Original Article

# Efficacy of Curcumin Mouth Wash on Gingival Inflammation in Patients with Chronic Gingivitis

*Chenar A. Mohammad*[1*]

## Abstract

**Objective:** For gingival disease, various modalities of treatment are available. Turmeric is a novel product obtained from plants that plays a vital role in treating gingival and periodontal diseases. This study aims to evaluate the anti-plaque and anti-inflammatory property effects of curcumin mouthwash (0.1%) on participants with plaque-induced gingivitis.

**Methods:** From eighty subjects, sixty participants with generalized gingivitis received meticulous scaling and polishing (S&P) and were divided randomly into 3 groups: Curcumin (CU), Chlorhexidine (CHX), and control (S&P) groups. Clinical parameters: PI, GI, BOP, and salivary IL-1β were assessed at baseline and after 4 weeks of therapy. The rest of the twenty patients with clinically healthy gingiva.

**Results:** The results showed a significant reduction in the mean values of PI, GI, BOP, and Il-1β levels after 4 weeks of therapy as compared to baseline in all 3 groups ($p \leq 0.05$), with the highest mean reduction of GI, BOP, and IL-1 β scores detected in the CU when compared to the CHX and S&P groups, and the highest mean reduction of PI scores detected in the CHX group in comparison to the CU and S&P groups ($p \leq 0.05$), with significant differences, existed between the 3 groups ($p \leq 0.05$).

**Conclusions:** CU was more effective than CHX when anti-inflammatory property effects were considered, while CHX was more effective than CU when anti-plaque property effects were considered. Therefore, CU mouthwash can be considered an effective alternative antigingivitis agent to CHX due to its anti-plaque and anti-inflammatory property effects.

**Keywords:** *Chlorhexidine, Curcumin, Gingivitis, IL-1 β, Mouthwash.*

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1. *Periodontics Department, College of Dentistry, Hawler Medical University, Erbil, Iraq.*

** Corresponding author: chenaranwar0@gmail.com.*

## Introduction

Periodontitis Gingivitis is a reversible chronic inflammatory disease limited to the gingiva without either attachment or alveolar bone loss, induced by the presence of microorganisms in the biofilm near the gingiva[1]. Dental plaque has been proved to be a paramount factor in the initiation and progression of gingival and periodontal diseases, and a direct relationship has been demonstrated between plaque levels and the severity of gingivitis[2]. The presence of bacterial lipopolysaccharides triggers the inflammatory response of the host, activating polymorphonuclear leukocytes and the secretion of inflammatory mediators such as cytokines and chemokines[3]. Among the inflammatory mediators, interleukin-1beta (IL-1$\beta$) presents the greatest correlation with the stage of periodontal disease, compared with other inflammatory mediators[4], and is considered a biomarker of periodontal disease.

Since bacterial plaque is the principal causative factor in gingival and periodontal diseases, the most rational methodology for the prevention of periodontal diseases would be regular effective removal of plaque by personal oral hygiene protocol, using procedures for plaque control that include mechanical and chemical means. Although mechanical plaque control methods are efficient in maintaining adequate levels of oral hygiene, studies have shown that patient compliance in following these methods is not adequate in a large population, and in order to overcome the shortcomings of mechanical plaque control methods, various chemotherapeutic agents have been employed and developed to control bacterial plaque, aimed at improving the efficacy of daily hygiene control measures[5]. Chemotherapeutic agents, including systemic antibiotics, antiseptic mouthwashes, as well as local drug delivery of antiseptics and antibiotics, and a host of modulating agents, have been used as an adjunct to the conventional periodontal therapy[5,6].

In the field of dentistry, Chlorhexidine (CHX) is regarded as the 'gold standard' anti-plaque treatment, is found to be particularly effective against gingivitis, and is widely used as an adjunct treatment for periodontitis; however, most practitioners do not recommend the long-term and daily use of CHX as a mouthwash, mainly because of its side effects, such as objectionable taste, tooth discoloration, desquamation, and soreness of oral mucosa[7]. Furthermore, synthetic antimicrobial agents and antibiotics are known to cause antimicrobial resistance, the emergence of previously uncommon infections, probably due to the inappropriate or widespread overuse of antimicrobials. Meanwhile, natural phytochemicals have been demonstrated to be good alternatives to such synthetic agents. The need to overcome the undesirable consequences associated with the wide-scale misuse of chemotherapeutic agents has therefore led to increased interest in plants with antibacterial and anti-inflammatory properties.

