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Anti-inflammatory activity of Euphorbia Tirucalli Mediated Selenium nanoparticles: An in vitro study

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ABSTRACT

Nanomaterials have become a promising commodity in numerous industries during the past decade, including cosmetics, healthcare, biomedicine, and food. Selenium, a "drug nanocarrier," has potent antibacterial, antioxidant, anti-cancer, and anti-inflammatory effects. This study is aimed at synthesizing selenium nanoparticles using Euphorbia tirucalli to characterize and evaluate the anti-inflammatory activity.

Aim: This study aims to evaluate the anti-inflammatory activity of the Euphorbia tirucalli stem extract mediated by selenium nanoparticles.

Materials and methods: E.tirucalli extract was prepared. The biosynthesis of selenium nanoparticles was measured using UV vis spectrometry at wavelengths of 300-600 nm and the anti-inflammatory activity was determined using Bovine Serum Albumin at 10, 20, 30, 40, and 50 µl.

Results: The inhibition percentage was maximum at 81.2 % at IC50 concentration. The percentage of inhibitions was higher than the standard values in all the concentrations.

Conclusion: Selenium nanoparticles induced aqueous stem extract of Euphorbia tirucalli showed a higher percentage of inhibition when compared to standard. Hence, this can be used in commercial medical products to reduce inflammation and swelling.

Keywords: *Anti-inflammatory activity, Aqueous extract of Euphorbia Tirucalli, Selenium Nanoparticle*

INTRODUCTION

In recent years, the area of interest in nanotechnology has grown significantly and is being researched worldwide. A particle called a nanoparticle has a size between 1 and 100 nm. (Website, no date a) Due to their small size (between 1 and 100 nm), increased surface area to volume ratio, high biological activity, and ability to interact with microbial cells, nanoparticles are recognized as an alternative to

antimicrobial drugs. (Website, no date b)(Raj Preeth et al., 2021) (Sushanthi and Department of Oral and Maxillofacial Surgery, Saveetha Dental College, SIMATS, Saveetha University, Chennai, 2021) Nanoparticles have recently been increasingly used for their anticancer, antioxidant, and drug delivery.('Nanoparticles: Properties, applications and toxicities', 2019)(Arjunkumar, 2018)The development of an alternative, eco-friendly, simple, and reliable synthetic process based on the reduction capability of specific molecules from natural organisms became essential due to the chemical toxic by-products.(Balasooriya et al., 2017)

Selenium nanoparticles (SeNPs), a type of metallic nanoparticle, are gaining importance due to their distinct physical, biological, and chemical characteristics.('Nanoparticles: Properties, applications and toxicities', 2019)(Ramamurthy and Professor and Head, Department of Periodontics, Saveetha dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, 162, PH Road, Chennai 600077, India., 2021) Selenium is one such critical trace element that is important for the functioning of humans and mammals.(Zambonino et al., 2021) Selenium nanoparticles are synthesized by various physical, chemical and biological agents. Ascorbic acid is used as a reducing agent in the simple procedure of synthesizing SeNP which produces nanometer-sized particles with a zero-oxidation condition. (Zambonino et al., 2021) Due to their low toxicity and enhanced absorption as compared with traditional selenium supplementation, selenium nanoparticles (SeNPs) are gaining interest for use as food supplements or therapeutic agents. Studies have shown that Se-NPs have a wide range of applications in medical diagnostics, are low in toxicity, and have good biological properties that include anti-inflammatory, anti-cancer, anti-cancer-causing agents, anti-viral, anti-fungal, and nano-biosensors.(Antony et al., 2021)(Website, no date c)Selenium nanoparticles have been studied in a variety of inflammatory-mediated diseases, including diabetes, cancer, nephropathy, and arthritis.(Sakthi and Department of Public Health Dentistry, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 2021) Bhonsle et al found that biosynthesized selenium nanoparticles synthesized using Pterocarpus santalinus extract had a potent anti-inflammatory.(Bhonsle et al., 2021) Cremonini et al concluded that synthesis of SeNPS and their anti-inflammatory activity against Stenotrophomonas maltophilia and Gram-positive Bacillus mycoides. (Website, no date d) From the above studies, it is concluded that selenium nanoparticles elicit anti-inflammatory activity entering the oral cavity mucous membrane and reducing swelling, and preventing diseases like cancer. (Website, no date e)

