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Original Article

Comparison of Cytotoxicity of Cetylpyridinium Chloride With Sodium Hypochlorite, Chlorhexidine and Halita as an Endodontic Irrigant Using MTT Assay

Zahra Aghazadeh[1], Parisa Falsafi[2], Milad Ghanizadeh[1], Zahra Mardani[3], Mahsa SarvCharandabi[1], Marzieh Aghazadeh[1*]

Abstract

Background: Given the limitations of the use of common endodontic irrigants such as sodium hypochlorite and chlorhexidine (CHX), researchers are seeking out new irrigants with less complications. The purpose of this study was to compare the cytotoxicity of cetylpyridinium chloride (CPC) with sodium hypochlorite, CHX and Halita as an endodontic irrigant using MTT assay.

Methods: In the present experimental study conducted from April 2016 to June 2018 in Tabriz University of Medical Sciences, cytotoxicity of CPC (0.05%), CHX (0.2%), sodium hypochlorite (2.5%) and Halita solutions was examined on human gingival fibroblast cell lines according to the standard MTT assay protocol. The solutions were diluted at ratios of 1, 0.1, 0.01 and 0.001. Thus, four concentrations of each solution were prepared and evaluated. Data were analyzed using descriptive statistical methods and paired *t* test, one-way ANOVA, repeated measures ANOVA, and post hoc tests. *P* value <0.05 was considered significance level.

Results: In the first 24 hours, the lowest cytotoxicity was observed for CHX (6.19 ± 3.10) and CPC (7.08 ± 3.04) at dilution of 0.001 and the highest cytotoxicity was observed for Halita solution (25.15 ± 7.02) and sodium hypochlorite (22.91 ± 7.77) at dilution of 0.01 ($P<0.05$). In total, the cytotoxicity of CPC at both concentrations and at all intervals was similar to CHX ($P>0.05$) and lower than two other solutions ($P<0.05$). At 24-hour interval, cytotoxicity of the solutions at both dilutions was lowest ($P<0.05$). At 48 and 72-hour intervals, the cytotoxicity of the solutions increased at both dilutions; however, there was no significant difference in mean cytotoxicity between 48- and 72-hour intervals ($P>0.05$).

Conclusions: All solutions, particularly at commercial doses, had some levels of cytotoxicity depending on time and dose. The cytotoxicity of CPC 0.05%, at all intervals and at the dilutions of 0.01 and 0.001, was similar to the cytotoxicity of CHX and lower than the cytotoxicity of sodium hypochlorite and Halita, and therefore CPC 0.05% can be replaced with CHX in the presence of favorable antibacterial effects.

***Correspondence to**
Marzieh Aghazadeh,
Address: Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran
Tel: +989143013953
Fax:+984133355921
Email: maghazadehbio@gmail.com

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Background

Microorganisms are the main cause of pulpal and periapical diseases, so that their complete elimination plays a major role in the success of endodontic treatment and their remaining plays the main role in the failure of endodontic treatment (1-3). Theoretically, the root canal filing causes loosening, tearing and separating the intracanal material and dentin cutting from the walls. But the discharge of this materials should be done by the pressure of the endodontic irrigant. Due to the complex anatomy of the canals, filing alone does not completely eliminate bacteria from the canal. Therefore, the ideal irrigant should have broad-spectrum antimicrobial properties and by slipping

Highlights

- At 24-hour interval, the lowest cytotoxicity was observed for chlorhexidine 0.2% and cetylpyridinium chloride 0.05% at dilution of 0.001.
- Cetylpyridinium chloride 0.05% cytotoxicity at both 0.01 and 0.001 dilutions and at all intervals was similar to chlorhexidine 0.2% and lower than two other solutions.
- In the first 24 hours, cytotoxicity of the solutions at both dilutions was lowest value. At 48 and 72 hours, the cytotoxicity of the solutions increased at both dilutions; however, there was no significant difference in mean cytotoxicity between 48- and 72-hour intervals.

