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CLINICAL AND ANTI-INFLAMMATORY EFFECTS of THYMUS EXTRACT MOUTHWASH in PATIENTS WITH GINGIVITIS

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ABSTRACT

Herbal medicines have been using in dentistry clinics for a long history for preventing growth of microorganisms and their effects on inflammation. This novel study aimed to evaluate *Thymus vulgaris* (*T. vulgaris*) mouthwash in the treatment of gingivitis in comparison with chlorhexidine mouthwash. Patients attended periodontics department of College of Dentistry at the University of Duhok with age ranges of 15-35 years old from both sexes (females =23, males=22) were included in the study. Following the preparation of alcoholic Thymus extract 5% mouth wash, the mouthwash pH was measured on days 3, 6, 9, 12, 15, 21 and 60. The patients were randomly divided into three groups of deionized water, T. vulgaris (5.00%) and chlorhexidine and rinsed twice daily for 21 days. Plaque index (PI), gingival index (GI) were assessed on days 0, 14 and 21 and salivary levels of interleukin-6 (IL-6) were assessed at baseline and day 21. There were not significant changes in pH of T. vulgaris extract mouthwash on different days. However, the treatment with *T. vulgaris* mouthwash decreased PI, GI and the levels of salivary IL-6 as compared with baseline (P=0.001). The results showed non significant differences between *T. vulgaris* and chlorhexidine mouth washes for PI, GI and IL-6 in all the days (P>0.05). The efficiency of T. vulgaris mouthwash was increased with increasing time (21 days vs 14 days). In conclusion, T. vulgaris mouthwash can be used as an alternative for chlorhexidine for treatment of gingivitis.

***KEYWORDS*:** Chlorhexidine, Gingivitis, Inflammation, Plaque index, *Thymus vulgaris*

INTRODUCTION

Gingivitis is formed due to the accumulation of microbial biofilms on surfaces of teeth (1). Poor and/or inadequate oral hygiene is a major factor for its predisposing and finally causing tooth losing (2). Gingivitis is also a reversible inflammatory process that is induced by the presence of microorganisms in the biofilm near the gingival margin (3). Bacterial lipopolysaccharides promote the inflammatory response of the host and activate polymorphonuclear leukocytes and the secretion of inflammatory mediators such as cytokines and chemokines (4, 5). Interleukin-6 (IL-6) is an important inflammatory cytokine that is involved in the inflammatory responses (6, 7). The therapeutic strategies for plaque-induced gingivitis aim to prevent and control of the plaque accumulation by improving the level of oral hygiene (8). Several mechanical methods have been used to eliminate the plaques such as tooth brushing, dental floss, tooth cleaning sticks, oral irrigators, and/or professional scaling and polishing. Such methods may not have a significant efficiency for controlling the formation of plaques (9). There is need to prepare and produce the antimicrobial agents for removing plaque-induced gingivitis. Several studies have recommended using antimicrobial mouth-washes in conjunction with mechanical oral hygiene methods for controlling the formation of plaques (10-12). The antimicrobial mouthwashes exhibit their effects against bacteria by disrupting the formation of plaque, preventing the bacterial growth and metabolism and also inhibiting the colonization of oral bacteria (13). Chlorhexidine has been extensively applied against a broad-spectrum of bacteria and known as a golden standard for controlling dental plaques (14). However, it has limitations such as coloration of teeth and tongue, disrupting teeth and tongue and negative effects on oral mucosa in long time uses (15). Undesirable side effects of chlorhexidine limit the long-term use and its acceptability by patients. There are requirement to research and find alternative for chlorhexidine with the maximum efficiency.

Herbal medicines have been used in dentistry clinics for a long history for preventing growth of microorganisms, decreasing the inflammation,

soothing irritation, and alleviating pain (16-18). Herbal mouthwashes have significant anti-inflammatory and antioxidant properties that can supply additional advantages compared with synthetic mouthwashes (19).

