

# SHORT-TERM EVALUATION OF A GREEN TEA EXTRACT-BASED MOUTHRINSE

## *Avaliação de curto prazo de um enxaguatório bucal à base de extrato de chá verde*

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## ABSTRACT

**Introduction:** mechanical hygiene is still the best method for the maintenance of oral health and prevention of biofilm formation. However, mouthrinses have been increasingly used as an adjunct support especially for post-operative conditions or when periodontal conditions require. In this way, a number of mouthrinses are available, presenting different active ingredients with predominant antimicrobial action. **Objectives:** the aim of the present study was to evaluate the topical action of a green-tea extract-based mouthwash on gingival tissue. **Materials and Methods:** a longitudinal double-blind comparison was performed with two groups of patients clinically evaluated and diagnosed as suffering from gingivitis (n= 40). The patients were divided into two groups, according to the mouthrinse used: GT – green tea (concentration of 20mg/ml), and P – placebo, 0.9% saline solution. Clinical examination of the mucosa, tooth staining, plaque and gingival indices were per-

formed before and 15 days after the continuous use of the products twice a day. **Results:** no mucosal or tasting alterations were found, neither tooth staining regardless the mouthrinse used. Statistical assessment did not detect differences on gingival indexes between the groups before and after mouthrinses use; however, plaque indexes were significant lower in patients of GT group, compared to the P group. **Conclusions:** the use of phytotherapeutic products with active ingredients should be investigated for biofilm control, responsible for the onset and progression of periodontal disease, as well as other various oral pathologies. Considering the period of evaluation proposed in this study, relevant decrease in initial phase of plaque formation could be observed with the green tea mouthrinse. Further long-term evaluation studies should be carried-out in order to elucidate its continuous effects on oral structures.

**Keywords:** Green tea. Mouthrinse. Periodontal disease. Inflammation.

## RESUMO

**Introdução:** higiene mecânica ainda é o melhor método para a manutenção da saúde bucal e prevenção da formação de biofilmes. No entanto, enxagues têm sido cada vez mais usado como um suporte auxiliar especialmente para as condições pós-operatórias ou quando as condições periodontais exigem. Desta forma, um número de bochechos estão disponíveis, apresentando diferentes ingredientes ativos com predominante ação antimicrobiana. **Objetivos:** o objetivo do presente estudo foi avaliar a ação tópica de um bochecho à base de extrato de chá verde no tecido gengival. **Materiais e Métodos:** a comparação longitudinal, duplo-cego, foi realizada com dois grupos de pacientes clinicamente avaliados e diagnosticados como portadores de gengivite (n = 40). Os pacientes foram divididos em dois grupos, de acordo com o enxaguatório oral usado: CV - chá verde (concentração de 20 mg / ml), e P - placebo, solução salina a 0,9%. O exame clínico da mucosa, coloração dos dentes, índice de placa e índice gengival foram realizados antes e 15 dias após o uso contínuo dos produtos duas vezes por dia. **Resultados:** nenhuma alteração na mucosa ou de degustação foi encontrada, assim como não foi identificada modificação na coloração do dente. A Avaliação estatística não detectou diferenças no índice gengival entre os grupos antes e depois de usar enxaguatórios. No entanto, índice de placa inferior foi menor de forma significativa nos pacientes do grupo CV, em comparação com o grupo P. **Conclusões:** o uso de produ-

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tos fitoterápicos com ingredientes ativos deve ser investigado para o controle de biofilme, responsável pelo aparecimento e progressão da doença periodontal. Considerando o período de avaliação proposto, a redução correspondente na fase inicial da formação de placa pôde ser observada com o enxaguatório à base de chá verde. Além disso, estudos de avaliação de longo prazo devem ser realizados, a fim de elucidar seu efeito contínuo em longo prazo sobre as estruturas orais.