[1]Department of Oral and Maxillofacial Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran. [2]Department of Oral and Maxillofacial Medicine, Faculty of Dentistry, Tabriz Islamic Azad University, Tabriz, Iran. [3]Department of Orthodontics, Faculty of Dentistry, Tabriz University of Medical sciences, Tabriz, Iran.

the canal inside, should facilitate the mechanical clearing process and the use of intracanal instruments. In addition, it should not have harmful effects for periapical tissues and does not interfere with peri-apex tissue repair (2,4-7). In studies, various materials at various concentrations have been used as intracanal irrigant, each of which have certain advantages and disadvantages. Sodium hypochlorite (NaOCl) has long been known as the most effective and ideal endodontic irrigant solution and has acceptable antimicrobial and tissue-solubility properties. But it has an unpleasant smell and taste, is very toxic when exposed to peri-apex tissue, and causes extensive tissue damage (5,8).

Chlorhexidine (CHX) is another substance with a cationic guanide base and has broad-spectrum antimicrobial properties. The important advantage of CHX is its use in perforations, open apexes and teeth which are difficult to be isolated. Another advantage of CHX is its use in patients who are sensitive to sodium hypochlorite. The most important disadvantage of CHX is lack of dissolving necrotic tissues and the possibility of allergic reaction in exposure to peri-apex tissue (6, 9).

Halita mouthwash contains 0.05% CHX, 0.05% cetylpyridinium chloride (CPC) and zinc lactate. Regarding the reduction of CHX concentrations in Halita mouthwash compared with CHX mouthwash (0.12%), it is expected that the undesirable feature of removing normal microflora in the mouth and color change due to its long-term use be lower (10).

CPC is a quaternary ammonium compound introduced for the first time by Schroeder. CPC is a cationic surfactant and has a broad-spectrum antimicrobial feature that causes the rapid removal of gram-positive pathogens and fungi. The cause of its antimicrobial activity is the degradation of cell membranes and the withdrawal of cytoplasm components (6, 11-13).

The search in the databases shows that so far few studies have been done on the antibacterial effects of CPC on *Enterococcus faecalis* (11), adding CPC to endodontic sealer in order to increase the antibacterial properties of sealers (14) and adding CPC to gutta-percha and creating antibacterial properties (15), but no study has yet been conducted on the use of cetylpyridinium as the endodontic irrigant, its possible adverse effects and its cytotoxicity compared with other common irrigants. Therefore, the aim of this study was to examine the cytotoxicity of CPC on fibroblast cells around the apex using MTT assay and compare it with those of sodium hypochlorite, CHX and Halita mouthwashes.

Methods

Study Design

This experimental study was carried out from April 2016 to June 2018 at Tabriz University of Medical Sciences. The human gingival fibroblast cell line was purchased from the National Cell Bank of Iran (NCBI) and transferred to the laboratory. Gingival fibroblast cells were then cultured in the flask 75 to a final count of 2 million. To ensure the cell count, 1 mL was stained with a Trypan blue flask and counted with Neubauer lam.

MTT Assay Procedure

MTT assay was used to evaluate the cytotoxicity of each of the solutions. According to the MTT standard protocol (16), 5000 fibroblast cells were poured into each well of 96 well-plate and 24 hours was allowed for the cells to stick on the well floor (cells were kept at 37°C and pH 7.4). The cell culture medium contained Dulbecco's Modified Eagle's Medium (DEME, Gibco, Grand Island, NY, USA) + 10% FBS (Fetal Bovine Serum) + 1X Ab (penicillin/streptomycin) (16). The 96-well plate was then divided into 4 equal parts, and each of the solutions was uniformly added to one of the 4 parts of the 96-well plate containing fibroblast cells.

