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ORIGINAL ARTICLE

The efficacy of *Salvadora persica* extract in the elimination of the intracanal smear layer: A SEM study

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KEYWORDS

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Abstract *Aim:* To evaluate the efficacy of an ethanolic *Salvadora persica* extract in removing the smear layer following a root canal procedure.

Methods: Sixty extracted, single-rooted human teeth were cleaned, shaped, and divided into four groups. Experimental groups 1 ($n = 20$) and 2 ($n = 20$) were irrigated with 1 mg/ml and 5 mg/ml *of S. persica*, respectively. The positive controls ($n = 10$) were irrigated with 17% ethylenediaminetetraacetic acid (EDTA), while the negative controls ($n = 10$) were irrigated with saline. Approximately 5 ml of the irrigating solution was delivered into the root canals for 5 min, and the final rinse was performed with 5 ml of 1% sodium hypochlorite. Scanning electron microscopy was used to evaluate the endodontic smear layer removal at the coronal, middle, and apical thirds of the specimens.

Results: A significant difference in smear layer removal between groups 1 and 2 at the coronal and middle thirds of the canal was observed, and no significant difference was seen between group 2 and the positive control at the coronal third. At the apical third, both concentrations of *S. persica* had similar effects and were less effective than the positive control in removing the smear layer.

Conclusion: The 5 mg/ml *S. persica* solution was significantly more effective than the 1 mg/ml solution. In addition, the 5 mg/ml *S. persica* solution was as effective as 17% EDTA in removing the smear layer from the coronal third of the canal wall.

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1. Introduction

Root canal treatment involves the chemo-mechanical removal of bacteria and infected pulp and dentin from within the root canal walls. Studies have shown that the mechanical root canal procedure leaves a smear layer covering the dentinal walls (McComb and Smith, 1975; Mader et al., 1984). Although there is controversy concerning whether to remove or retain the smear layer, some reports have suggested that there is no significant difference in leakage with or without the root smear

layer (Violich and Chandler, 2010). On the other hand, a recent systematic review and meta-analysis of *in vitro* leakage studies by Shahravan et al. (2007) concluded that smear layer removal improves the fluid-tight seal of the obturated root canal system.

There are a variety of smear layer removal methods, including chemical, ultrasonic, and laser techniques, but none have been found to be effective throughout the entire canal length (Violich and Chandler, 2010). The most common chemical method of smear layer removal is to use 15–17% ethylenediaminetetraacetic acid (EDTA). In addition, Baumgartner and Mader (1987) have shown that alternating irrigation with 15% EDTA and 5.25% NaOCl acts as an effective smear layer removal technique by flushing debris, acting as a tissue solvent and bactericidal agent, and lubricating the area.

The toothbrush tree, *Salvadora persica* (*S. persica*), locally called miswak, is a member of the Salvadoraceae family and has been used by many Islamic communities to supply toothbrushes. *S. persica* has been scientifically proven to be useful in the prevention of tooth decay even when used without any other tooth-cleaning methods (Salehi and Momeni Danaie, 2006). Studies have found that *S. persica* extract is somewhat comparable to other oral disinfectants and anti-plaque agents, such as triclosan and chlorhexidine gluconate, if used at sufficiently high concentrations (Almas, 2002; Almas et al., 2005). The World Health Organization (WHO) (1987) encouraged the use of *S. persica* in an international consensus report on oral hygiene and concluded that further research is needed to document the effects of *S. persica*. Likewise, clinical results have demonstrated a significant antimicrobial effect of aqueous and alcoholic *S. persica* extracts against aerobic and anaerobic bacteria when utilized as an irrigant during endodontic treatment (Al Salman et al., 2005; Al-Sabawi et al., 2007). However, there is not enough evidence to support the utilization of this extract as an irrigant solution in endodontic practice. Babay and Almas (1999) have compared instrumented human dentin treated with saline and aqueous and alcoholic *S. persica* extracts using different modes of application and exposure times. The results showed that burnishing the root dentin with saline and aqueous *S. persica* extract partially removed the smear layer, while the alcoholic extract completely removed the smear layer. On the other hand, Almas (2002) demonstrated that chlorhexidine (CHX) and aqueous *S. persica* extract had a similar effect on healthy dentin. Furthermore, *S. persica* opened more dentinal tubules in periodontally involved dentin.

