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ORIGINAL ARTICLE



# Assessment of cytotoxic and genotoxic effects of conventional and whitening kinds of toothpaste on oral mucosa cells

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**ABSTRACT**

**Objective:** This study aimed to evaluate possible DNA damages to oral epithelial cells exposed to whitening kinds of toothpaste considering the effect of conventional non-whitening toothpaste.

**Materials and methods:** Sixty volunteers were assigned into three experimental groups, each of them using a different regular toothpaste for the initial 2 months, followed by the use of whitening kind of toothpaste of the same brand for next 2 months. The oral epithelial cells were sampled prior and 30, 60, 90 and 120 days after the beginning of the use of tested kinds of toothpaste. Chromosomal damages were analyzed by micronucleus assay.

**Results:** For just one kind of tested whitening toothpaste was observed the significant increase in the number of micronucleated cells after 60 days of use compared values obtained 60 days of usage of conventional non-whitening toothpaste ($6.35 \pm 3.67$ and $2.8 \pm 1.91$; $p < .05$). There was no statistically significant difference in other micronucleus assay endpoints between tested types of toothpaste at either of the sampling times during the period of toothpaste application.

**Conclusions:** Based on the results, it can be concluded that the use of certain whitening kinds of toothpaste may cause a limited biologically insignificant genotoxic effect on buccal epithelial cells.

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Biocompatibility; DNA damage; micronucleus assay; oral epithelial cells; whitening toothpaste

## Introduction

Due to the growing aesthetic demands of modern society, the use of tooth whitening products has increased. Various at-home tooth bleaching products may be found on the market such as toothpaste, gels, rinses, gums, dentifrices, whitening strips or paint-on films. However, these self-applied bleaching treatments can be damaging, and there is a lack of clinical trials providing substantial scientific background awareness regarding biocompatibility of these whitening products [1].

Nowadays, whitening types of toothpaste are commonly used in many households. Whitening kinds of toothpaste are based on formulations with enhanced physical (mechanical) and chemical cleaning abilities claiming to remove and prevent extrinsic stains effectively. However, in some dentifrices chemicals that provide a bleaching effect are added; thus there are two particular subclasses—whitening toothpaste and bleaching toothpaste [2]. The performance of this whitening toothpaste is based on their size and rigidity of molecules of the added abrasive substance, which are more resilient than the stain molecules themselves. Typically, silica dioxide, hydrated silica dioxide, calcium carbonate, calcium phosphate dihydrate, calcium pyrophosphate, alumina oxide, perlite (70-75% silica dioxide) and sodium bicarbonate are the abrasive agents used in whitening toothpaste [2,3]. Surface stains can be reduced by adding various chemicals to toothpaste. Most of the dye molecules which are included in the pellicle contain proteins. Therefore, enzymes such as protease and papain produce a whitening effect. Sodium pyrophosphate, sodium tripolyphosphate, and other pyrophosphates can bind with enamel, dentin on tartar and absorb the stain molecules, creating a whitening effect [2]. In contrast to whitening toothpaste, bleaching toothpaste contain chemicals, most commonly that of hydrogen peroxide or calcium peroxide (Calprox). When peroxides come into contact with the tooth's surface or penetrate tooth tissue, they break down the stain molecule generating a bleaching effect. However, the concentration of peroxides added to toothpaste are low (usually 1% hydrogen peroxide or 0.5–0.7% calcium peroxide) [2,4]. Although in several studies whitening kinds of toothpaste that demonstrated to improve tooth color also exhibited adverse side effects. They can damage hard and soft tissue, which manifests either immediately or after prolonged exposure. Enamel and dentin abrasion are among the most severe side effects that can increase tooth sensitivity and gum irritation [2,4].

There is increasing effort to understand the size and significance of the impact of different lifestyle factors on genomic stability. Keratinized cells of buccal mucosa provide a barrier against potentially dangerous chemicals. If this barrier is passed, molecular structures and macromolecules of

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affected epithelial cells may be damaged. DNA as the molecule bearing the genes which warrants homeostatic cellular functioning is complex in physiology itself and is one of the most exceptional macromolecular structures in the preservation of integrity, which has been in focus of toxicological studies. Among cytogenetic methods used to evaluate the effect of lifestyle factors on genome integrity, the buccal micronucleus assay is a simple, painless and non-invasive method. It detects DNA damage at the chromosomal level that may lead to more severe genome instability causing a health risk [5].

