

COMPARATIVE CYTOTOXIC EFFECT OF COMMERCIAL MOUTHWASH AND GREEN TEA PAPAYA FORMULATION BASED MOUTHWASH - AN IN VITRO STUDY

Ashinie C¹, Balaji Ganesh S^{2*}, S.Rajesh Kumar³Taniya Mary Martin⁴,Meenakshi
Sundaram Kishore Kumar⁵

Authors:

¹Department of Periodontics,
Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai - 600077, India

²Reader,
Department of Periodontics,
Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai - 600077, India

³Professor,
Department of Pharmacology,
Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai - 600077, India

⁴Research Scholar
Saveetha Dental College and hospitals,
Department of Anatomy
Saveetha Institute of Medical and Technical
Sciences (SIMATS),
Saveetha University,
Chennai-600077,
TamilNadu, India

⁵Assistant Professor,
Department of Anatomy
Saveetha Dental College and hospitals,
Saveetha Institute of Medical and Technical
Sciences (SIMATS),
Saveetha University, Chennai-600077,
TamilNadu, India

Corresponding Author:

Balaji Ganesh S

Reader,

Department of Periodontics,

Saveetha Dental College and Hospital,

Saveetha Institute of Medical and Technical Sciences,

Saveetha University,

Chennai - 600077, India

KEYWORDS ABSTRACT

Cytotoxicity;Mout hwash;Papaya;Gre en tea

INTRODUCTION : An ideal mouthwash is one that doesn't cause allergies, doesn't discolor teeth or the oral mucosa, has anti-plaque and antibacterial qualities, doesn't have a lot of cytotoxic effects on cells, is environmentally friendly, and tastes good. The aim of the present study is to compare the cytotoxic effect of commercial mouthwash and green tea and papaya formulation based mouthwash.

MATERIALS AND METHODS :

The study was conducted in the month of August 2022 in Saveetha dental college and Hospitals. Papaya and green tea samples were collected and the samples were boiled, filtered and concentrated to prepare the extract. Both the extract were mixed together and 0.3g sucrose, 0.01 g SLS agent and sodium benzoate was added and mouthwash was prepared. Cytotoxic activity was compared with commercial chlorhexidine mouthwash using brine shrimp lethality assay. Salt water was prepared. 6 ELISA plates were taken with different concentrations and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours, the ELISA plates were observed and noted for the number of live nauplii present and calculated by using formula. The results were then tabulated and represented graphically

RESULTS : The cytotoxic activity of commercial mouthwash and green tea and papaya formulated mouthwash was compared. The number of live nauplii present in commercial mouthwash and papaya and green tea formulated mouthwash decreased on increasing the concentrations. But compared to commercial mouthwash, green tea and papaya formulated mouthwash had a higher number of nauplii present.

CONCLUSION : From the present study it was concluded that the papaya green tea formulated mouthwash showed lower cytotoxicity than commercial mouthwash

INTRODUCTION

People are more frequently affected by periodontal disorders. Plaque management is crucial in the prevention of periodontal disease. For the control of plaque, mechanical and chemical methods are popular nowadays¹. An inflammation of the teeth's supporting tissues is called periodontitis. Typically, it is a gradually damaging transformation that results in the loss of periodontal ligament and bone. Volatile substances such sulfur compounds, aromatic compounds, nitrogen-containing

compounds, amines, short-chain fatty acids, alcohols or phenyl compounds, aliphatic compounds, and ketones are responsible for the formation of halitosis². Halitosis is a problem for many people, impairing interpersonal interactions and leading to public stigma and personal pain. Mouthwashes with antimicrobial activity have been proven to be useful for lowering gingival inflammation and managing the colonization of oral bacteria, including periodontopathogens³. An ideal mouthwash is one that doesn't cause allergies, doesn't discolor teeth or the oral mucosa, has anti-plaque and antibacterial qualities⁴, doesn't have a lot of cytotoxic effects on cells, is environmentally friendly, and tastes good.⁵. Many mouthwashes have been introduced in order to fulfill all these qualities, one among them which is commonly used is chlorhexidine. Chlorhexidine (CHx) has been recommended as a mouthwash due to its therapeutic properties; nonetheless, it has a series of adverse side effects, prominent among them being cytotoxicity⁶.

