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EMBRYONIC TOXICOLOGY EVALUATION OF STRONTIUM NANOPARTICLES SYNTHESIZED USING DAUCUS CAROTA

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ABSTRACT

AIM:

To evaluate Embryonic toxicology evaluation of strontium nanoparticles synthesized using Daucus carota, by evaluating the hatching rate and vitality rate .

INTRODUCTION:

Daucus carota, commonly known as carrot, is a root vegetable belonging to the Apiaceae family. It is widely cultivated worldwide for its edible taproot and is rich in nutrients such as beta-carotene and fiber. Carrots have various culinary uses and are also valued for their potential health benefits.Nanotechnology has witnessed significant advancements in recent years, leading to the development of various nanoparticles for diverse applications. Strontium nanoparticles have garnered considerable attention owing to their potential applications in biomedicine and drug delivery. However, their potential toxicity raises concerns regarding their safe utilization, particularly during critical stages of development, such as embryogenesis. This study aims to assess the embryonic toxicological effects of strontium nanoparticles synthesized using Daucus carota extract.

MATERIAL AND METHODS:

Two grams of carrot powder was added to 100 ml of distilled water,was held in a heating mantle at 50–60 °C for 20 minutes; in another conical flask, 20 millimolar strontium chloride was extracted (by adding 50 ml of distilled water); the extract was then added to the NPs combination; it was then filtered using Whattman paper, and the final carrot powder extract was produced.

RESULTS:

The toxicology of the Daucus carota mediated strontium nanoparticles was studied in accordance with its effects on zebrafish embryos , Two graphs were plotted which represented the hatching rate of the embryos and further their vitality rate in increasing concentrations of the extract , the graphs were further analyzed and concluded that even as the concentrations increased , the hatching rate and vitality rate was not affected to a dangerous extent .

CONCLUSION:

The embryonic toxicology evaluation of strontium nanoparticles synthesized using Daucus carota is vital for understanding their safety and enabling responsible application in various fields. Further research is needed to explore mechanisms, modify properties, and assess long-term effects for sustainable utilization, since it is a noble method when compared to other extracts already in existence which showed significant toxicology at higher concentrations of the extract.

Keywords: Embryonic toxicology-Strontium nanoparticles-Daucus carota-Nanoparticle synthesis -Biocompatibility-Zebrafish model

INTRODUCTION:

Through a large portion of human history, plants have served as the basis for medicinal treatments. Traditional medicine of this kind is being used extensively today. The herbal items utilized in conventional treatment have grown in popularity. A human disease may be treated and prevented all over the world with herbal medications. The efficacy, security, acceptance, and cost of herbal medications are common benefits(Satish S et al 2018).The World Health Organization's (WHO) strategy, 2014–2023, aims to strengthen the role of traditional medicine, emphasizing the importance of promoting and including the utilization of medicinal plants in the health systems of its member countries(WHO traditional medicine strategies 2014-2023).In wealthy nations, there has been a resurgence of interest in the use of medicinal plants, which account for 80% of therapeutic approaches in underdeveloped nations. The bulk of the world's population (87.5%) treats health issues with traditional herbal therapy(1).In local communities all around the world, medicinal plants have long been used as a source of healing. Even yet, it is still crucial today since it serves as the primary healthcare option for almost 85% of the world's population(Pesic et al 2015(2)and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them(3,4).Plants have a variety of health advantages, including antibacterial, antioxidant, anticancer, hypolipidemic, cardiovascular, respiratory, immunological, anti-inflammatory, analgesic, antipyretic, and many other pharmacological actions(Al snafi et al 2015).The nutritious and therapeutic plant Daucus carota, sometimes known as wild carrot, is a member of the Umbelliferae genus. In traditional Persian medicine (TPM), this plant is known by the names Zardak and Gazar. The Wild Carrot is a biennial, cultivated plant that grows from 30 cm to 1 m tall. It has several pinnate, segmented, hairy leaves and a fusiform, often red root(Rosita Bahrami et al 2018). A phytochemical investigation revealed the presence of alkaloids, carbohydrates, chlorogenic acid, flavonoids, phenols, essential oil, terpenoids, and coumarin in the root of Daucus carota. The nutritional analysis of carrot juice revealed that it contained: 6.100 0.346% carbs, 1.167 0.153% crude fiber, 0.367 0.089% crude fat, and numerous vitamins and minerals. Studies on the plant's pharmacological properties showed that they included cytotoxic, antioxidant, antidiabetic, antimicrobial, smooth muscle relaxant, hypotensive effect and decrease intraocular pressure, antidepressant, memory-enhancing, anti-inflammatory, reproductive, wound healing and hear induction, among many other effects.(Ali et al. 2017).

