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The Effect of *Zataria multiflora* Boiss. Mouthwash on the Oral Microbial Load in Patients under Mechanical Ventilation: A Randomized Controlled Trial

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Abstract

This study aimed to examine the effect of *Zataria multiflora* Boiss. mouthwash on the microbial load of the oral cavity in patients under mechanical ventilation. This randomized controlled trial was conducted in 2019 on patients under mechanical ventilation. Sampling was performed using the consecutive method. Using a block randomization method, 90 patients were allocated to three equal groups of 30 to receive mouth care using chlorhexidine, *Z. multiflora*, or normal saline. Mouthwash was used three times a day for a week. Eight hours before and after the intervention, sterile samples of saliva were collected and cultured in the laboratory. Chi-square, Fisher's exact test, paired t and McNemar's tests, and analysis of variance were used to analyze the data. After the interventions, a significant difference in the microbial load was found between the three groups ($P < 0.021$). The Tukey post hoc test showed a significant difference between the group treated with *Z. multiflora* and those who received chlorhexidine (P value = 0.016). The frequency of patients with positive cultures was lower in the group treated with *Z. multiflora* and the differences were statistically significant in terms of Acinetobacter ($P = 0.01$) and *Klebsiella pneumonia* ($P = 0.02$). *Z. multiflora* mouthwash was effective in decreasing the microbial load of the oral cavity. This mouthwash can be used to reduce the microbial load of the oral cavity in patients under mechanical ventilation and reduce the risk of ventilator-associated pneumonia.

Keywords: Mouthwashes; *Zataria multiflora*; Essential oil; Colony count; Mechanical ventilation; Chlorhexidine

Introduction

Mouth care is a crucial part of nursing care in patients under mechanical ventilation [1]. There is a set of normal flora in the mouth; however, this flora can be dangerous for patients with a weak immune system. These floras include Gram-positive streptococci, a variety of viruses, and fungi, and remain stable in a healthy person. However, within 48 h of admission to the intensive care unit (ICU) and especially after tracheal intubation, the normal flora of the mouth changes to opportunistic and invasive agents such as Gram-negative bacilli and staphylococci [2,3]. In a study, 80 out of 110 ICU

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patients had bacterial colonization in their trachea during the first day of mechanical ventilation [4]. The presence of an endotracheal tube, and bacterial colonization in the oral cavity of patients under mechanical ventilation, can predispose them to ventilator-associated pneumonia [2], increase the length of their hospital stay, costs, and mortality [5]. Among all nosocomial infections, respiratory infections cause the highest mortality [6]. Therefore, more attention should be paid to oral care in patients under mechanical ventilation.

There is ample evidence of a link between ventilator-associated pneumonia and inadequate mouth care in ICU patients [1]. The rates of ventilator-associated pneumonia can be reduced by providing a rigorous and evidence-based caring protocol [7]. There is ample evidence of a link between ventilator-associated pneumonia and inadequate mouth care in ICU patients [1]. Some studies confirmed that mouth care using chlorhexidine diminishes the incidence of ventilator-associated pneumonia [1,2]. However, a study reported that the rate of ventilator-associated pneumonia was not significantly different between the patients who received mouthwash with 0.12% chlorhexidine and those who were treated with saline [8]. A systematic review also concluded that mouth care with chlorhexidine cannot reduce ventilator-associated pneumonia unless it is administered at 2% concentration or 4 times a day [9]. Therefore, some researchers have considered the use of other mouthwashes, especially those of plant origin [10-12].

Zataria multiflora boiss (Shirazi thyme) is an herb with antibacterial, antifungal, and antiviral properties and can be used as an antiseptic [13,14]. It is cultivated in Iran, Pakistan, and Afghanistan and has chemical and pharmacological properties similar to Thymus vulgaris, a well-known medicinal plant [15]. Its aerial parts, including its flower, are traditionally used for its antidiarrheal, anthelmintic, antiseptic, and analgesic properties. The essential oil of *Z. multiflora* contains significant amounts of phenols and flavonoids, thymol and carvacrol [16]. Studies have shown the anti-inflammatory and antibacterial effects of *Z. multiflora* [17-19], but few studies have used it as a mouthwash [13]. A study has also reported the beneficial effects of *Z. multiflora* on the reduction of insulin resistance [15]. In another study, the effect of an oral rinse made of a mixture of sage tea-thyme-peppermint in patients undergoing chemotherapy was assessed and showed its beneficial effects in alleviating oral mucositis [13]. Despite the antimicrobial and anti-inflammatory effects of *Z. multiflora* [13-19], little information is available on its effect as a mouthwash. Nurses have a crucial role in mouth care and in the prevention of ventilator-associated pneumonia [1]. Due to the controversies about the effects of chlorhexidine mouthwash, the question arises whether a mouthwash made of *Z. multiflora* can reduce the microbial load of the oral cavity? This study aimed to compare the effect of chlorhexidine mouthwash with a mouthwash made of *Z. multiflora* essential oil on the microbial load of the oral cavity in patients under mechanical ventilation.

