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RESEARCH ARTICLE
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Embryonic toxicology and antimicrobial potential of strontium nanoparticles synthesized using Oolong tea

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ABSTRACT

Background: Oolong tea due to the presence of flavonoids such as catechins, theaflavins, and TheaSinensis is used as a reducing agent to prepare strontium nanoparticles. When developing metal nanoparticles, the plant extracts-mediated technique is very effective and advantageous compared to employing other techniques.

Aim: To synthesize and evaluate the cytotoxicity, embryonic toxicology and antimicrobial properties of strontium nanoparticles mediated through oolong tea.

Materials and methods: Oolong tea extract was prepared and added with Strontium oxide to synthesize the strontium nanoparticles. The embryonic toxicity of the prepared nanoparticles is determined by assessing the toxicity to the hatching rate and viability rate of zebrafish embryos in different concentrations . The antimicrobial potential of the produced nanoparticles were assessed by determining the zone of inhibition against the common oral pathogens such as staph.aureus, strep.mutans, E.faecalis, candida. Albicans.

Result: There was also concentration dependent alterations in the hatching and viability rate of zebrafish. The anti-microbial assessment demonstrated the mean zone of inhibition of 23mm against Enterococcus faecalis, 33mm against Staphylococcus aureus, 23mm against Streptococcus mutans and 9mm against Candida albicans, by the end of fourth day.

Conclusion: Oolong tea shows strong antimicrobial activity when treated with strontium nanoparticles thus it can find a great application in preventive dentistry.

Keywords: *oolong tea, strontium nanoparticles,zebrafish, Embryonic toxicity, antimicrobial potential*

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INTRODUCTION

Oolong tea is a traditional Chinese tea. It's made from the leaves of the *Camellia sinensis* plant, the same plant which is used to make green tea and black tea. The only difference is how the oolong tea is processed. Oolong tea is made from the partially oxidized parts of the *Camellia sinensis* plant.Brewing techniques for oolong tea may vary.One of the most common methods used to brew oolong tea is to use a small steeping vessel , such as a gaiwan or a hiding clay teapot, with a higher than usual leaf to water ratio. Such methods are used in the gong function method for tea preparation, which involves multiple short steepings (Needham and Ronan 1978).Oolong Tea helps in preventing heart diseases, helps in weight loss and also helps in preventing cancer. Each cup of oolong tea consumed per day according to research was equated to a 4% lower risk, but the results were not significant. Oolong tea also helps in preventing cavities since the day contains natural fluoride.Hence consumption of less than 1 L of who long tea helps in preventing dental caries.(Smith 2016).

Incomplete knowledge exists on strontium's role in the human body. However, this element shares reactivity with calcium. These elements share similar biological and physical properties as a result. Strontium has been shown to substitute calcium in a number of physiological processes in animals, including muscle contraction, blood coagulation, and the release of certain hormones. However, these processes are not as effective as calcium ion induction. Strontium can also be taken up by bone cells, boosting bone density and reducing the incidence of osteopenia and osteoporosis. However, a high dose of Sr2+ given intravenously causes an increase in the kidneys' ability to eliminate Ca2+ ions, which culminates in hypocalcemia.

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).

When comparing a green approach to physical and chemical methods, it is important to take into account three factors: the solvent, the capping agent, and the reducing agent. When developing metal nanoparticles, the plant extracts-mediated technique is very effective and advantageous compared to employing microorganisms, where the cell maintenance period is constrained. To use nanoparticles in field applications, it is crucial to follow certain procedures, such as leaf extract preparation, phytochemical screening, and precursor preparation. These procedures involve the synthesis and characterization of nanoparticles.(Devatha and Thalla 2018).

Several researchers have investigated the positive benefits of oolong tea consumption on periodontal health, and these may be supported by the catechins' inhibitory activity on periodontal bacteria. Oolong tea catechin inhibits Prevotella gingivalis, Prevotella intermedia, and Prevotella nigrescens proliferation and adhesion to human buccal epithelial cells. Major tea polyphenols like EGCG, ECg, and gallocatechin gallate, which have 3-galloyl radial steric structures, prevent the production of dangerous end products from P. gingivalis. In a study, green tea catechin, EGCG, and ECg were found to inhibit P. gingivalis collagenase activity .

The aim of our study was to analyze the embryonic toxicology and antimicrobial potential of strontium nanoparticles in oolong tea.

MATERIALS AND METHODS

Ethical clearance was obtained from the Standard review board of Saveetha Dental College prior to the commencement of the study.Nano particle was effectively synthesized by the below mentioned procedure. The synthesized nanoparticle was subjected to toxicology and antimicrobial testing.