Several recent studies that assessed the bio-tolerance raised concerns regarding possible adverse effects induced by toothpaste [6–8]. The *in vitro* study showed that whitening kinds of toothpaste were more genotoxic to cells than the conventional ones [7]. However, till now none of the studies were evaluating the potential toxicity of different types of toothpaste in *in vivo* conditions. Thus, this study aimed to assess the genotoxic and cytotoxic effects of the use of and difference in severity of the effect between different non-whitening (conventional) and whitening kinds of toothpaste. An *in vivo* micronucleus assay in oral mucosal cells was applied. The null hypothesis tested the claim that there are more nuclear abnormalities in oral mucosal cells caused by using whitening kinds of toothpaste.

## Materials and methods

### *Subjects*

The study was conducted out at the Department of Restorative Dental Medicine and Endodontics of Study of Dental Medicine, School of Medicine, University of Split. It was approved by the universities Ethics Committee (study approval No. 2181-198-03-04-16-0007), and all participants were acquainted with the purpose of the survey, signing an informed consentient.

The study comprised of 60 young subjects—30 female and 30 male volunteers 23.27 ± 1.21 years of age (ranging from ages 21 to 26). The subjects were randomly assigned into three groups each consisting of 20 examinees. Each group brushed their teeth for the first 2 months with different conventional toothpaste and the following 2 months with various whitening toothpaste, but from the same manufacturer and of similar composition as the regular ones. The amount of 1 g (≈2 cm) of the tested toothpaste was applied twice daily, once in the morning and once in the evening, for a time of three minutes. The first group was given Kalodont Pro Care Extra Clean (Saponia, Croatia), a regular toothpaste for the first 2 months; followed by the Kalodont Pro Care Whitening (Saponia, Croatia) toothpaste with whitening effects for next 2 months. The second group used Sensodyne Fluoride (GlaxoSmithKline, UK), followed by Sensodyne Gentle Whitening (GlaxoSmithKline, UK). The third group applied Colgate Cavity Protection (Colgate—Palmolive Company, USA), followed by the Colgate Whitening (Colgate—Palmolive Company, USA) toothpaste. The compositions of the tested kinds of toothpaste, as provided by the manufacturers, are presented in Table 1. The respondents were not permitted to use any other chemical preparations for maintaining their oral hygiene (mouthwash, fluoridation preparations) at the time of the research.

Each participant filled out a questionnaire regarding their demographic characteristics (age, gender), lifestyle factors (smoking, alcohol consumption), personal factors (health status, use of medication, X-ray diagnostics), and dietary aspects. Individuals who smoked three or more cigarettes per day for at least 1 year were considered to be smokers. Persons who reported consumption of two or more units of alcohol three or more times a week were excluded from the study. Patients with oral lesions and a history of chronic

**Table 1.** Toothpaste, their respective manufacturers and their main components (active ingredients and inactive ingredients).

| Toothpaste | Active and inactive ingredients |
|---|---|
| Sensodyne Fluoride (GlaxoSmithKline, UK) | Active ingredients: Sodium Fluoride 0.315% w/w (1400 ppm $F^-$), Potassium Nitrate Inactive ingredients: Aqua, Sorbitol, Glycerin, Cocamidopropyl Betaine, Titanium Dioxide, Xanthan Gum, Aroma, Sodium Saccharin, Sodium Hydroxide, Sucralose, Trisodium Phosphate, Limonene, Hydrated Silica, Silica |
| Sensodyne Gentle Whitening (GlaxoSmithKline, UK) | Active ingredients: Sodium fluoride 0.306% w/w (1400 ppm $F^-$), Potassium Nitrate Inactive ingredients Aqua, Sorbitol, Glycerin, Pentasodium Triphosphate, PEG-6, Aroma, Titanium Dioxide, Cocamidopropyl Betaine, Sodium Methyl Cocoyl Taurate, Xanthan Gum, Sodium Hydroxide, Sodium Saccharin, Hydrated Silica |
| Colgate Cavity Protection (Colgate-Palmolive Company, USA) | Active ingredients: Sodium Monofluorophosphate 0.76% (1000 ppm $F^-$) Inactive ingredients: Dicalcium Phosphate Dihydrate, Water, Glycerin, Sorbitol, Sodium Lauryl Sulfate, Cellulose Gum, Flavor, Tetrapotassium Pyrophosphate, Sodium Saccharin |
| Colgate Whitening (Colgate-Palmolive Company, USA) | Active ingredients: Sodium Monofluorophosphate 1.1% (1450 ppm F\bar) Inactive ingredients: Aqua, Sorbitol, Sodium Lauryl Sulfate, Aroma, Cellulose Gum, Magnesium, Benzyl Alcohol, Sodium Saccharin, Cinnamal, Eugenol, Limonene, Aluminium Silicate, Sodium Carbonate, Calcium Carbonate, Hydrated Silica, Sodium Bicarbonate |
| Kalodont Pro Care Extra Clean (Saponia d.d., Croatia) | Active ingredients: Sodium Monofluorophosphate 0.76% (1000 ppm F\bar) Inactive ingredients: Aqua, Sorbitol, Dicalcium Phosphate, Glycerin, PEG-8, Sodium Lauryl Sulfate, Aroma, Xanthan Gum, Cocamidopropyl Betaine, Sodium Saccharin, DMDM Hydantoin, Limonene, Eugenol, CI 77891, Hydrated Silica |
| Kalodont Pro Care Whitening (Saponia d.d., Croatia) | Active ingredients: Sodium Monofluorophosphate 0.76% (1000 ppm F\bar) Inactive ingredients: Aqua, Sorbitol, Dicalcium Phosphate, Glycerin, PEG-8, Pentasodium Triphosphate, Sodium Lauryl Sulfate, Aroma, Disodium pyrophosphate, Xanthan Gum, Cocamidopropyl Betaine, Sodium Saccharin, Sodium Methylparaben, Limonene, Eugenol, CI 77891, Hydrated Silica |

