EFFICACY OF A COMBINED SEA SALT-BASED ORAL RINSE WITH XYLITOL AGAINST DENTAL PLAQUE, GINGIVITIS, AND SALIVARY *STREPTOCOCCUS MUTANS* LOAD

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Dental plaque is a biofilm which forms on the non-shedding surfaces of the oral cavity. If left untreated, the succession of dental plaque development can lead to serious complications, such as caries, gingivitis, and periodontitis. The control of dental plaque on tooth surfaces is vital for the prevention of dental plaque related diseases. In this context, antimicrobial agents may serve as a valuable complement to mechanical plaque removal. Therefore the present study was aimed to evaluate the action of a combined rinsing solution containing different antimicrobials (sea salt, xylitol and lysozyme) used individually, for the reduction of a salivary specific bacteria *(S. mutans)* colonizing oral environment.

To the Editor,

It is generally accepted that the development of dental plaque surrounding the tooth/gingiva interface is one of the major causes of gingival inflammation and caries (1-3). Chemical methods of reducing plaque, such as mouthwashes, are therefore appealing as they can provide significant benefits to patients who cannot maintain adequate mechanical plaque control (4, 5). Various chemical mouthwashes are available on the market, but are associated with side-effects such as immediate hypersensitivity reactions, toxicity, tooth staining, etc. (6).

Several studies have shown that natural products i.e. polyphenols and xylitol have biological activity most notably as a constituent of dietary products (2, 7). Frequent use of xylitol chewing gum has been shown to prevent caries, biofilm formation and *Streptococcus mutans (S. mutans)* level in the oral micro-environment. A wide range of experiments *in vitro* has shown that the majority of oral microorganisms cannot metabolise xylitol to acidic products (2, 5, 6). Nevertheless, xylitol in the forms of chewing gum and candies may not be practical for young children or elderly adults. In these situations, an aqueous solution or mouthrinse may be useful. However, clinical evidence of the efficacy of xylitol in mouthrinses is very limited (2).

Therefore, the aim of the present study was to compare the plaque control efficacy of the new formula H2Ocean Sea Salt Mouthwash on salivary

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S. mutans count, plaque and gingival scores of young adults.

MATERIALS AND METHODS

Subjects and study schedule

The present study was conducted at the Dentist Education Institute in collaboration with the Dental Institute of the City Unity College, Greece, from February 2018 to June 2018 as a double-blind parallel-group clinical trial. Twenty gingivitis patients between the ages of 18 and 25 years were enrolled in the study and all gave written informed consent in accordance with the Declaration of Helsinki .

The patients received a verbal description of the clinical protocol to be followed, and in order to have unbiased and accurate clinical data, a double-blind protocol was followed for enrolment of the patients in terms of treatment plan and further categorization into the study group. All the enrolled patients underwent scaling and polishing to get the baseline score to nil.

All the subjects were advised to brush twice daily for 5 min using a modified bass technique (which was demonstrated to each subject). Medium bristle tooth brushes and tooth paste were provided to each subject during the study course to maintain standardization.

Sample size and blinding

The subjects were then categorized into two treatment regime groups in groups. Group I (n = 10): combined mouth rinse containing sea salt and xylitol (H2Ocean Sea Salt Mouthrinse, a new mouthwash formula that contains sea salt, xylitol and lysozyme with no harsh chemicals, alcohol or fluoride). Group II (n = 10): placebo mouth rinse (physiologic saline solution - no mouthwash dilution added).

Subjects in both groups were instructed to rinse their mouth with 10 ml of mouthwash twice daily (for the first 30 days, then once per day for the following 60 days), after breakfast and again after lunch for 60 days for 1 min, and not to rinse with water thereafter for the following 30 min. Both the mouth rinses had similar bottle appearance.

Inclusion criteria

Patients aged between 18 and 25 years, and suffering from gingivitis with good to fair oral hygiene were selected for the study.

Exclusion criteria

Patients who presented less than 20 sound permanent teeth with a minimum of 5 teeth present in each arch quadrant, or who had received antibiotics or nonsteroidal antiinflammatory drugs (such as Ibuprofen) in the previous 9–11 weeks or periodontal treatment in the previous 6 months; pregnancy or breast-feeding; presenting artificial prosthesis; smoking or tobacco consumption in any form; suffering from any systemic diseases; female patients using intrauterine birth control devices or birth control pills; and subjects not willing to participate in the study.