Thymus vulgaris (*T. vulgaris*) is one of medicinal plants used for its antibacterial activities. It is an aromatic and flowering that belongs to the Lamiaceae family. Several compounds with antibacterial activities have identified in *T. vulgaris* extract with antimicrobial activity against pathogenic microbes and antioxidant properties such as thymol and carvacrol (20, 21). Antibacterial activities of *T. vulgaris* essential oil have previously reported (22, 23). In addition to antibacterial properties of *T. vulgaris*, it has anti-inflammatory properties (24-26).To the best of our knowledge, the effects of *T. vulgaris* on gingivitis have not been evaluated by plaque index (PI), gingival index (GI) and salivary IL-6 assessing. Thus the present study aimed to evaluate the effect of *T. vulgaris* mouthwash in the treatment of gingivitis and compare with chlorhexidine mouthwash.

MATERIALS AND METHODS

The preparation of T. vulgaris mouthwash

Aerial parts of *T. vulgaris* in the flowering stage were collected from different sites of the Zawita area, Duhok Governorate/Kurdistan Region of Iraq and transferred to the laboratory of the College of Agricultural Engineering science/Duhok University. 200 g of dry *T. vulgaris* plant was grounded with automatic miller (Adewo Company, China), then one liter of absolute ethanol (ethanol 99.8%) was added to it, macerated in a container sealed with paper foil to prevent losing the volatile solvent and kept at room temperature for 24 hours. At the end of this period, the contents were filtered using filter paper (No.1) into a beaker. The filtered solution was then concentrated by evaporating the solvent (ethanol) in a hot air oven at 40 $^{\circ}$C for 24 h. Then, all the solutions were evaporated and only T. vulgaris was remained in the bottom of the beaker. The (5%) mouthwash was prepared by solving 5% of *T. vulgaris* extract in a deionized water and the volume was increased to 100 mL (w/v) (27). The pH of mouthwash was determined with the help of a pH meter (Ecoscan, Singapore) on 3, 6, 9, 12, 15, 21 and 60 days after the preparation.

Patients

A clinical comparative study was conducted on patients with moderate to severe gingivitis from 15 Dec 2022 to 1 Jul 2023. The current study was confirmed by the Ethical Committee, College of Dentistry, Duhok University. Fifty-five patients of both sexes (females =23, males=22) with an average age of 15-35 years were included in the study. The study was commenced after receiving informed consent from all the participants.

All the participants responded a written case sheet for gender, age, mobile, address, history and other clinical information.

Criteria of patients selection

Inclusion criteria for the selection of patients were as follows: Systemically healthy individuals with moderate to severe gingivitis, age between (15-35) years from both genders, , lack of history of compromised medical status, non-pregnant or lactating females, lack of history of allergy to the thymus subspecies, lack of history of allergy to chlorhexidine, non-smoking and non-alcoholic.

Groups

The first group (*T. vulgaris*: F-6, M-9): The patients were asked to rinse for at least 1 min with 10 mL of *T. vulgaris* mouthwashes. The second group (Chlorhexidine group): (n=15; F-10, M-5) was asked to rinse for at least one minute with 10 mL of the Chlorhexidine mouthwash. The third group (deionized water group): (n=15; F-7, M-8) was asked to rinse only with 10 mL of deionized water for at least one minute. Mouthwashes and deionized water were applied twice daily, 30 minutes after tooth brushing for 21 days.

Clinical and IL-6 levels assessments

Clinical parameters of PI and GI were measured at baseline,14 and 21 days by a specialist and scored as follows*:*

For PI: Free of plaque (0), no plaque seen by naked eye, a film of plaque adhering to the free gingival margin and adjacent area of the tooth that can be recognized by running the probe or using disclosing agent (1), a thin to moderate accumulation of soft deposits within the gingival pocket or on the tooth gingival margin that can be seen with naked eye (2) and abundance of soft matter within the gingival pocket and/or on the tooth surface and gingival margin (3).

For GI: Absent of inflammation/normal gingiva (0), mild inflammation with slight change in color, slight edema, no bleeding on probing (1), moderate inflammation with

moderate glazing, redness, edema and hypertrophy and bleeding in probing (2) and severe inflammation, marked redness and hypertrophy ulceration and tendency to spontaneous bleeding (3).

