Original Article

Development of a High Penetration Safe Irrigant from *Withania somnifera*

Abstract

Background: Herbal medicine is regaining a strong position in health care by virtue of better safety and minimal toxicity as compared to conventional chemotherapies. It is making a place in dental care in the form of various toothpastes, lotions, and mouthwashes. It has been analyzed that very few discrete herbal irrigants have an action comparable to that of sodium hypochlorite. No data about any study on penetration depth and penetration area for herbal-based irrigants used in root canal treatment are available. **Aim:** The aim of this study was to assess the efficacy of *Withania somnifera* extracts by assessing its penetration depth and area inside dentinal tubules using a confocal laser scanning microscope. **Materials and Methods:** Freshly extracted 25 maxillary central incisors were divided into three groups: Group 1: *W. somnifera*; Group 2: sodium hypochlorite; and Group 3: distilled water. **Results:** *W. somnifera* showed better results with respect to penetration depth and area inside the dentinal tubules thansodium hypochlorite. **Conclusion:** From the study, it can be concluded that *W. somnifera* extract-based irrigant may be used as an alternative to sodium hypochlorite-based irrigant. It also shows that more herbal drugs need such types of screening and incorporation in dental practice to reduce the potential side effects of the conventional drugs used in daily practice.

Keywords: *Irrigants, sodium hypochlorite, Withania somnifera*

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Introduction

The paramount goal of an endodontic treatment is to restrain the build-out of apical periodontitis by eradicating the infected/or inflamed pulpal tissues and reinfection. The antibacterial effect of any irrigant is dependent on the degree of penetration depth and area covered by irrigant. Despite the realization that endodontic infections are biofilm mediated, clinical procedures have been focused more on the mechanical preparation of the root canals rather than the chemical debridement of the root canal anatomy.[1] Hence, to increase the effectiveness of root canal disinfection procedures, a combination of mechanical debridement with antibacterial irrigants has been recommended.[2] Hence, various chemical-based root canal irrigants like sodium hypochlorite as the gold standard for irrigation, have been in use for a successful endodontic treatment because of its ability to dissolve organic matter and high antimicrobial potential. However, certain major drawbacks such as irritant to periapical tissues, stains instruments, unpleasant taste, high toxicity, corrosion of instruments, inability to remove the smear layer, burning of surrounding tissues, and reduction in elastic modulus and flexural strength of dentin are associated with the use of sodium hypochlorite.[3] Hence, the dental fraternity is in search of some herbal-based root canal irrigants that can give desired results without exercising the above given drawbacks. During the past decade, there has been a sudden rise in clinical trials using herbal irrigants due to their high safety profile besides having physiologically beneficial active constituents that have curative, antioxidant, anti-inflammatory, and radical-scavenging properties.[4] Various natural plant extracts have been reported to exert antimicrobial properties and therapeutic effects, suggesting their potential to be used as an endodontic irrigant.[5]

For more than 5000 years, the complete plant or various portions of *Withania somnifera* have been employed in India's Ayurvedic and Unani medical systems for medicinal and therapeutic purposes, and the plant was reported as an official drug in the Indian Pharmacopoeia-1985 and a plant used in medicine from the time of Ayurveda.[6] It has

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been analyzed that very few discrete herbal irrigants have an action proportional to that of sodium hypochlorite. For this reason, in this study, 5% W/V extract of *W. somnifera* was evaluated on the basis of its penetration depth and area in the dentinal tubules at three levels – coronal, middle, and apical under confocal laser scanning microscope (CLSM) against 5.25% sodium hypochlorite being used as a positive control.

Materials and Methods

Specimen preparation

Twenty-five freshly extracted maxillary central incisors from 16- to 30-year-old patients were placed in normal saline solution. To ensure standardization, crown of each root was decoronated at Cemento-enamel junction (CEJ) using a double-sided diamond disc (0.15 mm × 22 mm). The apical portion of the root was resected to obtain a standardized length of 12 mm. In all 25 samples, the access preparation was done with high-speed sterilized round diamond bur (BR41, SS White bur) using water coolant. The root canals were subjected to the chemomechanical preparation using ProTaper instruments till F1 along with copious irrigation with 5% sodium hypochlorite and 17% Ethylenediamine-tetraacetic (EDTA) to remove the smear layer.