**Palavras-chave:** Chá verde. Enxaguatório. Doença periodontal. Inflamação.

## INTRODUCTION

The most common infectious diseases of the oral cavity are of bacterial etiology, such as tooth decays and periodontal disease (PETERSEN e OGAWA, 2005; WOLF e LAMSTER, 2011). Inflammation and bone loss are characteristics of periodontal disease presenting an initial response to anaerobic bacterial infection with a local inflammatory reaction, which sequentially activates the immune system (COCHRAN, 2008). The amplification of this response causes release of cytokines and other inflammatory mediators by gingival tissue (PRESHAW e TAYLOR, 2011). Advanced stages of periodontal disease are characterized by irreversible damage of periodontal fibers and bone tissue, leading to tooth loss. Bacteria such as *Prevotella spp* and *Porphyromonas gingivalis* are closely associated with periodontal disease (TAYLOR, HAMILTON-MILLER e STAPLETON, 2005).

Mechanical hygiene is still the best method for the maintenance of oral health and prevention of biofilm formation (ALVES *et al.*, 2010); however, mouthrinses have been increasingly used as an adjunct support especially for post-operative conditions or when periodontal conditions require. In this way, a number of mouthrinses are available, presenting different active ingredients with predominant antimicrobial action (ALLAKER e DOUGLAS, 2009).

Chlorexidine acts on Gram positive and negative bacteria, altering microbial adherence, increasing cell permeability with disruption of bacteria and precipitation or coagulation of cytoplasmic constituents (COENYE *et al.*, 2011). Mendes, Zenóbio e Pereira, (1995) stated that essential oils have beneficial effects for gingivitis because they decrease the synthesis of prostaglandins and found that mouthrinses containing essential oils reduced from 20 to 34% of the dental plaque and 28-34% of gingivitis. (GARCIA *et al.*, 2011) determined the in-

hibitory plaque effect of a 0.05% CPC (cetyl-pyridinium chloride) mouthrinse in de novo plaque formation in a 4-day non-brushing experimental model, reporting positive results with the reduction of plaque index.

Green tea, prepared from the mature leaves of *Camellia sinensis* plant is consumed in different parts of the world both as green and black tea. The chemical composition of green tea is complex, and the most abundant components are polyphenols, especially flavonoids such as catechins and catechin-gallate (VINSON, 2000). known not only because of their antioxidant, antimutagenic and anticarcinogenic effects (PRESHAW e TAYLOR, 2011), but also due to their anti-inflammatory and antimicrobial properties, especially on *Helicobacter pylori* (YEE, KOO e SZETO, 2002), Influenza and Herpes simplex viruses (YAM, SHAH e HAMILTON-MILLER, 1997). It has been shown that green tea catechins inhibit gingival inflammation (KRAHWINKEL e WILLERSHAUSEN, 2000), and present antibacterial action, contributing to the reduction of gingivitis (SAKANAKA, OKUBO e AKACHI, 1997). Green tea polyphenols inhibit the collagenase activity of oral bacteria and inhibit the activation of NF- $\kappa$ B (Nuclear Factor-kappa B), which is one of the key positive regulators of COX- 2 (cyclooxygenase 2) expression (BITU PINTO *et al.*, 2015). These substances also have the potential to diminish the damage caused by periodontal potent activity of proteases released by *Porphyromonas gingivalis* (MCKAY E BLUMBERG, 2002). It is reported that mouthrinses with a dilute solution of catechin reduce halitosis associated with periodontal disease, through modification of odorant sulphur components(KANEKO, SHIMANO e SUZUKI, 1993)

Once human clinical evidences are still limited, investigations become necessary to define the real magnitude of green tea health benefits, as well as its mechanisms of action. Thus, the purpose of this study was to evaluate the clinical action of a mouthrinse based on green tea extract in patients with periodontal disease, by the analysis of plaque and gingival indexes.