Materials having overall dimensions in the nanoscale, or under 100 nm, are known as nanoparticles. These materials have become significant actors in contemporary medicine in recent years, with uses ranging from contrast agents in medical imaging to carriers for delivering genes to specific cells. Due to their small size, nanoparticles differ from bulk materials in a number of ways, including chemical reactivity, energy absorption, and biological mobility(5).Some nanoparticles might be used in cutting-edge diagnostic tools, imaging and methodology, pharmaceutical goods, biomedical implants, and tissue engineering, as well as targeted medicines. Today, high toxicity medicines, such as chemotherapy-based cancer medications, may be provided with increased safety because to nanotechnology.(Abid Haleem et al. 2023(6)).The advantages of noble metal-based nanoparticles, which are important for medical applications, include high biocompatibility, stability and the possibility of large-scale production avoiding organic solvents and thus giving a positive effect on biological systems.(Bartosz et al 2018).The global metal nanoparticles market was valued at US$ 25,373.92 million in 2020 and is projected to reach US$ 81,567.38 million by 2028; it is expected to grow at a CAGR of 15.9% from 2021 to 2028(Dublin jan 2022).Metals like strontium (Sr) and hafnium (Hf) are becoming more well-known as potential trace elements(Rajaraman V et al. 2020). Strontium nanoparticles are spherical or nanoflake high surface area metal particles with properties that include stopping transmission of HIV and other viruses.. Nano strontium particles are available in Size range of 10 to 200 nm, which specific surface area in the 30-60 m^2/g range And also available as flakes with an average particle size of 2 to 10 microns range With a specific area of approximately 40 to 80 m^2 per gram(Karim et al 2022).However incomplete knowledge exists on strontium's role in the human body,but since this element shares reactivity with calcium and its chemical and physical are similar to calcium (Ca), it has been used in various research models. Studies have reported that Sr has been shown to promote osteoblast replication, differentiation and survival.To increase their biological activity and physicochemical characteristics, Sr has recently been added to tricalcium phosphate (TCP), hydroxyapatite (HA), and calcium phosphate cement (CPC).(Guo D et al 2009).

Zebrafish larvae have been popular in recent years as an animal model for research on the toxicity of nanomaterials.(Rajabi S et al 2015),this is due to their small size, transparent embryos, and simplicity of handling. Zebrafish can be used for a variety of toxicity testing, including behavioral assays, developmental toxicity tests, and acute toxicity tests. Acute toxicity studies entail exposing adult zebrafish to a drug at escalating doses for a brief time—usually 24 to 96 hours—while keeping an eye out for any fatalities or other negative effects.

These tests can reveal a substance's lethal dose (LD50), as well as any sub-lethal consequences such alterations in physiology or behavior.Zebrafish embryos are subjected to substances in developmental toxicity studies to check for developmental defects or mortality.They can also reveal whether a chemical increases the risk of birth abnormalities or other developmental problems. In behavioral assays, zebrafish are exposed to a chemical while being watched to see if their behavior changes, such as their swimming style, shoaling habits, or how they react to a given stimuli. These tests can reveal details about a substance's potential neurotoxicity or other potential consequences in their behaviour.(B Sumedha et al 2023). The study's objective is to evaluate Embryonic toxicology evaluation of strontium nanoparticles synthesized using Daucus carota, by evaluating the hatching rate and vitality rate .