To date, there has been no baseline information regarding the effectiveness of *S. persica* extract on smear layer removal following root canal instrumentation procedures. Therefore, the aim of this *in vitro* study was to evaluate the efficacy of ethanolic *S. persica* extracts in removing the smear layer after root canal instrumentation procedures using scanning electron microscopy.

2. Materials and methods

2.1. Preparation of ethanolic *S. persica* extracts

S. persica roots were collected from Almakhwah, which lies 45 km west of Albahah in the southern region of the Kingdom of Saudi Arabia. The roots were identified by a local expert on the plant, and a voucher specimen (#1745) was deposited at the herbarium center of the College of Pharmacy, King Saud University, for future reference. The fresh ground roots were extracted with 10% water in ethanol, which was then evaporated to dryness. The *S. persica* extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a 400 mg/ml stock solution; working concentrations of 1 and 5 mg/ml were prepared in sterile physiological saline for use as an irrigant.

2.2. Specimen preparation

Sixty extracted human anterior and premolar teeth, which had single, straight root canals and intact apices and were devoid of cracks and previous endodontic treatments, were collected and stored in saline throughout the study. The teeth were decoronated to obtain a standardized root length of 15 mm. The working lengths were measured by deducting 1 mm from the lengths recorded when the tip of a #15K-file was visible at the apical foramina. A glide path was confirmed with a #25K-file (Dentsply/Maillefer, Ballaigues, Switzerland). The root canal procedure was completed by using K3 Ni-Ti rotary instruments (SybronEnd, Glendora, CA, USA) according to the manufacturer's instructions until a #40/.06 file reached the working length. Each instrument was only used for the preparation of six canals. The root canals were flushed with 5 ml of a 2.5% NaOCl solution between the instrument changes using a disposable syringe with a 27-gauge needle (Monoject; Covidien LP, Deland, FL, USA) inserted at a distance of 2 mm from the working length without binding. The teeth were then randomly divided into experimental ($n = 40$) and control groups ($n = 20$). The experimental groups were subdivided into two equal groups (group 1 and group 2) according to the *S. persica* extract concentration. The canals in the experimental groups were irrigated for 5 min with 5 ml of the *S. persica* extract at concentrations of 1 mg/ml (group 1) or 5 mg/ml (group 2). The canals in the positive control group ($n = 10$) and the negative control group ($n = 10$) were irrigated for 5 min with 5 ml of 17% EDTA or saline, respectively.

The irrigation solutions were delivered in a passive manner using in and out movements via a sterile 27-guage needle (Monoject; Covidien LP, Deland, FL, USA) that penetrated to within 2 mm of the working length. The root canals then underwent a final flush with 5 ml of 1% NaOCl.

2.3. Scanning electron microscopy (SEM) evaluation

The roots were split longitudinally in the buccolingual plane to prepare the samples for SEM evaluation. The roots were grooved to three levels at 4, 8, and 12 mm from the root apices using a diamond bur to define the coronal, middle, and apical thirds. The specimens were left to dry overnight, mounted on copper stubs, coated with gold, and examined and photographed using a scanning electron microscope (Jeol, JSM, T330A, Electron Optical Laboratory, Tokyo, Japan) at an accelerating voltage of 25 kV. Digital photomicrographs at 1000× and 1500× were taken at the center of each third. Smear layer removal was evaluated using the three-point scoring system reported by Torabinejad et al. (2003a). This system measures the presence of the smear layer as follows: a score of (1) indicates the absence of a smear layer on the surface of

Table 1 Mean scores in the experimental and control groups according to the third.