Turmeric (Curcuma longa) is a member of the ginger family, (Zingiberaceae family) and is widely cultivated in India and Southeast Asia. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1E,6E-heptadiene-3,5-dione or diferuloylmethane) is a polyphenol derived from turmeric, is responsible for the yellow color of turmeric, and is obtained from turmeric by ethanol extraction. Various studies showed that CU has antimicrobial, antioxidant, and anti-inflammatory properties, along with its hepatoprotective, immunostimulant, antiseptic, and antimutagenic properties[8,9]. The anti-inflammatory effect of CU has been explored in the treatment of gingivitis, both as a local application in the form of a gel and as a mouthwash[10,11]. Although improvement in clinical parameters has been detected, the anti-inflammatory effects of CU on immunological parameters have not been studied yet in relation to gingivitis. Therefore, the aim of the study was to evaluate the anti-plaque and anti-inflammatory property effects of 0.1% CU mouthwash on clinical parameters (PI, GI, and BOP), as well as on pro-inflammatory IL-1$\beta$ levels in saliva of patients with moderate to severe gingivitis, and to conduct comparisons with a group using a commonly used chemical plaque control, namely 0.12% CHX mouthwash (Kin, Spain), and a group receiving S&P only, at baseline before and after 4 weeks of therapy.

## Patients and methods

### Design, setting and time of study

Clinical comparative study was carried out in the Periodontic Department / College of Dentistry - Hawler Medical University, and BIO Clinical Laboratory in Erbil city. The study period extended from March 2019 to September 2020. The study protocol was reviewed and approved by the institutional ethical committee of the College of Dentistry/Hawler Medical University, Erbil, Kurdistan Region, Iraq.

### Study samples

Eighty participants were recruited from outpatients in the Department of Periodontology, College of Dentistry, Hawler Medical University in Erbil city. The study protocol, duration, purposes, and the possible risk factors associated with the study were explained to all

participants, and informed written consents were signed by all participants before the conduction of the study.

Patient selection is based on the following inclusion criteria: both genders; twenty to forty-six years of age; individuals with ≥ 20 teeth; systemically healthy subjects; and cooperative patients who could be motivated to comply with further oral hygiene instructions. Meanwhile, the exclusion criteria for the study were: the active focus of infection other than gingivitis; gingival recession; a pocket of more than 3mm; history of clinical periodontal treatment for the previous 3 months; antibiotic/anti-inflammatory drug treatment within 6 months prior to the experiment; history of known allergy to chlorhexidine or CU mouth rinse; smoking; alcoholism; pregnancy; lactating mothers, post-menopausal females; mouth breathing habit; and users of orthodontic or prosthetic appliance. From total subjects, twenty volunteers with clinically healthy gingiva with no clinical sign of gingival inflammation or periodontitis were recruited for this study, to estimate the normal mean value of clinical and immunological parameters only.

The rest 60 subjects with generalized plaque-induced gingivitis met the following inclusion criteria: had moderate to severe gingivitis (mean GI = 1.1-2 in moderate to 2.1-3 in severe gingivitis), probing depth PD≤ 3 mm, and had no clinical attachment loss (CAL=0) or furcation involvements, were divided into three main studied groups, 20 in each.

Initially, the selected participants who satisfied the inclusion criteria received mechanical treatment for plaque-induced gingivitis that consisted of scaling (supra and subgingival scaling) and polishing, and then were divided randomly into three equal main groups:

1. (CU) group, which included 20 participants, was instructed to rinse with 10 ml of prepared curcumin mouthwash (0.1%) twice daily for 4 weeks.
2. (CHX) group, including 20 participants, were instructed to rinse with 10 ml of CHX mouthwash (0.12%) (Kin Gingival, Spain) twice daily for 4 weeks.
3. Control (S&P) group, which included 20 participants, was instructed to follow up without using any mouthwash.

In the (CU) and (CHX) groups, the mouthwashes were used twice daily (once in the morning and once in the evening before sleeping) for just one minute, after half-hour of tooth brushing, and the patients were instructed to refrain from eating or drinking for at least half-hour after rinsing. All mouthwashes provided to the chronic gingivitis subjects were given free from cost during the entire duration of the study. Furthermore, all patients were instructed to use the same type of toothbrush (soft-bristled toothbrush the same type of toothpaste (Colgate toothpaste with fluoride), and were instructed to use the same type of brushing technique (Bass method brushing technique) twice a day during the entire study. All participants were also provided with a sheet on which to record their compliance with instruction and their complaints (side effects) after using the mouthwashes. Subjective side effects included: taste acceptability, dryness/soreness, and burning sensation assessments, while objective side effects included: ulcer formation, staining of teeth, staining of the tongue, and allergy assessments. This sheet had to be filled by the participants daily after using the mouth wash. Then all sheets were checked by the investigator after 4 weeks of therapy.

