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JORGIANA SILVA DE ASSIS

ANÁLISE ANTIMICROBIANA DO FLAVONÓIDE EPIGALOCATEQUINA-3-GALATO COMO AGENTE DE LIMPEZA CAVITÁRIA EM DENTINA ARTIFICIALMENTE CARIADA.

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Aprovada em: __/__/__

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“...mas você partiu sem mim. Eu sei que estás em algum jardim entre as flores....”

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Muhammad Ali

RESUMO

O objetivo do presente estudo foi avaliar a eficácia do flavonóide epigalocatequina-3-galato (EGCG) nas concentrações de 0,5%, 1% e 2% como solução antimicrobiana em dentina artificialmente cariada, tendo a clorexidina 2% e a solução salina 0,9% como controles. Vinte e cinco blocos de dentina humana (4 mm x 4 mm) foram imersos por cinco dias em BHI-caldo inoculado com *Streptococcus mutans* UA159 no primeiro dia do experimento. No quinto dia do experimento, os blocos foram aleatoriamente distribuídos em cinco grupos: grupo I – controle negativo – solução salina 0,9%; grupo II – controle positivo – clorexidina 2%; grupo III – EGCG 0,5%; grupo IV – EGCG 1%; grupo V - EGCG 2%. Cada bloco recebeu tratamento de 15 µl da solução testada, que permaneceu em contato com o bloco por 60 segundos. Após os tratamentos, amostras dentinárias foram removidas com emprego de lâmina de bisturi e foram analisadas a partir da contagem de UFCs (unidades formadoras de colônias). Os experimentos foram realizados em triplicata e os dados, obtidos em UFCs foram convertidos em log base-10. Os testes estatísticos empregados foram análise de variância (ANOVA), seguida de Teste de Tukey. Não houve diferença estatística entre as concentrações de EGCG empregadas e a solução salina ($p > 0,05$). Além disso, não houve diferença estatística entre as concentrações de EGCG ($p > 0,05$). No entanto, houve diferença estatisticamente significativa entre a clorexidina e os demais grupos ($p < 0,05$). Conclui-se, a partir dos dados encontrados, que a substância investigada não deve ser empregada em preparos cavitários com a finalidade de eliminação de patógenos, visto que não se mostrou eficaz como antimicrobiano nas concentrações testadas.

Palavras-chave: Catequina, Dentina, *Streptococcus mutans*.

ABSTRACT

The aim of this study was to evaluate the efficacy of the flavonoid epigallocatechin-3-gallate (EGCG) in concentrations of 0.5%, 1% and 2% as an antimicrobial solution in artificially carious dentin, with 2% chlorhexidine and 0.9% saline solution as controls. Twenty-five slabs of human dentin (4 mm x 4 mm) were immersed for five days in Brain Heart Infusion broth (BHI-broth) inoculated at first day with *Streptococcus mutans* UA159 (Batch Culture Model). On the fifth day of the experiment, the blocks were randomly divided into five groups: group I - negative control - 0.9% saline solution, group II - positive control - 2% chlorhexidine, group III - 0.5% EGCG, group IV - 1% EGCG, group V - 2% EGCG. Each slab was subjected to 15 µl of the tested solution for 60 seconds. After treatments, artificially carious dentin was removed from the dentin slabs and analyzed by counting colony forming units (CFUs). All experiments were performed in triplicate and the data obtained in CFUs mean values converted to log base-10. The statistical tests used were analysis of variance (ANOVA) followed by Tukey test. There was no statistical difference between EGCG concentrations and saline ($p > 0.05$). Furthermore, there is no statistical difference between EGCG concentrations ($p > 0.05$). However, there was statistically significant difference between chlorhexidine and the other groups ($p < 0.05$). From the data obtained in this study, is possible to conclude that the substance tested is not effective on elimination of pathogen *S. mutans* when used in a concentration of 0.5%, 1% and 2% in artificially carious dentin.

