

# Efficacy of Mouthwash Containing Hydrogen Peroxide and Hyaluronic Acid on Biofilm-Induced Gingivitis: A 14 Days Randomized Clinical Trial

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## Abstract

**Background:** Dental biofilm is the primary cause of the periodontal disease. Hence, control of dental biofilm is crucial for maintaining oral health. **Objectives:** This study aims to investigate the anti-inflammatory and anti-plaque effectiveness of an oral solution containing hyaluronic acid (HA) and hydrogen peroxide ( $H_2O_2$ ), as well as to evaluate any potential adverse effects and obtain feedback from the participants involved. **Materials and Methods:** The present investigation employed a short-term, parallel, and double-masked design. The present study comprised a study group of 54 students who exhibited gingivitis induced by biofilm and were ultimately included in the analysis. The individuals were allocated randomly into three groups: [HA and  $H_2O_2$  (HA/ $H_2O_2$ ),  $n = 18$ ; chlorhexidine (CHX) group,  $n = 18$ ; and placebo group,  $n = 18$ ]. Clinical parameters (Gingival index and plaque index). Additionally, participants completed a visual analog scale-based assessment questionnaire. **Results:** There was a significant reduction in gingivitis in the HA/ $H_2O_2$  and CHX groups, but mouthwash with HA/ $H_2O_2$  had a greater effect size than CHX mouthwash. In addition, all interventions considerably decreased plaque outcomes, but CHX had a greater effect size than HA/ $H_2O_2$  mouthwash. The analysis of participant comments on the mouthwashes revealed that the HA/ $H_2O_2$  mouthwash was preferred over the CHX mouthwash. **Conclusions:** Mouthwashes containing ( $H_2O_2$ /HA) mouth rinse had significant anti-inflammatory properties and satisfactory levels of participant perception compared to the results associated with CHX use. However, it is less effective compared to CHX in reducing plaque. According to the findings of this research, mouthwashes containing (HA/ $H_2O_2$ ) might be utilized as an alternative to CHX.

**Keywords:** Gingivitis, hyaluronic acid, hydrogen peroxide, mouthwash

## INTRODUCTION

Biofilm-induced gingivitis is an inflammation of the gingivae that can cause bleeding (either on its own or after periodontal probing), gingival hypertrophy, erythema, or swelling of the affected tissues. Still, it does not lead to the loss of tooth-supporting tissue.<sup>[1]</sup> The most prevalent periodontal disease impacting people today is gingivitis, which has a high propensity to develop into a more serious periodontal disease if not treated early.<sup>[2,3]</sup> Nevertheless, treating of this kind of disease is essential as a preventative measure vs. more advanced periodontal disease, thereby maintain periodontal support and reducing the demand for more complex and expensive procedures.<sup>[4]</sup> Biofilm-induced gingivitis can be effectively

treated through measures such as motivation and oral hygiene instruction (OHI), professional mechanical plaque elimination, and anti-inflammatory products for oral hygiene.<sup>[5]</sup> CHX gluconate-containing mouthwashes have received the most attention as antimicrobial mouthwashes<sup>[6]</sup> and are the standard gold mouthwash used for chemical plaque control.<sup>[7]</sup> Unfortunately, CHX mouthwashes exhibits multiple unfavorable adverse

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reactions, including brown extrinsic staining of teeth, interference with taste function, bitter flavor, and promotion of calculus buildup.<sup>[8-10]</sup> These adverse effects necessitate the development of alternative mouthwashes with comparable efficacy, yet without these adverse effects.

