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Detection for toxigenic side effects of toothpaste

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Abstract---The most common daily home oral care product with Toothpastes like an important oral hygiene habit for tooth care with many benefits to dental and gingival health. In fact, the components is always found in toothpastes are almost binding agents, gelling, abrasive, humectant and surfactant, toothpaste components might cause bacterial resistance and damage fluoride as well as others problems in the presence or concentration of toothpastes components may cause an expected lesions. This research has taken care to evaluate possible oral bacterial fluoride resistance or DNA degradation to oral epithelial cells exposed for one kind of toothpaste has a high treating qualities considering in people uses .

Keyword---detection, toxigenic, toothpaste, DNA degradation.

Introduction

Mainly toothpaste components, are frequently used in removing plaque, due to their antimicrobial properties like professional dental treatments contain fluoride to prevents tooth decay with mouth washes and making more resistant to the action of acids. Although , fluoride like one component of toothpastes can stopped teeth decay beginning and ,other side its making teeth more resistant to the action of bacteria and foods acidity.[1]. Previous reports even show that in some cases. Bacteria have changed to have genetic modification abilities to withstand certain levels of fluoride [2], it mean Fluoride-resistant strains of several oral bacterial species, including *S. mutans*, *Streptococcus salivarius*, and *Streptococcus sanguinis* tested in laboratories [3]. wherever, several experiments have detected a fluoride-resistant strain is able to grow in an environment containing 400–1,000 ppm (21.1–52.6 mM) of fluoride, depending on the strain adaptation. Fluoride-resistance strains have tolerated at least three times higher than that which fluoride-sensitive strains could.[4]

There are many mechanisms of acquired and characteristics of fluoride-resistant strains , the mode of antimicrobial fluoride action against *S. mutans* must be known. One mechanism to do stable fluoride resistance is believed genetic modification such as chromosomal mutations. The studies was showed that such toothpastes produce a change in the topography of tooth enamel due to the increased abrasive ness, the accession of the biocompatibility of these toothpastes becomes particularly important. [5]. The presence of treating toothpaste components and different abrasives may cause alterations in the tooth surface. Addition , the components when exposure into contact with cells of the oral tissues at the long time of brushing it might be can caused cytotoxic and genotoxic effects . [6]. Genotoxic effects could detect when used cellular DNA analyses can be performed in vitro are able of detecting genetic damaged including breaks in the DNA strand, genetic mutations, chromosomal breakage and alterations of the DNA repair process and other genotoxicity .[7] This study emphases on Toothpaste effects should be tested for their biological behavior before being used in vitro and in vivo. For this purpose, One fluoride resistance gene was detected in oral *Streptococcus mutans AA* and experiments on animals tissues should be performed for genotoxicity lesion and bacterial chromosomal mutaions in long exposure uses period .

Materials and Methods

Identification of *S. mutans* resistance for toothpaste
Identification of fluoride-resistance-related gene in *S. mutans*

The fluoride resistant of *Streptococcus mutans* stains were examined by culturing to an optical density at (OD_{600}) (2 * 10^7 cells/ml) of the bacteria by streaking the nutrient agar plates containing gradient concentrations of sodium fluoride (500,1000,1500, 2000) ppm of sodium fluoride per ml) in order to show two different levels of fluoride resistance. Plates were incubated at 37°C for 48 hr., pretended colonies were indicated to positive result. The higher resistance colonies for fluoride was selected and inoculated in broth culture with concentration of sodium fluoride equal to 2000 ppm/ml, then bacteria were incubated until early exponential phase at 37C°. To evaluate bacterial proliferation at (OD_{600}) (4 * 10^5 cells/ml), single colonies detected like *S. mutans AA* was using *16S rRNA* gene was amplified by 28 cycles of denaturing at 94°C (30 s), annealing at 55°C (30 s), and elongation at 72°C (45 s) . [8]

Identification of *FR* gene in *Streptococcus mutans AA* strain

The resistance *Streptococcus mutans* colonies were selected from higher fluoride resistance concentration plates have 1500 ,2000 ppm/ml NaF, a DNA extraction was employed to amplify *FRA* gene by specific-gene primer by conventional (PCR) with *FR* gene primers (fig. 2):

F. (5'-CATATGCTTAATCCCCATCTAATGCT- '3)
R. (5'-GCATGCACTGATATTACTGGCTATTTA- '3).

The PCR reaction consist of (25 µl) PCR reaction mixture , it was begun with 4-min hold at 94°C denaturation, gene fragment was amplified with 32 cycles of

denaturing at 94°C (30 s), annealing at 55°C (30 s), and elongation at 72°C for 45 s . (fig.1). [9]

Oral epithelial tissues exposure for toothpaste solution

Toothpaste brand has a high treating qualities like solution was using for oral epithelial tissues of 3 rabbit samples exposure for 12 months , used for each samples 20 ml of 95% concentration of toothpaste solution [10] . Oral epithelial tissues were sampled in this period tested to detect chromosomal damages that they were analyzed by DNA extraction and separated on 1% agarose gel comparted with control (control is oral epithelial tissues was saving in 70% ethanol).[11]

The results

Fluoride resistance gene amplification in *S. mutans AA*

Streptococcus mutans AA was performed to confirm fluoride resistance gene by bacterial identification an amplification of specific Primers for conserved region of fluoride resistance genes were designed and were used for gene amplification with PCR, 1% agarose gel used for PCR products to separate on (Fig. 2), all results demonstrated that *S. mutans* had hypothetical gene band with 400bp.