Table I. *Baseline characteristics of study population.*

Population	Group 1	Group 2	Total
Total participants (N)	10	10	20
Males	4	3	7
Females	6	7	13
Mean age(years)	23.7	22.1	p = 0.29
Mean DMFT	2.16	2.18	p = 0.184

Clinical parameters

Clinical Recording Protocol Clinical parameters evaluated were gingival index (GI) and plaque index (PI), by the same blinded trained examiner at baseline and 30, 60 and 90 days. The measurements were recorded of central incisors, canines and second premolars of four quadrants (8). A mean value of each parameter was calculated in each pre- and post-rinse measurement.

Microbiologic examination

For microbial evaluation, saliva samples were collected by chewing on a piece of sterile paraffin wax in order to test whether there were variations in the numbers of bacteria due to the laboratory methods. A paired cultivation of 20 samples was carried out. Samples were transferred to the Microbiology Laboratory of University of Bari Aldo Moro, Italy, for further processing. The transport of samples to the lab was done via express courier and the use of tryptic-soy-serum-bacitracin-vancomycin transport media, and the samples were analyzed within 24 h (4). Then, 50 µL of the specimen were cultured on blood agar. After 48-hr incubation at 37°C, colony counts were enumerated. Total bacterial count was determined by visual counting and the latter was multiplied by 20 to express as colony forming units (CFU)/mL.

Statistical analysis

The statistical analyses were performed by using Two-way ANOVA, and Tukey's multiple comparisons test were performed to assess statistical differences using the GraphPad Prism software (version 7.0; San Diego, California, USA). The probability (p) of <0.05 was considered to indicate statistical significance.

RESULTS

The baseline characteristics of all the subjects who participated in the study are shown in Table I.

Clinical measurements

The clinical results were encouraging, and patients provided positive feedback on the use of this protocol. All subjects (N = 20) completed the trial, and there were no missing values. The amounts of mouthwashes used indicated good compliance with the instructions. No adverse events or side effects were reported or observed. The plaque and gingival scores for the test and control groups at the end of the experimental period are shown in Fig. 1 A-B.

Microbiologic examination

As reported in Table II, the mean reduction in colony forming units, before brushing and after H2Ocean Sea Salt Mouthwash were 87.5% and 32.5%, respectively, with a highly significant difference of 55.0% (P<0.001). Large variations in inter- and intra-individual measurements were observed. Both examinations showed a significant decrease from the baseline.

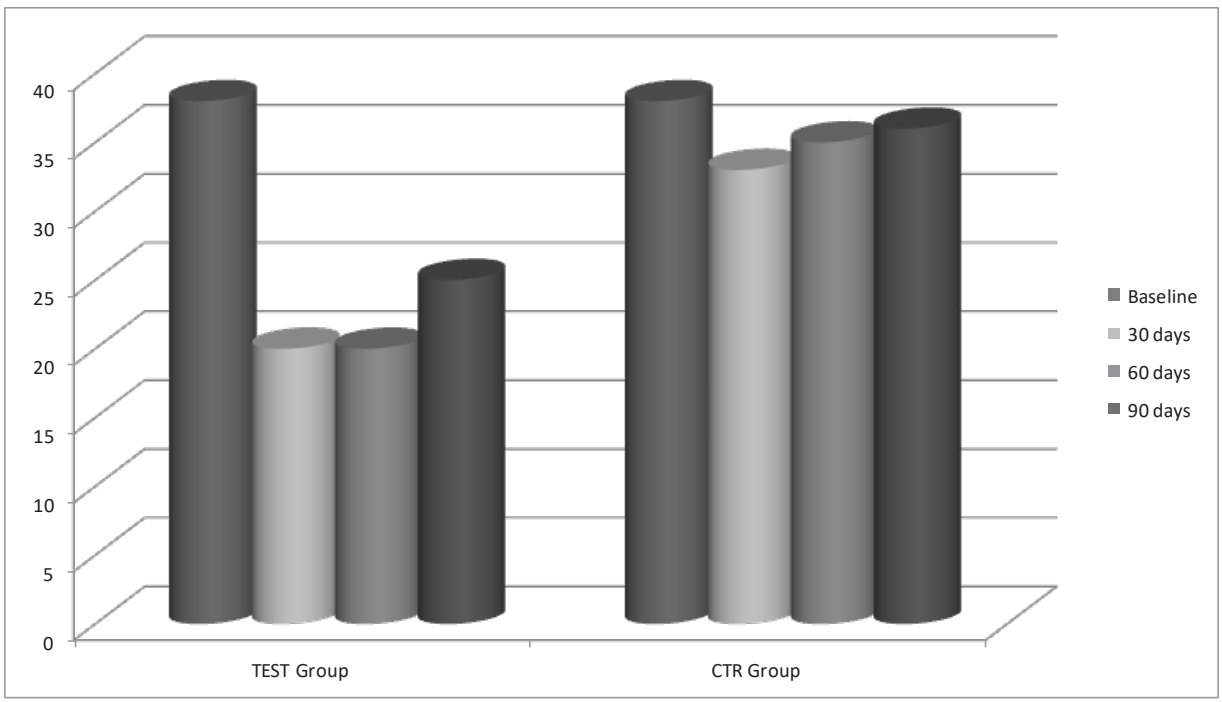
Compliance

Compliance with the course of the intake of the tested product from the participants was also documented. All subjects returned the medication bottles. All subjects from both groups (100%) completed the course of mouthwash protocol as indicated.