This research will investigate a mouth rinse containing hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hyaluronic acid (HA). H<sub>2</sub>O<sub>2</sub> is an oxygenating agent, has antimicrobial properties due to the oxygen it releases, and is effective against both gram-positive and gram-negative bacteria;<sup>[11]</sup> a recent study found that H<sub>2</sub>O<sub>2</sub> has anti-inflammatory and anti-plaque efficacy.<sup>[12]</sup> HA is a naturally occurring polysaccharide numerous human tissue,<sup>[13]</sup> including the umbilical cord, epidermis and synovial fluid in addition, periodontal ligament and gingiva contain HA, while the cementum and alveolar bone contain trace quantities.<sup>[14]</sup>

Consequently, the present investigation goals to compare the efficacy of mouthwash containing HA/H<sub>2</sub>O<sub>2</sub> to a control group (CHX) and distilled water. The objective of the 2-week investigation was to examine the potential anti-inflammatory and anti-plaque properties of HA/H<sub>2</sub>O<sub>2</sub> oral rinses as adjuncts to oral hygiene in managing gingivitis. Furthermore, an assessment of the feedback provided by the participants with respect to the utilization of each oral rinse employed.

## MATERIALS AND METHODS

### Study designs

The present study was a double-blind clinical trial with a parallel three-arm design, conducted at the Faculty of Dentistry, University of Karbala's, Karbala, Iraq, during March and April of 2023. We committed to the Helsinki Declaration for biomedical research involving human participants and the CONSORT 2010 Statement for documenting multiarm studies. Volunteers attended the hospital's dental clinics at the beginning and the end of the investigation (14-day period). The study obtained ethical approval from the Research Ethics Committee at the Faculty of Dentistry, University of Baghdad, under Protocol number 748622. This clinical trial was registered on Clinicaltrials.gov (US National Institute of Health Registry) in 2023 under protocol number NCT05787600.

### Study subjects

Participants were selected from undergraduate students seeking treatment at the hospital's dental clinics of Faculty of Dentistry, University of Karbala. An advertisement was placed in the reception room area inviting students to participate as volunteers in the research study. Individuals who met the predetermined criteria for inclusion were extended an invitation to take part in the present investigation.

The inclusion criteria included systemically healthy participants who were nonsmokers, had >20 natural teeth, who had been diagnosed with biofilm-induced generalized gingivitis (having >30% bleeding sites with no PPD > 3mm, intact periodontium, and no loss of periodontal attachment)<sup>[5]</sup>, without the presence of supra or subgingival calculus discovered by sight or through a periodontal probe, and regularly brushed their teeth. The exclusion criteria: patients unable to follow hygiene instructions, those having periodontitis, current usage of any oral rinse, smoking habitual behavior, presence of extensive neglected dental caries, wearing any dental appliances, periodontal therapy over the prior 6 months, antibiotic therapy 3 months prior to participating in the present trial, pregnant or breastfeeding women, and those having a history of allergic reactions to any of the items used in the present investigation.

After participant selection, the purpose of the investigation was conveyed to each prospective subject, and written permission was obtained. More instruction in OHI was not administered to preserve the subjects' existing oral hygiene routines.

### Calibration

Before initiating the investigation, calibration sessions were conducted to establish consistency between the primary examiner and a specialist in periodontics (interexaminer) and for self-assessment purposes (intraexaminer) were carried out using the teeth of five student volunteers (who were not included in the present study to avoid examiners' unconscious bias).

The calibration sessions were repeated until a kappa coefficient agreement level of >75% was achieved for categorical parameters such as plaque index (PI) and gingival index (GI).

### Study interventions

The clinical trial will employ a three-arm design, utilizing three different types of mouth rinses. The first rinse, KIN gingival from KIN, Spain, will contain 0.12% CHX and serve as the positive control. The second rinse will consist of distilled water with food coloring to ensure double-blind conditions and act as a placebo. Finally, the third rinse, Perhyal from BMG Pharma in Milan, Italy, will contain H<sub>2</sub>O<sub>2</sub> (1.80%) and HA (0.10%) as active ingredients, with the remaining 97.3% consisting of water and inert additives. This rinse will serve as the test intervention. The mouthwashes will be stored in matching impermeable bottles and randomly labeled by letter (A, B, and C) by another subject who does not take part in the clinical study. Thus, ensuring volunteer and investigator blindness throughout the clinical investigation. Decoding was carried out following the completion of the investigation.

## Clinical research design

A single experienced and calibrated examiner performed the subjects' selection, enrollment, and evaluation.

