



Cytotoxicity and antibacterial activity of bleached turmeric hydro-alcoholic extract *versus* sodium hypochlorite as an endodontic irrigant: *in vitro* study

Citotoxicidade e atividade antibacteriana do extrato hidroalcoólico de cúrcuma branqueado *versus* hipoclorito de sódio como irrigante endodôntico: estudo *in vitro*

Walaa Hassan SALEM¹ , Taheya Ahmed MOUSSA² , Nehal Lotfy Abou RAYA²

1 - Egyptian-Russian University, Faculty of Dentistry, Biomaterials Department. Cairo, Egypt.

2 - Cairo University, Faculty of Dentistry, Biomaterials Department. Cairo, Egypt.

ABSTRACT

Objective: To evaluate the cytotoxicity and antibacterial effect of bleached turmeric hydro-alcoholic extract compared to sodium hypochlorite as an endodontic irrigant. **Material and Methods:** Cytotoxicity was evaluated on human skin fibroblasts using MTT assay. The irrigants were tested at 1, 5- and 15-minutes time intervals. After contact time, MTT solution was added and well plates were incubated. After the incubation period, optical density was read and correlated with cell viability percent. Antibacterial efficiency was evaluated using direct contact test. Each endodontic irrigant was added to fresh *Enterococcus faecalis* suspension and brain heart infusion media then incubated for 48 hours. After incubation period, optical density readings were obtained and read by ELISA reader at 620 nm. **Results:** Results of cytotoxicity test revealed that bleached turmeric extract had significant higher cell viability percent than Sodium hypochlorite (NaOCl) at all time intervals ($p < 0.001$). However, in the intervention group, cell viability percent significantly decreased over time. Results of antibacterial test showed bacterial inhibition by both groups with non-significant difference between the two tested groups ($p < 0.05$). **Conclusion:** Bleached turmeric hydro-alcoholic extract can represent an herbal alternative endodontic irrigant to avoid the undesirable toxic effects of NaOCl due to its less cytotoxicity and prominent antibacterial effect against *Enterococcus faecalis*.

KEYWORDS

Antibacterial effect; Bleached turmeric; Cytotoxicity; Endodontic irrigation; Sodium hypochlorite.

RESUMO

Objetivo: Avaliar a citotoxicidade e o efeito antibacteriano do extrato hidroalcoólico de cúrcuma branqueado comparado ao hipoclorito de sódio como irrigante endodôntico. **Material e Métodos:** A citotoxicidade foi avaliada em fibroblastos de pele humana usando o ensaio MTT. Os irrigantes foram testados em intervalos de tempo de 1, 5 e 15 minutos. Após o tempo de contato, a solução de MTT foi adicionada e as placas de poços foram incubadas. Após o período de incubação, a densidade óptica foi lida e correlacionada com a porcentagem de viabilidade celular. A eficiência antibacteriana foi avaliada usando o teste de contato direto. Cada irrigante endodôntico foi adicionado à suspensão fresca de *Enterococcus faecalis* e ao meio de infusão cérebro-coração e então incubados por 48 horas. Após o período de incubação, as leituras de densidade óptica foram obtidas e lidas por leitor de ELISA a 620 nm. **Resultados:** Os resultados do teste de citotoxicidade revelaram que o extrato de cúrcuma branqueado apresentou porcentagem de viabilidade celular significativamente maior do que o hipoclorito de sódio (NaOCl) em todos os intervalos de tempo ($p < 0,001$). No entanto, no grupo de intervenção, a porcentagem de viabilidade celular diminuiu significativamente ao longo do tempo. Os resultados do teste antibacteriano mostraram inibição bacteriana por ambos os grupos com diferença não significativa entre os dois grupos testados ($p < 0,05$). **Conclusão:** O extrato hidroalcoólico de cúrcuma branqueado pode representar uma

alternativa fitoterápica irrigante endodôntico para evitar os efeitos tóxicos indesejáveis do NaOCl devido à sua menor citotoxicidade e efeito antibacteriano proeminente contra *Enterococcus faecalis*.

PALAVRAS-CHAVE

Efeito antibacteriano; Cúrcuma branqueada; Citotoxicidade; Irrigação endodôntica; Hipoclorito de sódio.

INTRODUCTION

Endodontic therapy aims to clean and disinfect the root canal system from all affected vital or necrotic pulp tissues, microorganisms and microbial by-products. The root canal system is highly complex and variable, thus limits the ability to clean and disinfect it predictably [1]. The use of various instrumentation techniques alone is not effective in producing bacteria-free root canal spaces. Therefore, the use of an endodontic irrigant along with mechanical instrumentation is mandatory to aid in disinfecting and lubricating the root canal, flushing out debris from the canal system, and dissolving organic and inorganic tissues [2].