Recently, Green fabrication of synthetic material has played a key role in enhancing human health care and the sustainment of nature. (Mathew et al., 2020) It has been reported that green synthesis is a better alternative for the production of non-toxic materials in contrast to harmful chemical compounds. (Jadoun et al., 2020) Using biomolecules involves mostly oxidation and reduction. Because of the medicinal properties, non toxic, low maintenance and easily available as well as ease of handling the plant is used for the synthesis of selenium nanoparticles.(Balasooriya et al., 2017) Euphorbia tirucalli L is a plant from the family of Euphorbiaceae and is an ornamental, widely grown as an unarmed shrub in India and Africa. (Website, no date f)(Abdel-Aty et al., 2019) Plant latex is used to cure gastrointestinal disorders, epilepsy, infertility, wounds, rheumatism, toothaches, hemorrhoids, tumors, and cancer. (Website, no date g)(Binckley and Zahra, 2022) Recent studies have shown a significant correlation between E. tirucalli whole plant methanol extracts and antioxidant activity, possibly as a result of their high phenolic content. (Binckley and Zahra, 2022)These extracts have been recognized as an excellent and widely available source of natural antioxidant activity. (Website, no date h)Furthermore, it has been reported to have antifungal, antibacterial, anthelmintic, diuretic, and sedative qualities. It also has anti-inflammatory and analgesic effects. (Website, no date g)(Antony et al., 2021).

A process of response to injury known as inflammation involves the deposition of cells and secretions in wounded tissues in order to prevent further damage. Although the main therapeutic agents for inflammation are steroids and non-steroidal anti-inflammatory drugs (NSAIDs), they can have serious adverse effects. Therefore, the production of new materials with comparable results and no side effects is required. Euphorbia

tirucalli extract was used in our study to produce selenium nanoparticles. The aim of this study was to synthesize, evaluate and characterize the anti-inflammatory activity of SeNPs through green methods. First novel study to report the Anti Inflammatory extract of Euphorbia tirucalli-mediated selenium nanoparticle through the Bovine Serum Albumin methods. (BSA)

MATERIALS AND METHODS

From the Institute of Saveetha Dental College and Hospital fresh stems of the Euphorbia Tirucalli plant were collected. The stem was cut into pieces and washed thrice with running tap water and once with distilled water. Then, it weighed approximately 15gm. It was grinded using a mortar and pestle to which 100 ml of distilled water was added. The solution was boiled at 70 degrees Celsius for 2 hours and allowed to cool. It was then filtered using Whatman no:1 filter paper.

Synthesis of SeNPs

60 mL of distilled water was used to dissolve 15 mM of selenium nanoparticles. 40 mL of Euphorbia tirucalli extract was then gradually added to the mixture. The reaction mixture was then kept at 400–600 rpm for 48–72 hours on a magnetic stirrer. The color change to brown indicates the preparation of the Se-NPs. Then, the selenium nanoparticles were kept for centrifugation at 8000 rpm for 20 min at room temperature for purifying purposes. The pellet was dried at 70°C in a hot air oven for 12 h. The dried pellet was grinded using mortar and pestle and the powder was stored in an airtight eppendorf tube for further use.

Characterization of Selenium Nanoparticles

To determine the optical properties of the E.tirucalli mediated selenium nanoparticles Double beam UV vis spectrometry at a wavelength of 300- 600 nm was used.

Anti Inflammatory activity of the E.tirucalli mediated SeNPs

Nanoparticles at serial dilution concentrations of 10, 20, 30, 40, and 50 µl were taken in 5 test tubes separately. To each test tube, 2 ml of 1% bovine serum albumin (BSA) was added. In test tubes containing 10, 20, 30, 40, and 50 µl of nanoparticles distilled water in quantities of 390, 380, 370, 360, and 350 µl was added. Diclofenac sodium was taken as standard (Reference drug) in five test tubes at concentrations of 10, 20, 30, 40, and 50 µl each. In each test tube, 2 ml of 1% BSA was added. The test tubes were incubated for 10 min at room temperature. They were then kept in a water bath at 55°C for 10 minutes. 2 ml of BSA solution was added to the test tube containing 2 ml of dimethyl sulfoxide as the control group. Using double UV vis spectrometry the absorbance was measured at 660 nm.

The percentage of inhibition was calculated by using the following formula Percentage Of Inhibition = Control Od - Sample Od/ Control Od X 100

RESULTS

Characterization of nanoparticles

Sodium selenite solution was combined with E.tirucalli during the formulation of selenium nanoparticles, but no chemical reaction was seen. A slight color change was noticed gradually at an interval of 24 hours. The bio-reduction of selenium ions using E.tirucalli stem extracts as a reducing agent and the stabilizing agent was seen by the increasing intensity of surface plasmon resonance/ absorption peak of selenium nanoparticles at 525 nm. The size of the metal nanoparticles and the medium's dielectric constant determines the surface plasmon resonance (SPR) absorption patterns.

Anti Inflammatory activity

Figure 2 represents the anti-inflammatory activity of E.tirucalli extract-mediated selenium nanoparticle extract at various concentrations against a standard value. It was found that the extract showed anti-inflammatory activity was higher at 40 and 50 µl with 75% and 80 % respectively. (table1)

FIGURE 1: Plant Euphorbia tirucalli and the preparation of aqueous plant stem E.tirucalli extract

TABLE 1: Indicates the antiinflammatory activity of SeNP induced Euphorbia tirucalli at various concentrations compared to the standard value.

