

Comparing the Effect of Herbal Mouthwash Made from *pistacia atlantica kurdica* with Chlorhexidine Mouthwash on Gingival Healing

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Abstract

Pistacia atlantica kurdica, belonging to the Anacardiaceae family, is mainly observed in the mountains of Western Iran. Given the importance of research and the critical role in improving patient health, this study was conducted to compare the effect of an herbal mouthwash derived from wild pistachio fruit with chlorhexidine (CHX) mouthwash on gingival healing following crown lengthening surgery. The present research was a clinical trial in which the statistical population of patients was randomly divided into five groups ($n = 10$) using chlorhexidine mouthwash, herbal mouthwash at concentrations of 1%, 5%, and 10%, and a control group receiving normal saline (NS). The shade-dried fruits were thoroughly ground using a laboratory mill. The resulting dried fruit powder was extracted using the Soxhlet extraction method at the Biotechnology and Medicinal Plants Research Center. For this study, an aqueous-alcoholic extract of the wild pistachio fruit was prepared according to previous studies. The extract was completely dried using a rotary evaporator apparatus at the research center, ensuring complete removal of the solvent. Using the prepared dry extract, the herbal mouthwashes of the wild pistachio fruit were prepared at concentrations of 1%, 5%, and 10%. Fifty individuals were included in this study. Regarding the Parotid Gland Swelling Index ($p = 0.225$) and the Modified Gingival Index (MGI) ($P = 0.656$), the differences between the groups were not statistically significant. According to the Kruskal-Wallis test, the difference in inflammation before and after treatment for the MGI was significant in each of the four intervention groups, and the level of inflammation decreased post-treatment. Furthermore, the difference in plaque levels before and after the use of mouthwashes was significant in each of the five groups, showing a reduction. In alignment with the findings, the application of the *pistacia atlantica kurdica* mouthwash led to gingival healing after crown lengthening surgery. Therefore, the prescription of this agent is recommended.

Keywords: *Pistacia atlantica Subsp, Kurdica*, Chlorhexidine, Gingival Hemorrhage, Surgery

Introduction

The mouth harbors a high degree of microbial diversity. Among these microorganisms, *enterococci* are frequently observed in many primary root canal infections associated with chronic apical

periodontitis. The normal oral flora is predominantly composed of Gram-positive *streptococci*, along with various viruses and fungi, which remain relatively stable in a healthy individual over time. However, the oral flora of Intensive Care Unit (ICU) patients shifts toward aggressive Gram-negative

agents and *staphylococci* within 48 hours, accumulating in the oral cavity [1-4]. The gingiva is a component of the masticatory mucosa that covers the cervical area of the teeth and the alveolar processes of the maxilla and mandible, acting as a robust barrier against the penetration of irritants into the periodontal tissues. Gingival recession can be localized or generalized and typically increases with age. It results from a combination of etiological and predisposing factors, including iatrogenic causes, bone breakdown, and improper tooth positioning within the dental arch [5-7].

Certain dental treatments, such as subgingival tooth preparation, impression techniques involving gingival retraction, restorations and crowns placed below the gingival margin, and improperly designed dental prostheses, can induce gingival inflammation [8]. Currently, the treatment of periodontal diseases is moving towards antimicrobial control. Due to the increasing microbial resistance associated with the concurrent use of antibiotics, there is a growing necessity not only to reduce antibiotic-based treatments, but also to increase preventive measures. In fact, the mechanical removal of supra- and subgingival calculus remains the most effective method for preventing gingival inflammation [9,10]. The healing process necessitates preventing wound infection and bacterial contamination of the surgical site (including periodontal and implant sites) while establishing a favorable environment. In the initial stages, inflammation often supersedes surgical wound healing, leading to complications. Despite the positive effects of mechanical control, a significant number of patients report discomfort and sensitivity in the treated areas. Therefore, various methods, including chemical control and the use of different types of mouthwashes, have been proposed [11,12].

