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Effect of hydrogen peroxide and hyaluronic acid in mouth rinse after third molar extraction: a triple-blind parallel randomized controlled clinical trial

E. BOCCALARI[1], S. KHIJMATGAR[2], C. OCCHIPINTI[2], M. DEL FABBRO[1,2], F. INCHINGOLO[3], G.M. TARTAGLIA[1,2,3]

[1]Department of Biomedical, Surgical, and Dental Science, University of Milan, Milan, Italy
[2]Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy
[3]Department of Interdisciplinary Medicine, University of Medicine Aldo Moro, Bari, Italy

E. Boccalari and S. Khijmatgar equally contributed as first authors

ABSTRACT. – OBJECTIVE: The study aimed to investigate the potential beneficial role of hydrogen peroxide (H_2O_2) and hyaluronic acid (HA) combination formulation in socket healing after third molar surgery. Biomaterials, including mouthwash formulations, were hypothesized to contribute to improved socket healing and reduced post-operative complications.

PATIENTS AND METHODS: A triple-blinded parallel randomized controlled clinical trial was conducted at a single-center dental hospital in Milan, Italy. The trial included 114 patients who underwent extraction of impacted, partially erupted, and completely erupted third molars. Patients were randomly assigned to three parallel groups: Group 1 (H_2O_2 and HA), Group 2 (placebo), and Group 3 (0.2% chlorhexidine). The trial was registered at ClinicalTrial.gov (registration number NCT04438434). The main outcome measures included various parameters related to socket healing, such as pain, inflammation, swelling, plaque index, bleeding index, granulation tissue, suppuration, re-epithelialization, bleeding upon palpation, odor, and taste alteration. Patients were followed up for 7 days.

RESULTS: All 114 enrolled patients completed the study, with no dropouts or loss to follow-up. The mean age of patients in the three groups differed (H_2O_2 and HA: 30.9±14.9; placebo: 27.6±13.1; 0.2% chlorhexidine: 23.05±10.16). Significant reductions ($p<0.001$) in visual analog scale (VAS) pain levels and other outcome measures were observed in the H_2O_2 and HA group compared to the placebo group. These findings suggest a positive effect of the H_2O_2 and HA combination on socket healing after the third molar surgery.

CONCLUSIONS: The study concludes that the combination of hydrogen peroxide and hyaluronic acid can be considered a potential mouthwash with beneficial effects on socket healing following third molar surgery. However, additional clinical trials are recommended to validate its effectiveness further and provide additional evidence supporting its use in clinical settings.

ClinicalTrial.gov: NCT04438434.

Key Words:
Alveolar ridge preservation, Chlorhexidine, Healing, Hyaluronic acid, Hydrogen peroxide, Inflammation, Pain, Plaque, Randomized clinical trial, RCT, Socket, Swelling, Triple blind.

Introduction

Healing of a surgical wound consists of three stages: inflammation, proliferation, and remodeling. The inflammatory stage takes place in the first five days and is characterized by a vascular response in which the process of hemostasis and clot formation is observed, which represents the substrate for the subsequent fibrinic organization. The cellular response is expressed through tissue infiltration of leukocyte elements, such as neutrophils and eosinophils, granulocytes, lymphocytes, and macrophages. The proliferative stage occurs in 5-14 days and consists of epithelial and connective tissue repair. The epithelium repair is carried out quickly by migration and proliferation of epithelial cells with consequent wound closure. The remodeling phase occurs after the 14th day and is characterized by the phenomenon of tissue contraction in which fibroblasts are replaced by similar cells but with contractile abilities, called

Corresponding Author: Gianluca Martino Tartaglia, Ph.D; e-mail: gianluca.tartaglia@unimi.it

myofibroblasts. The presence of these cells leads to the remodeling and reorganization of collagen fibers, which is completed after 6-7 weeks. Clinically, two different types of healing can also be distinguished: primary or secondary closure[1].

The healing of tissues in the oral cavity is also closely influenced by the presence of rich bacterial colonization, as well as the numerous mechanical stresses that the oral tissues are constantly subjected to[2,3]. To reduce the risk of super-infection, it is therefore recommended to use topical and systemic products that guarantee bacterial load control[4]. A report[5] has shown that postoperative complications are among the most important factors in ensuring patient satisfaction and perception of the quality of the surgical treatment received.