Characteristics of the solutions were as follows:

1. CPC 0.05% (Rojin Cosmetic Co., Iran)
2. NaOCl 2.5% (Golrang Co., Iran)
3. CHX digluconate 0.2% (Rojin Cosmetic Co., Iran)
4. Halita containing CHX digluconate 0.05%, CPC (CPC) 0.05% and zinc lactate 0.14% (Rojin Cosmetic Co., Iran)

The solutions were diluted at the ratios of 1, 0.1, 0.01 and 0.001. Thus, 4 different concentrations were prepared for each solution. A control group was also considered for each solution, in a way that all the steps of the experiment were carried out without adding the solution to the wells. At 24-, 48-, and 72-hour intervals, the stages of color change due to cell death via MTT and dimethyl sulfoxide (DMSO) were recorded according to MTT assay procedures (16).

The plates were evaluated separately and without interference. Because the fibroblast cells did not grow or proliferate, or their growth was very limited and that the culture medium was rich in DEME and FBS, the cell culture medium was responsive for 3 days and no replacement was required. Any apoptosis and cell death for various reasons other than the effect of toxic substances were appeared in the control group, and the device was initially calibrated with the resulting color change, and the changes in the colors of other wells were read with reference to the control group (16).

Statistical Analysis

The data were analyzed using descriptive statistics (mean ± SD), independent t-test, one-way ANOVA, repeated measure ANOVA, and Tukey's and Sidak post hoc tests in the SPSS version 16. *P* value less than 0.05 was considered significance level.

Results

In this study, first the mean cell viability for each of the solutions was calculated at four different concentrations

based on the following formula. Given that the cell viability was closer to the control group at dilutions of 0.001 and 0.01, these concentrations were considered as optimum concentrations and the results of the study were also examined and compared with reference to these two concentrations (Table 1).

Then, the cytotoxicity of the solutions was calculated according to the following formula and expressed in percentage (Table 2).

Repeated measure ANOVA was used to compare the mean cytotoxicity of the solutions studied over a period of 24 to 72 hours. The result of this test showed that over time, the mean cytotoxicity of the solutions was significantly increased (P<0.05). For paired comparison at each interval, Sidak post hoc test was used, whose results for dilutions of 0.01 and 0.001have been presented in Tables 3 and 4, respectively. Based on the results of this test, at 24-hour interval, the cytotoxicity of the solutions at both dilutions was lowest. At 48- and 72-hour intervals, the cytotoxicity of both dilutions increased but there was no statistically significant difference in mean cytotoxicity between the two intervals.

One-way ANOVA test was used to compare the mean cytotoxicity of the studied solutions at different intervals. The results of this test showed that there was a significant difference in cytotoxicity between different studied solutions at all three intervals ($P<0.05$). Tukey test was used for paired comparison of cytotoxicity of the solutions studied, whose results for dilutions of 0.01 and 0.001have been presented in Tables 5 and 6, respectively. Based on the results of this study, the cytotoxicity of CHX and CPC and the cytotoxicity of sodium hypochlorite and Halita were similar at all three intervals and both dilutions. In addition, the cytotoxicity of CHX and CPC was significantly lower than those of sodium hypochlorite and Halita at all three intervals and both dilutions.

To compare the cytotoxicity of the dilutions of 0.01 and 0.001 of the same solution, independent t-test was used. The result of this test showed that at 24-hour interval, cell cytotoxicity of all four solutions at dilution of 0.001

Table 1. Mean ± Standard Deviation of Cell Viability of Different Solutions at 24-, 48- and 72-Hour Intervals