Chlorhexidine (CHX) is widely used in the treatment of periodontal disease as a safe, non-toxic antiseptic agent. However, due to insufficient concentration, subgingival irrigation with CHX solutions, as well as controlled and sustained-release CHX gels, has not always demonstrated optimal efficacy in treating periodontitis [13-15]. Chlorhexidine has a significant effect on reducing microbial plaque, as well as aerobic and anaerobic bacteria. Side effects associated with this agent include brown tooth staining, altered taste perception, increased supragingival calculus formation, enhanced bacterial colonization, and a reduction in the natural oral microflora [16-18]. The limitations in systemic antibiotic prescription and the limited efficacy of administering them as mouthwashes or subgingival irrigants have led to these drugs being delivered using controlled and sustained-release technologies [4-6]. While the use of chlorhexidine reduces the incidence of pneumonia, its application offers no advantage in reducing mortality or shortening the length of hospital stay for patients admitted to the ICU [19,20]. Iranian oak (*Quercus brantii*), wild pistachio (*Pistacia atlantica* subsp. *mutica*), and wild almond are three of the most important forest species in Western Iran, forming the primary components of the Zagros forests across a wide range. Iranian oak is utilized in treating gastrointestinal disorders, diarrhea, burns, lacerations, and cancer [21]. Considering the vital role in improving the condition of patients undergoing periodontal treatment, the present study was designed with the aim of evaluating the potential of an herbal mouthwash extracted from wild pistachio fruit as an alternative to chlorhexidine in accelerating and improving gingival healing following a crown lengthening procedure.

Materials and Methods

The present study was a clinical trial in which the statistical population consisted of

patients randomly divided into five groups ($n = 10$ each) utilizing Chlorhexidine mouthwash, herbal mouthwash at concentrations of 1%, 5%, and 10%, and a control group receiving normal saline. All study variables were obtained through empirical observations and patient self-reporting via designed questionnaires. Empirical observations for measuring the research variables were meticulously recorded by trained personnel. The questionnaire was completed by the patients on a self-report basis. This study was conducted to investigate the effect of *Pistacia atlantica* fruit extract on gingival healing after crown lengthening surgery. To ensure random allocation and standardization among the samples across the five groups (chlorhexidine mouthwash, 1%, 5%, and 10% herbal mouthwashes, and the normal saline group), a final total of 50 participants was estimated, distributed equally with 10 samples per group. The samples were collected completely randomly from patients attending public and private dental clinics in Ilam Province. All study variables were obtained through empirical observations and patient self-reporting via designed questionnaires. Empirical observations for measuring the research variables were meticulously recorded by trained personnel. The study site was the Faculty of Dentistry at Ilam University

of Medical Sciences. The fruit of the wild pistachio tree was collected from the heights of the Kabir Kuh mountain range in Eastern Ilam Province, following authentication by an expert from the university's Medicinal Plants Research Center. The shade-dried fruits were thoroughly ground using a laboratory mill. The prepared dried fruit powder was subjected to extraction at the Biotechnology and Medicinal Plants Research Center using the Soxhlet extraction method. For this study, an aqueous-alcoholic extract of the wild pistachio fruit was prepared according to previous studies. The extracted material was completely dried using a rotary evaporator, ensuring complete removal of the solvent. Using the resulting dry extract, herbal mouthwashes of the wild pistachio fruit were prepared at concentrations of 1%, 5%, and 10%. Figure 1 shows the steps for preparing the solution from step one to step five.

Ethical Considerations and Statistical Methodology

The fruit of the wild pistachio plant (*Pistacia atlantica* subsp. *mutica*, implied) is edible, and its gum (resin) has traditional medicinal applications and is utilized in various forms, possessing significant uses in the food industry.



Figure 1 Steps for preparing the solution (from left to right)

Given its widespread consumption and past studies indicating a lack of toxic effects in humans, it was employed in this research as a mouthwash, meaning its impact was specifically studied topically. Nevertheless, a consent form was prepared for all participants, and the intervention performed was fully explained to them. To adhere to research ethics, a consent form was prepared for all study participants, and the intervention that was carried out was fully explained to them. The present study was conducted after obtaining the Ethics Code (IR.MEDILAM.REC.1403.114) from the Ethics Committee of Ilam University of Medical Sciences. This study was also registered in the Iranian Registry of Clinical Trials under the code IRCT202441126063859N1.

Data Analysis Method

Initially, a normality test was performed on the raw data. Normal data were analyzed using parametric methods, while non-normal data were analyzed using non-parametric methods. Given the nature of the acquired data, the analyses were conducted using SPSS software, version 22. Finally, statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney tests at a significance level of $\alpha = 0.05$.