**Clinical parameters assessment**

All participants underwent a full-mouth periodontal examination at baseline before therapy and after 4 weeks of therapy by a single specialist examiner. At baseline before therapy, the subjects were assessed clinically for PI, GI, and BOP. The thickness of plaque was measured according to the PI by Silness and Löe[12]. The extent and severity of gingival inflammation were measured according to the gingival index GI by Löe and Silness [13]. Finally, the presence or absence of bleeding on probing BOP was assessed according to Ainamo and Bay[14], by passing a Williamś periodontal probe gently along the inner surface wall of the gingival sulcus and noting bleeding after 30 seconds as either present (1) or absent (0). The surface sites of the measurements were the mesiobuccal/labial, distobuccal/labial, mid-buccal/labial, and midpalatal/lingual sites for all teeth. Following the measurement of the clinical parameters, scaling (supra and subgingival scaling) and polishing were performed for each patient with gingivitis in a single visit, and the patients were instructed to follow the mouthwash usage as per the instructions. The clinical parameters measurements were then assessed again at the end of the 4-week therapy period.

**Saliva sample collection and analysis**

Saliva samples were collected in the morning (between 9:00 and 11:00) according to the following procedure described by Navazesh[15]. The spitting method was used for the collection of un-stimulated saliva at the baseline before clinical parameters assessments and after 4 weeks of therapy, and in patients with clinically healthy gingiva. The samples were collected in disposable, sterilized plastic test tubes, then centrifuged immediately at 4000 rpm for 20 minutes to obtain clear supernatant, and finally stored at-20ºc for later estimation of IL-1β by ELISA technique. Salivary IL-1β levels were assessed using an enzyme-linked immunosorbent assay (Elisa technique) supplied by a specialized kit company (KOMA BIOTECH INC, USA).

**Preparation of Curcumin mouthwash**

Curcumin was prepared as a mouthwash according to Waghmare et al.'s method[16], by an expert pharmacologist, at the Department of Pharmacology/College of Pharmacy / Hawler Medical University/Erbil city/Iraq.

**Curcumin extraction and mouth wash preparation**

At first, the raw material, turmeric rhizome, was obtained from a bioorganic Indian grocery store.

In curcumin extraction, the turmeric root was ground into powder using a mortar and was air-dried to remove moisture in the ground powder. Then a known amount of turmeric powder (100 g) was weighed accurately and was soaked in 99% ethanol for 48 hrs. The filtrate that was procured was an even amber-colored ethanol solution. The ethanol was allowed to evaporate by air drying and was placed in a microwave at 200 F until a dry extract enriched in CU was obtained.

Preparation of the Curcumin mouthwash was in accordance with a previous study described by Waghmare et al. 2011[16]. This entailed dissolving 10 mg of curcumin extract in 100 ml of distilled water, then adding a flavoring agent (0.005% peppermint oil) and adjusting the pH to 4. The participants in the CU group were asked to gargle with 10 ml of mouthwash in 1:1 dilution with water twice a day, after half-hour of toothbrushing for 4 weeks.

**Statistical analysis**

Data were presented as numbers, mean, standard deviation, and degree of freedom. The data were statistically analyzed using the SPSS software package (version 22; SPSS Inc., Chicago, IL, USA). Normality of the data was tested using Kolmogorov-Smirnov. Z test (Wilcoxon Signed Ranks Test) was used to compare the mean values of PI, GI, BOP, and IL-1 beta of sixty participants between baseline and after 4 weeks of therapy. For each group, for the data with normal distribution, paired t-test was applied to compare the mean values of PI in the CU group, GI in the CHX group, BOP in CHX and S&P groups, between baseline and after 4 weeks of therapy, while for the data with non-normal distribution, Wilcoxon test was used to compare the mean values of IL-1$\beta$ in three studied groups, PI in CHX and S&P groups, and GI in CU and S&P groups between baseline and after 4 weeks of therapy. ANOVA test was applied for the comparison between the mean differences of three studied groups in regard to PI & IL-1$\beta$ parameters, accompanied by a post hoc test (LSD) for multiple pair groups comparison, while the Kruskal-Wallis H test was used for the comparison between the mean differences of three studied groups in regard to GI&BOP parameters, accompanied with Man –Whitney U test for the comparison between variable two groups. $P \leq 0.05$ was considered statistically significant.

