biosynthesised from *Capparis decidua* is a potent source of antioxidant activity with nil cytotoxic effects which can emerge as a better treatment for various diseases.

Comment [KayodeBab6]: At what concentration is best?
Is there any recommendation for further study.

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