In the baseline visit at the beginning, the measurement of clinical periodontal parameters for all teeth except wisdom, start with the GI, which is a tool that has been specifically developed to evaluate the degree and nature of gingival inflammation in a given individual or group. The periodontal tissue encircling the tooth is evaluated at four distinct locations: The aforementioned anatomical structures include the mesio-facial papilla, facial marginal gingiva, disto-facial papilla, and lingual marginal gingiva, as suggested by McClanahan *et al.*<sup>[15]</sup> followed by the modified Quigley Hein PI was utilized to quantify the amount of plaque present.<sup>[16]</sup> The data was collected on each tooth's labial/buccal and lingual/palatal surfaces, excluding third molars and any filled tooth surface. Using a calibrated periodontal probe, the distance from the gingival margin to the edge of the disclosed area was measured to the nearest 0.5 mm. The participants' scores were documented on a PI record form.

The first visit is after 14 days from the baseline visit, clinical periodontal parameters scoring (GI and PI) will be performed again as described at the baseline visit.

Investigators controlled the quantity of mouthwash used by providing a new bottle containing 140 mL for one patient for 1 week at the beginning of each week and returning the bottle from the previous week. The mouth rinse bottles will be retrieved, and the remaining liquid will be calculated to assess further the compliance with Oral rinse (the total volume per bottle over a period of 2 weeks will be 280 mL).

Upon completion of the trial, all participants were administered a questionnaire utilizing a visual analog scale (VAS) specifically formulated to evaluate their perceptions regarding the product they had utilized. Participants were instructed to mark a point on an uncalibrated line measuring 10 cm in length, with the negative extreme response of 0 located at the left end and the positive extreme response of 10 located at the right end. The methodology employed in this study included the integration of a questionnaire and data interpretation approach that was derived from a prior investigation.<sup>[17]</sup>

## Randomization

The examiner performed Simple Randomization for enrollment patients in three groups. Each group of participants will be allocated a letter (A, B, or C) matching the intervention. computer random number generator (Microsoft Excel 2016) was used to create random numbers, which were then used to rearrange the placement of each group and participants ( $n = 54$ ), who would be divided into designated groups; each group received 18 participants ( $n = 18$ ) according to a 1:1:1 allocation. Following that,

labeled bottles with the interventions were distributed to the patients, along with instructions for usage. Subjects were instructed to rinse for 1 min with 10 mL of undiluted mouth rinse 30 min after cleansing their teeth and abstain from consuming food or drink for 30 min after rinsing with mouth rinse. To prevent interference with the efficacy of the interventions, subjects were advised to cleanse their teeth twice daily with a 12-h interval between each session. Each participant utilized a toothbrush with filaments of soft hardness and toothpaste (COLGATE, Colgate-Palmolive, Mumbai, India). No adjustments were permitted to the subjects' tooth brushing method.

## Sample size

The sample size for the study was determined based on data from a prior investigation.<sup>[18]</sup> The previous study demonstrated that a new mouthwash containing HA/H<sub>2</sub>O<sub>2</sub> resulted in a significant reduction in gingival scores ( $41.2 \pm 16.05$ ) compared to a placebo ( $21.48 \pm 13.65$ ). Using G\*power (version 3.1.9.4), the sample size required to reject the null hypothesis between the HA/H<sub>2</sub>O<sub>2</sub> and placebo groups with a probability power of 0.95 and 0.05 type I error probability was determined to be 18 individuals per study arm (total = 54, including 16 calculated and 2 additional participants to account for potential dropouts).

## Statistical analysis

Data was described, analyzed, and presented using Statistical Package for the Social Science (version 21) (Chicago, IL). Cohen's kappa coefficient assessed investigator agreement. The Shapiro–Wilk test determined distribution normality. Intervention comparison employs GI and PI mean and standard deviation. The latter variables were compared intragroup using a parametric paired *t* test. A one-way analysis of variance (ANOVA) determined intervention differences. Tukey honestly significant difference (HSD) *posthoc* test was performed to evaluate whether each group of therapies differed significantly. Tukey HSD *posthoc* test was performed to assess the proportion of healthy participants. The questionnaire answers produced ordinal data, thus a nonparametric Kruskal–Wallis analysis was used to compare intervention responses. Dunn–Bonferroni multiple pairwise comparisons of mouthwash questions.  $P < 0.05$  was significant.

