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Original Research Article

Effects of herbal-based toothpastes on cell viability

Firdevs Kahvecioğlu[1]*, Hayriye Esra Ülker[2]
[1]Department of Pedodontics, [2]Department of Restorative Dentistry, Faculty of Dentistry, Selcuk University, Konya, Turkey

For correspondence: **Email: drfirdevskahveci@hotmail.com*

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Abstract

Purpose: *To evaluate the effects of herbal-based toothpastes with different contents on the viability of L929 cells.*
Methods: *Herbal-based toothpastes {Aloe vera - propolis herbal toothpaste, Group 1; Parodontax, Group 2; Toothpaste with miswak, propolis and tea tree extract, Group 3, Dent natural protective toothpaste with clay, Group 4; Gano fresh, Group 5} were diluted (1:1, 1:2, 1:4, 1:8, 1:16, and 1:32) in Dulbecco's modified Eagle's medium. L929 fibroblast cells were treated with the medium containing the herbal toothpastes for 2 min. Cell viability was assessed using methyl tetrazolium test. The viability of the negative control group was set at 100 %, and the percentage viability of all groups was determined accordingly.*
Results: *Cell viability was significantly reduced at all dilutions in Group 5 ($p = 0.00$). This trend was also observed in Group 4. All dilutions except 1:32 significantly affected cell viability ($p = 0.00$). In Group 2, only the 1:1 dilution showed a toxic effect ($p = 0.00$). The samples in Groups 1 and 3 did not show statistically significant cytotoxicity to L929 cells ($p > 0.05$).*
Conclusion: *Herbal toothpastes containing substances such as sodium lauryl sulfate (Group 5) and sodium benzoate (Groups 2 and 4) are cytotoxic towards L929 cells. Groups 1 and 3 did not contain detergents but contain potassium sorbate as a preservative; hence, they are not toxic.*

Keywords: *Cytotoxicity, Herbal toothpaste, Sodium lauryl sulfate, Sodium benzoate, Potassium sorbate*

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INTRODUCTION

The toothpaste is a necessary and integral part of an effective oral hygiene. Many types of toothpastes with different contents have been developed for different oral hygiene needs. Toothpastes are often used prophylactically for protection from dental caries, help remove biofilms from teeth and gums, add flavor to make brushing more enjoyable, remove unwanted odors in the mouth, strengthen teeth, and treat tooth sensitivity [1,2]. Toothpastes generally contain both active and passive ingredients. Active ingredients offer specific therapeutic benefits while inactive ingredients maintain the composition of the toothpaste and sensory appeal, such as taste and smell [3].

The toothpaste is the most accessible and widely used product for oral hygiene and health in all populations. In general, toothpaste can prevent dental caries, plaque, and gingivitis [2]. Local effects of toothpaste occur on hard tissues, particularly due to improper use. Hard tissue

damage can be seen as mechanical wear, which is most evident in the enamel and dentin. Additionally, soft tissue reactions may occur immediately or after prolonged exposure. Therefore, there is growing public interest in herbal toothpastes that may not contain harmful substances [3,4]. Herbal toothpastes can have many antimicrobial, antifungal, antiseptic, anti-inflammatory and analgesic effects [5]. Studies have mostly focused on the antimicrobial properties [6]. It is inaccurate to assume that herbal medicinal products are “safe” because they are obtained from “natural” sources [7]. Therefore, consumers need to be informed about the ingredients in products claimed to be “herbal.” Herbal toothpastes have different contents and therefore, the effects of these constituents in human including their cytotoxic effects should be evaluated. The purpose of this study was to evaluate the responses of L929 murine fibroblast cells to herbal-based toothpastes with different contents *in vitro*. The null hypothesis tested is that toothpastes containing different herbal ingredients do not have a significant effect on the viability of the L929 murine fibroblast cells.

EXPERIMENTAL

Materials

The tested herbal-based toothpastes are presented in Table 1. Herbal-based toothpastes were diluted in suitable medium to 50 %, homogenized using a vortex (WisemixVM-10; Daihan Scientific Co., Ltd., Seoul, South Korea), centrifuged (Hettich 320R Centrifuge, Germany), filter-sterilized, and immediately used in the experiments. The original extracts (1:1) were further diluted to 1:2, 1:4, 1:8, 1:16, and 1:32 in the medium (Figure 1).

