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Effect Of Agitation Of Salvadora Persica Extract And Sodium Hypochlorite Final Irrigating Solutions With Er,Cr:YSGG-Laser Using Radial- Firing Tips On Removal Of Smear Layer: A Scanning Electron Microscopic Evaluation

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Effect Of Agitation Of Salvadora Persica Extract And Sodium Hypochlorite Final Irrigating Solutions With Er,Cr:YSGG-Laser Using Radial-Firing Tips On Removal Of Smear Layer: A Scanning Electron Microscopic Evaluation

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Abstract

Introduction: to compare the effect of Er,Cr:YSGG-laser-activated and conventional irrigation techniques on intracanal smear layer elimination at the apical, middle, and coronal segments of root canal walls using environmental scanning electron microscope (ESEM).

Methods: Thirty-six freshly extracted human single-rooted single-canal anterior teeth were selected for this study. The root canals were conventionally accessed, then prepared by using ProTaper Next® system to size X4, 0.06 taper and divided into two equal groups (n = 18); syringe/needle- and laser-activated groups. Each group was further subdivided into three subgroups; saline, NaOCl, and salvadora P (6 teeth each). Er,Cr:YSGG-laser was emitted at pulse energy of 25 mJ, pulse width of 60 µs, pulse frequency of 50 Hz and delivered by a radial firing tip. Teeth were longitudinally sectioned for ESEM assessment. Data were collected and statistically analyzed by Kruskal-Wallis and Mann-Whitney tests.

Results: Er,Cr:YSGG-laser-activated generally showed better smear layer removal results than syringe/needle activated group. The statistical significant differences occurred between NaOCl and salvadora P subgroups at coronal and middle, and apical thirds, respectively. Salvadora P presented higher statistical smear layer removing-capacity than the other comparative subgroups at coronal third when Er:Cr:YSSG-laser-activated technique was used and at coronal and middle thirds when the conventional technique was applied.

Conclusion: Er,Cr:YSGG-laser-activated irrigation technique had a positive effect on removing of smear layer. Laser-activated Salvadora P was effectively removed smear layer from the entire root dentin while the conventionally-activated salvadora P was more potent at coronal and middle thirds.

Clinical significance: Er,Cr:YSGG-laser can effectively activate the intracanal irrigants.

Keywords: Er, Cr:YSGG laser, RFT, smear layer removal, root canal irrigation

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Introduction

Failure in root canal therapy is a result of the residual microorganisms settle inside the root canal space and wall during and after completing root canal therapy or due to the bacterial pathogens invades and re-colonize the filled canal system after treatment **(Peters O. et al., 2021)**. Therefore, the rationales of root canal therapy are optimizing the cleaning of the root canal system and the removal of root canal debris and completing dense, three-dimensional root canal obturation **(Peters O. et al., 2021)**. Because of mechanical preparation of the root canal unfortunately lefts 30–50% of the dentin

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wall of root canal untouched, in additional it creates smear layer consisting dentine fragments, pulp debris, and microorganisms, irrigation with disinfection agents should be applied during conventional endodontic treatment in order to get rid of utmost microorganisms and root canal debris for avoiding failure of accomplishing root canal procedure **(Jhajharia K. et al., 2015)**.

Numerous researchers have been investigated various chemical and natural components in different concentrations and application techniques for using them as intracanal irrigant **(Shravya, S. et al., 2020)**. Currently, 1-5.25% sodium hypochloride is considered the most common irrigant used for disinfection the root canal **(Singh M. et al., 2021)**. NaOCl irrigant is favorable since it is greatly able to dissolve necrotic dental pulp structures and organic constituents of the smear layer **(Shravya, S. et al., 2020)**. Besides, it significantly destroys the pathogens of root canal present in biofilm and in dentinal tubules **(Shravya, S. et al., 2020)**. Despite the advantageous properties of sodium hypochloride, this irrigant has a cytotoxic effect on vital biological structures leading to haemolysis, skin ulceration and necrosis **(Perotti S. et al., 2018)**. Additionally, it should be utilized along with another agent capable to remove inorganic elements of smear layer *such as 17% EDTA* which may increase time of endodontic procedure exacerbate operator's fatigue **(Shravya, S. et al., 2020)**.

Lately, several studies had concerned with herbal irrigants as they are more available, more cost effective, more limited for bacterial resistance, and lower cytotoxcity than chemical ones **(Shravya, S. et al., 2020)**. Antibacterial, antioxidant, anti-cariogenic characteristics of *Salvadora persica or pilu (S. persica)* as well its chelation actions encouraged the endodontists to examine its biochemical effects during cleansing and decontamination of root canal methods **(Shravya, S. et al., 2020)**. Literature revealed that applying of *S. persica* solutions have positive outcomes in the matter of smear layer removal, pulpal tissue dissolution, and antimicrobial activity **(Shravya, S. et al., 2020)**.

The anatomical complexity of root canal system impedes the intracanal irrigant for clean-up the areas of root canal system beyond the central body of the root canal **(Mancini, M. et al., 2022)**. Intracanal irrigant cannot reach lateral canals, canal fins, cul-de-sacs, canal isthmi without fluid agitation **(Mancini, M. et al., 2022)**. Residual bacterial pathogens and their by-product, and pulp tissue remnants existing at untouched zones of root canal system definitely cause persistent periradicular inflammation **(Yao Y. et al., 2021)**.The contemporary methods and systems of root canal agitation can be categorized into manual and machine-assisted agitation approaches **(Krongbaramee T. et al., 2020)**.

Apparently, lasers can be employed to decontaminate the infected root canal system with /without using intracanal irrigating solution **(Olivi G. and Olivi M., 2016)**. The conventional LAI was the first laser method employed to activate the intracanal irrigation solutions **(Olivi G. and Olivi M. 2016)**. Different laser wavelengths have been utilized for agitation the root canal irrigants **(Olivi G. and Olivi M. 2016)**. The medium infrared Erbium laser family (Er:YAG 2940 nm and Er,Cr:YSGG 2780 nm) are the most suitable laser irradiation wavelengths can be used for activating the intracanal irrigant due to their specific affinity to water **(Olivi G. and De Moor R., 2016)**.