Key Words: Catechin, Dentin, *Streptococcus mutans.*

SUMÁRIO

1 INTRODUÇÃO GERAL

O conceito de mínima intervenção ou odontologia minimamente invasiva (TYAS *et al*., 2000), amplamente aceito no mundo, preconiza que as zonas dentinárias mais profundas afetadas por cárie não necessitam ser completamente removidas, podendo, portanto, serem preservadas. As camadas mais superficiais, no entanto, apresentam-se irreversivelmente comprometidas, devendo, dessa maneira, ser removidas por completo (ZAVGORODNIY; ROHANIZADEH; SWAIN, 2008). Restaurações feitas com materiais adesivos dispensam preparos cavitários extensos, requerendo apenas a remoção da dentina infectada por cárie (TEN CATE, 2008).

Diversas propostas de pré-tratamento cavitário surgiram a partir do desenvolvimento de materiais resinosos e de técnicas que promovem adesão às estruturas dentárias. Substâncias com propriedades antimicrobianas têm sido empregadas como agentes de limpeza cavitária, previamente ao procedimento adesivo, a fim de evitar a colonização bacteriana na interface dente-restauração e o crescimento de bactérias remanescentes na cavidade preparada (COSTA; EDWARDS; HANKS, 2001).

Além de atuar contra microorganismos, uma solução de limpeza cavitária não deve interferir no mecanismo de adesão durante o procedimento restaurador (MEIERS; KRESIN, 1996) e deve apresentar pouco ou nenhum efeito tóxico às células pulpares, especialmente aos odontoblastos (SOUZA *et al*., 2006).

A clorexidina é considerada uma substância antimicrobiana padrão-ouro no controle de biofilmes, por conta da sua capacidade de reduzir significativamente os níveis de microorganismos orais. No entanto, estudos mostram que ela apresenta efeitos citotóxicos a vários tipos de células (HIDALGO; DOMINGUEZ, 2001; SOUZA *et al.*, 2007). É relevante, portanto, que se busque alternativas que agreguem os benefícios associados ao uso da clorexidina, mas que atendam aos requisitos de biocompatibilidade com as células pulpares.

Há um interesse crescente por componentes ativos derivados de produtos naturais, que possuam potencial terapêutico em medicina e odontologia (NEWMAN, 2008). *Camellia sinensis* é o nome científico da planta a partir da qual se origina o

chá verde, no qual são encontrados polifenóis, substâncias com uma série de ações benéficas à saúde, dentre as quais atividade anti-inflamatória, antimutagênica, contra diabetes, na prevenção do câncer e antimicrobiana (WU *et al*., 2009). Os polifenóis mais abundantes são os flavonóides tais como catequinas, catequinas-galato e proantrocianidinas (TAYLOR; HAMILTON-MILLER; STAPLENTON, 2005).

Os flavonoides mostram-se eficazes no combate a microorganismos orais, mais especificamente contra *S. mutans* (XU; ZHOU; WU, 2012; HIRASAWA; TAKADA; OTAKE, 2006), o qual é indicado como o principal agente etiológico da doença cárie; embora outros tipos de bactérias (notadamente *Lactobacillos* e *Actinomyces*) possam estar envolvidos (LOESCHE, 1986).

O flavonóide epigalocatequina-3-galato, presente em um percentual aproximado de 50% na constituição do chá verde (DALE; DANEEL; YU-DONG Z, 2006), interfere nos fatores de virulência associados à acidogenicidade e tem ação bactericida contra cultura planctônica e formação de biofilme (XU; ZHOU; WU, 2012).

Considerando que até o presente momento não há estudos sobre a ação antimicrobiana da epigalocatequina-3-galato como solução de limpeza cavitária, este trabalho tem por objetivo avaliar a eficácia desta substância nas concentrações de 0,5%, 1% e 2% como solução antimicrobiana em dentina humana cariada artificialmente.

2 PROPOSIÇÃO

O objetivo do presente estudo foi avaliar a ação antimicrobiana do flavonóide epigalocatequina-3-galato nas concentrações de 0,5%, 1% e 2%, quando empregado como solução de limpeza cavitária em dentina humana cariada artificialmente.

