PTEROCARPUS SANTA MEDIATED SYNTHESIS OF SELENIUM NANOPARTICLES AND ITS CYTOTOXIC EFFECT.

RUNNING TITLE: Synthesis of selenium nanoparticles and its cytotoxic effect

ABSTRACT:

Introduction: Selenium is an important trace element in the human body that plays a role in nutrition and medicine. Selenium research is gaining traction, not only because of its technical applications, but also because of its novel position in life sciences. The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts etc. *Pterocarpus santalinus*, with the common names red sanders, red saunders, red sandalwood, Rakt Chandan, and saunders wood, is a species of pterocarpus endemic to the southern Eastern ghats mountain range of south india. This tree is valued for the rich red colour of its wood. The aim of the present study is to find out the pterocarpus santa mediated synthesis of selenium nanoparticles and its cytotoxic effect.

Materials and methods: Selenium nanoparticles were synthesized using pterocarpus santa and its cytotoxic effect was done in brine shrimp and its lethality is checked.

Results: cytotoxic activity and the number of nauplii were all alive on the first day. After 24 hrs the number of nauplii were dead in all wells except in the 5µl well. The percentage of lethality increased from 50%,100%,100%,100%,100% in the 5µL to 80µL respectively.

Conclusion: pterocarpus Santa mediated synthesis of selenium nanoparticles showed better cytotoxic effect in brine shrimp lethality assay showing emergence of a drug candidate for future research.

Key words: Pterocarpus santa; brine shrimp; cytotoxic activity; selenium nanoparticles; lethality test

INTRODUCTION:

Nanotechnology, or the manufacture of structures and devices at the molecular stage, is a multidisciplinary research discipline that is rapidly developing.(1). There is an increasing interest in nanotechnology and its applications in a variety of fields, especially medicine for diagnostic, therapeutic, and research biomedical tools. It can be described as any method or technique for producing nanoscale materials with particle sizes ranging from 1 to 100 nanometers(2). Nanomaterials are now the most advanced, both in terms of scientific understanding and industrial applications. Nanoparticles were first studied a decade ago because of their size-dependent physical and chemical properties. They've now reached an era of commercial exploration (2,3).

Nanoparticles (NPs) are used to minimize toxicity, increase bioactivity, improve targeting, and monitor the release profile of the encapsulated moiety in a variety of ways. Inorganic NPs of metals such as Ag, Au, Ce, Fe, Se, Ti, and Zn hold a special position among NPs due to their unusual bioactivities in nanoforms. Selenium (Se) is an essential trace mineral.(4).Due to their low toxicity and high biocompatibility, selenium nanoparticles (SeNPs) have attracted attention and are widely accepted in biomedicine and food science(5). Several studies have shown that selenium nanoparticles have anticancer, antioxidant, antibacterial, and anti-biofilm properties in recent years. These nanoparticles have shown to have impressive antimicrobial activity against pathogenic bacteria, fungi, and yeast.(6).

Pterocarpus santalinus, also known as Red sanders, is a member of the Fabaceae family(7). The plant is predominantly used to treat skin disorders, oral diseases, cough, pyrexia, diarrhoea, dysentery, and hyper nervous activity, as well as acting as an anti-hemorrhagic, anti-inflammatory, anti-bacterial, anti-cancer, and hepatoprotective agent.(8). *Artemia* (brine shrimp) is zooplankton that is used to feed larval fishes (9). Artemia have one thing in common: they are extremely adaptable to hypersaline conditions like permanent salt lakes, coastal lagoons, and man-made salt pans. They play a crucial role in the food chain's energy flow in the marine environment. (10).

In the previous study (11) the anti oxidant, antimicrobial, anti diabetic, anti inflammatory and analgesic activities of extracts from stem wood of pterocarpus marsupium roxburgh was done. (12) had done a study on terpenoids of pterocarpus santalinus (isopterocarpolone, pterocarptriol and pterocarpdiolone)besides the known β-eudesmol, pterocarpol and cryptomeridiol. Their structures have been determined by spectral and chemical studies. The study conducted (13) used to check the cytotoxicity of selenium nanoparticles in rat dermal fibroblasts. Previous studies also state the similar effects (14).Our team has extensive knowledge and research experience

that has translated into high quality publications(15–19),(20),(21),(15),(22),(23),(24),(25)(17,26,27),(28–32) .

The aim of the present study is to synthesize extract of selenium nanoparticles using pterocarpus Santa and see its cytotoxic effect.

MATERIALS AND METHODS:

Green synthesis of Nanoparticle

The extract preparation was done by taking 0.5g of red sandal selenium mixed with 50ml of distilled water. The extract was boiled for 10mins at 55° Celsius and it had been filtered. Again sodium selenite was mixed in 50ml of distilled water and mixed with filtered pterocarpus santa extract. The red sandal selenium extract was kept in the shaker for an hour and reading was taken for its cytotoxic effect.

