



## CYTOTOXICITY OF NOVEL SODIUM HYPOCHLORITE BASED INTRA CANAL MEDICAMENT

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### ABSTRACT

**Aim:** The aim of this study was to compare the cytotoxicity of triple antibiotic paste, calcium hydroxide and sodium hypochlorite gel when used as intra-canal medicaments on cultured fibroblasts.

**Methodology:** Experimental groups were divided into three groups; Group (CaOH), Group (TAP) & Group (NaOCl). Cytotoxicity of tested materials was determined by the microculture tetrazolium (MTT) assay. Optical density representing the residual living cells was determined for each group at different concentrations at 2h, 4h, 24h and 48h. Data were then collected, tabulated and statistically analyzed

**Results:** Statistical analysis showed that the duration range from 2 hours up to 48 hours has no significant effect for each of the three tested medicaments. There was no significant difference between CaOH and NaOCl groups whereas TAP group recorded significantly lower viability results.

**Conclusion:** Under the condition of the current study, NaOCl gel is considered acceptable intracanal medicament regarding cytotoxicity in comparison to CaOH and Triple antibiotic paste.

### INTRODUCTION

Microorganisms found in the infected root canal space are colonizing either as free-floating planktonic cells or attached to each other or to the root canal walls to form biofilms<sup>(1)</sup>. Though planktonic microorganisms can be eradicated by different methods, the elimination of biofilm bacteria from the root canal remains a foremost task<sup>(2)</sup>. Although root canal sterilization is challenging, it is commonly

accomplished by chemomechanical procedures with the assistance of antimicrobial irrigation solutions with or without intracanal medications between visits<sup>(3)</sup>. Mechanical preparation can considerably lessen intracanal bacteria but does not totally eradicate them<sup>(4)</sup>. The chemical microbial control phase uses antimicrobial agents during and after instrumentation. On average, 40% to 60% of root canals have no cultivable bacteria after mechanical

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root canal instrumentation with sodium hypochlorite (NaOCl) solution <sup>(5)</sup>

Consequently, efforts to eradicate microorganisms could link with achieving effective disinfection. Placement of intracanal medicaments such as calcium hydroxide is frequently suggested as it has high pH that alters the bacterial lipopolysaccharides in the cell wall <sup>(6)</sup>. However, it has been shown that this high pH is not maintained <sup>(7)</sup>. Furthermore, the increase in pH, can enhance the bacterial attachment to collagen fibers of dentine thus protecting them from the disinfection processes <sup>(8)</sup>. Also, it can decrease the strength of dentine, even with short span use <sup>(9)</sup> and it has low ability to penetrate dental tubules <sup>(10)</sup>.

Antibiotics are used as an adjunct to endodontic treatment but its ineffectiveness in systemic route of administration has led to the intracanal application to increase its efficacy <sup>(11)</sup>. Hoshino et al <sup>(12)</sup> suggested sterilizing infected root canals by topical use of mixture of ciprofloxacin, metronidazole and minocycline. After that, several studies reported the antimicrobial efficacy of this mixture against the pathogens commonly found inside the root canal system including *E. faecalis* <sup>(13,14)</sup>.

Continuous advances had led to a new formulation of sodium hypochlorite. This comes in the form of gel with 2.2% concentration in a trial to minimize the well-known side effects of sodium hypochlorite. This helped in increasing the viscosity keeping it within the confinement of the root canal, minimizing its toxic effect to the periapical tissues after accidental leakage or extrusion.

As the ultimate goal in endodontic therapy is the healing and regeneration of periradicular tissues and because intra-canal medicaments can come in contact with periapical tissues, thus, in addition to having good antibacterial ability, they also should be biocompatible <sup>(15)</sup>.

To our knowledge, no sufficient data are available regarding the biocompatibility of these medi-

caments to periradicular tissues. To launch biocompatibility and safety in the use of such materials, it is necessary to conduct a diversity of tests that analyze some parameters such as in vitro cytotoxicity.

Hence, this study was undertaken to compare the cytotoxicity of triple antibiotic mixture, calcium hydroxide and sodium hypochlorite gel when used as intra-canal medicaments on cultured fibroblasts. The null hypothesis was that there is no difference in the parameters of toxicity in irrigating materials evaluated.