The salivary concentration of IL-6 was assessed at baseline and on day 21. The patients was requested to non-eat and non-drink anything one hour before saliva collection. Un stimulated saliva samples were collected in a plain tube for 30 min, centrifuged for 10 min at 2000 rpm to get clear saliva and stored at -20 ºC in a refrigerator . Salivary IL-6 was measured with the help of an Enzyme-Linked Immunosorbent Assay (ELISA) device, a Sandwich-ELISA kit (Boster, USA), according to the manufacturer's instructions. The results were expressed as pg/mL.

Data analysis

SPSS program (version 23) was used to analyze the obtained data. The data were reported as a mean and standard deviation. ANOVA test was follow by Duncan's Multiple analysis range test. Paired sample t-test was used to compare within groups. The data for gender were compared with Kruskal-Wallis test. P-value at <0.05 was considered significant. The Figures were illustrated by Graph Pad Prism (Version of 6.07).

RESULTS

Descriptive data

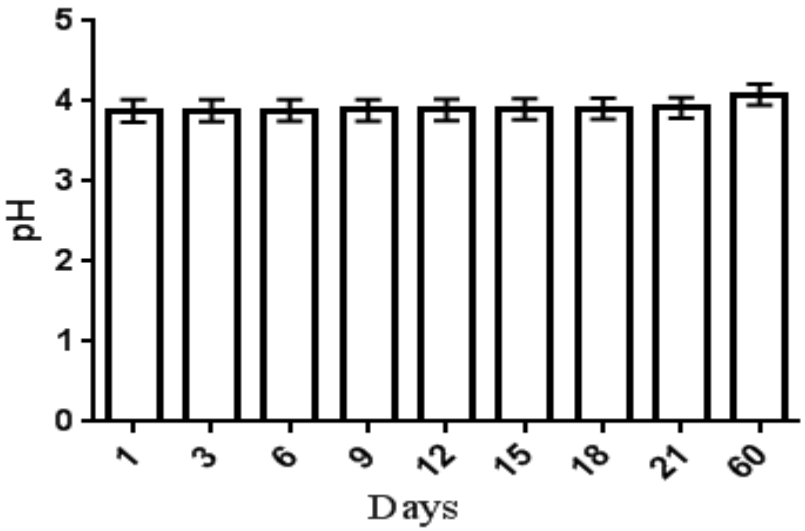
The mean age in T. vulgaris, Chlorhexidine and deionized water groups were 25.85 ± 5.00 year, 23.93 ± 5.76 year and 25.80 ± 5.54 year, respectively that did not show significant differences [df=2, F=0.596, P=0.556] (Table 1). There were not significant differences between groups for gender (P=0.763).

Table (1):-The mean age of patients

Groups	T. vulgaris	Chlorhexidine	deionized water	P-value
Age (year)	25.85 ± 5.00	23.93 ± 5.76	25.80 ± 5.54	0.556

Thyme mouthwash pH

Figure 1 illustrates the results for thyme mouthwash pH on different days. As the results show, significant differences were seen among days for pH [df=8, F=0.928, P=0.510].



Fig(1):- T. vulgaris mouthwash pH on different days

PI

Table 2 illustrates the results for PI in different groups on baseline ,14 and 21days. There was no significant differences between groups in baseline (P>0.05), while significant differences were observed on days 14 (P=0.001) and 21 (P=0.001).

Table (2): - The mean values of PI in different groups in baseline, 14 and 21 days.

	Baseline		14		21	
Groups	**Mean± SD**	**P-value**	**Mean± SD**	**P-value**	**Mean± SD**	**P-value**
T. vulgaris	**1.79±0.10**	**0.513**	**1.54 ±0.05**	**0.846**	**1.21 ±0.10**	**0.696**
Chlorhexidine	**1.76±0.12**	**NS**	**1.55±0.06**	**NS**	**1.22 ±0.04**	**NS**
T. vulgaris	**1.79±0.10**	**0.834**	**1.54 ±0.05**	**0.001**	**1.21 ±0.10**	**0.001**
Deionized water	**1.80±0.09**	**NS**	**1.72±0.07**	*******	**1.78±0.08**	*******
Chlorhexidine	**1.76±0.12**	**0.384**	**1.55±0.06**	**0.001**	**1.22 ±0.04**	**0.001**
Deionized water	**1.80±0.09**	**NS**	**1.72±0.07**	*******	1.71±0.08	*******