A conventional tip of Erbium family lasers induces laser emission from their end o intracanl irrigant without capability to deliver laser energy laterally to dentinal wall of root canal. In contrast, more recent radial firing tip can permit some lateral laser emission which can laterally irradiate the root surfaces and surrounding intracanal irrigating fluid solution **(Olivi G. and Olivi M. 2016)**.

Actually, few studies had been carried out to evaluate the solution of *S. persica* extract regarding its ability to eliminate the smear layer. Also, there are no studies have investigated the conventional laser-assisted irrigation method using Er,Cr:YSGG-laser with a radial-firing tip on smear layer removing-capacity of the *S. persica* extract irrigating solution. Consequently, this study aimed to microscopically assess the agitation of *S. persica* extract and sodium hypochlorite final irrigating solutions with Er,Cr:YSGG-laser using radial-firing tip on smear layer removal and dentinal tubules opening by environmental scanning electron microscope (ESEM). The null hypothesis was that there are no differences in smear layer removal with different intracanal irrigating solutions as well as irrigant activation techniques will be used.

Materials & Methods

Ethical approval:

This in-vitro study was approved by the Scientific Research Ethics Committee of National Institute of Laser Enhanced Sciences (NILES), Cairo University, Egypt.

Sample size calculation:

Sample size calculation was executed by using R statistical package, version 3.3.1 (21-0-2016) copyright (C) 2016, based on **(Bolhari B. et al., 2014)**. Therefore, 6 samples for each group were found to be enough, with an 80% power and a significance level of 0.05.

Preparation of S. persica extracts solution:

Preparation of *Salvadora persica* extracts was performed in accordance to **(Balto H. et al., 2012; Balto H. et al., 2015)** studies. Therefore, 10% water in ethanol was utilized as a solvent to extract *Salvadora persica* juice from fresh ground roots of Arak shrub **(Balto H. et al., 2012; Balto H. et al., 2015)**. Cryodesiccation method was applied to entirely take out the 10% water in ethanol from *Salvadora persica extracts* **(Balto H. et al., 2012; Balto H. et al., 2015)**. Then, the extracted product was dissolved in dimethyl sulfoxide (DMSO) **(Balto H. et al., 2012; Balto H. et al., 2015)**. For recruiting *Salvadora persica* extracts in irrigating the root canal system, a sterile physiological saline at a pH of 7.4.was used to obtain *Salvadora persica* extract with working concentrations of 5 mg/ml **(Balto H. et al., 2015)**.

Samples'selection, grouping and preparation:

A total thirty-six freshly extracted human anterior teeth were selected for this study. Teeth were clinically intact and free of dental caries, restoration and crack **(Ayranci L. et al., 2016; Majumdar Y. et al., 2021)**. Also, teeth roots were radiographically closed-apex, single-rooted without resorption or calcification **(Ayranci L. et al., 2016; Majumdar Y. et al., 2021)**. The apical curvature of teeth roots doesn't than 20 degree **(Ayranci L. et al., 2016; Majumdar Y. et al., 2021)**.

Teeth surfaces had been cleaned with ultrasonic scaler and washed with tap water **(Obeid M. and Nagy M., 2015)** before they were stored in sodium chloride 0.9 W/V (normal saline) **(Adl A. et al., 2019)**.

Root canal therapy of all teeth was stared with standard access cavity preparation of each tooth **(Dagher J. et al., 2019)**. These prepared cavities were performed by using a high-speed contra-angle handpiece connected to a tapered diamond bur **(Dagher J. et al., 2019)**. Next a size#10, sterile, stainless-steel, hand K-file was inserted inside root canal space for measuring the apical potency **(Kanaan C. et al., 2020)**. When the k-file tip searched to the same level of apical foramen, the apical potency was determined **(Kanaan C. et al., 2020)**.

Instrumentation of root canal was achieved by ProTaper Next® files (Dentsply Maillefer, NewYork, USA) up to size# x4**(Abd-Elgawad R. and Fayyad D., 2017)**. The working length of mechanical preparation of each root canal was 0.5 mm short of its apical potency **(Majumdar T. et al., 2021)**. The engine-driven files were employed at torque of 2N/m and rotational speed of 300 rpm **(Abd-Elgawad R. and Fayyad D., 2017)**. Each NiTi file was used for three canals only **(Elemam R. et al., 2016)**. Distilled water was utilized as a root canal irrigant after each instrumentation procedure.After that, distilled water root canal irrigation had been applied before the instrumented root canals were subjected to final flush **(Kaushal R. et al., 2020)**.

Subsequently, teeth were randomly allocated to two groups (18 teeth in each) in accordance to the irrigating technique of the root canal: conventional syringe/needle irrigation technique (group I) and 2780-nm Er,Cr:YSGG laser-assisted irrigation technique (group II). Teeth of each group were further subdivided into three subgroups of 6 teeth each in respect with the type of the final intracanal irrigant: sodium chloride 0.9 W/V, normal saline solution (subgroup A), 5% sodium hypochlorite solution (subgroup B) and a solution of *Salvadora Persica L extract* (subgroup C).

5 ml of each examined intracanal irrigant was gradually applied for one minute **(Nakamura V. et al., 2013; Shravya, S. et al., 2020; Murugesan K. et al., 2022)**. During conventional syringe/needle irrigation technique, the side-vented needle (Monoject, Sherwood Medical, Switzerland) was placed with the root canal at 1 mm shorter than its working length **(Tan L. et al., 2022)**.