To date, sodium hypochlorite (NaOCl) is considered the gold standard for endodontic irrigants due to its potent antimicrobial activity and its ability to dissolve the organic tissues. NaOCl has a broad antibacterial spectrum and is sporicidal and viricidal. It has the ability to dissolve both necrotic and vital pulp tissues by breaking down proteins into amino acids. These valuable properties have encouraged the use of liquid NaOCl as a basic irrigation solution in endodontics since the early 1920s [3].

The bactericidal ability of NaOCl results from the formation of hypochlorous acid (HOCl), when in contact with organic debris. The resultant HOCl exerts its effects by oxidizing sulfhydryl groups within bacterial enzyme systems, thereby disrupting the metabolism of the microorganism, thus killing the bacterial cells [4].

Although NaOCl has valuable advantages, it also has major disadvantages such as cytotoxic and genotoxic effects on adjacent vital tissues, which means that, especially at high concentrations, this chemical agent may dissolve both vital and necrotic pulp remnants indistinguishably and be toxic to periapical tissues in case of extrusion through the apical foramen to the periradicular space. Another disadvantage of NaOCl is that it decreases the mechanical resistance of dentin by causing deterioration of collagen and proteoglycans [5]. These drawbacks created a

continuous need to find a safer alternative to NaOCl for endodontic irrigation.

The safety concerns, side effects, constant increase in antibiotic resistance and ineffectiveness of conventional drug formulations has prompted investigators to shift to herbal alternatives.

Herbal products are considered the best alternative for their lower side effects and less microbial resistance, provided that they can fulfil the major requirements to be used as endodontic irrigants.

Curcuma longa, commonly called turmeric belongs to ginger family, is a native of Southeast Asia and cultivated mainly in India. It has been shown to have a wide spectrum of actions like anti-inflammatory, antioxidant, antibacterial, antifungal, antiprotozoal, and antiviral activities. Components of turmeric are named curcuminoids [curcumin (feruloyl methane), desmethoxycurcumin, and bisdemethoxycurcumin]. These components are polyphenols with a strong antioxidant function. Curcumin, the most important fraction is responsible for the biological activities of turmeric [6].

The antibacterial effect of turmeric is attributed to the presence of polyphenols that affect the selective permeability of the bacterial plasma membrane leading to the leakage of intracellular substances and damage of the bacterial cell [7]

Curcumin can be extracted by an aqueous solution, alcoholic or hydro-alcoholic solutions. Previous studies has shown that hydro-alcoholic extract of curcumin had more potent antibacterial effect against *E. faecalis* compared to aqueous extract [8].

However, the major problem of curcumin or turmeric is its yellow color that was proven to have a staining effect on dental restorations and the surrounding oral tissues [9,10]. Therefore, in this study, bleaching of turmeric powder was performed to overcome the staining effect of the original turmeric color.

The bleaching technique of turmeric was previously described to be used as a topical acne cream [11]. The bleaching process implies contacting turmeric powder with a strong bleaching agent such as NaOCl for sufficient time followed by thorough washing with distilled water.

The aim of this research was to evaluate the cytotoxicity and the antibacterial activity of the bleached turmeric hydro-alcoholic extract as a natural alternative of herbal origin compared to NaOCl to be used as endodontic irrigant solution.

The null hypothesis of the present study stated that there would be no difference regarding the cytotoxic potential and antibacterial action of bleached turmeric hydro-alcoholic extract and NaOCl.

MATERIAL AND METHODS

The materials and reagents used are listed in Table I.

Preparation of bleached turmeric extract

Bleaching of turmeric powder

Bleaching of turmeric powder was done according to the method described by Nguyen [11]. One gram of turmeric powder (Puritan's Pride, USA) was added to 10 ml of household bleach (Clorox) in a sealed test tube, then vortexed (Hwashin Technology Co.-250 VM). The solution was then centrifuged at 4000 rpm for 2 minutes to separate the liquid from the powder. Bleaching time of 3 minutes was found to be the optimum

duration to produce properly bleached turmeric powder. After the bleaching process, turmeric powder was washed to remove the residues of the bleaching agent. Distilled water was added to the bleached powder, then vortexed to ensure all powder particles were contacted with water to ensure proper washing. The suspension was centrifuged at 4000 rpm for 2 minutes. The washing process was repeated until the pH value of the washing water became neutral (= 7) determined by digital pH meter (Jenway, Cole-Parmer Ltd, UK).

