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ABSTRACT

**PHARMACOTOXICOLOGICAL EVALUATION OF
COMPOUNDS WITH THERAPEUTIC POTENTIAL IN
ORAL PATHOLOGIES**

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SUMMARY

INTRODUCTION

The global prevalence of dental caries, colloquially known as tooth decay or cavities, persists as a prominent and consequential concern within the realm of public health. Dental caries stands as one of the most pervasive chronic ailments, afflicting individuals across the age spectrum, with a particular proclivity for children, adolescents, and the elderly. This incidence arises from a complex interplay of factors, predominantly encompassing the dynamic interactions between oral microorganisms, fermentable carbohydrates, and susceptible dental surfaces.

Compounded by inadequate oral hygiene practices, a diet characterized by high sugar content, and socio-economic determinants, the predisposition to dental caries is heightened. Despite advancements in preventive strategies, such as the introduction of fluoridated water, dental sealants, and enhanced oral health education, dental caries continue to affect a considerable segment of the population, giving rise to discomfort and pain, and imposing substantial economic burdens on healthcare systems. A vigilant approach to oral care, routine dental examinations, and comprehensive public health initiatives must persist as pivotal endeavors in mitigating the incidence of dental caries while fostering overall oral health. Due to the high incidence of unpleasant adverse reactions to synthetic compounds, natural products tend to gradually replace conventional treatment, as they can be just as potent and cause fewer, milder adverse effects. Researchers use several methods

to measure the effectiveness and safety profile of these compounds, and employing standard techniques also contributes to progress across all medical disciplines.

The relationship between dental caries and oral carcinogenesis, particularly bone neoplasms, has been rigorously examined in the scientific literature. Dental caries indirectly influences oral cancer etiology through mechanisms involving chronic inflammation, oral mucosal disruption, changes in the oral microbiome, and long-term exposure to potential carcinogens. While the direct causal inferences to bone malignancies within the oral cavity remain infrequent, the shared behavioral risk factors, such as tobacco use and alcohol consumption, highlight the importance of early dental intervention and the adoption of a health-promoting lifestyle. These measures are crucial for mitigating the collective risks associated with dental caries and oral cancer.

The first part of this thesis presents information from the recent specialized literature about oral pathologies, the causes that lead to the appearance of the main oral diseases, and the impact of these diseases on society. Later, in the second chapter, a series of natural and synthetic compounds with applicability in oral diseases were addressed. The therapeutic actions and adverse effects of the most well-known compounds in dental medicine were synthesized.

The special part, with personal contributions, included a detailed *in vitro* and *in ovo* analysis of sodium fluoride (NaF), xylitol (Xyl), and eugenol (EUG) on cellular behavior and chorioallantoic membrane.

AIM AND OUTLINE

The studies comprehensively evaluated different parameters, including cell viability and morphology, effects on confluency, nuclei for the natural compound EUG, and in addition action on actin filaments, as well as caspases 3/7 and 9 enzymatic activities and expression levels of anti-apoptotic and pro-apoptotic genes

for NaF and Xyl. Furthermore, both studies examined the irritant effects of these substances on the hen's egg chorioallantoic membrane.

RESULTS

STUDY I. COMPREHENSIVE IN VITRO AND IN OVO ASSESSMENT OF CYTOTOXICITY: UNRAVELING THE IMPACT OF SODIUM FLUORIDE, XYLITOL, AND THEIR SYNERGISTIC ASSOCIATIONS IN DENTAL PRODUCTS

The data revealed that the lowest NaF concentration of 0.05% significantly reduced cell viability, reaching a maximum decline of 58%. As concentrations increased, the cytotoxic effect lessened while remaining discernible, with cell viability approximately at 87% at the 0.5% concentration. Conversely, Xyl demonstrated an opposing effect on HaCaT cell viability. The lowest evaluated concentration of Xyl (0.1%) significantly increased cell viability to approximately 136%, and at 1%, the increase was approximately 109% (Figure 1).