Group third	Group 1 (1 mg/ml)	Group 2 (5 mg/ml)	+ve (EDTA)	−ve (Saline)	*p*-Value
Coronal	56.50	46.50	46.50	110.50	< 0.0001
Middle	65.30	49.20	30.50	103.50	< 0.0001
Apical	60.92	61.83	24.50	93.00	< 0.0001

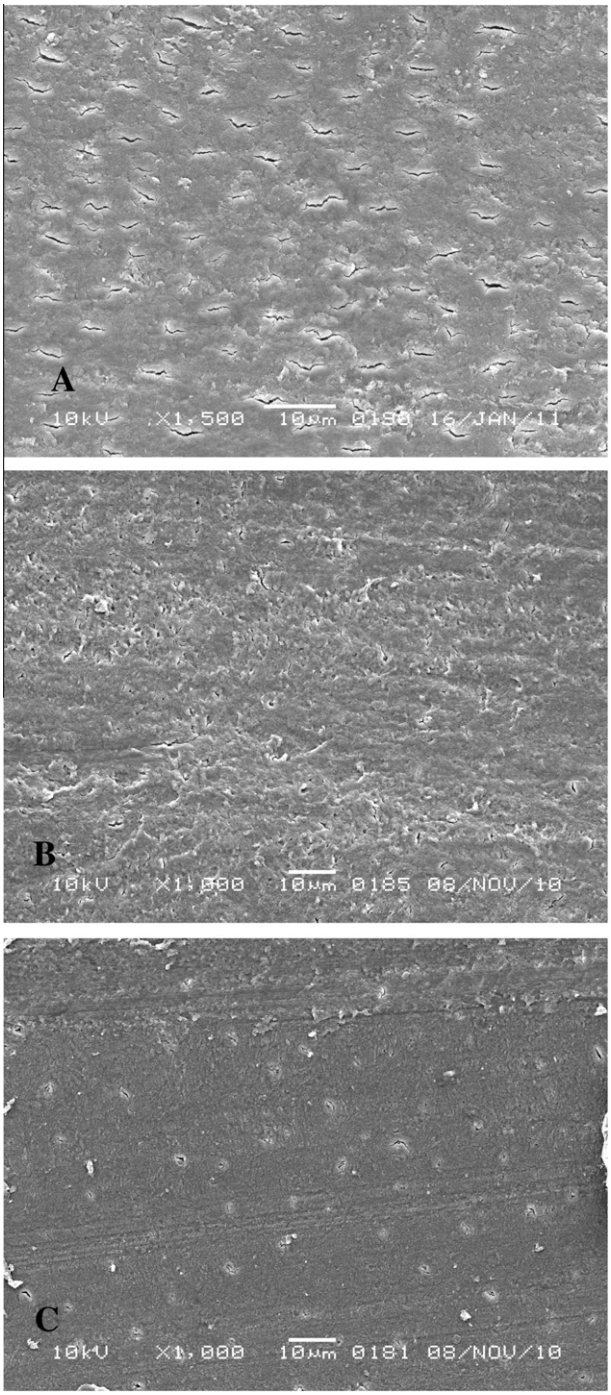

Figure 1 The effect of irrigating with saline for 5 min followed by 1% NaOCl. A typical amorphous smear layer is observed throughout the entire length of the root canal: the coronal (A), middle (B), and apical (C) thirds (1000–1500×).

the root canal and that all tubules are clean and open; a score of (2) indicates a moderate smear layer on the tubules but no smear layer on the surface of the root canal; and a score of (3) indicates a heavy smear layer that covers the root canal surface and the tubules. The scoring procedure was implemented by two examiners who performed the blinded evaluations independently after a joint examination of 20 specimens for calibration purposes. The reliability of the intraexaminer and interexaminer results was verified by using the kappa test. The score data for the presence or absence of the smear layer were statistically analyzed by Kruskall–Wallis analysis with subsequent pair-wise comparisons of the individual groups. All statistical analyses were set with a significance level of $p < 0.05$.

3. Results

The intraexaminer and interexaminer reproducibilities were excellent for both observers with values greater than or equal to 0.97 for the different groups. Table 1 shows the mean score ranks at each level. The negative control group had a heavy smear layer, and the dentinal tubules were not visible throughout the entire length (Fig. 1). In contrast, the positive controls revealed that the dentinal tubules were open, and no smear layer was noted on the surfaces of root canals irrigated with 17% EDTA (Fig. 2). The two tested ethanolic *S. persica* extract concentrations were effective in removing the smear layer from the coronal and middle thirds of the root canal wall. However, the 5 mg/ml *S. persica* solution was significantly more effective than the 1 mg/ml solution. In addition, the 5 mg/ml *S. persica* solution was as effective as 17% EDTA in removing the smear layer from the coronal third of the canal wall. At the apical third, both *S. persica* concentrations had similar effects and were less effective than EDTA in removing the smear layer. Representative photomicrographs of the experimental group at each third are shown in Figs. 3 and 4.

