International Endodontic Journal

doi:10.1111/j.1365-2591.2010.01794.x

A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament

A. E. Badr[1], N. Omar[2] & F. A. Badria[3]

[1]Department of Conservative Dentistry and Endodontics, Faculty of Dentistry; [2]Department of Medical Microbiology and Immunology, Faculty of Medicine; and [3]Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

Abstract

Badr AE, Omar N, Badria F. A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. *International Endodontic Journal*, **44**, 51–58, 2011.

Aim To evaluate the antibacterial and cytotoxic effects of Liquorice as a root canal medicament and to compare its action to the commonly used root canal medicament calcium hydroxide $Ca(OH)_2$.

Methodology The antibacterial effect of Liquorice and $Ca(OH)_2$ either separately or in combination was investigated against *Enterococcus faecalis*. Agar-well diffusion methods, broth microdilution tests and biofilm susceptibility assays were used to determine the antibacterial activity. Human periodontal ligament fibroblast tissue culture was used to assess the cytotoxicity of the preparations under investigation.

Results Liquorice extract either by itself or in combination with $Ca(OH)_2$ had a significant inhibitory effect against *Enterococcus faecalis* compared with that of $Ca(OH)_2$ alone. The use of Liquorice extract followed by Liquorice/$Ca(OH)_2$ mixture retained significantly more viable periodontal ligament cells than $Ca(OH)_2$, which had a strong lethal effect on the cells.

Conclusion Liquorice extract either separately or as Liquorice/$Ca(OH)_2$ mixture had a potent bactericidal effect against *Enterococcus faecalis* and retained compatibility with fibroblasts in tissue culture compared to the commonly used root canal medicament $Ca(OH)_2$.

Keywords: antibacterial, calcium hydroxide, cytotoxicity, *Enterococcus faecalis*, Liquorice.

Received 14 April 2009; accepted 27 July 2010

Introduction

Microorganisms play a key role in pulpitis, infection-mediated necrosis and their extension to the periapical tissue (Kakehashi *et al.* 1965). A fundamental aim of root canal treatment is to eliminate bacterial infection from the root canal system (Sjögren *et al.* 1997). This cannot be accomplished solely by chemomechanical preparation of the root canal system because microorganisms remain in the root canal and the dentinal tubules after root canal instrumentation (Barnett *et al.* 1985). Thus, the use of antimicrobial dressings after canal preparation is generally recommended (Sjögren *et al.* 1991). Such medicaments must have the greatest possible antimicrobial efficacy against the various bacterial species in the infected root canal, without causing irritation to the periapical tissue. Calcium hydroxide suspensions in paste form have been shown to meet these demands to a high degree (Siqueira & Lopes 1999). Therefore, $Ca(OH)_2$ is presently considered the intracanal medication of choice. However, specific microorganisms such as *Enterococcus faecalis (E. faecalis)* have shown resistance against $Ca(OH)_2$ (Evans *et al.* 2002). The species *E. faecalis* has been recovered in a high proportion of cases with post-treatment disease; approximately one-third of the canals of root filled teeth with persistent periapical lesions (Molander *et al.* 1998, Sundqvist *et al.* 1998). It

Correspondence: Dr. Amany El-Said Badr, Faculty of Dentistry, Mansoura University, Mansoura, Egypt (Tel.: +2 0127859696; e-mail: amanybadr@hotmail.com).

is possible that *E. faecalis* is related to the failure but definitive evidence is currently lacking (Nair *et al.* 1990).

There is limited research to explain the dominant presence of *E. faecalis* in such failed cases. The organism is easily destroyed when grown in the laboratory, but becomes resistant when present in the root canal system (Shih *et al.* 1970). The supposition is that *E. faecalis* undergoes changes whilst in the root canal environment, possibly through activation of virulence factors that make it more resistant (Distel *et al.* 2002, Williamson *et al.* 2009). Hems *et al.*(2005) and Abdullah *et al.* (2005) indicated that it is harder to eliminate *E. faecalis* in biofilm than in planktonic suspension *ex vivo*. Kayaoglu *et al.* (2009) have shown that collagen, the main component of dentine, associated with *E. faecalis* was found to increase the tolerance of the bacterium to $Ca(OH)_2$. Recognizing the association of *E. faecalis* with failed root canal treatment may eventually lead to evidence of its role and thence to development of strategies to control such infections (Molander *et al.* 1998, Sundqvist *et al.* 1998).

