

Effect of Three Commercial Mouth Rinses on Epithelial Cells: an in Vitro Study

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Abstract: Aim: The aim of the present study was to determine the cytotoxic effect of three commercial mouth washes (chlorhexidine, persica and Irsha) upon Hela cells. Methods and Material: For determination cytotoxicity of Irsha, Chlorhexidine and Persica, uninfected cells were grown in the absence and presence of various dilutions (1:2 to 1:128) of these mouth wash at different times (1,2,5,15 and 60 minutes). Results: In this study, three mouth washes show cytotoxic effect on cultured cells, at commercially available concentration an even diluted and Irsha was the most toxic one. Cytotoxicity of three mouth washes reduced with decreasing concentration. Conclusion: Our results showed that all three solutions were toxic to Hela cells. On the basis of these results, we suggest that clinical application of these mouthwashes should be limited.

Key words: Irsha, Chlorhexidine, Persica, cytotoxicity, mouthwash.

INTRODUCTION

Good oral health has a major influence on one's general quality of life and well-being (Halawany, H.S., 2012). The use of mechanical control alone to maintenance oral health has been challenged because it is considered to be a rather time-consuming and most importantly, insufficient activity (Barnett, M.L., 2003). In this regard, numerous mouthwashes are available for use as part of a daily oral hygiene routine. The formulation contains actives that may inhibit microbial growth and enzymatic reactions or may react directly with volatile sulfur compounds to reduce their levels in the mouth (Saad, S., 2011). Among them the most commonly used mouth rinses in Iran, consist of chlorhexidine (CHX), persica (driven from *Salvadora Persica* Plant) and Irsha.

The aim of the present study was to determine the cytotoxic effect of these mouth washes upon Hela cells.

MATERIALS AND METHODS

Cell Culture:

Hela cells (epithelial cells) was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (GIBCO) containing 100 Ug/ml penicillin and 100 Ug/ml streptomycin. For determination cytotoxicity of Irsha, Chlorhexidine and Persica, uninfected cells were grown in the absence and presence of various concentrations (1:2 to 1:128) of these mouth wash at different times (1, 2, 5, 15 and 60 minutes). Then, discarded the mouth wash and replaced with DMEM which incubated at 37°C for two days. Afterwards, the cells were exposed to trypan blue, loaded into hemocytometer, and the number of viable cells (unstained) was counted using low power of microscope. The number of cells per ml was then calculated. Chi-Squared test was used for statistical analysis. The level of significance was set at 0.05.

Results:

In this study, three mouth washes show cytotoxic effect on cultured cells, at commercially available concentration an even diluted (to 1:32 diluted), but at the diluting concentration of 1:8, Irsha had more cytotoxicity than the other two mouth washes (P=0.00) and at 1:32 diluting concentration Persica was less toxic than CHX significantly (the contact time was longer). (P=0.03).

Cytotoxicity of three mouth washes reduced with decreasing concentration.

(Figure 1).

Group 1=persica mouth wash

2-Irsha

3-Chlorhexidine

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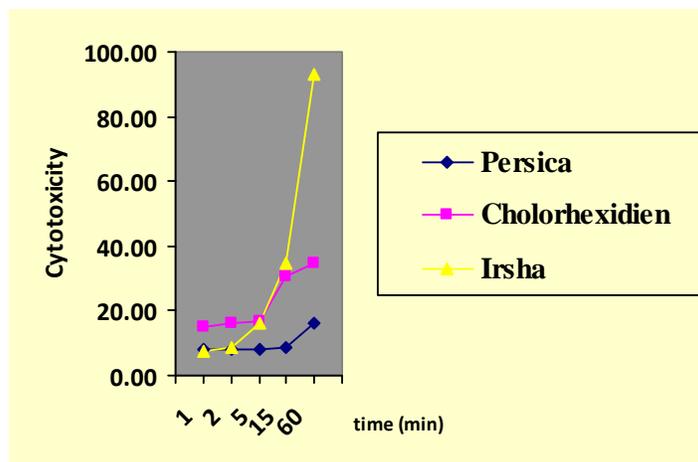


Fig. 1: Comparison of mouthwashes cytotoxicity in different concentration at different times.

Discussion:

In this study Irsha mouth wash was the most toxic to Hela cells.