		24 hours		48 hours		72 hours	
Solution	Dilution ratio	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
CHX	1	.398	.070	.225	.105	.147	.023
	0.1	.416	.030	.143	.007	.187	.021
	0.01	.832	.047	.672	.113	.668	.047
	0.001	.938	.030	.691	.138	.709	.137
	Control	.959	.035	.763	.070	.741	.036
CPC	1	.352	.069	.267	.149	.119	.024
	0.1	.538	.220	.382	.052	.184	.016
	0.01	.829	.071	.675	.126	.653	.118
	0.001	.929	.030	.689	.116	.696	.142
	Control	.977	.016	.734	.775	.764	.043
HAL	1	.318	.053	.236	.030	.157	.033
	0.1	.408	.059	.369	.144	.207	.015
	0.01	.748	.070	.601	.102	.587	.070
	0.001	.860	.053	.613	.124	.583	.172
	Control	.966	.072	.736	.098	.703	.054
NaoCL	1	.264	.017	.213	.051	.107	.013
	0.1	.403	.054	.324	.051	.123	.024
	0.01	.770	.077	.606	.107	.589	.082
	0.001	.877	.031	.620	.100	.594	.177
	Control	.897	.026	.675	.037	.664	.037

Table 2. Mean ± Standard Deviation of Cytotoxicity (Percentage) of the Solutions at 24-, 48- and 72-Hour Intervals

	24 Hours			48 Hours			72 Hours		
Solution	0.01Dilution	0.001 Dilution	*P* Value*	0.01 Dilution	0.001 Dilution	*P* Value*	0.01 Dilution	0.001 Dilution	*P* Value*
CPC	17.04±7.11	7.08±3.04	<0.0001	32.41±12.66	31.05±11.67	0.70	34.64±11.83	30.33±14.21	0.25
NaoCL	22.91±7.77	12.29±3.10	<0.0001	39.37±10.71	37.97±10.04	0.64	41.02±8.26	40.51±17.75	0.89
CHX	16.72±4.79	6.19±3.01	<0.0001	32.73±11.33	30.84±13.83	0.60	33.18±4.79	29.08±13.77	0.17
HAL	25.15±7.02	13.93±5.34	<0.0001	39.82±10.23	38.64±12.48	0.72	41.28±7.09	41.66±17.28	0.92

* Independent sample t test.

Table 3. The Results of Sidak Post Hoc Test to Compare the Mean Cytotoxicity (Percentage) of the Studied Solutions at Dilution of 0.01 Over a Period of 24 to 72 Hours

	(I) time	(J) time	Mean Difference (I-J)	Standard Error	Sig.(a)	95% CI for Difference(a)	
						Lower Bound	Upper Bound
CPC	24 h	48 h	15.37	2.96	<0.0001	9.40	21.33
		72 h	17.6	2.81	<0.0001	11.92	23.27
	48 h	72 h	2.23	3.53	0.53	-4.88	9.34
NaOCL	24 h	48 h	16.46	2.70	<0.0001	11.02	21.89
		72 h	18.11	2.31	<0.0001	13.45	22.76
	48 h	72 h	1.65	2.76	0.55	-3.90	7.20
CHX	24 h	48 h	16.01	2.51	<0.0001	10/95	21.06
		72 h	16.46	1.38	<0.0001	13.67	19.24
	48 h	72 h	0.45	2.51	0.85	-4.60	5.50
Halita	24 h	48 h	14.67	2.53	<0.0001	9.57	19.76
		72 h	16.13	2.03	<0.0001	12.03	20.23
	48 h	72 h	1.46	2.54	0.56	-3.65	6.57

Table 4. The Results of Sidak Post Hoc Test to Compare the Mean Cytotoxicity (Percentage) of the Studied Solutions at Dilution of 0.001 Over a Period of 24 to 72 Hours