3 CAPÍTULOS

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-graduação da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato (EM ANEXO). Por se tratar de pesquisa envolvendo seres humanos, ou partes deles, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de ética em pesquisa da Faculdade de Medicina da Universidade Federal do Ceará, tendo sido aprovado sob o parecer n° 329632 (EM ANEXO). Assim sendo, esta dissertação de mestrado é composta de um capítulo contendo um artigo científico que deverá ser submetido para publicação no periódico “Archives of Oral Biology”, após ter sido previamente analisado e corrigido por corretor da língua inglesa, conforme descrito abaixo:

- ✓ Capitulo 1

  ***In vitro* antimicrobial effect of a catechin as a cleaning agent in artificially carious dentin.**

  Running title: Catechin as a cavity cleanser in dentin.

  Assis JS, Rodrigues LKA, Lima JPM, Araújo RL, Santiago SL.

In vitro antimicrobial effect of a catechin as a cleaning agent in artificially carious dentin.

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ABSTRACT

Objectives: The aim of this study was to evaluate the efficacy of the flavonoid epigallocatechin-3-gallate (EGCG) in concentrations of 0.5%, 1% and 2% as an antimicrobial solution in artificially carious dentin.

Design: Twenty-five blocks of human dentin (4 mm x 4 mm) were immersed for five days in Brain Heart Infusion broth (BHI-broth) inoculated at first day with *Streptococcus mutans* UA159 (Batch Culture Model). On the fifth day of the experiment, the blocks were randomly divided into five groups: group I - negative control - 0.9% saline solution, group II - positive control - 2% chlorhexidine, group III - 0.5% EGCG, group IV - 1% EGCG, group V - 2% EGCG. Each slab was subjected to 15 µl of the tested solution for 60 seconds. After treatments, carious dentin was removed from the dentin slab and analyzed by counting colony forming units (CFU's). All experiments were performed in triplicate. Data were analyzed by ANOVA followed by a Tukey's test.

Results: There was no statistical difference between EGCG concentrations and saline solution ($p > 0.05$). Furthermore, there is no statistical difference between EGCG concentrations ($p > 0.05$). However, there was statistically significant difference between chlorhexidine and the other groups ($p < 0.05$).

Conclusion: From the data obtained in this study, is possible to conclude that the substance tested is not effective on elimination of pathogen *S. mutans* when used in a concentration of 0.5%, 1% and 2% in artificially carious dentin.

Key Words: Catechin, Dentin, *Streptococcus mutans.*

INTRODUCTION

Caries develops at different depths in dentin structure. The more superficial layers (infected layer) typically are quite changed, which makes them not likely to be remineralized. However, in the deeper layers (affected layer) the cross-banded ultra structure of the collagen matrix is preserved, although there has been demineralization. Thus, the elimination of pathogens present in the affected dentin ensure the preservation of this substrate, especially in the areas where the collagen is not changed by the carious process [4]. Bactericide substances can be a viable option for dentin disinfection. Therefore, the use of antimicrobial solutions for cleaning cavities has been recommended [5].

Chlorhexidine is considered a gold standard agent to control biofilms due to its ability to significantly decrease the levels of oral microorganisms. However, an ideal cavity cleanser should also present a low toxicity or preferably no toxic effects to the pulp cells, especially to odontoblasts [6]. Lessa et al. demonstrated that chlorhexidine presents cytotoxic effects on a variety of cell lines [7], similar to different chemical agents indicated for use as cavity cleansers [8]. It is important, therefore, to look forward natural alternatives [9] that add the satisfactory performance in the elimination of pathogens and that meet the requirements of biocompatibility with pulp cells.

Leaves from *Camellia sinensis* contain polyphenolic components with activity against a wide spectrum of microbes. The most abundant components in green tea are polyphenols, in particular flavonoids such as the catechins, catechin gallates and proanthocyanidins [10]. This polyphenolic components exhibit antimutagenic, anti-inflammatory, antidiabetic, cancer-preventive and antimicrobial properties [11,12,13,14,15].

Over 50% of green tea is composed by Epigallocatechin-3-gallate, and many researchers suggests that it is the main flavonoid responsible for most of the potential health benefits attributed to green tea intake [10,16,17]. The effects of green tea polyphenols, more specifically against *S. mutans*, have been previously shown [18,19,20]. Epigallocatechin-3-gallate is indicated as the major polyphenol component in tea that interferes on virulence factors associated with microorganisms acidogenity and acidurity [20].