Because root canal medicaments can come in contact with periapical tissue, in addition to having good antibacterial ability, they must also be biocompatible. In selecting root canal medicaments, it is necessary to consider their therapeutic benefits against their potential cytotoxic effects. Ideal root canal medicaments should have strong antibacterial properties and minimal cytotoxic effect on the host tissues. Herbs have been used in clinical medicine for thousands of years. However, it is only in recent times that researchers have been able to employ scientific methods to prove the efficacy of many of these herbs and to provide a better understanding of their mechanisms of action (Graf 2000). Liquorice is the most commonly used crude drug and flavouring agent in kampo medicines (traditional Chinese medicines modified in Japan) (Fukai *et al.* 2002). Liquorice is 'generally recognized as safe' in the USA (van Gelderen *et al.* 2000). A number of pharmaceutical effects of Liquorice are known, e.g. anti-inflammatory (Shibata 2000, Bodet *et al.* 2008), antiviral (Bean 2002) and anticarcinogenic (Lee *et al.* 2007, 2008).

In dental studies, Segal *et al.* (1985) studied the effect of Liquorice on the growth and adherence to the cariogenic *Streptococcus mutans* (*S. mutans*). They found that bacterial growth and adherence (plaque formation) was markedly inhibited. However, other researchers have found no difference in plaque accumulation (Söderling *et al.* 2006). In a previous anti-inflammatory study, Shoreibah *et al.* (2000) compared the efficacy of Liquorice extract to corticosteroid triamcinolone acetonide in the treatment of oral *Lichen planus* (OLP). They reported that Liquorice extract was as effective as triamcinolone acetonide but safer and may be used as an alternative treatment for OLP. Badria & Zidan (2004) studied the antibacterial activity of Liquorice and glycyrrhizin on different strains of *S. mutans* and also studied their effects on the adherence of *S. mutans* to glass. Liquorice extract exhibited a more profound activity in both adherence and antibacterial assays than that of glycyrrhizin. Burgess *et al.* (2008) studied the treatment of aphthous ulceration using Liquorice extract and suggested its efficacy in reducing pain and enhancing healing.

The aim of this study was to evaluate the efficacy of Liquorice extract and $Ca(OH)_2$ either separately or in a mixture against *E. faecalis* grown as different phenotypes (planktonic and biofilm) as well as to assess their biocompatibility using fibroblasts of dental origin.

Materials and methods

Liquorice extract [standardized extract as 7.5% glycyrrhizin (>95% purity), high-performance liquid chromatography] was obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University. The 60% $Ca(OH)_2$ (pH 12.3) (El-Naser Co. for chemicals, Cairo, Egypt) was made up by mixing 60 mg $Ca(OH)_2$ powder with 100 μL distilled water. A combination of 60% $Ca(OH)_2$ and Liquorice extract (1 : 1 v/v) was also used in this study.

Antibacterial test

The antibacterial activity of three preparations was evaluated against a clinical isolate of a Gram-positive facultative anaerobic microorganism *E. faecalis* using agar diffusion, broth microdilution and biofilm susceptibility tests. The selected *E. faecalis* strain (stored in-house culture collection bank of the Microbiology Department and coded EF-MD-5-08)was isolated from an infected root canal associated with periapical disease by plating on 5% Sheep Blood agar, Mac-conkey agar and Bile aesculin azide agar (Biomérieux, Marcy l'Etoile, France). Extensive phenotypic and physiological characterization was performed to validate identity as suggested by Facklam *et al.* (1989). The tests for phenotypic identification of strains to genus level included: glucose fermentation test, reaction to streptococcal group D antiserum, Bile-aesculin reaction,

growth in 6.5% NaCl broth and Vancomycin resistance using a non-standardized heavy inoculum spread over one-half of a Trypticase soy agar-5% sheep blood plate (BBL Microbiology Systems, Cockeysville, MD, USA).

Agar-well diffusion method

Bacterial suspensions in phosphate-buffered solution were made at concentrations of 0.5 MacFarland. Petri plates with 20 mL of Muller-Hinton agar (Oxoid, Unipath Ltd, Basingstoke, UK) were inoculated with 0.1 mL of the microbial suspensions, and sterile swabs used to spread the medium evenly. Wells (5 mm deep, 4 mm in diameter) were punched in each agar plate using a sterile stainless steel borer (three wells/plates). Each well was filled with 30 μL of the test material separately. The plates were maintained for 2 h at room temperature and then incubated at 37 °C for 48 h in an atmosphere of nitrogen (80%), hydrogen (10%) and carbon dioxide (10%). Zones of microbial inhibition around the wells containing the test materials were measured and recorded after the incubation. The inhibitory zone was considered to be the shortest distance (mm) from the outer margin of the well to the initial point of microbial growth. All assays were performed under aseptic conditions and in six replicates. The data were analysed for differences using ANOVA and Tukey's test.

