Effectiveness of Oxidative Potential Water as an Irrigant in Pulpectomized Primary Teeth

Valdez-Gonzalez C * / Mendez-Gonzalez V ** / Torre-Delgadillo G *** / Flores-Reyes H **** / Gaitan-Fonseca C ***** / Pozos-Guillen AJ ******

Objective: *The aim of this study was to evaluate the effectiveness of oxidative potential water (OPW) as an irrigating solution in reducing bacterial loading in necrotic pulpectomized primary teeth.* ***Study design:*** *Forty necrotic teeth were included, 20 irrigated with OPW (experimental group) and 20 with 1% NaOCl (control group); in both groups, 2 microbiological samples from within the canals were taken with a sterile paper point, the first before irrigation (immediately before opening the crown), and the second after instrumentation and final irrigation (before filling). All samples were evaluated by McFarland´s scale.* ***Results:*** *After the samples were analyzed before and after irrigation in the control group, there was a significant decrease in bacterial load, as in the experimental group ($P < 0.0001$). When both groups were compared post irrigation, no significant difference was observed ($P = 0.1519$).* ***Conclusion:*** *The OPW was as effective as the NaOCl and is suggested as an alternative for irrigating after pulpectomy of necrotic primary teeth.*

Keywords: *irrigating solution, oxidative potential water, primary teeth, pulpectomy*
J Clin Pediatr Dent 37(1): 31–36, 2012

INTRODUCTION

One of the most challenging objectives of pediatric dentistry is the preservation of the integrity of the primary arches until they are naturally exfoliated.[1]

* Concepcion Valdez-Gonzalez, DDS, Resident Pediatric Dentistry Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
** Veronica Mendez-Gonzalez, DDS, MS, Associate Professor, Endodontics Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
*** Gabriela Torre-Delgadillo, DDS, MS, Associate Professor, Pediatric Dentistry Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
**** Hector Flores-Reyes, DDS, MS, PhD, Associate Professor, Endodontics Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
***** Cesar Gaitan-Fonseca, DDS, MS, Associate Professor, Centro de Ciencias de la Salud, Universidad Autónoma de Aguascalientes, México.
****** Amaury J Pozos-Guillen, DDS, MS, PhD, Associate Professor, Pediatric Dentistry Postgraduate Program and Endodontics Postgraduate Program Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.

Send all correspondence to Amaury de Jesús Pozos Guillén, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava #2, Zona Universitaria, C.P.78290; San Luis Potosí, SLP. Mexico.

Tel: 52 (444) 8262357 X 114
Fax: 52 (444) 8139743.

E-mail: apozos@uaslp.mx.

The main purpose of pulp treatment of primary teeth is to preserve pulp vitality; however, if the infectious or inflammatory process is so advanced that it is not possible to carry out conservative pulp treatment, then the ideal treatment is pulpectomy. The objective of pulpectomy is to reduce or eliminate microorganisms through appropriate chemomechanical disinfection of the root canal system by means of mechanical instrumentation and profuse irrigation.[2] The ideal irrigating solution should remove necrotic pulp remnants, tissue fluid, bacteria, mummified tissue slices, living tissue, and instrumentation products; in addition, it should be able to remove organic and inorganic substances, have low toxic potential, be biocompatible with oral tissues, have excellent antimicrobial action, and have the ability to remove the smear layer.[3]

A number of solutions have been used for irrigating root canals both during and after primary root canal preparation. Sodium hypochlorite (NaOCl) is one of the most popular irrigating solutions and is widely used as endodontic irrigant due to its antibacterial activity and its ability to dissolve necrotic tissue remnants. Despite these features, its use has been controversial because of its citotoxicity and potential risk of injury to permanent tooth germs when it reaches the periapical tissues.[4] Chlorhexidine gluconate has been suggested as an effective irrigating solution for pulpectomy treatment of necrotic primary teeth, having shown a reduction of intracanal bacterial loading.[5] Physiological saline solution has also been used as an irrigant because of its availability and low cost. It is able to remove debris and lubricate the canal during instrumentation, but it has no

antimicrobial activity and does not dissolve organic material.[6]