Methods

Trial design

The design of the present study was a randomized controlled trial with two arms, parallel and 1:1 allocation ratio.

Participants

This study was conducted from Mar 21 to Aug 23, 2019, on patients who received mechanical ventilation in the ICUs of an educational hospital in Kashan, Iran. Eligibility criteria were an age of 18 years or over, being under mechanical ventilation, and having no history of an allergic reaction to *Z. multiflora*. The exclusion criteria for the study included being pregnant, a history of allergic reactions to mouthwash, asthma, allergic rhinitis and dermatitis, radiation therapy, immunosuppressive, autoimmune, and malignant diseases, coagulopathy, using medications (including corticosteroids and anticoagulant medications), re-intubation, oral mucositis, advanced periodontal disease, pulmonary and systemic infections, endotracheal extubation during the study, converting from an endotracheal tube to a tracheostomy during the study, and transferring the patient from ICU to another hospital ward before the end of the study.

Interventions

Z. multiflora essential oil, chlorhexidine 0.2%, or sterile normal saline were used for mouthwash. The *Z. multiflora* essential oil was purchased from the Barij Essence Company, Kashan, Iran, as a 10% ethanol solution in 100 mL dark bottles (with 27.99 mg/dL of thymol, pH: 5.03). The bottles were stored at 5 to 25 ° C after each use. Chlorhexidine 0.2% was purchased from the World Health Company, Iran. In the control group, only normal saline was used for mouthwash. Mouthwash was performed three times a day (i.e. 8 am, 2 pm, and 10 pm) for a week [20]. First, the head of the bed was elevated so that the patient was placed in a semi-Fowler's position. Then, the nurse who performed the procedure washed her hands and wore sterile gloves. Next, the patient's oral cavity and throat were rinsed using cotton swabs and 20 mL of normal saline, and then the oral cavity was suctioned. Subsequently, the mouthwash (i.e. 1 mL of *Z. multiflora* essential oil along with 4 mL of distilled water; 5 mL of chlorhexidine; or 5 mL of normal saline) was poured into the patient's mouth and entire surfaces of the mouth, throat, gums, and teeth were swabbed with mouthwash for 6 minutes so that all the surfaces were impregnated with the mouthwash. Finally, a sterile suction catheter was used to remove the solution from the patient's mouth.

Outcomes

Preliminary outcome of this study included measuring the microbial load of the mouth in three groups of patients under mechanical ventilation after interventions. Also, determining the type of microbial agents was a secondary outcome. A two-part instrument was used to gather the study data. The first part included items on the patient's age and sex, number of days having an endotracheal tube, length of ICU stay, antibiotic use, and comorbidities. The second part was a checklist for recording the laboratory results and included the name of the cultured bacterium and the number of colonies. The face validity of the instrument was confirmed by the faculty members of Kashan University of Medical Sciences.

Eight hours before and after the intervention, the researcher used sterile swabs to collect a sample of saliva from the bottom of the throat (small tongue), placed it inside a sterile glass test tube, and sent it immediately to the laboratory of the Shahid Beheshti Medical Center, Kashan, Iran. All samples were cultured in a blood agar medium at 37 ° C for 48 h and then the results were read. Using a flashlight, two researchers observed the oral cavity of all patients once a day for any complications (such as mucositis, sores, or redness). No complications were found during the study.

Sample size

Ninety patients under mechanical ventilation were consecutively recruited and allocated randomly to three equal groups of 30 (Figure 1). The sample size was calculated using the results of an earlier study [21]. Then, with a confidence level of 95%, a power of 80%, a δ of 0.36, and a measurement error of 0.28 (i.e. d = 0.28), the needed sample size in each group was estimated at 30 (Equation 1).