Preparation of *Withania somnifera*

Ripened plants of *W. somnifera* were obtained from the net house of the Department of Biotechnology, Guru Nanak Dev University, Amritsar. Ethanol extraction was used for further experiment. The roots and leaves of *W. somnifera* were gently washed with tap water 2–3 times, followed by a washing with distilled water and finally by ethanol wash and then conceded to dry for overnight at 50°C. The desiccated roots and leaves were completely milled to a coarse powder. For extract preparation, 50.0 g of dried plant materials (root and leaves) was extracted with 150 ml ethanol and sonicated at 25°C for 45 min. The supernatant was collected and the residue was further extracted twice (2 × 150 ml). These aliquots were then pooled and vacuum dried using vacuum-rotary evaporator. The dried residues were reconstituted in ethanol to get the final concentration of 5% (W/V).

Samples were randomly divided into three experimental groups – Group 1 – 5% *W. somnifera*, Group 2 – 5% sodium hypochlorite, and Group 3 – distilled water. The experimental irrigants were merged with 0.1% rhodamine B dye (Sigma-Aldrich, St. Louis, MO) as a fluorescence tracer for analysis under CLSM. The samples were irrigated with 15 ml of each experimental irrigant and the irrigant was delivered through the root canals until it appeared from the apex and up to the cervical line. Then, the access cavities were sealed with a temporary filling material (Cavit W; 3M ESPE, Seefeld, Germany), and the samples were incubated at 37°C for 24 h before analysis. The samples were sliced perpendicular to the long axis of the tooth at three levels of cervical, middle, and apical third of 1 mm thickness using hard-tissue microtome. All specimens were mounted onto glass slides and were studied under CLSM (LSM Pascal; Carl Zeiss, Jena, Germany) using the 543 nm wavelength of a helium laser under 2.5× (numerical aperture = 0.12.). Surface tension was also measured as per standard methods.

Results

The maximum penetration depth and the area were recorded in *W. somnifera* group at all three levels as compared to the gold standard of the endodontic irrigant sodium hypochlorite and distilled water [Table 1]. According to statistical analysis, the differences among all the irrigants at all three levels were highly significant for both penetration depth and area (at 0.1% probability level). The results demonstrated that the maximum penetration depth and area of 410.4 μm and 104.93 × 10^3 μm^2, respectively, were seen at the coronal level in Group 1 [Figure 1]. The penetration depth and area increased from the apical to the coronal third; the values recorded at the apical and middle third were statistically significantly lower ($P < 0.0001$) than those measured at the coronal level. Distilled water showed minimum penetration depth and area at the three levels compared to the other irrigants [Figure 1]. The penetration depth and area were highest at all the levels in the *W. somnifera* group when compared to sodium hypochlorite [Figure 1]. The surface tension of the extracts of *W. somnifera* was found to be 20.2 compared to sodium hypochlorite with the surface tension of 46.7 mJ/m^2.

Discussion

In recent times, there seems to be an accentuated increase in the branch of dentistry to opt for the use of herbal extracts for eliminating caries, dental plaque, and root canal irrigants. Herbal medicines such as *W. somnifera*, *tulsi*, garlic extract, Triphala, *Curcuma longa*, clove oil, neem (*Azadirachta indica*) extract, *Aloe vera*, *Terminalia chebula*, propolis, and *Salvia officinalis* have been tried by many researchers with relative efficacy.[7,8] It has also been reported that due to their high antimicrobial activity, biocompatibility, anti-inflammatory, and antioxidant properties, herbal or natural products are gaining popularity in today's era.[9] Several studies have described a safe, natural, and powerful antioxidant compounds in Ashwagandha[10,11] that elevate the levels of three naturally occurring antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase.[12] The roots of *W. somnifera* have withanolides, and a group of steroidal lactones, which attribute to its pharmacological effects that are believed to account for its extraordinary medicinal properties. Ashwagandha is also considered an adaptogen, facilitating the ability to withstand stressors, and has antioxidant properties as well. The crude ethanol root extract of *W. somnifera* holds excellent potential as an