Until this date, no previous studies have tested the antimicrobial action of epigallocatechin-3-gallate as a cavity cleanser, although some researchers have demonstrated the bactericidal effects of this agent in planktonic [19] and biofilm oral culture [18,19]. Thus, the purpose of this work was to evaluate the efficacy of this catechin at concentrations of 0.5%, 1% and 2% as an antimicrobial solution in artificially carious dentin. The null hypothesis to be tested was that the epigallocatechin-3-gallate is not effective in reducing *Streptococcus mutans* in artificially carious dentin.

MATERIALS AND METHODS

Experimental Design

This study was randomized, comprising five groups: group I - negative control - 0.9% saline solution, group II - positive control - 2% chlorhexidine, group III - 0.5% EGCG, group IV - 1% EGCG, group V - 2% EGCG. The teeth were randomily allocated to the 5 test groups, with 5 experimental units per group, according to a computer generated randomization list. The experimental design was performed in triplicate in order to reduce the inherent bias related to microbiological procedures.

Specimen Preparation

Fifty caries-free third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the local Research and Ethics Committee (Protocol # 329.632). The teeth were stored in a 0.01 % (v/v) thymol solution for a month or less and remained refrigerated until use.

Teeth were fixed in acrylic devices (40 x 40 x 5 mm) and cut using a water-cooled diamond saw mounted in a cutting machine (IsoMet Low Speed Saw, Buehler, Ilinois USA) in order to obtain dentin slabs (4 mm x 4 mm). The plane occlusal surfaces obtained were assessed by examination under a microscope, at 40X magnification, to ensure complete removal of enamel. Only the occlusal dentin surface was used and the other surfaces were protected with resistant acid varnish (Risqué, São Paulo, SP, Brazil), resulting in an area of 16 mm^2 that served as a microbial surface on which caries lesions were produced. Afterwards, the slabs were

mounted on metal appliances, autoclaved (121°C, 15 min) [21] and stored in 100% humidity.

Production of caries lesions in dentin

After sterilization, the dental slabs were removed from the distilled water and immersed in sterile brain-heart infusion broth (BHI CM0225 Oxoid LTD, SP, Brazil) containing 5% (w/v) sucrose. All BHI-containing 24-well polystyrene plates, except those that served as contamination controls, were inoculated with 0.1 ml [2 x 10^8 colony-forming unit (CFU) ml^{-1}] of an overnight culture of *S. mutans* UA159. After 18 hours, Gram test was used to verify the existence of *S. mutans* only. A specific optical density was determined using a spectrophotometer (Ultrospec 1100 *pro*, Amershanm Biosciences) and used for all samples to adjust the inoculum to the same cell number. Inoculation of each BHI-containing 24-well polystyrene plates was performed only on the first day. During five consecutive days, the dentin specimens were transferred mounted on metal appliances into fresh medium every 24 h. At each transfer time, samples of the cultures were streaked onto BHI agar plates and incubated at 37°C in an atmosphere of 5% CO_2 in order to check purity [22].

Microbiological analysis

A 0.9% saline solution (NaCl) was prepared at a ratio of 0.9 g/100ml and previously autoclaved (121°C, 15 min). Chlorhexidine digluconate at 20% (v/v) was diluted to give 2% chlorhexidine digluconate (v/v) and, because it is an organic substance was sterilized in filter. Epigallocatechin-3-gallate from green tea - EGCG, 95% (wt/wt) (Sigma-Aldrich Corp. St. Louis, MO) was dissolved into distillated water at concentration of 20 mg/ml. Then the EGCG/distillated water was diluted at concentrations of 10 mg/ml and 5 mg/ml, obtaining in this way the three different concentrations used in this study.

After a five-day experimental period, the biofilm formed over the slabs was removed and the caries dentin was exposed. The slabs were randomly allocated to five different treatment groups with five specimens per group (Table 1).