Preparation of hydro-alcoholic extract of bleached turmeric

Preparation of the extract was done as described by Hegde et al. [12]. Fifty grams of bleached turmeric powder were placed in a glass container, then 70 ml of distilled water and 30 ml of ethanol (70:30) were added to the powder to obtain hydro-alcoholic extract of bleached turmeric. The glass container was properly sealed to prevent the evaporation of the solution, then was kept to stand for 7 days with frequent stirring at room temperature ($24 \pm 1^\circ\text{C}$). Afterwards, the suspension was filtered to separate the powder from the liquid. After complete filtration, the liquid solution was stored in sealed test tubes and kept refrigerated (-3°C).

Sample size calculation method

Sample size for cytotoxicity test was calculated according to Karkehabadi et al. [13], who found the effect size to be (1.854). Sample

Table I - Materials and reagents used in this study

Material/Reagent	Manufacturer	Lot No.
Turmeric powder	Puritan's Pride, USA	198239
Clorox household bleach	Clorox Egypt-FMCG in 10 th of Ramadan City-Egypt	1336
99.9% Ethanol	Sphinx chemicals, Egypt	19006
Distilled water	Prepared in Research lab, faculty of pharmacy, Nahda University in BeniSuef	
Human skin fibroblasts cell line	VACSERA, Egypt	HFD4
RPMI-1640	Bio west, USA	MS009E
Fetal Bovine Serum (FBS)	Bio west, USA	S000S1810
Phosphate Buffer Saline (PBS)	Sigma, USA	SL8W7829
Dimethyl Sulfoxide (DMSO)	Alpha Chemika, India	D7012
Pure cultures of <i>Enterococcus faecalis</i>	American type Culture Collection (ATCC)	29212
Bile Aesculin Agar	Oxoid Ltd, United Kingdom	1964919
Brain Heart Infusion (BHI)	Oxoid Ltd, United Kingdom	1520234

size was calculated using G*Power version 3.1.9.2 for sample size analysis at $\alpha=0.05$ and 80% power, which yielded a sample size of 6 samples per group. Number of samples prepared per group was 7 to gain extra power.

To calculate antibacterial effect against *E. faecalis*, the effect size was found to be (1.489) according to Oliveira et al. [14]. Sample size was calculated using G*Power version 3.1.9.2 for sample size analysis at $\alpha=0.05$ and 80% power, which yielded a sample size of 9 samples per group. Ten samples per group were prepared to gain extra power.

Groups were coded according to the type of irrigant as follows:

Group 1: Bleached turmeric hydro-alcoholic extract

Group 2: 2.5% NaOCl

Cytotoxicity test using MTT assay

Evaluation of cytotoxicity was done on human fibroblasts cell line (HFD4, VACSERA, Egypt) using MTT assay. A total number of 42 samples were divided according to the test groups (bleached turmeric extract and 2.5% NaOCl).

Cells were cultured in 75 cm³ tissue culture flasks (Griner, Germany) using RPMI-1640 (Bio west, USA) and supplemented with 10% fetal bovine serum (FBS) (Bio west, USA) as culture medium. Cells were grown in 5% CO₂ in air-humidified incubator at 37°C (Jouan, France). The medium was replaced every 3 days, and cells were passaged when they were ~80% confluent. Confluent monolayer cultures were modified using 0.25% Trypsin / EDTA (VACSERA, Egypt). The cell pellets were washed with sterile phosphate buffer saline (PBS) (Sigma-Aldrich, USA) and cells were then suspended in fresh culture medium. At the time of the experiment, cells were randomly distributed in two 96-well plates. Cells were observed by an inverted binocular microscope (Carl Zeiss, Germany) to confirm their presence in wells. After that, cells were subjected to the tested irrigant solutions. This method was described in previous studies [13].

Twenty-one samples were assigned for each group (n=21). Groups were coded according to the type of irrigant as follows:

Group 1: Bleached turmeric hydro-alcoholic extract

Group 2: 2.5% NaOCl

Each group was further divided into three subgroups where seven replicates (n= 7) were tested at different time intervals 1, 5 and 15 minutes. An amount of 100 μ l of each irrigant solution (Bleached turmeric hydro-alcoholic extract or 2.5% NaOCl according to the assigned study group) was added to the cells. A number of 7 wells were used for each subgroup. After completion of each time interval, 250 μ l of saline were added to the wells to stop the action of irrigant. The solution was then aspirated and discarded. Control group of untreated cells was taken as reference for each irrigant group.