## Ethical approval

All of the research participants were informed prior to sample collection, and permission was obtained using a consent form with reference number 748 so that the tests could be carried out and the findings published (with the number and date on December 28, 2022).

## RESULTS

Seventy-five individuals were evaluated for eligibility, and 54 were chosen after the inclusion and exclusion criteria were employed. Consequently, the total number

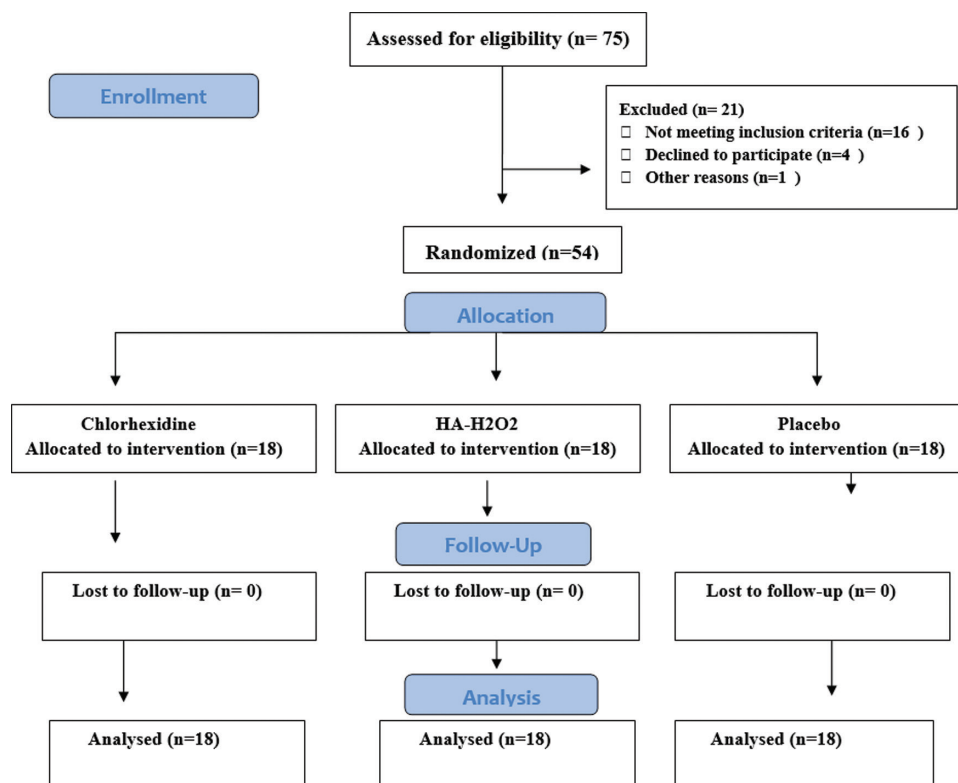


Figure 1: CONSORT 2010 path diagram participants

Table 1: Demographic variables of the study groups

Variables	H <sub>2</sub> O <sub>2</sub> /HA (n = 18)	CHX (n = 18)	Placebo (n = 18)	P value
Male/female	8/10	8/10	7/11	0.927*
Mean age ± SD	21.11 ± 0.832	21.22 ± 0.878	21.16 ± 0.924	0.931**

P value > 0.05 is nonsignificant, SD: standard deviation,

\*Chi- square test,

\*\*ANOVA test

of individuals accessible for the final analysis was 54, as shown in Figure 1. Participants’ baseline demographic data are reported in Table 1.

### GI

At baseline, nonsignificant differences were detected among the groups ( $P > 0.068$ ).

On day 14, The mean GI values were significantly decreased following  $0.994 \pm 0.080$ ,  $0.706 \pm 0.130$ , and  $2.100 \pm 0.114$  for HA/H<sub>2</sub>O<sub>2</sub>, CHX, and placebo, respectively, with statistically significant differences among groups. The effect sizes were higher (9.332) in HA/H<sub>2</sub>O<sub>2</sub> than in CHX (9.323), with significant differences when comparing each pair of groups, as shown in Table 2.