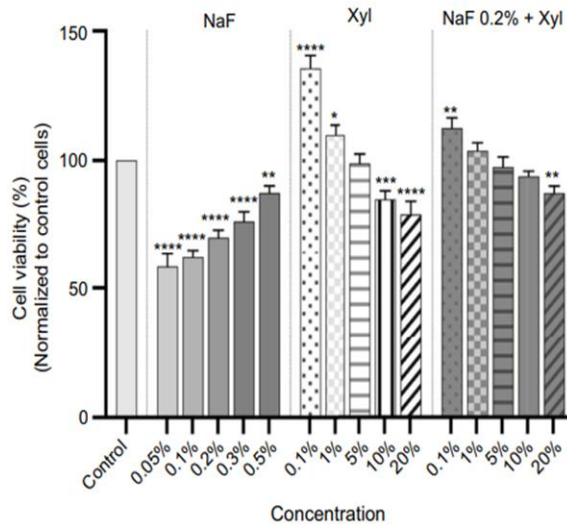


Figure 1. Analyses of the cytotoxic effects of NaF at various concentrations, Xyl at various concentrations, and a combination of 0.2% NaF with various concentrations of Xyl on HaCaT cells following a 24-h treatment period.

A comparable pattern manifested in the context of the osteosarcoma cell line, SAOS-2. Treatment with NaF at this cellular level resulted in an augmentation in cell viability corresponding to the escalating concentration. Specifically, at the highest concentration of 0.5%, a notable increase in cell viability, approximating 124%, was documented. Concurrently, Xyl exhibited a dose-dependent reduction in cell viability. The initial two concentrations of Xyl examined did not produce a noteworthy decline in the percentage of viable cells (Figure 2).

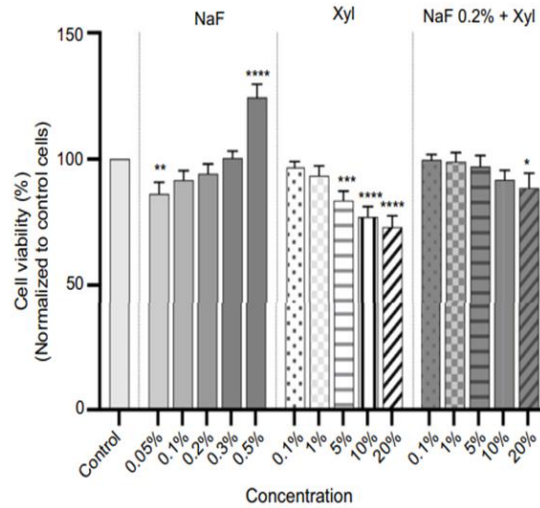


Figure 2. Analyses of the cytotoxic effects of NaF at various concentrations, Xyl at various concentrations, and a combination of 0.2% NaF with various concentrations of Xyl on SAOS-2 cells following a 24-h treatment period.

In HaCaT cells, NaF caused significant changes in nuclear structure, leading to a reduction in the size and number of nuclei and chromatin condensation. NaF also triggered reorganization and strong condensation of actin filaments. In contrast, Xyl at 5% did not notably affect nuclear or actin filament structure, except for some nuclear fragmentation. The combination of NaF and Xyl led to only minor chromatin condensation, with cell organelles remaining similar to those in untreated cells (Figure 3).