Microdilution test

The *E. faecalis* inoculum was prepared at the concentration of 10^{-2} CFU mL^{-1}, starting from a 0.5 McFarland (or 10^{8} CFU mL^{-1}) prepared from fresh colonies of bacteria. The microdilution test was performed in a sterile microplate. Initially, each microplate well received 200 μL of brain heart broth, followed by a 100 μL of the test material to the first well and serial three-fold dilution. The sample concentrations used for the test were 1000, 333, 111, 37, 12.3, 4.1 μg mL^{-1}. Each well received 100 μL of the standardized inoculum. The tests were carried out in duplicate. Microplates were incubated at 37 °C for 48 h. Because of the difficulty in visually reading growth in the wells and determination of minimal inhibitory concentrations, the outcome measure of minimum bactericidal concentration (MBC) was used instead. This was achieved by dispensing 25 μL from each well onto blood agar plates. The plates were incubated at 37 °C for 48 h in an atmosphere of nitrogen (80%), hydrogen (10%) and carbon dioxide (10%) (Schoenknecht *et al.* 1991).

Biofilm susceptibility assay

Phosphate-buffered saline (PBS) was used as a control agent to compare the three test preparations against *E. faecalis* biofilms that were grown on cellulose nitrate membrane filters. Overnight cultures of *E. faecalis* were diluted 1 : 100 in fresh brain heart infusion broth (Oxoid, Unipath Ltd, Basingstoke, UK) and grown with shaking for 2 h at 37 °C to a final density of 1.0×106 CFU mL^{-1} and an aliquot of 20 μL was seeded onto cellulose nitrate membrane filters (0.2 μm pore size, 13 mm diameter; Whatman International Ltd, Maidstone, UK) which were on the surface of agar plates. The method used here was modified from that described by Spratt *et al.* (2001). Plates containing membranes were then incubated for 48 h at 37 °C in an aerobic atmosphere.

Each biofilm-containing membrane was completely covered with 1 mL of the test medications and incubated for 48 h at 37 °C. PBS-covered biofilm membranes served as a control. The membrane filters were then carefully transferred to neutralizing broth and vortexed for 1 min to resuspend the organisms. Suspensions were 10-fold diluted, seeded onto 5% sheep blood agar medium plates and the colony-forming units (CFU) counted after 48 h of aerobic incubation at 37 °C. Four replicates were performed for each antimicrobial agent and control group, the mean and standard deviations were calculated.

Cytotoxicity assay

Cell culture

Periodontal ligament cells (PDL) cells were obtained from teeth extracted for orthodontic purposes as approved by the Ethics Committee of Mansoura University; written informed consent was obtained from the patient. Fibroblasts were grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% foetal bovine serum and antibiotics (10 000 units of penicillin-G per mL, 10 mg of streptomycin per mL and 20 mmol L^{-1} of L-glutamine) (Sigma-Aldrich GmbH, Steinheim, Germany).

Growth measurement

Periodontal ligament cells were plated at a density of 3–4×104 cells mL^{-1} and dispensed into 96-well culture plate with 100 μL of DMEM medium per well and incubated at 37 °C supplemented with 5% CO_2 for 48 h to allow attachment of the fibroblasts to the bottom of the wells. After 48 h, the test medications [Liquorice

extract, 60% $Ca(OH)_2$ and mix of both in equal volume] were added separately to each well in 100 μL volume and incubated at 37 °C with 5% CO_2 for 48 h. At the end of the incubation period, the culture medium was aspirated and 100 μL of formaldehyde phosphate-buffered saline (FPBS) was added to each culture well for 2 h. Then, FPBS was removed and each well was rinsed thoroughly with distilled water and 100 μL of 0.23% of trypan blue (w/v) was added to each well to stain the non-viable cells. After 3 h, the trypan blue was removed and the cells were rinsed thoroughly with distilled water. Viable cells were counted under an inverted microscope (Olympus, 1 × 71, Japan) and expressed as a percentage. The number of cells was expressed by averaging of triplicate readings in each well. The results were statistically analysed with an ANOVA and LSD test.

