

ANTICARIOGENIC ACTIVITY OF AZADIRACHTA INDICA AND ZINGIBER OFFICINALE FORMULATION MEDIATED IRON OXIDE NANOPARTICLES**Sai Sreekar Reddy¹, Dr. Abirami Arthanari^{2*} and Dr. Rajesh Kumar³**¹Department of Forensic Odontology, ²Senior Lecturer, Department of Forensic Odontology and ³Professor, Department of Pharmacology

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai - 600077

ABSTRACT**INTRODUCTION:**

To better understand the metabolic process and its impact on the human body, traditional medicine and evidence-based medicine are being combined in global health and medical practice. (1) Use of supplementary therapies like phytotherapy is one illustration. (2) To begin, *Azadirachta indica* has been widely recognized for its antimicrobial, anti-inflammatory, and antioxidant properties. *Zingiber officinale*, on the other hand, ginger contains active compounds like gingerols and shogaols, which exhibit antibacterial effects against various oral pathogens, including *S. mutans*. The combination of *Azadirachta indica*, *Zingiber officinale*, and iron oxide nanoparticles in a formulation offers several advantages for oral health.

AIM: To evaluate the anticariogenic activity of *azadirachta indica* v *Zingiber officinale* formulation mediated iron oxide nanoparticles.

MATERIALS AND METHODS

The antimicrobial activity of *azadirachta indica* and *zingiber officinale* was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*azadirachta indica* and *zingiber officinale*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip.

RESULTS

A 1 mL aliquot of the bacterial and fungal suspension (*Streptococcus mutans*, *Lactobacillus sp*, *Staphylococcus aureus*, *Candida albicans*) was added to 9 mL of Mueller Hinton broth containing the FeO NPs at a concentration of 25 µg, 50 µg, 100 µg. The final microbial concentration was approximately 106 CFU/mL.

Keywords: Anticariogenic activity, *azadirachta indica*, *zingiber officinale*, iron oxide nanoparticles, oral pathogens, sterilization, inflammation, room temperature.

INTRODUCTION

Around the world, dental caries, also referred to as tooth decay or cavities, continues to be a major problem. One of the most adaptable medicinal plants with a wide spectrum of biological activity, ginger, the rhizome of *Zingiber officinale*, species of the ginger family (Zingiberaceae), has a long history of medicinal usage for more than 2000 years. It is also a common condiment for various cuisines and beverages. (3) Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% Carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. (4) The ginger plant is a suppressant erect perennial. Ginger grows from one to three feet tall and is just as well-liked. Today in India, just as then, the stem is encircled by the sheathing bases of medicine. The two-ranked leaves are made from ginger rhizomes. Traditional yellowish, purple-lipped blossoms have showy medicinal as a carminative, antipyrexia, and greenish yellow bracts beneath a club-like spike of spice in food and beverages. Unfortunately, cultivation of ginger and rheumatism treatment rarely produces blooms. The bronchitis ginger. It is made up of the plant's 13. (Underground stems), which include thick, scaly rhizomes that are used to treat digestive issues. (5) Iron oxide nanoparticles are highly reactive by nature and have a tendency to aggregate,

which results in a loss of reactivity. However, the chemical synthesis of iron oxide nanoparticles has drawbacks, including the use of hazardous chemicals, formation of hazardous byproducts, contamination from precursor chemicals, and the resultant iron oxide nanoparticles' low biocompatibility [29]. Common processes for producing iron oxide nanoparticles, such as thermal decomposition and hydrothermal techniques, also required high temperatures, high pressure, and a lot of hazardous and expensive organic solvents.(6)

Due to their innate antibacterial and therapeutic qualities, natural items and conventional treatments have been thoroughly researched for their ability to prevent tooth caries. Neem (*Azadirachta indica*) and ginger (*Zingiber officinale*) have long been employed in a variety of pharmaceutical compositions. Aim of this study is to evaluate the anticariogenic activity of *skyahiachte indica v Zingiler dfeinal* formulation mediated iron oxide nanoparticles.

MATERIALS AND METHODS

The antimicrobial activity of *azadirachta indica* and *zingiber officinale* was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*azadirachta indica* and *zingiber officinale*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip.

The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of CuO NPs . An antibiotic (e.g., Bacteria-Amoxyrite, Fungi- Flucanazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated.

RESULTS

Figure 1: A 1 mL aliquot of the bacterial and fungal suspension (*Streptococcus mutans*, *Lactobacillus* sp, *Staphylococcus aureus*, *Candida albicans*) was added to 9 mL of Mueller Hinton broth containing the FeO NPs at a concentration of 25 µg, 50 µg, 100 µg. The final microbial concentration was approximately 106 CFU/mL



Figure 2: The mixture was then incubated at 37°C with shaking at 200 rpm for varied time intervals (0,4,6,8,10,12, &24hr).Then the percentage of dead cells is calculated at wavelength of 600nm at regular time intervals.