NS=Non-significant
***= Significant at P<0.001

The results did not show significant differences between all the groups in baseline (P>0.05). However, significant differences were not seen between T. vulgaris and Chlorhexidine groups on all the days (P>0.05). The T. vulgaris and Chlorhexidine groups showed significant differences with deionized water group on days 14 (P=0.001) and 21 (P=0.001). The man values of PI were more significantly decreased in T. vulgaris and Chlorhexidine groups as compared to deionized water group (P=0.001).

Figure 2 shows significant differences between groups on different days. The results show decrease in PI with the passing time in T. vulgaris and chlorhexidine groups compared with baseline.

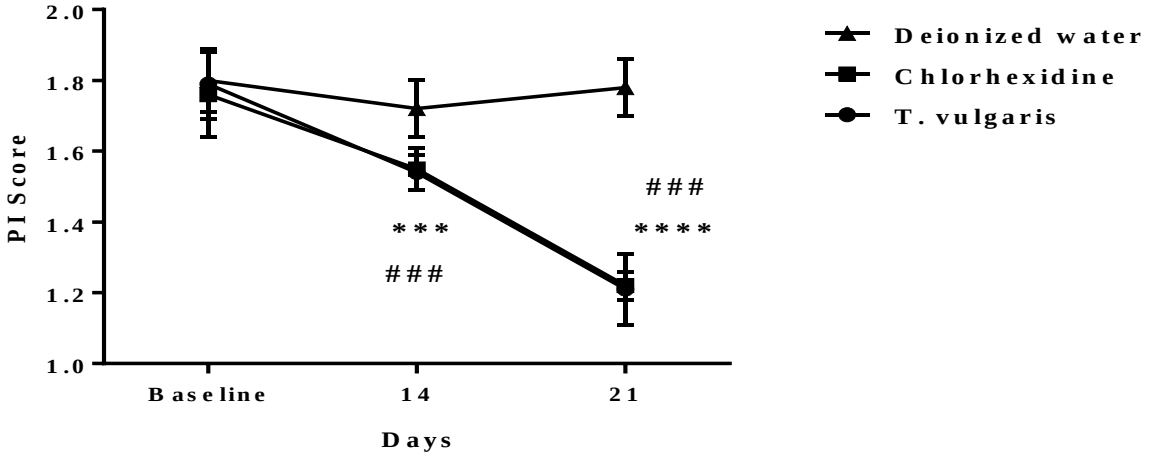


Fig.(2):- Comparison of PI in baseline and follow-up periods.
***=Significant differences between other groups with deionized water in the same day
###= Significant differences between T. vulgaris and chlorhexidine on days 14 and 21 compared with baseline

GI

Table 3 illustrates the results for GI in different groups on baseline and days 14 and 21. There were no significant differences between groups in baseline (P=0.305), while significant differences were observed on days 14 (P=0.001) and 21 (P=0.001).

Table (3):-The mean values of GI in different groups in baseline and days 14 and 21

	Baseline		14		21	
Groups	Mean± SD	P-value	Mean± SD	P-value	Mean± SD	P-value
T. vulgaris	1.86±0.24	0.153	1.42 ±0.09	0.245	1.20 ±0.06	0.0696
Chlorhexidine	1.75±0.11	NS	1.47±0.13	NS	1.23 ±0.06	NS
T. vulgaris	1.86±0.24	0.548	1.42 ±0.09	0.001	1.20 ±0.06	0.001
Deionized water	1.81±0.15	NS	1.78±0.10	***	1.81±0.06	***
Chlorhexidine	**1.75±0.11**	**0.260**	**1.47±0.13**	**0.001**	**1.23 ±0.06**	0.001
Deionized water	**1.81±0.15**	**NS**	**1.78±0.10**	*******	**1.81±0.06**	***

NS=Non-significant
***= Significant at P<0.001

The results did not show any significant differences between all the groups in baseline (P>0.05). However, significant differences were not seen between T. vulgaris and Chlorhexidine groups on all the days (P>0.05). However, T. vulgaris and Chlorhexidine groups showed significant differences with deionized water group on days 14 (P=0.001) and 21 (P=0.001). The results showed that the man values of GI were more significantly decreased in T. vulgaris and Chlorhexidine groups as compared to deionized water group (P=0.001).