Results

Agar diffusion test

Liquorice extract had the largest mean inhibition zone 3.97 ± 0.24 mm and thus showed the most significant microbial inhibition of the all materials tested, and it was followed by the Liquorice/$Ca(OH)_2$ mixture with a mean 2.56 ± 0.015 mm; $Ca(OH)_2$ had the least bacterial growth inhibition with a mean 0.97 ± 0.017 mm. The difference between the three experimental groups was statistically significant ($P < 0.05$) (Table 1, Fig. 1).

Microdilution test

The minimal bactericidal concentration of Liquorice extract against *E. faecalis* was 12.3 μg mL^{-1}. All dilutions used for $Ca(OH)_2$ had only some degree of growth inhibition.

Table 1 Mean area of the zones of microbial growth inhibition (in mm) ($n = 6$) provided by licorice, $Ca(OH)_2$ and licorice/$Ca(OH)_2$ mixture against *E. faecalis*

Group	Mean	SD	Significance
Licorice	3.97[a]	0.24	$P = 0.00$
$Ca(OH)_2$	0.96[b]	0.017	
Licorice/$Ca(OH)_2$ (1 : 1v/v)	2.56[c]	0.015	

Values of means within a column followed by the different letters are significantly different ($P < 0.05$), SD = Standard Deviation.

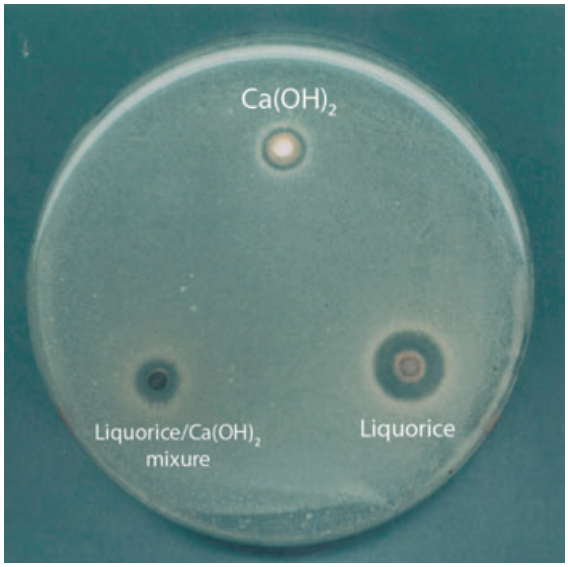

Figure 1 Inhibition zones of Liquorice extract, $Ca(OH)_2$ and Liquorice/$Ca(OH)_2$ mixture.

Biofilm susceptibility assay

Comparison of the susceptibility of the *E. faecalis* biofilms to the various antimicrobial agents is shown in Fig. 2. Liquorice extract and Liquorice mixed with $Ca(OH)_2$ were equally effective against *E. faecalis* achieving 100% kill. Although there was some killing with $Ca(OH)_2$, it left in excess of 106 ± 0.0816 × 106 CFUs (marginal antimicrobial effects).

Cytotoxicity assay

Treatment of PDL fibroblasts with Liquorice extract resulted in 87% ± 1.91 cell viability, whilst 60% $Ca(OH)_2$ resulted in 100% killing of the PDL fibroblasts. The viability of PDL cells was 58% ± 1.73 when treated with the mixture of $Ca(OH)_2$ and Liquorice extract (Figs 3 and 4). The difference between the three experimental groups was statistically significant ($P < 0.05$).

Discussion

The use of a biocompatible intracanal medicament possessing antimicrobial properties between appointments may reduce or eliminate bacteria in the root canal system and increase the success of root canal treatment (Byström *et al.* 1985). Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of

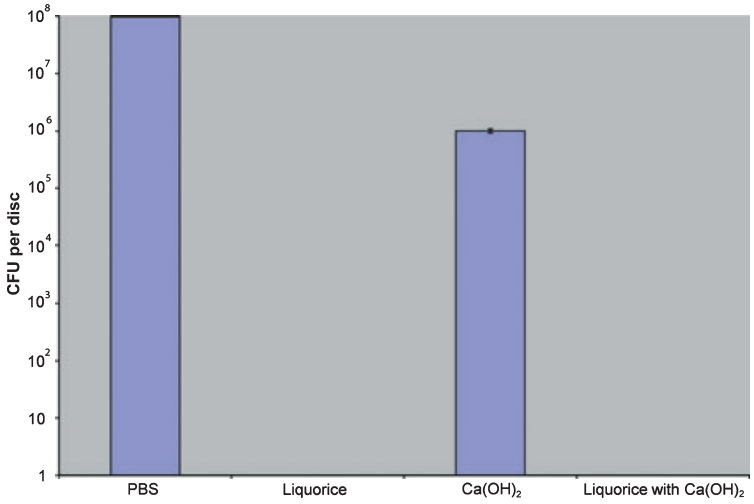

Figure 2 Susceptibility of *Enterococcus faecalis* biofilms to the PBS phosphate-buffered saline (control), Liquorice extract, $Ca(OH)_2$ and Liquorice/$Ca(OH)_2$ mixtures (n = 4).