Results

In this study, 50 individuals with a mean age of 43.2 ± 10.5 years and an age range of 24 to 55 years were included, consisting of 31

women (60% of the study population, with a mean age of 45.6 ± 8.5 years) and 21 men (40% of the study population, with a mean age of 35.6 ± 12.5 years). The age and gender composition of the study groups is detailed in [Table 1](#).

According to [Table 2](#), regarding the taste quality of the mouthwash based on Kruskal-Wallis test analysis, there was a statistically significant difference between the HM group and CHX, and the HM group and the HM 10% group ($p=0.005$). Also, regarding the parotid gland swelling index ($p=0.225$) and the Modified Gingival Index (MGI), the difference between the groups was not significant ($P=0.656$). According to the Kruskal-Wallis statistical test, the difference in inflammation before and after treatment for the Modified Gingival Index (MGI) was significant in each of the four groups, and the amount of inflammation decreased after treatment. According to the results of the Kruskal-Wallis test in the comparison between groups in terms of tongue surface discoloration, the difference between the HM group and CHX, the HM group and HM 10%, the CHX group and NS, and the NS group and HM 10% was significant ($P=0.000$). The mouthwash taste preference index was only compared between the two groups HM 10% and CHX. According to the Mann-Whitney test, the difference between the two groups was significant ($P<0.001$). According to the Kruskal-Wallis test, in terms of the Plaque Index (PI), only the difference between the CHX and NS groups was significant ($P=0.001$) ([Table 2](#)).

Table 1. Mean and standard deviation of age and frequency distribution of gender in the studied groups

Group	Mean age	Number of men	Number of women
Herbal mouthwash 1 percent	38.4 ± 13.07	6	4
Herbal mouthwash 5 percent	45.6 ± 14.03	4	6
Herbal mouthwash 10 percent	41.06 ± 11.78	3	7
Chlorhexidine mouthwash	38 ± 10.56	5	5
Normal saline	40 ± 10.56	3	7
P-value	0.605	0.203	0.109

Table 2. Mean rank of mouthwash taste, parotid gland swelling index, modified gingival index, and tongue surface discoloration index in different groups

Groups	Mean of rank measurement traits			
	Tongue surface discoloration Index	Modified gingival index	Parotid gland swelling index	Mouthwash taste index
Herbal mouthwash 1 percent	22.12	35.5	29.50	41.13
Herbal mouthwash 5 percent	21.5	35.3	30.12	42.14
Herbal mouthwash 10 percent	25.62	37.42	34.18	45.33
Chlorhexidine mouthwash	46.19	32.54	35.02	22.15
Normal saline	21.43	28.35	29.02	29.51
P-value	<0.001	0.657	0.225	0.005

Also, according to the Wilcoxon statistical test, the difference in plaque levels before and after using the mouthwash was significant in each of the five groups, and it had decreased. Regarding the intensity and extent of staining, the data were non-normal and were analyzed using non-parametric methods and the Kruskal-Wallis test. According to the results of this test, the differences between the HM and CHX groups, the HM and HCHX groups, the CHX and NS groups, and the HCHX and NS groups were significant (Table 2).

Table 3 compares the performance of four main indices between the mouthwashes based on mean rank. In the taste preference index, chlorhexidine (CHX) had the most desirable taste with the lowest mean rank (14.22), while herbal mouthwashes (HM) ranked higher. Regarding the plaque index, the results are surprising: normal saline (NS) performed best with a rank of 18.05, and CHX performed the worst with a very high rank of 45.63. Herbal mouthwashes also showed better performance than NS and much better than CHX with ranks around 27. In terms of tooth discoloration (intensity and extent), CHX had the worst results by a wide margin (47.56 and 46.62). In contrast, all concentrations of herbal mouthwash (with ranks below 17.3) caused the least amount of tooth discoloration. These data clearly confirm the superiority of herbal mouthwashes in preventing

discoloration and their adequate performance in plaque control compared to chlorhexidine.