Given the wide chemical diversity present in various species of plants, animals, and microorganisms, nature is a significant source of new prospects for medicinal chemicals^{4,7}. This chemical variety frequently represents self-defense mechanisms that indicate the tactics used to eliminate predators.⁸. Currently, many substances originating from plants are successfully used in chemotherapy for cancer. In fact, numerous studies have stressed the significance of plants as a source of novel anticancer drugs. Over 50% of approved medications are derived from natural substances, according to a study of the number of chemotherapeutic agents and their sources⁹. Mineral elements are abundantly present in the green leaves of papaya. It has been demonstrated that the polar extracts of papaya possess a variety of biological activities, including an anti-inflammatory action, analgesic qualities, the ability to heal wounds, anticancer capabilities, and an immunomodulatory effect¹⁰. Scientific evidence of papaya demonstrated against cancer cells also have been documented in various in vitro studies.

Naturally papaya contains high amounts of antioxidants and studies have also proved that rinsing with 10% *Carica papaya* L. seeds extract mouthwash was able to reduce dental plaque score in patients with gingivitis¹¹. Green tea also has many health benefits and previous study was done on this and concluded that Continuous use of green tea catechin on a daily basis may be a useful and practical method for the prevention of periodontal disease, but should be carried out with caution to avoid side-effects¹². The beneficial effects of consuming green tea on periodontal health have been researched by a number of writers, and they may be supported by the catechins in green tea's inhibitory effects on periodontal bacteria. *Prevotella gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* proliferation and attachment to human buccal epithelial cells are inhibited by green tea catechin¹³. The present study includes preparation of green tea and papaya formulated mouthwash and compares its cytotoxic activity against commercial mouthwash. The aim of the present study is to compare the cytotoxic activity of commercial mouthwash and papaya green tea formulated mouthwash.

MATERIALS AND METHODS

MOUTHWASH PREPARATION

Samples of papaya and green tea were gathered. A sample of crushed green tea leaf and crushed papaya seeds were used as the sample. 2g of each sample was boiled for 10 minutes at 75 degrees Celsius. To make the green tea and papaya extract, the boiled samples are filtered and concentrated.

Mouthwash was made by combining both extracts, adding 0.3 g of sugar, 0.01g of SLS agent, and sodium benzoate. The cytotoxic activity of the prepared mouthwash was compared to that of commercial chlorhexidine mouthwash.

BRINE SHRIMP LETHALITY ASSAY:

Salt water preparation :

200ml of distilled water was used to dissolve 2g of iodine-free salt.

6 ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5 μ L, 10 μ L, 20 μ L, 40 μ L, 80 μ L and control). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for the number of live nauplii present and calculated using the following formula, number of dead nauplii/number of dead nauplii+number of live nauplii \times 100. The results were then tabulated and represented graphically

RESULTS

The cytotoxic activity of commercial mouthwash and green tea and papaya formulated mouthwash was compared. The concentrations of the mouthwashes were increased as 5 μ L, 10 μ L, 20 μ L, 40 μ L and 80 μ L. The number of live nauplii present in commercial mouthwash and papaya and green tea formulated mouthwash decreased on increasing the concentrations. The number of live nauplii present in commercial mouthwash at 5 μ L was 7, at 10 and 20 μ L it was 6, at 40 μ L it was 5 and at 80 μ L it was only 4 nauplii present. In papaya and green tea formulated mouthwash at 5, 10 and 20 μ L the number of live nauplii was 9 and at 40 μ L it was 8 and at 80 μ L the number of live nauplii was 6. But compared to commercial mouthwash, green tea and papaya formulated mouthwash had a higher number of nauplii present.