MTT solution was prepared by dissolving 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (Sigma-Aldrich, USA) in PBS (0.5mg/ml). After that, 50 μ l were added to each well then, the plates were incubated at 37°C under 5% CO₂ and 95% humidity for 4 hours (Thermo Electron Co, USA). After the incubation period, the supernatant solution was gently evacuated from each well and replaced with 50 μ l of dimethyl sulfoxide (DMSO) solution (Alpha Chemika, India). After dissolution of formazan crystals, optical density was read using Elisa reader at wavelength 570nm (ELX-800, Biotek, USA). The intensity of color generated was correlated with the percentage of viable cells. The obtained optical density readings were tabulated and statistically analyzed. Also, cell viability percent for each group was determined according to the following equation:

$$\text{Cell viability \%} = \frac{\text{mean optical density of test solution}}{\text{mean optical density of cell control}} \times 100 \quad (1)$$

Antibacterial test against *E. faecalis* using direct contact test

Pure cultures of *Enterococcus faecalis* (ATCC 29212) were grown on bile aesculin (Oxoid Ltd, United Kingdom) supplemented with 1.5% (wt./vol) agar (Bio sharp, Hirono, Japan), then incubated aerobically at 37 C for 24 hours. Afterwards, a single colony of *E. faecalis* from a bile aesculin plate was collected and suspended in sterile Brain Heart Infusion (BHI) (Oxoid Ltd, United Kingdom) broth at 37 C. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale (1.5 \times 10⁸ cfu/ml).

Direct contact test was done to evaluate antibacterial efficiency according to the method described by Souza et al. [15]. The tested solutions were put in two test tubes and named as follows: group 1 (bleached hydro-alcoholic turmeric solution) and group 2 (2.5% NaOCl solution). One milliliter of each solution was placed on the bottom of the individual test tube. Two milliliters of the bacterial suspension were added to the solution, then vortexed (Vortex mixer, MoBio, USA) for 5 minutes. Half ml of each tube was transferred to a tube containing 1 ml of BHI. Ten samples were made from each solution. Then, 100 μ l were transferred from each group to 96 well plate to be subjected to optical density readings. Optical density readings were obtained at wavelength 620 nm by Multiskan Elisa reader (Thermofisher scientific, USA) before incubation as a baseline record (T_0). Then, all tubes were inserted in an incubator (Mettler, Germany) at 37°C for 48 hours, to obtain optical density readings again (T_{48}). Optical density readings of the media alone, cell suspension alone and each irrigant sample alone were also determined to ensure that bacterial inhibition was related to the antibacterial activity of the endodontic irrigant. The resulted readings were tabulated and statistically analyzed.

Statistical analysis

Data were coded and analyzed using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were summarized using mean and standard deviation. Comparisons between groups were done using unpaired t-test [16]. For comparison of serial measurements within each group paired t-test and repeated measures ANOVA were used [17]. P-values less than 0.05 were considered as statistically significant. Data were explored for normality using Kolmogorov-Smirnov test.

RESULTS

Cytotoxicity test

At all-time intervals of testing, bleached turmeric hydro-alcoholic extract showed significant positive results than NaOCl group indicating less cytotoxic effect as indicated by the unpaired t-test in Table II (P-value < 0.001). The cell viability percent significantly decreased over time in turmeric group (P-value < 0.001). There was no significant difference in cell viability percent of NaOCl group over time (P-value 0.228). The calculated cell viability percent in both groups is shown in Table III.

Antibacterial test

Both groups showed bacterial inhibition manifested by decreased optical density values, with non-significant difference between them (Table IV).

The bacterial growth media and the solutions of the tested irrigation samples showed minimum mean values of optical density after incubation. On the other hand, mean optical density value of bacterial suspension increased after incubation.

DISCUSSION

The rationale of the present study was the need to find a safe alternative to NaOCl to avoid its detrimental cytotoxic effects. Herbal products represent the best alternative if they can fulfil the other requirements to be used as an endodontic irrigant.