Anti Inflammatory activity of the extract	
conc in ul	% of activity
10µl	2.031
20µl	1.547
30µl	1.560
40µl	1.569
50 µl	1.378

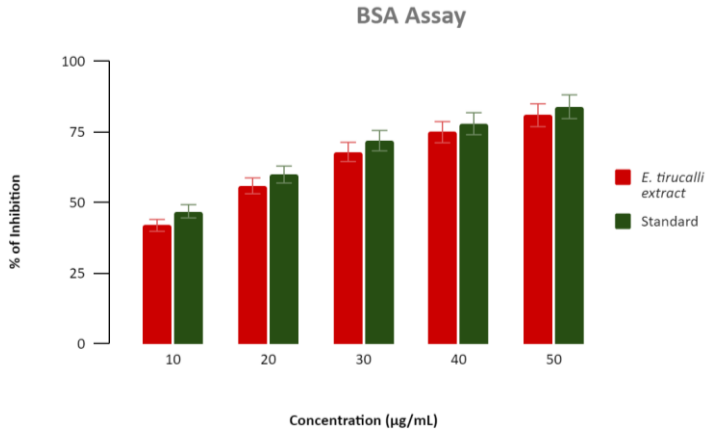

FIGURE 2: Graph indicated the antiinflammatory activity of SeNP-induced euphorbia tirucalli at various concentrations compared to the standard value.

DISCUSSION

In the recent century, the use of contemporary interdisciplinary scientific methodologies, such as metabolomics and nanoscience, has grown increasingly important in drug discovery.(Website, no date i) In the limelight of contemporary nanotechnology is the synthesis of selenium nanoparticles. The use of plant extracts in the biosynthesis of nanoparticles is currently being investigated. However, the toxic chemicals used in the chemical synthesis of NPs limited their application in medicine. To meet this requirement, green synthesis was done which was simple, cost effective, less harmful when compared to the chemical methods. (Sundaram, Nandhakumar and Haseena Banu, 2019) Selenium nanoparticles (SeNP) are more beneficial because of their nontoxicity,

biocompatibility, drug and antibacterial activities.Therefore, this study was done to evaluate the anti-inflammatory activities of SeNps induced with E.tirucalli extract.

There has been a significant increase in the exploration of nanoparticles for the treatment of various infections, cancer, inflammation, and other oxidative stress-related disorders. (Sundaram, Nandhakumar and Haseena Banu, 2019) Treatment with melatonin-SeNPs reduced splenocyte proliferation, proinflammatory cytokines, and chronic liver impairments. (Sundaram, Nandhakumar and Haseena Banu, 2019)('Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism', 2018). A recent study demonstrated that the green synthesis of selenium nanoparticles with the Capparis decidua possessed an effective anti-inflammatory activity.(Madhumitha et al., 2021)

Our study results, the maximum peak absorbance rate was at 525 nm using Double UV vis spectrometry showed a color change which was visually observed, which was in accordance with previous studies reported by Francis et al with an absorbance rate of 660 nm.(Website, no date j) This can be due to extract involved in the reduction of selenium ions and stabilization of SeNPs by phytochemicals in selenium nanoparticles. (Kirupagaran*, Saritha and Bhuvaneswari, 2016)

In the present study, SeNPs-mediated E. tirucalli extract showed increased anti-inflammatory activity with an increasing maximum at 81.2 % at IC^{50} concentration, when compared to the standard reference drug diclofenac, demonstrating that this formulation may be beneficial for treating inflammatory diseases. These results were similar to the previous study reported which showed an increase in anti-inflammatory activity by synthesis of selenium nanoparticles utilizing green sources such as clove and cumin as antiinflammatory agents. at 50µl (92.3%) and 40µl (85%) respectively. (Website, no date k; Francis et al., 2020; Saivarshine et al., 2021) Excessive production of ROS and RNS results in DNA breakage, lipid peroxidation, protein oxidation, and nitration, which ultimately affects enzyme activity, gene expression, and cell membrane structures. ROS-induced inflammation is mediated by the selenium nanoparticles through the formation of selenocysteine-specific tRNA. (Rehman, John and Bhatti, 2021) It has also been demonstrated that the Euphorbia tirucalli plant extract induced inhibition of intracellular interleukin-2 (IL-2) and interferon-gamma (IFN- γ)and decrease in CD4+ and CD8+ T Cells by flow cytometry (Bhonsle et al., 2021)

From the present study, it was found that E.Tirucalli-mediated SeNPs have a potential antiinflammatory activity at increasing concentrations which explains its usage in density as it adopts anti-inflammation mechanisms when penetrating the mucous membrane of the oral cavity and thereby suppressing swelling. (Website, no date l; Benjamaa et al., 2022) Hence this formulation can be utilized in treatments against inflammation, infection, and cancer.

CONCLUSION

It is concluded that the E.Tirucalli mediated Selenium nanoparticles can be used as a potential anti-inflammatory agent in the future for various infectious disorders. This can also be used for targeting various infections with minimal side effects and designing new drugs.

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CONFLICT OF INTEREST

None

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