Equation 1. Sample size calculation

$$n = \frac{2(z_{1-\alpha/2} + z_{1-\beta})^2 \delta^2}{d^2} = 30$$

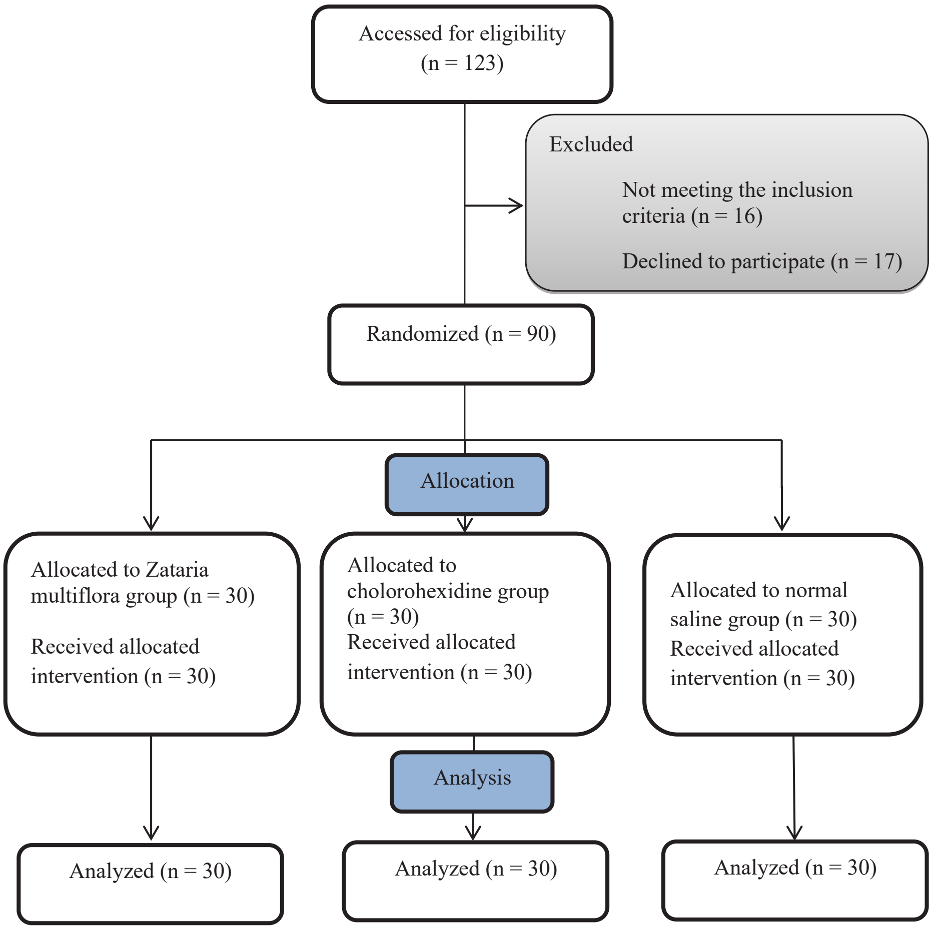

Figure 1. The study flow diagram

Randomization

Using an online randomizer software (https://www.sealedenvelope.com/simple-randomiser), letters A, B, and C were assigned to the *Z. multiflora*, chlorhexidine 0.2%, and normal saline groups, respectively.

Allocation concealment mechanism

Random allocation was performed through block randomization with a block size of six. The samples were then entered into the groups according to the letters specified in the blocks. The corresponding author was in charge of the random allocation sequence and the first author enrolled the participants and performed the interventions.

Blinding

The present study was double-blind. The statistical and laboratory analyst did not know the study groups. They knew the names of the groups only by the letters A, B, and C and were completely unaware of the intervention performed for each group.

Statistical methods

The data were analyzed with the SPSS software (v. 13.0). The data were described via the measures of descriptive statistics such as frequency, percent, mean, and standard deviation. The normality of quantitative data was assessed by the Kolmogorov-Smirnov test. The Chi-square test was used to compare the frequency of sex in the three groups. Fisher's exact test was used to compare the frequency of antibiotic use in the three groups. Analysis of variance was also used to compare the mean age, length of hospital stay, duration of intubation, and microbial load before and after the intervention in the three groups. Paired t-test was used to compare the mean microbial load before and after the intervention in each group. McNemar's test was used to compare the frequency of the type of microbes in the three groups. The level of significance was set at < 0.05.