MATERIALS AND METHODS

The current study was undertaken by Saveetha dental college and the study was conducted for a period of three months.

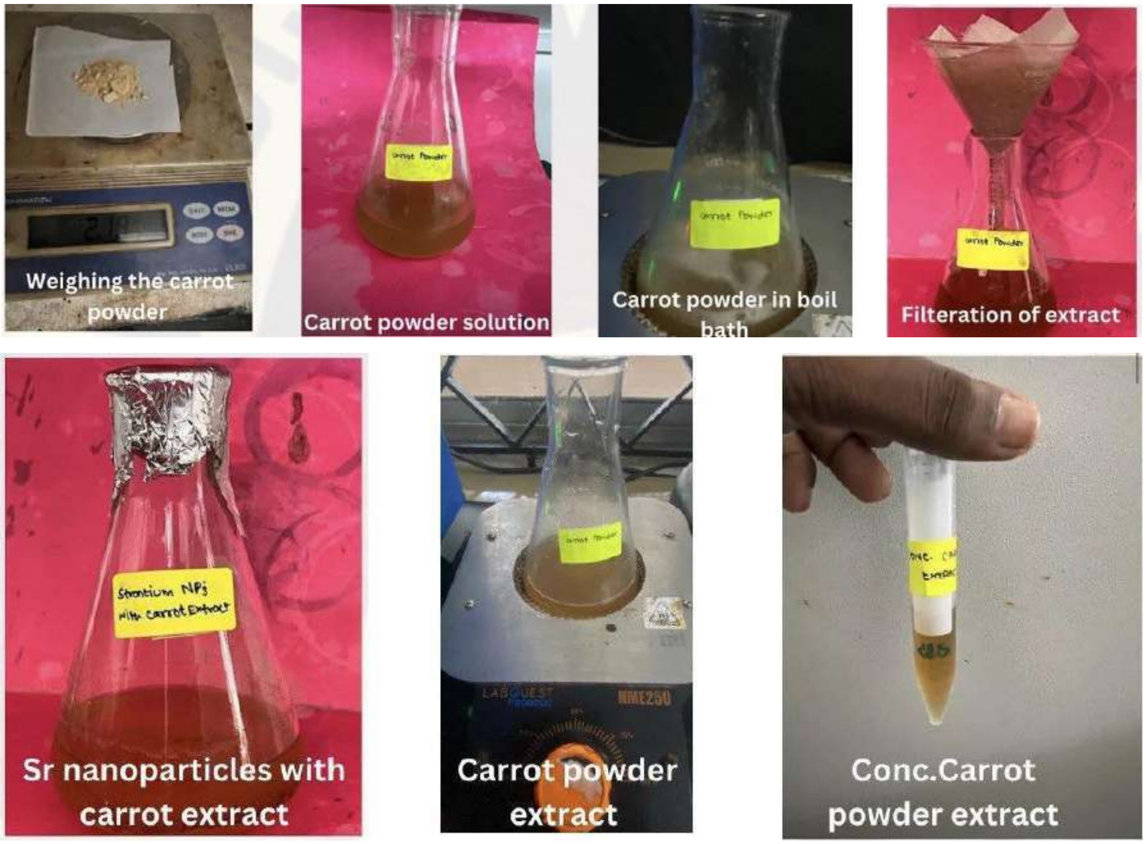
Zebrafish Embryonic Toxicology Evaluation of Strontium Nanoparticles