All dentin specimens were treated for 60 seconds with 15 µl of each solution and dried with an absorbent paper. Afterwards, carious dentin was collected from slabs using a #15 scalpel blade [23]. The dentin samples were weighed in pre-

weighed microcentrifuge tubes and 0.9% saline solution (NaCl) was added in the rate of 0.1ml of solution per 1mg of dentin. The tubes were agitated during 1 min in a Disrupter Genie Cell Disruptor (Scientific Industries, Ciencor) with three 0.1 mm diameter glass beads to detach the bacterial cells. Subsequently the suspension was serially diluted (1:10 to 1:100,000) with 0.9% saline solution (NaCl). Samples were plated in triplicate on BHI agar, and incubated for 48 h at 37°C in a 5% CO_2 atmosphere [24,25]. Representative colonies with typical morphology of *S. mutans* were counted after 48 hours using a colony counter and the results were expressed in CFU/mg of dentin.

Statistical Analysis

All experiments were performed in triplicate and the data obtained in CFUs mean values were converted to log base-10, because they did not meet the necessary assumptions initially. The differences between the experimental groups and control groups were analyzed by BioEstat 5.0. One-way analysis of variance (ANOVA) was performed and followed by a post hoc Tukey test to compare the antimicrobial efficacy between groups. The level of significance was set at $p < 0.05$.

RESULTS

No significant difference between mean values were found for EGCG treatment in different concentrations when compared to saline group ($p > 0.05$), and there was no statistical difference between EGCG concentrations ($p > 0.05$). Only CHX group presented statistically different results from the others, as seen in Figure 1 ($p < 0.05$).

DISCUSSION

There is a growing interest in active components derived from natural products, which have therapeutic potential in medicine and dentistry [26]. In addition to the benefits already reported and well described in the literature, flavonoids have been reported in studies from different areas in dentistry [15,27,28,29,30].

Many researchers have investigated the effect of catechins on the growth of *S. mutans*. According to Xu et al. [20], discrepant results of antimicrobial activity of Epigalocatechin-3-gallate can be found, depending on the type of bacterial organization. In planktonic cells, MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values in a BHI medium were 625 µg/ml and >1,250 µg/ml, respectively. Moreover, epigallocatechin-3-gallate inhibited the formation of *S. mutans* biofilm in BHI medium with a $MBIC_{50}$ (the lowest flavonoid concentration that showed at least 50% inhibition of the formation of biofilms compared with control) of 312.5 µg/ml. In the data presented by Mankovskaia et al. [30], the MIC values for epigallocatechin-3-gallate against *S. Mutans* were within reference ranges of 31.25 µg/ml to 625 µg/ml, depending on the bacterial strain and culture medium. Du et al. [31] concluded that antibacterial activity of the dental adhesives was increased after 200 µg/ml or higher concentration EGCG incorporated. Hirasawa et al. [18] demonstrated that more than 2000 µg/ml of epigallocatechin-3-gallate is require to a mouth-rinsing be effective against *S. mutans* presents in dental plaque. These researches suggest that epigallocatechin-3-gallate may represent a natural agent against dental biofilm.

The concentrations in which catechins are effective as an antimicrobial agent are wide and one of the causes is the application of different methods of determining bacterial susceptibility. Usually, levels of epigallocatechin-3-gallate to inhibit bacterial culture in planktonic levels are lower than in oral biofilm. This is associated with the fact that in planktonic bacteria cultures find themselves free in suspension, whereas in the biofilms there is an ecosystem structured and dynamic, which acts coordinately. These types of microorganism organization present distinct properties [42,43]. Biofilms are composed of a variety of species mechanisms that establish communication between them, including transfer of genetic material, as virulence traits or bacterial resistance faster than that occurring in culture planktonic [43]. Such characters favors these structures develop resistance to many therapeutic agents [32].

Epigallocatechin-3-gallate at concentrations of 0.5%, 1% and 2% was not effective as a cleaning solution on carious dentin in eliminating the pathogen. The ineffectiveness in reducing or eliminating *S. mutans* can be attributed to interactions formed between the tea catechins and the collagen fibrils. Proteins present on the

surface dentin that remained of the nutrient-rich BHI medium may bind or even precipitate catechins and the binding may jeopardize their antimicrobial activity [18,30]. Therefore, it can be suggested that there were interactions between catechin and specific sites in the molecular structure of collagen. More studies are needed to confirm this hypothesis.