Figure 3 shows significant differences between groups on different days. The results show decrease in PI with the passing time in T. vulgaris and chlorhexidine groups compared with baseline.

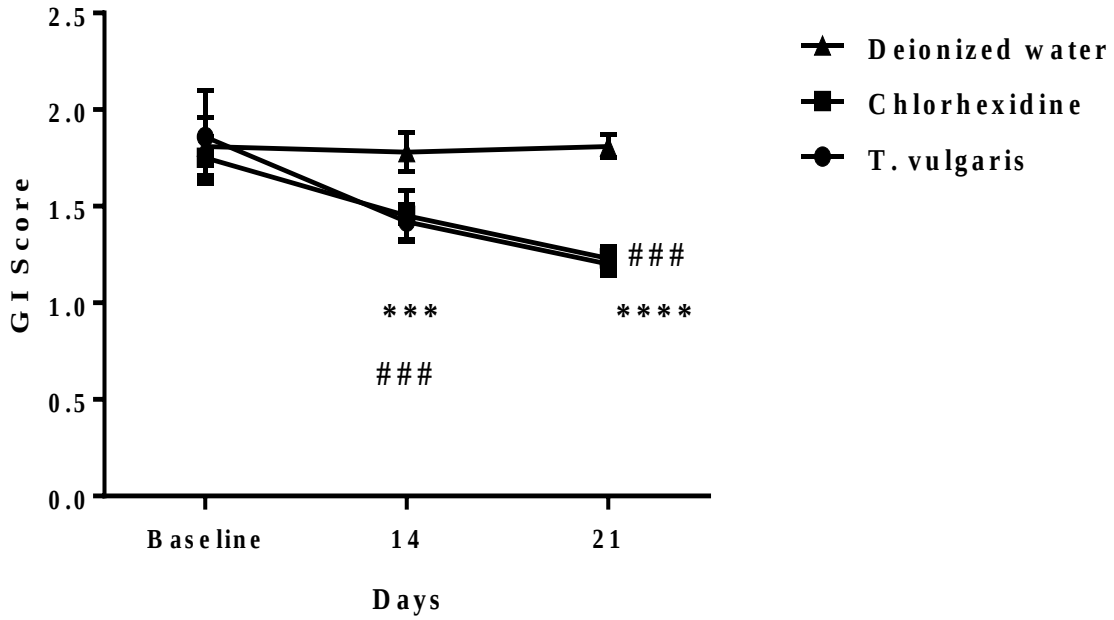


Fig.(3):- The results for comparison of GI in baseline and follow-up periods. ***=Significant differences between other groups with deionized water in the same day ###= Significant differences between T. vulgaris and chlorhexidine on days 14 and 21 compared with baseline

IL-6 levels

Table 4 shows the results for levels of IL-6 in different groups before and after intervention. There were no significant differences between groups in baseline (P=0.880), while significant differences were observed in follow-up period (P=0.001).

Table (4):-The results for the levels of IL-6 in different groups before and after intervention

	Baseline		**Follow-up**	
Groups	Mean± SD	P-value	Mean± SD	P-value
T. vulgaris	19.89±2.18	0.657	15.03 ±1.17	0.559
Chlorhexidine	19.53±2.09	NS	15.41±2.10	NS
T. vulgaris	19.89±2.18	0.726	15.03 ±1.17	0.001
Deionized water	19.64±1.44	NS	19.52±1.75	***
Chlorhexidine	19.53±2.09	0.863	15.41±2.10	0.001
Deionized water	19.64±1.44	NS	19.52±1.75	***

NS=Non-significant
***= Significant at P<0.001

The results did not show significant differences between all the groups in baseline (P>0.05). Furthermore, significant differences were not seen between T. vulgaris and Chlorhexidine groups at follow-up period (P>0.05). The T. vulgaris and Chlorhexidine groups showed significant differences with deionized water group in follow-up (P=0.001). The results showed lower levels of IL-6 in T. vulgaris and Chlorhexidine groups compared

with deionized water group (P=0.001). Thus, the treatment with T. vulgaris and Chlorhexidine mouthwashes decreased the levels of IL-6 in follow-up period.

