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Cytotoxicity of toothpastes used for gum health

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Abstract:Objective: The aim of this study was to evaluate the cytotoxic effects of 4 different toothpaste on the L929 mouse fibroblast cells.**Materials and Methods: Cytotoxicity of** Colgate Total Pro Gum Health, Ipana Pro Expert Clinic Line Gum Protection, Paradontax Compelete Protection and Paradontax Extra Fresh toothpastes evaluated by XTT assay on L929 cells **Results:** Different degrees of cytotoxicity were observed in toothpastes. Mean survival rate of L929 cells exposed to Colgate Pro Gum Health in wells were 57,25%, 56,71% for Ipana Pro Expert Clinic Line Gum Protection, 45,05% for Paradontax Complete Protection and 37,49% for Paradontax Extra Fresh. **Coclusion:**Toothpastes, marketed for gum health are cytotoxic. Paradontax Complete Protection and Paradontax Extra Fresh more cytotoxic than Colgate Total Pro Gum Health and Ipana Pro Expert Clinic Line Gum Protection to L929 cells. We recommend manufacturers to review the contents of their toothpastes.

Keywords:*toothpastes, biocompatibility, cytotoxicity, XTT, L929*

I. INTRODUCTION

Dental biofilm should be effectively removed to preserve oral health because it is defined as a critical factor in the etiology of caries, gingivitis and periodontitis(1-3). Daily tooth brushing with toothpaste and using dental floss is the most frequently recommended method to remove supragingival dental biofilm (4).

Although toothpastes are oral hygiene products that are used with toothbrushes to prevent tooth decay and maintain gingiva health, they can also be used for gum diseases, tooth whitening, prevention of teeth formation, halitosis and dentin sensitivity.Gum health toothpaste fights plaque by breaking it apart and killing plaque bacteria. This helps prevent gingivitis, which can lead to bleeding gums (5).

Considering the multifactorial effects of today's toothpastes, we can say that these toothpastes contain a lot of ingredients. Improper use of toothpastes with toothbrushes can lead to tooth wear, gingival recession, and consequently dentine sensitivity(6). On the other hand, some substances in the toothpastes may cause adverse effects such as inflammation, desquamation, aphthous ulcers and allergy in oral tissues(7-11).

The aim of this study was to evaluate the cytotoxic effects of four different toothpaste, which are marketed under the name of gum care, on the cytotoxic effects of L929 mouse fibroblast cells.

II. MATERIALS AND METHODS

L-929 cells were cultivated in DMEM (Dulbecco's modified Eagle medium) supplemented with 10% FBS (fetal bovine serum), penicillin (150 IU/mL), and streptomycin (150 µg/mL) at 37°C and 5% CO_2. The L-929 cells were seeded at a density of 2×10^4 into each well of a 96-well plate and incubated for 24 hours at 37°C.

The following toothpastes were used: Colgate Total Pro Gum Health, Ipana Pro Expert Clinic Line Gum Protection, Paradontax Compelete Protection, Paradontax Extra Fresh. 14 wells were used for each toothpaste. Toothpastes were diluted in serum-free medium (50 w/v%) and were shaken vigorously, filter sterilized, and used immediately in the experiments. Then the cell cultures were exposed to 100 of toothpaste mixture or medium (as a negative control) for 2 min.

Cell cultures were washed with PBS (Phosphate Buffered Saline), fixed with 1% glutardialdehyde and 200 µl freshly prepared XTT solution was added to each well and incubated for 2 h at 37°C.The spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments) was used to measure the cell culture plates at a wavelength of 460 nm.The readings obtained from the control group wells were averaged. Readings from the wells of tested toothpastes were proportioned to this control group average value.