## Results

**Patients background**

A total of eighty individuals of both sexes were recruited for the study with a mean age of 33.28 ± 5.715 years. 55 subjects were male (61.1%) and 25 subjects were female (38.9%). Of the total number, twenty subjects with normal healthy gingiva with a mean age of 33.25±5.893 years. The rest of the sixty subjects with gingivitis (moderate to severe type) with a mean age of 33.28±5.705 years. Among these, 43 were male (71.7%) and 17 were female (28.3%) as shown in Table 1.

**Clinical and immunological parameters results**

Table 2 shows that the mean values of PI, GI, BOP, and pro-inflammatory cytokine IL-1 $\beta$ level among the 60 participants with plaque-induced gingivitis at baseline before therapy were: 2.100±0.230, 2.062±0.154, 68.763±5.280, 17.646±0.543, respectively. Then these values were significantly reduced to 0.553±0.187, 0.745±0.166, 25.234±5.723, and 12.097±0.981, respectively, after 4 weeks of therapy ($P \leq 0.05$).

Table 3 shows that the mean values of PI in the first CU, second CHX, and the third S&P groups were significantly highly decreased after 4 weeks of therapy as compared to baseline ($P \leq 0.05$), and the mean difference of PI from baseline to 4 weeks (mean improvement of PI scores) was highest in the second CHX group (1.745±.232), followed by the first CU

group (1.530±0.270), and the least was in the S&P group (1.365±0.187). Regarding GI and BOP, the mean values were also highly significantly reduced after 4 weeks of therapy as compared to baseline in all three studied groups (*P≤0.05)*, and the mean differences from baseline to 4 weeks for GI and BOP scores (mean improvement of GI and BOP scores) were highly detected in the first CU group (1.472±0.134) (48.921±4.148), followed by the second CHX group (1.345±0.145) (44.369±4.865), and then the third S&P group (1.134±0.183) (37.298±6.232), respectively. Finally, for IL-1*β*, the mean values were also highly significantly reduced after 4 weeks of therapy as compared to baseline in all three studied groups (*P≤0.05)*, with the highest mean difference in IL-1*β* levels (best mean improvement of IL-1 *β* scores), was detected in the CU group (5.837±0.218 pg/ml), followed by S&P group (5.413±0.672 pg/ml), and CHX (5.398±0.627 pg/ml) group.

Meanwhile, for the comparison between the mean differences of three studied groups in regard to PI, and IL-1β, using one way-Anova test, Table 4 revealed significant differences between the mean differences of the three studied groups *(P≤0.05*), accompanied by significant differences in the mean improvements between two groups(*P≤0.05),* with the exception of the comparison between CHX and S&P group in regard to IL-1*β (P >0.05).* Also, significant differences between the 3 groups in regard to GI and BOP(*P≤0.05*), using the Kruskal-Wallis H test, accompanied by significant differences in the mean improvements between the 2 groups *(P≤0.05).*

Regarding participants' complaints, no subjective side effects were detected in CU group, while a tolerable taste was detected in 2 participants using CHX mouthwash after 7 days (Table 5). Additionally, staining of the teeth was seen in 3 participants from the CHX group (in the lingual surface of lower incisors) after 21 days of mouth rinsing, while no complaints were observed in the CU group.

Table 1: Age and gender distribution of participants.

| Variables | Levels | CU+CHX+NC groups | | Healthy gingiva | | All groups | |
|---|---|---|---|---|---|---|---|
| | | N. | % | N. | % | N. | % |
| Age | 20 - 28 | 16 | 26.7 | 6 | 30.0 | 22 | 27.5 |
| | 29 - 37 | 32 | 53.3 | 7 | 35.0 | 39 | 48.8 |
| | 38 - 46 | 12 | 20.0 | 7 | 35.0 | 19 | 23.8 |
| | Total | 60 | 100.0 | 20 | 100.0 | 80 | 100.0 |
| | Mean ±S.D | 33.28±5.705 | | 33.25±5.893 | | 33.28 ± 5.715 | |
| Gender | Male | 43 | 71.7 | 12 | 60.0 | 55 | 61.1% |
| | Female | 17 | 28.3 | 8 | 40.0 | 25 | 38.9% |
| | Total | 60 | 100.0 | 20 | 100.0 | 80 | 100% |

N: number of samples; %: percentage; S.D: standard deviation.