Figure 4 shows the results for comparison of IL-6 in baseline and follow-up periods. The results showed significant differences for T. vulgaris and Chlorhexidine groups in baseline and follow-up (P=0.001) while significant differences were not seen in deionized water in baseline and follow-up periods (P=0.880). The treatment with T. vulgaris and Chlorhexidine mouthwashes decreased the levels of IL-6.

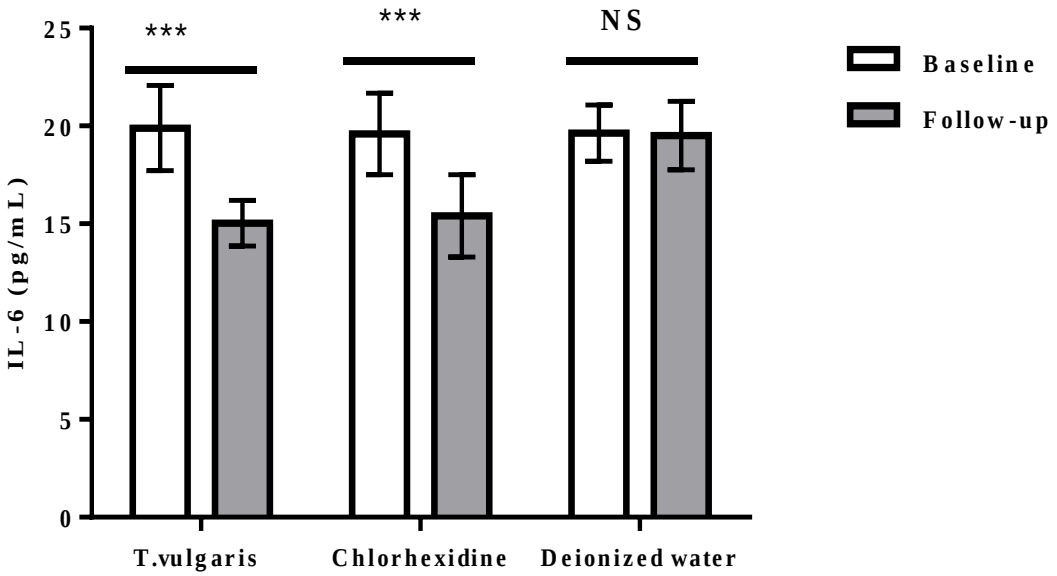


Fig.(4):- Comparison of IL-6 in baseline and follow-up periods. NS=non significant; ***=significant at P<0.001

DISCUSSION

This study aimed to evaluate clinical and anti-inflammatory effects of thymus extract mouthwash in patients with gingivitis. The results did not show significant changes in T. vulgaris mouthwashes pH. The results are in agreement with other studies for pH of *Libidibia ferrea* mouthwashes during a 60-day period (28). The lack of change in pH is a satisfactory factor. The pH= 4 is appropriate for removing the inflammation and plaques. The results show T. vulgaris mouthwash is appropriate for obtaining a desirable pH. Our findings in other parts also confirmed that mouthwash pH is appropriate for affecting clinical properties. In addition, patients did not report any side effect of T. vulgaris mouthwash during the application. It confirms that safety of T. vulgaris mouthwash.

The results showed a significant decrease in the mean scores of PI in the treatment groups. Similar with our findings, a meta-analysis showed that herbal mouthwashes and chlorhexidine decrease plaques in the treatment of gingivitis (14). Another studies have confirmed anti-plaque activity of herbal mouthwashes (29, 30). The mechanisms of action for the herbal mouthwashes against plaques have not been still elucidated due to the inherent complex nature of botanical products. The possible mechanisms of herbal mouthwashes could be attributed to active compounds that prevent the growth and adhesion of oral microorganisms and subside inflammatory cytokines such as IL-6 (31, 32). In the current study, application of T. vulgaris decreased the levels of IL-6 that is a reason for decreased plaque. IL-6 is an inflammatory marker and the decrease in IL-6 is parallel with the decrease in plaque. The results also showed better efficiency of T. vulgaris on day 21 compared with day 14. It means that T. vulgaris mouthwash needs a longer time for influencing plaques. However, it could compete with chlorhexidine.

