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Anti-Oxidant Efficacy of Ginger Containing Dentifrice in the Control of Biofilm Induced Gingivitis. (Randomized Parallel Clinical Trial)

Zainab Zuhair Mohammed*

Maha Abdul Aziz Ahmed**

*M.Sc. student of college of dentistry, Baghdad University , Department of periodental dentistry, Iraq, E-mail:- zainabalalawi1987@gmail.com.

**Professor at college of Dentistry,Baghdad university, Department of periodental dentistry, Iraq, E-mail:- Maha.abidalaziz@avic.uobaghdad.edu.iq, ORCID: https://orcid.org/0000-0002-1658-4210.

Abstract

Background: Gingivitis induced by biofilm is one of the most prevalent periodontal diseases. Due to their antimicrobial, anti-inflammatory, antioxidant, and other multipotent effects, a number of traditional medicinal plants are revered as pharmaceutical alternatives.

Aim: The aim of the current study was to assess the antioxidant efficacy of ginger containing dentifrice in control patients with biofilm induced gingivitis.

Materials and methods: In a double-blind, randomized, parallel clinical trial, one hundred adults suffering from gingivitis caused by dental biofilm were split into two groups and given either a ginger-containing or a control dentifrice to use for three months. Clinically, periodontal assessment and saliva collection were performed at the baseline visit and after three months of toothpaste use in order to determine the superoxide dismutase level.

Results: Ginger containing dentifrices showed a significantly improved in plaque (0.216+0.069) and bleeding on probing (7.818+7.339) index scores. In addition, a significant increase (22.082+1.510) in the level of SOD antioxidant after 3months compared with control dentifrice. **Conclusion:** Compared to conventional dentifrices, herbal dentifrices demonstrated promising outcomes.

Key Words: Dental plaque, Ginger, Gingivitis, Dentifrices, Antioxidant.

Introduction

Dental biofilm-induced gingivitis is an inflammatory condition of the gingiva characterized by bleeding, redness, and edema. It is frequently caused by the accumulation of hazardous bacteria in the gingival crevice, which causes an inflammatory response in the gingival tissues (Akram et al. 2020). Several clinical trials have shown that brushing teeth with herbal dentifrices reduces supra-gingival plaque and gingivitis (Janakiram and Dye 2020) .

Ginger was one of these active botanical compounds. Ginger (Zingiber Officinale) is a medicinal plant that has been used extensively throughout the globe and is a popular seasoning and condiment in Asia. Ginger contains gingerol, shogaol, hydrocarbons, and oleoresins, which are phenolic compounds with functional and pharmacological properties such as antioxidants, anti-inflammatory, and immunomodulatory, which aid in combating oral microorganisms and preventing oral diseases and gingivitis. Dried ginger is pungent due to the presence of shogaols, specifically (6)-shogaol, which are dehydrated gingerols (Deshpande et al. 2021).

Periodontal disease occurs when there is an imbalance between the host's immune response, the microbial biofilm and its by-products. Frequently, an imbalance between antioxidant defense systems that protect and repair vital tissue cells and reactive oxygen species (ROS) occurs. Chronic exposure to ROS can initiate pathological responses such as periodontal disease. Antioxidants are substances that protect body tissues and counteract the detrimental effects of oxidation. " (Najah et al. 2017).

Super oxide dismutase SOD is the first line of defense in antioxidant reactions against reactive oxygen species (ROS) as it converts superoxide anion to hydrogen peroxide within gingival fibroblasts. SOD is localized within human periodontal ligaments (Thomas et al. 2014).

Researchers have shown that saliva may be used to detect and diagnose a wide range of illnesses, both in the body and in the mouth (Roi et al. 2019). The salivary marker levels could be used to differentiate between those with robust periodontium and those with periodontal disease (Becerik et al. 2012).

This research set out to assess and contrast the efficacy of herbal dentifrice containing ginger versus Colgate total dentifrice in controlling plaque and gingivitis, as well as in measuring clinical periodontal parameters (mQHPI and BOP). Antioxidant effect determined by salivary SOD measurement.