## MATERIALS AND METHODS

Eighty patients were selected to participate in this study, performed as a longitudinal double-blind comparison with two balanced groups (40 patients each), who used a mouthrinse based on green tea extract and a placebo. This work was approved by the Ethics Committee of Sacred Heart University under protocol number 048/10. Pa-

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tients were informed about their participation in the study and signed an informed consent.

Individuals were included in this study according the following criteria: minimum of 16 natural teeth including at least four molars and clinical evidence of gingivitis (gingival index score > 0). Criteria of exclusion included acute tooth decays, severe or uncontrolled systemic conditions, pregnancy or nursing, use of any antibiotic medication or anti-inflammatory drug during and / or at least seven days before the beginning of the research. No guidance on brushing and flossing technique has been given to the patient, in order that patients maintained the usual standard of oral hygiene. No basic periodontal therapy was instituted, either.

The initial tests were made by examining general health, oral soft tissue, dental stains, plaque index (LANG *et al.*, 1982) and gingival index (LOE, 1967). The examination included assessment of gums, hard and soft palate, oropharynx, lips and buccal mucosa, tongue, floor of mouth and lips. The area, size and severity of injuries that could eventually appear were registered, as well as whether the injury could be related to the product used. All participants underwent a thorough dental prophylaxis in order to reset the index plaque, received the mouthrinse and instructions to use the product (10 ml of mouthwash for 30 seconds, twice daily, preferably morning and night). The formulation of the mouthwash used in this study contains green tea extract (20mg/ml) (LEE *et al.*, 2004), based on an alcohol-free and without dye. The placebo was formulated in the same way, except for the absence of green tea extract. After 7 days of product use, patients were invited again and the tests repeated.

The data obtained in the plaque and gingival indexes were expressed as percentages, that is, the sum of the faces with the presence of plaque or bleeding, respectively, divided by the total number of dental surfaces of each patient. The percentages of the groups were compared using Student's t-test considering  $p < 0.05$ . Other data followed a descriptive analysis.

## RESULTS

At the end of the experiment, a total of 74 patients were evaluated, aged between 18 and 61 years, since six patients did not show up for the second examination resulting in 37 patients for each group. Clinical examinations were performed, and the obtained periodontal scores are shown in Tables 1 and 2. Statistically significant reduction in plaque index between the initial and final periods of using mouth-

wash ( $p < 0.05$ ) was observed, although no gingival index alteration was detected. None of the patients presented mucosal lesions compatible with the use of the drug or placebo, nor change in tasting or dental staining.

Table 1 - Index of plaque in the patients who utilized mouthwash with and without green tea extract.

PI (%) $\pm$ SD	Whit green tea	No green tea
decreasing rates in the periods before and after use of mouthwash	20,15 $\pm$ 5,2*	9 $\pm$ 2,9

Decrease in the percentage of plaque index after the use of mouthwash with and without green tea extract. PI = plaque index, GI = gingival index, SD = standard deviation \* statistically significant. ("T" Student test,  $p < 0.05$ ).

Table 2 - Gingival index in the patients who utilized mouthwash with and without green tea extract.

GI (%) $\pm$ SD	Whit green tea	No green tea
decreasing rates in the periods before and after use of mouthwash	7,45 $\pm$ 3,7	5,65 $\pm$ 2,5

Decrease in the percentage of gingival index after the use of mouthwash with and without green tea extract. PI = plaque index, GI = gingival index, SD = standard deviation. ("T" Student test,  $p > 0.05$ ).

## DISCUSSION

The initial response to the accumulation of plaque is an inflammatory reaction that activates the innate immune system, resulting in the release of cytokines and other mediators, spreading by gum tissues. This inflammatory process can progress apically and cause destruction of tooth supporting tissues (COCHRAN, 2008).

