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ORIGINAL ARTICLE

EFFECT OF ADDING FLUORIDE TO *CAMELLIA SINENSIS* AGAINST DENTIN EROSION GENERATED BY NON-BACTERIAL ACIDS

Efecto de la adición de flúor a la *Camellia sinensis* frente a la erosión de dentina generada por ácidos no bacterianos

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ABSTRACT

Introduction: Natural products are an option to be used in different conditions in the oral cavity, such as *Camellia sinensis,* which due to its different properties would be beneficial in the erosion of the dental surface.

Objetive: Determinar el efecto del extracto de *Camellia sinensis* (*C. sinensis*) adicionado con flúor frente a la acción erosiva de ácidos no bacterianos en la superficie de la dentina humana

Materials and Methods: Quasi-experimental, prospective, longitudinal, and *in vitro* study, consisting of 50 samples of human dentin, which were classified into 5 study groups: distilled water, hydrochloric acid, 2% *C. sinensis* extract, sodium fluoride. 2% and 2% *C. sinensis* extract added with 2% sodium fluoride. The surface roughness of each sample was evaluated with the SRT6200 digital roughness meter, obtaining a total average roughness and the measurements were carried out in two moments.

Results: It was observed that the 2% *C. sinensis* extract, the 2% sodium fluoride, and the *C. sinensis* extract added with 2% sodium fluoride, showed a variation in surface roughness between before and after, which was not significant ($p<0.05$) in all cases. It should be noted that the *C. sinensis* plus sodium fluoride group is the one that obtained the best variation in the mean surface roughness than the other groups studied.

Conclusions: The 2% *C. sinensis* extract, 2% sodium fluoride, and the combination of both compounds demonstrated an inhibitory effect against the erosive action of hydrochloric acid (0.01 M) on the dentin surface, not presenting a statistically significant difference in the results.

Keywords: *Camellia sinensis; Tooth erosion; Fluorides; Dentina; Surface properties; Prospective studies.*

RESUMEN

Introducción: Los productos naturales son una opción para ser usados en diferentes afecciones en la cavidad bucal, como lo podría ser la *Camellia sinensis*, que por sus diferentes propiedades sería de beneficio en la erosión de la superficie dental.

Objetivo: Determinar el efecto del extracto de *Camellia sinensis (C. sinensis)* adicionado con flúor frente a la acción erosiva de ácidos no bacterianos en la superficie de la dentina humana.

Materiales y Métodos: Estudio cuasi experimental, prospectivo, longitudinal e *in vitro*, constituido por 50 muestras de dentina de dientes permanentes, que se clasificaron en 5 grupos de estudio: Agua destilada, ácido clorhídrico, extracto de *C. sinensis* a 2%, fluoruro de sodio al 2% y extracto de *C. sinensis* a 2% adicionado con fluoruro de sodio al 2%. Se evaluó la rugosidad superficial de cada muestra con el rugosímetro digital SRT6200, obteniéndose una rugosidad media total y se realizaron las mediciones en dos momentos.

Resultado: Se observó que el extracto de *C. sinensis* al 2%, el fluoruro de sodio al 2% y el extracto de *C. sinensis* adicionado con fluoruro de sodio al 2%, presentaron una variación de la rugosidad superficial entre el antes y el después, no siendo esta significativa ($p<0.05$) en todos los casos. Cabe resaltar que el grupo *C. sinensis* más fluoruro de sodio, es el que obtuvo una mejor variación de la media de rugosidad superficial, que los otros grupos estudiados.

Conclusión: El extracto de *C. sinensis* al 2%, el fluoruro de sodio al 2% y la combinación de ambos compuestos demostraron un efecto inhibitorio frente a la acción erosiva del ácido clorhídrico (0,01 M) en la superficie de dentina, no presentando una diferencia estadísticamente significativa en los resultados.