Parallel with findings for PI, the results showed that T. vulgaris mouthwash decreased GI. Based on findings, T. vulgaris mouthwash lowered moderate inflammation and had similar effects with chlorhexidine. Several studies have reported the efficiency of herbal mouthwashes and chlorhexidine on GI (33, 34). The efficiency

of herbal mouthwashes on GI could be attributed to their effects on inflammatory responses and also their antibacterial activities (35). In this study, T. vulgaris mouthwash decreased the levels of IL-6 that confirms its anti-inflammatory properties. In addition, it could show higher effects in a longer time that confirms the need a longer time for affecting on inflammation.

This novel study was conducted on mouthwash of T. vulgaris extract in gingivitis mainly due to its anti-inflammatory effects of the extract. The results confirmed anti-inflammatory of the T. vulgaris mouthwash and showed a decrease in levels of IL-6 before and after intervention. IL-6 is a good marker for inflammatory responses due to its role in promoting human periodontal disease tissues (36), biofilm–gingival interface (37) and modulating in immune system (38). The findings are in agreement with another study reported the effects of *Polygonum cuspidatum* mouthwash (39). The current results are also in agreement with

a study reported the efficiency of plant essential oils on the levels of IL-6 in a murine model (40). Our findings are also in agreement with a study revealed the effects of canes and pomace mouthwashes on levels of IL-6 (41). The T. vulgaris mouthwash showed effects similar with chlorhexidine for the inflammatory responses. Chlorhexidine is known to have anti-inflammatory properties (42, 43). The results show appropriate anti-inflammatory properties of T. vulgaris. IL-6 may have a major role in the pathogenesis of gingivitis. The decrease in levels of IL-6 could be attributed to lower degree of inflammation (44). Microbial inflammatory responses in the mouth lead to produce plaque in the teeth and gums that results in plaque calcifying to form a scale on the surface of the teeth. Plaque and breeding grounds for oral bacteria result in the inflammatory gum diseases such as gingivitis (45). The plant extracts have antimicrobial activities against the oral bacteria by preventing the production of inflammation-related factors at a safe concentration (39, 46, 47). In addition, T. vulgaris decreases the inflammatory responses by modulation in several factors such as nitric oxide and other macrophage-related factors (48). Active compound of thymol decreases the levels of the cytokines and the expression of pro-inflammatory cytokines (25).

Studies have reported phenolic compounds in alcoholic extract of T. vulgaris such as gallic acid, rutin, naringin, hesperidin, rosmarinic acid, cinnamic acid, quercetin, etc that participate in anti-inflammatory responses (49-51). In sum, T. vulgaris decreased the levels of IL-6 and the inflammation. The results for GI showed that T. vulgaris decreases moderate inflammation at baseline to mild inflammation on day 21. The results confirm anti-inflammatory properties of T. vulgaris and also its ability for competing with chlorhexidine. In sum, T. vulgaris could compete with Chlorhexidine as a chemical agent and did not show significant differences. We did not observe any toxicity of Chlorhexidine during 21 days.

In this study, a short time period was considered to evaluate the effects of T. vulgaris mouthwash on IL-6 levels, PI and GI that is a major limitation. A 6-month period is required to evaluate the mouthwashes on IL-6 levels, PI and GI. In addition to anti-inflammatory responses on IL-6, microbiological assessment on plaque and/or saliva of the participants was not assessed that is another limitation for the current study. Microbiological responses are required to be evaluated in future.

CONCLUSIONS

In conclusion, T. vulgaris mouthwash could significantly decrease the mean levels of salivary IL-6 and clinical parameters (PI and GI) as chlorhexidine. So it can be used for the treatment of gingivitis. However, the results are promising and opened a window for further studies in relation with T. vulgaris.

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