Table 2: Comparison of the mean overall PI, GI, BOP, and IL-1 *β* values among participants with chronic gingivitis at baseline, and after 4 weeks of therapy, using the Z test (Wilcoxon Signed Ranks Test).

| Groups | Index | Time | N | Mean ± SD | Z test | P-Value |
|---|---|---|---|---|---|---|
| All 3 groups | PI | Base line | 60 | 2.100±0.230 | -6.746 | P<0.001 |
| | | 4weeks | 60 | 0.553±0.187 | | |
| | GI | Base line | 60 | 2.062±0.154 | -6.743 | P<0.001 |
| | | 4weeks | 60 | 0.745±0.166 | | |
| | BOP% | Base line | 60 | 68.763±5.280 | -7.738 | P<0.001 |
| | | 4weeks | 60 | 25.234±5.723 | | |
| | IL-1β pg/ml | Base line | 60 | 17.646±0.543 | -6.739 | P<0.001 |
| | | 4weeks | 60 | 12.097±0.981 | | |

Table 3: Comparison of the mean value of PI, GI, BOP, and IL-1 beta between baseline and after 4 weeks of therapy for each group with the mean differences from baseline to 4 weeks.

| Index | groups | Time | N | Mean ± SD | Mean differences (base line-4weeks) | test | P-Value |
|---|---|---|---|---|---|---|---|
| PI | CU | Baseline | 20 | 2.125±0.273 | 1.530± 0.270 | 25.325′ | P<0.001 |
| | | 4 weeks | 20 | 0.595±0.193 | | | |
| | CHX | Baseline | 20 | 2.150±0.259 | 1.745±0.232 | -3.932″ | P<0.001 |
| | | 4 weeks | 20 | 0.405±0.119 | | | |
| | S&P | Baseline | 20 | 2.025±0.121 | 1.365±0.187 | -3.938″ | P<0.001 |
| | | 4 weeks | 20 | 0.660±0.143 | | | |
| GI | CU | Baseline | 20 | 2.073±0.133 | 1.472±0.134 | -3.937″ | P<0.001 |
| | | 4 weeks | 20 | 0.601±0.087 | | | |
| | CHX | Baseline | 20 | 2.070±0.153 | 1.345±0.145 | 41.482′ | P<0.001 |
| | | 4 weeks | 20 | 0.725±0.094 | | | |
| | S&P | Baseline | 20 | 2.045±0.181 | 1.134±0.183 | -3.929″ | P<0.001 |
| | | 4 weeks | 20 | 0.911±0.134 | | | |
| BOP (%) | CU | Baseline | 20 | 69.114±4.539 | 48.921±4.148 | -3.923″ | P<0.001 |
| | | 4 weeks | 20 | 20.193±2.889 | | | |
| | CHX | Baseline | 20 | 69.074±5.090 | 44.369±4.865 | 40.784′ | P<0.001 |
| | | 4 weeks | 20 | 24.704±3.173 | | | |
| | S&P | Baseline | 20 | 68.104±6.285 | 37.298±6.232 | 26.764′ | P<0.001 |
| | | 4 weeks | 20 | 30.806±4.857 | | | |
| IL-1β (Pg/ml) | CU | Baseline | 20 | 17.502±0.539 | 5.837±0.218 | -3.937″ | P<0.001 |
| | | 4 weeks | 20 | 11.665±0.533 | | | |
| | CHX | Baseline | 20 | 17.763±0.577 | 5.398±0.627 | -3.921″ | P<0.001 |
| | | 4 weeks | 20 | 12.365±1.069 | | | |
| | S&P | Baseline | 20 | 17.676±0.508 | 5.413± 0.672 | -3.925″ | P<0.001 |
| | | 4 weeks | 20 | 12.263±1..124 | | | |

Table 4: Comparison between the three studied groups using Anova test for PI and IL-1 $\beta$ / or Kruskal-Wallis H test for GI and BOP, accompanied with post hoc test (LSD)/or Mann-Whitney U test to compare the mean differences between multiple 2 groups.

| Index | Groups | N. | Mean difference ±SD | P-Value (3 groups) | Sig. | Groups | P-Value (2 groups) |
|---|---|---|---|---|---|---|---|
| PI | A) CU | 20 | 1.530±0.270 | 13.430‘ | P<0.001 | B×A | 0.005" (HS) |
| | B) CHX | 20 | 1.745±0.233 | | | C×A | 0.029" (S) |
| | C) S&P | 20 | 1.365±0.187 | | | C×B | <0.001" (HS) |
| | Total | 60 | 1.547±0.277 | | | | |
| IL-1β (pg/ml) | A) CU | 20 | 5.838±0.218 | 4.173‘ | 0.02 | B×A | 0.014" (S) |
| | B) CHX | 20 | 5.398±0.628 | | | C×A | 0.017" (S) |
| | C) S&P | 20 | 5.414±0.673 | | | C×B | 0.929 " (NS) |
| | Total | 60 | 5.550±0.575 | | | | |
| GI | A) CU | 20 | 1.472±0.134 | 23.970▸ | <0.001 | B×A | 0.013▸▸ (S) |
| | B) CHX | 20 | 1.345±0.145 | | | C×A | <0.001 ▸▸(HS) |
| | C) S&P | 20 | 1.134±0.184 | | | C×B | <0.001▸▸ (HS) |
| | Total | 60 | 1.317±0.208 | | | | |
| BOP % | A) CU | 20 | 48.922±4.148 | 25.819▸ | <0.001 | B×A | 0.007▸▸ (HS) |
| | B) CHX | 20 | 44.370±4.865 | | | C×A | <0.001▸▸ (HS) |
| | C) S&P | 20 | 37.298±6.232 | | | C×B | <0.001▸▸ (HS) |
| | Total | 60 | 43.530±6.995 | | | | |