Among the various biological effects of green tea, its inhibitory effect during the inflammatory process on Tumor Necrosis Factor -  $\alpha$  (TNF- $\alpha$ ) gene expression; this occurs mediated through inhibition of activation of transcription factor kappa  $\beta$  (NF-kappa  $\beta$ ) and proteic1 activator (AP -1) (SUEOKA *et al.*, 2001). In addition, some studies have reported its antimicrobial action (WU e WEI, 2002), a feature that would aid in the control of bacterial biofilms.

A number of studies have been reported the action of the green tea by its ingestion (ISOBE *et al.*, 1989; KUBO, MURO e HIMEJIMA,

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1992; GOTO, KANAYA e NISHIKAWA, 1998). Therefore, in these situations, the performance of its chemical components occurs systemically. In the present work, a mouthrinse based on extracts of the green tea for topical use was investigated. (MUKHTAR e AHMAD, 2000) showed that topical application of green tea polyphenols prior to radiation therapy resulted in protection against tumor promotion, reducing the number and size of the tumors in rats when compared to the control group. With respect to the use of tea for periodontal disease control, (HIRASAWA *et al.*, 2002) found significant reductions in markers of gingivitis after using local application of slow release for a period of eight weeks. Although no gingival index reduction was observed in the present study, (AWADALLA *et al.*, 2011) examined 25 patients that used a 2% green tea mouthrinse for five minutes, resulting in a statistically significant reduction the gingival index. Kushiya *et al.*, (2009) evaluated the gingival index, using the systemic route of application of green tea. The patients ingested various doses of tea during the day providing a decrease in the rate analysis, in a dose dependent response.

No significant change in the parameters that assess the presence of gingival inflammation was observed in this study, that may be explained by the limited period of use and the lack of basic periodontal therapy. The period of mouthwash used was determined considering that besides the natural source of the green tea, it should be indicated for specific conditions, and not for continuous use. Some substances can be harmful to the oral mucosa cells being dose and time-dependent (POGGI *et al.*, 2003).

Moreover, other studies have also chose the period of seven days to conduct the evaluation of plaque formation in an early stage (HERNANDEZ-COTT *et al.*, 2009; LOTUFO *et al.*, 2009). Zambon *et al.*, 1989 evaluated the use of mouthrinses with antimicrobial solutions for 7, 14 and 28 days, and noted that the highest levels of plaque reduction (28.9%) occurred after 7 days of use.

Otake *et al.*, (1991) showed that green tea extract in a concentration of 100mg / L causes a substantial inhibition in the initial adhesion of *S. mutans* to the tooth surface, essential step for the development of a mature plaque with periodontal pathology features. In addition to interfering with the initial adhesion of *S. mutans* to tooth surface, Blanco *et al.*, (2005) observed that epigalato-catechin, found in green tea extract interferes with the polysaccharides that form the glycocalyx, operates breaking the interactions between bacteria or between the cell wall and extracellular matrix that accumulates. Also epigalato-catechins bind to peptidoglycan, breaking the integrity of bacterial cell wall, interfering with the initial phase of biofilm

formation, which requires hydrophobic interactions between the cell wall of bacteria and the surface to be colonized. The influence on the mechanisms of plaque formation may be responsible for the decline, around 20%, statistically significant, the plaque index observed in this work. Importantly, this reduction was observed without any change in the way the patient's hygiene was performed.

Taste changes, allergic processes and staining were not observed with the use of this extract, although they are often reported with other mouthrinses (MYSTIKOS *et al.*, 2011). The characteristics presented by the mouthrinse proposed in this paper showed satisfactory results, since it decrease the amount of plaque formation, which can lead, in a long-term period of analysis, to a reduction in the signs of gingival inflammation.

New products with active plant-based extracts should be investigated for the control of dental plaque, responsible for the onset and progression of various oral diseases, especially periodontal disease. This focus should be based not only on its curative but also preventive action, since a formulation that does not cause undesirable effects to the other structures of the oral cavity is possible. Another advantage when indicating phytotherapeutic products would be its reduction in cost, making it accessible to a larger number of people.

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