In the present study, we implemented a method for bleaching of the turmeric powder to avoid the possible staining effect of its yellow color. The aim of the work was to compare bleached turmeric extract to NaOCl as an endodontic irrigant regarding cytotoxicity, antibacterial activity

Table II - Mean optical density values of cell viability of the tested groups at different time intervals

	Group 1 Intervention		Group 2 Control		P value
	Mean	SD	Mean	SD	
Optical density 1 min	0.214	0.019	0.042	0.010	<0.001
Optical density 5 min	0.172	0.026	0.035	0.006	<0.001
Optical density 15 min	0.128	0.006	0.040	0.008	<0.001

Table III - Cell viability percent of the two investigated groups

Time	Group 1 Intervention	Group 2 Control
1 minute	58.4%	14.8%
5 minutes	46.42%	11.56%
15 minutes	36.36%	12.3%

Table IV - Mean optical density values for antibacterial activity of the tested groups

Group 1		Group 2		P value
Mean	SD	Mean	SD	
0.006	0.002	0.008	0.005	0.796

Bleaching of the turmeric powder was done to avoid yellow staining effect of non-bleached turmeric to the surrounding restorations and oral tissues. The bleaching process could be done by sodium hypochlorite or sodium perborate. In the present study we used sodium hypochlorite (Clorox) for the ease of use and availability.

The solvent of the turmeric extract was hydro-alcoholic (70% water-30% ethanol) as the active ingredient curcumin was proved not to lose its active potential when extracted in ethanolic solvent. Besides, the active component of turmeric is insoluble in water. Therefore, hydro-alcoholic extract was chosen according to various studies stated that ethanol is a good solvent for turmeric with better efficacy extracting the active components compared to aqueous extract [8,12,18], in addition, the gradient percent of the solvent and the concentration of turmeric in the solvent (70:30) with proven antibacterial effect against *E.faecalis* comparable to NaOCl were used according to the method described by Chaitanya et al. [8]. On the other hand, NaOCl was used in concentration 2.5% as it is the most commonly used concentration in the clinical situation to minimize its high toxic effect [19].

Cytotoxicity was assessed by MTT assay as it is a sensitive and quantitative method for the detection of cellular activity, easy to use and highly reproducible. Cytotoxicity was evaluated after time intervals 1, 5 and 15 minutes according to a previous study by Karkehehadi et al. [13] where these time intervals mimic the clinical situation for how long the irrigation solution comes in contact to the adjacent periapical tissues during an endodontic treatment. Dermal fibroblasts were chosen for the test because they have almost the

same fundamental characteristics as intraoral fibroblasts [20]. This method is a colorimetric assay that depends on the reduction of MTT, a yellow water-soluble tetrazolium dye, primarily by the mitochondrial dehydrogenases, to purple colored formazan crystals. MTT formazan is insoluble in water thus it could not be detected for colorimetric measurement. Therefore, an organic solvent such as dimethyl sulfoxide (DMSO) is required to solubilize the crystals [21]. The formazan product is then analyzed and quantified spectrophotometrically at a specific wavelength. Higher values of optical density indicate more cellular viability.

In the present study, NaOCl showed low cell viability percent at all tested time intervals (1, 5 and 15 minutes), where the cell viability percent was 14.8%, 12.3% and 11.56% respectively. These results revealed that NaOCl is highly cytotoxic to fibroblasts with inverse relation between exposure time and cell viability in agreement with previous studies [13,22-24].

However, on the other hand, cell viability percent in the bleached turmeric group was significantly higher than NaOCl group at all time intervals, where it was 58.4%, 46.42% and 36.36% at time intervals 1, 5 and 15 minutes respectively. These findings prove that bleached turmeric hydro-alcoholic extract is safer and have less cytotoxic potential than NaOCl as confirmed by Indi and Kulkarni [25] in 2016 using hemolysis test.

The significant decrease in cell viability percent by time exhibited by the bleached turmeric group could be attributed to the effect of ethanol in the hydro-alcoholic extract [26]. It was explained in previous studies that ethanol concentration above 25-30% cause cellular basement membrane alterations. These alterations include disruptions, discontinuities and irregularities detected by electron microscopy [27]. It was also concluded that ethanol affects cellular viability in a dose and time-dependent manner [28].

On the other hand, evaluation of antibacterial activity was tested against *E.faecalis* because it is the most commonly isolated pathogen in persistent root canal infections [29]. Direct contact test was used as a standardized protocol developed by Weiss et al. [30] as it is independent on diffusion and physical properties of tested materials. It is based on determining the turbidity of bacterial growth in 96-well plate. The major advantage of this method is its sensitivity as optical detection systems allow detection of

subtle changes in bacterial growth and eliminated the inaccuracy of manual readings [31].

Base line optical density readings were used to compare it with those after incubation. Optical density of bacterial suspension only was read to ensure that there was bacterial outgrowth after incubation. Also, optical densities of the media only and tested materials only were read to ensure that they are free from bacteria. The previous measures together ensure that bacterial inhibition after incubation against the tested material was related only to its antibacterial effect. Absolute ethanol was not tested as previously published data revealed it has no effect against *E.faecalis* [18,32].