4. Discussion

Root canal instrumentation procedures produce a layer of organic and inorganic material called the smear layer. This layer can form two zones: the first zone is 1–2 µm thick and consists of organic matter and dentine particles, and the second zone extends into dentinal tubules to a depth of 40 µm (smear plugs) and is formed largely of dentine chips (Mader et al., 1984). Unfortunately, no irrigation solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer. Presently, sodium hypochlorite can be combined with EDTA to offer bactericidal, solvent, and chelating actions (Baumgartner and Mader, 1987).

The clinical interest of *S. persica* arises from a number of mechanisms, including its acidic and antimicrobial properties.

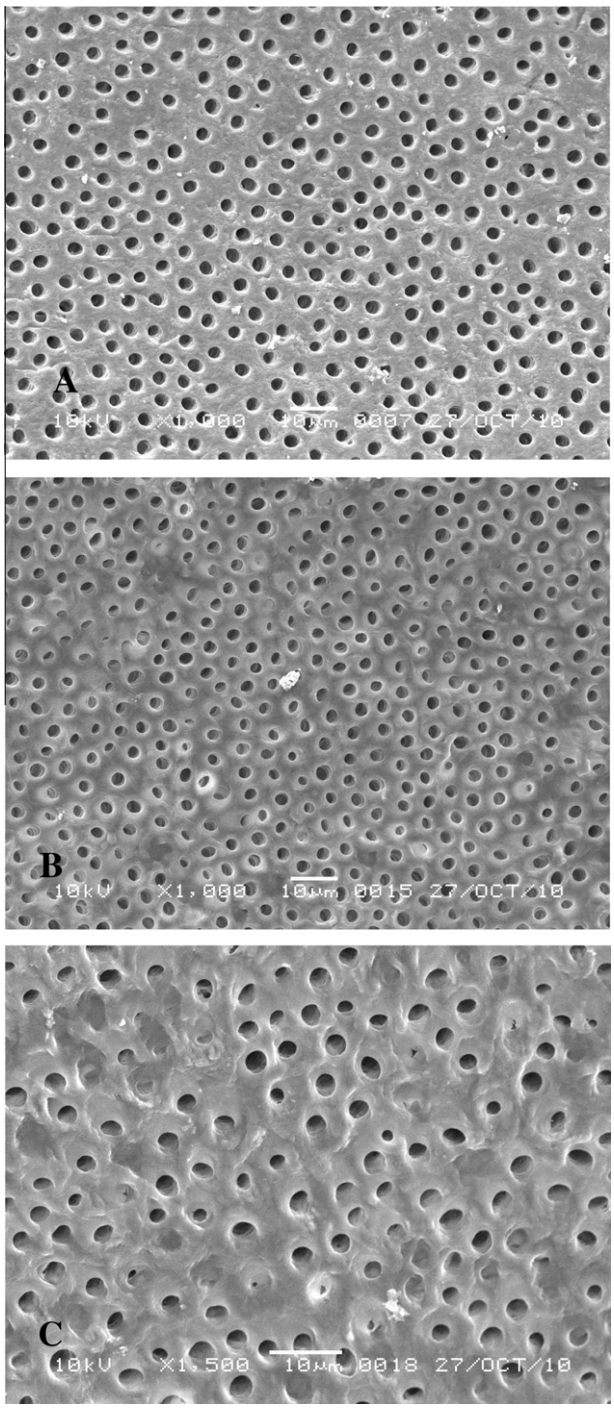

Figure 2 The effect of irrigating with 17% EDTA for 5 min followed by 1% NaOCl. The smear layer is completely removed and all of the tubule openings are clearly visible on the coronal (A), middle (B), and apical (C) thirds (1000–1500×).