Topical treatment can be prescribed in addition to antibiotics and anti-inflammatory agents at the time of oral surgery. Numerous products characterized by the presence of peculiar components like hyaluronic acid (HA), hydrogen peroxide (H_2O_2), chitosan, chlorhexidine, lincomycin, etc., have been tested *in vitro* and *in vivo*, with variable results regarding the actual improvement of healing and reduction of post-surgical complications[6-8].

Oral rinse contributes to the reduction of pain, inflammation, and bleeding of the gums due to dental hygiene maintenance, extraction treatment, and post-oral surgery. We hypothesized that HA and H_2O_2 combination may reduce the occurrence of side effects and postoperative complications after extraction of impacted third molars. H_2O_2 is a strong oxidizing agent, effective against a wide range of micro-organisms, with high antibacterial, antiviral, and anti-mycotic action. It may act from the very first stages of the healing process, facilitating hemostasis by different mechanisms that include activation of different tissue growth factors, promotion of platelet aggregation, and regulation of contractility and barrier function of endothelial cells[9]. Subsequently, with the establishment of the inflammatory phase, neutrophils and macrophages may sustain the bactericidal activity and eliminate most microorganisms through the formation of proteases and elastases. The activity of these granulocytes is also promoted in the presence of H_2O_2[10,11]. In the next phase of cell proliferation, H_2O_2 participates in two other important processes: it promotes the mobility of keratinocytes that will migrate from adjacent tissues to participate directly in tissue regeneration and promotes angiogenesis[12,13].

HA is one of the main components of the extracellular matrix and is widely distributed in different tissues such as skin, synovial fluid, cartilage, tendons, eyes, and most body fluids. This long-chain molecule with high moisturizing and anti-inflammatory properties provides a reticular structure barrier against pathogens and inflammatory cytokines. HA has been extensively studied and used in different branches of medicine. However, its effects in oral surgery, particularly in socket healing, are still poorly known[8,14]. On the other hand, as previously stated, H_2O_2 has an important antibacterial, anti-viral, and anti-mycotic action. Finally, it promotes healing, but there is limited understanding in the literature regarding the effectiveness of a combination of HA and H_2O_2 that would facilitate healing. Therefore, the objective of this study was to compare HA and H_2O_2 combination with placebo and 0.2% chlorhexidine mouthwash and follow up for 7 days.

We chose to compare it with the golden standard chlorhexidine 0.2% because this type of concentration is most commonly used for severe gum problems and/or pre-operative surgical preparation to achieve greater antimicrobial action. Among the considered variables, there were the accumulation of plaque both at the gingival level and at the suture thread, the organoleptic point of view (smell and taste), and, therefore, the degree of acceptance of the product, as well as any side effects.

Patients and Methods

This study conforms to the Consolidated Standards of Reporting Trials guidelines. The protocol of this clinical trial was registered on ClinicalTrials.gov (number NCT04438434) and can be accessed at the following link: https://clinicaltrials.gov/ct2/show/NCT04438434. This research, involving human participants and human data, was performed in accordance with the Declaration of Helsinki.

Study Design and Population

This was a three-arm, triple-blinded, parallel-group 7-day study with a random allocation of subjects in three groups, each using a different mouthwash. The test product was a mouth rinse containing a combination of hydrogen peroxide (1.80%, H_2O_2 5.15% at 130 volumes) and sodium hyaluronate (0.10%) with the remaining part being water (97%) and inert additives (1.1%) (BMG Pharma, Milan, Italy). The water-based placebo was a mouthwash containing 98.55% water plus a few inert additives, and the positive control was a commercial 0.2% chlorhexidine mouthwash

(Corsodyl, GlaxoSmithKline, Brentford, UK). Ethics approval was granted by the Institutional Ethics Committee of the University of Milan, Italy (Prot. No. 22/19, approved on the 20th of May, 2019). Informed consent was obtained from all subjects and/or their legal guardian(s) included in this study. Patients were selected consecutively at the IRCCS "Ca" Granda Ospedale Maggiore Policlinico di Milano - UOC Maxillo Facial Surgery and Dentistry, according to the following criteria:

Inclusion Criteria

- Systemically healthy subjects between 12 and 50 years of age.
- Acceptance of informed consent.
- The need to perform the extraction of a third molar in total or partial bone inclusion[15].