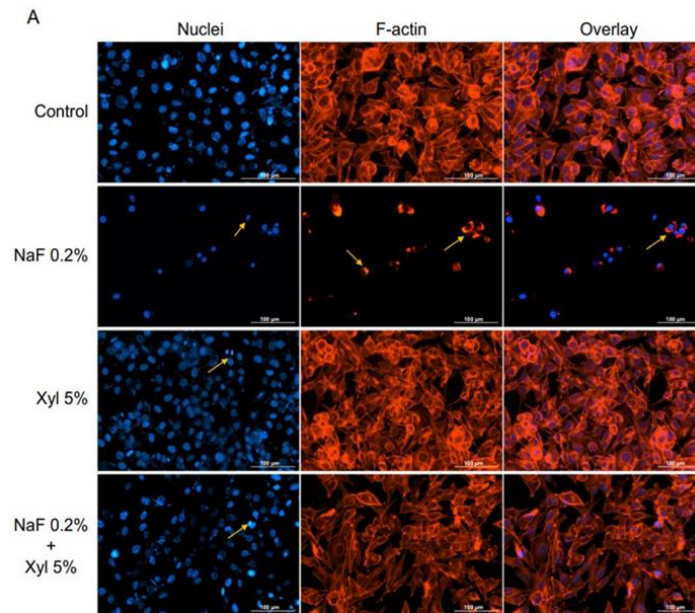


Figure 3. The immunofluorescence technique employed to examine changes in nuclear and actin filaments structures in HaCaT cells, following treatment with sub-cytotoxic concentrations of NaF, Xyl, and their combination.

In SAOS-2 cells, NaF at 0.2% caused slight chromatin condensation and reorganization of actin filaments, but these changes were not significantly different from the control cells. In contrast, Xyl induced more pronounced changes, including chromatin condensation, nuclear fragmentation, formation of apoptotic bodies, and reorganization of actin filaments into a peripheral ring. When NaF and Xyl were combined, they caused mild condensation of nuclei and actin filaments, though the effects were less intense compared to Xyl alone (Figure 4).

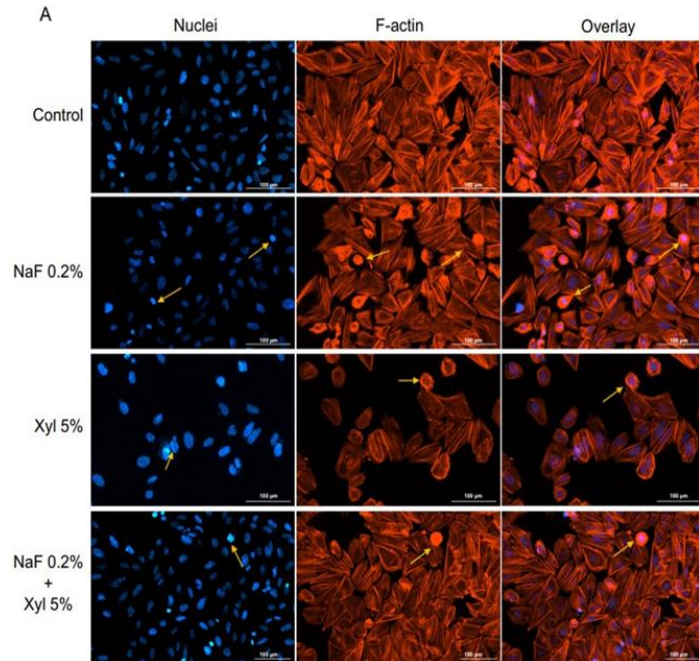


Figure 4. The immunofluorescence technique employed to examine changes in nuclear and actin filaments structures in SAOS-2 cells, following treatment with sub-cytotoxic concentrations of NaF, Xyl, and their combination.

The application of NaF to the vascular plexus elicited manifestations of vascular irritation, characterized by vascular lysis and microhemorrhage. In contrast, Xyl did not induce substantial alterations but rather induced a mild dilation of blood vessels (Figure 5).

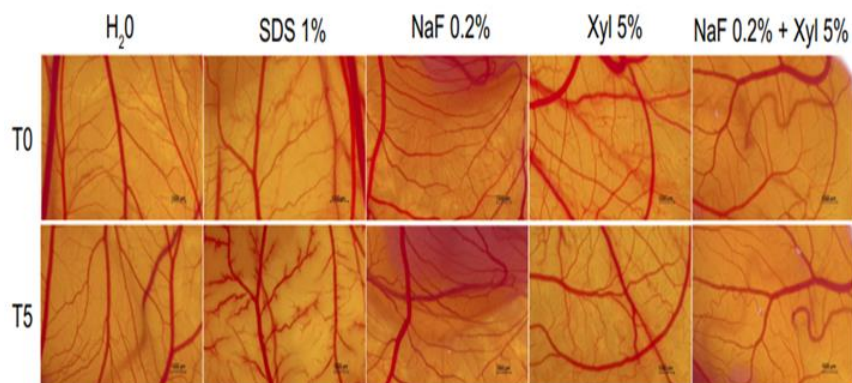


Figure 5. Evaluation of the irritant potential of NaF, Xyl, and their combination (NaF + Xyl) utilizing the HET-CAM assay.