DISCUSSION

Maintaining proper oral hygiene is one of the most important components of keeping healthy, and it is attainable with a variety of oral cleansing products¹⁴. Mouthwashes are one such agent, but many people are unaware that synthetic mouthwashes can destroy dangerous germs while simultaneously eradicating the natural flora in your mouth, aggravating the issue¹⁵. In addition, the alcohol in commercial mouthwashes will dry out your mouth¹⁶. Therefore, the goal of this research is to create mouthwash from natural ingredients. So the present study focuses on preparation of mouthwash with naturally occurring products. The commercial mouthwash is compared to prepared papaya green tea formulated mouthwash and the cytotoxicity also dependent on concentration of mouthwash, similar results was seen in study by¹⁷ that the cytotoxicity of mouthwashes was found to be dependent on the exposure time and on the concentration. In the present study commercial mouthwash had higher cytotoxicity similarly a study was done comparing the commercial chlorhexidine mouthwash and citronella oil on prosthetic materials and concluded that Citronella had a lower cytotoxic effect and a higher action compared to commercial mouthwash¹⁸. An earlier study that examined the cytotoxic effects of mouthwash made with blue tea extract came to the same conclusion, though the degree of the difference was smaller at higher

doses, that blue tea extract-based mouthwash demonstrated cytotoxic activity at varied concentrations.¹⁹.

Similar mouthwashes from herbal extracts were prepared and cytotoxic activity was analyzed as done in study where mouthwash prepared using extract of juca and results stated that The mouthwash from juca extract did not promote cytotoxic effect in human fibroblasts²⁰. New herbal products with medicinal properties like Vetiver leaves have been used to make mouthwash and its cytotoxic activity was analyzed and the results stated that the synthesized product is non-toxic in nature through cytotoxic tests but before employing this innovative product as mouthwash in patients for the treatment of periodontal diseases, more research must be done in regards to the effectiveness and substantivity of plaque inhibitory effects²¹. Similarly ethanolic extract of *Abies webbiana* based preparation of mouthwash revealed that it possessed less cytotoxic effect against brine shrimp which are within normal limits⁷.

The World Health Organization (WHO) estimates that 80% of individuals only use herbal medicine for their most basic needs. Over 25% of medicines in both developed and developing nations are solely made from plants and their derivatives. Numerous herbal extracts have been used as tooth cleaners in dentistry for a long time. Around the world, the usage of herbal medicines is steadily rising^{3,22}. The cytotoxic activity of papaya and green tea formulated mouthwash was analyzed, in future other activities like antioxidant activity, antimicrobial and antioxidant activity of the mouthwash can be analyzed and compared with commercial mouthwash so a herbal mouthwash can be used in future for periodontal disease as mouthwashes formulated from herbal products shows less side effects.

CONCLUSION

From the present study it was concluded that the papaya green tea formulated mouthwash showed lower cytotoxicity than commercial mouthwash and further studies can be done in future to use this mouthwash as treatment for periodontal disease. The increasing use of natural materials in dental products and the lack of studies in the literature describing the efficacy and safety of herbal products indicate the need for additional research of this kind. This can also be used as a viable alternative to the synthetic products used in the field of dentistry.

ACKNOWLEDGEMENT

The authors of this study are thankful to Saveetha dental college and hospitals for giving an opportunity to carry out this project.

CONFLICT OF INTEREST

The authors of this study declared that there is no conflict of interest.