The NaOCl group showed significant antibacterial activity (manifested by low mean optical density value = 0.05). The strong antibacterial effect of NaOCl is attributed to the formation of hypochlorous acid (HOCl) when NaOCl ionizes in water. HOCl adversely affects the vital functions of the bacterial cell through oxidation of the bacterial enzymes leading to the formation of disulfide linkages and interruption of the bacterial metabolic reactions [1]. Nevertheless, its cytotoxicity is inevitable affecting viable periapical cells.

There was no difference between bleached turmeric group and NaOCl group regarding antibacterial effect against *E.faecalis* (P value > 0.05). Neelakantan et al. in 2011 [33], Sinha et al. in 2015 [34], and Dhariwal et al. in 2016 [35] also reported that there was no significant difference between NaOCl (34 ± 4) and non-bleached turmeric (32 ± 4) in elimination of *E.faecalis* in spite of using different method: agar diffusion. The antibacterial effect of turmeric is attributed to the presence of polyphenols that affect the selective permeability of the bacterial plasma membrane leading to the leakage of intracellular substances and damage of the bacterial cell [7]. Absolute ethanol was not selected as a group in the present study as previously published data revealed it has no effect against *E.faecalis* [32,34], which ensures that the bacterial inhibition results from the bleached turmeric extract not the ethanol. However, there is disagreement with Neelakantan et al. in 2013 [36] Hegde and Kesaria in 2013 [37] and Chaitanya et al. in 2016 [8] who compared the antibacterial activity of NaOCl to aqueous extract of turmeric using agar diffusion method, where they reported that NaOCl showed larger

zone of inhibition (34 ± 4 mm) than turmeric extract (12 ± 4). This disagreement could be attributed = [to the type of the used turmeric extract, where in the above-mentioned studies, turmeric aqueous extract was used while in the present study we used turmeric hydroalcoholic extract.

The minimum values of optical density of the bacterial growth media and solutions of the tested irrigants after incubation indicated they were free of bacterial growth. However, the mean optical density of bacterial suspension increased after incubation which proved the bacteria is capable of growth and the optical density readings were due to the effect of irrigant.

CONCLUSION

Based on the limits of the current study, it could be concluded that bleached turmeric hydro-alcoholic extract can be considered as an alternative herbal endodontic irrigant to avoid the undesirable toxic effects of NaOCl due to its less cytotoxicity and prominent antibacterial effect against *E.faecalis*.

LIMITATIONS

The encountered limitations of the present study were lack of availability of normal oral fibroblasts cell lines (gingival or periodontal cell lines) to be accurately mimicking the oral conditions. Also, lack of funding which limits further testing of the material on other properties.

Author's Contributions

WHS: Conceived and planned the experiments, performed the experiments and wrote the manuscript with input from all authors. TAM: Designed the model, supervised overall direction and planning, and contributed to the interpretation of the results. NLAR: Conceived and planned the experiments supervised the experimental part, manuscript writing and interpretation of data.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Regulatory Statement

The regulatory statement is not applicable as this is *in vitro* study and no human subjects were included in this study.