Fish Maintenance and NPs Exposure

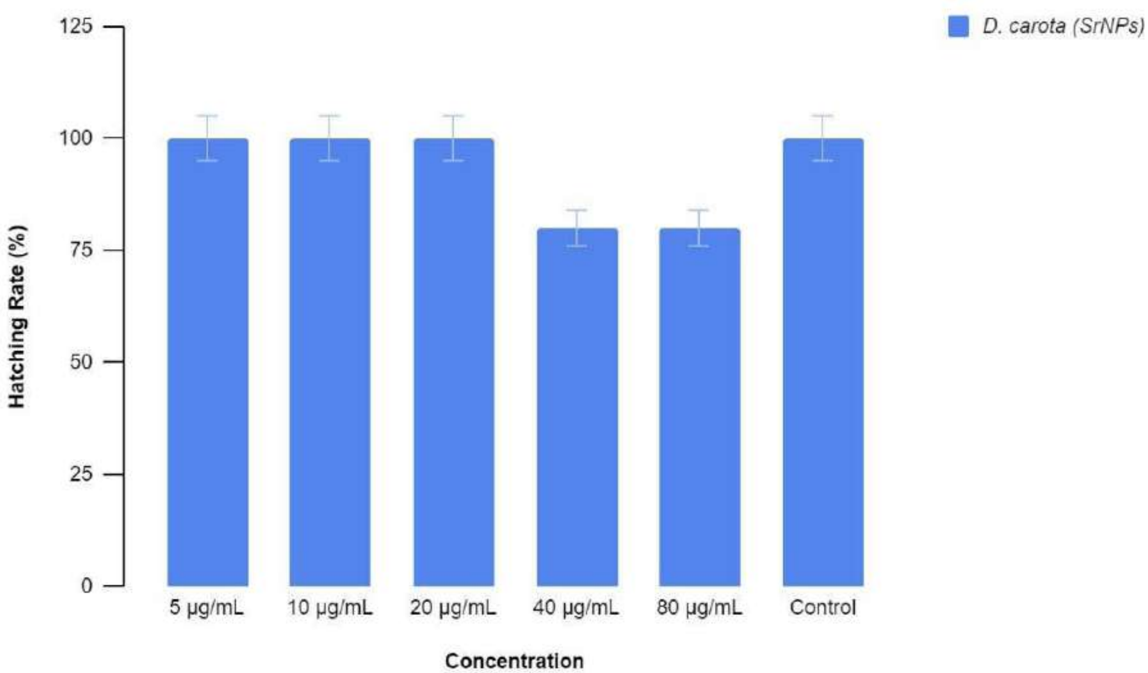
Wild-type zebrafish (Danio rerio) were acquired from local Indian vendors and were housed in individual tanks under controlled conditions of temperature (280±20C), light/dark cycle (14:10 h), and pH (6.8–8.5). The fishes were fed with commercially available dry blood worms or optimum food twice a day. Zebrafish embryos were obtained by crossing one female and three males per breeding tank, and viable eggs were collected and rinsed at least three times with freshly prepared E3 medium without methylene blue. The study involved the placement of fertilized eggs in culture plates of varying well sizes (6, 12, and 24 wells) with 20 embryos per 2mL solution per well. The experimental treatment and control groups were replicated three times. To prepare the experimental treatment, a stock suspension of TCF-SrNPs with five different concentrations was freshly made and added directly to the E3 medium. The solution was sonicated for 15 minutes to disperse the nanoparticles while maintaining a pH range of 7.2-7.3.Healthy fertilized embryos were exposed to different concentrations of SrNPs ranging from 0 to 1000 µg/L for 24 to 96 hours post fertilization. The SrNPs were added to the E3 medium in which the embryos were incubated. Control groups were also included in the experiment.Dead embryos were removed from the nanoparticles exposed groups every 12 hours. All experimental plates were wrapped in foil to exclude light and maintained at 28°C.

Zebrafish embryo evaluation

Throughout the exposure period following fertilization, the developmental stages of Zebrafish embryos were monitored using a stereo microscope. The embryos were subjected to various concentrations of silver nanoparticles (0, 100, 250, 500, 750, and 1000 µg/L) for 24-78 hpf. Embryonic mortality and hatching rates were assessed at 24-hour intervals. The study endpoints included embryo/hatchling mortality, hatching rate, and the identification and documentation of any malformations among the embryos and larvae in both control and treatment groups. Photographs of malformed embryos were captured using a COSLAB - Model: HL-10A light microscope, and the percentage of abnormal embryos was recorded every 24 hours.