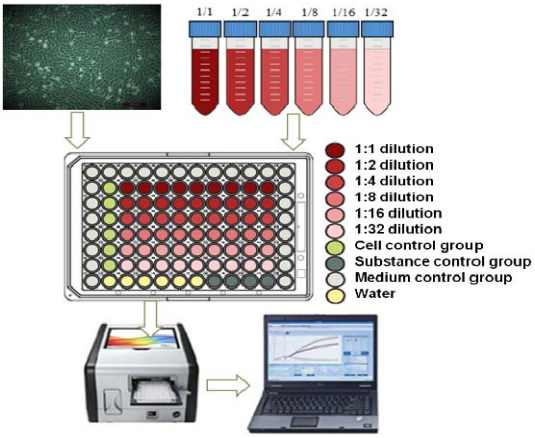

Figure 1: Experimental design

Herbal ingredients of each group

Group 1: *Aloe barbadensis* gel, *Salvadore persica* powder, Carrageenan, Propolis cera, Tea tree extract

Group 2: Mint, Mirra, Chamomile, Sage, Ratania, *Echinacea* herbs, and sodium bicarbonate

Group 3: *Salvadore persica* extract, Propolis, Menthol, Tea tree extract

Group 4: *Commiphora myrrha* extract, *Krameria triadra* extract, Propolis cera, *Citrus grandis* extract, *Camellia sinensis* extract, *Chamomille recutita* extract, Menthol, *Stevia rebaudiana* leaf powder, Alcloxa, *Vitis vinifera* seed oil

Group 5: *Ganoderma lucidum* extract

Cytotoxicity testing using L929 cells

The L929 murine fibroblast cells (ATCC CCL 1) were cultivated in 10 % fetal bovine serum, streptomycin (150 µg/mL), and penicillin (150 IU/mL) supplemented with Dulbecco’s modified Eagle’s medium in a humidified atmosphere with 5 % CO_2 at 37 °C. The L929 cells were seeded onto 96-well plates at a density of 2×10^4 cells, incubated for 24 h, and exposed to 100 µL of the toothpaste dilutions. Serum-free cell culture medium was used as a negative control. After 2 min, cell survival was evaluated by assessing enzyme activity using 3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide (MTT) assay, where mitochondrial dehydrogenases in living cells reduce the yellow tetrazolium salt MTT to blue formazan. After incubation, the cells were exposed to freshly prepared MTT solution (0.5 mg/mL) for 2 h at 37 °C. The blue formazan precipitate was then dissolved in dimethyl sulfoxide in a shaker for 30 min at 23 °C. The absorption was determined using a spectrophotometer at 540 nm (Epoch BioTek, Winooski, VT, USA). Three independent experiments were performed in four wells (n = 12 per group). The viability of the negative control group was calculated as 100 %, and the percentage viability of all groups was determined accordingly (Figure 1).

Statistical analysis

Statistical analyses were performed using IBM SPSS (version 22.0; IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to test the normal distribution of the data. Statistically significant differences between the tested groups were determined using one-way analysis of variance. Statistical differences between the

groups were compared using the post-hoc Tukey test with $p < 0.05$ considered statistically significant.

RESULTS

The cytotoxicity findings obtained using the herbal toothpastes are summarized in Figure 2. The cell survival values of L929 fibroblasts are expressed as a percentage of cell viability, with untreated cells acting as a negative control (normalized to 100 % cell viability). The L929 fibroblast cell line was exposed to toothpaste extracts for 2 min. Cell viability at all dilutions in Groups 1 and 3 was not significantly different from that of the negative control group ($p > 0.05$). All dilutions of group 5 significantly reduced the cell viability ($p = 0.00$). The same trend was observed in Group 4; all dilutions, except the 1:32 dilution, significantly reduced cell viability ($p = 0.00$). In Group 2, only the 1:1 dilution showed a toxic effect ($p = 0.00$).