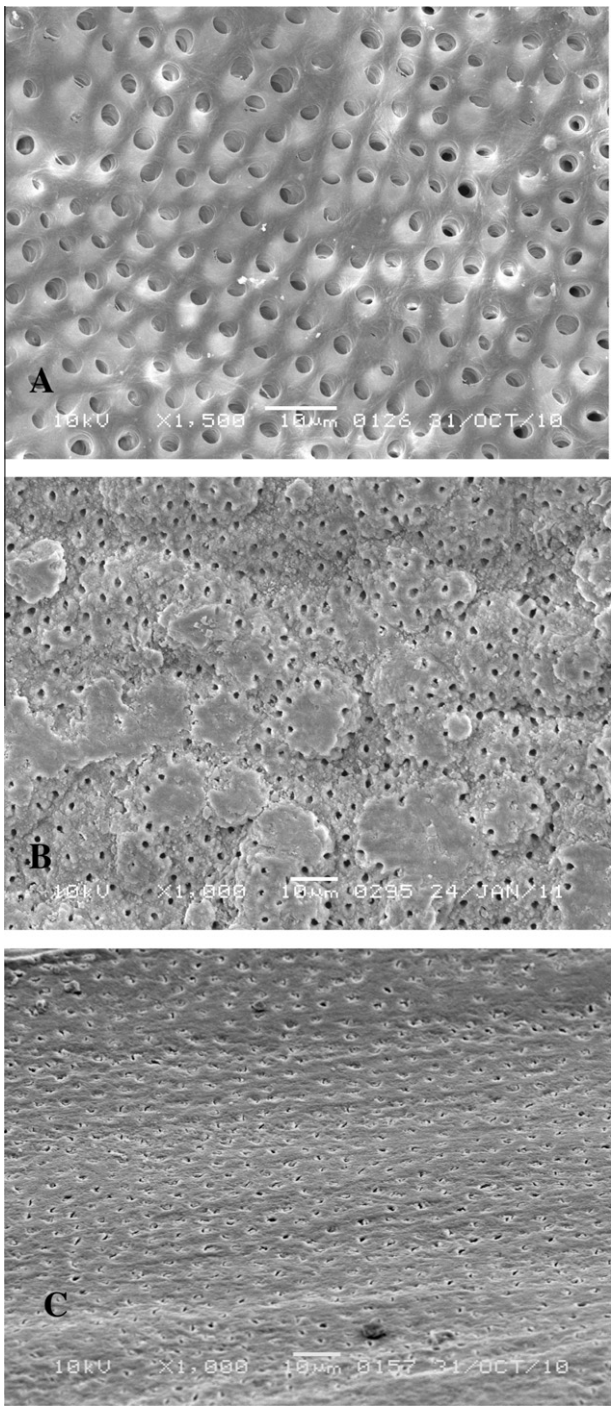

Figure 3 The effect of irrigating with a 1 mg/ml *S. persica* solution for 5 min followed by 1% NaOCl. The smear layer is removed and the tubule openings are visible on the coronal third (A) (1500×). A moderate amount of smear layer is observed in the middle third (B) (1000×). The smear layer is seen to occlude the openings of the dentinal tubules in the apical third (C) (1000×).

By the isolation of the active ingredient from *S. persica*, Wolinsky and Sote (1983) found that limonoid had a great antimicrobial activity against various Gram positive and Gram negative microorganisms. Phytochemical investigation revealed that it contains oleic, linolic, and stearic acids. Among the compounds identified are esters of fatty acids and aromatic acids and some terpenoids (Abd El Rahman et al., 2003). Nevertheless, the antimicrobial and cleaning effects of *S. persica* may be attributed to various chemicals contained in its extracts, such as sodium chloride and potassium chloride, as well as salvadourea, salvadorine, saponins, tannins, vitamin C, silica, and resin (Darout et al., 2000). To the best of our knowledge, this is the first study that evaluates the ability of