Regarding 2780-nm Er,Cr:YSGG laser-assisted irrigation technique laser beam was emitted at pulse energy of 25 mJ, pulse width of 60 µs, pulse frequency of 50 Hz **(Al-Mafrachi R., 2019)** for 20 second in cycle using Er,Cr:YSGG-laser device (Waterlase iPlus, Biolase Technology, Irvine, CA, USA). Total time of laser irradiation was 60 sec: 20 second (time of irradiation) X 3 (number of irradiation cycles). Thewater and air sprays of the laser device were shut off **(Peeters H. et al., 2018)**. The photon energy was delivered in contact mode with the intacanal irrigant through 415-µm-diameter, 21-mm-length radial firing tip (RFT) (Waterlase laser tip RF3-21, Biolase, Technology, Irvine, CA, USA) with 0.85 calibration factor **(Al-Mafrachi R., 2019)**. In each irradiation cycle, RFT tip had been firstly positioned within the root canal space at 2 mm short of root apex **(Al-Mafrachi R., 2019)** then it was helicoidally moved in a speed of 1mm/s towards coronally direction **(Al-Mafrachi R., 2019)**. The irradiation cycle was identically repeated three times.

After that, the root canals were subjected to distilled water (5 ml) flush using side-vent needle & plastic syringe **(Vineetha C. et al, 2022)** and were dried with ProTaper Next® paper point x4.

(Table 1): Laser specifications, parameters and technique used in this study

Laser type specifications & parameters	Intrinsic properties	Laser device	Waterlase, Biolase, USA
		Laser type (Active medium)	Er,Cr:YSGG
		Laser wavelength	2780 nm
		Emission type	Free running pulse
		Delivery system	Contact tip
		Pulse shape (energy distribution)	Gaussian
	Adjustable parameters	Pulse energy	25 mJ
		Pulse duration	60 µs
		Pulse repetition rate (Pulse frequency)	50 Hz
		Activation time per session	20 sec
		No. of activation sessions	3
		Total activation time	60 sec
Tip type specifications	Intrinsic specifications	Tip type	(RFT3-21) radial firing tip with 0.85 calibration factor
		Tip diameter	415 µm
		Tip length	21 mm

SEM evaluation:

Firstly, a single absorbent paper point size# X4 was properly seated inside the root canal **(Khaord P. et al., 2015)**. Teeth were longitudinal sectioned into two halves. The samples' sectioning was carried out onthe proximal aspects parallel to long axis of tooth root **(Chatterjee S. et al., 2021)** using a low-speed diamond water-cooled diamond saw (Isomet 4000, Buehler Ltd, Lake Bluff, IL, USA).

The coronal, middle and apical thirds of dentinal wall of the sections were microscopically examined by environmental scanning electron microscopeESEM (FEI Quanta 250 FEG, Berlin, Germany) with an electron accelerating voltage of 20 KVat a standard magnification of ×2000 **(Sukhbir K. et al., 2019; Kharouf N. et al. 2020; Sharma S. et al., 2020**; **Mohamed N. et al., 2022)** for measuring the smear layer removing-capacity and the presence of dentinal tubules' opening.

Two well-trained qualified evaluators independently assessed the FESEM images **(Alakshar A. et al., 2020)**. SEM evaluations were carried out with the 5- point method of scoring by means of numerical assessment scale established by **(Hülsmann M. et al., 1997)**.The scores were as follows- Score 1: no smear layer was revealed on the root canal surface besides the orifices of dentinal tubules were clearly opened; Score 2: diminutive amount of smear layer was revealed on the root canal surface, additionally some open dentinal tubule were present; Score 3: homogenous smear layer along approximately the whole dentinal wall on root canal, in addition a small number of open dentinal tubules were observed; Score 4: the complete dentinal wall of root canal covered with a homogenous smear layer with no open dentinal tubules was found; Score 5: a heavy, homogeneous smear layer covering the whole dentinal wall of root canal with no open dentinal tubules was seen **(Hülsmann M. et al., 1997)**.

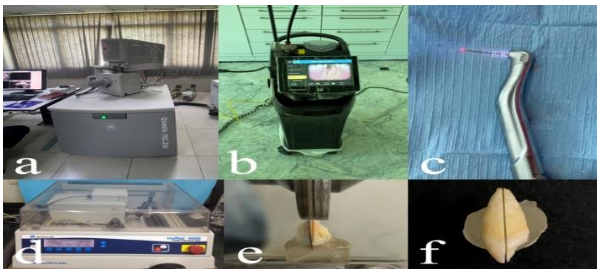

Figure (1): showed the ESEM **(a)**, Er,Cr:YSGG laser device **(b)**, RFT attached with a handpiece**(c)** and cutting machine **(d)** used in this study. Additionally, it presented a sample during **(e)** and after sectioning **(f)**.

Statistical analysis

Data of ESEM assessment were recorded and analyzed using SPSS *(Statistical Packages for the Social Sciences 26.0, IBM, Armonk, NY, USA)*.. Cohen's kappa coefficient was utilized to determine inter- & intra-evaluators reliability. Also, Kolmogorov-Smirnov and Shapiro-Wilk tests employed to check the study data normality. Moreover, Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney test were conducted to statistically analyze the numeric data of the examined groups in accordance to the intracanal irrigant type and the examined root segment considering the level of statistical significance was set at 0.05.

Results

Analysis of data extracted from this in-vitro study demonstrated that the mean values of smear layer score of the samples that were irrigated with Er,Cr,YSGG-laser-activated NaOCl (group II, subgroup B) salvadora P (group II, subgroup C) irrigants recorded lower values than those that were received the conventional syringe/needle irrigations with the same intracanal irrigant; NaOCl (group I, subgroup B) and salvadora P (group I, subgroup C) at different examined root segments; coronal, middle, and apical root thirds. There were statistical significant differences between two groups when they had been activated NaOCl (subgroup B) and salvadora P (subgroup C) intracanal irrigants. These significant differences occurred at coronal and middle root thirds of NaOCl irrigant's specimens and at apical root third of salvadora P irrgant's samples *(Table 2)*.
Regarding the results of the examined root canal irrigants at each root segment, there were statistical significant differences between the root canal irrigants activated by conventional syringe/needle technique (group I) at coronal third (between saline; subgroup A and NaOCl; subgroup B) and middle third (between NaOCl; subgroup B and salvadora P; subgroup C)*(Tables 3 and 4)*.
There were statistical differences between Er,Cr:YSGG-laser-activated root canal irrigants (group II) in all evaluated root segments. These statistical differences occurred between saline (subgroup I) and salvadora P (subgroup III) at coronal, middle and apical root thirds *(Tables 3 and 4)*.
Concerning the results of the examined intracanal irrigants measured at different root segments; coronal, middle and apical root thirds, there were statistical differences between the smear layer scores recorded between the tested root canal irrigants at the different root segments whether the irrigants activated by conventionally (group I) orlaser-activated (group II) irrigation techniqueexcept the three segments of Er,Cr:YSGG-laser activated irrigant *(Tables 5 and 6)*.