DISCUSSION

The use of herbal medicines and hygiene products continues to spread rapidly worldwide, with many turning to them for health reasons [7]. There is no professional consensus regarding the use of herbal dental products. The results of this study indicate that natural ingredients used in toothpaste may show toxic effects, which may increase in the presence of certain ingredients, specifically sodium lauryl sulfate (SLS). Additionally, sodium benzoate-containing toothpastes have toxic effects. In the current study, the toothpastes in Groups 1 and 3 were not cytotoxic, while the other groups showed cytotoxic effects on L929 cells, partially proving our hypothesis.

Ganofresh contains the extract of *Ganoderma lucidum*, which is a traditional medicinal fungus widely used in tumor therapies. However, Ganofresh toothpaste contains SLS and was cytotoxic to cells in this study for all the concentrations tested. Similarly, Cvikl *et al* found that SLS-containing toothpastes were cytotoxic to L929 fibroblasts [8].

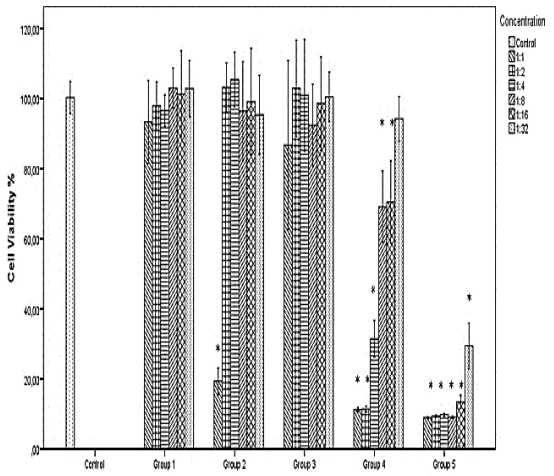

Figure 2: Effects of the original extract (1:1) and concentrations (1:2, 1:4, 1:8, 1:16, and 1:32) of herbal-based toothpastes on the viability of L929 cells 2 min post-exposure. *Indicates statistically significant difference compared to the control group ($p < 0.01$)

Table 1: Chemical composition of herbal-based toothpastes

Herbal-based toothpaste	Contents
Group 1: *Aloe vera* propolis herbal toothpaste	Calcium carbonate, *Aloe barbadensis* gel, Sorbitol, Glycerin, Sodium silicate, *Salvadore persica* powder, Carrageenan, Propolis cera, Decyl glucoside, Aroma (Flower), Menthol, Tea tree extract, Potassium sorbital
Group 2: Parodontax	Sodium bicarbonate, Glycerin, Cocamidopropyl betaine, Alcohol, *Krameria triadra* Extract, *Echinacea purpurea* flower/leaf/stem juice, Alcohol denat., Xanthan gum, *Chamomilla recutita* extract, *Commiphora myrrha* extract, Sodium fluoride, Sodium saccharin, Sodium benzoate, *Salvia officinalis* oil, *Mentha piperita* oil, *Mentra arvensis* oil, Limonene, Cl 77491
Group 3: Toothpaste with miswak, propolis and tea tree extract	Calcium carbonate, Sorbitol, Glycerin, *Salvadore persica* extract, Propolis, Decyl Glucoside, Aroma, Menthol, Tea tree extract, Potassium sorbate
Group 4: Dent natural protective toothpaste, full whiteness protection with clay	Calcium carbonate, Sorbitol, Propylene glycol, Peg-400, Hydrated silica, Disodium phosphate, Glycerin, Xanthan gum, Shea butter, amidopropyl betaine, Aroma, Clay, Titanium dioxide, Sodium bicarbonate, *Salvia officinalis* extract, *Commiphora myrrha* extract, *Krameria triadra* extract, Propolis cera, *Citrus grandis* extract, *Camellia sinensis* extract, *Chamomille recutita* extract, Menthol, *Stevia rebaudiana* leaf powder, Alcloxa, *Vitis vinifera* seed oil, Sodium benzoate
Group 5: Gano fresh	Sorbitol, Glycerin, Silica powder, Sodium lauryl sarcosinate, Mint flavor, Xanthan gum, Sodium lauryl sulfate, *Ganoderma lucidum* extract, Methyl paraben, Brilliant blue, Quinoline yellow

Moore *et al* reported that detergents, the key component of toothpastes, are associated with *in vitro* cell membrane disruption, which is consistent with the findings of other *in vitro* studies that revealed that incubation with SLS for 2 min reduced TERT-1 keratinocyte viability [9]. Ghapanchi *et al* investigated the *in vitro* cytotoxicity of 16 types of commercially available toothpastes on primary epithelial cells, and all the SLS-containing tested toothpastes showed various toxic effects on cultured cells [1].