	(I) time	(J) time	Mean Difference (I-J)	Standard Error	Sig.(a)	95% CI for Difference(a)	
						Lower Bound	Upper Bound
CPC	24 h	48 h	23.97	2.46	<0.0001	19.01	28.92
		72 h	23.24	2.96	<0.0001	17.26	29.21
	48 h	72 h	-0.73	3.75	0.84	-8.28	6.82
NaOCL	24 h	48 h	25.68	2.14	<0.0001	21.36	29.99
		72 h	28.22	3.67	<0.0001	20.81	35.62
	48 h	72 h	2.54	4.16	0.54	-5.83	10.91
CHX	24 h	48 h	24.65	2.88	<0.0001	18.83	30.46
		72 h	22.89	2.87	<0.0001	17.09	28.68
	48 h	72 h	-1.76	3.98	0.66	-9.77	6.25
Halita	24 h	48 h	24.71	2.77	<0.0001	19.13	30.28
		72 h	27.73	3.69	<0.0001	20.29	35.16
	48 h	72 h	3.02	4.35	0.49	-5.73	11.77

was lower than that of the same solution at dilution of 0.01 ($P<0.0001$) (Table 2). At 48- and 72-hour intervals, there was no significant difference in mean cytotoxicity at dilutions of 0.01 and 0.001 for each solution.

Discussion

The main purpose of endodontic treatments is sterilization of root canal and its 3D tubular network, in which mechanical instrumentation, antimicrobial irrigants and intracanal drugs are used to achieve this goal. The mechanical cleansing alone cannot clean the root canal sufficiently, and the irrigants must be used to remove the microorganisms. The ideal endodontic irrigant should eliminate bacteria, dissolve necrosis tissues, slip off the canal and remove the smear layer (1-3).

The toxicity of endodontic irrigants is important because damage to the periapical tissue could delay wound healing (17).

With the aim of comparing the cytotoxicity of CPC 0.05% solution with sodium hypochlorite 2.5%, CHX 0.2% and Halita solutions (containing CPC 0.05% and CHX 0.05% and zinc lactate 0.14%) at two dilutions of 0.01 and 0.001, at 24-hour interval, which is of particular importance in the inflammatory reactions surrounding apex to the irrigating substances and solutions, the lowest cytotoxicity was obtained for CHX (6.19 ± 3.10) and CPC (7.08 ± 3.04) at the dilution of 0.001, and the highest cytotoxicity was obtained for Halita solution (25.15 ± 7.02) and sodium hypochlorite (22.91 ± 7.77) at dilution of 0.01. Generally, CPC cytotoxicity at both concentrations and at all intervals was similar to that of CHX and lower than those of 2 other solutions. Although CHX and NaOCl are used routinely in endodontic treatments, there are still concerns about their use.

The CHX molecule structure has a systemic risk, since it is likely to break down into para-chloroaniline, which is a reactive byproduct and can have reactive oxygen species (11, 18).

NaOCl has long been known as the most effective and ideal endodontic irrigant and has acceptable antimicrobial

Table 5. The Results of Tukey Test to Compare the Mean Cytotoxicity (Percentage) of the Studied Solutions at Dilution of 0.01 at 24-, 48- and 72-Hour Intervals

Time	(I) Group	(J) Group	Mean Difference (I-J)	Standard Error	Sig.	95% CI	
						Lower Bound	Upper Bound
24 Hours	CPC	NaOCL	5.87	2.15	0.008	1.54	10.19
		CHX	-0.32	1.75	0.85	-3.84	3.20
		Halita	8.11	2.04	0.0002	4.005	12.21
	NaOCL	CHX	-6.19	1.88	0.0019	-9.98	-2.4
		Halita	2.24	2.13	0.30	-2.06	6.54
	CHX	Halita	8.43	1.73	<0.0001	4.93	11.92
48 Hours	CPC	NaOCL	6.96	3.38	0.04	0.14	13.77
		CHX	0.32	3.46	0.92	-6.66	7.30
		Halita	7.41	3.32	0.03	0.72	14.09
	NaOCL	CHX	-6.64	3.18	0.04	-13.04	-0.23
		Halita	0.45	3.02	0.88	-5.63	6.53
	CHX	Halita	7.09	3.11	0.02	0.81	13.36
72 Hours	CPC	NaOCL	6.38	2.94	0.03	0.452	12.30
		CHX	-1.46	2.60	0.5	-6.70	3.78
		Halita	6.64	2.81	**0.02**	0.97	12.30
	NaOCL	CHX	-7.84	1.94	0.0002	-11.76	-3.91
		Halita	0.26	2.22	0.90	-4.21	4.73
	CHX	Halita	8.1	1.74	<0.0001	4.58	11.61