Dentin submitted to cariogenic challenge seems to be more resistant to antimicrobial therapy. In the development of the dental biofilms, salivary proteins and glycoproteins on oral surfaces and other organisms play a decisive role in bacterial adhesion [44]. Dentin is present as a tubular, porous mineralized tissue composed primarily of hydroxyapatite and collagen fibrils [45]. Collagen type I, the main organic component of dentin, is recognized by streptococci that bind to collagen and may facilitate the bacterial adhesion and tissue penetration [46]. Therefore, EGCG concentrations used in this study are shown greater than those used in studies of culture planktonic and biofilm.

This study aimed to evaluate the antimicrobial activity of the flavonoid directly applied in carious dentin, which has not yet investigated in the literature. In order to perform the cariogenic challenge, we employed the microbiological method, in which the dentinal slabs remained immersed for five days in the nutrient medium inoculated with *S. mutans*. The production of *in vitro* caries-like lesions may be achieved in different ways, but the microbiological method presents a pattern of collagen degradation morphologically more similar to natural lesions, with an evident infected layer in the dentin caries lesion simulated by that method [33].

The use of chlorhexidine has been employed on caries prevention [34]. This substance has been studied for over twenty years and is considered the most potent therapeutic agent against oral biofilms. This is why the majority of studies investigating the efficacy of new antimicrobial drugs employs chlorhexidine as a positive control [35].

Besides antimicrobial activity, catechins, especially epigallocatechin-3-gallate, have been identified as specific inhibitors of matrix metalloproteinases (MMPs) [36,37]. These enzymes are a class of zinc-and-calcium-dependent endopeptidases present in dentin matrix [38]. They are responsible for degrade the incompletely resin-infiltrated collagen fibrils [39,40]. Santiago et al. [41] found that the

catechin remained in contact with dentin for 60 s may preserve resin-dentin bond-strength over time. Therefore, we choose a time of application of 60 s, which it cannot affect the longevity of restorative procedures.

The absence, until this date, of specific answers about the mechanism of EGCG anti-cariogenic points to the need for further research in this direction. There are numerous studies on the anti-cariogenic potential of the flavonoid. However, there is a great variability in the conditions and methods employed, which makes it difficult to attribute this potential to a specific factor or a set of elements.

In conclusion, the data obtained indicate that EGCG should not be used as a cleaning agent cavity in concentrations proposed by this study because it was not efficient in reducing *S. mutans* levels.

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TABLES

TABLE 1: Groups and treatments performed.

GROUP	TREATMENT
G I (n= 5)	**0.9% Saline solution**
G II (n= 5)	**2% Chlorhexidine**
G III (n= 5)	**0.5% EGCG**
G IV (n= 5)	**1% EGCG**
G V (n= 5)	**2% EGCG**