Materials and Methods

Subject Sample

The study approval number was (Ref. 532, 17/04/2022, Project # 532622, College of Dentistry, University of Baghdad). Patients agreed to participate in the trial after receiving comprehensive information about its purpose and signing an informed consent form. Subjects had to be between the ages of 20 and 35, have good overall health, have generalized dental biofilm induced gingivitis, and have at least 20 remaining natural teeth to be included in the study. Exclusion criteria included: current use of any mouthwash; use of antibiotics within 2 months prior or during the study; history of hypersensitivity to any item used in the current study; recent tooth extraction; current smoking or alcohol use; presence of extensive untreated dental caries; presence

of soft and hard palate lesions; and use of orthodontic appliances.

Study Design

A double-blind, parallel, randomized clinical investigation was conducted on 100 patients for this study, had generalized dental biofilm induced gingivitis (having ≥30% bleeding sites with no probing pocket depth PPD >3 mm, intact periodontium and no loss of periodontal attachment)(Murakami et al. 2018) from both sexes. The control toothpaste with active ingredient: Stannous fluoride 0.454% (0.15% w/v fluoride ion) and intervention toothpaste with 3%ginger as active ingredient were used in this study. Each intervention had a code (A or B) on a plain gray covering to verify the proper masking of the item from the examiner and the participants; this was performed by a subject who was not a part of this study. The intervention was randomly allocated to the participants by using a random number generator in Excel software. They were divided into two groups (50 for each group) according to intervention intake:

The ginger containing (intervention) [group A] or (control) [group B] toothpastes with modified bass technique method (Disyam 1987) and Oral B toothbrush (medium size) for tooth brushing supplied by the examiner.

Clinical Periodontal Parameters Measurement

Clinical measurement was performed by Bleeding on probing BOP (Löe and Silness 1963) and Plaque Index mQHPI (Modified Quigley and Hein) (Turesky 1970), with six surfaces of each tooth were examined. Clinically, periodontal parameters was examined using the University of Michigan O probe with Williams markings. The parameters included assessment of bleeding tendency (bleeding on probing index), and assessment of plaque deposit ((modified Quigley Hein plaque index, (mQHPI) and fluorescein disclosing tablets) (Newman et al. 2018).

Clinical Calibration

The accuracy and reproducibility of the examiner for clinical periodontal parameters (BOP and mQHPI) were assessed by inter and intra examiner calibration. Inter examiner calibration; scores were recorded by the researcher under supervision of a senior supervisor as described by (Hefti and Preshaw 2012)

For intra examiner calibration, the periodontal parameters for 5 subjects from Baghdad dental Teaching Hospital of college of Dentistry\University of Baghdad were measured twice by the researcher with two-hours interval between the two measurements to do the standardization and alignment exercise and to get an acceptable level of agreement (> 0.75) kappa test (Organization 2013).

Clinical Trial

At baseline (visit 1), after participants were selected with biofilm induced gingivitis, unstimulated saliva sample collection was done. The study was made at the morning, so all the included samples were recorded at the same period from 10 AM to 12 PM (Abdulbaqi, Himratul-Aznita, and Baharuddin 2016) . After that, measure the clinical periodontal parameters (mQHPI and BOP). At first BOP was taken using a periodontal probe to measure soft tissue state and bleeding response. Then participants were given disclosing tab chewed and moved around all teeth for 30 seconds then spit it for mQHPI

measurement. Finally, the participants were given either intervention or control toothpaste and instructed to use it twice daily (in the morning and before bed) for three months.

Motivation and instruction about using interdental aids and brushing with modified Bass technique were provided. Scaling and polishing were done for all of participants at baseline visit. Participants received directions to use a sufficient amount of tooth paste (along all the superior surface of tooth brush) and to brush for a full two minutes. At visit 2, saliva collection had been taken from the participants from 10 AM to 12 PM and clinical periodontal parameters were measured. the codes were declared at the end of the study (after 3 months), then data analysis were begun.

Salivary Samples Collection

Prior to the clinical periodontal assessment, unstimulated whole saliva was collected using a revised protocol (Syndergaard et al. 2014) and all samples were taken at the same period from 10 AM to 12 PM. Subjects had been asked to avoid brushing, eating, drinking except water, or chewing gum 2 hours prior to saliva collection. Saliva was collected after subjects washed their mouths with tap water (10 mL) for 30 seconds and expectorated. A minimum of 1 mL of the subject's unstimulated saliva was expectorated into a test tube., Saliva sample had been collected from the sterile tube with micropipette 600 µl into Eppendorf tube and kept in cooling box after that, it putted in centrifuge for 15 min with 3000rpm to remove cell debris at 4 °C, the supernatant collected by micropipette and kept in -20°C deep freeze until the biochemical analysis by Enzyme-linked immunosorbent assays ELIZA kit (Zhu et al. 2019) for SOD levels at 450 nm. Commercially available ELISA kits purchased from MyBioSource, California, USA, was used for determining protein levels in the salivary samples. The results were reported as Nano gram per milliliter (ng/mL).