SOURCE OF FUNDING

The current study is funded by

- Saveetha Dental College,
- Saveetha Institute of Medical and Technical Science, Saveetha University
- RR Acrotech, Thiruvanamallai district, Tamilnadu

REFERENCES

1. Newman MG, Takei HH, Carranza FA. *Carranza's Clinical Periodontology*. W B Saunders Company, 2002.
2. Rangaraju V, Mousin S, Babu H, et al. Efficacy of Carica papaya seed extract on periodontitis: A clinico-microbiological study. *International Journal of Oral Care and Research* 2019; 7: 35.
3. Wennström J, Lindhe J. The effect of mouthrinses on parameters characterizing human periodontal disease. *J Clin Periodontol* 1986; 13: 86–93.
4. Chatterjee S, Ramamurthy J. Evaluation of Antimicrobial and Cytotoxic Activity of Nanoformulated Chamomile and Green Tea-Based Mouthwash: An In Vitro Study. *Cureus*; 16. Epub ahead of print 2 April 2024. DOI: 10.7759/cureus.57470.
5. Shafiee HA, Motamedi MHK, Mina M, et al. Evaluation of cytotoxic effects of Anbarnesa on fibroblast L929: Can it be used as a mouthwash? *Anc Sci Life* 2014; 33: 203–207.
6. Hidalgo E, Dominguez C. Mechanisms underlying chlorhexidine-induced cytotoxicity. *Toxicol In Vitro* 2001; 15: 271–276.
7. Timothy CN, Thaslima Nandhini JS, Varghese SS, et al. Abies webbiana Ethanolic Extract Based Mouthwash and Its Antimicrobial and Cytotoxic Effect. *Journal of Pharmaceutical Research International* 2021; 371–385.
8. Rocha A da, da Rocha A. Natural products in anticancer therapy. *Current Opinion in Pharmacology* 2001; 1: 364–369.
9. Ribeiro SS, de Jesus AM, dos Anjos CS, et al. Evaluation of the cytotoxic activity of some Brazilian medicinal plants. *Planta Med* 2012; 78: 1601–1606.
10. Husin F, Ya'akob H, Rashid SNA, et al. Cytotoxicity study and antioxidant activity of crude extracts and SPE fractions from Carica papaya leaves. *Biocatalysis and Agricultural Biotechnology* 2019; 19: 101130.
11. J M, Méndez J. Carica Papaya Mouthwash for Reducing Dental Plaque. *SunText Review of Dental Sciences*; 01. Epub ahead of print 2020. DOI: 10.51737/2766-4996.2020.002.
12. Chatterjee A, Saluja M, Agarwal G, et al. Green tea: A boon for periodontal and general health. *J Indian Soc Periodontol* 2012; 16: 161–167.
13. Sakanaka S, Aizawa M, Kim M, et al. Inhibitory Effects of Green Tea Polyphenols on Growth and Cellular Adherence of an Oral Bacterium, *Porphyromonas gingivalis*. *Bioscience, Biotechnology, and Biochemistry* 1996; 60: 745–749.
14. Sindhusa VB, Rajasekar A, Sindhusa VB. Efficacy of Oxygen-Enriched Mouthwash as a