Recently a solution called oxidative potential water (OPW), or electrochemically activated water (ECA), was introduced as an irrigating solution.[7] This electrolytic solution has important characteristics, including a pH of 2.3–2.7 and an oxidation-reduction potential (ORP) greater than +1100 mV.[8] It has bactericidal efficacy against *Streptococcus sobrinus, Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetecomitans, Fusobacterium nucleatum, Escherichia coli, Pseudomonas aeruginosa, Baccillus subtilis,* methicillin-resistant *Staphylococcus aureus, Enterococcus faecalis,* and *Candida albicans*.[9,10] It also has important features useful as an endodontic irrigant because of its ability to remove the smear layer and leave the dentinal tubules permeable; further, it has been shown to be less cytotoxic than EDTA or sodium hypochlorite.[11] For these reasons, OPW has been considered an alternative endodontic irrigant in permanent teeth; however, its use in primary teeth has not been evaluated. In this context, the aim of this study was to evaluate the effectiveness of OPW in reducing bacterial loading as an irrigating solution in necrotic pulpectomized primary teeth.

MATERIALS AND METHOD

This study was a double-blinded, randomized, placebo-controlled, crossover clinical trial conducted in accordance with the Declaration of Helsinki. The ethics committee of our institution approved this study, whose objective was explained to the parents/legal guardians and for which written informed consent was obtained. This controlled, randomized clinical trial included 40 patients of both sexes between 3 and 8 years old. Inclusion criteria were as follows: primary teeth (anterior or posterior) containing at least one necrotic pulp canal, abscess, or sinus tract; presence of radiolucent area(s) in furcation or periapical region; at least two thirds of root remaining; carious lesion(s) without direct exposure to the oral environment; sufficient tooth structure to support a rubber dam; sufficient isolation and sterility control in the operative field to demonstrate no bacterial growth. Patients who had received antibiotics up to 2 weeks prior to the sampling, having any systemic compromise, or having any non restorable teeth, perforated pulpal floor, excessive mobility, or pathological root resorption were excluded. Contaminated microbiological samples were eliminated from the study. Forty canal treatments were performed in necrotic primary teeth; 20 belonged to the experimental group, which were irrigated with OPW, and 20 to the control group, which were irrigated with 1% NaOCl. All treatments were performed in a single visit. Sampling of patients was made by a non probabilistic method (consecutive cases), and irrigant selection in each case was assigned by simple randomization in blocks with matching to 4.

Prereduced thioglycolate tubes, supplemented with hemin (5 mg L-1) and menadione (1 mg L-1) (Oxoid Ltd, Basingstoke, Hampshire, UK), were used as transport and growth media owing to their capacity to maintain the vitality of sampled bacteria.[12]

Isolation and Operative Field Disinfection

The study procedure was performed by a single pediatric dentist; periapical radiographs of the selected teeth were taken using a standard paralleling technique. After antisepsis of the oral cavity, local anesthesia was induced using an inferior alveolar nerve block for the mandibular teeth and infiltration (palatal and buccal) for the maxillary teeth. Each treated tooth was cleaned with pumice and isolated with a rubber dam. Provisit (Casa Idea, SA de CV, Mexico) was placed along the tooth-rubber dam interface to prevent leakage of saliva into the operative field. To disinfect the operative field, we followed the protocol previously described.[13] Briefly, the tooth crown, surrounding rubber dam, and clamp were swabbed with 30% H_2O_2 (Fermont, Productos Quimicos, Monterrey, Mexico), followed by 5.25% NaOCl for 1 minute each; both solutions were inactivated with 10% sodium thiosulfate. Disinfection control samples were taken with sterile cotton pellets from the coronal surface of the tooth, rubber dam, and clamp, and immediately inoculated on blood agar plates (BBL, Becton Dickinson, Cuautitlan, Mexico). The samples were then transferred to an aerobic incubator at 37ºC for 48 hours. The gross carious tissue was removed with a sterile round carbide bur (No. 3) cooled with sterile saline solution. The cavity and field were again disinfected as above. Then the pulp ceiling was withdrawn using a new bur of the same size, a sterile cotton pellet was placed on the floor of the pulp chamber to prevent penetration of disinfectants into the canals, and the root canal was accessed.