Sample Size

Using G power 3.0.10 (Program written by Franz-Faul, Universitatit Kiel, Germany) with power of study=90%, alpha error of probability=0.05 two sided, assume effect size of Cohen D is 0.8 (large effect size), with two groups, adding 10 % as drop out with all these condition the sample size 50 subjects for each group (Chuan and Penyelidikan 2006). Cohen D is: Small =0.2, medium=0.5, large=0.8

Statistical Analysis

Data analysis were performed using Statistical Package for social Science (SPSS version -22, Chicago, Illionis, USA). Statistical analyses included descriptive Analysis, (mean and Standard Deviation), and Inferential analysis which include independent sample T test, Paired T test (for mean percentage of the bleeding surfaces), Shapiro Wilk test (for normality test) and Kappa test (for clinical calibrations). Level of significance when p value less than 0.05

Results

Participants' ages ranged from 20 to 35, with a mean of 25.615 ± 4.073, who were 55 males and ٤5 females. Results showed that all studied variables were normally distributed among groups and time using Shapiro Wilk test at p >0.05.

The statistical analysis (by using T paired test) shows that the mean indexes of the clinical periodontal parameters (BOP and mQHPI) were decreased and SOD were

significantly increased after 3 months in both groups (p=0.000) compared to that at baseline with high rate of significance in intervention group(**A**) more than that in group (**B**). Effects size were more in group **A** than group **B** for all clinical parameters and SOD biomarker. The reduction in the means difference of all parameters after tooth brushing with ginger containing toothpaste were statistically significant (p=0.000) compared to control group. While the mean difference of SOD biomarker was not significant (p=0.084).

Discussion

There were no differences between study groups in terms of periodontal clinical parameters, similar to other studies (Hassan, Abdul, and Ahmed, n.d.). No differences between immunological markers at baseline, it means no influence on future outcomes.

This study revealed that the means of mQHPI and BOP were significantly decreased in both control and ginger containing groups at 2nd visit compared to the 1st visit, with highly significant reduction in group A. This finding indicates that ginger containing toothpaste is superior to control toothpaste in preventing plaque buildup and gingival inflammation.

The antibacterial components in control toothpaste are 0.3% triclosan and 2.0% PVM/MA copolymer. For both Gram-positive/-negative bacteria, triclosan is an effective antibacterial agent. Essential amino acids are not absorbed when triclosan concentrations are at a bacteriostatic level. Triclosan, when used in bactericidal quantities, disrupts the cytoplasmic membrane, resulting in the release of cellular contents (Kraivaphan and Amornchat 2017)

In the microbiologic analysis, Tieli and his colleagues (Faria et al. 2021) exhibited that 0.5% Zingiber *officinal* essential oil (ZOEO) mouthwash showed antibacterial efficacy against *Streptococcus mutans*. Both dental biofilm and gingival bleeding were effectively managed by the ZOEO. Another double blinded clinical trial (Mahyari et al. 2016) in which polyherbal mouthwash containing extracts of *Z. officinale* was used and showed that *Z. officinale* was effective in the treatment of gingivitis and its efficacy was comparable to that of chlorhexidine mouthwash in reducing gingivitis through decreasing mQHPI index and bleeding on probing index . Numerous studies revealed that ginger antimicrobial effects primarily were due to the existence of oxygenated mono and sesquiterpenes phenolic constituents (shogaol and gingerol), which are lipid-soluble phenol compounds mostly isolated from the root of ginger. Most of the phenols can change the cell permeability because they are protein denaturing agents s, which may lead to swelling and rupture of the bacterial cells (Sana'a and Ahmed 2017).

