Antimicrobial and Cytotoxic Effect of Ginger and Stevia Herbal Formulation Based Mouthwash - An *In-vitro* Study

G. Preethi[1], Balaji Ganesh S*[2], Rajesh Kumar S[3]

[1]Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu, chennai

[2]Reader, Department of Periodontics, Saveetha Dental college and Hospitals, Saveetha institute of Medical and Technical Sciences, Saveetha University, Chennai 77, India

[3]Associate Professor, Department of Pharmacology, Saveetha Dental college and Hospitals, Saveetha institute of Medical and Technical Sciences, Saveetha University, Chennai 77, India

Corresponding Author: Dr. Balaji Ganesh. S, Reader, Department of Periodontics, Saveetha Dental college and Hospitals, Saveetha institute of Medical and Technical Sciences, Saveetha university,
Chennai 77, India
Email id: balajiganeshs.sdc@saveetha.com

Abstract:

Introduction: Nanomedicine, the use of nanotechnology for medical purposes, is characterized by the management, prevention, and treatment of diseases through the use of nanoparticles. Canker sores, as well as any oral or gum discomfort or inflammation, can be treated with a general mouthwash. Thus the aim of this study was to evaluate the antimicrobial and cytotoxic effect of ginger and stevia herbal formulation based mouthwash. **Materials and Methods:** Cytotoxic activity was evaluated by adding 5ML of artificial seawater to each 6 wells of ELISA plate and 10 nauplii were added to each well. Then, 5 different concentrations of prepared extract were introduced to each of the 5 wells and control containing only seawater was taken in one well. Antimicrobial activity of respective nanoparticles against the strains staphylococcus mutans, Aureus and lactobacillus. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. **Results and discussion:** The tests for cytotoxic activity were assessed by using brine shrimps. Ten nauplii were placed in each of 5 wells with one control group. The nanoparticle's concentrations were 5µl, 10µl, 20µl, 40µl and 80µl. At incubation time after 24hours, 100% of the nauplii were present in all concentrations. At incubation time after 48 hours, 100% of nauplii was present at the concentration of 5µl. 90% of the nauplii were present at the concentration of 10µl. 80% of the nauplii were present at the concentration of 20µl and 40µl. 60% of the nauplii were present at the concentration of 80µl. Also ginger and stevia herbal formulations showed good antimicrobial activity against S.mutans, S. Aureus and lactobacillus as the concentration of micro filters increased, the zone of inhibition also increased. **Conclusion:** The observation of our study, an increased concentration of ginger and stevia herbal formulation was found to be toxic to the nauplii as the number of nauplii present in higher concentrations of herbal formulation was decreased. Also, as the concentration of the nanoparticle increased, the antioxidant activity also increased. Thus, ginger and stevia herbal formulation-based mouthwash showed cytotoxic effect and antimicrobial effect.

Keywords: Antimicrobial ; cytotoxic activity ; stevia ; nauplii ; antioxidant activity

Introduction:

We now use nanotechnology into our daily lives. Through the use of an integrated strategy, this technology has been utilised in many other industries. More and more products and applications with nanomaterials or at least claims based on nanotechnology become available. The same thing takes place in pharmaceutical research (2). Our research today includes the use of nanotechnology in the creation of the newest medications, and present in the international organization (EU), it has been designated as a key enabling technology, capable of delivering novel and creative medical treatments to address unmet medical requirements (3). Nanomedicine, the use of nanotechnology for medical purposes, is characterized by the management, prevention, and treatment of diseases through the use of nanoparticles. The various scientific and international regulatory bodies disagree on what constitutes a nanomaterial, nevertheless. These characteristics significantly expand a number of opportunities for drug development, but some safety concerns have also surfaced (1). Some examples of the physicochemical characteristics of the nanoformulation that could change the absorption, distribution, elimination, and metabolism of the drug include its propensity to more readily pass biological barriers, its toxicity, and its resistance to environmental degradation.

Commercial mouthwashes typically contain a preservative like sodium benzoate to maintain freshness. Many newer brands on the commercial market today are alcohol-free and include odor-removal ingredients like oxidizers as well as odor-prevention ingredients like zinc ion technology to keep the symptoms of gingivitis, canker sores, swollen, painful, or inflamed gums, sore mouth, inflamed or ulcerated throat, oral infections, calculus, and bleeding gums under control. Canker sores, as well as any oral or gum discomfort or inflammation, can be treated with a general mouthwash for bad breath (7). Stevia, also known as Stevia rebaudiana, is a member of the Asteraceae family's Chrysanthemum subfamily. Many of them are commonly known by their scientific names, such as angiospermous yellowwood stevia, honey leaf plant, sweet chrysanthemum, sugar leaf, etc. Brazil and Argentina are its native habitats in northeastern South American nations. It is currently grown in several parts of the world, including North American nations, a region of Asia, and Europe. It is perhaps easiest to describe this as a supply of natural sweeteners (9). It serves as a non-nutritive supplement sweetener and flavorer. A sweetener without nutritional value has extremely few or no calories. Stevia is used as a healthy alternative to sugar in many dishes and beverages. Stevia will be an excellent replacement for sugar. Approximately forty times as much stevia leaf (10).