Table 6. The Results of Tukey Test to Compare the Mean Cytotoxicity (Percentage) of the Studied Solutions at Dilution of 0.001 at 24-, 48- and 72-Hour Intervals

Time	(I) Group	(J) Group	Mean Difference (I-J)	Standard Error	Sig.	95% CI	
						Lower Bound	Upper Bound
24 Hours	CPC	NaOCL	5.21	0.88	<0.0001	3.42	6.99
		CHX	-0.89	0.87	0.31	-2.64	0.86
		Halita	6.85	1.25	<0.0001	4.32	9.37
	NaOCL	CHX	-6.1	088	<0.0001	-7.87	-4.32
		Halita	1.64	1.26	0.19	-0.89	4.17
	CHX	Halita	7.74	1.25	<0.0001	5.22	10.25
48 Hours	CPC	NaOCL	6.92	3.14	0.03	0.59	13.24
		CHX	-0.21	3.69	0.95	-7.64	7.22
		Halita	7.59	3.48	0.03	0.57	14.61
	NaOCL	CHX	-7.13	3.48	0.04	-14.15	-0.10
		Halita	0.67	3.27	0.83	-5.91	7.25
	CHX	Halita	7.8	3.80	0.04	0.14	15.45
72 Hours	CPC	NaOCL	10.19	4.64	0.03	0.84	19.53
		CHX	-1.24	4.03	0.76	-9.37	6.89
		Halita	11.34	4.56	**0.01**	2.14	20.53
	NaOCL	CHX	-11.43	4.58	0.01	-20.66	-2.2
		Halita	1.15	5.05	0.82	-9.02	11.32
	CHX	Halita	12.58	4.51	0.007	3.50	21.65

and tissue solubility properties, but its unpleasant odor and taste and its toxicity have limited its use (5, 8).

Therefore, researchers are seeking out irrigants with desirable antibacterial properties and fewer side effects. In this study, CPC as 4-ammonium compound had low cytotoxicity similar to CHX. This is inconsistent with previous studies supporting its high antimicrobial properties. For example, in the study of Estrela et al, the results of the agar diffusion test showed that CPC had similar antibacterial activity to that of CHX and better than that of 2.5% NaOCl (11).

Regarding Halita mouthwash, it can be argued that because this mouthwash is a combination of CPC 0.05%, CHX 0.05% and zinc lactate 0.14%, its higher cytotoxicity than the solutions CPC and CHX alone seems logical.

In the present study, the cytotoxicity of irrigants

increased over time. At 24-hour interval, which is the longest time of the presence of the irrigant substance in the area around the apex, the cytotoxicity of all solutions was the highest; however, the cytotoxicity of CPC was still similar to that of CHX at both concentrations and was lower than those of two other solutions.

In one study, Müller et al evaluated 12 mouthwashes for antibacterial and cytotoxic properties. They reported that mouthwashes containing 0.05% CPC and 0.2% CHX over a 24-hour period had high cytotoxicity and moderate to high antibacterial activity (19). In the present study, CPC 0.05% and CHX 0.2% solutions had high cytotoxicity as well, but their cytotoxicity decreased at dilutions 0.01 and 0.001.

Fromm-Dornieden et al in a study showed that CPC at a concentration of more than 0.003% had cytotoxic properties against human keratinocytes and L929 fibroblast cells (20), which is consistent with the results of the present study.

According to the study of Karkehabadi et al, EDTA, QMix, CHX, NaOCl and MTAD solutions had cytotoxicity in human periodontal ligament cells in ascending order, which were time-dependent (17).