Discussion

Considering the vital role of improving the condition of patients under periodontal treatment, the present study was designed with the aim of evaluating the potential of a herbal mouthwash extracted from wild pistachio fruit as an alternative to chlorhexidine, in accelerating and improving the process of gingival healing after crown lengthening surgery. *Pistacia atlantica kurdica* from the Anacardiaceae family is mostly observed in the mountainous range of Western Iran. Its husk and fruit are mostly known to have therapeutic effects, including anti-diarrheal properties, nerve strengthening, hypoglycemic, and antimicrobial effects [22-24]. Due to the extensive local use of wild pistachio and the scarcity of related research, and considering the studies proving the antioxidant properties and the presence of saturated and unsaturated fatty acids in *pistacia atlantica kurdica*, its role in promoting patient health is important [25-28]. Gingival healing is an important necessity in dentistry. In recent years, the determination of different parts of the masticatory mucosa, especially gingival thickness, has received attention from periodontists both epidemiologically and therapeutically.

Table 3. Mean rank of mouthwash taste index, plaque index, tooth discoloration intensity index, and tooth discoloration extent index in different groups

Groups	Mean of rank measurement traits			
	Tooth discoloration extent index	Tooth discoloration severity index	Plaque index	Mouthwash flavor preference Index
Herbal mouthwash 1 percent	16.3	16.1	28.03	24.48
Herbal mouthwash 5 percent	16.71	16.89	27.56	25.51
Herbal mouthwash 10 percent	16.97	17.29	26.45	27.6
Chlorhexidine mouthwash	46.62	47.56	45.63	14.22
Normal saline	19.44	21.38	18.05	--
<i>P</i> -value	<0.001	< 0.001	0.001	<0.001

Periodontal inflammatory diseases are among the most common chronic inflammatory diseases that can affect any individual at any age [7,29,30]. The cause of gingival inflammation is the presence of opportunistic bacteria that naturally exist in the mouth. Gingivitis is a multifactorial disease that is influenced by various factors. For this reason, the evaluation of risk factors for chronic diseases is of special importance [31-33].

In the study by Ali Mohammadi *et al.*, where two herbal mouthwashes and chlorhexidine were tested on gingival healing after surgery, the researched patients were randomly divided into four groups. The researched groups included the herbal mouthwash group, the chlorhexidine mouthwash group, and the Normal Saline group. According to the findings, the herbal mouthwash caused improvement in gingival inflammation indices. On the other hand, chlorhexidine caused improvement in the GI and PI indices. Also, no complications were observed in the herbal mouthwash group [34]. In the study by Banihashem *et al.*, the effects of mouthwash on gingival inflammation in chronic periodontitis patients were studied, where they were randomly divided into three groups of ten. According to the findings, the anti-inflammatory mouthwash is comparable to chlorhexidine and yields greater improvement in some aspects [10]. The results of the

present research confirm previous findings in two key areas. The study by Kamali *et al.* [35] showed that the occurrence of discoloration caused by 0.2% chlorhexidine after periodontal surgery was 6.6 times higher than Persica; this rate is in direct consistency with our findings regarding the discoloring side effects of CHX. Furthermore, the significant difference in reported taste between chlorhexidine and the *Terminalia chebula* mouthwash by Gupta *et al.* [36] is consistent with the results observed in our current study. One of the limitations of this study is the small sample size. Therefore, further studies with a larger sample size are recommended.

Conclusion

The most important advantage of the herbal groups, besides maintaining effectiveness, is in reducing aesthetic complications, including tooth and tongue discoloration. At the same time, a better taste was experienced, without the swelling of the parotid gland being affected. The proven reduction of plaque and inflammation indices in all groups confirms the general therapeutic efficiency of these products. Therefore, herbal mouthwashes can be introduced as an alternative and highly tolerable therapeutic option for Chlorhexidine, especially in cases where patient acceptance is a factor. The use of these formulations will significantly

contribute to maintaining oral health and improving the user experience.

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Conflict of Interest

No conflict of interest was reported by the authors in this work.

Ethical Considerations

In order to comply with ethical standards in research, a consent form was nevertheless prepared for all study participants, and the intervention was fully explained. The present study was conducted after obtaining the creative code (IR.MEDILAM.REC.1403.114) from the Ethics Committee of Ilam University of Medical Sciences. This study has been registered with the Iranian Trial Registration Center under the code IRCT202441126063859N1.

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