STUDY II. EUGENOL - A NATURAL ALTERNATIVE IN DENTISTRY: AN IN VITRO AND IN OVO BIOSAFETY ASSESSMENT

EUG acted more intensely on JB6 Cl 41-5a cells showing that they are more sensitive to the compound compared to HaCaT cells, thus at the highest concentration tested (100 µg/ml) the results showed a percentage of 55% viable cells for JB6 Cl 41-5a, while for HaCaT cell viability did not decrease below 78% (Figure 6).

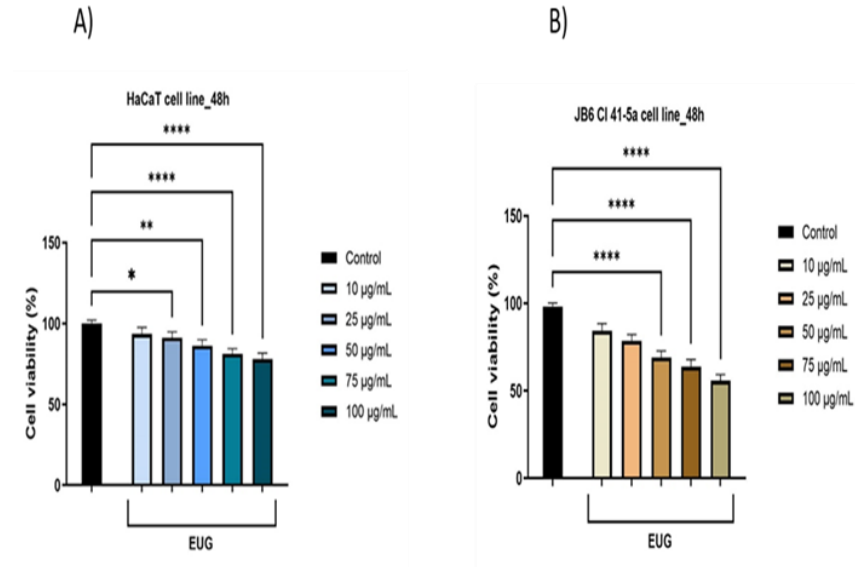


Figure 6. In vitro assessment of EUG (10, 25, 50, 75, and 100 µg/mL) impact on the viability of HaCaT and JB6 Cl 41-5a cells after 24 h of treatment by applying the MTT test.

As can be seen in Figure 7, for HaCaT cells, at the highest concentration tested a shrinking of the cells can be observed in places, but the morphologic appearance is not considerably affected. For JB6 Cl 41-5a cells, more intense action of EUG can be observed by the presence of debris, shrinkage, or elongation of the cell shape in places at the highest concentrations. Moreover, for JB6 Cl 41-5a cells, a reduction in confluency can also be observed with increasing doses.