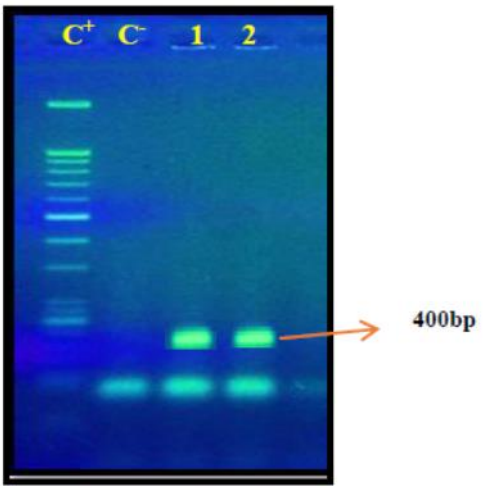

Figure 1. Agarose gel electrophoresis for amplification of *FR* gene of *S. mutans* on (1%) agarose gel, 70 voltage for 90 minutes. Lane (C^+): DNA marker Ladder(100bp). Lane (C^-): Amplicon *FRA* gene primer with *S. salivarious* . Lane (1,2): Amplicon of *FRA* gene (125bp) with resistance *S. mutans strains*.

Cytogenetic damage

The comet test was more sensitive than the other tests, showing extracted DNA are damaged in slight bands and decline amounts of oral epithelia tissue for each

3 rabbit samples after have saved at 10 ml concentrated toothpaste solution for 12 months compared with control. (fig.2,3).

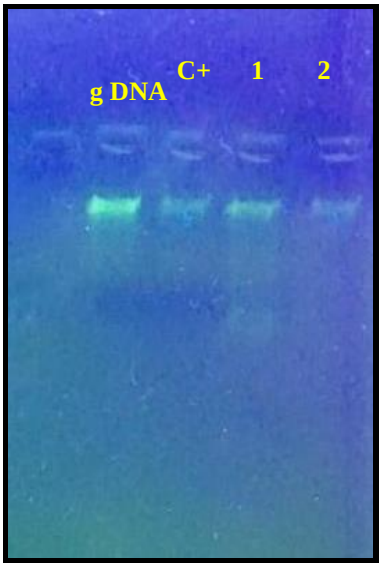

Figure 2. Agarose gel electrophoresis for DNA extracting on (1%) agarose gel, 70 voltage for 90 minutes. (C^+) lane : Bright band consist of rabbit oral epithalial tissues was saved in 70% ethanol for 12 months {control} . Lane (1,2,3): Slight bands of extracted gDNA dameging of rabbit oral epithalial tissues after exposre in 10 ml concentrated toothpaste solution for 12 months

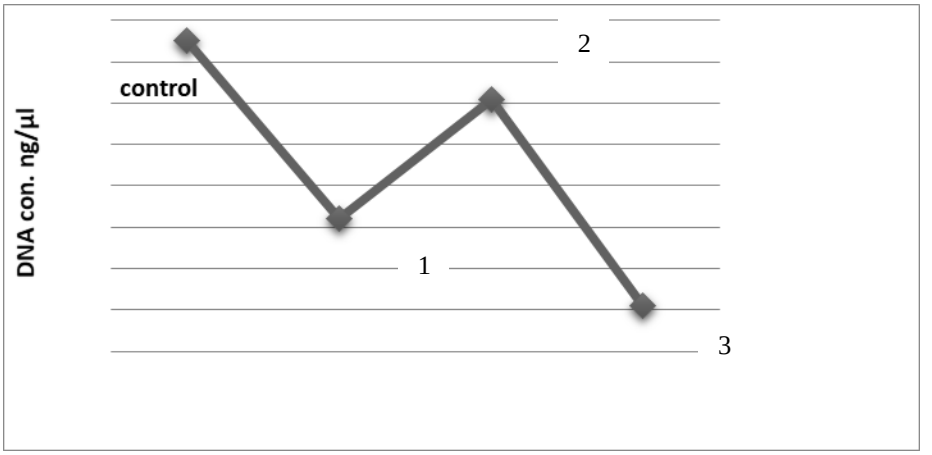

Figure 3. extracted DNA quantity ng/µl . : (control) amount of extracted DNA from oral epithalial tissues of rabbit was saved in 70% ethanol for 12 months . samples (1,2,3) extracted DNA become less amounts of oral epithalial tissues of rabbits after exposre in 10 ml concentrated toothpaste solution for 12 months compared with control .