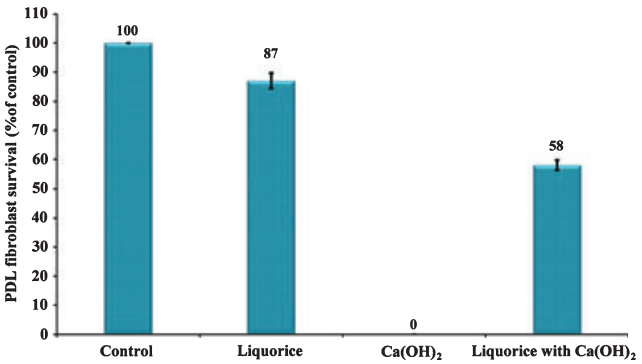

Figure 3 The survival of the periodontal ligament fibroblasts (PDL) after treatment with the tested preparations (n = 6).

Liquorice root. This compound represents a mixture of potassium-calcium magnesium salts of glycyrrhizic acid that varies within a 2–25% range. Amongst the natural saponins, glycyrrhizic acid is a molecule composed of a hydrophilic part, two molecules of glucuronic acid and a hydrophobic fragment, glycyrrhetic acid (Olukoga & Donaldson 1998). Laboratory tests of any kind are only the first step in a study of the effectiveness and biocompatibility of intracanal medicaments. *E. faecalis* was chosen as the test organism because it is associated with persistent apical inflammation in clinical situations (Fabricius *et al.* 1982). It has been used extensively in endodontic research because it has been found in infected canals and has been associated with failed root canal treatment (Sundqvist *et al.* 1998). Sixty per cent concentration of $Ca(OH)_2$ was selected because it was recommended by Blanscet *et al.* (2008) for use as an endodontic interappointment medicament. They studied whether varying the per cent (by weight) of aqueous $Ca(OH)_2$ had an effect on its antibacterial efficacy against common endodontic pathogens. They indicated that formulation 50% to 60% aqueous $Ca(OH)_2$ resulted in the greatest overall bacterial inhibition of endodontic pathogens studied including *E. faecalis*. In the present study, the trials of mixing varying percentages of $Ca(OH)_2$ resulted in the most manageable mixture at 60% aqueous $Ca(OH)_2$.

Based on the average of the zones of bacterial growth inhibition in the agar diffusion test, Liquorice had the strongest antibacterial activity against *E. faecalis* followed by the Liquorice/$Ca(OH)_2$ mixture. $Ca(OH)_2$ showed direct contact inhibition only.

It has been proposed that in the process of evaluating antibacterial properties of dental materials, more than one assay method should be used (Shalhav *et al.* 1997). For this reason, the microdilution test was performed to determine the extent of antimicrobial activity and to measure the MBC. The diffusion test gave a qualitative value of bacterial sensitivity, and the dilution test gave