Toothpaste and manufacturer	**Ingredients**
Colgate Total Pro Gum Health *Colgate-Palmolive Company*	purified water, glycerol, silica dental type, sorbitol liquid (70%) non-crystalising, Poly (methyl vinyl ether) maleic acid, sodium hydroxide (25% solution), sodium laurylsulfate, mint flavour (contains propylene glycol), carmellose sodium, titanium dioxide (E171), Iota carrageenan, saccharin sodium, sodium fluoride 0.32% w/w (1450ppm F^-), Triclosan 0.3%w/w
Ipana Pro Expert Clinic Line Gum Protection *Procter & Gamble GmbH*	aqua, glycerin, hydrated silica, sodium hexametaphosphate, PEG-6, propylene glycol, zinc lactate, sodium gluconate, CI 77891, sodium lauryl sulfate, silica, aroma, sodium saccharin, Chondrus crispus powder, trisodium phosphate, stannous chloride, xanthan gum, stannous flüoride (1100 ppm F^-), sodium floride (350 ppm F^-).
Paradontax Complete Protection *GlaxoSmithKline plc*	aqua, glycerin, sodium bicarbonate, hydrated silica, sodium lauryl sulphate, aroma, xanthan gum, cocamidopropy betaine, sodium saccharin, titanium dioxide, steviol glycosides, limonene, Cl 77941, sodium floride(1400ppm F^-)
Paradontax Extra Fresh *GlaxoSmithKline plc*	aqua, glycerin, sodium bicarbonate, alcohol, cocamidopropyl betaine, Mentha arvensis oil, Mentha piperita oil, xanthan gum, Echinacea purpurea flower/leaf/stem juice, Krameria triandra extract, aroma, chamomilla recutita extract, Salvia officinalis oil, Commiphora myrrha extract, sodium saccharin, limonene, linalool, Cl 77491, sodium floride (1400 ppm F^-)

Table 1: Tested toothpastes, manufacturers and its ingredients.

Statistical analyses were performed with IBM SPSS Statistics (v. 25). The Shapiro–Wilk test was used to evaluate the homogeneity of variables. One-way analysis of variance and post hoc Tukey's tests were used to compare VHN and RN data. The Kruskal–Wallis and Mann–Whitney U-tests were used to analyze cytotoxicity data. The significance level was set to ($p<0.05$).

III. RESULTS

Different degrees of cytotoxicity results were determined in the experiments. The distribution of data obtained from our study is shown in the Fig. 1.

Mean survival rate of L929 cells exposed to Colgate Pro Gum Health in wells were 57,25% (min %41,63, max %78,41). The mixture was statistically different from the negative control group.

Mean survival rate of L929 cells exposed toIpana Pro Expert Clinic Line Gum Protection in wells were 56,71% (min %32,34, max %79,75). The mixture was statistically different from the negative control group.

Mean survival rate of L929 cells exposed to Paradontax Complete Protection in wells were 45,05% (min %36,14, max %43,87). The mixture was statistically different from the negative control group.

Mean survival rateof L929 cells exposed toParadontax Extra Fresh in wells were 37,49% (min %20,36, max %56,76). The mixture was statistically different from the negative control group.