health conditions were also excluded from the study, as well as those who had been exposed to materials used in orthodontics or removable and fixed prosthodontics [9].

### Sample collection

Samples of epithelial cells were collected from each participant using the swab technique; immediately before they started using the tested toothpaste (T0 – referent), and after 30 days (T1) of toothpaste usage without whitening effect, 60 days (T2) of usage of toothpaste without whitening effect, 90 days (T3), i.e. 30 days of usage of toothpaste with whitening effect and 120 days (T4), i.e. 60 days of usage of toothpaste with whitening effect.

One hour prior to sampling, the participants were asked to refrain from smoking, eating, and drinking alcohol. After rinsing, the oral cavity three times with tepid water to remove exfoliated cells, a swab was taken by gently brushing the buccal mucosa bilaterally with a cytobrush (Cytobrush Plus, GmbH, Dietramszell-Linden, Germany) and then the samples were applied to coded laboratory glass slides prewarmed at 37 °C.

### Micronucleus assay in buccal epithelial cells

The cells applied on microscopic slides were allowed to air-dry and then fixed in methanol (80%v/v) at 4 °C for 20 min. Staining was carried out with 5% Giemsa solution for 10 minutes. Afterwards, the slides were rinsed with aqua distillate and air-dried. An analysis was done under a light microscope with a 400 × magnification, while each micronucleus and other nuclear anomalies were additionally verified under 1000 × magnification. Two replicated slides were prepared for each subject, and 2000 epithelium cells per preparation were scored for each sampling time. Frequencies of nuclear abnormalities other than micronuclei, such as binucleated, condensed chromatin, karyorrhexis, karyolysis, nuclear buds and "broken eggs," were also evaluated and classified according to Tolbert et al. [10].

In order for a micronucleus to be calculated as such, it has to meet following conditions: (a) it must consist of nuclear material; (b) it must be completely separated from the parent nucleus; (c) it must be less than 1/3 of the diameter of associated nuclei; (d) it must be smooth, oval or round shaped; (e) it must be on the same plane of focus and (f) it must be of the same color, texture and refraction as the main nucleus. Cells with two nuclei were considered to be binucleated. Nuclear anomalies, such as karyorrhexis (nuclear disintegration), karyolysis (dissolution of the nucleus), nuclear buds (precursors of micronuclei) and nucleoplasmic bridges (nuclei that appear cinched) were recorded separately [9].

### Statistical analysis

Statistical analysis was completed by using Statistica 13 software package (Dell Software, CA, USA). By using descriptive statistical analysis, the basic statistical parameters (mean, standard error, standard deviation, and relative standard deviation, median and minimum and maximum values) were determined. The differences in the number of micronuclei and other nuclear anomalies between different sampling times for each group and between the groups of examinees were tested by ANOVA and Student–Newman–Keuls *post hoc* tests using a general linear model procedure. General regression model (GRM) from linear/nonlinear modeling method and canonical correlation analysis were used for the assessment of the influence of the predictor variables (age, gender, state of health and use of medication, X-ray exposure and dietary habits) onto dependent variables (micronucleus, binucleated cells, nucleoplasmic bridges, nuclear buds, pyknosis, condensed chromatin, karyolysis and karyorrhexis). The results of GRM are expressed in the form of Pareto charts. A significant level of 0.05 was considered as the cut-off criterion of significant impact.

## Results

Basic statistical parameters were used to analyze results of the micronucleus assay in buccal epithelial cells, before and during the course of testing the various kinds of toothpaste (Colgate Cavity Protection and Colgate Whitening, Kalodont Pro Care Extra Clean and Kalodont Pro Care Whitening, Sensodyne Fluoride and Sensodyne Gentle Whitening), which are shown in Figures 1 and 2.