Conclusions

The lesion effects of toothpastes have been examined on multiple genetic modification terms . This work was showed *Streptococcus mutans AA* is fluoride-resistant strain was selective in in vitro used culturing for mother strain in media with high concentrations of fluoride to detect cariogenic bacterial species detects to fluoride resistance development in this specie through identify one *FRA* gene analyzed like had hypothetical gene to showed chromosomal mutations lead to fluoride-resistant strains.[12]. As well as , based on the results of other toothpaste genotoxicity, it can be concluded in the use of certain higher treating quality kinds of toothpaste may cause a limited biologically insignificant genotoxicity effect on oral epithelial cells pretended in DNA damage at normal doses .[12,13]

References

1. "Assessment of cytotoxic and genotoxic effects of conventional and whitening kinds of
2. Antonija Tadin, Lidia Gavic, Ana Zeravica, Klara Ugrin, Nada Galic, Davor Zeljezic.
3. Atheer A. M., Mohammed L.,Wsnaa J. M. Exploration of a new Fluoride resistance gene (*FRAM gene*) in oral *Streptococcus mutans MAW* . Research Journal of Pharmacy and Technology . 2021 ;14(1).
4. Burne, R. A., and Marquis, R. E. . Alkali production by oral bacteria and protection against dental caries. *FEMS Microbiol. Lett.*2000 ; 193, 1–6.
5. Cytotoxic Effects of Different Detergent- Containing Children's Toothpastes on Human
6. Darling, A. E., Mau, B., and Perna, N. T. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* ,2010 l5:e11147.
7. Deepak S., Yihong Li, and page W. C. Identification of Unique Bacterial Gene Segments from Streptococcus mutans with Potential Relevance to Dental Caries by Subtraction DNA Hybridization. J. of clinical microbiology, 2005 ;43(7) : 3508–3511.
8. Del Real G., Saldaña-Velasco FR , Sánchez-de la R. SV, Ortiz-García YM, Morales-Velazquez G, Gómez-Meda BC, Zúñiga-González GM, Sánchez-Parada MG, Zamora-Perez In vivo evaluation of the genotoxicity and oxidative damage in individuals exposed to 10% hydrogen peroxide whitening strips. AL.Clin Oral Investig. 2019 ;23(7):3033-3046.
9. Gingival Epithelial Cells", BMC Oral Health (2022) ;22:66
10. Jannan G., Fereshteh K., Afagh M., Sara Poorshahidi, E. S, Fahimeh R, Hooman K, and Samira J. *In Vitro* Comparison of Cytotoxic and Antibacterial Effects of 16 Commercial Toothpastes . J Int Oral Health. 2015 ; 7(3): 39–43.
11. Jeremy A. H. , Jason M. T., and Peter M. M. Fluorides and Other Preventive Strategies for Tooth Decay. Dent Clin North Am. 2018; 62(2): 207–234.
12. Liao Y, Brandt BW, Li J, Crielaard W, Van Loveren C, Deng DM. Fluoride resistance in *Streptococcus mutans*: a mini review.. J Oral Microbiol. 2017 ; 6;9(1):1344509
13. Liao Y, Yang J, Brandt BW, Li J, Crielaard W, van Loveren C, Deng DM. Genetic Loci Associated With Fluoride Resistance in *Streptococcus mutans*. Front Microbiol. 2018 ; 11;9:3093

14. Sigmar de Mello R, Tabata do Prado S . , Felipe de Souza M. , Ayla Macyelle de O. C , Samira E.and Afonso C Toxicity and effect of whitening toothpastes on enamel surface Braz Oral Res, 2021 ; 12;35:e025.
15. Sinem Birant, Yazgul Duran, Tunc Akkoc, Figen Seymen. "In Vitro Investigation of the
16. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
17. Tabatabaei MH, Mahounak FS, Asgari N, Moradi Z. Cytotoxicity of the Ingredients of Commonly Used Toothpastes and Mouthwashes on Human Gingival Fibroblasts. Front Dent. 2019 ;16(6):450-457
18. toothpaste on oral mucosa cells", Acta Odontologica Scandinavica, 2017 ; 76 (1): 64-70
19. Xiaochen Men, Yukie Shibata, Toru Takeshita,Yoshihisa Yamashita. "Identification of Anion Channels Responsible for Fluoride Resistance in Oral Streptococci", PLOS ONE, 2016; 11(11): e0165900.
20. Yuriah, S., Kartini, F., & Isnaeni, Y. (2022). Experiences of women with preeclampsia. International Journal of Health & Medical Sciences, 5(3), 201-210. https://doi.org/10.21744/ijhms.v5n3.1901