Ethical consideration

The Ethics Committee of Kashan University of Medical Sciences approved this study (Ethics code: IR.KAUMS.NUHEPM.REC.1397.050). Permissions were also sought from the officials in the hospital and ICUs. The objectives of the study were explained to the patients' first-degree relatives and their written consent was obtained. Patients' relatives were assured that no risk from the investigation would threaten their patients, the study imposes them no costs, the data would be confidential, and that they have the right to withdraw from the study whenever they wished. They were also assured that attending or not participating in the study would not affect their patient's treatment.

Results

A total of 123 patients, 16 patients not meeting inclusion criteria, and family members of 17 patients were not satisfied with their patient's participation in the study. Of all patients studied, 56 (60%) were males. The mean ICU stay of patients was 10.45 ± 14.94 days. A majority of the patients in all three groups had received antibiotics during their ICU stay. The between-group differences respecting participants' demographic and clinical characteristics were not statistically significant (Table 1). The mean microbial load of the three groups was not significantly different before the intervention. However, after the interventions, a significant difference in the microbial load of the oral cavity was found between the three groups (P < 0.021, Table 2). The Tukey post hoc test showed a significant difference between the group treated with *Z. multiflora* and those who received chlorhexidine (P value = 0.016). The microbial load of the oral cavity increased in the groups treated with normal saline and chlorhexidine, however, the load of microbes decreased in the group treated with *Z. multiflora*, al-

Table 1. Between-group comparisons of the participants' characteristics

Variables		Zataria multiflora n (%)	Chlorhexidine n (%)	Normal saline n (%)	P-value
Sex	Male	22 (73.3)	20 (66.7)	14 (46.7)	0.71[a]
	Female	8 (26.7)	10 (33.3)	16 (53.3)	
Receiving antibiotics	Yes	30 (100)	26 (86.7)	28 (93.3)	0.20[b]
	No	0	4 (13.3)	2 (6.7)	
Having other comorbidities	Yes	9 (30)	10 (33.3)	8 (27.3)	0.68[a]
	No	21 (70)	20 (66.7)	22 (73.3)	
		Mean±SD	Mean±SD	Mean±SD	
Length of ICU stay, Day		11.03 ± 3.10	7.90 ± 0.97	13.37 ± 3.33	0.58*
Age, Year		50.66 ± 4.68	53.07 ± 5.04	60.66 ± 4.28	0.42*
Duration of intubation, Day		7.40 ± 1.30	7.07 ± 0.96	6.70 ± 0.62	0.96*

[a] Chi-square test; [b] Fisher's Exact test; *ANOVA

Table 2. The mean microbial load of the three groups before and after the intervention

Group	Before		After		
	Mean	Standard deviation	Mean	Standard deviation	P value (paired t-test)
Total	102758.44	57157.52	110750.71	127601.27	0.581
Normal saline	100334.67	36240.88	106667.33	36512.82	0.161
Chlorhexidine	124345.60	67120.95	150509.67	209331.39	0.535
Zataria multiflora	84213.23	57870.61	76225.97	48676.37	0.541
P value (ANOVA)		0.072		0.021	

though the within-group differences were not statistically significant (Table 2).

As the McNemar's test revealed, after the intervention, the number of patients with positive cultures was considerably lower in the group treated with *Z. multiflora* and the differences were statistically significant in terms of *Acinetobacter* (P = 0.01) and *Klebsiella pneumonia* (P = 0.02). Also in the normal saline and chlorhexidine groups, the results of this test showed that there was no statistically significant difference between before and after the intervention for the frequency of patients infected with *E. coli*, *Staphylococcus aureus*, *Acinetobacter*, and *Klebsiella pneumonia* (P > 0.05; Table 3).

Table 3. The frequency and the type of microbes found in the bacterial cultures of the three study groups, before and after the intervention

Groups		E. coli	Staph[a]	Acinto[b]	Kleb[c]
Normal saline	Before	2 (6.7)	1 (3.3)	17 (56.7)	9 (30)
	After	3 (10)	1 (3.3)	18 (60)	11 (36.7)
P*		0.5	0.99	0.99	0.62
Chlorhexidine	Before	1 (3.3)	6 (20)	18(60)	9(30)
	After	4 (13.3)	6 (20)	16(53.3)	12(40)
P		0.37	0.99	0.77	0.45
Zataria multiflora	Before	3 (10)	4 (13.3)	11(36.7)	17(56.7)
	After	1 (3.3)	1 (3.3)	4(13.3)	8(26.7)
P		0.5	0.68	0.01	0.02

*McNemar's test; n (%); [a] Staphylococcus aureus; [b] Acinetobacter; [c] Klebsiella pneumonia