The difference between the groups which were subdivided according to exposure time and used toothpaste, and tested for following parameters: the number of micronucleated and binucleated cells, cells with nucleoplasmic bridges, nuclear buds, pyknosis, condensed chromatin and karyorrhexis and karyolysis was established by using the analysis of variance. There were no statistically significant differences in micronucleus assay endpoints between the tested different brands' toothpaste at either of the sampling times, during the period of toothpaste application.

In epithelial cells of participants brushing the teeth with Colgate toothpaste we observed statistically significant differences in the frequency of micronuclei, between samples T2 sampling time (day 60 of usage of Colgate Cavity Protection), compared to T4 sampling time (60 days of usage of Colgate Whitening toothpaste); $p < .05$ (Figure 1).

The general model of regression has been applied in order to assess the extent of effect on the subjects' demographic and anamnestic variables (age, gender, physical activity, smoking and alcohol consumption, use of medication, X-ray exposure, and dietary habits) on the number of micronuclei, binuclear cells, number of nuclear buds and nucleoplasmic bridges, karyolysis and karyorrhexis and condensation of the chromatin. Results of the analysis are presented in Figures 3 and 4. None of the tested factors were found to exhibit noteworthy influences on the occurrence of karyolysis, pyknosis and karyorrhexis. Recreational physical activity more than three times a week significantly affected micronucleus frequency ($p = .041$). Regular, everyday fruit consumption also significantly influenced the existence of cells with condensed

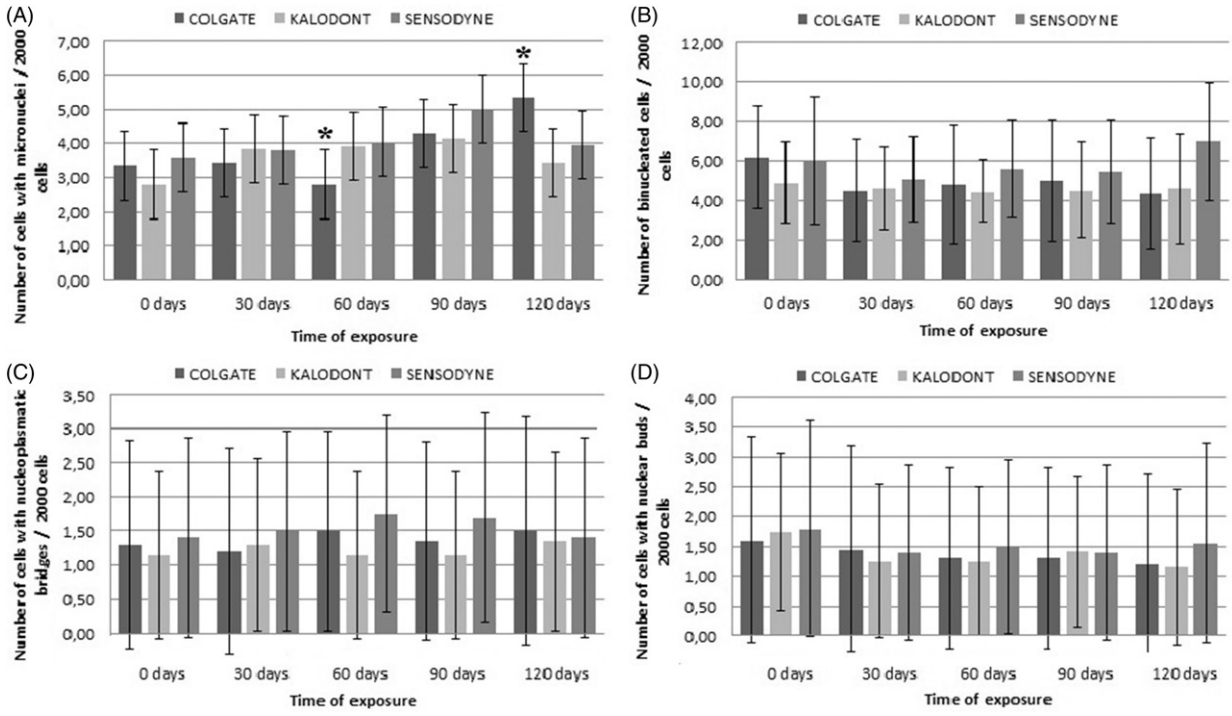


**Figure 1.** The frequency of cells with micronuclei, binuclear cells, nucleoplasmic bridges and nuclear buds in 2000 buccal epithelial cells of participants ($n = 20$/toothpaste combination) for each time-point of measurement. Mean values are expressed as columns, while error bars represent standard deviations. *Denotes statistically significant values ($p < .05$) between tested sampling times.