### PI

At the start of the study, nonsignificant differences ( $P > 0.05$ ) were detected in mean PI between the groups. On

day 14, The mean PI values were significantly decreased, following  $1.012 \pm 0.104$ ,  $0.875 \pm 0.0013$ , and  $2.244 \pm 0.120$  for HA + H<sub>2</sub>O<sub>2</sub>, CHX, and placebo, respectively, with statistically significant differences in comparison among groups. Still, the effect sizes were higher in the CHX group (17.387) than in the HA + H<sub>2</sub>O<sub>2</sub> group (11.198); hence, the comparison revealed a significant difference between them as well as between each of them with placebo, as shown [Table 3].

### Satisfaction questionnaire responses

Table 4 summarizes the answers provided by the participants to the questionnaire. Regarding the taste of (Q1), subjects rated the (H<sub>2</sub>O<sub>2</sub>/HA) rinse (VAS 6.667) as having a markedly better taste ( $P = 0.000$ ) than the CHX rinse (VAS 3.833). The answers provided by the subjects about the durability of taste after use (Q2) revealed that CHX (VAS 4.889) significantly increased taste

**Table 2: Statistical analysis of GI for study groups and intervals**

	HA/H <sub>2</sub> O <sub>2</sub>	0.12% CHX	Placebo	P value*
	Mean ± SD	Mean ± SD	Mean ± SD	
Baseline	2.244 ± 0.120	2.206 ± 0.121	2.294 ± 0.094	0.068
14 days	0.994 ± 0.080	0.706 ± 0.130	2.100 ± 0.114	<b>0.000</b>
Paired <i>t</i> test	39.593	39.557	5.596	
<i>P</i> value**	0.000	0.000	<b>0.000</b>	
Effect size	9.332	9.323	<b>1.319</b>	
<b>Multiple comparisons</b>				
(I) Groups	(J) Groups	Mean difference (I–J)		<i>P</i> value***
HA/H <sub>2</sub> O <sub>2</sub>	0.12% CHX	0.289		<b>0.000</b>
	Placebo	–1.1060		<b>0.000</b>
0.12% CHX	Placebo	–1.394		<b>0.000</b>

\*One-way ANOVA test,

\*\*paired *t* test,

\*\*\*Tukey HSD test

**Table 3: Statistical analysis of PI for study groups and intervals**

	HA/H <sub>2</sub> O <sub>2</sub> (mean ± SD)	0.12% CHX (mean ± SD)	Placebo (mean ± SD)	P value*
Baseline	2.456 ± 0.110	2.506 ± 0.094	2.400 ± 0.124	0.087
14 days	1.012 ± 0.104	0.875 ± 0.013	2.244 ± 0.120	<b>0.000</b>
Paired <i>t</i> test	47.510	73.771	6.710	
<i>P</i> value**	0.000	0.000	<b>0.000</b>	
Effect size	11.1983	17.3879	1.5816	
<b>Multiple comparisons</b>				
(I) Groups	(J) Groups	Mean difference (I–J)		<i>P</i> value***
HA/H <sub>2</sub> O <sub>2</sub>	0.12% CHX	0.137		<b>0.000</b>
	Placebo	–1.233		<b>0.000</b>
0.12% CHX	Placebo	–1.369		<b>0.000</b>

\*One-way ANOVA test,

\*\*paired *t* test,

\*\*\*Tukey HSD test

persistence compared to placebo (VAS 7.056;  $P = 0.001$ ) and (H<sub>2</sub>O<sub>2</sub>/HA) (VAS 6.833;  $P = 0.002$ ) mouth rinses. Individuals were questioned regarding changes in taste (Q3), and the findings revealed no difference among the (H<sub>2</sub>O<sub>2</sub>/HA), CHX, and placebo groups (5.167, 5.222, and 4.944, respectively). The response of the participants to the rinsing intervals for CHX (VAS 4.944) and (H<sub>2</sub>O<sub>2</sub>/HA) (VAS 5.111) mouthwashes was substantially higher ( $P < 0.05$ ) than that of the placebo (VAS 6.722) (Q4). In terms of the efficacy of mouth rinses in reducing biofilm dental plaque, the ratings for all interventions were significantly higher ( $P < 0.05$ ) compared to the placebo group. However, there were no significant differences observed between the various types of mouth rinses (Q5) as per the participants' feedback.