### Methodology

All tests were done by Cancer Biology Department (National Cancer Institute, Cairo University). Experimental groups were divided into three groups; Group (CaOH), Group (TAP) & Group (NaOCl). Group (CaOH) in which Calcium hydroxide and Iodoform paste (Metapex®, Meta Biomed Co., Ltd, Korea) was tested. Group (TAP) in which triple antibiotic paste was prepared using metronidazole (500-mg tablets [Flagyl 500 mg; Aventis, Cairo, Egypt]), ciprofloxacin (250-mg tablets [Ciprocin 250 mg; EPICO, Cairo, Egypt]) and doxycycline (100-mg capsules [Vibramycin; Pfizer, Cairo, Egypt]). The doxycycline capsule content was evacuated in a sterile mortar; a tablet of metronidazole and a tablet of ciprofloxacin were crushed and ground into homogenous powder in the same mortar using a pestle. Saline drops were added and mixed using the pestle until a creamy paste was achieved. Group NaOCl in which 2.2% sodium hypochlorite gel was tested. Control group; Cells in culture environment and phosphate-buffered saline (PBS).

Cytotoxicity of tested materials was determined by the microculture tetrazolium (MTT) assay which is based on the protocol described for the first time by Mosmann <sup>(16)</sup>, where sterile filtrates were prepared by soaking test materials in the cell culture medium for 7 days. Extracts were two-fold diluted in a descending order using modified Eagle's medium (MEM) and added to plates containing fibroblasts taken from normal fibroblast cell line "BHK". The

later was obtained frozen in liquid nitrogen from the American type culture collection (ATCC, Manassas, USA) and were maintained by serial sub-culturing in the National cancer institute, Cairo, Egypt. The cell line was grown as "monolayer culture" in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 units/ml penicillin and 2mg streptomycin.

These cells were seeded at a density of  $2 \times 10^4$  cells/well in a 96-well plate in Dulbecco modified Eagle medium (Gibco, Grand Island, NY) supplemented with 100 mg/mL streptomycin, and 100 mg/mL penicillin, 10% fetal bovine serum (Sigma-Aldrich, St Louis, MO) at 37° C in an incubator under pressure air atmosphere containing 5% CO<sub>2</sub>, and incubated for 24 hrs. Confluent cells were detached with 0.25% trypsin and 0.05% EDTA for 5 minutes, and aliquots of separated cells were subcultured. Cells were seeded in 24-well plates ( $1 \times 10^5$  cells/well). After overnight attachment, cells were then exposed to either culture media (negative control) or test material and incubated. Treated cells were microscopically examined for 2, 4, 24 and 48 hours post treatment. Treatment Media was discarded at the decided time intervals. Plates were washed with phosphate buffered saline (PBS) and MTT dissolved dye was dispensed as 25  $\mu$ l / well at a final concentration of 0.5mg/ml. Plates were incubated at 37°C for 3 hrs. MTT staining buffer was decanted, plates were washed using normal saline twice 200 $\mu$ L/well. Washing buffer was decanted and 50  $\mu$ l / well DMSO (dimethylsulphoxide) were dispensed in each well. Plates were incu-

bated as previous for 2 hrs. Optical density (OD) was recorded at 570 nm using Eliza reader. Optical density representing the residual living cells was determined according the following equation:

Viability percentage =

$$\frac{\text{Optical density (OD) of treated cells}}{\text{Optical density (OD) of control cells}}$$

× Number of negative control cells ( $10^4$  cells/0.1ml).

Data were collected, tabulated and statistically analyzed using SPSS (SPSS statistical package 19, IBM Corporation 1, Armonk, NY, USA), Two way ANOVA was performed and Student-Newman Keuls post-hoc test was done for pair-wise comparison.

## RESULTS

The cells viability obtained from each experiment were calculated as a percentage of cell proliferation for each dose. Concentration-Viability related curves were then generated by plotting the percentage inhibition of cell proliferation at each concentration tested for different periods Tables(1) Figures (1-4).

Statistical analysis showed that the duration range from 2 hours up to 48 hours hasno significant effect for each of the three tested medicaments.