Collection of Microbiological Samples (Pre and Post irrigation)

Once the canals were exposed and, after the canal's length was estimated using the preoperative periapical radiograph, the first microbiological sample was obtained from inside the canal (pre irrigation); then 3 sterile absorbent paper points of a size compatible with the root canal diameter were sequentially placed for 30 seconds. If the canal was dry, then a small amount of sterile saline was used to wet the canal before the points were inserted. The retrieved paper points were immediately placed into the tube with thioglycolate. After sample collection, the teeth were given conventional pulpectomy treatment. The usual instrumentation was done with FlexoFiles (Dentsply Maillefer, Balaigues, Switzerland), together with 1 irrigation of 0.5 mL of the selected solution between each filing. At the end of the instrumentation and before filling, the canal was irrigated for the last time and dried. At that time, a second microbiological sample was acquired from the same canal, as previously described, with another 3 paper points. Finally, the canal was filled with an iodoform paste (Vitapex®), and a postoperative X-ray was taken.

Laboratory Procedures

Pre- and post irrigation samples were incubated for 24 hours at 37°C in an anaerobic chamber (85% N_2, 10% H_2, 5% CO_2) (Coy Laboratory Products, Grass Lake, MI, USA). Later, the bacteria present in these samples were counted by means of the turbidimetry method, through McFarland's scale pattern. This method estimates the number of bacteria in suspension (as CFU/mL) according to the degree of turbidity or density displayed by the different values of the scale.[14] In addition, samples of the operative field (tooth crown, rubber dam, and clamp) in blood agar were also assessed, using the same method, to verify complete disinfection of the field before elimination of the carious tissue. Before the microbiological phase of this research, McFarland's measurement scale of 10 degrees was developed in the laboratory.

Preparation of OPW

The OPW was produced by an electrolytic process in an electrochemical cell, which was divided into 2 compartments (cathode/anode) separated by a cationic membrane, the latter to avoid passage of ions from 1 compartment to another in the solution, maintaining the proton concentration (H+) and oxidized species on the anionic side. Later, 2 electrodes, 1 of titanium oxide (anode), and the other of titanium settled (cathode). Then 500 mL 1% NaOCl was placed in each compartment and a voltage was applied. Two electrodes were placed within the compartment of the anode, in order to register ORP (Pt-Ag/AgCl) and pH (Ag/AgCl) during the electrolytic activation of the solution, until reaching the properties required for the OPW: +1100 mV pH and ORP greater than 2.7.[8,9]

Statistical Analysis

Intragroup and intergroup comparisons (pre irrigation samples vs. post irrigation samples) were performed. A nonparametric statistical analysis (Mann-Whitney *U* test) was carried out for analyzing the differences between groups. Alpha level was set at 0.05. The JMP IN v. 4.0.1(SAS Institute Inc, Cary, NC, USA) statistical program was used to analyze the data.

RESULTS

In all, 40 canal treatments were performed on primary necrotic teeth in pediatric patients whose average age was 5.5 years. Of these, 80 microbiological samples were obtained: 40 from the experimental group (20 before and 20 after irrigation) and 40 from the control group (20 before and 20 after irrigation).

Basal conditions (pre irrigation) were comparable in both groups in relation to the bacterial load. The number of colony-forming units (CFU)/mL from the pre irrigation samples were quantified and compared. The comparative statistical analysis exhibited a *P* value of 0.5589 (Mann-Whitney *U* test), indicating that there was no statistical difference between (homogeneous) groups under basal conditions.

In the pre-irrigation samples corresponding to the OPW group, a median of 1.65×10^9 CFU/mL (range, 9×10^8–2.1×10^9) with a mean of $1.63 \times 10^9 \pm 4.18 \times 10^8$ CFU/mL was obtained. In the same experimental group for post-irrigation samples, a median of 0 CFU/mL (range $0 – 5 \times 10^8$), with a mean of $5 \times 10^7 \pm 1.53 \times 10^8$ CFU/mL, was obtained. The difference between the bacterial load after irrigation with OPW was statistically significant ($P < 0.0001$, Mann-Whitney *U* test) (Figure 1). For the control group (1% NaOCl) pre-irrigation samples, a median of 1.5×10^9 CFU/mL (range $1 \times 10^9 – 2.1 \times 10^9$) with a mean of $1.56 \times 10^9 \pm 3.36 \times 10^8$ CFU/mL was obtained. For the post irrigation samples, a median and mean of 0 CFU/mL were obtained. The difference between bacterial load after irrigation with 1% NaOCl was statistically significant ($P < 0.0001$, Mann-Whitney *U* test) (Figure 2). Finally, the antimicrobial activity of both irrigating solutions used in the study was compared by quantifying the bacterial CFU/mL after irrigation. The analysis demonstrated no statistically significant difference between 1% NaOCl and OPW ($P = 0.1519$, Mann-Whitney *U* test).