Table (2): The mean, standard deviation (SD) and p-value of smear layer scores of different groups conventional and laser-activated irrigation techniques using saline, NaOCl and salvadora P at coronal, middle, and apical thirds.

Subgroups / Groups		Coronal third		Middle third		Apical third	
		Conventional	Er,Cr:YSGG	Conventional	Er,Cr:YSGG	Conventional	Er,Cr:YSGG
Saline	N	6	6	6	6	6	6
	Mean	1.833	2	2.833	2	4	4.5
	SD	±0.983	±0.894	±0.408	±0.894	±1.095	±0.837
	Mean Rank	6.17	6.83	8.17	4.83	5.75	7.25
	Sum of Ranks	37	41	49	29	34.5	43.5
	Mann-Whiteny U	16		8		13.5	
	Wilcoxon W	37		29		34.5	
	Z value	-0.341		-1.805		-0.822	
	P-value	0.733		0.071		0.411	
NaOCl	N	6	6	6	6	6	6
	Mean	3.167	1.333	3.333	2.333	4.333	3
	SD	±0.408	±0.516	±0.516	±0.817	±0.516	±1.673
	Mean Rank	9.5	3.5	8.5	4.5	8	5
	Sum of Ranks	57	21	51	27	48	30
	Mann-Whiteny U	0.000		6		9	
	Wilcoxon W	21		51		30	
	Z value	-3.052		-2.345		-1.554	
	P-value	0.002*		0.019*		0.120	
Salvadora P	N	6	6	6	6	6	6
	Mean	1.667	1	1.167	1	4.5	2.5
	SD	±0.517	±0	±0.408	±0	±0.548	±1.378
	Mean Rank	8.5	4.5	7	6	8.75	4.25
	Sum of Ranks	51	27	42	36	52	25
	Mann-Whiteny U	6		15		4.5	
	Wilcoxon W	27		36		25.5	
	Z value	-2.345		-1.000		-2.233	
	P-value	0.019*		0.317		0.026*	

(*) values had statistically significant difference at ($P<0.05$).

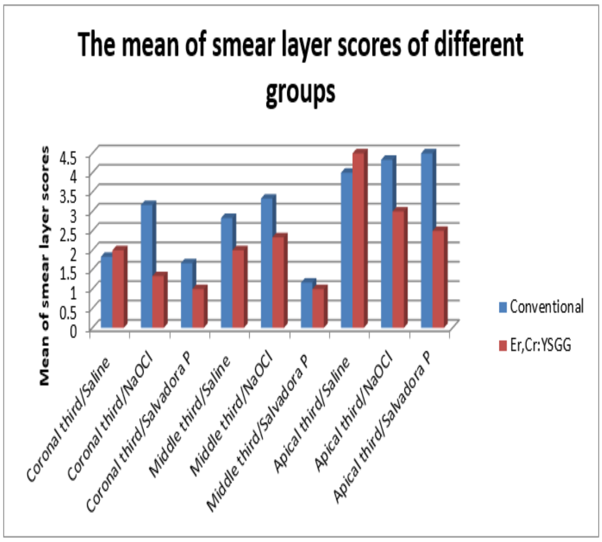

Figure (2): The mean of smear layer scores in different groups; conventional and laser-activated irrigation technique groups using saline, NaOCl and salvadora P at coronal, middle, and apical thirds.

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Table (3): The mean, standard deviation (SD) and kruskal-Wallis statistics of smear layer scores of different subgroup; saline, NaOCl and salvadoraP at coronal, middle, and apical thirds.

Group (Irrigation technique)	Root segment	Subgroup (Irrigant type)	N	Mean	SD	Mean Rank of score	Kruskal-Wallis statistics: Kruskal-Wallis H	Degree of freedom	P-value
Conventional	Coronal	Saline	6	1.833	0.983	7.5	9.516	2	0.009*
		NaOCl	6	3.167	0.408	14.67			
		Salvadora P	6	1.667	0.516	6.33			
	Middle	Saline	6	2.833	0.408	11.08	13.886	2	0.001*
		NaOCl	6	3.333	0.516	13.83			
		Salvadora P	6	1.167	0.408	3.58			
	Apical	Saline	6	4	1.095	8.25	0.773	2	0.680
		NaOCl	6	4.333	0.516	9.5			
		Salvadora P	6	4.5	0.548	10.75			
Er,Cr:YSGG	Coronal	Saline	6	2	0.894	12.83	6.139	2	0.046*
		NaOCl	6	1.333	0.516	9.17			
		Salvadora P	6	1	0	6.5			
	Middle	Saline	6	2	0.894	12.17	5.566	2	0.062
		NaOCl	6	1.667	0.817	10.33			
		Salvadora P	6	1	0	6			
	Apical	Saline	6	4.5	0.836	13.5	5.583	2	0.061
		NaOCl	6	3	1.673	8.25			
		Salvadora P	6	2.5	1.378	6.75			

(*) values had statistically significant difference at (P<0.05).

Table (4): Mann whiteny U statistics of smear layer scores of different subgroup; saline, NaOCl and salvadora P at coronal, middle, and apical thirds.