Table 1: Mean values of penetration depth and mean values of penetration area in respect of different regions within each of three irrigants

Sample	Irrigant	*n*	Mean	SD	CV (%)	95% CL		99% CL	
						Lower	Upper	Lower	Upper
Mean values of penetration depth (µm)									
Coronal	*W. somnifera*	10	302.7	65.60	21.67	255.8	349.6	235.3	370.1
	Sodium hypochlorite	10	280.9	103.5	36.85	206.9	354.9	174.5	387.3
	Distilled water	5	84.70	9.60	11.33	72.8	96.6	64.9	104.5
Middle	*W. somnifera*	10	193.2	35.50	18.37	167.8	218.6	156.7	229.7
	Sodium hypochlorite	10	135.8	34.90	25.70	110.8	160.8	99.9	171.7
	Distilled water	5	49.80	6.60	13.25	41.6	58.0	36.2	63.4
Apical	*W. somnifera*	10	130.2	40.90	31.41	100.9	159.5	88.2	172.2
	Sodium hypochlorite	10	89.90	12.70	14.13	80.8	99.0	76.8	103.0
	Distilled water	5	28.60	0.5	1.75	28.0	29.2	27.6	29.6
Mean values of penetration area ($\times 10^3$ micro m^2)									
Coronal	*W. somnifera*	10	85.19	13.40	15.73	75.60	94.78	71.42	98.96
	Sodium hypochlorite	10	72.16	11.23	15.56	64.13	80.19	60.62	83.7
	Distilled water	5	42.21	2.43	5.76	39.19	45.23	37.21	47.21
Middle	*W. somnifera*	10	44.03	23.55	53.49	27.18	60.88	19.83	68.23
	Sodium hypochlorite	10	29.97	7.13	23.79	24.87	35.07	22.64	37.3
	Distilled water	5	22.01	18.09	82.19	−0.45#	44.47	−15.24	59.26
Apical	*W. somnifera*	10	15.85	5.46	34.45	11.94	19.76	10.24	21.46
	Sodium hypochlorite	10	14.63	7.27	49.69	9.43	19.83	7.16	22.1
	Distilled water	5	13.64	4.05	29.69	8.61	18.67	5.3	21.98

W. somnifera: Withania somnifera; n: Number of observations; SD: Standard deviation; CL: Confidence limits; CV: Coefficient of variation