- Pre-procedural Mouth Rinse Against Oral Microbes Produced During Ultrasonic Scaling. *Cureus*; 15. Epub ahead of print 21 November 2023. DOI: 10.7759/cureus.49164.
15. Shanmugam R, Govindharaj S, Arunkumar P, et al. Preparation of a Herbal Mouthwash With Lemongrass and Mint-Mediated Zinc Oxide Nanoparticles and Evaluation of Its Antimicrobial and Cytotoxic Properties. *Cureus* 2024; 16: e53671.
 16. Kon K, Rai M. *Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches*. Academic Press, 2016.
 17. Coelho AS, Laranjo M, Gonçalves AC, et al. Cytotoxic effects of a chlorhexidine mouthwash and of an enzymatic mouthwash on human gingival fibroblasts. *Odontology* 2020; 108: 260–270.
 18. Cunha BG, Duque C, Caiaffa KS, et al. Cytotoxicity and antimicrobial effects of citronella oil (*Cymbopogon nardus*) and commercial mouthwashes on *S. aureus* and *C. albicans* biofilms in prosthetic materials. *Archives of Oral Biology* 2020; 109: 104577.
 19. Rajasekar A, Lecturer S, Department of Periodontics, et al. Blue Tea Extract Based Preparation Of Mouthwash And Its Cytotoxic Activity. *International Journal of Dentistry and Oral Science* 2014; 4620–4623.
 20. Venâncio GN, de Souza WM, Sampaio FC, et al. CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY OF MOUTHWASH OBTAINED FROM THE EXTRACT OF LIBIDIBIA FERREA MART / CITOTOXICIDADE E ATIVIDADE ANTIMICROBIANA DE ENXAGUATÓRIO BUCAL OBTIDO DO EXTRATO DE LIBIDIBIA FERREA MART. *Brazilian Journal of Development* 2020; 6: 69828–69841.
 21. Evaluation of Cytotoxic Activity of *Chrysopogon Zizanioides* Mouthwash - An In Vitro Study. *International Journal of Pharmaceutical Research*; 12. Epub ahead of print 2020. DOI: 10.31838/ijpr/2020.sp1.431.
 22. S. S, Prabakar J, Indiran MA, et al. Assessment of Cytotoxic and Antioxidant Effects of *Rosmarinus officinalis* and Fluoride containing Dentifrice - An In vitro Study. *Research Journal of Pharmacy and Technology* 2021; 3779–3784.