A. Preparation of the strontium nanoparticles

Fresh *Camellia sinensis* leaves were collected, washed under running water, and then washed once more with double-distilled water. To make the sample's aqueous extract, 10g of newly gathered leaves were boiled with 100 ml of distilled water at 60°C for around 20 minutes, or until the color of the aqueous solution changed from watery to light yellow. The extract was then warmed to room temperature, put through a filter with paper, and used in additional tests. Strontium oxide was mixed with the extract for the production of nanoparticles, and a magnetic

stirrer was used to mix the fluid continuously. The solution was rapidly agitated for 5 to 6 hours at about 150°C once the mixture had completely dissolved. The supernatant was taken out and thrown away once the solution had been cooled to room temperature.The resulting pale white solid product was thoroughly washed, centrifuged twice at 4500 rpm for 15 minutes, and then dried at 80°C for 7-8 hours.

B. Assessment of Embryonic toxicology of Zebrafish

Zebrafish (Danio rerio) have become a popular model organism for studying toxicity and drug discovery due to their small size, transparency of embryos, and ease of handling. There are several methods to assess toxicity using zebrafish, including acute toxicity tests, developmental toxicity tests, and behavioral assays.Acute toxicity tests involve exposing adult zebrafish to increasing concentrations of a substance for a short period, typically 24-96 hours, and monitoring for mortality or other adverse effects. These tests can provide information on the lethal dose (LD50) of a substance, as well as sub-lethal effects such as changes in behavior or physiology.Developmental toxicity tests involve exposing zebrafish embryos to a substance during early development and monitoring for developmental abnormalities or mortality. These tests can provide information on the potential for a substance to cause birth defects or other developmental issues.Behavioral assays involve exposing zebrafish to a substance and monitoring for changes in behavior such as swimming speed, shoaling behavior, or response to stimuli. These tests can provide information on the potential neurotoxicity or other behavioral effects of a substance.

In all of these tests, zebrafish are typically housed in specialized tanks with controlled temperature, light, and water conditions to ensure consistent results. The tests can be conducted using individual zebrafish or in groups, depending on the specific research question being addressed.

C. Evaluation of Antimicrobial Activity of strontium nanoparticle derived from oolong tea

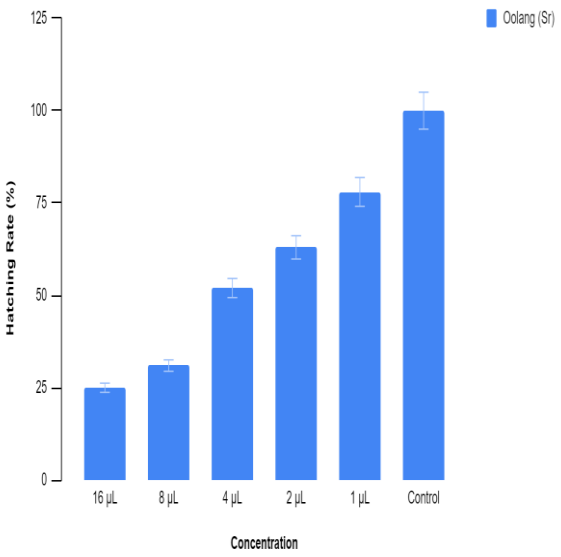
Antibacterial activity: Relative nanoparticles' antibacterial activity against the strains of E. faecalis, Staphylococcus aureus, Candida albicans, and Streptococcus mutans. In order to identify the zone of inhibition, Mueller Hinton Agar was used. For 15 minutes at 121 degrees Celsius, Mueller hinton agar was prepared and sterilized. Sterilized plates were filled with media, which was then left to solidify. A 9mm sterile polystyrene tip was used to cut the wells, and the test organisms were then swabbed. Different concentrations of nanoparticles (25 L, 50 L, and 100 L) were loaded, and a conventional antibiotic, amoxyrite, was added in the fourth well. At 37 °C, the plates were incubated for 24 hours. The zones of inhibition were measured following the incubation period.

Assessment of Antifungal activity: Agar well diffusion experiment uses the pathogen Candida albicans as a test pathogen. The fungus medium is made with Rose Bengal Agar. After test organisms were swabbed into the prepared and sterilized medium, different concentrations of nanoparticles (25 l, 50 l, and 100 l) were added to the wells, and in the fourth well, the standard antibiotic fluconazole was loaded. For 48–72 hours, the plates were incubated at 37°C. The zone of inhibition was assessed following the incubation period.