PI: plaque index;IL-1β: Interlukine 1beta ; GI : gingival index; BOP:bleeding on probing ;CU:curcumin;CHX: chlorhexidine; S&P:scaling and polishing ; SD: standard deviation ; N: number; Sig: significancy ; P: probability ; ‘= *Anova test ;* "= post hoc test (LSD) ; ▸ = Kruskal-Wallis H test ; ▸▸= Mann-Whitney U test ; $P \leq 0.05$ was considered as significant and $P>0.05$was considered as non-significant.

Table 5: Subjective and objective side effects records.

| Group | N. | Taste acceptability | | | Burning | | Dryness/Soreness | |
|---|---|---|---|---|---|---|---|---|
| | | Accep-table | Toler-able | Unaccep-table | Absent | Present | Absent | Present |
| CU | 20 | 20 | 0 | 0 | 20 | 0 | 20 | 0 |
| CHX | 20 | 18 | 2 | 0 | 20 | 0 | 20 | 0 |

| | | Ulcer | | Teeth staining | | Tongue staining | | Allergy | |
|---|---|---|---|---|---|---|---|---|---|
| | | Present | Absent | Present | Absent | Present | Absent | Present | Absent |
| CU | 20 | 0 | 20 | 0 | 20 | 0 | 20 | 0 | 20 |
| CHX | 20 | 0 | 20 | 3 | 20 | 0 | 20 | 0 | 20 |

N: number of participants.

## Discussion

### Clinical findings

Regarding the anti-plaque property effect, the mean values of PI were measured at baseline and after 4 weeks in all three studied groups. In the CU group, the mean value of PI was significantly reduced after 4 weeks of therapy as compared to baseline *(P ≤0.05)*. This may have been due to the anti-plaque property effect of curcumin mouthwash. Our results related to curcumin are similar to those studies carried out by Waghmare et al.[16], Chatergee et al.[17], and Arunachalam et al.[18], where turmeric was used as a mouthwash. Curcumin has demonstrated a wide range of activities, including antimicrobial, anti-inflammatory, and antioxidant agents. The lipophilic nature of the molecule allows rapid permeability of the cell membrane, and the reduction in the plaque level at the end of the 4 weeks follow-up period could also be attributed to the bactericidal property of the curcumin herbal product. The curcumin mimics various events that happen during the apoptosis process by causing changes in the function and structure of the cell membrane[19]. The antibacterial effect of CU is well documented and involves the activity of many microorganisms[20]. Mohammed et al.[21] have shown that the antimicrobial activity of CU may be useful for controlling dental biofilms, and Waghmare et al.[16] have reported a significant reduction in the total microbial count with the use of turmeric mouthwash, making it a suitable candidate as an anti-plaque agent.

For the second CHX group, the mean value of PI was also significantly reduced after 4 weeks of therapy as compared to baseline ($P \leq 0.05$), indicating that chlorhexidine has anti-plaque activity effects. This result was to be expected since CHX is regarded as the 'gold standard of chemical plaque control and is found to be particularly effective against gingivitis. Similar results were obtained by Leyes et al.[22], who compared the effect of chlorhexidine with and without alcohol along with placebo, by Waghmare et al.[16], who used 0.2% chlorhexidine gluconate with base alcohol, Grundemann et al.[23], who used chlorhexidine alone(0.12%) and chlorhexidine in combination with an oxidizing agent (sodium perborate monohydrate), Chatterjee et al.[17], Arunachalam et al.[18], who used CHX gluconate in a concentration of 0.2%, and Francetti et al.[24], all the previous studies reported anti-plaque activities of CHX.