FIGURES AND TABLE

Figure 1



Figure 2



Figure 3



Figure 4

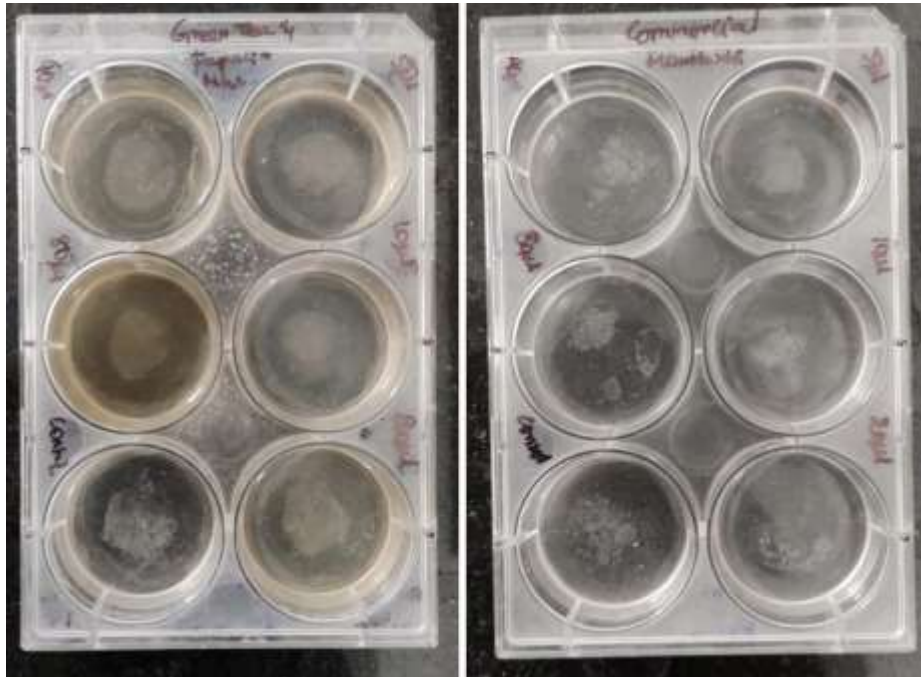


Figure 5

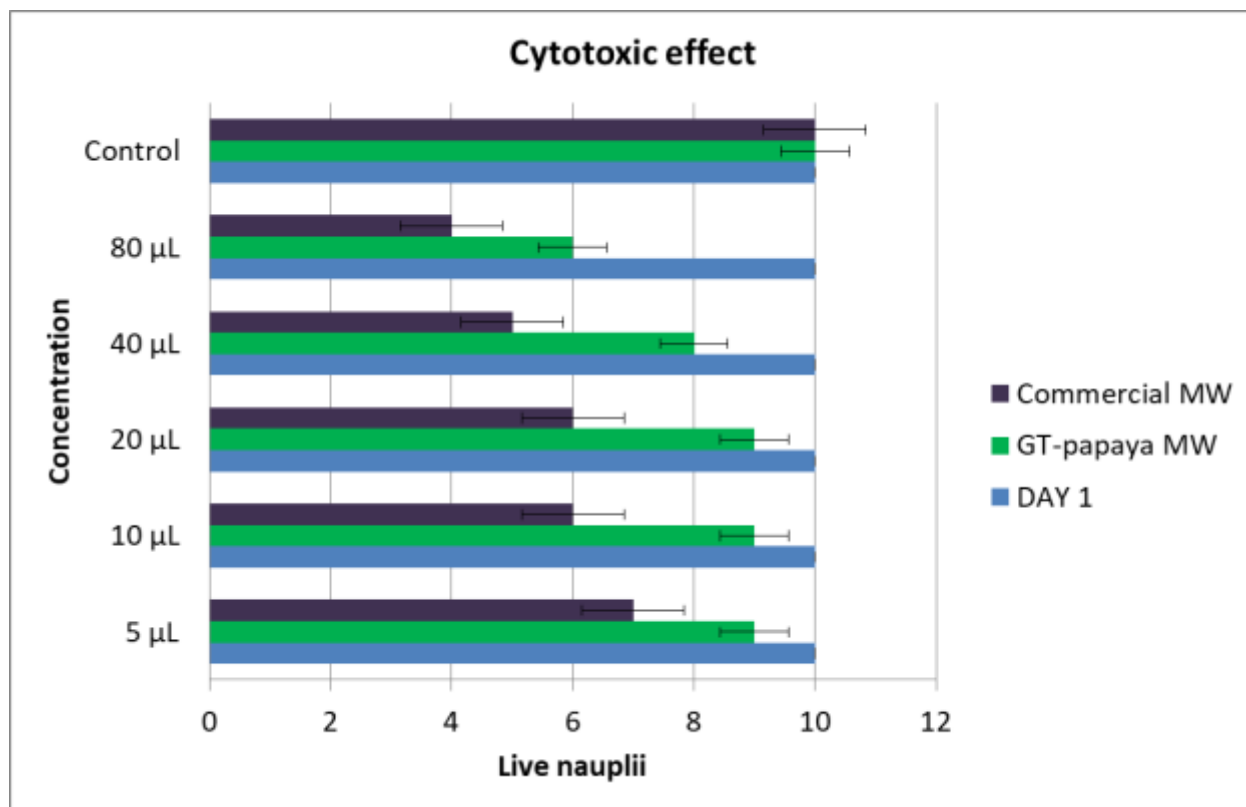


Table 1

Concentration	Green tea and papaya formulated mouthwash	Commercial Mouthwash
5μL	9	7
10μL	9	6
20μL	9	6
40μL	8	5
80μL	6	4
Control	10	10

Titles for figures and table

Figure 1 : Green tea and Papaya leaf sample

Figure 2 : Filtrate of Green tea and Papaya leaf sample

Figure 3 : Green tea and Papaya extract

Figure 4 : Cytotoxic activity of green tea and papaya leaf mouthwash and commercial mouthwash

Figure 5 : Graph representing the comparison of the cytotoxic effect of commercial mouthwash and papaya green tea formulated mouthwash. X axis represents the number of live nauplii and Y axis represents concentration of sample. Violet colour represents commercial mouthwash and green colour represents papaya green tea formulated mouthwash.

Table 1 : Represents the comparison of live nauplii present in commercial mouthwash and green tea papaya formulated mouthwash in various concentrations