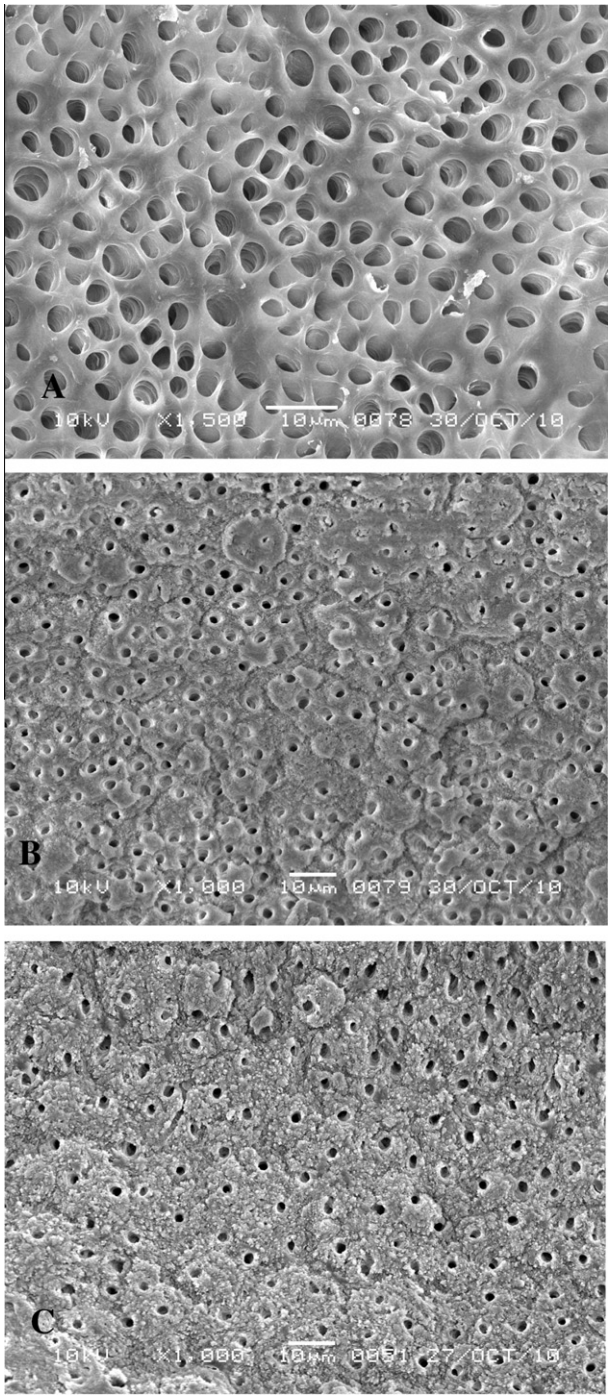

Figure 4 The effect of irrigating with a 5 mg/ml *S. persica* solution for 5 min followed by 1% NaOCl. The smear layer is completely removed and all of the tubule openings are clearly visible on the coronal (A) and middle (B) thirds. Excessive erosion of the intertubular and peritubular dentin is seen at the coronal third, which leads to tubular aperture conjugation and tubular diameters widening (1500–1000×). The smear layer is seen to occlude the openings of some of the dentinal tubules in the apical third (C) (1000×).

S. persica to remove the smear layer following root canal instrumentation procedures.

Extracts were obtained by using the appropriate solvent on a dry plant. Thus, the active compounds were dissolved in the solvent used. The extraction process has been well studied to respect the integrity of the active molecules. Total extracts of *S. persica* prepared in water, phosphate-buffered saline (PBS), and organic solvents have been tested for their antibacterial (Al Salman et al., 2005; Al-Sabawi et al., 2007; Babay and Almas, 1999) and anti-fungal activities (Al-Bagieh et al., 1994). In this study, an ethanolic *S. persica* extract solution was used because it has been reported to have a significant antimicrobial effect against aerobic and anaerobic bacteria when used as a root canal irrigant (Al-Sabawi et al., 2007). Other studies have indicated that an alcoholic *S. persica* solution can remove the smear layer from periodontally involved dentin (Babay and Almas, 1999; Almas, 2002). The accepted application time of the chelating solution, as supported by the literature, is between 1 and 5 min (Yamada et al., 1983; Torabinejad et al., 2003b). Thus, irrigation for 5 min with *S. persica* extract followed by a final rinse with NaOCl was adopted for this study. Because the objective of this study was to evaluate the efficacy of the irrigants rather than the efficacy of root canal irrigation (Torabinejad et al., 2003a,b), an ''open-system'' design (Tay et al., 2010) with an unsealed root apex (Baumgartner and Mader, 1987), which permitted air and vapor communication between the external environment and the canal space, was implemented in this study. The apical preparation of each canal was enlarged to #40/.06 to allow deep cleaning and penetration of the solution to the apical third of the root canal.