Group (Irrigation technique)	Root segment	Area of comparison (Irrigant types)		N	Mean Rank	Sum of Ranks	Mann-Whiteny U	Wilcoxon W	Z value	P-value
Conventional	Coronal	Saline- NaOCl	Saline	6	4.33	26	5	26	-2.342	0.019*
			NaOCl	6	8.67	52				
		Saline-Salvadora P	Saline	6	6.67	40	17	38	-0.173	0.863
			Salvadora P	6	6.33	38				
		NaOCl-Salvadora P	NaOCl	6	9.5	57	0	21	-3.052	0.002*
			Salvadora P	6	3.5	21				
	Middle	Saline- NaOCl	Saline	6	5.17	31	10	31	-1.687	0.092
			NaOCl	6	7.83	47				
		Saline-Salvadora P	Saline	6	9.24	56.5	5	21	-3.028	0.002*
			Salvadora P	6	3.58	21.5				
		NaOCl-Salvadora P	NaOCl	6	9.5	57	0	21	-3.052	0.002*
			Salvadora P	6	3.5	21				
	Apical	Saline- NaOCl	Saline	6	6	36	15	36	-0.512	0.609
			NaOCl	6	7	42				
		Saline-Salvadora P	Saline	6	5.75	34.5	13.5	34.5	-0.783	0.434
			Salvadora P	6	7.25	43.5				
		NaOCl-Salvadora P	NaOCl	6	6	36	15	36	-0.561	0.575
			Salvadora P	6	7	42				
Er,Cr:YSGG	Coronal	Saline- NaOCl	Saline	6	7.83	47	10	31	-1.398	0.162
			NaOCl	6	5.17	31				
		Saline-Salvadora P	Saline	6	8.5	51	6	27	-2.298	0.022*
			Salvadora P	6	4.5	27				
		NaOCl-Salvadora P	NaOCl	6	7.5	45	12	33	-1.483	0.138
			Salvadora P	6	5.5	33				
	Middle	Saline- NaOCl	Saline	6	7.17	43	14	35	-0.682	0.495
			NaOCl	6	5.83	35				
		Saline-Salvadora P	Saline	6	12.5	112.5	13.5	58	-2.298	0.022*
			Salvadora P	6	6.5	58.5				
		NaOCl-Salvadora P	NaOCl	6	8	48	9	30	-1.897	0.058
			Salvadora P	6	5	30				
	Apical	Saline- NaOCl	Saline	6	8.25	49.5	7.5	28.5	-1.763	0.078
			NaOCl	6	4.75	28.5				
		Saline-Salvadora P	Saline	6	8.75	4.5	4.5	25	-2.263	0.024*
			Salvadora P	6	4.25	25.5				
		NaOCl-Salvadora P	NaOCl	6	7	42	15	36	-0.490	0.624
			Salvadora P	6	6	36				

(*) values had statistically significant difference at (P<0.05).

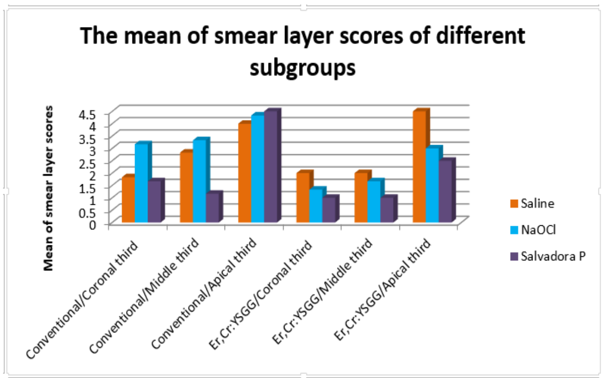

Figure (3): The mean of smear layer scores in different subgroups; saline, NaOCl and salvadora P at coronal, middle, and apical thirds.

Table (5): The mean, standard deviation (SD) and kruskal-Wallis statistics of smear layer scores of different root canal segments; coronal, middle, and apical

Group (Irrigation technique)	Subgroup (Irrigant type)	Root segment	N	Mean	SD	Mean Rank of score	Kruskal-Wallis statistics: Kruskal-Wallis H	Degree of freedom	P-value
Conventional	Saline	Coronal	6	1.833	0.983	5.25	9.270	2	0.010*
		Middle	6	2.833	0.408	9.5			
		Apical	6	4	1.095	13.75			
	NaOCl	Coronal	6	3.167	0.408	6.33	9.888	2	0.007*
		Middle	6	3.333	0.516	7.67			
		Apical	6	4.333	0.516	14.5			
	Salvadora P	Coronal	6	1.667	0.516	8	13.485	2	0.001*
		Middle	6	1.167	0.408	5			
		Apical	6	4.5	0.548	15.5			
Er,Cr:YSGG	Saline	Coronal	6	2	0.894	6.67	3.781	2	0.005*
		Middle	6	2	0.837	6.67			
		Apical	6	4.5	1.465	15.17			
	NaOCl	Coronal	6	1.333	0.516	7.17	3.781	2	0.151
		Middle	6	1.667	0.817	8.75			
		Apical	6	3	1.673	12.58			
	Salvadora P	Coronal	6	1	0	7	12.729	2	0.002*
		Middle	6	1	0	7			
		Apical	6	2.5	1.378	14.5			

(*) values had statistically significant difference at (P<0.05).

Table (6): Mann whiteny U statistics of smear layer scores of different root canal segments; coronal, middle, and apical.

Group (Irrigation technique)	Subgroup (Irrigant type)	Area of comparison (Root segments)		N	Mean Rank	Sum of Ranks	Mann-Whiteny U	Wilcoxon W	Z value	P-value
Conventional	Saline	Coronal-Middle	Coronal	6	4.75	28.5	7.5	28.5	-1.896	0.058
			Middle	6	8.25	49.5				
		Coronal-Apical	Coronal	6	4	24	3	24	-2.529	0.011*
			Apical	6	9	54				
		Middle-Apical	Middle	6	4.75	28.5	7.5	28.5	-2.021	0.043*
			Apical	6	8.25	49.5				
	NaOCl	Coronal-Middle	Coronal	6	6	36	15	36	-0.638	0.523
			Middle	6	7	42				
		Coronal-Apical	Coronal	6	3.83	23	2	23	-2.768	0.006*
			Apical	6	9.17	55				
		Middle-Apical	Middle	6	4.17	25	4	25	-2.447	0.014*
			Apical	6	8.83	53				
	Salvadora P	Coronal-Middle	Coronal	6	8	48	9	30	-1.682	0.093*
			Middle	6	5	30				
		Coronal-Apical	Coronal	6	3.5	21	0	21	-2.983	0.003*
			Apical	6	9.5	57				
		Middle-Apical	Middle	6	3.5	21	0	21	-3.035	0.002*
			Apical	6	9.5	57				
Er,Cr:YSGG	Saline	Coronal-Middle	Coronal	6	6.5	39	18	39	0	1
			Middle	6	6.5	39				
		Coronal-Apical	Coronal	6	3.67	22	1	22	-2.802	0.005*
			Apical	6	9.33	56				
		Middle-Apical	Middle	6	3.67	22	1	22	-2.802	0.005*
			Apical	6	9.33	65				
	NaOCl	Coronal-Middle	Coronal	6	5.83	35	14	35	-0.73	0.465
			Middle	6	7.17	43				
		Coronal-Apical	Coronal	6	4.83	29	8	29	-1.716	0.086
			Apical	6	8.17	49				
		Middle-Apical	Middle	6	5.08	30.5	9.5	30.5	-1.419	0.156
			Apical	6	7.92	47.5				
	Salvadora P	Coronal-Middle	Coronal	6	6.5	39	18	39	0	1
			Middle	6	6.5	39				
		Coronal-Apical	Coronal	6	4	24	3	24	-2.702	0.007*
			Apical	6	9	54				
		Middle-Apical	Middle	6	4	24	3	24	-2.702	0.007*
			Apical	6	9	54				
			Apical	6	9.17	55				