In a study conducted by Bajrami et al, the cytotoxicity of 2% CHX and 3% NaOCl solution on rat ligamental fibroblast cells was examined by WST-1 assay. At concentration of 100 µL/mL and 24-hour interval, both solutions were highly cytotoxic, but CHX toxicity was less than that of NaOCl, which is consistent with the present study. In contrast to the results of the present study, the NaOCl toxicity was reported to be higher than that of CHX at 48 and 72-hour intervals (21). This inconsistency in results could be due to the difference in the concentrations of studied solutions, the evaluation method and the type of cells studied. In the study of Oncag et al, the cytotoxicity of 2% CHX injected into the subcutaneous tissue of the rat was less than that of 5.25% sodium hypochlorite (22).

Based on the results of this study, all of the solutions studied at commercial concentrations were toxic to fibroblast cells. Therefore, it is better to use these solutions with caution in patients who have the potential of the passage of irrigant through the canal to periapical tissues, such as patients with open apex. However, the dilutions of 0.01 and 0.001 of these solutions, particularly, of chloride 0.05% and CHX 0.2%, had very low cytotoxicity that was close to that of the control group.

However, in future studies, it is necessary to evaluate the minimum inhibitory concentration of the solutions on bacterial and fungal species to determine if there is antibacterial and antifungal effect in the safe concentrations obtained in this study. It should also be noted that *in vivo*, the materials are neutralized quickly and removed by phagocytes and the lymphatic and vascular systems, so they are less harmful in clinical conditions than at same concentrations in *in vitro* studies. *In vitro* measurements of toxicity are only at cell level, so the results of the present study cannot be compared with the results of *in vivo* studies directly (21). Future studies should be conducted on root canal irrigants in animals and then in humans to assess their cytotoxicity and *in vivo* biocompatibility.

Conclusions

In the present study, all solutions, particularly at commercial doses, had some degrees of cytotoxicity depending on time and dose. The cytotoxicity of CPC 0.05% at all intervals and at the dilutions of 0.01 and 0.001 was similar to that of CHX and less than those of sodium hypochlorite and Halita, and therefore CPC 0.05% can be replaced with CHX in the presence of favorable antibacterial effects.

Authors' Contribution

ZA, MA and PF contributed to the design and conception of the study as well as the revision of the prepared manuscript. MG, ZM and MSC carried out the literature search and data collection. MG, MA and PF analyzed the data and drafted the manuscript. All the authors have read and approved the final manuscript..

Ethical Statement

This study was conducted in accordance with the Helsinki Declaration of 1975, which was revised in 2002. The procedure of the study was approved by the Ethics Committee of the Tabriz University of Medical Sciences.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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References

1. Siqueira JF Jr, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-301.e3. doi: 10.1016/j.joen.2008.07.028.
2. Basmaci F, Oztan MD, Kiyan M. Ex vivo evaluation of various instrumentation techniques and irrigants in reducing E. faecalis within root canals. Int Endod J. 2013;46(9):823-30. doi: 10.1111/iej.12067.
3. Maria Ferrer-Luque C, Teresa Arias-Moliz M, Ruiz-Linares M, Elena Martinez Garcia M, Baca P. Residual activity of cetrimide and chlorhexidine on Enterococcus faecalis-infected root canals. Int J Oral Sci. 2014;6(1):46-9. doi: 10.1038/ijos.2013.95.
4. Andrabi SM, Kumar A, Kumar Tewari R, Kumar Mishra S, Iftekhar H. An in vitro SEM study on the effectiveness of smear layer removal of four different irrigations. Iran Endod J. 2012;7(4):171-6.
5. Dammaschke T, Jung N, Harks I, Schafer E. The effect of different root canal medicaments on the elimination of Enterococcus faecalis ex vivo. Eur J Dent. 2013;7(4):442-8. doi: 10.4103/1305-7456.120683.