## DISCUSSION

The present investigation aimed to examine the 14-day interval of anti-inflammatory and anti-plaque influence

of a marketplace-available mouthwash (H<sub>2</sub>O<sub>2</sub>/HA) in participants with generalized gingivitis. Both of these constituents have been separately examined *in vitro* and *in vivo*, and their attributes are widely recognized.<sup>[11,17,19]</sup> The agents were utilized as a supplementary measure to mechanical dental debridement while keeping the subjects' regular oral hygiene practices unaltered. The study additionally included an assessment of potential adverse effects associated with the use of mouth rinses, which was conducted through a questionnaire administered to the participants upon completion of the study. Randomized controlled trials offer more robust evidence for chemotherapeutic agents for chemical plaque control.<sup>[20]</sup> As a positive control, CHX, the gold standard for anti-plaque and anti-gingivitis mouthwash, was used in this trial. This study's negative control consisted of using a mouthwash containing distilled water in addition to brushing and OHI. Brushing only with OHI vs. using distilled water (placebo) mouthwash without OHI could be the subject of debate. This decision was warranted by the nature of the

**Table 4: Responses to the questionnaire in relation to the interventions by VAS**

Question and response	H <sub>2</sub> O <sub>2</sub> /HA (mean ± SD)	0.12% CHX (mean ± SD)	Placebo (mean ± SD)	Kruskal–Wallis	P value*
(1) How was the product's taste? 0 = very bad to 10 = very good	6.66 ± 1.37	3.83 ± 1.38	5.72 ± 1.27	24.50	<b>0.000</b>
(2) After rinsing, how long did the flavor stay in the mouth? 0 = very long to 10 = very short	6.83 ± 1.33	4.88 ± 1.53	7.05 ± 1.51	16.96	<b>0.000</b>
(3) How was your taste of food and drinks affected? 0 = positive effect to 10 = negative effect	5.16 ± 0.98	5.22 ± 0.87	4.94 ± 0.87	0.63	0.729
(4) What are your thoughts on the rinsing duration? 0 = very long and 10 = very short	5.11 ± 1.02	4.94 ± 0.87	6.72 ± 1.52	15.21	<b>0.000</b>
(5) How did you feel about the plaque reduction? 0 = insufficient to 10 = very efficient	5.50 ± 1.04	6.61 ± 0.97	3.05 ± 0.80	38.13	<b>0.000</b>
Comparisons**	0.12% CHX	Placebo	0.12% CHX–H <sub>2</sub> O <sub>2</sub> /HA	Placebo–HA	
Q1		0.005	0.000	0.230	
Q2		0.001	0.002	0.999	
Q4		0.002	0.999	<b>0.003</b>	
Q5		0.000	0.051	<b>0.000</b>	

\*Kruskal–Wallis test,

\*\*multiple pairwise comparisons of questions between mouthwashes by Dunn–Bonferroni method

trial to assure standardization and blindness to eliminate any potential bias, which necessitated the use of a uniform design for each group.

The justification for this investigation was based on a previously published pilot study that discovered the need to conduct a clinical trial to compare the efficacy of this new formulation with a CHX mouthwash.<sup>[18]</sup>

