Original Article

Evaluation of pH and Chlorine Content of a Novel Herbal Sodium Hypochlorite for Root Canal Disinfection: An Experimental *In vitro* Study

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Abstract

Introduction: Sodium hypochlorite (NaOCl) is widely used endodontic irrigant with its array of advantages and disadvantages. Addition of herbal agents to NaOCl might help in enhancing its efficacy. **Materials and Methods:** NaOCl was prepared using chlorinated lime, anhydrous sodium carbonate, and distilled water to obtain 6% fresh solution. Then, this solution was evaluated for its pH and chlorine content. The fresh herbal extract of *Cymbopogon citratus* (lemongrass), *Mentha piperita* (peppermint), and *Ocimum sanctum* (tulsi) was prepared from plant leaves. After which, various permutation and combinations were made, and it was found that 9:1 ratio was the most effective combination. The pH and percentage of free chlorine were evaluated and analyzed. **Results:** No statistical significant difference was found in pH content. However, significant difference was found between the groups for chlorine content ($P < 0.05$) of herbal NaOCl. **Conclusion:** The current study presents a simplified method for preparation of herbal irrigating solution.

Keywords: *Chlorine content, Cymbopogon citratus, herbal irrigants, Mentha piperita, Ocimum sanctum, pH, sodium hypochlorite*

Introduction

Irrigating solutions aims toward elimination of microorganisms, debris, and tissue remnants from the complex root canal system.[1] Numerous endodontic irrigants have been proposed for the clinical use,[2] of which sodium hypochlorite (NaOCl) is one of the most preferred irrigants[3] because of its excellent nonspecific proteolytic and antimicrobial activity.[4] However, none of the irrigating solutions is regarded as optimal.[5] NaOCl is available from two main sources, pharmaceutical supplies or commercially available household bleaches, of which latter source is most commonly used.[6] It acts by chloramination, amino acid neutralization, and saponification reaction that leads to antimicrobial effect and tissue dissolution process.[7]

Although NaOCl has many favorable properties, it lacks chemical stability. This inherent instability of NaOCl solutions is because of light, air, change in pH, and organic and inorganic contaminants.[8] Considering this instability, ineffectiveness, and shelf life of NaOCl, it was investigated by numerous researchers.[9-11] Studies have found that NaOCl remained stable for 23 months[9] and 10 weeks[12] at a different concentration of <1% and 5.25%, respectively. However, the current literature states that for effective root canal disinfection and antimicrobial efficacy, fresh NaOCl solution shall be prepared.

Further, it was also recommended to store NaOCl in amber color glass bottle. The instability of NaOCl is because of decomposition of hypochlorite ions to chlorate and chlorine ions. The decomposition is dependent on pH and concentration of hypochlorite ion. The NaOCl solution is most stable at pH 11 and above. As the pH decreases from 11 to 7, decomposition also increases and is highest at pH 7. A study concluded that <6% available chlorine, at pH of 11 or higher, should have an acceptable shelf life when stored at <30°C temperature.[13] Thus, the optimum pH and chlorine content of NaOCl solution are very important for its effectiveness.

The desirable properties of NaOCl are dissolution of pulpal tissue and removal of smear layer and provide bactericidal/bacteriostatic effect.[14] Nevertheless, the undesirable properties are

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tissue toxicity, allergic potential, eye damage, and inability to remove smear layer and do not eradicate microorganisms from the infected root canals, risk of emphysema on overfilling, discoloration of clothes, and disagreeable smell and taste.[15-17] Considering these undesirable properties, safety concerns and ineffectiveness, newer herbal endodontic irrigants would be an alternative. The advantages of using herbs as antimicrobials agents are minimal side effects, cost-effective, better patient tolerance and lastly are renewable in nature.[18] Incorporation of herbal agents in NaOCl might help in reducing its undesirable properties. The agents, such as perfumes, surfactants, and thickening agents, may be added in a freshly prepared NaOCl solution with pH 12–14.[6]

Cymbopogon citratus (CC; lemongrass) is a tropical plant present in South and Southeast Asia. The leaves and essential oil can be used for medicinal purpose. It acts as a stimulant, carminative, depressant, analgesic, cytoprotective, anti-inflammatory, antipyretic, antibacterial, and antifungal agent.[19] It was found that CC has potent amount of anticandidal activity when compared to chlorhexidine gluconate.[20] It shows antimicrobial activity against standard and clinical strains of streptococci[21] and is attributed to the presence of citral compound that inhibits biofilms formation.[22]

Mentha piperita (MP; peppermint) leaves, leaf extract, oil, and leaf water can be used for relieving irritation and inflammation.[23] It also showed antibacterial effect by inhibiting the proliferation of Staphylococci,[24] antifungal action against Candida species, and an antibiofilm property.[25]

Ocimum sanctum (OS; tulsi) is used as a medicinal herb since ancient times and has wide range of properties such as anti-inflammatory, analgesic, antipyretic, antiulcer, antidiabetic, antioxidant, and anticancer activity.[26] The antimicrobial activity is attributed the presence of ursolic acid and carvacrol.[27] It is effective against *Escherichia coli*, *Klebsiella*, *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Proteus*.[28,29]

Considering the above-mentioned properties of herbal agents, this experimental *in vitro* study aims to prepare herbal NaOCl solution using MP, OS, and CC without altering the pH and chlorine content.