(*) values had statistically significant difference at (P<0.05).

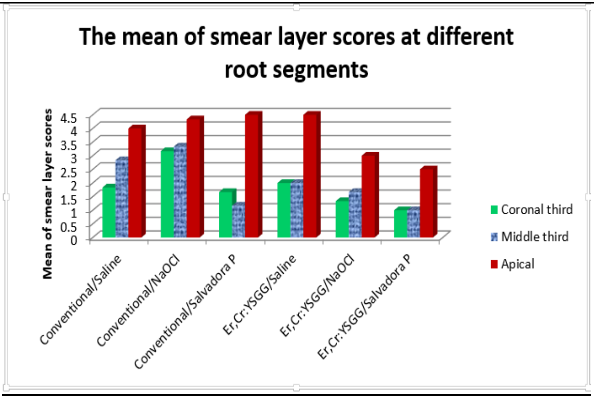

Figure (4): The mean of smear layer scores in root canal segments; coronal, middle, and apical thirds.

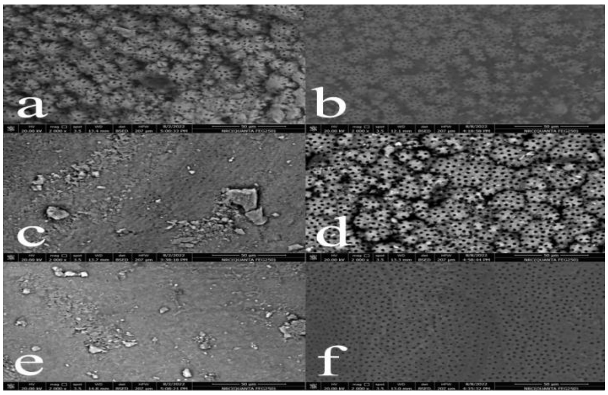

Figure (5): SEM pictures of using conventionally (group I) and Er,Cr:YSGG–laser-activated saline (group II) techniques showed that there was no statistical differences between the two groups; group I **(a)** and group II **(b)** when using at coronal third. The picture exhibited that there were statistical differences between the two groups; group I **(c)** and group II **(d)** when using NaOCl at middle third. Also, there were statistical differences between the two groups; group I **(e)** and group II**(f)** when using salvadora P at apical third.

All available data are submitted with the results section of the main script.

Discussion

Root canal mechanical instrumentation utilizing hand or engine-driven endodontic files always creates an unavoidable thin layer covering the prepared root dentin and occluding the cut dentinal tubules known as smear layer **(Bhagwat S. et al., 2016)**. Microscopic dentin chips, dental pulpal soft tissue residue, biological cellular relics can be found in smear layer **(Bhagwat S. et al., 2016)**. Remove of smear layer after applying the endodontic files is mandatory for accomplishing successful endodontic treatment **(Bhagwat S. et al., 2016)**. In fact, ineffective cleaning of root canal walls dramatically reduces dentin permeability **(Bolhari B. et al., 2014; Bhagwat S. et al., 2016)**, sealing ability and adaption of root canal gutta percha and sealer **(Bolhari B. et al., 2014)**, and diffusion of intracanal medicaments **(Bhagwat S. et al., 2016)**. Therefore, various root canal irrigating solutions and techniques have been developed and assessed for getting rid of smear layer to maximize the root canal wall cleaning and disinfection **(Bolhari B et al., 2014; Bhagwat S. et al., 2016)**.

Since the smear layer comprises two different forms of matter; organic and inorganic, utilizing of both organic and inorganic solvents *such as: NaOCl and EDTA* is carried out for total eliminating of smear layer **(Bhagwat S. et al., 2016)**. However, operating the alternate use of EDTA and NaOCl can prolong the duration of endodontic visit increasing the endodontist's fatigue. Furthermore, NaOCl can generate adverse biological reactions for example: heamolysis, cytotoxicity, and skin necrosis and ulceration **(Perotti S. et al., 2018)**. Thus, several researchers have been lately concerned with other root canal irrigating solutions **(Shravya, S. et al., 2020)**. Base on many in-vitro studies, using of solutions of herbal extracts such as *Salvadora persica or pilu (S. persica)* can be acted as promising intracanal irrgants due to their encouraging influences on biologic structures: Antibacterial, antioxidant, anti-cariogenic & chelation actions **(Shravya, S. et al., 2020)**. Also, they are more available, more cost effective, more limited for bacterial resistance and lower cytotoxcity than chemical ones **(Shravya, S. et al., 2020)**. At present, literature showed that employment of *Salvadora persica or pilu (S. persica)* solutions in endodontics have positive outcomes in the matter of smear layer removal, pulpal tissue dissolution, and antimicrobial activity **(Shravya, S. et al., 2020)**.