Our study's results showed a statistically significant difference in the mean of GI with the positive control, the negative control, and the baseline after 14 days from use. The significant result of our study in term of GI is explained by the synergistic effect of two active components (HA and H<sub>2</sub>O<sub>2</sub>), hence the HA, which act as an anti-inflammatory effect due to its scavenging effect on prostaglandins, matrix metalloproteinases, and the mediators of inflammation.<sup>[21]</sup> In addition to, the anti-inflammatory effect of H<sub>2</sub>O<sub>2</sub> which significantly reduces gingivitis.<sup>[12]</sup> This outcome is comparable to the pilot study of Boccari *et al.*<sup>[18]</sup> which revealed a statistically significant difference between the placebo and test groups regarding the GI at 21 days. In addition, findings are consistent with one of the few previously published reports analyzing a formulation containing HA; Abdulkareem *et al.*<sup>[17]</sup> discovered that 0.025% HA induced a substantial reduction in gingival inflammation compared to baseline, the placebo and 0.12% CHX mouth rinse. On the other hand, the findings are in accordance with one of the few publications that have been published previously that investigates a formulation that contains H<sub>2</sub>O<sub>2</sub>. Yaneva *et al.*<sup>[12]</sup> conducted a study in which a group of mouthwashes, including H<sub>2</sub>O<sub>2</sub>, were tested over a period of 21 days. The results indicated that the H<sub>2</sub>O<sub>2</sub> group exhibited the lowest

reported values of the GI, thereby demonstrating its efficacy in reducing gingival inflammation as compared to the other groups.

Regarding, CHX mouthwash exhibited a statistically significant reduction in the mean of GI, which is in accordance with previous studies that have investigated the impact of CHX on reducing the mean of GI among individuals with gingivitis. The study conducted by Abed *et al.*<sup>[22]</sup> revealed that the utilization of CHX mouthwash over a period of 7 days resulted in a decrease in the mean GI among individuals with gingivitis. This may be due to CHX's antimicrobial activity against Gram-positive, Gram-negative, yeast, and fungi, as well as good OHIs and motivation.<sup>[23]</sup>

PI was the secondary outcome variable, and the QHI-s modified by Turesky *et al.*<sup>[16]</sup> was chosen to assess the PI score. The results of this study revealed a statistically significant decrease in the mean value of PI in the H<sub>2</sub>O<sub>2</sub>/HA rinse group compared with baseline data. The outcome may be attributable to the synergistic antibacterial capacity of two active agents (HA and H<sub>2</sub>O<sub>2</sub>). For instance, *in vitro*, HA mouthwash significantly inhibited the growth of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* and *in vivo* experiment, HA has plaque growth suppression efficacy equivalent to CHX in a 4-day plaques regrowth paradigm.<sup>[19]</sup> In addition, the potent oxidant hydroxyl radical of H<sub>2</sub>O<sub>2</sub> reacts easily with macromolecules like membrane lipids and DNA, killing bacteria.<sup>[24]</sup>

These results are consistent with one of the few published articles evaluating a formulation containing HA. After 7 days, Abdulkareem *et al.*<sup>[17]</sup> reported a

significant plaque reduction in 0.025% HA participants. In addition, Research conducted by Rodrigues *et al.*<sup>[19]</sup> found Hyaluronan (0.025%) mouthwash was shown to be equivalent to CHX (0.2%) in preventing plaque growth *in vivo*. In contrast, a study conducted by Boccalari *et al.*<sup>[18]</sup> examined the effects of a H<sub>2</sub>O<sub>2</sub>/HA rinse compared to a placebo on PI. The results indicated that there were no statistically significant changes between the two groups after 21 days of use. Explained by detailed instructions for routine oral hygiene, leading to adequate mechanical action of tooth brushing. However, low plaque and gingivitis scores at follow-up indicated not optimal oral hygiene levels, potentially masking the effects of the test mouth rinse and resulting in the lack of significance in PI levels in the sample. In addition, the Differences in our research methodology from Boccalari *et al.*<sup>[18]</sup> such as rinse time (30 vs. 60s), might explain the variance.