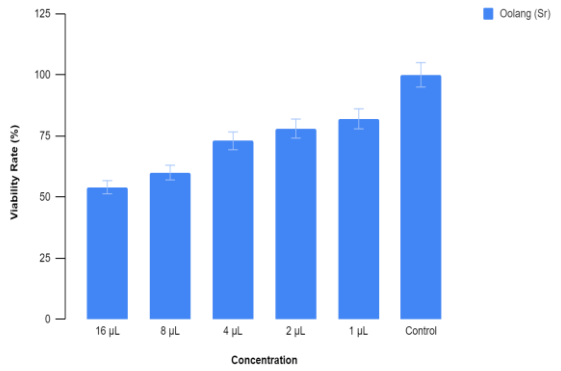
RESULTS

Embryonic toxicology Study (graphs)

Graph -1

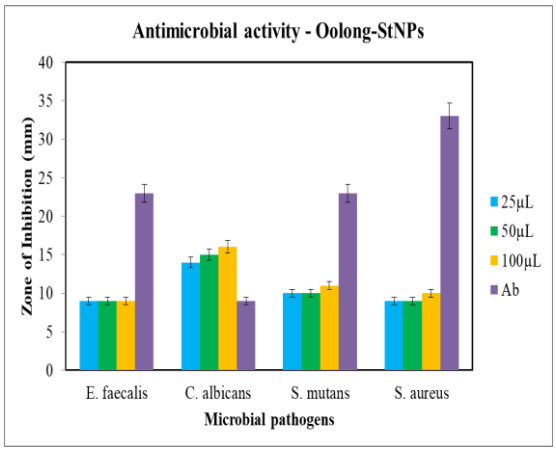


The graph shows the relation between hatching rate (%) of zebra fish with decreasing concentrations for oolong tea+ Sr nanoparticles mixture.



The graph shows the comparison between viability rate of oolong tea+ Sr nanoparticle mixture with decreasing concentrations.

Antimicrobial activity table Zone of inhibition against each organism
Graph-1



The graph-1 shows the comparison of the zone of inhibition in mm of different microbes such as Enterococcus faecalis, Candida albicans, Streptococcus mutans and staphylococcus aureus taken at different concentrations.

DISCUSSION

Strontium-conjugated nanomaterials have antimicrobial activity and effectively remove toxic pollutants from industrial wastewater. Strontium nanoparticles are used for targeted drug delivery and can induce a long-lasting immune response, so they can act as a good immunotherapy tool. Applications of strontium nanoparticles have also been found in diabetics**,** where they can control the release of insulin and thus regulate the pathophysiology of diabetes. Strontium nanoparticles are also used in wastewater treatment, agriculture, and as gas sensors to detect several toxic gasses.

Green synthesis of strontium nanoparticles can also be done with other varieties of tea such as black tea, green tea, white tea, etc but we have have preferred oolong tea for our study because of the presence of certain antioxidants and alkaloids specific to oolong tea.

Polyphenols are antioxidants that most people choose to consume when drinking tea for health reasons. Some of the polyphenols in oolong tea are theaflavins, thearubigins and EGCG, which are responsible for many of the health benefits of oolong tea. Polyphenols in oolong tea can help treat digestive problems, weight management, diabetes, neurodegenerative diseases, and heart disease. They also have strong anti-cancer properties. It also contains various minerals such as carotene, calcium, copper, selenium and vitamins A, B, C, E and K. In addition, oolong tea contains non-toxic alkaloids such as folic acid and niacinamide. Because oolong tea is semi-fermented, it also contains various polyphenolic compounds that provide additional health benefits.

From the graphs obtained from plotting concentration and zone of inhibition we have found that Oolong tea, like other types of tea, contains polyphenols and catechins that have been shown to have antimicrobial properties. Studies have demonstrated that oolong tea can inhibit the growth of various microorganisms, including bacteria, viruses, and fungi.For example, one study found that oolong tea extract was effective in inhibiting the growth of four strains of bacteria, including Staphylococcus aureus and Escherichia coli. Another study demonstrated that oolong tea extract had antiviral activity against the influenza virus.Oolong tea has also been found to have antifungal properties. In a study, oolong tea inhibited the growth of Candida albicans, a common yeast that can cause infections in humans.Overall, these findings suggest that oolong tea may have potential as a natural antimicrobial agent. In our study we have determined the optimal concentration of oolong tea required for antimicrobial activity and the mechanisms by which it exerts its effects.