Exclusion Criteria

- Periodontal treatment within the last 3 months.
- Use of mouth rinses, local or general medication, within the last month.
- Allergy to mouth rinse ingredients.
- Presence of systemic and chronic diseases, immunocompromised patients.
- Ongoing orthodontic treatment, including removable maintenance appliances.
- Extensive intrinsic teeth staining.
- Pregnant patients.
- Poor oral hygiene.
- Inability to provide consent.
- Inability to follow post-intervention hygiene instructions.
- Regular smokers and alcohol consumers.

Each patient received a thorough explanation regarding the protocol and the research objectives, and then voluntarily signed a dedicated consent form. In the case of minors, the consent of the parents or guardians was collected.

Randomization and Allocation Concealment

All eligible participants were equally randomized into one of the three groups (Figure 1). A stratified randomization method was used to balance the covariates in this clinical study. This was done by producing a separate block for each combination of covariates, and subjects were assigned to the appropriate block. Covariates in this study were age (<30 and ≥30 years) and tooth extraction complexity (simple extraction: the operator uses the elevator to loosen up the tooth, followed by forceps to remove it completely; surgical extraction: it is performed by the dental surgeon only in cases where simple extraction is not feasible). After all subjects were identified and assigned into blocks, simple randomization was performed within each block to assign subjects to one of the groups. The products have been packaged in such a way that they were not recognizable either by the operator or by the patient (blinded). Each package has been assigned a code that, in turn, will refer to the type of the product. The association between the code and the product type was predetermined by dedicated software, Research Randomizer[16], and was kept by another operator not involved in the treatment. The operator who delivered the product was informed of the type only at the end of the treatment.

The data collected in pseudonymized form were entered into a dedicated database, where they were meticulously checked for completeness, consistency, and plausibility before undergoing statistical analysis. To guarantee privacy, in compliance with current legislation, the data associated with the individual subjects were used in a confidential manner by the researchers and staff in charge. A numerical code was assigned to each patient. Only the principal investigator had the key to disclose the correspondence between the code and the personal and sensitive patient data. Data sharing between the researchers was carried out in pseudonymized form.

Clinical Evaluation

All included subjects were examined and treated by specialized medical personnel who had a minimum of 15 years of experience in assessment, diagnosis, treatment planning, and management of oral surgery cases. Patients underwent professional oral hygiene and mechanical debridement three days before the extraction. Then, patients were instructed to perform correct oral hygiene maneuvers.

Patients underwent an oral health assessment session performed by qualified personnel both at the beginning and during treatment. During the examination, the same trained professional collected data related to the subject's history, such as dietary habits, medication use, oral hygiene habits, and evaluation of the state of health of the oral cavity, with particular reference to the area adjacent to the extraction.

On the day of surgery, the operator opened a closed opaque envelope containing the allocation indication. Extraction was performed by an experienced practitioner with a minimum of 15 years of experience, following standard guidelines for extraction of impacted teeth. The difficulty level