Palabras Clave: *Camellia sinensis; Erosión de los dientes, Fluoruros; Dentina; Propiedades de superficie; Estudios prospectivos.*

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INTRODUCTION

Teeth consist of three primary hard tissues —enamel, dentin, and cementum—and one soft tissue known as pulp. Enamel, primarily composed of 94% calcium phosphate (hydroxyapatite) and 4% organic substances, forms the outer layer.

Dentin, compromising most of the tooth, approximately 50% mineral material, 35% organic material (type I collagen), and 15% water.[1] The process of demineralization-remineralization is a dynamic and ongoing cycle influenced by dietary intake. Upon metabolization, foods produce acids that interact with the enamel surface, releasing calcium and phosphate ions. This interaction modifies the crystalline structure of hydroxyapatite, rendering it more vulnerable to remineralization. Disruptions and imbalance in the demineralization-remineralization cycle lead to the loss of the hard tissues of the teeth.[2,3]

In dental literature, various chronic destructive processes aside from dental caries are documented, resulting in the irreversible loss of the external surface of the tooth. These conditions are collectively referred to as non-carious lesions. Factors contributing to their formation include biocorrosion (erosion), friction (abrasion), and occlusal stress (abfraction).[4,5]

Dental erosion, or biocorrosion, is a painless chronic condition characterized by the gradual pathological loss of hard dental tissue, chemically etched by nonbacterial acids onto the tooth surface. These acids can originate from extrinsic sources (acidic drinks, foods, and mouthwashes), as well as intrinsic ones (gastric acids).[4,5]
Intrinsic sources of erosive acids, such as hydrochloric acid, are very frequently observed in patients suffering from recurrent vomiting, regurgitation, or gastroesophageal reflux.[6] Saliva (pH, viscosity, flow, composition, buffer capacity) plays a crucial role in mitigating dental erosion. Its ions facilitate the remineralization of demineralized dental tissue, thereby helping to prevent the onset of dental erosion.[3] The biocorrosion of dentin is a complex process that begins with the dissolution of its mineral component, exposing the organic matrix to degradation by bacteria and enzymes of the human body, such as matrix metalloproteinases (MMPs).
The enzymatic removal of the demineralized organic matrix by collagenases significantly enhances the demineralization process by restricting ionic diffusion in and out of the demineralized surface. Despite its resistance to mechanical removal by brushing, this layer is vulnerable to degradation by MMPs present in dentin and saliva, thereby accelerating the progression of dentin erosion.[6]

In the management and prevention of biocorrosion, sodium fluoride (NaF) exhibits a protective effect against biocorrosive attacks. Concentrations as low as 1.23% NaF, applied for only one minute, protect dentin from collagenase degradation *in vitro*. The use of stannous fluoride (SnF_2) and titanium tetrafluoride (TiF_4) in toothpastes prevents the loss of enamel and dentin due to biocorrosion and abrasion wear. This protective effect is enhanced when these compounds are combined with NaF.[3]
Studies on natural products like *Camellia sinensis (C. sinensis)*, green tea, have identified compounds such as epigallocatechin gallate, derivatives of theaflavin gallate, and various phenolic compounds. These findings suggest that these compounds could be beneficial in preventing dentin erosion caused by acidic substances.[6,7]

Camellia sinensis is a woody species capable of living over 100 years and reaching heights of approximately 15 meters in its natural habitat.[8,9] It thrives in acidic soils with good drainage and flourishes best in warm climates with ample

rainfall, typically between 150 to 300 cm (60 to 200 inches) annually.[10,11] In its natural environment, the green tea plant appears as a small tree with solitary yellow and white axial flowers that grow either individually or in clusters of up to three.
These flowers, measuring 2.5 to 3.5 cm in diameter, have between 6 and 8 petals, the outer ones are sepaloid and inner ones obovate in shape.[12-15] Plants from the Theaceae family, like *C. sinensis*, contain naturally low concentrations of fluoride in their leaves and infusions.[16]