FIGURES

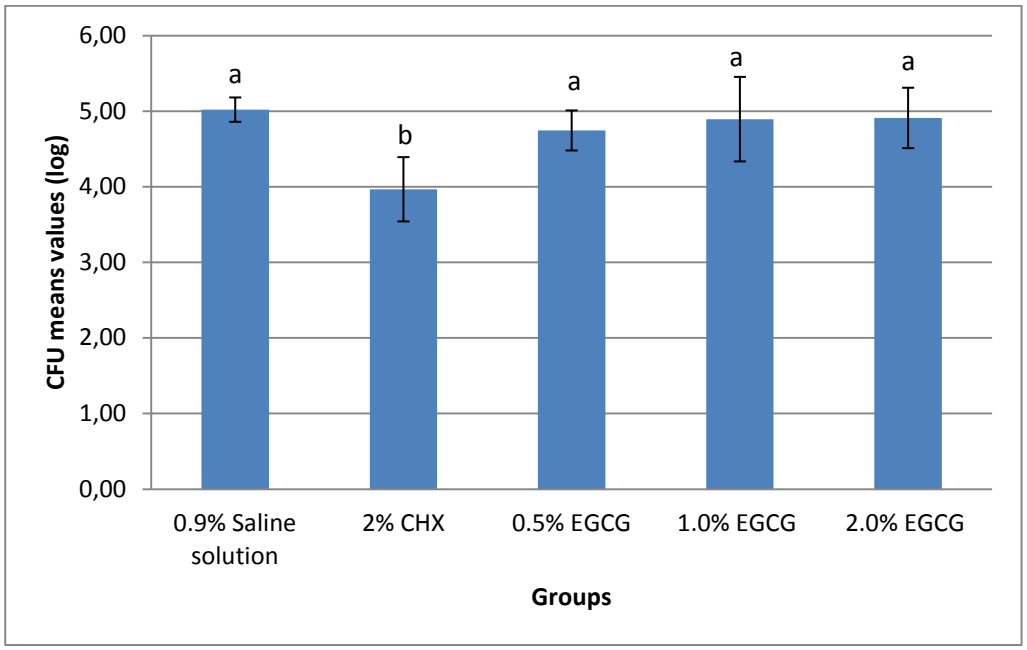

FIGURE 1 – CFU Mean values and standard deviations obtained from the average of triplicates for each group

4 CONCLUSÃO GERAL

Conclui-se, a partir dos resultados obtidos, que a epigalocatequina-3-galato não apresenta-se eficaz na redução ou eliminação de *S. mutans* em dentina cariada, nas concentrações de 0,5%, 1% e 2%. Esta substância não deve, portanto, ser empregada como solução de limpeza cavitária nas concentrações acima descritas.

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ANEXO A - TERMO DE DOAÇÃO DE DENTES

Pelo presente instrumento que atende às exigências legais, o Sr. (a) _____, após ter tomado conhecimento do protocolo de pesquisa **"ANÁLISE ANTIMICROBIANA DO FLAVONÓIDE EPIGALOCATEQUINA-3-GALATO COMO AGENTE DE LIMPEZA CAVITÁRIA EM DENTINA ARTIFICIALMENTE CARIADA"**, que tem como objetivo avaliar o efeito do flavonóide epigalocatequina-3-galato como agente de limpeza cavitária em dentina cariada, vem na melhor forma de direito DOAR à CD JORGIANA SILVA DE ASSIS __ dentes, declarando, sob as penas da lei, que os dentes objeto da presente doação foram extraídos por indicação terapêutica, cujos históricos circunstanciados fazem parte dos prontuários dos pacientes de quem se originam.

Data:___/___/___

Assinatura:_____

RG: _____

ANEXO B - ARTIGO 46 DO REGIMENTO INTERNO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA (PPGO) DA UNIVERSIDADE FEDERAL DO CEARÁ

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ANEXO C - NORMAS PARA PUBLICAÇÃO EM PERIÓDICO

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As titles frequently stand alone in indexes, bibliographic journals etc., and indexing of papers is, to an increasing extent, becoming computerized from key words in the titles, it is important that titles should be as concise and informative as possible. Thus the animal species to which the observations refer should always be given and it is desirable to indicate the type of method on which the observations are based, e.g. chemical, bacteriological, electron-microscopic, histochemical, etc. A "running title" of not more than 40 letters and spaces must also be supplied. A keyword index must be supplied for each paper.

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This should be a succinct statement of the problem investigated within the context of a brief review of the relevant literature. Literature directly relevant to any inferences or argument presented in the Discussion should in general be reserved for that section. The introduction may conclude with the reason for doing the work but should not state what was done nor the findings.

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• Stating that P=0.000 (a figure which is generated by some computer packages). The correct statement (in this case) is P<0.0005.

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In general, *Archives of Oral Biology* will use the recommended SI (Systeme Internationale) units and symbols. The use of the litre, usually better written in full, in place of SI dm^3 and ml^3 in place of SI cm, will continue to be accepted. For details of the SI symbols, authors are referred to: Symbols, Signs and Abbreviations (1969) by the Royal Society of Metric and Decimal Systems in Council of Biology

Abbreviations
As *Archives of Oral Biology* is a journal with a multidisciplinary readership, abbreviations, except those universally understood such as mm, g, min. u.v., w/v and those listed below, should be avoided if possible. Examples of abbreviations which may be used without definition: ADP, AMP, ATP, DEAE-cellulose, DNA, RNA, EDTA, EMG, tris.
Other abbreviations used to improve legibility should be listed as a footnote on the title page. Chemical symbols may be used for elements, groups and simple compounds, but excessive use should be avoided. Abbreviations other than the above should not be used in titles.