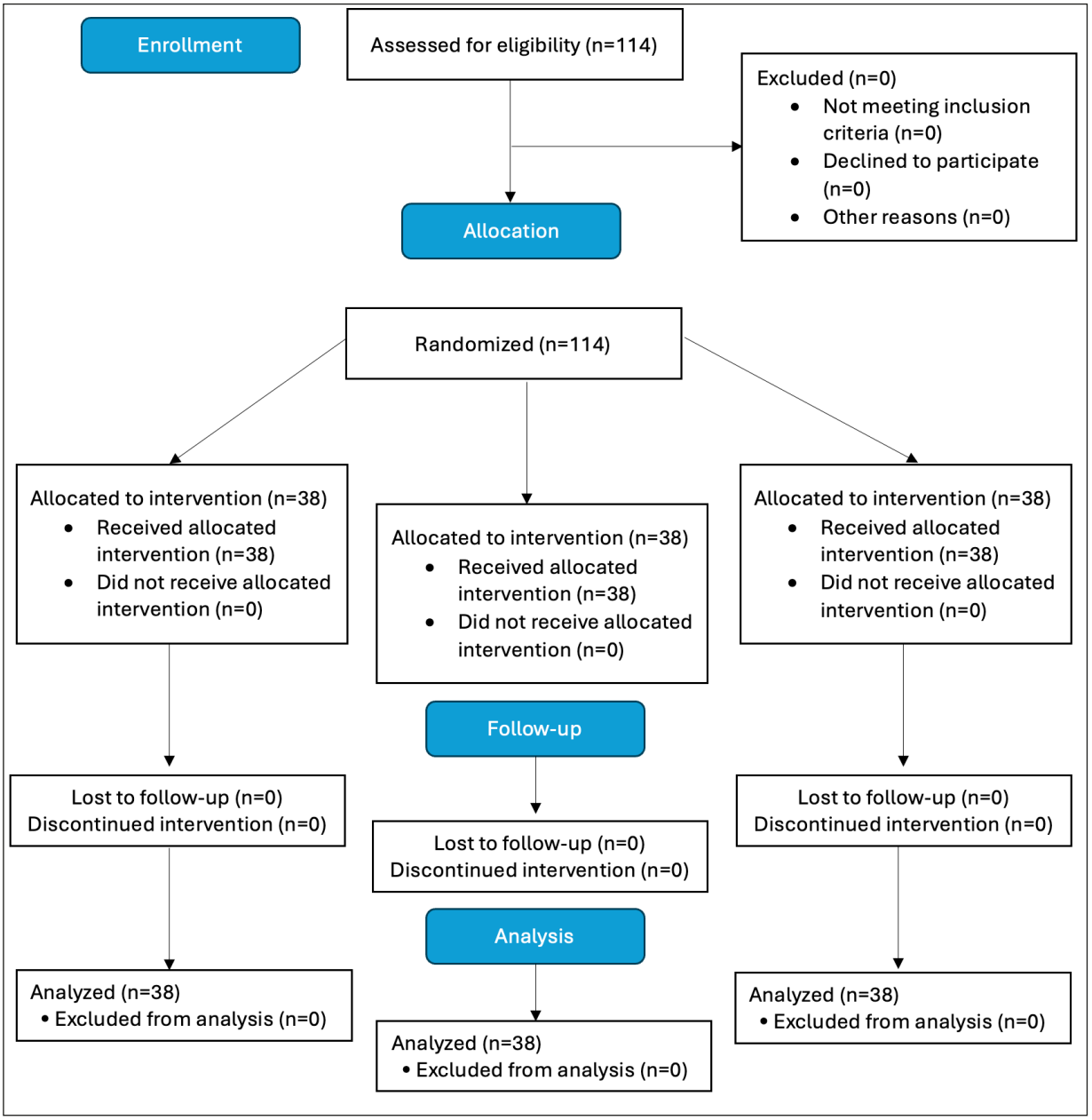

Figure 1. Consort 2010 flow diagram.

of the intervention was assessed based on the Pederson classification[17]. 1-2 scores indicate no difficulty, 3-4 indicate slight difficulty, 5-6 moderate difficulty, and 7-10 very difficult.

Once the element was removed, a suture with detached stitches was applied using a 3/0 silk thread. From a pharmacological point of view, the patient was prescribed antibiotic therapy (amoxicillin 1 g) to be taken twice a day for seven days, and pain/anti-inflammatory therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) (Brufen 800 mg) was used only on the day of surgery. The patients would be prescribed additional analgesics only in cases of severe or not tolerable pain. We tried to avoid the use of concomitant and rescue analgesics during the period of study, which could influence the VAS score[18]. The number of analgesic tablets consumed by each patient was noted.

The treatment included rinsing (10 ml) with the study products (H_2O_2/HA, 0.2% chlorhexidine mouthwash, or placebo) three times a day, after meals and after normal oral hygiene procedures, for one week. Patients who experienced allergic

reactions or hypersensitivity due to the use of the products were advised to discontinue the products, consult a physician to assess symptoms, and undergo alternative therapies if necessary. Subjects were then reassessed after 7 days. At the end of the treatment period, each individual bottle containing the assigned mouthwash was weighted, using a scale with 0.1 g sensitivity, to make sure that each patient used the correct amount of the product. Patients who did not strictly follow the protocol assigned in the post-treatment period were excluded. Patients were instructed to report any side effects arising during the 7 days post-extraction, and, in this case, they were told to come back for a check-up and suspend the use of the product. Patients were asked to report twice a day (morning and evening) in a daily diary any pain/malaise perceived starting from the day of the surgery (T0) for seven consecutive days using a reference scale (T1).