On the other hand, the present study observed a statistically significant decrease in the mean PI upon administration of CHX mouthwash due to CHX acts as an anti-plaque agent primarily by suppressing bacterial multiplication.<sup>[25]</sup> This finding is consistent with prior research exploring the effects of CHX on reducing the mean PI in individuals diagnosed with gingivitis. According to Abdulkareem *et al.*<sup>[17]</sup>'s research, CHX demonstrated the greatest effectiveness as a mouthwash for preventing plaque. In a comparison of H<sub>2</sub>O<sub>2</sub>/HA and CHX mouthwashes, the current findings revealed that CHX mouthwash had a greater impact on PI reduction than H<sub>2</sub>O<sub>2</sub>/HA mouthwash. In addition, these results demonstrated a statistically significant difference between H<sub>2</sub>O<sub>2</sub>/HA mouthwash and CHX in terms of GI reduction at day 14.

On the other hand, the third (placebo) group in both GI and PI showed positive results. The anticipated decrease in GI and PI scores within the placebo group can be attributed to the patients' desire to maintain most effective oral hygiene. The Hawthorne effect, a well-documented phenomenon, may have played a role in the observed improvements in both study groups. This effect could be attributed to the heightened awareness of oral health care among participants, as well as the regular monitoring provided by weekly control visits.<sup>[26,27]</sup> These factors likely contributed to the overall positive outcomes observed in the study.

The study employed the VAS to assess patient satisfaction toward the therapies. The questionnaire included questions on the sensory perception of the products, specifically focusing on taste, the duration of taste perception after use, any changes in taste experienced, the time required for rinsing the products, and the participants' assessment of the mouthwashes' effectiveness in reducing dental plaque.

It is generally established that CHX-based mouthwashes modify taste sense in a time-dependent manner. The bitter taste sensation rises while the salty taste decreases, which

might be due to epithelial ion transport-based interference of CHX with particular taste receptors.<sup>[28]</sup>

The study's findings suggest that the responses of the participants to taste and its duration following CHX usage are consistent with the results of previous research investigations.<sup>[17,29]</sup>

The majority of the participants expressed satisfaction with the flavor of H<sub>2</sub>O<sub>2</sub>/HA. The satisfaction of participants with (HA/H<sub>2</sub>O<sub>2</sub>) was compatible with the result of Boccalari *et al.*<sup>[18]</sup> who found H<sub>2</sub>O<sub>2</sub>/HA mouth rinse palatable. In addition, Abdulkareem *et al.*<sup>[17]</sup> found that feedback regarding mouthiness revealed that the subjects preferred the HA mouthwash to the other mouthwashes. Regarding, the rinsing time associated with alcohol-containing mouthwashes was rated as relatively lengthy (H<sub>2</sub>O<sub>2</sub>/HA and CHX), probably this is due to the alcohol's searing effect, which gave a sense of a prolonged cleansing period, which was reported to be shorter in the case of the mouthwash containing distilled water. This result was comparable with the outcomes of a separate trial involving a mouthwash containing CHX.<sup>[30]</sup> In general, effective periodontal interventions not only facilitate the well-being of the periodontium but also significantly improve the quality of life associated with oral health and positively influence patients' perspectives for a considerable duration following the completion of treatment.<sup>[31]</sup>

The trial's shortcomings were a short (14-day) evaluation interval, and the intervention group did not contain instances with supra-sub-calculus, severe gingival inflammation, and no healthy control subject. Furthermore, the trial was restricted to students aged 18 to 23; applying inclusion and exclusion criteria mitigated this limitation as much as feasible. Therefore, it is necessary to conduct a more comprehensive assessment over a longer period of time, with a larger sample size and a diverse range of ages and severity levels of periodontal disease.

## CONCLUSION

Mouthwashes containing (H<sub>2</sub>O<sub>2</sub>/HA) mouth rinse have anti-inflammatory and anti-plaque properties. Furthermore, satisfactory levels of participant perception compared to the results associated with CHX use. According to the findings of this research, mouthwashes containing (H<sub>2</sub>O<sub>2</sub>/HA) might be utilized as an alternative to CHX and may be used for daily regular oral hygiene practises.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## Ethics approval and consent to participate

This study was approved by the Ethics Board of the Baghdad University School of Dentistry and corresponded to the Helsinki and Tokyo standards for human research (Reference No.: 748). Informed consent was taken from every participant.

## Availability of data and material

The data used to support the findings of this trail are available from the corresponding author upon request.

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