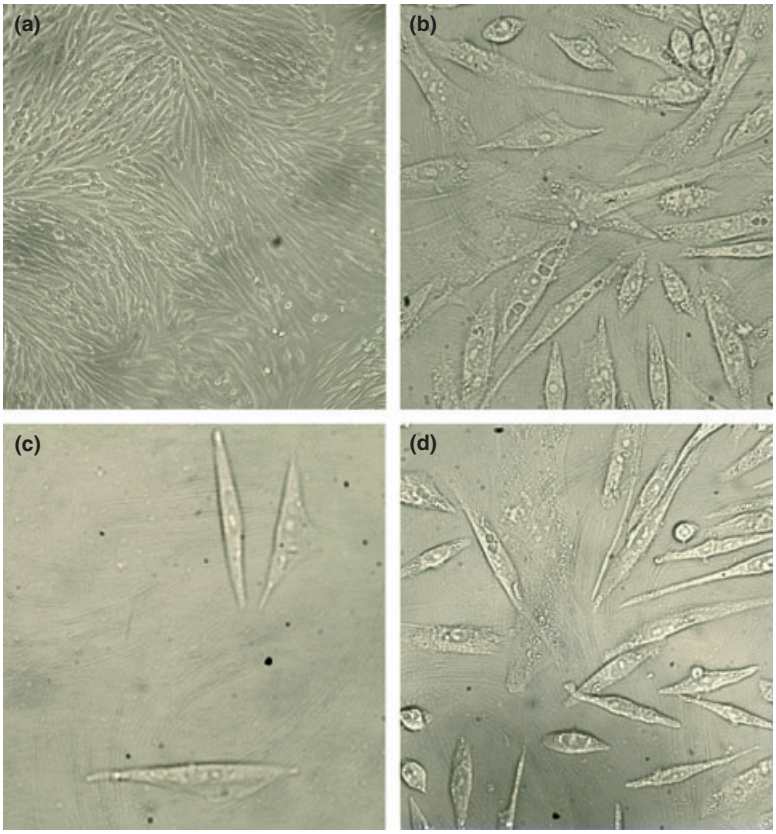

Figure 4 Representative phase micrograph of periodontal ligament fibroblasts (PDL) of the control group and of those treated with the three studied preparations. (a) Control cells, untreated PDL cells, (100% viability); (b) PDL cells treated with Liquorice (87% viability); (c) PDL cells treated with $Ca(OH)_2$ (0% viability); (d) PDL cells treated with Liquorice/$Ca(OH)_2$ mixture (58% viability).

quantitative results. Comparison of the agents showed that Liquorice was effective against *E. faecalis* at a relatively moderate concentration (12.3 μg mL^{-1}), whilst MBC for $Ca(OH)_2$ could not be determined as none of the dilutions used in the test were effective against *E. faecalis*.

A number of approaches have been used to test the effectiveness of antimicrobial agents in the laboratory. Shih *et al.* (1970) used two resistant strains, *Enterococcus faecalis* and *Staphylococcus aureus*, and found the bacteria were easily killed when incubated in broth culture with a concentration of sodium hypochlorite as low as 1 : 1000. However, in the same study, when the infected tooth model was used, even full strength sodium hypochlorite failed to eradicate the bacteria completely in all canals. The difference was attributed to the opportunity for contact between microorganisms and the irrigant. As a result, the conditions used for determining MBC for bacteria in fluid suspension may not reflect the *in vivo* conditions where bacteria grow as biofilms on tooth surfaces. A biofilm is defined as an aggregation of bacteria associated with a surface, embedded in an extracellular matrix of polysaccharide. The biofilm phenotype differs from their planktonic counterparts in their lower susceptibility to antimicrobial killing.

The biofilm results of this study revealed that Liquorice extract and Liquorice/$Ca(OH)_2$ mixture were completely (100% kills) effective against *E. faecalis*, whilst $Ca(OH)_2$ alone had only a marginal antimicrobial effect. The weak antibacterial effect of $Ca(OH)_2$ against *E. faecalis* in this study is in agreement with some studies (Safavi *et al.* 1990, Peters *et al.* 2002). The antimicrobial effect of Liquorice extract against *E. faecalis*, may be related to the Glycyrrhizin (Badria *et al.* 2003). The mode of action of antibacterial effects of saponins seems to involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial population density (Killeen *et al.* 1998). The flavonoid content of Liquorice extract is also a strong inhibitor of oxygen consumption in bacterial cells; the site of inhibition is thought to be between Co Q and cytochrome C in the bacterial respiratory electron transport chain (Haraguchi *et al.* 1998).

In the present study, the biological compatibility of Liquorice extract and $Ca(OH)_2$ was examined sepa-

rately and in combination with each other by laboratory methods. Various cell lines (normal diploid and transformed or anaploid cell lines) are commonly used in cytotoxicity evaluation (Azar *et al.* 2000). However, the normal diploid cells (e.g. fibroblasts), because of the relative similarity to *in vivo* conditions, are preferred to the transformed cells. In this study, the cytotoxicity was evaluated by measuring the cell growth *via* cell counting. The cells used in this study were of dental origin, putatively making the assay more relevant.

Liquorice showed greater biocompatibility with fibroblasts cells compared to $Ca(OH)_2$, which was severely toxic to the cells. A mixture of Liquorice and $Ca(OH)_2$ showed moderate cytotoxicity. The test implied a relative tolerability of Liquorice to fibroblasts at a relatively high concentration. This may be explained on the basis of the pentacyclic triterpenoid structure, which may resemble the phospholipid bilayer of the cell in containing polar (hydrophilic moiety) and non-polar (hydrophobic moiety) components (Shibata 2000). The Liquorice extract retains a slight acidic pH 6, whilst $Ca(OH)_2$ is a very polar and strong alkali (pH 12). The high toxicity of $Ca(OH)_2$ is in accordance with published data (Gordon *et al.* 1985, Tronstad *et al.* 1988).