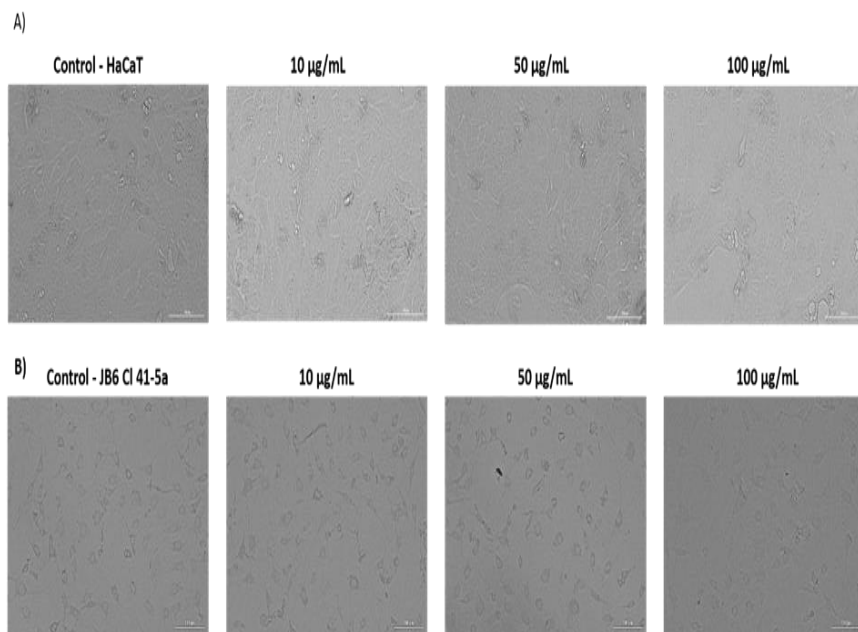


Figure 7. The morphological aspect of HaCaT and JB6 Cl 41-5a cells after 24 h of treatment with EUG (10, 50 and 100 µg/mL).

According to the results obtained via the HET-CAM assay (Figure 8), EUG is included in the category of non-irritant samples. As expected for the controls used in the experiment, SDS 1% caused severe damage and is categorized as a severely irritating sample, while H₂O is non-irritating.

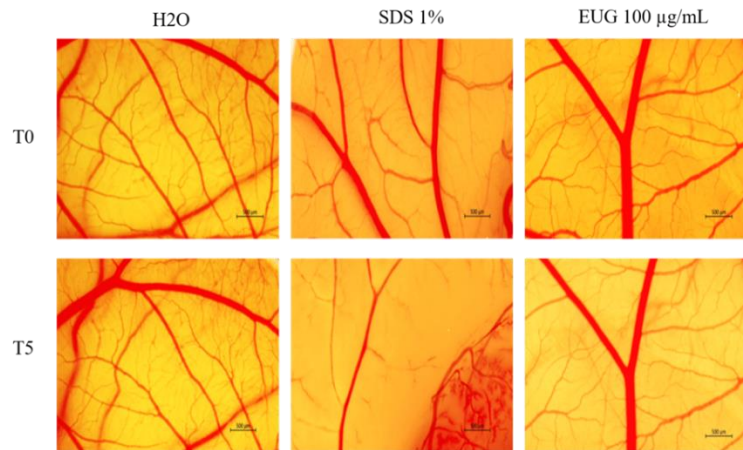


Figure 8. Stereoscopic images of the HET-CAM method illustrating the initial effect (T0) and at 5 min (T5) after the application of the samples: negative control (H₂O), positive control (SDS 1%), and EUG 100 µg/mL. The scale bars indicate 500 µm.

CONCLUSIONS

In recent decades, dental health products containing fluoride have been widely used to prevent tooth decay and promote oral hygiene. However, concerns about the potential toxic effects of fluoride exposure have led to ongoing research.

The first study examined the effects of NaF and Xyl on HaCaT and SAOS-2 cells. In HaCaT cells, NaF reduced cell proliferation and induced apoptosis-related morphological changes at low concentrations, while Xyl showed dose-dependent cytotoxic effects. The combination of NaF and Xyl decreased cell viability, and NaF affected caspase activity and pro-apoptotic gene expression. In SAOS-2 cells, NaF increased proliferation at high concentrations, while Xyl had cytotoxic effects. In chorioallantoic membrane experiments, NaF caused irritant effects, suggesting vascular risks.

The second study investigated EUG, which did not cause cytotoxicity in HaCaT cells but sensitized JB6 Cl 41-5a cells in a dose-dependent manner. In the HET-CAM assay, EUG was classified as a non-irritant.

The studies emphasize that while herbal treatments are often considered safe, they are not without adverse effects. More research and funding are needed to assess the safety and efficacy of natural and synthetic compounds in dentistry.