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In-Vitro Cytotoxicity of White Vinegar: Effects of Different Concentrations

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Abstract

This study aims to compare the cytotoxic effects of different concentration of white vinegar, that easy to apply, unexpensive and effective on microorganisms.

The cells L929 mouse skin fibroblasts were used for the experiments. Cytotoxicity was evaluated colourimetrically by MTT [4,5-dimethylthiazol-2-yl-2,5 diphenyltetrazolium bromide; thiazolyl blue] proliferation assay.

White vinegar was found to be moderately cytotoxic at its all concentrations, in the 10-minutes incubation period. While the other concentrations were moderately cytotoxic, only 100% white vinegar concentration was found to be severely cytotoxic, in the 24-hours incubation period.

It was observed that white vinegar was moderately cytotoxic on L929 fibroblast at all concentrations, in the 10-minutes incubation period

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1. Introduction

White vinegar is a sour and astringent liquid composed of acetic acid (Vijayakumar & Wolf-Hall, 2002a). Acetic acid which is a component of white vinegar has a disinfecting effect (Vijayakumar & Wolf-Hall, 2002b). White vinegar, which is inexpensive, is readily available on the market and appears to have antimicrobial potential (Pinto, Neves, Leão & Jorge, 2008).

White vinegar has different uses in dentistry, especially the disinfection of removable denture (Basson, Quick & Thomas, 1992). A high amount of

plaque accumulation can be seen in removable denture, therefore; oral hygiene and denture hygiene is important for these patients. For patients who cannot adequately maintain denture hygiene, the use of a newly made denture can be disappointing (Suresan et al., 2016). Although there are many solutions, pastes and powders used in denture cleaning, there is no consensus on their effectiveness (Jagger & Harrison, 1995). Although mechanical and chemical methods are given for the cleaning of denture (Yildirim-Bicer, Peker, Akca & Celik, 2014), chemical ones are effective and easy to use (Nakamoto, Tamamoto & Hamada; Ünlü, Altay & Sahmali,1996). White vinegar is an alternative chemical disinfectant for dentures. Basson, et al. have reported that undiluted white vinegar solutions in killing adherent microorganisms when white vinegar was used for denture disinfection (Basson, Quick & Thomas, 1992).

Another use of white vinegar is to disinfect extracted human teeth. Because extracted teeth are used during dental education and disinfection of these teeth is important in terms of cross-infection (Tijare et al., 2014). They are considered a potential source for bloodborne pathogens (Dominici, Eleazer, Clark, Staat, Scheetz, 2001). Sterilization methods such as chemical heat, microwave, radiation, and autoclave are recommended to prevent cross-contamination (DeWald, 1997; Viana, Machado, Giampaolo, Pavarina, Vergani, 2010). Although, using these methods are effective, they are neither practical for dental education nor research to use routinely. The white vinegar has been reported to be as a best disinfectant medium for them (Tijare et al., 2014).

Except for limited number of studies examining the disinfection effect (Vijayakumar & Wolf-Hall, 2002a; Pinto, Neves, Leão & Jorge, 2008; Yildirim-Bicer, Peker, Akca & Celik, 2014; Tijare et al., 2014, Nascimento, Silva, Catanozi, & Silva, 2003), no published article was found that evaluated biocompatibility and cytotoxicity of white vinegar. So, the cytotoxic effects of different concentration of white vinegar, that easy to apply, unexpensive and effective on microorganisms was evaluated in this study.

2. Materials and Methods

The cells used for the experiments were obtained from Cell Culture Collection (HÜKÜK, Ankara). They were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS). The cells were grown in a T75 flask, passaged at 37°C under a humidified 5% CO_2, and maintained for three passages. When the cells were at 90% confluency as a monolayer, they were detached with trypsin/EDTA (0.025% trypsin / 0.02% ethylenediaminetetraacetic acid). After incubated for 2–5 min at 37°C and used to prepare cell suspension for cell inoculation. The L929 cell suspension was dispensed 100 µl per well onto 96-well cell culture plates after preparing at a concentration of $4x10^4$ cells mL^{-1}. The multiwell plates were incubated at 37°C, with 5% CO_2 in the air for 24 h. After 24 h, the culture was examined microscopically for growth and sterility. The equal volumes (100 µl) of the different concentrations (50%, 60%, 70%, 80%, 90%, and 100%) of white vinegar were added to each well after removing the culture medium. In control wells, 100 µl DMEM was added.