Possible non-carbohydrate organic compound is stevioside. Hence, it lacks the qualities that plant products and alternatives to carbohydrates. Rebaudioside-A and other stevia extracts have been discovered to be 300 times sweeter than sugar. In addition to having almost no calories, Stevia is also notable for its great temperature resistance and lack of fermentation (12). Stevia will be used orally for medicinal purposes such as reducing vital signs, treating polygenic condition, heartburn, high blood acid levels, weight loss, stimulating the digestive system, and treating water retention.

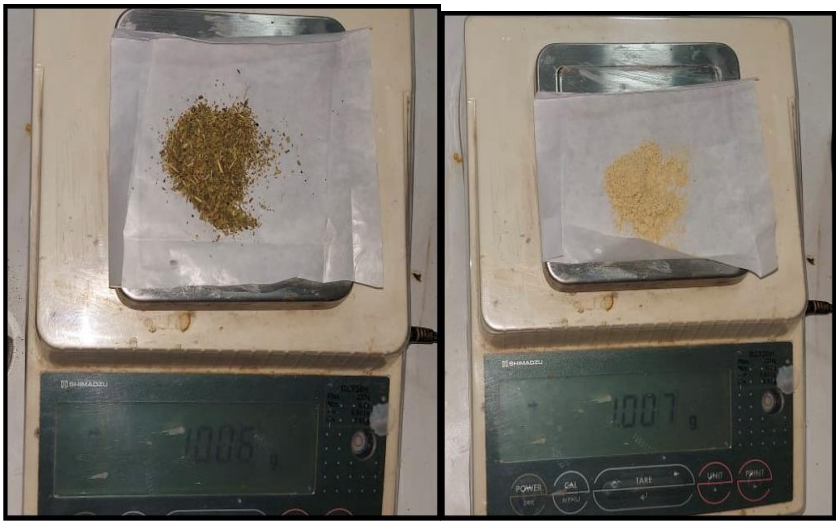
Because it doesn't contain carbohydrates, stevia prevents the growth of the bacterium mutans bacteria, which is thought to be a cause of tooth decay and cavities .

In the earlier investigation, selenium nanoparticles with garlic oil were synthesised. It can be inferred that selenium nanoparticles produced by garlic oil have good cytotoxicity at high doses. In a different study, it was straightforward and easy to create selenium nanoparticles via green synthesis (14). The study found that selenium nanoparticles mediated by coriander oleoresin showed effective anti-inflammatory properties. A brine shrimp lethality assay was used to assess the cytotoxicity. The brine shrimp showed no cytotoxicity against the iron nanoparticles. Future drug discovery for a variety of ailments can leverage it. It can be used as toothpaste and mouthwash in dentistry. This cost-effective, practical, and safe synthesis made from plant extract must be employed in a variety of dental and medical fields (16). Thus the aim of this study was to evaluate the antimicrobial and cytotoxic effect of ginger and stevia herbal formulation based mouthwash.

Materials and Methods :

Preparation of plant extract :

Stevia and ginger diffusa leaves were collected from the local fields and were dried in a shady place. After getting dried, they were crushed and made into a powder using a grinder or a mixer. Then a hundred ml of distilled water is taken in a measuring cylinder and 1 g of ginger (Figure 1b) and stevia (Figure 1b) is measured and taken using a weighing machine. The solution is labeled (Figure 2a) and heated by using a machine called the heating mantle. The temperature of the heating mantle was set to 50 degrees Celsius. The time taken to hear it is about 5 to 10 minutes.



a) Stevia. b) Ginger

Figure 1 : Preparation of plant extract

a)ginger and stevia extract **b)** centrifuged extract

Figure 2 : Preparation of plant extract

Cytotoxic activity:

A six well Elisa plate was taken and 5ML of artificial seawater was added to each 6 wells and 10 nauplii were added to each well. Then, 5 different concentrations of prepared extract were introduced to each of the 5 wells and control containing only seawater was taken in one well (Figure 3).

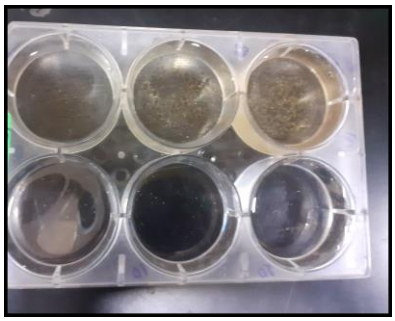


Figure 3 : Isolation of brine shrimps and incorporation of nanoparticle

Antimicrobial activity:

Antimicrobial activity of respective nanoparticles against the strains staphylococcus mutans, Aureus and lactobacillus. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller Hinton agar was prepared and sterilized for 15minutes at 121 degree Celsius. Media poured into sterilisation plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and test organisms were swabbed. The nanoparticles with different concentrations were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37 degree Celsius. After the incubation time the zone of inhibition was measured (Figure 4).