Chemical disinfection procedure of root canal system traditionally performed by means of conventional syringe/needle method. On the other hand, this method unable to achieve utmost root canal sterilization because of its limitation to efficiently displace the strongly attached dentin-cutting precipitations, bacteria, and pulpal soft tissue remnant from dentin walls of root canals particularly apical one third of root canal**(Amato M. et al., 2011; Rasheed S. and Jawad H., 2022)**. Hence, improving the fluid flow of intracanal irrigant by means of manual or engine-driven is obligatory **(Krongbaramee T. et al., 2020)**.

As photon energy of laser irradiation had proved its competence to effectively agitate the root canal irrigating solutions, many infrared laser wavelengths were examined to be a proper tool for optimizing root canal cleaning and disinfecting procedure **(Olivi G. and Olivi M. 2016)**. During laser-activated irrigation, laser beam should be operated in proper parameters and accurately applied on specific intracanal irrigant depending on evidence-based protocols. Incorrect laser beam settings and technique can induce higher thermal laser-tissue interaction causing reversible injuries to periradicular biological structures **(Olivi G. and Olivi M. 2016)**.

Currently, the wavelengths of mid-infrared Erbium family (2780 nm, Er,Cr:YSSG and 2940 nm, Er:YAG lasers) are the most suitable laser irradiation wavelengths can be utilized for activating the water-based intracanal irrigant due to their specific affinity to water **(Olivi G. and De Moor R., 2016)**.

Er,Cr:YSSG-laser-activated irrigation generates shockwave-like impact on inrtracanalirrigant using conventional flat or more recent radial firing tip (RFT) by means of laser/irrigant photomecnanical interaction **(Jezeršek M. et al., 2020)**. Contrary to flat tips, radial firing tips can permit some lateral laser emission which can laterally irradiate the few areas of root surfaces and more surrounding intracanal irrigating fluid solution **(Olivi G. and Olivi M. 2016)**.

Actually, few studies had been carried out to evaluate the solution of *S. persica* extract regarding its ability to eliminate the smear layer. Also, there are no studies have investigated the conventional laser-assisted irrigation method using Er,Cr:YSGG-laser with a radial-firing tip on smear layer removing-capacity of the *S. persica* extract irrigating solution. Consequently, this study aimed to microscopically assess the agitation of *S. persica* extract and sodium hypochlorite final irrigating solutions with Er, Cr:YSGG-laser using radial-firing tip on smear layer removal and dentinal tubules opening by scanning electron microscope (SEM). The null hypothesis was that there are no differences in smear layer removal with different intracanal irrigating solutions used.

Selecting of human single-canal, single-root anterior dentitions had no or minimal root curvature (less than 20°) was done for achieving high standardization of the study specimens. Additionally, the preparation of the conventionally accessed root canal continued up to size# x4 Protaper Next file to guarantee the research work standardization. Furthermore, other variable factors; the volume of root-canal irrigating solution and total irrigation time were standardized for each root canal examined in this study; 5 ml and 60 sec, respectively **(Alakshar A. et al., 2020)**.

Selecting teeth with straight or minimal curved (less than 20°) root canal allow introducing of the needle tip as well as laser delivery tip into root canal to full application length without facing complexities or difficulties **(Alakshar A. et al., 2020)**.

Teeth were cleaned & shaped with rotary files till size# x4 to achieve suitable root canal preparation allowing a good enough flushing and diffusion of the examined root canal irrigating solutions with no risk for occurrence apical overpreparation and other intracanal iatrogenic errors **(Haupt F. et al., 2020)**.

Each NiTi file was used for three canals only to avoid bluntness of cutting edges of the file and occurrence of microcrack defects on its surface **(Elemam R. et al., 2016)**.

Subablative photon energy levels of Er, Cr:YSGG laser beam were selected in accordance to **(Al-Mafrachi R., 2019)** for this study. Subablative Er,Cr:YSGG laser should be applied during laser-activated irrigation technique to avoid possibly reversible periiradicular damage which may happen as a result of unwanted higher thermal laser/irrigant interaction **(Keskin G. and Çiloğlu M. et al., 2021)**.

The photon energy was delivered in contact mode with the intacanal irrigant through 415-μm-diameter, 21-mm-length radial firing tip (RFT3-21) to laterally irradiate the intracanal irrigants and root dentin because the laser beam is refracted out from this tip at a highly divergent angle **(Verdaasdonk R., 2021)**.

SEM evaluating technique was recruited to powerfully assess the smear layer precipitated on dentin root and the presence of dentinal tubules' opening at coronal, middle, & apical one-thirds of root canal even though its preparation considers a destructive approach to specimens **(Alakshar A. et al., 2020)**.

The sectioning of teeth was achieved by low-speed precision cutting saw for minimizing the specimen's deformity during its preparation for SEM.

A qualified practioner microscopically photographed the root canal segments at a magnification of x2000 which is a suitable power to assess and capture smear layer scores of root dentin **(Sukhbir K. et al., 2019; Kharouf N. et al. 2020; Sharma S. et al., 2020**; **Alakshar A. et al., 2020; Mohamed N. et al., 2022)**. Two well-trained qualified evaluators independently assessed the SEM images based on Hűlsmann-score system for strengthening the findings' reliability and evading the human bias **(Alakshar A. et al., 2020)**.