We found only one research which studies this mouth wash and in this study, its antimicrobial effect was assessed (Vahabi, S., 2011).

Irsha contains different components such as alcohol, glisirin, sodium lauryl sulfate (SLS), benzoic acid, alantion and PUM/MA benzoate.

Triclosan and SLS are antimicrobial agents used, both singularly and in combination, in dentifrices and mouth-rinses. Studies by Waaler *et al* (1994) with human volunteers showed that the adverse side-effects induced by SLS in mouth-rinses i.e desquamation of oral epithelium and a burning sensation, were lessened by the addition of triclosan.

Babich et al showed that SLS induced vacuolization in gingival fibroblast (Babich, H., J.P. Babich, 1997). Cytotoxic activity of this mouth wash may probably due to SLS.

In the present study, it was found that persica at commercially available concentration is cytotoxic to fibroblast cells.

There was limited study which investigates cytotoxic effect of *Salvadora persica*. In 1983, Mohammad and Turner evaluated the cytotoxic potential of the *S. Persica* plant and it's components on oral tissues. Their results demonstrated no cytotoxic effect of freshly cut *S. Persica* miswak, but showed that the same plants contained harmful components if used after 24h (Mohammad, A., J.E. Turner, 1983).

Dormani et al investigated the effect of direct administration of high doses of *S. Persica* Miswak extract to mice and found some minor side effects on males and females reproductive system (Darmani, H., 2003).

In a recent study, similar to our result, it was shown that 1-h exposure to as low as 0.1 Persica solution induced irreversible cytotoxic effects on the cells involved in the wound healing process. However, diluting solution with Fatal Cuff Serum (FCS), offered protection from drug toxicity.

They suggested that reduction in the cytotoxic effect of Persica in the presence of FCS is probably due to the binding of potent toxic components of the mouthwash to serum proteins.

It seems that the toxic compounds of Persica solution exert their effects through irreversible binding to cellular proteins there by disturbing their function (Saeed Rajabalian, 2009).

In our study, similar to Rajabalian et al cytotoxic effect of persica mouth wash reduce with serial dilution, with the least toxic effect at 1:16, which may be due to interaction between FBS and Persica.

S. Persica contains different components such as indole, alkaloids, flavanoids, the sulphur-containing compound tropaedin, triterpenses, and phytosterol (Ohtani, K., 1992; Akhtar, M.S., M. Ajmal, 1981). Cytotoxic activity of this mouthwash is probably due to alkanoid and flavanoids.

Oral CLX mouth rinses have been effective in decreasing plaque formation and controlling gingivitis. It's positive charge at physiological PH, which produces non-specific binding to the negatively-charged membrane phospholipids of bacteria, this cause an alteration in bacterial osmotic equilibrium, with potassium and phosphorus leakage. As the CLX concentration increases, cytoplasmic contents precipitate, triggering cell death (Hidalgo, E., C. Dominguez, 2001).

Several studies have shown that CLX has toxic effects on a variety of eukaryotic cells (Giannelli, M., 2008). In this study CHX was cytotoxic to Hela cells in a concentration and contact time dependent manner.

Several studies suggest that CHX may also have adverse effects on oral tissues and cells at the concentrations used clinically. Cabral *et al* (Cabral, C.T., M.H. Fernandes, 2007) have reported that CHX has cytotoxic activity on cultured alveolar bone and gingival epithelial cells. CHX is able to induce primary DNA

damage in leukocytes and oral mucosal cells of rats treated daily with this compound (Ribeiro, D.A., 2004) and exert genotoxic side effects on epithelial and blood cells when used for mouth rinsing in clinical trials (Eren, K., 2002).

Carlin et al demonstrated that chlorhexidine (0.12 %) was able to induce cytogenetic damage as depicted by the increase of micronucleus frequency in peripheral blood cells after continuous exposure (Carlin, V., 2012).

The intrinsic mechanism underlying CHX-induced cytotoxicity in eukaryotic cells is, however, still unknown. It has been proposed that CHX inhibits mitochondrial activity protein and DNA synthesis and cell proliferation causing cell death by ATP depletion (Chang, Y.C., 2001; Aria, G., 2009).

Our results showed that all three solutions were toxic to cultured fibroblast, Irsha being the most cytotoxic. On the basis of these results, we suggest that clinical application of these mouthwashes should be limited.