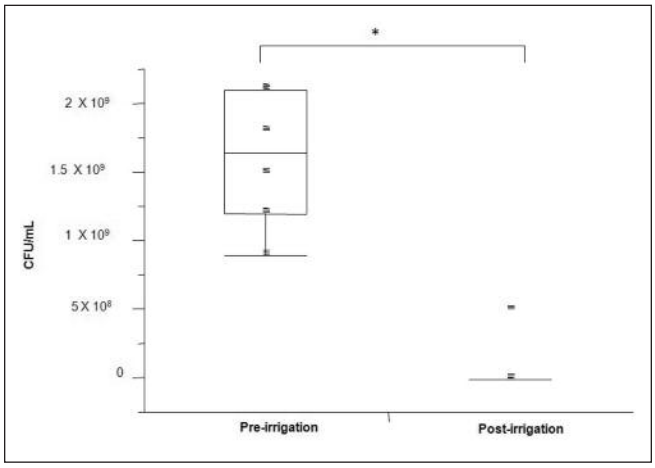

Figure 1. Boxplots comparing bacterial quantifications before and after irrigation in the experimental group (oxidative potential water). *Statistically significant ($P < 0.0001$, Mann-Whitney *U* test).

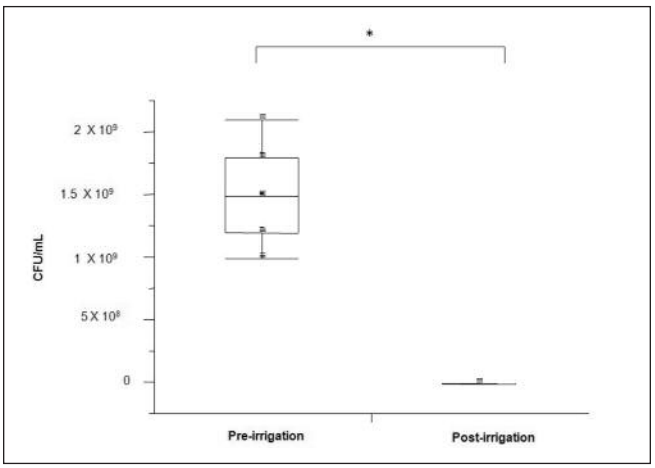

Figure 2. Boxplots comparing bacterial quantifications before and after irrigation in the control group (1% sodium hypochlorite). *Statistically significant ($P < 0.0001$, Mann-Whitney *U* test).

DISCUSSION

Pulpectomy in primary teeth is still considered a controversial procedure because of, among other things, the complex canal system characteristic of these teeth. Success in endodontic treatment depends largely on the eradication of bacteria through proper instrumentation and debridement of the pulp canals and irrigation with antiseptic solutions.[4,5,15] One of the goals of endodontic treatment is to eliminate bacteria and its products. Bacteria found in the root space can be removed by mechanical instrumentation; however, in certain situations due to the complex anatomy of the root canal, organic debris and bacteria in the dentinal tubules cannot be removed even after careful instrumentation. In these cases, the use of irrigating solutions is essential to minimize the bacterial load and remove organic tissue remnants.[16]

Anaerobic bacteria, mainly gram negative, have been identified in necrotic primary teeth; facultative mircroorganisms such as *E faecalis, S aureus*, and *C albicans* are considered highly resistant species and therefore possible causes of failed root canal treatment.[17] According to Brook,[18] endodontic lesions in deciduous teeth are primarily or secondarily caused by infectious bacteria, and the bacteria most commonly identified in irreversibly inflamed pulps, pulp necrosis, and dentoalveolar abscesses are *Prevotella, Porphyromonas, Fusobacterium, and Peptostreptococcus spp*. Da Silva *et al*[19] and Ledezma *et al*[20] reported the existence of combinations of several species of bacteria, with a predominance of anaerobic bacteria. They concluded that the microbiota in primary root canals having necrotic pulps and periapical lesions are similar to those found in permanent teeth, so that the endodontic treatment should be similar for both dentitions. Therefore, it is essential that dentists be aware of the microbiota of these teeth so that the appropriate antimicrobial agent can be used to eliminate these pathogens.