Materials and Methods

Preparation of fresh solution 6% sodium hypochlorite

About 24 gm of chlorinated lime (KR chemicals, India) and 17 gm of anhydrous sodium carbonate (Merck Specialities Pvt. Ltd., Mumbai, India) were mixed in 100 ml distal water to obtain fresh 6% NaOCl solution. This solution was centrifuged at 5000 rpm for 5 min and filtered using Whatman paper no. 0. This filtered solution was evaluated for its chlorine content and pH using iodometric titration method and digital pH meter (Eutech Instruments, pH meter, pH 1500, CyberScan), respectively. The percentage of free chlorine content and pH was analyzed for 20 such freshly prepared samples (Group 1; control).

Preparation of herbal extracts

The fresh aerial parts of MP (peppermint; Group 2), OS (tulsi; Group 3), and LC (lemongrass; Group 4) were collected from the local forest area (Nagpur, Maharashtra, India). About 100 gm of each plant leaves was washed and cut into small pieces. They were immersed in 1000 ml Milli-Q water (BioAGE Direct Ultra, Punjab, India) at 80°C for about 1 h with constant stirring. This is known as decoction method or hot water extraction by heating. The obtained solution was allowed to cooldown and filtered by gauze. The filtered solution was concentrated *in vacuo* (25°C, SONAR Buchitype) to get the 10% (w/v) extracts.

Preparation of herbal solution

The herbal NaOCl was prepared by adding herbal extracts to 6% NaOCl. Various permutation and combinations were made such that it does not affect the basic properties. It was observed that 9:1 ratio (9 ml of NaOCl and 1 ml of herbal extracts) was found to be effective combination. Twenty such samples were prepared by adding herbal extracts; MP (peppermint; Group 2), OS (tulsi; Group 3), and CC (lemongrass; Group 4). The pH and percentage of chlorine were analyzed for 20 such samples for each group (Groups 2, 3, and 4).

Results

The data were descriptively analyzed using SPSS for windows (Microsoft, Chicago, IL, USA). The mean and standard deviation for chlorine content and pH were calculated [Table 1]. A significant result was seen in between the groups with the chlorine content of herbal NaOCl ($P = 0.001$) [Table 2]. The intergroup comparison for chlorine content was compared by Bonferroni test, and the result showed that statistically significant difference was found between Group 1 and Group 4 ($P = 0.013$), Group 2 and Group 3 ($P = 0.026$), and Group 3 and Group 4 ($P = 0.002$) [Table 3].

Discussion

Commercially, NaOCl is produced by passing chlorine gas through sodium hydroxide solution.[30] However, currently, it is manufactured by electrolysis of sodium chloride solution.[6] Although it is available in pharmaceutical and commercial (household) preparation, its use is dependent on handling and packaging of the product. It is used, if safe in contact with vital tissues.[6] Being a strong oxidizing and hydrolyzing agent, it has bactericidal and proteolytic activity; however, it is highly toxic to living tissue except keratinized epithelia.[12] This toxicity is obviated by

confining the NaOCl solution to the pulp chamber and root canal using rubber dam and appropriate irrigating techniques. Nevertheless, considering advantages of NaOCl as an endodontic irrigant, this is the first study to evaluate the pH and chlorine content after addition of herbal extract to NaOCl producing herbal NaOCl.

The herbal extracts were prepared using decoction method to acquire a concentration of 10% (W/V). Various permutations and combinations were made to achieve a successful ratio of NaOCl solution and extracts such that it does not alter pH and chlorine content. In this study, 6% NaOCl solution was prepared as it was stated that among various test solutions, this percentage was able to render bacteria nonviable and physically eliminates biofilms.[31]

In this study, the percentage of free chlorine was determined using iodometric chemical analysis. This is a manual sensitive method and employee's colorimetric technique with many dilutions required to reach the titration itself; however, it comes as sensible, simple, and most widely used no disposal and toxicity issues.[32]