In the present study, two of the tested toothpaste groups (Groups 2 and 4) contained sodium benzoate. These toothpastes exhibited varying degrees of cytotoxicity. Sodium benzoate is a preservative and fungistatic/bacteriostatic agent used under acidic conditions [10]. Mpountoukas *et al* have studied the cytotoxic, genotoxic, and cytostatic potentials of sodium benzoate in human peripheral blood cells *in vitro*. They concluded that sodium benzoate did not induce cytotoxicity and was nongenotoxic at low concentrations [11] thus supporting the results of this study this reveals that no toxic effects were observed in groups 2 and 4 at low concentrations. In another study, it was observed that the effects of sodium benzoate on cultured human peripheral lymphocytes decreased and the mitotic index values and chromosome aberrations increased in a dose-and time-dependent manner [12]. Extracts of toothpastes from other groups (Groups 1 and 3) did not affect L929 cell viability. Notably, the toothpastes of these groups did not contain any detergents. Another remarkable feature of these two toothpastes is the presence of potassium sorbate (potassium salt of sorbic acid), which is frequently used as a preservative in the food industry. It is used as a fungistatic and bacteriostatic agent in various processed food products, such as cheese, fish, and baked foods, and cigarettes [13]. Mohammadzadeh-Aghdash *et al* reported that potassium sorbate did not show significant cytotoxic or genotoxic effects and are safe for use in the food industry at low concentrations [14]. The results of this present study indicate the promising potential for preservatives, such as potassium sorbate, to be used as substitute for sodium benzoate.

Cell culture tests to determine dental material-induced cell damage with varied cell types have been reported in the literature [1,8,17,18]. In this study, the cytotoxic reaction of L929 cells after exposure to toothpastes was evaluated using the standard MTT assay, which is a well-established method for analyzing cell viability. In the MTT assay, cell proliferation and viability are assessed by measuring the physiological state of cell mitochondria. Mitochondrial dehydrogenases in living cells reduce the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide) to blue formazan, which is retained in the cell. The formation of formazan crystals correlates well with living cell numbers. To measure the absorbance, the formazan must be dissolved. For this, dimethylsulfoxide and isopropanol were used in this study. The dissolved formazan color was measured by spectrophotometry at 540 nm. The reduction of tetrazolium compounds to formazan compounds occurs through mitochondrial activity; therefore, dead cells cannot perform this reaction [15]. The MTT test is a suitable *in vitro* method to evaluate the cytotoxicity of dental materials [16]. Recent *in vitro* studies on cell viability, cytotoxicity, and genotoxicity have revealed potential adverse effects of toothpaste ingredients [1,8,17,18]. However, the oral cavity environment differs from *in vivo* conditions, and factors such as the presence of saliva, blood flow, mucus layer, creatinine levels, and bacterial flora can protect the oral environment from harmful effects [17].

Further studies are required to investigate the relevance of in vitro studies to the target tissues and environment of the oral cavity. However, the cell culture is a convenient tool for studying the mechanisms of variable reactions. As an alternative, data from cytotoxicity tests, cell culture models, or implantation studies should be used to determine the biocompatibility of toothpaste.

CONCLUSION

Herbal toothpastes containing substances such as sodium lauryl sulphate and sodium benzoate show cytotoxic effects while toothpastes that do not contain detergents but rather contain potassium sorbate as preservative are not cytotoxic. This study provides guidance on whether potentially biocompatible ingredients could be found in herbal toothpastes. Therefore, when choosing toothpastes, including those with herbal ingredients, it is important for consumers to pay attention to their ingredients.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by them.

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