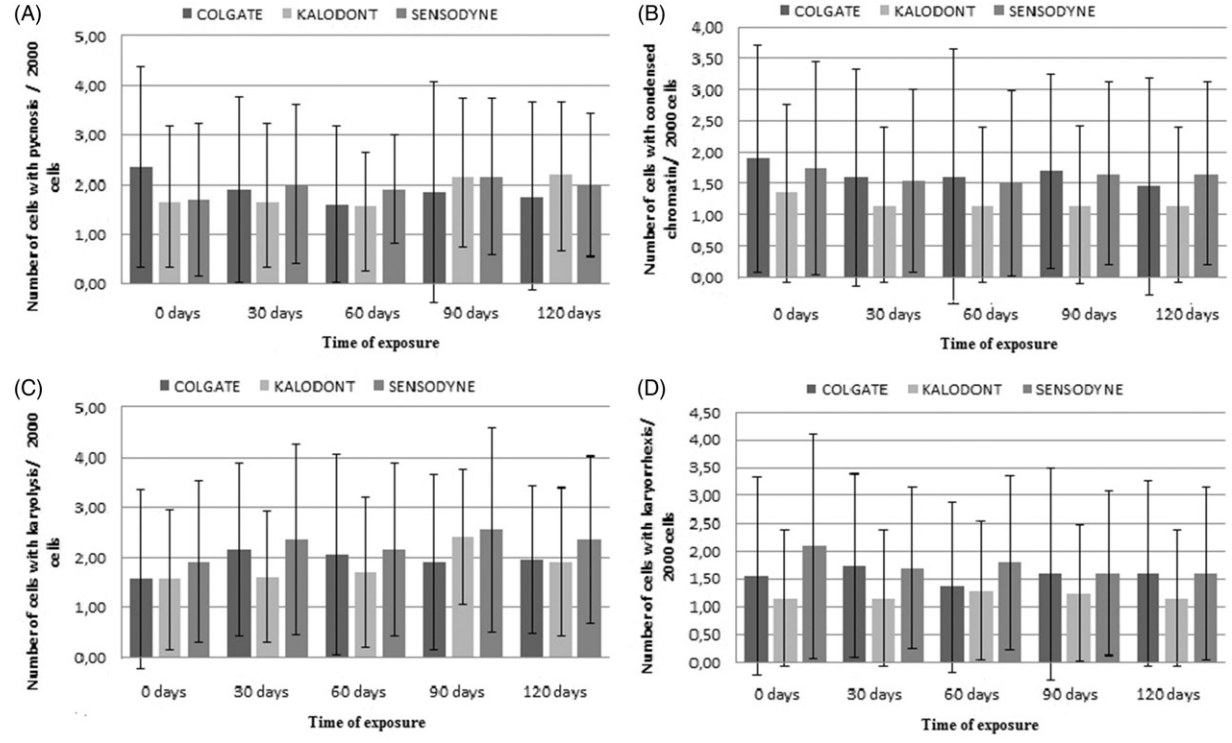


**Figure 2.** The frequency of cells with pycnosis, condensed chromatin, karyolysis and karyorrhexis in 2000 buccal epithelial cells of participants ($n = 20$/toothpaste combination) for each time-point of measurement. Mean values are expressed as columns, while error bars represent standard deviations.

chromatin ($p = .001$). Alcohol consumption meaningfully affected the incidence of nuclear buds ($p = .005$), while smoking showed a statistically substantial connection to the number of nucleoplasmic bridges ($p = .027$) and binuclear cells ($p = .003$).

## Discussion

This study aimed o to evaluate the biocompatibility of non-whitening (regular) and whitening kinds of toothpaste by using *in vivo* micronucleus assay in oral epithelial cells.