Superoxide dismutase SOD was elevated in the intervention group compared to the control group; this may be due to the antioxidant effect of ginger. Ginger supplementation improved inflammation, oxidative status, and periodontal health in intervention patients, according to a study (Zare Javid et al. 2019). J. Yang. and his colleagues (Yang et al. 2018) showed that gingerol compounds [6], [8], [10] and [6]-shogaol exhibited significant scavenging abilities against superoxide radical, hydroxyl radical and reactive oxygen species (ROS). The free radical scavenging activity could be increased with increasing concentration because of its high anti-oxidant capacity

(Dugasani et al. 2010). ROS accumulation lead to cellular destruction through lipid peroxidation(Kang and Yang 2020). However, ginger enhance a rescue mechanism through the enzyme paraoxonase-1 that reduce lipid oxidation (LDL) (Carnuta et al. 2018) .The powerful antioxidant ginger component (6-gingerol) raise the Beclin1 expression promoting endothelial cells autophagy and inhibits PI3K/AKT/mTOR pathway signaling without affecting cell cycle(Santos Braga 2019). Significantly increased levels of antioxidant enzymes such as SOD, protective against the effect of ROS, were linked to ginger consumption, while inflammatory mediator levels were found to be significantly reduced (Zare Javid et al. 2019). The limitations of this research included that microbiological analysis was not conducted and the lack of control to the participants' diet during the study duration. Other limitations that no feedback about the acceptability of the participants to the taste of the dentifrices was recorded.

Conclusion: Brushing with ginger toothpaste twice day by day can reduce dental plaque massing and bleeding on probing following three months withoutadverse effects. There was a positive increase in salivary SOD levels after brushing with ginger toothpaste which is a sign of less periodontal destruction.

Suggestions

1 - Long-term clinical study with same design could be undertaken for better evaluation of the usefulness of this natural product.

2 - The actual mechanism involved behind the antimicrobial activity of ginger containing tooth paste needs to be researched using the same study design.

Source of Funding. Self-funding

Conflict of Interest. There is no conflict of interest.

Trial Registration. This study was registered in ClinicalTrials.gov as NCT05868200 in May 19,2023.

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Table 1: Descriptive and statistical analysis of mQHPI between groups and intervals.

	Toothpastes					
	Ginger containing TP.		**Control TP.**			
	Mean	**SD**	**Mean**	**SD**	**T test**	**P value**
Visit 1 (baseline)	**1.219**	**.271**	**1.365**	**0.211**	**1.954**	**0.058**
Visit 2 (after 3 months)	**0.216**	**0.069**	**0.841**	**0.120**	**28.514**	**0.000**
Paired T test	**15.061**		**24.554**			
P value	**0.000**		**0.000**			
Effect size	**3.882**		**2.381**			
Mean difference	**1.002**	**0.268**	**0.424**	**0.210**	**8.836**	**0.000**

TP : Toothpaste.

SD: Standerd deviation.

Table 2: Descriptive and statistical analysis of BOP between groups and intervals.

	Toothpastes					
	Ginger containing TP.		**Control TP.**			
	Mean	**SD**	**Mean**	**SD**	**T test**	**P value**
Visit 1 (baseline	**47.822**	**4.758**	**48.154**	**5.549**	**0.332**	**0.644**
Visit 2 (after 3 months	**7.818**	**7.339**	**36.276**	**3.179**	**18.825**	**0.000**
Paired T test	**16.441**		**34.429**			
P value	**0.000**		**0.000**			
Effect size	**5.449**		**2.517**			
Mean difference	**41.014**	**7.533**	**13.188**	**5.027**	**19.479**	**0.000**

TP : Toothpaste.

SD: Standerd deviation.

Table 3: Descriptive and statistical analysis of SOD between groups and intervals.

	Toothpastes					
	Ginger containing TP.		**Control TP.**			
	Mean	**SD**	**Mean**	**SD**	**T test**	**P value**
Visit 1 (baseline	**11.224**	**2.463**	**7.417**	**2.640**	**1.957**	**0.054**
Visit 2 (after 3 months	**22.082**	**1.510**	**17.091**	**1.557**	**14.553**	**0.000**
Paired T test	**18.955**		**24.506**			
P value	**0.000**		**0.000**			
Effect size	**3.387**		**2.997**			
Mean difference	**10.858**	**2.802**	**9.675**	**3.228**	**1.750**	**0.084**

TP : Toothpaste.

SD: Standerd deviation.