Bacterial nomenclature
Organisms should be referred to by their scientific names according to the binomial system. When first mentioned the name should be spelt in full and in italics. Afterwards the genus should be abbreviated to its initial letter, e.g. '*S. aureus*' not '*Staph. aureus*'. If abbreviation is likely to cause confusion or render the intended meaning unclear, the names of microbes should be spelt in full. Only those names which were included in the Approved List of Bacterial Names, *Int J Syst Bacteriol* 1980; 30: 225?420 and those which have been validly published in the *Int J Syst Bacteriol* since 1 January 1980 have standing in nomenclature. If there is good reason to use a name that does not have standing in nomenclature, the names should be enclosed in quotation marks and an appropriate statement concerning the nomenclatural status of the name should be made in the text (for an example see *Int J Syst Bacteriol* 1980; 30: 547?556). When the genus alone is used as a noun or adjective, use lower case Roman not italic, e.g.'organisms were staphylococci' and 'streptococcal infection'. If the genus is specifically referred to use italics e.g. 'organisms of the genus *Staphylococcus*'. For genus in plural, use lower case roman e.g. 'salmonellae'; plurals may be anglicized e.g.'salmonellas'. For trivial names, use lower case Roman e.g. 'meningococcus'.

Numbers, measurements and statistics.
Numbers one to nine are spelled out unless they are measurements (e.g.5 ml). Numbers greater than nine are spelled out if they begin a sentence, or when clarity requires it. Numbers above and including 10 000 have a space, not a comma. A decimal point is preceded by a number or cypher e.g. '0.5'. Decimal points in columns should be aligned vertically. Dates are usually provided in full: 14 April 1949. Measurements may be expressed in SI or non-metric units. Use 10 ml/h rather than $ml.h^{-1}$ or ml per h.

Drugs
These should be referred to by their approved and not proprietary names; for guidance, see the British National Formulary. Where it is desirable to indicate a particular brand of preparation, the proprietary name and source should be given in parentheses after the proper name, e.g. testicular hyaluronidase (Testovase, Bovine Enterprises Ltd, London, UK).

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ANEXO D - PARECER CONSUBSTANCIADO DO COMITÊ DE ÉTICA EM PESQUISA (CEP)

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DO EPIGALOCATEQUINA-3-GALATO NA DESINFECÇÃO E RESISTÊNCIA DE UNIÃO DE DENTINA CARIADA A UM SISTEMA ADESIVO

Pesquisador: Jorgiana Silva de Assis

Área Temática:

Versão: 2

CAAE: 08450913.4.0000.5054

Instituição Proponente: Departamento de Odontologia Restauradora

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 329.632

Data da Relatoria: 04/04/2013

Apresentação do Projeto:

Comumente, os pesquisadores têm investigado a qualidade e a durabilidade dos sistemas adesivos em relação ao substrato dentinário não cariado. No entanto, na prática clínica, por vezes a adesão se desenvolve em dentina cariada. Tendo em vista a possibilidade de na técnica úmida ¿ associada aos adesivos de condicionamento total ¿ haver a possibilidade da incompleta penetração desse componente na dentina desmineralizada, os sistemas autocondicionantes surgiram como uma tentativa de eliminar a discrepância entre a profundidade da dentina desmineralizada e a penetração do adesivo. A durabilidade adesiva é essencial para a longevidade das restaurações, visto que o processo de degradação pode enfraquecer a adesão e conduzir a falhas entre a estrutura dentária e o material restaurador. Neste trabalho, estruturado em duas fases (modelo microbiológico e resistência de união), será avaliado o efeito antimicrobiano da epigalocatequina-3-galato(EGCG) nas concentrações de 0,1%. 0,01% e 0,001% em dentina cariada; em seguida, avaliar-se-á o efeito da EGCG na resistência de união de dentina cariada a um sistema adesivo autocondicionante. Serão utilizados 120 terceiros molares humanos hígidos, e extraídos por razões que não envolvem essa pesquisa. Na primeira fase da pesquisa Os dentes serão fixados com cera pegajosa (Kota Ind. E Com. Ltda, São Paulo,

Endereço: Rua Cel. Nunes de Melo, 1127
Bairro: Rodolfo Teófilo **CEP:** 60.430-270
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