Little research has been done on the embryonic toxicology of oolong tea using zebrafish. However, previous studies have investigated the effects of another type of tea on embryonic

development and toxicity in zebrafish. For example, one study found that exposure to high concentrations of green tea extract during early embryonic development caused abnormal development and growth retardation in zebrafish embryos. Another study examined the effects of black tea on zebrafish embryos and found that high doses of black tea caused developmental delays, increased mortality and reduced hatchability. Although there is no direct evidence for the fetal toxicology of zebrafish oolong tea, it is possible. that high concentrations of oolong tea can also have similar effects on zebrafish embryonic development. However, further research is needed to determine the optimal concentration of oolong tea and the duration of exposure required to produce toxicity. It is worth noting that the concentration and duration of exposure to oolong tea required to cause zebrafish embryotoxicity is likely. are much higher than what people usually consume when drinking tea. In general, moderate consumption of oolong tea is considered safe for human consumption.

The first two graphs were plotted for testing the embryonic toxicity of the sample using zebrafish. The graph plotted for conc. Vs. Hatching rate showed that with decrease in concentration the hatching rate increased. The graph which was plotted for conc. Vs. Viability rate showed that as concentration decreased, viability rate increased.The graphs obtained show that the oolong tea(Sr) sample shows strong antimicrobial activity and has passed the embryonic toxicity test. While the research results that have been discussed suggest that oolong tea may be effective against many micro organisms there are certain issues that need to be addressed concerning these results. (8(Chou, Lin, and Chung 1999))Agencies such as the Clinical And Laboratory Standards Institute (CLSI) In the United States, have strict protocols for the determination of antimicrobial susceptibility. Hopefully, in the future, researchers will be able to study the effects of oolong tea on infections in humans.(9(Wu et al. 2007))This type of research is the critical part of determining the antimicrobial capabilities of oolong tea. It might possibly be incorporated into research with other antimicrobial compounds. Perhaps naturopathic practitioners could begin to collect data on patients using oolong tea. With emerging multidrug-resistant organisms and the lack of effective new antimicrobial drugs which are being produced, we cannot afford to ignore the potential of oolong tea.(10(Zhang, Qi, and Mine 2019)).

The next graph obtained shows that C albicans have the largest zone of inhibition among the other microbes studied. The antimicrobial potential of C albicans was found to be better than the standard antibiotic taken for comparison. For Staphylococcus aureus, streptococcus mutans And Enterococcus faecalis the antimicrobial potential was found to be lower than the standard antibiotic. But in all the four microbes the antimicrobial potential was found to increase with increase in concentration.

In comparison to the earlier part of the century, nanoparticle production has improved quickly in recent years. Large-scale nanoparticle synthesis using traditional physical and chemical methods takes less time, but toxic substances are used as capping agents to ensure stability. Until recently, standard techniques were used to make nanoparticles.

These methods damaged the environment since dangerous compounds were used, which is why they were used. The Green Synthesis technique was created to do away with the use of such hazardous chemicals, and it is now utilized all over the world. It's an environmentally friendly and cost-effective solution.(11(Thangavelu et al. 2022))

This study employed strontium and oolong tea, which is the key factor contributing to this feature and has been proved in research to be an effective antibacterial, with promising outcomes. Specific bacteria are killed when they come into contact with a mixture of strontium and oolong tea because the ions generated from the copper surface cause internal oxidative stress in the bacterial cell wall. Although this phenomenon has long been known, interest in it among experts has recently increased. For the aforementioned occurrence, the term "contact killing" was coined. In the year 2008, copper was designated as the first antibacterial metal by the United States Environmental Protection Agency (US EPA). A major advantage of the combination of strontium and oolong tea as an antimicrobial agent is its low level of bacterial resistance. (12(Andhare et al. 2022)

(Our team has extensive knowledge and research experience that has translate into high quality publications(Vishnu Prasad et al. 2018; Ramesh

Kumar et al. 2011; Ganapathy et al. 2022; Arumugam et al. 2021; Mohanavel et al. 2020; Muthukrishnan 2021; Chellapa et al. 2020; Markov et al. 2021; Felicita 2017; Uthrakumar et al. 2010)).

CONCLUSION

Within the limits of this particular study, we can say that Oolong tea shows strong antimicrobial activity when treated with strontium nanoparticles thus it can find a great application in preventive dentistry.

AUTHOR CONTRIBUTIONS

Study design, Conceptualisation – Dr.S.Sandhya, Dr. R.Ramya, Dr.Rajeshkumar shanmugam

Data verification, manuscript drafting – B.Sumedha, Dr.rajeshkumar shanmugam, Dr.Suganya paneerselvam, Dr.S.Sandhya

Literature search, survey, experimental data collection, analysis, manuscript writing – B.Sumedha

Manuscript review – Dr.R.Ramya , Dr.S.Sandhya, Dr. Pratibha Ramani

CONFLICT OF INTEREST

There is no conflict of interest.

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