Our study also revealed that the best improvement in the mean values of PI scores (The highest mean difference from baseline to 4 weeks) was detected in the CHX group followed by the CU group. This superior antiplaque property effect of chlorhexidine gluconate could be due to chlorhexidine having the property of substantivity (The high surface substantivity of CHX enables it to remove high concentrations of microorganisms). It could also be due to the action of chlorhexidine at different levels of plaque formation, the property of slow-release, and the concentration used was 0. 2%. Although curcumin showed an antiplaque property effect, it was observed to be slightly less effective in comparison with chlorhexidine, and this might be due to the dilution of curcumin which was followed in the present study. These results are similar to the results obtained by other studies.[16,20,25], which found a larger mean reduction of PI in the CHX group than in the curcumin group when curcumin and chlorhexidine were used as a mouthwash[16,20] or in gel form[25]. However, in contrast to our study, Arunachalam et al.[18] reported that there was no significant difference between the CHX and CU groups after 4 weeks of therapy, and denoted that both were comparable in terms of the anti-plaque property. The

difference between our and those results could be due to the limited number of participants (10 in each group) in that study, which may have influenced the results.

Regarding the anti-inflammatory property effect, clinically, the mean values of GI and BOP were measured at baseline and after 4 weeks in all three studied groups. In the CHX group, statistically significant reductions in the mean values of GI and BOP were detected after 4 weeks of therapy as compared to baseline before therapy, which may have been due to the anti-inflammatory property effects of CHX. Similar to the findings of the present study, studies carried out by[16-18,22,23,26] reported a significant reduction of gingival inflammation (GI) using chlorhexidine gluconate.

Regarding the CU group, significant reductions in the mean values of GI and BOP were also reported after 4 weeks of therapy as compared to baseline ($p \leq 0.05$). This may have been due to the anti-inflammatory property effect of CU, which inhibits inflammatory mediator, tumor necrosis factor TNF-dependent NFκB activation, reducing the production of reactive oxygen species ROS[27].

Arora et al.[28] evaluated the anti-inflammatory property of turmeric and, similar to our study, found a significant reduction in gingival inflammation ($p<0.01$). Similar results were also obtained in studies carried out by Srimal and Deodhar[29] and Ghatak and Basu[30]. In the above studies, the anti-inflammatory effect was observed in relation to turmeric but not in relation to the gingiva. In our study, the anti-inflammatory property effects of curcumin mouthwash on the gingiva were evaluated clinically using GI and BOP, and immunologically using salivary IL-1 beta level. These results are nearly similar to studies[16,17,25], that reported the anti-inflammatory action of turmeric on clinical parameters using GI and the sulcus bleeding index SBI in the cases of Chatterjee et al.[17] and Singh et al.[25], and GI in the cases of Waghmare et al.[16] and Arunachalam et al.[18].

The possible mechanism of action of curcumin as an anti-inflammatory agent could be due to the inhibitory action on prostaglandin synthesis E2 (PGE2) and a strong stabilizing action on the lysosomal membranes[31], and could also be due to the inhibitory action of inflammatory mediators of arachidonic acid metabolism. CU selectively inhibits the synthesis of PGE2 and thromboxane, while not affecting the synthesis of prostacyclin[27]. Curcumin, by virtue of its anti-inflammatory property, reduces inflammatory mediators and causes shrinkage by reducing inflammatory edema and vascular engorgement of connective tissue[32]. It has also been shown that curcumin incorporated in collagen, which acts as a supportive matrix for the slow release of curcumin, increases wound reduction, and enhances cellular proliferation[33].

However, the best improvement in the mean values of GI and BOP scores (the highest mean difference from base line to 4 weeks) was detected in the CU group, followed by the CHX group, indicating that both types of mouthwash were effective in reducing the gingival inflammation but with non-equal results, since better anti-inflammatory properties were detected clinically in the CU than the CHX group. Our results are in concordance with the finding by Arunachalam et al.[18] reported that CU mouthwash had better anti-inflammatory effects than CHX mouthwash, based on a more significant reduction of GI and reactive oxidative metabolites ROMs in the CU group after 4 weeks of therapy. Mali et al.[10] reported improvement in results in clinical indices for both CU and CHX types of mouthwash, while Suhag et al.[32] reported that 1% CU solution could resolve signs and symptoms of inflammation better than CHX and saline groups when used as a subgingival irrigant. In contrast to our study, another study[16] reported that both curcumin and CHX types of mouthwash were equally effective in reducing the gingival inflammation (GI) from baseline to 14 days and their results were explained as due to their anti-inflammatory properties. Meanwhile, another study [25], reported that the means of GI and sulcus bleeding index SBI were reduced after 2 and 3 weeks in both curcumin and CHX groups, but higher reductions were detected in the CHX group as compared to the curcumin group. The difference in results between that study and our study could be due to the use of different formulations – gel in that study versus mouthwash in our study.