6. Kudiyirickal MG, Ivancakova R. Antimicrobial agents used in endodontic treatment. Acta Medica (Hradec Kralove). 2008;51(1):3-12.
7. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98. doi: 10.1016/j.joen.2005.09.014.
8. Khademi A, Usefian E, Feizianfard M. Tissue dissolving ability of several endodontic irrigants on bovine pulp tissue. Iran Endod J. 2007;2(2):65-8.
9. Sajjan P, Laxminarayan N, Kar PP, Sajjanar M. Chlorhexidine as an Antimicrobial Agent in Dentistry–A Review. Oral Health Dent Manag. 2016;15(2):93-100.
10. Roldan S, Winkel EG, Herrera D, Sanz M, Van Winkelhoff AJ. The effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc lactate on the microflora of oral halitosis patients: a dual-centre, double-blind placebo-controlled study. J Clin Periodontol. 2003;30(5):427-34.
11. Estrela C, Sousa-Neto MD, Alves DR, Alencar AH, Santos TO, Pecora JD. A preliminary study of the antibacterial potential of cetylpyridinium chloride in root canals infected by *E. faecalis*. Braz Dent J. 2012;23(6):645-53.
12. Raangs GC, Winkel EG, van Winkelhoff AJ. In vitro antimicrobial effects of two antihalitosis mouth rinses on oral pathogens and human tongue microbiota. Int J Dent Hyg. 2013;11(3):203-7. doi: 10.1111/idh.12014.
13. Sreenivasan PK, Haraszthy VI, Zambon JJ. Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. Lett Appl Microbiol. 2013;56(1):14-20. doi: 10.1111/lam.12008.
14. Gjorgievska E, Apostolska S, Dimkov A, Nicholson JW, Kaftandzieva A. Incorporation of antimicrobial agents can be used to enhance the antibacterial effect of endodontic sealers. Dent Mater. 2013;29(3):e29-34. doi: 10.1016/j.dental.2012.10.002.
15. Tomino M, Nagano K, Hayashi T, Kuroki K, Kawai T. Antimicrobial efficacy of gutta-percha supplemented with cetylpyridinium chloride. J Oral Sci. 2016;58(2):277-82. doi: 10.2334/josnusd.15-0620.
16. van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. Methods Mol Biol. 2011;731:237-45. doi: 10.1007/978-1-61779-080-5_20.
17. Karkehabadi H, Yousefifakhr H, Zadsirjan S. Cytotoxicity of endodontic irrigants on human periodontal ligament cells. Iran Endod J. 2018;13(3):390-4. doi: 10.22037/iej.v13i3.20438.
18. Barbin LE, Estrela C, Guedes DF, Spano JC, Sousa-Neto MD, Pecora JD. Detection of para-chloroaniline, reactive oxygen species, and 1-chloro-4-nitrobenzene in high concentrations of chlorhexidine and in a mixture of chlorhexidine and calcium hydroxide. J Endod. 2013;39(5):664-8. doi: 10.1016/j.joen.2012.10.018.
19. Müller HD, Eick S, Moritz A, Lussi A, Gruber R. Cytotoxicity and antimicrobial activity of oral rinses in vitro. Biomed Res Int. 2017;2017:4019723. doi: 10.1155/2017/4019723.
20. Fromm-Dornieden C, Rembe JD, Schafer N, Bohm J, Stuermer EK. Cetylpyridinium chloride and miramistin as antiseptic substances in chronic wound management - prospects and limitations. J Med Microbiol. 2015;64(Pt 4):407-14. doi: 10.1099/jmm.0.000034.
21. Bajrami D, Hoxha V, Gorduysus O, Muftuoglu S, Zeybek ND, Kucukkaya S. Cytotoxic effect of endodontic irrigants in vitro. Med Sci Monit Basic Res. 2014;20:22-6. doi: 10.12659/msmbr.890247.
22. Oncag O, Hosgor M, Hilmioglu S, Zekioglu O, Eronat C, Burhanoglu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J. 2003;36(6):423-32.