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REFERENCES

- Akhtar, M.S., M. Ajmal, 1981. Significant of chewing-sticks (Mishawaka) in oral hygiene from a pharmacological viewpoint. *J. Pak. Med. Assoc.*, 31: 89-95.
- Aria, G., C.R. Cardoso, R.E. Larson, J.S. Silva, M.A. Rossi, 2009. Mechanism underlying chlorhexidine induced cytotoxicity. *Toxicol. Appl. Pharmacol.*, 23: 256-65.
- Babich, H., J.P. Babich, 1997. Sodiumlaurylsulfate and triclosan: in vitro cytotoxicity studies with gingival cells. *Toxicol Lett.*, 91: 189-96.
- Barnett, M.L., 2003. Role of therapeutic antimicrobials mouthrinses in clinical practice. *J Am.Dent. Assoc.*, 134: 699-704.
- Cabral, C.T., M.H. Fernandes, 2007. In vitro comparison of chlorhexidine and povidone-iodine on the long-term proliferation and functional activity of human alveolar bone cells. *Clin. Oral. Investig.*, 11: 155-64.
- Carlin, V., M.A. Matsumoto, P.P. Saraiva, A. Artioli, C.T. Oshima, D.A. Ribeiro, 2012. Cytogenetic damage induced by mouthrinses formulations in vivo and in vitro. *Clin Oral. Investig.*, 16: 813-20.
- Chang, Y.C., F.M. Huang, K.W. Tai, M.Y. Chou, 2001. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod.*, 92: 446-50.
- Darmani, H., A.S. Al-Hiyasat, A.M. Elbetieha, A. Alkofahi, 2003. The effect of an extract of *Salvadora Persica* (Miswak, Chewing Stick) on fertility of male and female mice. *Phytomedicine*, 10: 63-5.
- Eren, K., N. Ozmeriç, S. Sardaş, 2002. Monitoring of buccal epithelial cells by alkaline comet assay (single cell gel electrophoresis technique) in cytogenetic evaluation of chlorhexidine. *Clin. Oral. Investig.*, 6: 150-4.
- Giannelli, M., F. Chellini, M. Margheri, P. Tonelli, A. Tani, 2008. Effect of chlorhexidine digluconate on different cell types: A molecular and ultrastructural investigation. *Toxicol. In Vitro.*, 22: 308-17.
- Halawany, H.S., 2012. A review on miswak (*Salvadora persica*) and its effect on various aspect of oral health. *The Saudi Dental Journal*, 24: 63-69.
- Hidalgo, E., C. Dominguez, 2001. Mechanism underlying chlorhexidine-induced cytotoxicity. *Toxicol. In Vitro.*, 15: 271-6.
- Mohammad, A., J.E. Turner, 1983. In vitro evaluation of Saudi Arabian tooth brush tree (*Salvadora Persica*). *Odontostomatol Trop.*, 6: 145-8.
- Ohtani, K., R. Kasai, K. Yamasaki, O. Tanaka, M.S. Kamel, M.H. Assaf, *et al.*, 1992. Lignanglycosides from stems of *Salvadora persica*. *Phytochemistry*, 31: 2469-71.
- Ribeiro, D.A., A.P. Bazo, C.A. da Silva Franchi, M.E. Marques, D.M. Salvadori, 2004. Chlorhexidine induces DNA damage in rat peripheral leukocytes and oral mucosal cells. *J. Periodontal. Res.*, 39: 358-61.
- Saad, S., J. Greenman, H. Shaw, 2011. Comparative effects of various commercially available mouthrinse formulation on oral malodor. *Oral. Dis.*, 17: 180-6.
- Saeed Rajabalian, Mohammad Mohammadi, Behrooz Mozaffari, 2009. Cytotoxicity evaluation of *Persica* mouthwash on cultured human and mouse cell lines in the presence and absence of fetal calf serum. *Indian J. Dent. Res.*, 20: 169-73.
- Vahabi, S., E. Najafi, S. Alizadeh, 2011. In vitro antimicrobial effects of some herbal essences against oral pathogens. *J. Med.Plants Res.*, 19: 4870-8.
- Waler, S.M., G. Rölla, V. Kjaerheim, 1994. Triclosan-containing mouthwashes-does the nature of the solvent influence their clinical effect? *Scand J Dent Res.*, 102: 46-9.