Figure 4 : Antimicrobial activity using S.mutans, S. Aureus and lactobacillus.

Results :

Brine shrimp lethality assay is an important test in the study of toxicity that gives us information on the cytotoxic effect shown by a bioactive molecule to cells. For various concentrations of Stevia and Neem-derived nanoparticles, the viability of the nauplii was examined. The tests for cytotoxic activity were assessed by using brine shrimps. Ten nauplii were placed in each of 5 wells with one control group. The nanoparticle's concentrations were 5µl, 10µl, 20µl, 40µl and 80µl. At incubation time after 24hours, 100% of the nauplii were present in all concentrations (Table 1). At incubation time after 48 hours, 100% of nauplii was present at the concentration of 5µl. 90% of the nauplii were present at the concentration of 10µl. 80% of the nauplii were present at the concentration of 20µl and 40µl. 60% of the nauplii were present at the concentration of 80µl (Table 2). Also ginger and stevia herbal formulations showed good antimicrobial activity against S.mutans, S. Aureus and lactobacillus as the concentration of micro filters increased, the zone of inhibition also increased (Table 3).

Table 1 : The above table depicts the number and percentage of live shrimps after 24 hours of incubation

Concentration	5µl	10µl	20µl	40µl	80µl	control
No. of live shrimps	10	10	10	10	10	10
% of live shrimps	100	100	100	100	100	10

Table 2 : The above table depicts the number and percentage of live shrimps after 48 hours of incubation

Concentration	5µl	10µl	20µl	40µl	80µl	control
No. of live shrimps	10	9	8	8	7	10
% of live shrimps	100	90	80	80	70	10

Table 3 : This table shows the zone of inhibition of microbial pathogens in different concentrations of micro filter

Organism	25µl	50µl	100µL
S. mutants	16 mm	17mm	20mm
S. aureus	15mm	16mm	21mm
Lactobacillus	17mm	18mm	20mm

Discussion :

One of the well-known causes of inflammatory disorders is the denaturation of tissue protein. Therefore, the creation of anti-inflammatory drugs can make use of substances that can block protein denaturation. The management of excessive inflammation, which is typically brought on by a variety of immune system defensive pathways, may be helped by this broader inhibitory function (6). Plaque-related illnesses are the most prevalent ailments of tooth-supporting systems. It caused inflammatory changes in the periodontium and gingiva. It has been established that bacterial infections are what cause periodontal disorders. For a long time, herbal medications made from botanical sources have been used in dentistry to inhibit bacteria, lessen swelling, calm irritation, and ease pain (11). A similar study reported that the antibacterial activity of produced mouthwash against various microorganisms at 25 L was noticeably lower than the norm, however there was an increase in the antimicrobial property that was concentration dependent. At all concentrations, it was discovered that the mouthwash's cytotoxic effect was within acceptable bounds. The cytotoxic effect was shown to be minimal at low concentrations (17). Another study reported the antimicrobial activity of herbs where the sizes of the inhibitory zones were found to range from 7 to 59 mm. When pure essential oil samples were applied to microorganism specimens, the bacterial static and cidal activity was typically 50% and higher (5)

The present study was done on shrimps. A better understanding would have been obtained if it was an animal study. More shrimps could have been added and performed the various activities with plant extract. Ginger and stevia herbal formulation could be used for future nanomedicine

applications in treating various periodontal conditions. A significant variety of herbal mouthwashes have reportedly produced good results in the management of plaque and gingivitis (11).

Herbal mouthwashes are created and prepared with essential oils and plant extracts from phytotherapeutic plants that include a variety of active ingredients such as tannins, sterols, and catechins (8). The mixture of natural ingredients in the herb- or plant-derived products often has mild curative effects. Herbal mouthwashes can have additional anti-inflammatory and antioxidant qualities, which could further aid gingival health, in contrast to the antibacterial processes provided by synthetic chemicals . In the future, we can research larger volumes of material under in vivo conditions.

Conclusion :

The observation of our study, an increased concentration of ginger and stevia herbal formulation was found to be toxic to the nauplii as the number of nauplii present in higher concentrations of herbal formulation was decreased. Also as the concentration of the nanoparticle increased, the antimicrobial activity also increased. Thus ginger and stevia herbal formulation based mouthwash showed cytotoxic effect and antimicrobial effect.

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CONFLICT OF INTEREST

The author declares that there were no conflicts of interests in the present study.

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