Furthermore, compounds found in *C. sinensis* such as epigallocatechin gallate, theaflavin and theaflavin digallate exhibit inhibitory activity against enzymes that degrade collagen, as demonstrated in the study by Passos *et al*.[6] Their research suggests that *C. sinensis* can reduce the erosive effects of hydrochloric acid on dentin surfaces affected by erosion.[6,17]
Considering the qualities of *C. sinensis* and its MMP inhibitory activity, and the potential enhancement through increased fluoride content, when these two components are present in dentin, the resultant compound could significantly amplify its effectiveness against erosive action of non-bacterial acids. Therefore, this study aims to determine the effect of adding fluoride to *C. sinensis* against dentin erosion caused by non-bacterial acids.

MATERIALS AND METHODS

The present study employs a quasi-experimental, prospective, longitudinal design, conducted *in vitro*. Approval was obtained from the Research Unit of the School of Dentistry, Universidad Nacional Mayor de San Marcos, Lima, Perú. The research was conducted from October to December 2021.
The sample comprised eighteen human premolar teeth extracted for orthodontic purposes, selected through non-probabilistic sampling. These teeth were completely healthy, free of dental caries, restorations, and dental malformations. Each premolar was divided into 03 dentin fragments, resulting in a total of 54 dentin sample bodies. The 50 most suitable fragments were selected and allocated to each group (Figure 1A):

Distilled water (DW) negative control group
Ten dentin sample bodies, control group, distilled water was applied.

Hydrochloric acid (HCL) positive control group
Ten dentin sample bodies, positive control group, with application of 0.01M HCl.

Experimental groups
2% *Camellia sinensis* extract group (CS)
Ten dentin sample bodies with application of 0.01M HCl and 2% ethanolic extract of *C. sinensis*.

2% sodium fluoride group (FNA)
Ten dentin sample bodies with application of 0.01M HCl and 2% sodium fluoride.

2% CS extract + 2% FNA (CSF) group
Ten dentin sample bodies with application of 0.01M HCl and 2% sodium fluoride added to the 2% ethanolic extract of *C. sinensis*.

The first variable, type of solutions, was analyzed by applying solutions to the surface of the dentin. Chemicals such as hydrochloric acid (0.01 M), 2% sodium fluoride, and artificial saliva were used. The artificial saliva was prepared according to the formula: 1.5 mM of sodium chloride (NaCl), 0.9 mM of sodium biphosphate (NaH_2PO_4), and 0.15 mM of potassium chloride (KCl) at pH 7.0.[18]

The second variable was the erosive effect on the surface of human dentin, which is calculated as the variation in roughness on the dentin surface before and after the application of HCl and different solutions.

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The evaluation of the initial surface roughness (before the application of HCl and different solutions) was done sequentially in four areas of the sample surface, with these four readings the arithmetic mean, also called mean roughness (Ra), was calculated. An SRT6200 digital roughness meter (M&A INSTRUMENTS INC.) was used and then the data collection form was filled out (Figure 1B).

The dentin samples were immersed in artificial saliva for 1 hour to facilitate the formation of an acquired film, simulating the oral environment. Between applications, the samples were stored in artificial saliva to promote remineralization, following the method described by Passos *et al.*[6]:

DW GROUP

The samples were soaked in 20 ml of distilled water for 2 minutes (negative control).

HCL GROUP

The samples were soaked in 20 ml of 0.01 M HCl for 30 seconds (positive control).

CS GROUP

The samples were subjected to 20 ml of 0.01 M HCl for 30 seconds, then they were soaked in 20 ml of 2% ethanolic extract of *C. sinensis* for 2 minutes.