In the current study, pH and chlorine content of the herbal NaOCl solution were evaluated as it interferes with the cell metabolism and decomposition of hypochlorite ions, respectively. This presence of chlorate and chlorine ions that are decomposed from hypochlorite ions is pH dependent. In this study, the mean pH and percentage of chlorine content were in the range of 5.9–6.1 and 11.9–12.07, respectively [Table 1]. The above-mentioned findings suggest that the solutions have acceptable shelf life.[6] These findings were in consonance with other study that stated that the solutions with <6% available chlorine with a pH 11 or higher have an acceptable shelf life.[13] The results of this study stated that the pH and chlorine content of herbal NaOCl have approximately similar value to that of control group (Group 1). Nevertheless, it is stated that the bactericidal activity is greater at acidic pH (<6) and shelf life is greater at alkaline pH (>11).[8,33] The decomposition rate of NaOCl is pH dependent that increases rapidly from pH 11–7. The result showed that there is no change in pH; however, statistical significant difference was found with the percentage of free chlorine in herbal NaOCl [Table 2]. These findings were in consonance with the study by Rosen and Zhu, where no significant decrease in pH of NaOCl was found with different surfactants (mint, lemon, osmanthus flowers, pineapple, and vanilla essence).[34]

Multiple comparisons were done between the groups using Bonferroni test with mean difference for chlorine content [Table 3]. When Group 1 (control) was compared with Groups 2, 3, and 4, statistical significant difference was found between control and Group 4 (P = 0.013). However, when Group 2 was compared with Groups 3 and 4, statistical significant difference was found between Groups 2 and 3 (P = 0.026). Similarly, statistically

Table 1: Descriptive analysis

Descriptive analysis	*n*	Mean	SD	SE
pH				
1 (control)	20	12.0515	0.22335	0.04994
2	20	12.0790	0.09862	0.02205
3	20	12.0380	0.05634	0.01260
4	20	11.9855	0.23157	0.05178
Chlorine content				
1 (control)	20	6.1500	0.36635	0.08192
2	20	5.9775	0.09872	0.02207
3	20	6.1950	0.24954	0.05580
4	20	5.9145	0.11404	0.02550

Group 1: pH and chlorine content for freshly prepared sodium hypochlorite; Group 2: pH and chlorine content for NaOCl + *Mentha piperita*; Group 3: pH and chlorine content for NaOCl + *Ocimum sanctum*; Group 4: pH and chlorine content for NaOCl + *Cymbopogon citratus*. SE: Standard error; SD: Standard deviation

Table 2: Two-way ANOVA test was applied

	ANOVA				
	Sum of squares	df	Mean square	Fisher test	*P*
pH					
Between groups	0.092	3	0.031	1.058	0.372
Within groups	2.212	76	0.029		
Total	2.304	79			
Chlorine content					
Between groups	1.086	3	0.362	6.605	0.001
Within groups	4.165	76	0.055		
Total	5.251	79			

Table 3: Multiple comparison using Bonferroni test

Multiple comparisons (Bonferroni)					
Dependent variable	Group (I)	Group (J)	Mean difference (I−J)	SE	*P*
Chlorine content	1 (control)	2	0.17250	0.07403	0.135
		3	−0.04500	0.07403	1.000
		4	0.23550*	0.07403	0.013
	2	3	−0.21750*	0.07403	0.026
		4	0.06300	0.07403	1.000
	3	4	0.28050*	0.07403	0.002

*The mean difference is signifi cant at the 0.05 level. SE: Standard error.

significant difference was found between Groups 3 and 4 (P = 0.002). Thus, with the above-mentioned results, we can state that chlorine content was approximately similar for Group 1 (control) and Group 3.

The herbal agents used in the present study were found to be effective against endodontic pathogens. Mathew *et al*. found that the MP along with other herbal agents (EndoPam) was effective against *E. faecalis* for endodontic irrigation when compared with 5.25% NaOCl and 2% chlorhexidine.[35] Other study showed that the herbal extracts (neem, OS) had significant inhibitory effects against *E. faecalis* as compared to 2% chlorhexidine gluconate.[36] Another study concluded that CC has potent anticandidal activity compared to chlorhexidine gluconate.[20] It also suppresses the activity of *Streptococcus mutans* with no cytotoxicity.[37] Thus, addition of herbal extract in NaOCl would further enhance its effectiveness against endodontic pathogens. Hence, these can be used alternatively as endodontic irrigants/medicaments.

Conclusion

Within the limitations of this study, it can be concluded that the herbal NaOCl has effective chlorine content and pH when compared to the control. Preparation of fresh herbal NaOCl can be implemented in institutional setups and clinical practice to attain more success in endodontic therapy. Further studies are required for evaluating the antibacterial efficacy and properties of herbal NaOCl.

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Conflicts of interest

There are no conflicts of interest.

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