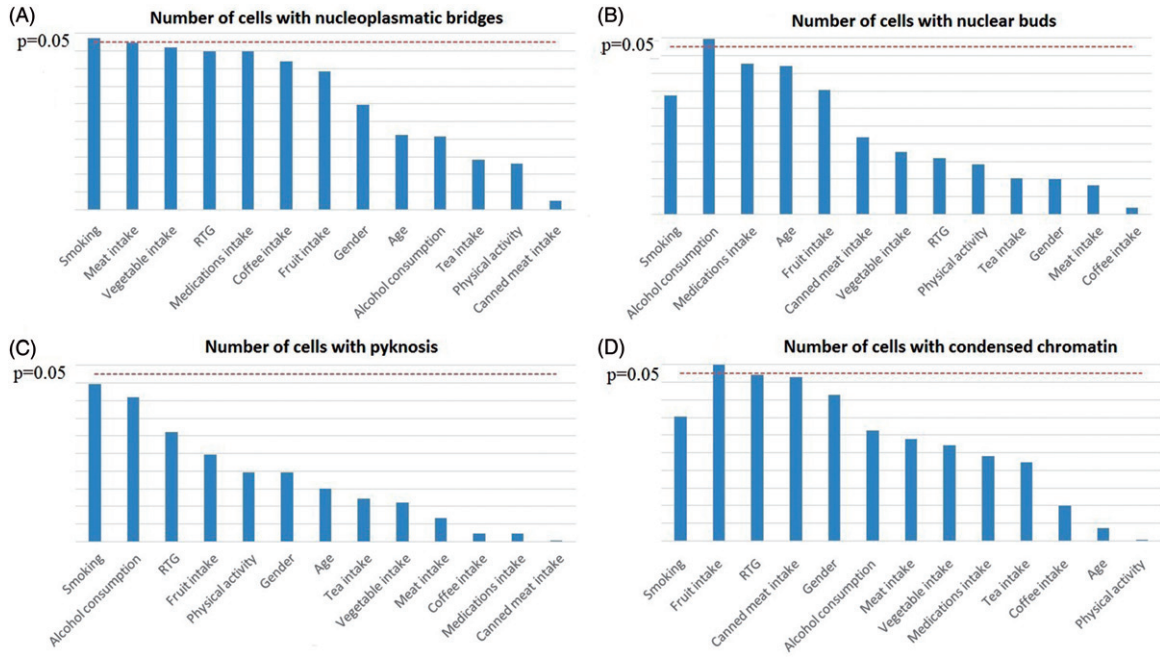


**Figure 3.** Multiple regression analysis results. Significant dependence of measured cytogenetic endpoints (number of cells with nucleoplasmic bridges, nuclear buds, pyknosis, and condensed chromatin) on buccal cells and demographic and lifestyle factors as possible predictors.

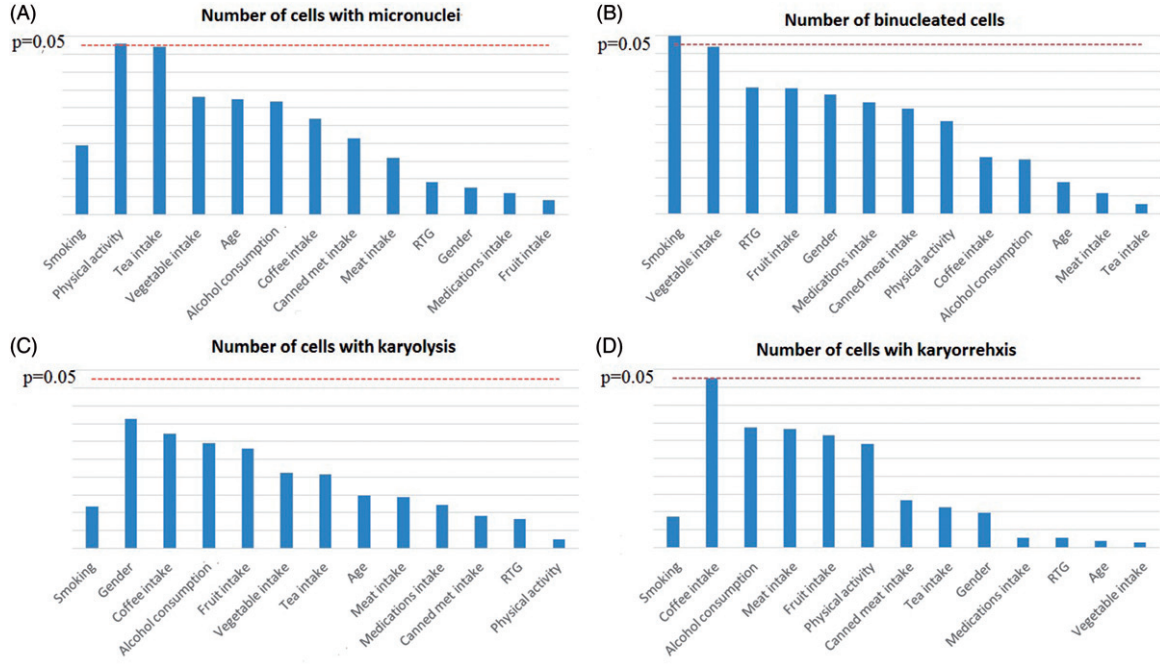


**Figure 4.** Multiple regression analysis results. Significant dependence of measured cytogenetic endpoints (number of cells with micronuclei, nuclear buds, karyolysis and karyorrexis) on buccal cells and demographic and lifestyle factors as possible predictors.