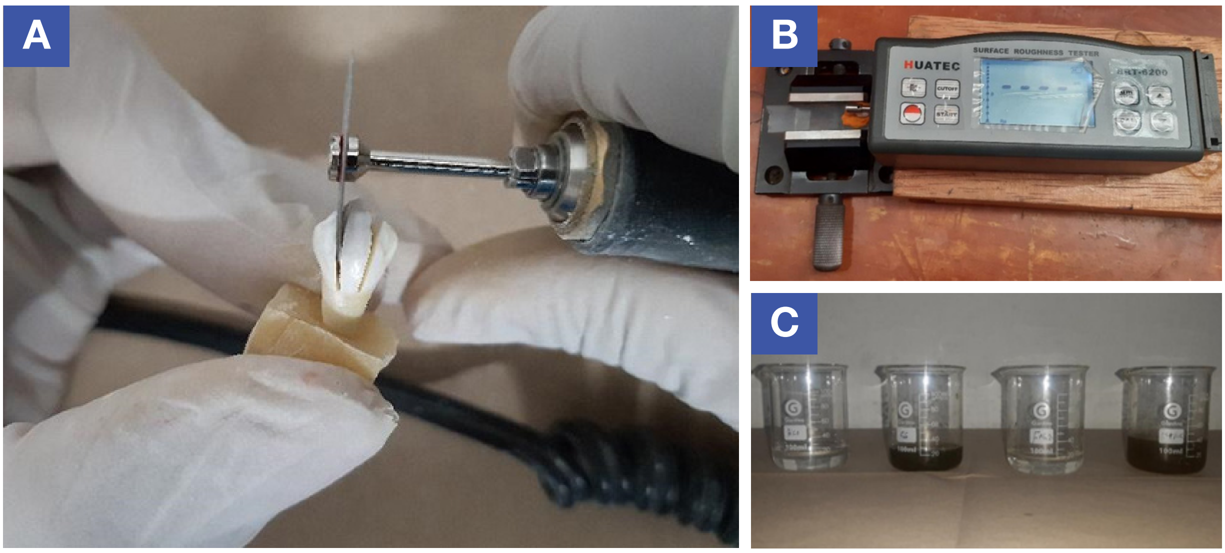
FNA GROUP

The samples were subjected to 20 ml of 0.01 M HCl for 30 seconds, then they were soaked in 20 ml of 2% sodium fluoride for 2 minutes.

CSF GROUP

The samples were subjected to 20 ml of 0.01 M HCl for 30 seconds, then they were soaked in 20 ml of 2% ethanolic extract of *C. sinensis* added with 2% sodium fluoride for 2 minutes. In the study, this sequence was applied five times a day for three days. Once the exposure cycle between days was completed, each group of samples was cleaned with gauze and stored in its respective sterile container with physiological saline (Figure 1C).
Subsequently, the four areas of the dentin surface on each sample were measured, and the

Figure 1. Overall sample preparation and processing.



A: Tooth sectioning process.
B: Composite resin disc with stratified technique (enamel + dentin) from Palfique LX5® – TOKUYAMA (G2) in roughness meter.
C: Samples being subjected to their respective erosive cycle.

Figure 2. Comparison of pre-test and post-test mean surface roughness.