Discussion

The results of the current study showed that the mean bacterial load of the oral cavity was increasing in patients who received mouth care with normal saline or chlorhexidine. However, the mean microbial load of the oral cavity was significantly lower in those who have been treated with *Zataria multiflora*. These findings denote that *Zataria multiflora* mouthwash was more effective than other mouthwashes used in this study in reducing the microbial load of the oral cavity in patients under mechanical ventilation. An earlier study by Baptista et al. showed the increase in the bacterial load of the oral cavity in patients with prolonged use of mechanical ventilation and concluded that bacteria may migrate rapidly from the mouth and upper airways during orotracheal intubation. This migration might contribute to the pathogenesis of ventilator-associated pneumonia [22]. Jazayeri et al. studied the in-vitro effects of essential oils of Thymus eriocalyx and Thymus kotschyanus [23]. Consistent with our results, they have reported that the essential oils of these two herbs can inhibit the growth of Candida Albicans. Yadegar et al. have also reported that the ethanol extract of *Zataria multiflora* was effective in inhibiting the growth of methicillin-resistant Staphylococcus [24]. Another study has also reported that the essential oil of Thymus vulgaris has considerable antifungal and antibacterial effects [25]. Two other studies also compared the effects of chlorhexidine and some other herbal mouthwashes containing Persica [26] or Aloe Vera [27] and reported that the herbal mouthwashes were more effective than chlorhexidine both in inhibiting the growth of Candida Albicans [26] and in the reduction of dental plaque [28].

The effect of *Zataria multiflora* essential oil on the microbial load of the oral cavity can be attributable to its antiseptic effects and to the fact that it contains considerable amounts of ingredients such as thymol and carvacrol [29,30]. Due to its lipophilic properties, thymol accumulates in the bacterial membranes and finally destructs them [31].

In the present study, the number of patients with positive cultures was considerably lower in the group treated with *Zataria multiflora* and the differences were statistically significant in terms of *Acinetobacter* and *Klebsiella pneumonia*. Some previous studies have also shown that *Z. multiflora* essential oil reduces the growth of *Acinetobacter* [17,32], *E. coli* [25,33-37], *Klebsiella pneumonia* [38,39], and *Staphylococcus aureus* [17,40-42]. Methicillin-resistant Staphylococcus is a common cause of nosocomial infections in patients under mechanical ventilation [43]. *Acinetobacter* is also one of the opportunistic agents and a majority of its infections affect the respiratory tract of hospitalized patients, especially those admitted to ICUs. It can cause pneumonia in immunocompromised people and patients of ICUs. Infections caused by *Acinetobacter* are of great concern because of multidrug resistance to this pathogen [17]. Despite advances in medical technology and the use of mouthwashes such as chlorhexidine 0.2%, ventilator-associated pneumonia is still among the most common problems in ICUs patients [44], causing mortality of more than 70% [45]. Therefore, the prevention of ventilator-associated pneumonia is a top priority in all patients under mechanical ventilation [46]. The use of effective mouthwashes such as *Z. multiflora* can help reduce the microbial load of the oral cavity which then would decrease the risk of ventilator-associated pneumonia.

Conclusion

This study suggests the effectiveness of *Z. multiflora* essential oil mouthwash on decreasing the microbial load of the oral cavity and on inhibiting the growth of bacteria such as *Acinetobacter*, *E. coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia*. Therefore, nurses can use this mouthwash to reduce the microbial load of the oral cavity in patients under mechanical ventilation and reduce the risk of ventilator-associated pneumonia. In the present study, we only focused on the effect of *Z. multiflora* essential oil mouthwash on the microbial load of the oral cavity but did not assess the rate of ventilator-associated pneumonia. Further studies should be performed to examine the effect of *Z. multiflora* mouthwash on the incidence of ventilator-associated pneumonia in patients under mechanical ventilation. This study was conducted in a hospital and therefore, the results might not be generalized. Larger, multicenter clinical trials are suggested. Also, other limitations of the study may include the possible effects of confounding variables that could not be controlled, and the existence of ethanol in *Z. multiflora* mouthwash that can interfere with the results.

Trial registration

IRCT, 20100124003146N6. Registered 10 Mars 2019, https://www.irct.ir/user/trial/37111/view

Conflict of Interests

The authors declare no conflict of interest. The funding body had no role in the design of the study and collection, analysis, and interpretation of data, and in writing the manuscript.

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