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2.1. MTT

Cytotoxicity was evaluated colorimetrically by MTT proliferation assay. MTT assay was performed as described in the method of Berridge et al. (Berridge, Herst & Tan, 2005). The culture medium of cells was aspirated, and 100 µl fresh DMEM medium and 13 µl of MTT solution (5 mg/mL) was added to each well. Ninety-six well culture plates were left at 37°C in a humidified 5% CO^2 in the dark for four hours incubation. The supernatant was aspirated, and 100 µl/well of isopropyl alcohol was added after incubation. The absorbance at 570 nm was measured using a UV–visible spectrophotometer (Molecular Device Corp, USA), and the results were compared with the control group. The average of three independent experiments determined the effects. The cell viability was calculated according to the following equation

Cell viability (%) = [OD570 treated cells ⁄ OD570 control cells] × 100

Meriç et al. have rated the cytotoxicity based on cell viability relative to controls as; non-cytotoxic >90% cell viability, slightly cytotoxic =60–90% cell viability, moderately cytotoxic =30–59% cell viability, and severely cytotoxic =<30% cell viability (Meriç, Dahl & Ruyter, 2008).

3. Statistical analysis

Statistical analysis was performed with the SPSS version 22.0 software (SPSS Inc., Chicago, Illinois, USA). Firstly, data were submitted to Kolmogorov-Smirnov and Levene's test to determine normality and homogeneity, respectively. The values of the number of viable cells were subjected to analysis of variance (ANOVA), with two factors to determine whether there were statistical differences between groups. The significance level was *p=0.05*.

4. Results

Table 1 and Figure 1 present the means and standard deviations of cells viability in percentages compared to the control group, according to the different concentration of white vinegar and incubation periods.

White vinegar was found to be moderately cytotoxic at its all concentrations, in the 10-minutes incubation period. While the other concentrations were moderately cytotoxic, only 100% white vinegar concentration was found to be severely cytotoxic, in the 24-hours incubation period. The analysis of variance showed no significant effect of the studied factors on cell viability.

5. Discussion

In-vitro cytotoxicity of white vinegar in different concentration (%50, %60, %70, %80, %90, %100) on L-929 mouse fibroblasts for 10 minutes and 24 hours of incubation periods was compared in the present study. While white vinegar at only 100% concentration was severely cytotoxic in the 24-hours incubation period, it was moderately cytotoxic in all other concentrations and in both incubation periods.

All patients using a prosthetic restoration, especially in patients with systemic disease, should be informed of pathologies associated with the microbial plaque on the restoration to preserve oral health and prevent potential complications (Le Bars, Kouadio, N'goran, Badran, Soueidan, 2015; Yang, Zhang, Chai, Chen,

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Zhang, 2014; Preshaw et al., 2011). Various pathogens exist in the oral cavity, and this microbial reservoir can cause a variety of infections (Preshaw et al., 2011). In healthy individuals, yeast and bacteria can be colonized in the mouth (Ribeiro et al., 2012) and using prosthesis and poor hygiene can increase the bacterial colonization (Lyon, da Costa, Totti, Munhoz, de Resende, 2006).

On the other hand, it is very important for patients to clean their prostheses. Removable dentures are cleaned with brushes associated with some kind of detergent, soap or dentifrice (Coelho, Sousa & Dare, 2004). Mechanical cleaning has been found insufficient for cleaning on dentures (Palenik & Miller, 1984; Salles, Macedo, Fernandes, Silva-Lovato, Paranhos, 2007). It has stated that this may be due to irregularities on the dentures (Kulak, Arikan & Kazazoglu, 1997; Davenport, 1972). In addition to mechanical cleaning, chemical solutions are required for a good prosthesis cleaning. In this respect, it is important to evaluate the cytotoxicity of the chemical solutions to be used. White vinegar in 50% and 100% concentrations was used frequently for disinfection (Da Silva, 2008). A previous study has found that the white vinegar effective for *Candida albicans* on acrylic resin (Yildirim-Bicer, Peker, Akca & Celik, 2014). It has been known that patients over the age of 60 who use full dentures and live in shelters do not have prosthetic cleaning habits; in addition, denture cleaning agents may be expensive for these patients (Schou, Wight & Cumming, 1987). Therefore, we evaluated the cytotoxicity of white vinegar, which has proven antimicrobial activity, is inexpensive and easy to apply.