A prospective longitudinal clinical study was conducted over a four month period. Three groups of examinees for first 2 months used different standard toothpaste and for the following 2 months consumed whitening toothpaste from the same manufacturer. Oral mucosa samples were taken before the commencing the study, after 1 and 2 months of usage of their particular toothpaste. As per our knowledge, this is the first such longitudinal *in vivo* study evaluating the biocompatibility of various kinds' toothpaste involving voluntary participants. Our null hypothesis presuming that whitening types of toothpaste will exhibit a more pronounced effect in epithelial cells was partially confirmed since their limited genotoxic effect was demonstrated.

Toothpaste may contain chemicals that can have an adverse effect on oral tissue in humans. Their components are considered potentially harmful, and the risk of adverse effects is dependent on the concentration and duration of their contact with living tissue. Due to frequent application of toothpaste that occurs regularly over an extended period, it suggests that these dentifrices must be highly

biocompatible, which can be assessed by conducting an epidemiological study. The micronucleus assay in cells of buccal exfoliates, one of the most convenient cytogenetic methods that permit in such studies the elucidation of the effects of potential genotoxic effect in a particular target tissue. Since it is a noninvasive technique and was proved to show high correlation with systemic health risks regarding carcinogenesis, it is highly recommended for evaluation of chromosome damage in the oral cavity [11,12]. The cells which are most prone to genotoxic damage are mitotically active mesenchymal cells in the basal layer of oral epithelium. In the course of mitosis primary chromosome damage is transduced and at the end appearing as micronucleus. Buccal cells maintain themselves by a continuous cell renewal, whereby new cells are produced in the basal layer by mitosis migrating to the surface and replacing those that are shed [13]. The optimal timing is between seven and 21 days after exposure, which is the period needed for micronucleus or other nuclear anomalies to appear in exfoliated cells. The peak expression may vary depending on the effects of toxic agents on the basal cell turnover rate [14,15].

When applying a testing method to the buccal epithelium, it is important to consider that the oral cavity is a multifactorial environment and since each bears his/her particular biological variation, results of *in vivo* studies are not simple to interpret. To avoid the influence of interindividual variations, each subject's endpoint status at the initial point of the study is used as the self-referent value, and its status is monitored with each subject, at different time points over the research period [9].

All three regular kinds of toothpaste without the effect of whitening tested in the present study did not induce the significant increase in the number of micronuclei compared to referent swab that was taken immediately before the first use of the specific toothpaste. The highest number of cells with micronuclei was detected after 60 days of the use of Colgate Whitening toothpaste compared to samples were taken 60 days following the usage of Colgate Cavity Protection ($6.35 \pm 3.67$ and $2.8 \pm 1.91$; $p < .05$). There was no statistically significant difference in other micronucleus assay endpoints between the same types of toothpaste tested at different sampling times, or between the various kinds of the toothpaste in the same period of toothpaste application. Regarding the composition of tested kinds of toothpaste, Sensodyne and Kalodont toothpaste contain only hydrated silica abrasive particles, while the Colgate Whiting toothpaste besides containing these particles, also consists of aluminum silicate, sodium carbonate, calcium carbonate, hydrated silica and sodium bicarbonate. These additional components may be indicative of the most prominent effect of the use of Colgate Whitening on micronuclei status. In contrast to the Colgate Whitening toothpaste, Colgate Cavity Protection does not contain abrasive particles. Different cytotoxicity and genotoxicity of toothpaste may be attributed to the effect of various toothpaste components, a mixture of which, in the final dentifrice, may result in synergistic or even an additive effect [6]. Cytotoxic action of different toothpaste components *in vitro* was reported by several groups of authors [6,16,17]. Thus, a difference in the formulations of tested kinds of toothpaste may be responsible for the difference in the severity of induced adverse effects; however, manufacturers do not willingly provide their detailed composition in its entirety.

The present results are to a great extent in agreement with those obtained by Camarago et al. [7]. The authors reported that whitening types of toothpaste exhibit a higher genotoxic effect in gingival human fibroblasts cells *in vitro* compared to the regular ones. Different kinds of toothpaste tested in that particular study reduced cell survival. Colgate Whitening reduced the percentage of viable cells below 5% and led to the two-fold increase in the number of micronuclei. Compared to the conventional Colgate toothpaste, the Colgate Whitening toothpaste induced a single-fold increase in the number of micronuclei.