Several studies have compared the properties of currently used endodontic irrigants. Most of these studies have been carried out on permanent teeth. Few have reported on their antimicrobial effect on primary root canals. To our knowledge, although there have been studies of the antibacterial efficacy of OPW as an irrigating solution in permanent teeth, there are no reports on the effects of OPW on primary teeth. Marais and Williams evaluated the antimicrobial effectiveness of ECA, finding that ECA was effective against all bacteria, but the reduction in the number of bacteria was not statistically significant when compared with NaOCl.[21] Gulabivala *et al* evaluated the effectiveness of ECA in the debridement of *E faecalis* biofilm in root canals of extracted of permanent teeth and found that it was highly effective as an antimicrobial endodontic irrigant, but there was no significant difference when compared with NaOCl.[22] Their results were similar to those obtained in the present study; however, unlike in their studies, our, bacteria were obtained directly from necrotic root canals of primary teeth during pulpectomy treatment.

Rossi-Fedele *et al* examined the antimicrobial effect of super oxidized water (SOW) and 4% NaOCl in 15 teeth of a bovine model that were infected with strains of *E faecalis*. It was shown that under the conditions of the study, SOW had higher antimicrobial activity when it was used as an endodontic irrigant compared with saline solution, but not when compared with NaOCl. They conclude that the solution is capable of producing a decrease in bacterial load in spite of being less effective than NaOCl.[23]

Gaitan *et al* studied in vitro the antimicrobial effect of OPW against *E. faecalis* strains obtained directly from infected root canals, demonstrating that OPW has an antimicrobial effect.[8] Kiura *et al* evaluated the antimicrobial effect of OPW on *P. aeruginosa, B. subtilis, M. bovis, and M. tuberculosis* strains, demonstrating that OPW has an antimicrobial effect in the 2 NaOCl concentrations used (0.05% and 0.3%) against these strains.[24] Serper *et al* conducted a study showing that OPW is less toxic than EDTA or 5.25% NaOCl.[25] Morita *et al* demonstrated the systemic effects of OPW on the gastrointestinal tract and periodontal tissues by ingestion. Histologically, they found no abnormalities in the tissues, concluding that drinking the solution has no systemic effect.[26]

CONCLUSION

Although more controlled clinical studies are needed to support the effectiveness of OPW as an irrigant solution. The results exhibited in this study are highly promising in terms of being a feasible alternative for irrigating after pulpectomy of necrotic primary teeth.

ACKNOWLEGMENT

The authors thank Norman Wahl for his assistance in editing the manuscript. This work was partially supported by PIFI 2010-11 and CONACYT 103311, 162651 grants.