Results for overall cell viability were compared for the three medicaments CaOH, NaOCl gel and TAP, MTT results showed insignificant difference between CaOH and NaOCl groups wheareas TAP group recorded significantly lower viability results.

TABLE (1) Percentage of cell viability for the three tested groups at different observation periods.

	2h	4h	24h	48h	
Group (CaOH)	78.23±16.87% <sup>aA</sup>	78.12±16.9% <sup>aA</sup>	76.15±16.2% <sup>aA</sup>	89.26±6.12% <sup>aA</sup>	P=0.14
Group (NaOCl)	76.5±15.7% <sup>aA</sup>	77.17±16.5% <sup>aA</sup>	80.17±15.2% <sup>aA</sup>	80.2±17.5% <sup>aA</sup>	P=0.31
Group (TAP)	54.5±29.3% <sup>bA</sup>	53±30.4% <sup>bA</sup>	61.83±28.7% <sup>bA</sup>	65.67±23% <sup>bA</sup>	P=0.095
	P=0.0062	P=0.018	P=0.0031	P=0.011	

*Different lower-case letters indicate significant difference between different groups. Different capital letters indicate significant difference within the same group at different subgroups. P≤0.05*

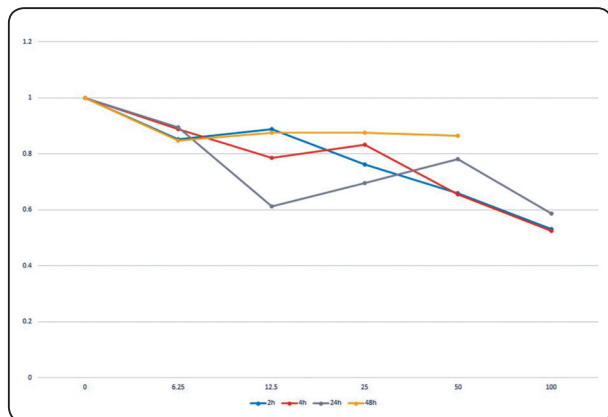


Fig. (1) Dose-viability plots of Group (CaOH) at 2h, 4h, 24h and 48h.

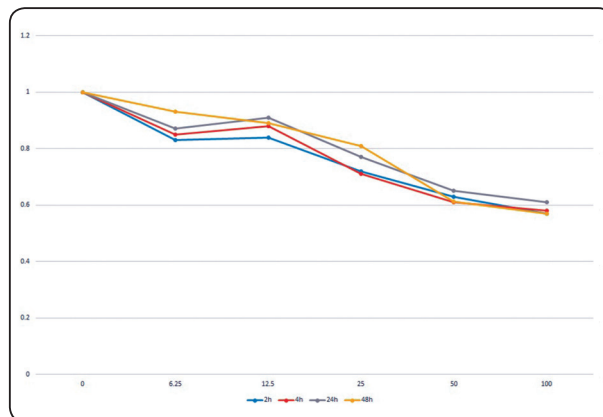


Fig. (2) Dose-viability plots of Group (NaOCl) at 2h, 4h, 24h and 48h.

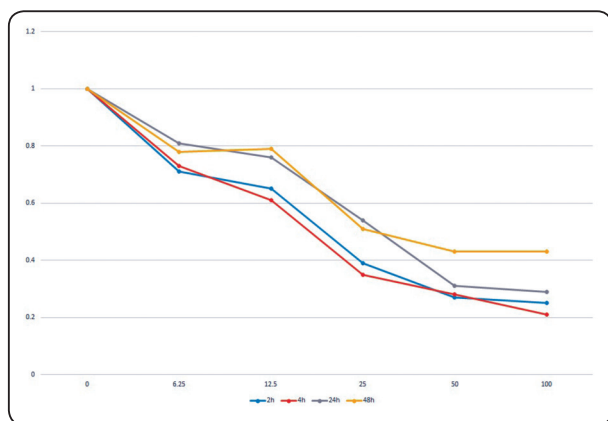


Fig. (3) Dose-viability plots of Group (TAP) at 2h, 4h, 24h and 48h.