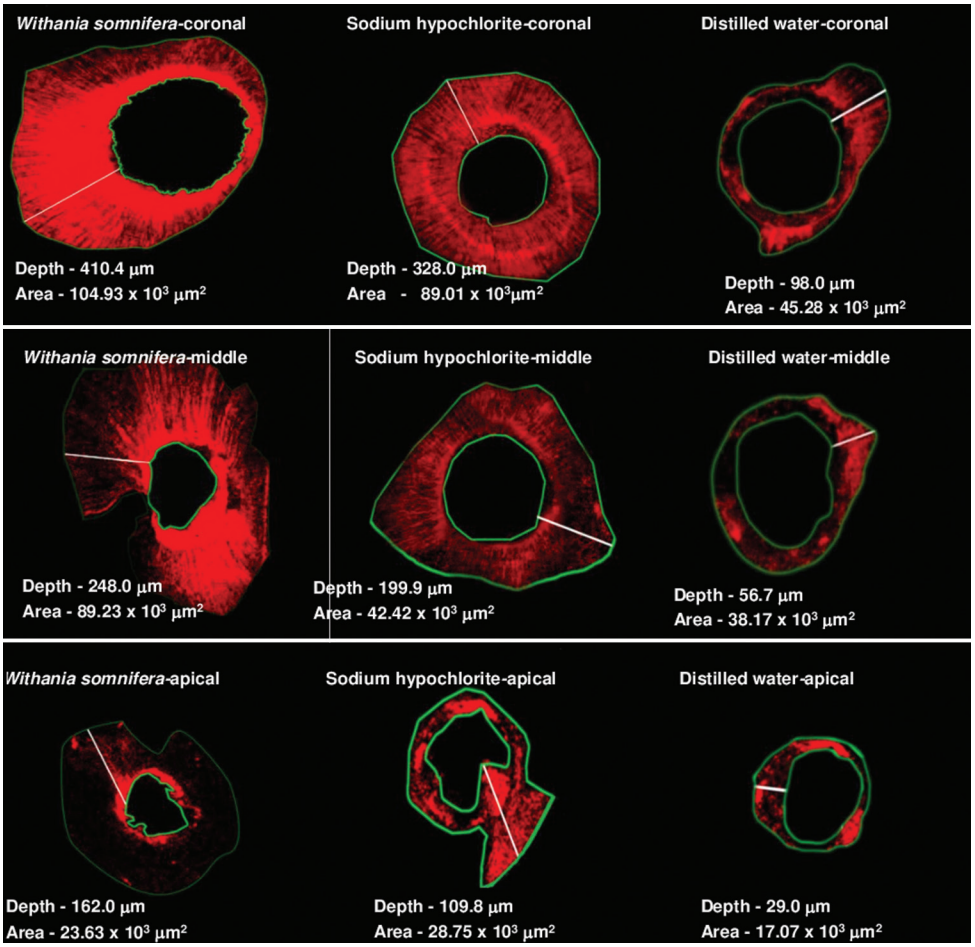

Figure 1: Confocal images showing coronal, middle, and apical sections of penetration depth and area of *Withania somnifera*; sodium hypochlorite and distilled water

antibacterial agent and it was analyzed that the ethanolic extract was more effective in inhibiting pathogens as compared to chloroform and aqueous root extracts by agar diffusion method using respective solvents as a negative control.[13]

In the present work, the irrigants were evaluated on the basis of penetration depth and area in the dentinal tubules under confocal laser microscope using rhodamine B dye. It has already been ruled out that the penetration depth and area are not affected by the use of rhodamine dye.[14] In this present study, the penetration depth and area for sodium hypochlorite varied significantly between coronal and apical third. These results are in accordance with the study.[15] At the coronal and middle third, *W. somnifera* showed a highly significant difference (at 0.1% probability level) compared to sodium hypochlorite and distilled water, whereas at apical third, all the paired comparisons were statistically nonsignificant. *W. somnifera* showed better penetrability into the dentinal tubules as compared to the synthetic irrigant, i.e., sodium hypochlorite. *W. somnifera* also demonstrated good antimicrobial activity against *E. faecalis* and has been shown to be effective against microorganisms and reported to possess anti-inflammatory properties, thereby preventing protein denaturation.[16] A better penetration depth and area of the *W. somnifera* extracts can be due to the very low surface tension of the extract solution as compared to the surface tension of the sodium hypochlorite solution, as observed in the present study.

Conclusion

The aim of this study was to analyze the efficacy of *W. somnifera* extract by comparing its penetration depth and area into the dentinal tubules against gold standard sodium hypochlorite. As low surface tension is of primary importance in the penetration of endodontic irrigants into inaccessible areas and the results of the present study also established that *W. somnifera* extract showed better results than sodium hypochlorite in reaching higher penetration depth and area. Hence, herbal drugs must be incorporated into dental practice to reduce the potential side effects of conventional drugs used in daily practice.

Ethical statement

The study was approved by the institutional Ethical Committee, Letter No- (2194/IDSR/2017 dated 18/11/2017). Sri guru ram das institute of dental sciences and research, Amritsar.

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Conflicts of interest

There are no conflicts of interest.

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