REFERENCES

- Topbas C, Adiguzel O. Endodontic irrigation solutions: a review. *J Dent App J Dent App*. 2018;2017(7):25-32.
- Vineet AS, Rajesh M, Sonali K, Mukesh P. A contemporary overview of endodontic irrigants – a review. *J Dent App J Dent App*. 2014;1(1):105-15.
- Mohammadi Z, Abbott PV. Antimicrobial substantivity of root canal irrigants and medicaments: a review. *Aust Endod J*. 2009;35(3):131-9. <http://dx.doi.org/10.1111/j.1747-4477.2009.00164.x>. PMID:19961451.
- Hülsmann M, Hahn W. Complications during root canal irrigation-literature review and case reports. *Int Endod J*. 2000;33(3):186-93. <http://dx.doi.org/10.1046/j.1365-2591.2000.00303.x>. PMID:11307434.
- Bosch-Aranda ML, Canalda-Sahli C, Figueiredo R, Gay-Escoda C, Alkahtani A, Alkahtany SM, et al. Managing sodium hypochlorite accidents: the reality of toxicity. *J Clin Exp Dent*. 2012;72(6):271.
- Sharma A. Turmeric - its applications in dentistry. *J Adv Pharm Technol Res*. 2016;3(1):1-4. PMID:26955602.
- Soligo LT, Lodi E, Farina AP, Souza MA, Vidal CMP, Cecchin D. Antibacterial efficacy of synthetic and natural-derived novel endodontic irrigant solutions. *Braz Dent J*. 2018;29(5):459-64. <http://dx.doi.org/10.1590/0103-6440201802172>. PMID:30517444.
- Chaitanya BV, Somisetty KV, Diwan A, Pasha S, Shetty N, Reddy Y, et al. Comparison of antibacterial efficacy of turmeric extract, morinda citrifolia and 3% sodium hypochlorite on *Enterococcus faecalis*: an in-vitro study. *J Clin Diagn Res*. 2016;10(10):ZC55-7. <http://dx.doi.org/10.7860/JCDR/2016/19718.8650>. PMID:27891459.
- Bindal P, Bindal U, Dabbagh A, Ramanathan A, Ginjupalli K. Comparative effects of turmeric, coffee, and chewable tobacco on the color stability of tooth-colored restorative materials. *Br Dent J*. 2015;219(12):570-570. <http://dx.doi.org/10.1038/sj.bdj.2015.953>.
- Malhotra N, Shenoy RP, Acharya S, Shenoy R, Mayya S. Effect of three indigenous food stains on resin-based micro-hybrid and nanocomposite. *J Esthet Restor Dent*. 2011;23(4):250-7. <http://dx.doi.org/10.1111/j.1708-8240.2011.00431.x>. PMID:21806757.
- Nguyen VB. Turmeric for treating health ailments. United States Patent US-6048533-A. 2000. p. 233-5.
- Hegde N, Shetty S, Yelapure M, Patil A. Evaluation of antimicrobial activity of aqueous and hydro-alcoholic curcuma longa extracts against endodontic pathogens. *IOSR J Pharm*. 2012;2(2):192-8. <http://dx.doi.org/10.9790/3013-0220192198>.
- Karkehabadi H, Yousefifakhr H, Zadsirjan S. Cytotoxicity of endodontic irrigants on human periodontal ligament cells. *Iran Endod J*. 2018;13(3):390-4. PMID:30083212.
- Oliveira DP, Barbizam JVB, Trope M, Teixeira FB. In vitro antibacterial efficacy of endodontic irrigants against *Enterococcus faecalis*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology*. 2007;103(5):702-6. <http://dx.doi.org/10.1016/j.tripleo.2006.11.007>. PMID:17368057.
- Souza MA, Dalla Lana D, Gabrielli E, Ribeiro MB, Miyagaki DC, Cecchin D. Effectiveness of final decontamination protocols against *Enterococcus faecalis* and its influence on bond strength of filling material to root canal dentin. *Photodiagnosis Photodyn Ther*. 2017;17:92-7. <http://dx.doi.org/10.1016/j.pdpdt.2016.11.004>. PMID:27866000.
- Chan YH. Biostatistics102: quantitative data – parametric & non-parametric tests. *Singapore Med J*. 2003;44(8):391-6. PMID:14700417.
- Chan Y. Biostatistics301: repeated measurement analysis. *Singapore Med J*. 2004;45(8):354-68. PMID:15284929.
- Joy Sinha D, D S Nandha K, Jaiswal N, Vasudeva A, Prabha Tyagi S, Pratap Singh U. Antibacterial effect of *Azadirachta indica* (Neem) or *Curcuma longa* (Turmeric) against *Enterococcus faecalis* compared with that of 5% sodium hypochlorite or 2% chlorhexidine in vitro. *Bull Tokyo Dent Coll*. 2017;58(2):103-9. <http://dx.doi.org/10.2209/tdcpublication.2015-0029>. PMID:28724858.
- Bosch-Aranda ML, Canalda-Sahli C, Figueiredo R, Gay-Escoda C. Complications following an accidental sodium hypochlorite extrusion: a report of two cases. *J Clin Exp Dent*. 2012;4(3):e194-8. <http://dx.doi.org/10.4317/jced.50767>. PMID:24558554.
- Ebisawa K, Kato R, Okada M, Sugimura T, Latif MA, Hori Y, et al. Gingival and dermal fibroblasts: their similarities and differences revealed from gene expression. *J Biosci Bioeng*. 2011;11(3):255-8. <http://dx.doi.org/10.1016/j.jbiosc.2010.11.014>. PMID:21288768.
- Aslantürk ÖS. In vitro cytotoxicity and cell viability assays: principles, advantages, and disadvantages. In: Larramendy ML, Soloneski S, editors. *Genotoxicity: a predictable risk to our actual world*. London: IntechOpen; 2018. p. 64-82. <http://dx.doi.org/10.5772/intechopen.71923>.
- Farhad Mollashahi N, Saberi E, Karkehabadi H. Evaluation of cytotoxic effects of various endodontic irrigation solutions on the survival of stem cell of human apical papilla. *Iran Endod J*. 2016;11(4):293-7. PMID:27790258.
- Heling I, Rotstein I, Dinur T, Szwec-Levine Y, Steinberg D. Bactericidal and cytotoxic effects of sodium hypochlorite and sodium dichloroisocyanurate solutions in vitro. *J Endod*. 2001;27(4):278-80. <http://dx.doi.org/10.1097/00004770-200104000-00009>. PMID:11485267.
- Singh A, Kakkar P, Pant AB. Comparative evaluation of cytotoxic effects of MTAD and sodium hypochlorite using lactate dehydrogenase and trypan blue assays : an in vitro study. *Saudi Endod J*. 2018;8:189-95.
- Indi S, Kulkarni S. Cytotoxic analysis of herbal root canal irrigants at cellular level. *Int J Res Sci Innov*. 2016;3(6):50-4.
- Tapani E, Taavitsainen M, Lindros K, Vehmas T, Lehtonen E. Toxicity of ethanol in low concentrations: experimental evaluation in cell culture. *Acta Radiol*. 1996;37(6):923-6. <http://dx.doi.org/10.3109/02841859609175470>. PMID:8995467.
- Wyganowska-Świątkowska M, Nowak A, Paszyńska E, Grzech-Lesniak K. Ethanol influence on gingival fibroblasts – A real-time in vitro study. *Ann Agric Environ Med*. 2018;25(4):647-50. <http://dx.doi.org/10.26444/aaem/78696>. PMID:30586975.
- Chen CC, Chang JH, Lee JB, Javier J, Azar DT. Human corneal epithelial cell viability and morphology after dilute alcohol exposure. *Invest Ophthalmol Vis Sci*. 2002;43(8):2593-602. PMID:12147590.
- Vinothkumar TS, Rubin M, Balaji L, Kandaswamy D. In vitro evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase

chain reaction. *J Conserv Dent*. 2013;16(2):167-70. <http://dx.doi.org/10.4103/0972-0707.108208>. PMID:23716972.

30. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. *Endod Dent Traumatol*. 1996;12(4):179-84. <http://dx.doi.org/10.1111/j.1600-9657.1996.tb00511.x>. PMID:9028181.
31. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis*. 2009;49(11):1749-55. <http://dx.doi.org/10.1086/647952>. PMID:19857164.
32. Ghonmode WN, Balsaraf OD, Tambe VH, Saujanya KP, Patil AK, Kakde DD. Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *E. faecalis*: an *in vitro* study. *J Int Oral Health*. 2013;5(6):61-6. PMID:24453446.
33. Neelakantan P, Subbarao C, Subbarao CV. Analysis of antibacterial activity of curcumin against enterococcus fecalis. *Int J Curr Res Rev*. 2011;03(09):37-41.
34. Sinha DJ, Vasudeva A, Jaiswal N, Garg P, Tyagi SP, Singh J. Antibacterial efficacy of Melaleuca alternifolia (Tea tree oil), Curcuma longa (Turmeric), 2% chlorhexidine, and 5% sodium hypochlorite against Enterococcus faecalis: an *in vitro* study. *Saudi Endod J*. 2015;5(3):182-6. <http://dx.doi.org/10.4103/1658-5984.163623>.
35. Dhariwal NS, Hugar S, Harakuni S, Sogi S, Assudani H, Mistry L. A comparative evaluation of antibacterial effectiveness of sodium hypochlorite, Curcuma longa, and Camellia sinensis as irrigating solutions on isolated anaerobic bacteria from infected primary teeth. *J Indian Soc Pedod Prev Dent*. 2016;34(2):165-71. <http://dx.doi.org/10.4103/0970-4388.180447>. PMID:27080968.
36. Neelakantan P, Subbarao C, Sharma S, Subbarao CV, Garcia-Godoy F, Gutmann JL. Effectiveness of curcumin against enterococcus faecalis biofilm. *Acta Odontol Scand*. 2013;71(6):1453-7. <http://dx.doi.org/10.3109/00016357.2013.69627>. PMID:23394209.
37. Hegde V, Kesaria DP. Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against *E. Faecalis* and *C. Albicans* - An *in vitro* study. *Endodontology*. 2013;25(2):38-45. <http://dx.doi.org/10.4103/0970-7212.352328>.

Nehal Lotfy Abou Raya
(Corresponding address)

Cairo University, Faculty of Dentistry, Biomaterials Department, Cairo, Egypt.
Email: nl.abouraya@dentistry.cu.edu.eg

Date submitted: 2022 June 26
Accept submission: 2022 Oct 03