Based on the single-species model used, this study found the Liquorice to have good potential as a root canal medicament and may well represent progress in the development of more biocompatible endodontic materials. Although calcium hydroxide is suitable as intracanal medicament, it may not be a universal intracanal medication, because it is not equally effective against all bacteria found in the root canal (Gomes *et al.* 2002). Further studies are required for clinical application of the results of this *in vitro* study.

References

Abdullah M, Ng YL, Gulabivala K, Moles DR, Spratt DA (2005) Susceptibilities of two *Enterococcus faecalis* phenotypes to root canal medications. *Journal of Endodontics* **31**, 30–6.

Azar NG, Heidari M, Bahrami ZS, Shokri F (2000) *In vitro* cytotoxicity of a new epoxy resin root canal sealer. *Journal of Endodontics* **26**, 462–5.

Badria FA, Zidan OA (2004) Natural products for dental caries prevention. *Journal of Medicinal Food* **7**, 381–4.

Badria FA, Houssen WE, El-Nashar EM, Said SA (2003) Biochemical and histopathological evaluation of glycyrrhizin and boswellia carterii extract on rat liver injury. *Biosciences, Biotechnology Research Asia* **1**, 93–6.

Barnett F, Trope M, Khoja M, Tronstad L (1985) Bacteriologic status of the root canal after sonic, ultrasonic and hand instrumentation. *Endodontics & Dental Traumatology* **1**, 228–31.

Bean P (2002) The use of alternative medicine in the treatment of hepatitis C. *American Clinical Laboratory* **21**, 19–21.

Blanscet ML, Tordik PA, Goodell GG (2008) An agar diffusion comparison of the antimicrobial effect of calcium hydroxide at five different concentrations with three different vehicles. *Journal of Endodontics* **34**, 1246–8.

Bodet C, La VD, Gafner S, Bergeron C, Grenier D (2008) A Liquorice extract reduces lipopolysaccharide-induced proinflammatory cytokine secretion by macrophages and whole blood. *Journal of Periodontology* **79**, 1752–61.

Burgess JA, van der Ven PF, Martin M, Sherman J, Haley J (2008) Review of over-the-counter treatments for aphthous ulceration and results from use of a dissolving oral patch containing glycyrrhiza complex herbal extract. *The Journal of Contemporary Dental Practice* **9**, 88–98.

Byström A, Claesson R, Sundqvist G (1985) The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol, and calcium hydroxide in the treatment of infected root canals. *Endodontics & Dental Traumatology* **1**, 170–5.

Distel JW, Hatton JF, Gillespie MJ (2002) Biofilm formation in medicated root canals. *Journal of Endodontics* **28**, 689–93.

Evans M, Davies JK, Sundqvist G, Figdor D (2002) Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. *International Endodontic Journal* **35**, 221–8.

Fabricius L, Dahlén G, Holm SE, Möller AJ (1982) Influence of combinations of oral bacteria on periapical tissues of monkeys. *Scandinavian Journal of Dental Research* **90**, 200–6.

Facklam R, Hollis D, Collins MD (1989) Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. *Journal of Clinical Microbiology* **27**, 724–30.

Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T (2002) Anti-Helicobacter pylori flavonoids from Liquorice extract. *Life Sciences* **71**, 1449–63.

van Gelderen CE, Bijlsma JA, van Dokkum W, Savelkoul TJ (2000) Glycyrrhizic acid: the assessment of a no effect level. *Human & Experimental Toxicology* **19**, 434–9.

Gomes BP, Ferraz CC, Zaia AA, Teixeira FB (2002) Microbial susceptibility to calcium hydroxide pastes and their vehicles. *Journal of Endodontics* **11**, 758–61.

Gordon TM, Ranly DM, Boyan BD (1985) The effects of calcium hydroxide on bovine pulp tissue: variations in pH and calcium concentration. *Journal of Endodontics* **11**, 156–60.

Graf J (2000) Herbal anti-inflammatory agents for skin disease. *Skin Therapy Letter* **5**, 3–5.

Haraguchi H, Tanimoto K, Tamura X, Mizutani K, Kinoshita T (1998) Mode of antibacterial action of retrochalcones from Glycyrrhiza inflata. *Phytochemistry* **48**, 125–9.

Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA (2005) An *in vitro* evaluation of the ability of ozone to kill a strain of *Enterococcus faecalis*. *International Endodontic Journal* **38**, 22–9.

Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery, Oral Medicine, and Oral Pathology* **20**, 340–9.

Kayaoglu G, Erten H, Bodrumlu E, Ørstavik D (2009) The resistance of collagen-associated, planktonic cells of *Enterococcus faecalis* to calcium hydroxide. *Journal of Endodontics* **35**, 46–9.

Killeen G, Madigan C, Connolly C, Walsh G, Clark C, Hynes M, Timmins B, James P, Headon D, Power R (1998) Antimicrobial saponins of *Yucca schidigera* and the implications of their *in vitro* properties for their *in vivo* impact. *Journal of Agricultural and Food Chemistry* **46**, 3178–86.

Lee CK, Park KK, Lim SS, Park JH, Chung WY (2007) Effects of the Liquorice extract against tumor growth and cisplatin-induced toxicity in a mouse xenograft model of colon cancer. *Biological & Pharmaceutical Bulletin* **30**, 2191–5.

Lee S, Oh HM, Lim WB *et al.* (2008) Gene induction by glycyrol to apoptosis through endonuclease G in tumor cells and prediction of oncogene function by microarray analysis. *Anti-Cancer Drugs* **19**, 503–15.

Molander A, Reit C, Dahlén G, Kvist T (1998) Microbiological status of rootfilled teeth with apical periodontitis. *International Endodontic Journal* **31**, 1–7.

Nair R, Sjögren U, Krey G, Kahnberg KE, Sundqvist G (1990) Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesion: a long-term light and electron microscopic follow-up study. *Journal of Endodontics* **16**, 580–8.

Olukoga A, Donaldson D (1998) Historical perspectives on health. The history of liquorice: the plant, its extract, cultivation, and commercialisation and etymology. *The Journal of the Royal Society for the Promotion of Health* **118**, 300–4.

Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR (2002) Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *International Endodontic Journal* **35**, 13–21.

Safavi KE, Spangberg LS, Langeland K (1990) Root canal dentinal tubule disinfection. *Journal of Endodontics* **16**, 207–10.

Schoenknecht FD, Sabath LD, Thornsberry C (1991) Susceptibility special tests. In: Lennette EH *et al.* eds. *Manual of Clinical Microbiology*, 4th edn. Washington, D. C.: American Society for Microbiology, pp. 1000–8.

Segal R, Pisanty S, Wormser R, Azaz E, Sela MN (1985) Anticariogenic activity of Liquorice and glycyrrhizine I: Inhibition of in vitro plaque formation by Streptococcus mutans. *Journal of Pharmaceutical Sciences* **74**, 79–81.

Shalhav M, Fuss Z, Weiss EI (1997) In vitro antibacterial activity of a glass ionomer endodontic sealer. *Journal of Endodontics* **23**, 616–9.

Shibata S (2000) A drug over the millennia: Pharmacognosy, chemistry, and pharmacology of Liquorice. *Yakugaku Zasshi* **120**, 849–62.

Shih M, Marshall FJ, Rosen SR (1970) The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. *Oral Surgery, Oral Medicine, and Oral Pathology* **29**, 613–9.

Shoreibah EA, Soliman OA, Badria FA, Abdel-Gawad AH (2000) Efficacy of topical delivery of corticosteroid versus glycyrrhizin in the treatment of Lichen planus using bioadhesive patches. *Egyptian Dental Journal* **46**, 2105–13.

Siqueira JF Jr, Lopes HP (1999) Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *International Endodontic Journal* **32**, 361–9.

Sjögren U, Figdor D, Spangberg L, Sundqvist G (1991) The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. *International Endodontic Journal* **24**, 119–25.

Sjögren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root canal filling on the outcome of endodontic treatment of teeth with apical periodontists. *International Endodontic Journal* **30**, 297–306.

Söderling E, Karjalainen S, Lille M, Maukonen J, Saarela M, Autio K (2006) The effect of liquorice extract-containing starch gel on the amount and microbial composition of plaque. *Clinical Oral Investigations* **10**, 108–13.

Spratt DA, Pratten J, Wilson M, Gulabivala K (2001) An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. *International Endodontic Journal* **34**, 300–7.

Sundqvist G, Figdor D, Persson S, Sjögren U (1998) Microbiological analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **85**, 86–93.

Tronstad L, Barnett F, Flax M (1988) Solubility and biocompatibility of calcium hydroxide containing root canal sealers. *Endodontic Dental Traumatology* **4**, 152–9.

Williamson AE, Cardon JW, Drake DR (2009) Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*. *Journal of Endodontics* **35**, 95–7.