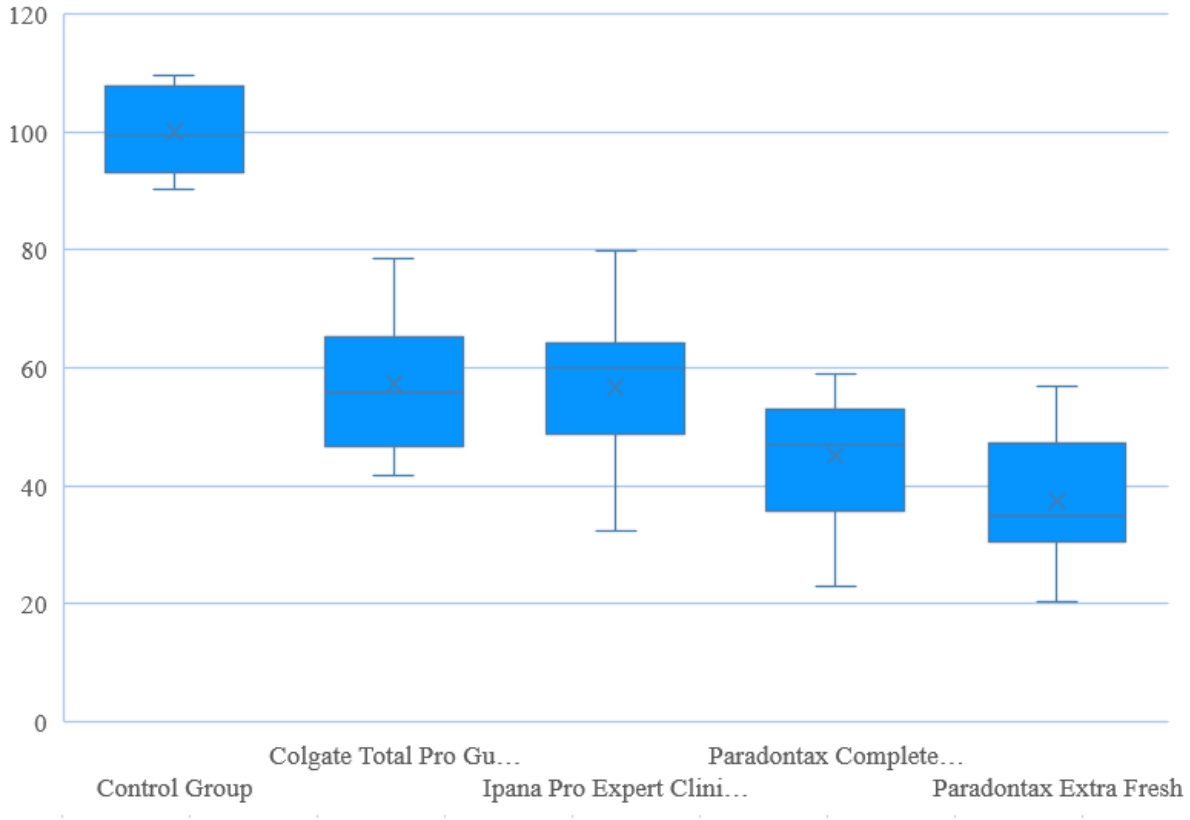

Figure 1: Distrubition of percentage of the viable cells in wells.

IV. DISCUSSION

In ancient times, toothpastes or more accurately toothpowders made of powdered bone, eggshells, pumice and herbs came without much change until the 19th century.In the early 1800s, glycerin was added to the dental powders to form toothpastes.In 1824, for the first time, soap was added to the toothpastes to increase the cleaning efficiency and was then replaced with sodium lauryl sulfate.For the first time in 1873, toothpastes began mass production produced by Colgate and sold in jars.. Later in 1892, toothpasteshave entered into squeezable tubes for firs time by Dr. Washington Sheffield.(12).

Today's toothpastes help to provide oral hygiene, but they also become multifactorial products that can be used in tooth whitening, to prevent tartar formation, to eliminate bad breath and dentin sensitivity. In order to achieve these effects, many different ingredients have been introduced into the toothpastes(12).

Although toothpastes have very beneficial effects such as helping to remove dental biofilm, increasing intra-oral pH, and having antibacterial and anti-carious effects, they also have possible adverse effects(5). Sodium lauryl sulphate used as detergent in toothpastes may cause desquamation in the oral mucosa and aphthous ulcer and allergy(8, 13, 14).Toothpastes are intended for topical use but may be swallowed by children, especially by under six years of old who have not fully developed the swallowing reflex, and fluorosis may develop due to fluoride in the content of toothpastes(15). Fluoride also can interact with a wide range of cellular processessuch as, proliferation and migration, respiration, ion transport, apoptosis/necrosis, and oxidative stress, and that these mechanism are involved in a wide variety of signaling pathways(16). Triclosan,

an antibacterial agent, has been shown to affect thyroid and estrogen metabolism in animal experiments.(17, 18). Essential oils, (peppermint, anethole, cinnamon, cloves etc.) can cause cheilitis or circumoral dermatitis (19). Rarely allergic rhinitis (20) or asthma (9) may occur. Therefore, toothpastes should be tested for their biological behavior before being used clinically.

Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy (21). Although toothpastes are considered as cosmetic products rather than dental materials, they still interact with living tissues and therefore these products should be biocompatible.Biocompatibility can be measured with 3 types of biologic tests: in vitro tests, animal tests, and usage tests. In vitro tests have the advantages of being experimentally controllable, repeatable, fast, relatively inexpensive, and relatively simple. In addition, these tests generally avoid the ethical and legal issues that surround the use of animals and humans for testing. (22).

Continuous cell lines frequently used in in vitro cytotoxicity studies are mouse fibroblasts (L929, 3T3) or human epithelial cells (HeLa)(23). L929 cells respond similarly to human fibroblast cells against ions released from dental materials(24).Therefore, in our study, L929 mouse fibroblasts were selected for use in cell cultures.To evaluate the cytotoxicity of dental materials LDH (lactate dehydrogenase) test, WST-1 [2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium]assay, MTT [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide]assay, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay, resazurin reduction test or fluorescence tests can be used(25).The MTT test has been shown to be a suitable in vitro method for assessing the cytotoxicity of dental materials(26).It has therefore become a standard test commonly used to assess the cytotoxicity of new biomaterials. For the XTT test, a different tetrazolium reduction test developed later, the formazan thawing step in the MTT assay was eliminated. Thus, it is possible to make cytotoxicity tests faster and easier (27, 28). Due to these advantages, XTT assay method was used to evaluate the cytotoxic effects of the related materials in our study.

Our results show that all of the toothpastes marketed for the gum health we tested are cytotoxic on L929 cells.($p<0,05$). The degree of cytotoxicity may vary among toothpastes.The minimum survival rate of the toothpastes for the gum health we tested belongs to Paradontax Extra Fresh.Mean survival rate of L929 cells treated with Paradontax Extra Fresh in wells is 37,49%. For Paradontax Complete Protection, mean survival rate is 45,05%. However, as a result of the statistical evaluation, it was seen that there was no significant difference in cytotoxicity between these two toothpaste. Mean survival rate of L929 cells treated with Ipana Pro Expert Clinic Line Gum Protection in wells is 56,71%. The difference between Ipana Pro Expert Clinic Line Gum Protection and Paradontax toothpastes (Extra Fresh, Complete Protection) is statistically significant, and Ipana Pro Expert Clinic Line Gum Protection is more biocompatible. Mean survival rate of L929 cells treated with Colgate Pro Gum Health in wells is 57,25%.Statistical analysis revealed no significant difference between Colgate Total Pro Gum Health and Ipana Pro Expert Clinic Line Gum Protection.

The cytotoxicity of toothpastes has also been the subject of some previous research.Cvikl et al. stated that the toxicities of toothpastes were in close contact with the detergents they contained and that toothpastes containing sodium lauryl sulphate and amine fluoride were more cytotoxic(29).Only Parodontax Extra Fresh does not contain sodium lauryl sulphate from the toothpastes we use in our study.The lowest survival rate was seen in the cell culture group treated with Paradontax Extra Fresh toothpaste. (37,49%). However, there was no statistically significant difference between the Parodontax Extra Fresh and thesodium lauryl sulphate containing Parodontax Complete Protection (45.05%) ($p>0,05$). The difference between the contents of these two toothpaste is shown in Table 1.This suggests that cytotoxicity of toothpastes may also be affected by contents other than sodium lauryl sulphate.

The clinical relevance of the in vitro data presented has to be interpreted with caution. Oral cavity condition differs from in vitro status and many factors such as saliva, mucus layer, creatine levels, blood flow, and normal flora can influence the oral cavity protection from harmful materials(30).

V. CONCLUSION

Within the limitations of this study, it can be concluded that the tested toothpastes, marketed for gum health are cytotoxic. We recommend that this type of pastes should not be kept in the mouth for long periods of time.

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