Table II. *Colony-forming units (CFUs) before and after treatment.*

	Before	**After**	**Difference**
Range	65-110	25-40	-
Mean	87.5	32.5	55.0
"p" Value	-	-	<0.001

A



B

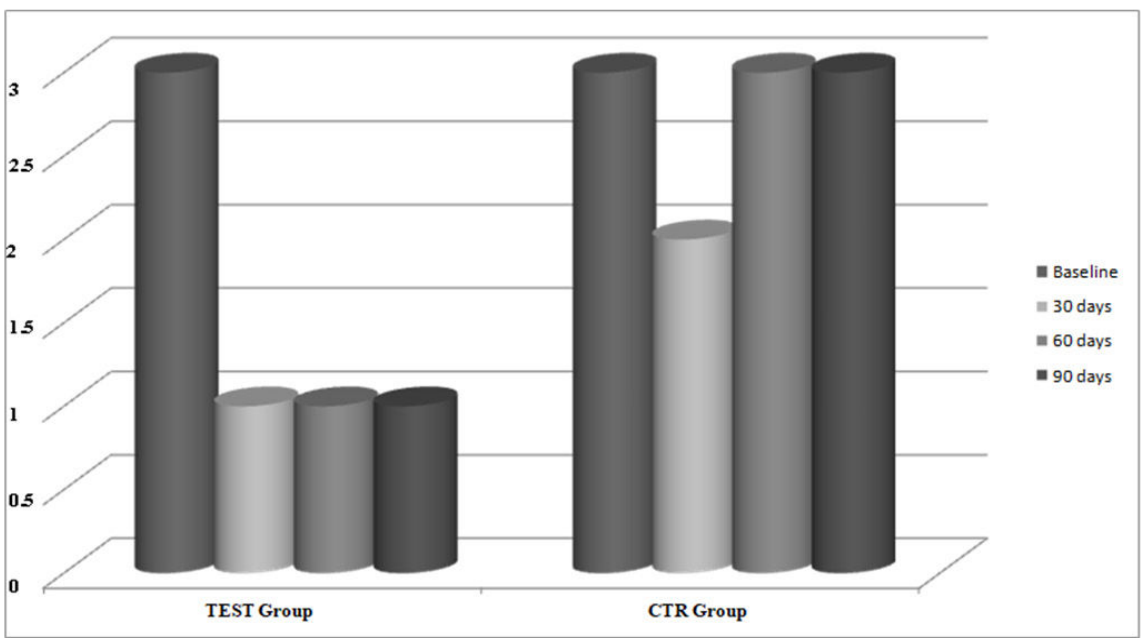


Fig. 1. *Plaque (**A**) and Gingival (**B**) scores for the test and control groups at the end of the study period.*

DISCUSSION

Microbial biofilms are complex communities of bacteria present in the environment and human body (1). If not removed regularly and adequately, dental plaque matures into a pathogenic bacterial complex which can lead to caries, gingivitis, periodontitis and peri-implantitis, ultimately resulting in impaired oral function (9-11).

Although different mechanical tooth cleaning methods are considered the most reliable means of plaque control worldwide, various chemotherapeutic agents have always been sought as adjuvants (4). Thus, mouth rinses are frequently recommended for chemical control of dental biofilm, especially in areas which are inaccessible to tooth brushing (3).

The findings of the present study are in accordance with Shyama et al. (12) who assessed the effect of xylitol candies on gingival indices in physically disabled pupils, and discovered a statistically significant reduction in gingival index scores of the study participants when they consumed xylitol candies thrice daily.

However, this research was a relatively short clinical study and the findings may be different to a prolonged one. Studies of longer duration with cross-over study design and wash-out period in gingivitis patients would have been more authenticating as they would eliminate the bias of viable host relating to the use of H2Ocean Sea Salt Mouthwash. Finally, it should be noted that natural agents such as mouth rinses are not substitutes for through brushing and flossing, but they should be used as adjunct.

A larger-scale, longer-term clinical trial using mouthwashes is needed which could demonstrate the feasibility of H2Ocean Sea Salt Mouthwash in different populations with different dietary and oral hygiene patterns, and establish the minimum daily frequency of use.

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