In their study, Torrado et al. [16] tested on mouse fibroblasts cells L929 *in vitro* cytotoxicity of Crest Extra-Whitening toothpaste. The toothpaste led to cell-cycle inhibition beyond 50%. However, cytotoxicity did not increase with concerning the duration of treatment. Ghapanachi et al. [17] also evaluated cytotoxicity *in vitro* of 16 commercial toothpaste in primary epithelial cells of the oral cavity and HeLa cell line. All tested types of toothpaste induced cytotoxic effect but at different magnitudes. Cytotoxicity of toothpaste significantly increased with the increase of exposure from 1 to 5 min. Bruno et al. [6] reported a cytotoxic effect of Colgate Total 12 Clean Mint, Colgate Luminous White, Oral B Limpeza and Closeup Ação Profunda in human gingival fibroblasts *in vitro*. Cell viability in treated cultures ranged between 16 and 21%. The extent of the effect did not significantly differ between tested kinds of toothpaste. Another Brazilian group of authors conducted similar assessment [18]. They evaluated cytotoxicity in widely available conventional toothpaste on human gingival fibroblasts *in vitro* by MTT assay. The Sensodyne dentifrices did not significantly affect cell viability, compared to the negative control cultures treated with distilled water (60 to 68%). However, other types of toothpaste, including Colgate Total 12, showed high cytotoxicity with the decrease in cell viability below 50% of the control [18].

Sodium lauryl sulfate (SLS) is known as one of the most toxic substances in toothpaste. It is used as a detergent and cosmetics–health agent. Frequent contact with this chemical may lead to multiple allergic and toxic reactions. Repeated long-term ingestion of SLS may also exert a carcinogenetic effect [19]. The researcher conducted by Gerckens et al. [20,21] affirmed that SLS is toxic for mucosal cells and efficiently causes epithelial desquamation. Neppelberg et al. [22] demonstrated the direct association between SLS and induction of cell death in epithelial cells. It should be noted that four out of six of toothpaste tested in the present study contained SLS (Kalodont Pro Care Extra Clean, Kalodont Pro Care Whitening, Colgate Cavity Protection and Colgate Whitening). Considering the lack of cytotoxic and genotoxic effects of the Kalodont toothpaste we can assume that the content of this detergent is low.

Other toothpaste components such as sodium monofluorophosphate, silicon dioxide, hydrated silica, sodium benzoate, preservatives, colors, flavors, and essences might also exhibit toxic effects [17,23]. Colgate Whitening toothpaste contains

the highest amount of fluoride components (sodium monofluorophosphate 1.1%), which combined with abrasive particles and sodium lauryl sulfate turned this toothpaste to seem least biocompatible. We monitored the effect of the use of this toothpaste over a period of 60 days, but it may be speculated that the micronucleus incidence would fall to referent level with prolonged use. Our results are likely to differ from those obtained by *in vitro* studies due to numerous factors such as saliva, a mucus layer, creatine levels, blood flow, and oral flora, which can influence the efficiency of epithelial cell protection against harmful materials [6].

In addition to this, there is a broad spectrum of other factors characterizing each of the participants taking part in the study that may modulate the effect of the evaluated substance or complex mixtures such as dentifrices on micronucleus assay endpoints. These include, but are not exclusive to demographic factors (age, gender), lifestyle (smoking, alcohol, occupation, folate and vitamins intake), and disease susceptibility (medication, X-ray diagnostics) [24]. In this present study, there was no significant difference in regards to the ratio of male and female subjects and age between the groups utilizing the various brands of toothpaste tested. Canonical correlation analysis indicated that of all considered confounding factors smoking and alcohol consumption may be associated with the outcome of the assessment. In subjects who were more frequently consummating alcohol we detected a significantly higher number of cells with nuclear buds ($p = .005$), and in smokers increased the number of cells with nucleoplasmic bridge ($p = .027$) and binuclear cells ($p = .003$) was observed.

This study has certain limitations to it. Before commencing the study, the participants used different kinds of toothpaste of their own choice, albeit, it would be preferable that they all were used the same kind of toothpaste over a longer period how would T0 sampling time (before the beginning of used tested kinds of toothpaste) be as homogeneous. The results could conclusively be more detailed, had the research been done with the same toothpaste over a longer space of time (e.g. at least 6 months), to monitor the long-term biological effect.

Results of this study indicated that whitening toothpaste might exhibit a limited, biologically insignificant genotoxic effect on buccal epithelial cells. Thus, additional efforts should be made to avoid the use of potentially toxic substances or at least to keep their content as low as possible. Additionally, further studies are required, comprising a higher number of examinees with a longer longitudinal follow-up to additionally determine any other possible side effects of these oral hygiene products.

## Disclosure statement

The authors report no conflicts of interest.

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