Fig-1 : Synthesis of P.santa Mediated Selenium nanoparticle

BRINE SHRIMP LETHALITY ASSAY:

Salt water preparation :

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10µL,20µL,40µL,80µL). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the plates were observed and noted for number of live nauplii present and calculated by using following formula,

Number of dead nauplii/number of dead nauplii+number of live nauplii×100

RESULTS:

On the first day of the cytotoxic action, all of the nauplii were alive. After 24 hrs the number of nauplii were dead in all wells except in the 5µl well. The percentage of lethality increased from 50%,100%,100%,100%,100% in the 5µL,10µL,20µL,40µL,80µL respectively (Figure 2).

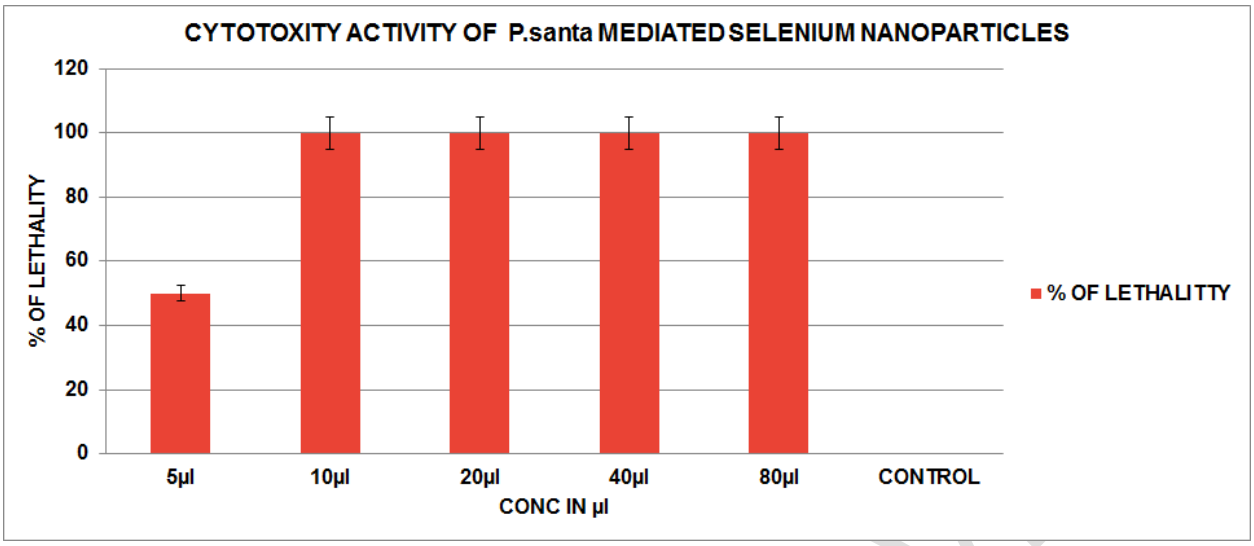

Figure-2 : The given figure represents the cytotoxic activity of brine shrimp using selenium nanoparticle extract where the X axis represents the concentration in µl and the Y axis represents the % of lethality of the brine shrimp nauplii, data implies as mean±SEM

DISCUSSION:

Pterocarpus santa mediated synthesis of selenium nanoparticles extract has a positive outcome in this present study. It has shown that it has a potent effect on cytotoxic activity. Many studies have been conducted in this activity but with other nanoparticles like silver nanoparticles etc were used(33). The advantage of the selenium nanoparticle is that it is cost- efficient, high yield in reactions and less time consuming. Protein, peptides, and a number of other reducing agents are used to produce various forms of Se nanoparticles.(34). The study done by (35) evaluated the antioxidant and cytotoxic effect of selenium nanoparticles (Se NPs) biosynthesized by a newly isolated marine bacterial strain bacillus sp. MSh-1.

In contrast to other selenospecies, Se nanoparticles have been identified as novel compounds with excellent antioxidant properties and lower toxicity.(36). Because of their low toxicity and high stability, selenium nanoparticles (SeNPs) are widely accepted and recommended for use in a variety of scientific disciplines.(37). A pharmacological dose of SeNPs could lead to cytotoxicity and induce cell death through apoptosis and extrinsic pathways contributing to SeNP-induced apoptosis in BRL cells.(38) . In a rodent model, the use of SeNPs significantly decreases the death caused by acute Se toxicity by up to four times. Furthermore, as demonstrated by biomarkers of hepatotoxicity, the liver injuries associated with high doses of Se are significantly decreased when SeNPs are used.(4).

The brine shrimp lethality bioassay is a straightforward cytotoxicity test for bioactive chemicals with a high throughput. It is based on the ability of test compounds to destroy brine shrimp, a basic zoological organism (Artemia salina).(39).

Previously our institution has done several studies which include (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53)

The major limitation of the study is that it is conducted *in vitro,* so it cannot be assumed that the results of the cytotoxic activity could be translated into clinical effectiveness. In future studies , *in vivo* studies are recommended with people's recommendation values as well.

CONCLUSION:

In the present study the brine shrimp nauplii showed that the pterocarpus santa mediated synthesis of the selenium nanoparticles showed better cytotoxic activity as the number of nauplii showed the percentage of the lethality by the increased concentration of the selenium nanoparticles extract(54-63).The Pterocarpus santa mediated synthesis of selenium nanoparticles showed potential cytotoxic effect in brine shrimp lethality assay showing emergence of a drug candidate for future research.

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