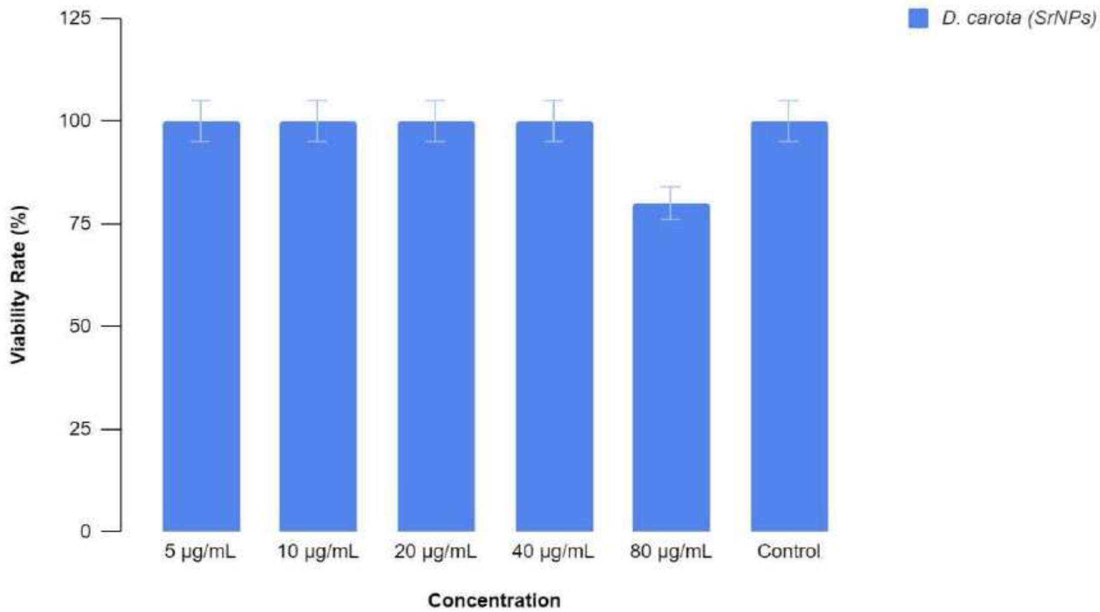


RESULTS



Graph 1: Relation between hatching rate (%) of zebrafish with increasing concentrations for Dacus carota Sr nanoparticles mixture.

The hatching rate of zebrafish embryos was found to be the highest when the concentration was 5 µg/ml - 20 µg/ml and as the concentration increased from there onwards, the hatching rate decreased.



Graph2: Comparison between viability rate of Dacus Carota Sr nanoparticle mixture with increasing concentrations.

Similarly, the viability rate was greatest in and equal in all concentrations from 5 µg/ml - 40 µg/ml and the viability of the embryos decreased only at 80 µg/ml

DISCUSSION

From the above graphs The percentage of eggs that successfully hatch shows a correlation, with increasing levels of Dacus carota Sr nanoparticles mixture. As the concentration of nanoparticles goes up the ability of the eggs to hatch decreases. This indicates that there may be an impact of these nanoparticles on the development of embryos. It is crucial to conduct studies to understand the underlying mechanisms behind this relationship.The rate at which Dacus carota Sr nanoparticle mixture remains viable noticeably decreases as concentrations increase. This decline suggests that toxicity is dependent on concentration highlighting the need for evaluating nanoparticle safety. Additional research is necessary to clarify the mechanisms driving this trend and assess any health implications.

Nanomaterials are becoming a part of our daily lives and cannot be completely avoided when exposed to humans or the environment.