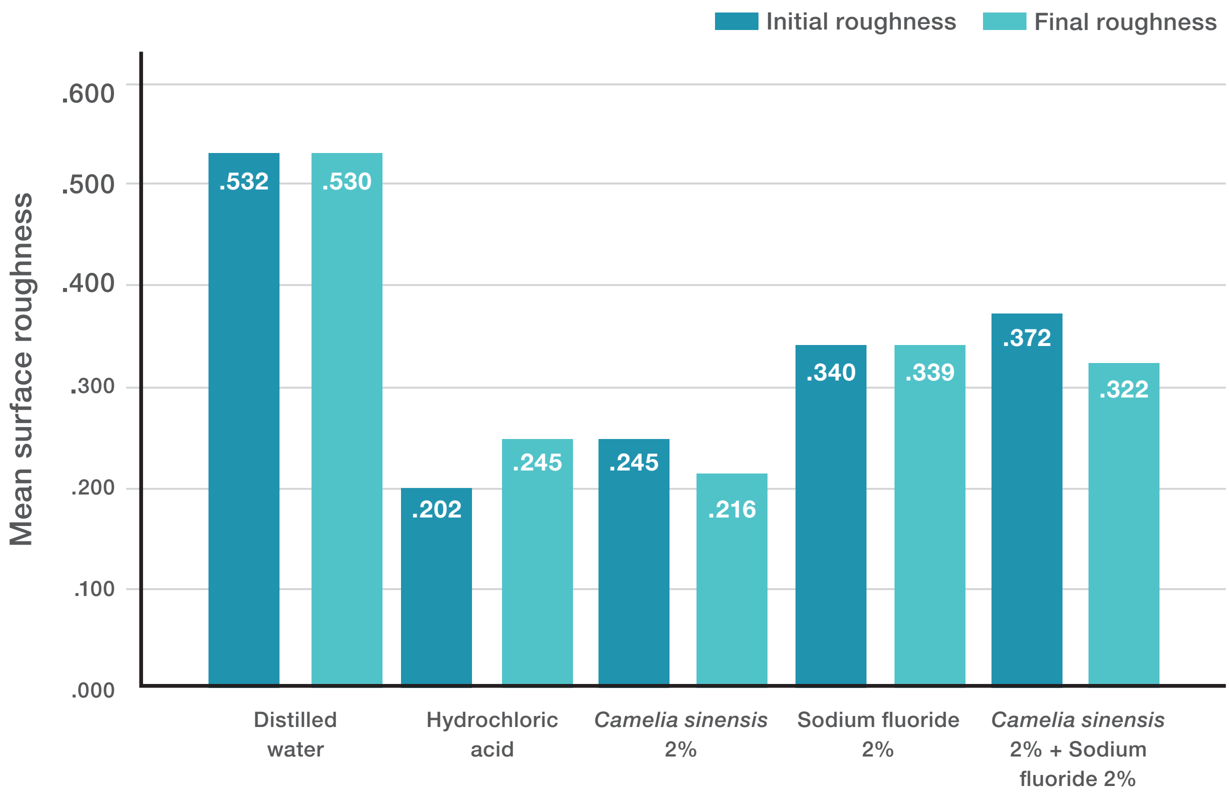


Table 1. Comparison and variation of the mean surface roughness according to the moment before and after for each experimental group; Wilcoxon test of the results before and after for each experimental group.

	Moment	Mean Surface Roughness (µm)	Variation of Mean Surface Roughness (µm)	Wilcoxon test asymptotic significance (bilateral)
Distilled water group (Control -)	Before	0.5320	0.0022	1.000
	After	0.5298		
Hydrochloric acid group (Control +)	Before	0.1516	- 0.1436	0.005
	After	0.2952		
2% *Camelia sinensis* extract group	Before	0.2451	0.0291	0.959
	After	0.2160		
2% Sodium fluoride group	Before	0.3400	0.0016	0.799
	After	0.3384		
2% *Camelia sinensis* extract +	Before	0.3721	0.0501	0.508
2% Sodium fluoride group	After	0.3220		

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final mean roughness was determined (after the application of HCl and various solutions). These values were recorded and compared with the initial mean roughness results. Data processing involved descriptive and inferential statistical tests, specifically the Wilcoxon test with a significance level set at $p<0.05$. Statistical analysis was conducted using the SPSS version 21 software package.

RESULTS

Table 1 shows the mean roughness values before and after the application of HCl and other solutions. In relation to the distilled water group (negative control), the measurements were 0.5320 µm and 0.5298 µm, for the hydrochloric acid group (positive control), they were 0.1516 µm and 0.2952 µm.

For the 2% *C. sinensis* extract group, they were 0.2451 µm and 0.2160 µm, for the 2% sodium fluoride group, they were 0.3400 µm and 0.3384 µm; and for the 2% CS extract + 2% FNa group, they were 0.3721 µm and 0.3220 µm, respectively. The 2% CS extract group+ 2% FNa shows a greater variation in the mean surface roughness, with a result of 0.0501 µm over the 2% *C. sinensis* extract group (0.0235 µm) and the 2% sodium fluoride group (0.0016 µm).