**Immunological findings**

Regarding the anti-inflammatory property effect of CU, the mean value of inflammatory marker IL-1β was measured at baseline and after 4 weeks in all three studied groups, In our study, the results showed high mean levels of inflammatory cytokine Il-1$\beta$ in the saliva of plaque-induced gingivitis participants at baseline before therapy in all three studied groups, which then significantly reduced after 4 weeks of therapy *($P \leq 0.05$)*, with the best mean reduction of IL-1$\beta$ level from baseline to 4 weeks detected in the CU group in comparison to the CHX and control groups *($P>0.05$)*.

Since inflammatory cytokines are secreted in response to inflammatory and infectious stimuli, increased levels of inflammatory cytokines have been detected in the saliva of patients with plaque-induced gingivitis, and the presence of this inflammatory marker in the saliva is important to determine the presence, risk, and transition phase between healthy gingiva and gingivitis[34]. Therefore, the presence of elevated levels of IL-1β is related to the pathogenesis and progression of periodontal disease, because the secretion of this cytokine requires the exposure of cellular alterations or stress signals triggering the activation of inflammatory cells, which mediate the action of caspase-1, essential for IL-1$\beta$ activation[35]. In general, IL-1$\beta$ plays an important role in the neutrophil migration capacity by stimulating the spread of these inflammatory cells through the blood vessels, and reducing the concentration of this cytokine may decrease the need for migration. The inflammatory process can be reduced, either by mechanically removing the biofilm[34], which results in reducing gingival inflammation, as in the S&P group, or by using 0.2% CHX mouth rinse or curcumin mouth rinse as an adjunct to mechanical therapy for better plaque control.

Another reason for the superior salivary IL-1$\beta$ reduction in the curcumin group after 4 weeks of therapy could be that curcumin is known to possess potent immune-modulating agents which down-regulate the expression of the cyclooxygenase-2 enzyme (an enzyme that catalyzes the synthesis of PGs and is linked to most forms of inflammation, including periodontitis) and inhibit the expression of pro-inflammatory cytokines. The downregulation of various inflammatory cytokines such as TNF, IL-1, IL-6, IL-8, interferon, and some other chemokines is also carried out by curcumin[36]. Moreover, another study reported that the anti-inflammatory mechanism of action of CU could be due to the blockage of arachidonic acid metabolism, namely, (1) inhibition of arachidonic acid metabolism through lipoxygenase and scavenging of free-radicals generated in this pathway; and (2) decreased expression of inflammatory cytokines: interleukin (IL)-1b, IL-6, and tumor necrosis factor-alpha[37].

Additionally, in our study, reduction of PI, GI, BOP, and IL-1 beta levels were also detected in the S&P group after 4 weeks of mechanical therapy (scaling and polishing alone) as compared to baseline before therapy. This could have been due to patients' motivation to maintain good oral hygiene. Furthermore, proper mechanical plaque control could have resulted in the resolution of gingival inflammation, This result is very similar to that in a study performed by Türkoğlu[38], who reported improvement in the clinical parameters PI, GI, sulcus bleeding index SBI, calculus index, and pocket depth PD after 4 weeks of rinsing with placebo in one group, and CHX in the other group, while gingival crevicular fluid GCF IL-1alpha and IL-1Ra levels remained unchanged after 4 weeks.

Regarding patients' compliance and complaints, in the CU group, there was good compliance with no adverse effects, while in the CHX group, a bitter taste was noticed by two participants, and tooth staining was experienced by three participants. However, a limitation of the study was the short duration of the clinical trial and the limited sample size. Additional studies are required to evaluate the anti-plaque and anti-inflammatory property effects of CU for a longer duration of time, using large sample size, and different concentrations.

## Conclusions

Curcumin CU mouthwash, as an anti-inflammatory agent, was found to be more effective in the reduction of gingival inflammation than CHX mouthwash, as was demonstrated clinically throughout more significant reduction of GI, and BOP, and immunologically throughout more significant reduction of inflammatory mediator IL-1$\beta$. Meanwhile, chlorhexidine mouthwash was more effective than CU mouthwash in terms of anti-plaque effect, as was demonstrated clinically throughout a larger reduction of PI scores after 4 weeks of therapy. Therefore, like chlorhexidine mouthwash, curcumin mouthwash can be used for effective prevention of plaque, gingival inflammation, and treatment of gingivitis, but without the unfavorable side effects of bitter taste and teeth staining found with CHX.

## Conflicts of Interest

No conflicts of interest are reported.

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