Metal oxide nanoparticles have recently attracted a lot of attention for use in biomedical tissue engineering and implants, notably orthopedic and dental implants.(Nikolova et al. 2020).The embryonic zebrafish has shown great potential as an in vivo model for the toxicity of nanomaterials. The chorion and vitelline membranes, as well as the perivitelline space between them, make up the two membranes that make up zebrafish eggs. The chorion's pore canals have a diameter of 0.5 to 0.7 meters(Lee KJ et al 2007).Larger nanoparticle sizes may obstruct the chorion pore canals, but smaller nanoparticle sizes may be able to do so. There will be very little penetration through the pore since the metal nanoparticles used in this study range in size from 50 to 100 nm. Nanomaterials with strontium conjugation exhibit antibacterial properties and efficiently filter out hazardous contaminants from industrial effluent.Strontium nanoparticles can be utilized as an effective immunotherapy tool since they are employed for targeted medication administration and can trigger an immune response that lasts for a long time.(Sumedha et al 2023).(3)

The findings of the study by Harini Sri et al. in 2022 strongly suggest that increasing the concentration of strontium nanoparticles may result in acute toxicity and developmental deformities, particularly given the differences in the toxic concentration in the zebrafish model, a nonmammalian lower vertebrate and human cell. Additional toxicity studies are necessary to define the effects of various dosages and determine a particular concentration for further clinical trials.(17)

Oolong tea was synthesized using strontium nanoparticles in another study conducted by B Sumedha et al 2023, that looked at how it affected zebrafish embryos. It was discovered that high doses of oolong tea cause developmental delays, increased mortality, and decreased hatchability. Although there is no direct evidence for the fetal toxicology of zebrafish, the results of the present study show that high concentrations of Dacus carota do not have similar effects on the development of zebrafish embryos, as the hatching rate didn't significantly decrease as the concentration increased and the vitality rate was at its highest at all but the concentration of 80 micrograms/ml.The ideal concentration of Dacus carota-mediated strontium nanoparticles and the length of time necessary to cause toxicity, however, still require more study.(18)

CONCLUSION:

In conclusion, this study provides significant insights into the embryonic toxicology evaluation of strontium nanoparticles synthesized using Daucus carota extract. The use of Daucus carota as a green and sustainable source for nanoparticle synthesis is advantageous, as it offers biocompatible nanoparticles that hold promise for various biomedical applications. However, the potential toxicity of these nanoparticles necessitates rigorous assessment, especially during embryogenesis.

Our findings demonstrate that exposure to strontium nanoparticles even at higher concentrations of the extract led to no significant changes in hatching rate and vitality rate until it reached its maximum concentrations, when compared to other studies done to evaluate possible toxicology with zebrafish embryos, which showed increased mortality rates and the occurrence of significant developmental abnormalities as the concentration of extract increased, Daucus carota mediated strontium nanoparticles did not show such results even as the concentration increased . Therefore further toxicology studies and enchantment of Daucus carota mediated strontium nanoparticles is necessary to establish it as potential tool as a non toxic tool in nanotechnology.

CONFLICT OF INTEREST:

The author reported the conflict of interest while performing this study to be nil.

ETHICAL CLEARANCE:

Ethical approval number not needed because it is an in-vitro study.

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REFERENCES:

1. N. Chandra Mohana, H.C. Yashavantha Rao, D. Rakshith, P.R. Mithun, B.R. Nuthan, S. Satish,Omics based approach for biodiscovery of microbial natural products in antibiotic resistance era,Journal of Genetic Engineering and Biotechnology,Volume 16, Issue 1,2018,Pages 1-8,ISSN 1687-157X, https://doi.org/10.1016/j.jgeb.2018.01.006.

2. Parveen A, Parveen B, Parveen R, Ahmad S. Challenges and guidelines for clinical trials of herbal drugs. J Pharm Bioallied Sci. 2015 Oct-Dec;7(4):329-33. doi: 10.4103/0975-7406.168035. PMID: 26681895; PMCID: PMC4678978.

3. Pešić M. (2015). Development of natural product drugs in a sustainable manner. Brief for United Nations Global Sustainable Development Report 2015. Available at: https://sustainabledevelopment.un.org/content/documents/6544118_Pesic_Development%20of%20natural%20product%20drugs%20in%20a%20%20sustainable%20manner.pdf. (Accessed August 15, 2018).