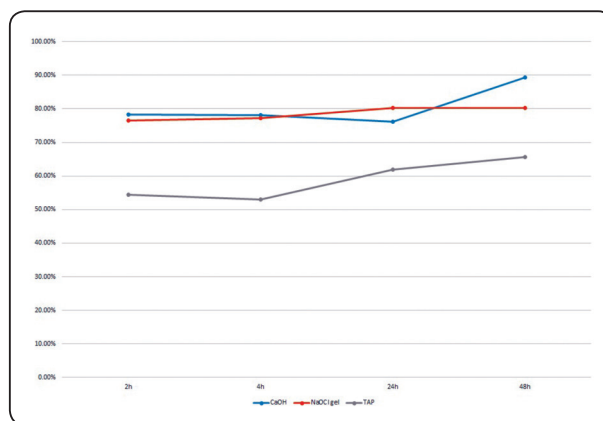


Fig. (4) overall cell viability of the three tested groups at 2h, 4h, 24h and 48h.

**DISCUSSION**

Although cleaning and shaping of root canal are effective in reducing the main bacterial load, yet remnant bacteria might remain in dentinal defects and crevices causing reinfection of the root canal.

A variety of intracanalmedicaments have been advocated for inter-appointment disinfection such as Calcium hydroxide, Chlorohexidine and recently triple antibiotic paste.

In this study, a sodium-hypochlorite based gel and Triple antibiotic paste were compared to Calcium hydroxide which is considered the gold standard as an intracanal medicament.

Sodium hypochlorite solution has been always recommended as irrigating solution during root canal instrumentation because of its well-known broad spectrum bactericidal actionas well as its unique organic matter dissolutionability<sup>(6,17,18)</sup>

Cytotoxicity was considered one of the main issues against the use of NaOCl as an intracanal medicament. Sodium hypochlorite has an alkaline pH 11–12. Upon tissue contact, nitrogen, formaldehyde and acetaldehyde are formed within a short time due to peptide links breakage resulting in protein dissolution<sup>(19)</sup>

The gel form factor <sup>(20)</sup> was an encouraging factor for testing the material due to its significant

low flow properties compared to that used as a regular irrigating solution. The form used contains Lauramine Oxide & Myristamine Oxide which serve as stabilizer and thickener. Potassium Iodide is also included and considered as synergistic disinfection<sup>(21)</sup> with the main ingredient; sodium hypochlorite. Sodium Cocoate is included as a surfactant<sup>(22)</sup> for lowering the surface tension and hence better dentin wetting. And finally, Sodium Hydroxide is included as an alkali for pH adjustment and might help in buffering the acidity due to pre-existing inflammation.

Triple antibiotic paste was recently introduced as an opponent due its outmost disinfecting efficacy as described by Hoshino et al<sup>(12)</sup> and Sato et al<sup>(13)</sup> and hence got widely spread popularity in regenerative Endodontics<sup>(23)</sup>. However, it still poses a significant cytotoxic effect<sup>(24)</sup>.

The results of our study showed that there was no significant difference between the tested groups, CaOH, and NaOCl. This might indicate an acceptable biologic reaction of NaOCl gel comparison with CaOH.

By analyzing the concentration-viability plot for all tested medicaments, It can be concluded that logically the increased concentration is associated with more cytotoxic effect with lower cell viability. Regarding the time factor, the average peak for the cytotoxic effect with all

Bogovic et al<sup>(25)</sup> studied CaOH cytotoxicity on rat fibroblasts, they found that Calcium Hydroxide has high cytotoxicity but still known to be biocompatible. On the contrary, Miranda et al<sup>(26)</sup> stated that the cytotoxicity of CaOH based on cell lysis rather than the percentage of viable cells. They found CaOH to be grade 2 (mild) where grade 2 cytotoxicity was defined as cell lysis at 5 mm distance and severe cytotoxicity was defined as lysis at 10 mm distance from the specimen. Wang et al. in 2007<sup>(27)</sup> evaluated the cytotoxicity of intracanal medicaments and concluded that CH had slight cytotoxicity

## CONCLUSIONS

Under the condition of the current study, NaOCl gel is considered acceptable intracanal medicament regarding cytotoxicity in comparison to CaOH and Triple antibiotic paste. Further research is needed to assess the effect of the NaOCl gel on root canal dentin

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