Although there was a significant difference in smear layer removal between the experimental groups, 1 mg/ml of *S. persica* extract was able to partially remove the smear layer from the coronal and middle thirds of the root canal. Alternatively, 5 mg/ml of *S. persica* extract was as effective as 17% EDTA in removing the canal-wall smear layers from the coronal third. It is possible that the larger size of the canals in these thirds (compared with the apical third) allowed for improved circulation and action of the irrigation solution, making the removal of the smear layer possible. These results agree with those of others, who also have observed an effective cleaning action on these thirds, even when different quantities of solutions and irrigation times were employed (Baumgartner and Mader, 1987; Abbott et al., 1991). At the apical third, both the concentrations of *S. persica* extract had similar effects and were less effective than EDTA in removing the smear layer. These results were consistent with the general finding from the endodontic literature that the apical third of the canal is more difficult to clean (Goldman et al., 1982; Barkhordar et al., 1997; O'Connell et al., 2000; Calt and Serper, 2000). However, these results could be due to tubular sclerosis, which is most pronounced in the apical third of the root canal (Vasiliadis et al., 1983). The results of this study are nearly consistent with the findings of Babay and Almas (1999) who observed that alcoholic *S. persica* extracts completely removed the smear layer. However, the modes of application, exposure times, and sample preparation were different. In addition, Babay and Almas (1999) have used dentin root surface blocks (not including the apical third of the root).

The ability of *S. persica* to remove the smear layer may be attributed to its acid content (stearic acid), which may react with calcium in the dentin and act as a chelating agent. Additionally, this ability may be due to the low pH of the alcohol-

derived extract. For example, the 1 mg/ml solution has a pH value of 5.2, and the 5 mg/ml solution has a pH value of 4.7. The weak hydrogen bonds that link the alcohol to the collagen of the smear layer can be easily broken, leading to separation of the smear layer from the dentin surface and exposing the tubules (Babay and Almas, 1999). Register and Burdick (1975) suggested that the range of acid penetration depended on the pH and application time of the acid solution used. This could justify the increased etching effects with the 5 mg/ml *S. persica* solution compared with the 1 mg/ml solution. However, this finding contradicts that of Blomlof and LindsKog (1995) who found that etching at neutral pH is equal if not more efficient than with the agents at low pH in exposing collagen fibrils on dentin surfaces. Branstrom and Johnson (1974) have reported that the smear layer is only removed by demineralizing solutions, which may suggest that alcoholic *S. persica* extracts have chelating properties. In addition, the inability of saline to remove the smear layer may be explained by the astringent action of saline on the smear layer (Babay and Almas, 1999).

Although the evaluation of erosion was beyond the aim of this study, it was noticed that slight erosion of the dentinal walls in groups 1 and 2 may have been due to a prolonged application time or the large volume of *S. persica* solution. Therefore, further investigations should be performed to clarify the ideal volume and application time for irrigation after the root canal instrumentation procedures. While studies have indicated that the combination of EDTA and NaOCl causes severe erosion of canal wall surfaces (Baumgartner and Mader, 1987; Calt and Serper, 2000; Niu et al., 2002), the application time and the volume of EDTA used in this study caused slight alterations in the dentin structure, mainly at the middle and apical thirds. Such observations may be related to the ''open-system'' design utilized in this study.

Based on the previous findings (Al-Sabawi et al., 2007; Babay and Almas, 1999; Abo Al-Samh and Al-Nazhan, 1997) and the current results, *S. persica* extract has the potential to be used as an irrigant solution due to its biocompatibility, antibacterial properties, and chelating effects. While the current data look promising, further studies are recommended to provide results that could justify the clinical application of *S. persica* extract in endodontics.

5. Conclusion

Within the limitations of this *in vitro* study, the 5 mg/ml ethanolic *S. persica* extract solution was significantly more effective than the 1 mg/ml solution at the coronal and middle thirds. Furthermore, the 5 mg/ml *S. persica* solution was as effective as 17% EDTA in removing the smear layer from the coronal third of the canal wall. At the apical third, both the concentrations of *S. persica* had similar effects and were less effective than EDTA in removing the smear layer.

Ethical Statement

There is no ethical issue regarding this study.

Conflict of interest

No conflict of interest declared.

Acknowledgments

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