DISCUSSION

From previous study Ginger is also having significant Hepatoprotective activity. The bromobenzene (BB)-induced hepatotoxicity comes from its reactive metabolites. The efficacy of different doses of ginger (*Zingiber officinale* Rose.) extract in alleviating hepatotoxicity was investigated. (7) Regular brushing with toothpaste containing neem reduces plaque buildup, prevents cavities, and improves the immune system for general dental health. Neem extract mouthwash can be used often to alleviate halitosis and reduce gingival issues.(8)Every day, the liver is subjected to a wide range of biological insults. To maintain hepatic homeostasis and avoid damage from absorbed endotoxins, cytoprotective enzymes, such as antioxidant and carcinogen-detoxification enzymes, must be activated. In order to protect the liver from harm, nuclear factor erythroid 2-related factor 2 (Nrf2) transcriptionally regulates the gene expression of a variety of cytoprotective enzymes.(9)The study's findings conclusively show that iron oxide nanoparticles mediated by *Azadirachta indica* and *Zingiber officinale* have strong anticariogenic effect. The mixture successfully slowed the growth of cariogenic bacteria due to its strong antibacterial characteristics. (11) Additionally, the nanoparticles showed the capacity to prevent the development of bacterial biofilms, a crucial element in the emergence of dental caries. The formulation also shown encouraging effects on enamel remineralization, suggesting its potential to enhance tooth toughness and durability.(12)

LIMITATIONS

The study may have concentrated on a particular kind of cariogenic bacteria, dental caries are caused by a varied microbial community. It is important to take into account a wider range of cariogenic bacteria when evaluating how well the formulation defends against different infections.

Future Scope

To increase the effectiveness of the formulation, its potential as an adjuvant therapy to currently used caries prevention techniques, such as fluoride treatments, should be investigated.

The performance of the formulation could be improved by future study focusing on IONPs' physicochemical features optimization to optimize their distribution and targeting to particular oral locations.

CONCLUSION

The study investigates the potential of iron oxide nanoparticles mediated by *Azadirachta indica* and *Zingiber officinale* as a novel strategy to prevent dental caries. Although the early results are encouraging, it is crucial to recognize the research's limitations. To confirm the formulation's effectiveness and safety in human beings, more research is required, including clinical trials and in vivo studies. If effective, this organic and biocompatible formulation could support already used dental care methods, giving people an additional way to preserve their oral health and possibly lowering the prevalence of dental caries. The study lays the groundwork for additional research in this area, where nanotechnology and herbal medicine intersect to address issues with oral health.

Conflict of interest: Nil

Duration of the study: 3 months

Ethical clearance number: Since it is in-vitro study ethical clearance is not needed.

Author contribution: All the authors contributed equally in this study.

Fundings: Balaji Dental Clinic Palamaner 517408 and Saveetha Dental College and Hospitals.

REFERENCE

- 1) Islas JF, Acosta E, Zuca G, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B, Moreno-Cuevas JE. An overview of Neem (*Azadirachta indica*) and its potential impact on health. *Journal of Functional Foods*. 2020 Nov 1;74:104171.
- 2) Islas JF, Acosta E, Zuca G, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B, Moreno-Cuevas JE. An overview of Neem (*Azadirachta indica*) and its potential impact on health. *Journal of Functional Foods*. 2020 Nov 1;74:104171.
- 3) Dhanik J, Arya N, Nand V. A review on *Zingiber officinale*. *Journal of Pharmacognosy and phytochemistry*. 2017;6(3):174-84.
- 4) Banerjee S, Mullick HI, Banerjee J, Ghosh A. *Zingiber officinale*: 'a natural gold'. *Int J Pharmaceutical Bio-Sci*. 2011;2:283-94.
- 5) Yoganasimhan SN. *Medicinal Plants of India*, Vol. 1, Interline Publishing Private2. Limited: 645, (1996).
- 6) Zambri ND, Taib NI, Abdul Latif F, Mohamed Z. Utilization of neem leaf extract on biosynthesis of iron oxide nanoparticles. *Molecules*. 2019 Oct 22;24(20):3803.
- 7) Banerjee, S., Mullick, H.I., Banerjee, J. and Ghosh, A., 2011. *Zingiber officinale*: 'a natural gold'. *Int J Pharmaceutical Bio-Sci*, 2, pp.283-94.
- 8) Lakshmi T, Krishnan V, Rajendran R, Madhusudhanan N. *Azadirachta indica*: A herbal panacea in dentistry—An update. *Pharmacognosy reviews*. 2015 Jan;9(17):41.
- 9) Zhuang X, Deng ZB, Mu J, Zhang L, Yan J, Miller D, Feng W, McClain CJ, Zhang HG. Ginger-derived nanoparticles protect against alcohol-induced liver damage. *Journal of extracellular vesicles*. 2015 Jan 1;4(1):28713.
- 10) Hwang JK, Shim JS, Chung JY. Anticariogenic activity of some tropical medicinal plants against *Streptococcus mutans*. *Fitoterapia*. 2004 Sep 1;75(6):596-8.
- 11) Sneha S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from *Capparis decidua* against *Escherichia coli* and *Lactobacillus* Species. *Research Journal of Pharmacy and Technology*. 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773
- 12) Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of *Kaempferia galanga* rhizome using different solvent systems. *J Adv Pharm Technol Res*. 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr_189_22.