The statistical analysis of the quantitative variables was conducted using the non-parametric Wilcoxon test due to the related nature of the samples. It was observed that only the hydrochloric acid group exhibited a statistically significant difference with $p<0.05$. The values shown in Figure 4 indicate that there were no statistically significant differences in the mean roughness measurements among the distilled water group, 2% CS extract + 2% FNa, 2% *C. sinensis* extract, and 2% sodium fluoride.

DISCUSSION

Camellia sinensis contains components such as epigallocatechin gallate (EGCG), gallocatechin (GC), and catechin gallate (CG).[19,20] Epigallo-catechin-3-gallate, theaflavin and theaflavin digallate exhibit inhibitory activity on enzymes that degrade collagen. According to Passos *et al.,*[6] these active compounds can inhibit matrix metalloproteinases (MMP), thereby reducing erosive attacks on tooth surfaces. Additionally, Magalhaes *et al.,*[21] suggest that future research should explore the combined effects of fluoride and MMP inhibitors on dentin demineralization.

Consequently, to advance this research, the effect of alcoholic extract of *C. sinensis* combined with sodium fluoride against erosive action from non-bacterial acids on human dentin surfaces was studied. We evaluated dentin mineral loss using profilometry, a precise technique widely employed in dentin erosion studies.[6, 21]

When comparing groups treated with 2% ethanolic extract of *C. sinensis,* 2% sodium fluoride, and a combination of both compounds, no significant differences were found among them.

These three groups exhibited a similar effect, showing no progression of erosive effects from hydrochloric acid. However, a statistically significant difference was observed between the before and after moments in the hydrochloric acid group. These findings align with those of Magalhaes *et al.,*[21] who used a 0.61% extract of *C. sinensis* and found it to be equally effective as 0.12% chlorhexidine in reducing dentin erosion under conditions simulating oral cavity environments. Both compounds were similarly effective to a 250 ppm fluoride solution.

The present study demonstrated that the application of 2% ethanolic extract of *C. sinensis*, 2% sodium fluoride, and the combination of

2% ethanolic extract of *Camellia sinensis* with the addition of 2% sodium fluoride yielded comparable results.

Compared to the positive control, there was a notable increase in erosive action caused by hydrochloric acid on the dentin surface. Similar studies such as those by Passos *et al.,*[6] Magalhaes *et al.,*[20] and De Moraes *et al.,*[22] have also studied the protective effects of *C. sinensis* against erosive damage from non-bacterial acids. Therefore, the use of *C. sinensis* could serve as a natural and viable alternative for reducing erosive wear during short acid exposures.

CONCLUSION

Based on the findings of this research, it can be concluded that a 2% extract of *C. sinensis*, 2% sodium fluoride, and their combination demonstrated inhibitory effects against the erosive action of hydrochloric acid (0.01 M) on human dentin surfaces. The combination of fluoride and green tea products may provide enhanced protection against erosive damage from non-bacterial acids. Further studies on this topic should be carried out in the future. It is crucial to conduct both *in vitro* and *in vivo* research to explore the application of *C. sinensis* extract combined with sodium fluoride and its impact on the MMPs present in human dentin affected by erosive non-bacterial acids.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICS APPROVAL

Study approval was obtained from the Research Unit of the School of Dentistry, Universidad Nacional Mayor de San Marcos. Perú.

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AUTHORS' CONTRIBUTIONS

Reyes-Mansilla R: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; visualization; writing – original draft; writing.

Ramos-Perfecto D: Conceptualization; formal analysis; methodology; project management; writing – original draft; writing, review.

Maita-Véliz L: Methodology; formal analysis.

López-Pagan E: Formal analysis; review.

Maita-Castañeda L: Review.

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