REFERENCES

1. Cox ST Jr, Hembree JH Jr, McKnight JP. The bactericidal potential of various endodontic materials for primary teeth. Oral Surg Oral Med Oral Pathol, 45: 947–54, 1978.
2. Fuks AB. Pulp therapy for the primary and young permanent dentition. Dent Clin North Am, 44: 571–96, 2000.
3. Pappen F, Bolzani L, Rodríguez S, Amaral MR, Tanumaru M. Efecto antimicrobiano de soluciones irrigadoras utilizadas en endodoncia. Rev Estomatol Herediana, 13: 9–11, 2003.
4. Onçağ O, Hoşgör M, Hilmioğlu S, Zekioğlu O, Eronat C, Burhanoğlu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J, 36: 423–32, 2003.
5. Ruiz-Esparza CL, Garrocho-Rangel A, Gonzalez-Amaro AM, Flores-Reyes H, Pozos-Guillen AJ. Reduction in bacterial loading using 2% chlorhexidine as an irrigant in pulpectomized deciduous teeth. A preliminary report. J Clin Pediatr Dent, 35: 265–70, 2010.
6. Razi RS. Pulp therapy in the primary dentition. NY State Dent J, 65: 18–22, 1999.
7. Hata G, Hayami S, Weine FS, Toda T. Effectiveness of oxidative potential water as a root canal irrigant. Int Endod J, 34: 308–17, 2001.
8. Gaitán CI, Gonzalez AM, Cruz R, Flores HE, Pozos-Guillen AJ. Efecto antimicrobiano del agua potencialmente oxidativa. Rev Odontol Mex, 13: 224–8, 2009.
9. Thorn RM, Lee SW, Robinson GM, Greenman J, Reynolds DM. Electrochemically activated solutions: evidence for antimicrobial efficacy and applications in healthcare environments. Eur J Clin Microbiol Infect Dis, 31: 64–53, 2012.

10. Yamada K, Yama M, Takaku Y, Kakizawa T, Kimizuka R, Okuda K, Kato T. Antimicrobial activity of super-oxidised water against oral microorganisms. Arc Oral Biol, 55: 397–400, 2010.
11. Hata G. Removal of smear layer in the root canal using oxidative potential water. J Endod, 22: 643–5, 1996.
12. Carlsson J, Sundqvist G. Evaluation of methods of transport and cultivation of bacterial specimens from infected dental root canals. Oral Surg Oral Med Oral Pathol, 49: 451–4, 1980.
13. Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: A randomized clinical trial. J Endod, 33: 114–8, 2007.
14. McFarland J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc, 49: 1176–8, 1907.
15. Zehnder M. Root canal irrigants. J Endod, 32: 389–98, 2006.
16. Gomes-Filho JE, Aurélio KG, Costa MM, Bernabé FE. Comparison of the biocompatibility of different root canal irrigants. J Appl Oral Sci, 16: 137–44, 2008.
17. Gomes BP, Lilley JD, Drucker DB. Associations of endodontic symptoms and signs with particular combinations of specific bacteria. Int Endod J, 29: 69–75, 1996.
18. Brook I. Microbiology and management of endodontic infections in children. J Clin Pediatr Dent, 28: 18–25, 2003.
19. Da Silva LA, Nelson-Filho P, Faria G, Souza-Gugelmin MC, Ito IY. Bacterial profile in primary teeth with necrotic pulp and periapical lesions. Braz Dent J, 17: 144–8, 2006.
20. Ledezma-Rasillo G, Flores-Reyes H, Gonzalez-Amaro AM, Garrocho-Rangel A, Ruiz-Rodriguez MS, Pozos-Guillen AJ. Identification of cultivable microorganisms from primary teeth with necrotic pulps. J Clin Pediatr Dent, 34: 329–34, 2010.
21. Marais JT, Williams WP. Antimicrobial effectiveness of electrochemically activated water as an endodontic irrigation solution. Int Endod J, 34: 23–43, 2001.
22. Gulabivala K, Stock JR, Lewsey JD, Ghori S, Ng YL, Spratt DA. Effectiveness of electrochemically activated water as an irrigant in an infected tooth model. Int Endod J, 37: 624–31, 2004.
23. Rossi-Fedele G, Figueiredo JA, Steier L, Canullo L, Steier G, Roberts A. Evaluation of the antimicrobial effect of super-oxidized water (Sterilox) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model. J Appl Sci, 18: 498–502, 2010.
24. Kiura H, Sano K, Morimatsu S, Nakano T, Morita C, Yamaguchi M, Maeda T, Katsuoka Y. Bacterial activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. J Microbiol Methods, 49: 285–93, 2002.
25. Serper A, Calt S, Dogan AL, Guc D, Ozcelik B, Kuraner T. Comparison of the cytotoxic effects and smear layer removing capacity of oxidative potential water, NaOCl and EDTA. J Oral Sci, 43: 233–8, 2001.
26. Morita C, Nishida T, Ito K. Biological toxicity of acid electrolyzed functional water: Effect of oral administration on mouse digestive tract and changes in body weight. Arch Oral Biol, 56: 359–66, 2011.