Based on the obtained results from this study, thesignificant differences between conventional syringe/needle (group I) and Er,Cr,YSGG-laser-activated (group II, subgroup B) irrigation techniques are denoted that the efficiency of Er,Cr:YSGG-laser-activated technique was better than the conventional syringe/needle technique for eliminating the smear layer from the entire root dentin. The finding of Er,Cr:YSGG-laser-activated technique accomplishes superior smear layer cleaning results compared to conventional syringe/needle technique were reported in several previous studies *such as***(George R. et al., 2008; DiVito E. et al., 2012; Sabari M., 2012; Murugesan M. et al., 2013; Madhusudhana K. et al., 2016; Wang X. et al. 2017; Montero-Miralles P. et al., 2018**; **Ozbay Y. and Erdemir A., 2018; Al-baker H. et al., 2021)**

These findings may be ascribed to that the physical effect of using pulsed Er,Cr:YSSG on intracanal irrigant. **(van der Sluis L. et al., 2016)** When the incident photon energy of Er,Cr:YSGG laser is absorbed by root canal irrigant solution during laser-activated irrigation technique, the water molecule are increasingly heated-up and exploded creating large vapor bubble at the end of the inserted optical fiber of the delivery laser system. **(van der Sluis L. et al., 2016)** Then, the generated vapor bubble in irrigant solution is collapsed inducing pulling the irrigant fluid towards the bubble center allowing better irrigant flow inside the root canal and more agitation **(van der Sluis L. et al., 2016; Nagahashi T. et al., 2022)**. Moreover, once collapsing of vapor bubble is completed, shock waves (numerous small cavitation bubbles) occur. **(Nagahashi T. et al., 2022)**. Cavitation effect of laser increases the shearing stresses on root dentin and enhances the cleaning effect of the laser-activated root canal irrigant. **(Nagahashi T. et al., 2022)**.

In contrast to salvadora P and NaOCl intracanal irrigants results, Er,Cr:YSGG laser-activated saline irrigant solution (group II, subgroup A) recorded higher mean values of smear layer score compared to those conventionally activated by syringe/needle technique (group I, subgroup A) at coronal and apical root thirds. This may be related to insufficient laser energy absorbed by saline irrigant at coronal and apical two-thirds. At middle one-third laser-activated saline irrigant solutions showed better smear layer eliminating capacity than conventionally activated ones.

Regarding the results of the examined intracanal irrigants at root apical region, Er,Cr:YSGG laser-activated irrigants (group II, subgroup A and B) showed more potent cleaning effect on dentinal walls of root canal proving that radial firing tip was properly researched the apical one-third and being in contact with the more intracanal irrigant permitting additional laser energy absorption, further laser cavitation and better smear layer elimination. These results are coincided with **(George R. et al., 2008;DiVito E. et al., 2012; Murugesan M. Sabari, et al., 2013)**

On the other hand, SEM evaluation of this study revealed that using of Er,Cr:YSGG subablative parameters were positively influenced the smear layer cleaning efficacy of the root canal irrigant associated with no thermal collateral damage **(George R. et al., 2008; DiVito E. et al., 2012; Sabari M., 2012; Murugesan M. et al., 2013; Madhusudhana K. et al., 2016; Wang X. et al. 2017; Montero-Miralles P. et al., 2018)** or unwanted removal of the dentin root **(Montero-Miralles P. et al., 2018)**.

Concerning the effect of root segment on smear layer eliminating capacity of the intracanal irrigants, the mean value of smear layer score generally increased towards the root apex when using the same activation method; conventional syringe/needle (group I) or Er,Cr:YSGG-laser-activated irrigation techniques (group II) for the same intracanal irrigant; saline (subgroup A), NaOCl (subgroup B) & salvadora P (subgroup C). There were statistical differences between the smear layer scores recorded at different root segments for each intracanal irrigants except for laser-activated NaOCl irrigant (group II, subgroup B). This is indicated that the smear layer removal efficacy by activated intractacanal irrigant gradually reduced in apical direction. This may be attributed to insufficient root canal irrigant that had been in contact with root dentin increases as the distance between root canal region and the root canal orifice is far particularly in apical one-third. Moreover, the complexity of apical one-third of root canal anatomy **(Montero-Miralles P. et al., 2018**; **Rasheed S. and Jawad H., 2022)** and its greater extent of tubular sclerosis **(Ayranci L. et al., 2016)** can negatively affect the smear layer removal potency of root canal irrigants. These results are in accordance with those of previous studies **(Nakamura V. et al., 2013; Zand V. et al., 2014; Ozbay Y. and Erdemir A., 2018; Hora B. et al., 2021)**

However, the study findings showed that the mean value of smear layer score recorded at middle one-third of root canal was lower than that measured at coronal one-third after using salvadora P as an intracanal irrigant activated by conventional method (group I, subgroup C). Furthermore, the mean value of smear layer score of the same intracanal irrigant; salvadora P activated by Er,Cr:YSGG laser (group II, subgroup C) at coronal and middle two thirds was identical.This may be due to salvadora P irrigant made a higher or same contact with root dentin of middle one-third in comparison to that of coronal one-third.

Regarding the results of the examined intracanal irrigants measured at different root segments; coronal, middle and apical root thirds, salvadora P root canal irrigant recorded lesser smear layer score mean than other irrigants at coronal and middle one-third whether it had been conventionally activated (group I, subgroup C) or activated by Er,Cr:YSGG laser (group II, subgroup C). The statistical differences between the smear layer scores recorded between the conventionally and laser-activated root canal irrigants at the same root segments means that salvadora P is more efficient for removing smear layer than NaOCl and saline from coronal and middle two-thirds' walls of root canal. Conversely, the recorded mean of smear layer score of conventionally activated salvadora P (group I, subgroup C) at apical one-third was the highest between all evaluated subgroups (group I, subgroup A and B) with no statistical difference was occurred between them. This signifies that smear layer removing-capacity of conventionally activated salvadora P at the apical root dentin can be less powerful than the other examined irrigants; NaOCl& saline. In contrast, Er,Cr:YSGG-laser-activated salvadora P root canal irrigant recorded lower smear layer score mean than other laser-activated irrigants at apical root region. This implies that smear layer removing-capacity of active salvadora P at the apical root dentin was the best compared to other examined irrigants and recommends that salvadora P solution is the intracanal irrigant of choice in case Er,Cr:YSGG-laser-activated irrigation technique will be applied.

Conclusions

Under the conditions of this study, employing Er,Cr:YSGG pulsed laser (25 mJ, 50 Hz, air and water spray off) at short pulse duration (60 µsfor activating the intracanal irrigants to remove root canal smear layer was more valuable than using conventional syringe/needle activation technique. Salvadora P intracanal irrigant was the most effectual in removing smear layer from the entire root canal wall particularly when it was activated by Er,Cr:YSGG laser. Hence, this may be considered a helpful protocol for elimination smear layer during a root canal therapy.

Conflict of interest:

The author declares no potential conflicts of interest

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