4. Bauer A., Brönstrup M. (2014). Industrial natural product chemistry for drug discovery and development. Natural Prod. Rep. 31 (1), 35–60. 10.1039/C3NP70058E [PubMed] [CrossRef] [Google Scholar]

5. Al-Snafi, A. E. (2017). Nutritional and therapeutic importance of Daucus carota-A review. IOSR Journal of Pharmacy, 7(2), 72-88.

6. Rosita Bahrami, Ali Ghobadi , Nasim Behnoud, Elham Akhtari(;2018)Medicinal Properties of Daucus carota in Traditional Persian Medicine and Modern Phytotherapy ; Biochem Tech (2018) Special Issue (2): 107-114.

7. Abid Haleem, Mohd Javaid, Ravi Pratap Singh, Shanay Rab, Rajiv Suman, Applications of nanotechnology in medical field: a brief review,Global Health Journal, Volume 7, Issue 2,2023,Pages 70-77,ISSN 2414-6447, https://doi.org/10.1016/j.glohj.2023.02.008.

8. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. Int J Nanomedicine. 2007;2(2):129-41. PMID: 17722542; PMCID: PMC2673971.

9. Sanjog Agarwal, Subhabrata Maiti, S. Rajeshkumar, Vatika Agarwal, Madhura Deshmukh, & Dhanraj Ganapathy. (2022). Green synthesis and Characterization of Strontium And Zirconium Nanoparticles from Green tea leaf Extracts and Studying their Antimicrobial Activity and antiinflammatory activity against Oral Pathogens. Journal of Pharmaceutical Negative Results, 539–543. https://doi.org/10.47750/pnr.2022.13.S07.073

10. Harini Sri, Subhabrata Maiti, Vinay Sivasamy, S. Rajeshkumar. (2022). Cytotoxicity Of Strontium and Hafnium Containing Bone Graft in The Embryonic Development of Zebrafish - An Animal Study. Journal of Pharmaceutical Negative Results, 2292–2297. Retrieved from https://pnrjournal.com/index.php/home/article/view/3705

11. Karim ME, Chowdhury EH. PEGylated Strontium Sulfite Nanoparticles with Spontaneously Formed Surface-Embedded Protein Corona Restrict Off-Target Distribution and Accelerate Breast Tumour-Selective Delivery of siRNA. Journal of Functional Biomaterials. 2022; 13(4):211. https://doi.org/10.3390/jfb13040211

12. Dagang Guo, Kewei Xu, Xiaoyun Zhao, Yong Han, Development of a strontium-containing hydroxyapatite bone cement, Biomaterials,Volume 26, Issue 19,2005,Pages 4073-4083,ISSN 0142-9612, https://doi.org/10.1016/j.biomaterials.2004.10.032.

13. Rajabi S, Ramazani A, Hamidi M, Naji T. Artemia salina as a model organism in toxicity assessment of nanoparticles. Daru. 2015 Feb 24;23:20.

14. Rajaraman V, Nallaswamy D, Ganapathy DM, Kachhara S. Osseointegration of hafnium when compared to titanium - A structured review. Open Dent J. 2021 Apr 16;15(1):137-44.

15. Nikolova MP, Chavali MS. Metal Oxide Nanoparticles as Biomedical Materials. Biomimetics. 2020 Jun 8;5(2):27.

16. B.Sumedha, Sandhya Sundar, Rajesh kumar Shanmugam, Ramya Ramadoss, Suganya Paneerselvem, & Pratibha Ramani. (2023). Embryonic toxicology and antimicrobial potential of strontium nanoparticles synthesized using Oolong tea. Journal of Population Therapeutics and Clinical Pharmacology, 30(15), 516–522. https://doi.org/10.47750/jptcp.2023.30.15.058

17. Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.

18. Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. Gulf J Oncolog. 2021 Sep;1(37):36-41.