Currently, the most widely used cytotoxicity determination method is the MTT test. It was developed in 1983 by Mossman and it's called the 'gold standard' of cell viability (Mosmann, 1983) and how many viable cells are remaining at the end of the experiment is important (Riss, 2016). The starting point for assessing biocompatibility is usually cell cultures. This system minimizes the effect of confounding variables (Lefebvre & Schuster, 1994; Lefebvre, Knoernschild & Schuster, 1994). Therefore, in this study, MTT method was used for cytotoxicity assessment.

Estrela et al. have evaluated the use of white vinegar as an irrigation solution and an intracanal drug for microbial elimination from canines with apical periodontitis (Estrela, Holland, Bernabé, Souza, Estrela, 2004). It was observed that 40% of the samples treated with white vinegar were elimination of microbes (Estrela, Holland, Bernabé, Souza, Estrela, 2004). In addition to the studies on microbial elimination, the cytotoxic effect of white vinegar on L-929 fibroblast cells was evaluated in this study. White vinegar was found to be moderately cytotoxic on L-929 fibroblast at all concentrations in the 10-minutes incubation period. In addition, white vinegar was found to be severely cytotoxic only for 100% concentration while moderately cytotoxic at other concentrations, in the 24-hours incubation period. Therefore, the usage time of white vinegar can be limited to 10 minutes and vinegar can be used in low concentrations. This result is important for easy to apply and cheap white vinegar. However, it should not be used in direct oral applications.

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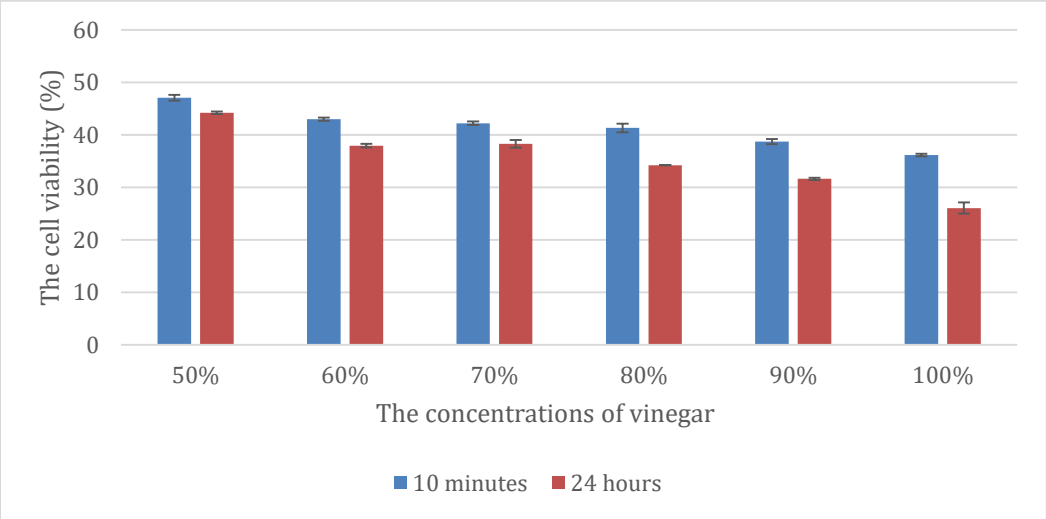
Table 1. Mean and standard deviation (SD) of cell viability (%) compared to control groups

The concentration of white vinegar	The incubation periods	
	10 minutes Mean ± Standart deviation	24 hours Mean ± Standart deviation
50%	47,09627 ± 0,531726	44,20977 ± 0,248313
60%	43,01183 ± 0,285016	37,96193 ± 0,338105
70%	42,21666 ± 0,33884	38,32738 ± 0,74106
80%	41,32691 ± 0,819789	34,23032 ± 0,036859
90%	38,74167 ± 0,500832	31,63857 ± 0,232794
100%	36,16390 ± 0,229656	26,07016 ± 1,071497

Figure 1. Mean and standard deviation (SD) of cell viability (%) compared to control groups



Conclusion

As a result of this study, it was observed that white vinegar was moderately cytotoxic on L929 fibroblast at all concentrations, in the 10-minutes incubation period. Only 100% concentration of white vinegar was found to be severely cytotoxic, while other concentrations of white vinegar were to be moderately cytotoxic, in the 24-hours incubation period.

For further studies, the cytotoxicity of white vinegar can be improved and supported by in vivo studies. Because the cells themselves can give different answers in the living environment.

Conflicts of interest

The authors declare no conflicts of interest.

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