The pain assessment scale used was the Visual Analogue Assessment (VAS), a one-dimensional scale represented by a 10 cm long segment, at the end of which there are two pain parameters: absent (left) and maximum pain (right). The examination was conducted by asking the patient to indicate the amount of perceived pain with an X-mark along the segment. All the other parameters were evaluated at T0 (day 0) and T1 (day 7). The time frame for the symptoms to reside after tooth extraction was 3-4 days. Therefore, a follow-up of 7 days was considered.

The Landry et al[19] index was chosen for healing assessment, considering the changes made by Pippi et al[20] in 2015. In particular, seven parameters were evaluated, assigning to each one a value equal to 1 or 0. The total score indicated the degree of healing. The parameters evaluated are described in Table I.

Moreover, the bleeding index (BI) and plaque index (PI) were assessed at T0 and T1. The first is to evaluate bleeding through a modified version of the Mombelli semi-qualitative bleeding index (MSI). This index is assessed using the WHO periodontal probe, attributing 4 different codes for each site observed: 0 absence of bleeding; 1 presence of bleeding on probing without redness and edema; 2 presence of bleeding on the poll with redness and edema; 3 spontaneous bleeding[18].

The second index evaluates plaque according to the O'Leary method expressed in percentages. This index considers 4 surfaces at the gum line: the cheek side, the tongue side, the front side, and the back side[21]. This index was assessed after the use of plaque staining tablet, attributing 4 different codes for each observed site: code 0: absence of plaque; code 1: 1/3 of the dental surface covered with plaque; code 2: 2/3 of the dental surface covered with plaque; code 3: greater than 2/3 of the dental surface covered with plaque.

At T1, before removing the stitches, the presence of plaque through the Silness and Loe[22] plaque index (PI) was also assessed on the suture thread. The overview of the outcome measures of the healing index is illustrated in Table I.

The odor (smell) was measured by asking patients about their experience and severity of odor loss between 0-10 (0=normal and 10=complete loss of odor). Similarly, taste sensation was measured using a 0-10 scale (0=normal taste and 10=loss of taste).

To ensure the validity and reliability of our outcome measurements and clinical findings in our randomized controlled clinical trial, we used standardized methods such as VAS scores, plaque index, bleeding index, granulation tissue, etc., validated in previous studies[23-25]. We followed established protocols for their use and employed well-established criteria for the diagnosis of the condition under study and assessment of clinical findings. To minimize experimental error, we used standard operating procedures and had the examiner trained according to the protocol. We also minimized measurement error by using calibrated instruments, ensuring consistent lighting and visibility during the examination, and having independent and experienced examiners assess the patients for consistency and accuracy of the diagnosis and clinical findings.

Table I. Outcome measures for healing index assessment.

Redness of the area/mucous membrane	Presence (Score 0)	Absence (Score 1)
Granulation tissue	Presence (Score 0)	Absence (Score 1)
Suppuration	Presence (Score 0)	Absence (Score 1)
Swelling	Presence (Score 0)	Absence (Score 1)
Re-epithelization	Partial (Score 0)	Complete (Score 1)
Bleeding	Presence (Score 0)	Absence (Score 1)
Pain on palpation	Presence (Score 0)	Absence (Score 1)

Table II. Demographics of participants.

	H_2O_2/HA group	Placebo group	CHX group
Number of study participants	38	38	38
Males (N, %)	27 (55.10%)	24 (52.17%)	9 (15.0%)
Age in years (Mean, SD)	30.9, 14.9	27.6, 13.1	23.05, 10.16

N=number; %=percentage; SD=standard deviation. CHX=chlorhexidine; H_2O_2=hydrogen peroxide; HA=hyaluronic acid.

Statistical Analysis

A difference of at least 2.7 healing index score was considered clinically significant. Based on preliminary tests, within the control group (2 weeks), mean and standard deviation healing index of 1.9±1.0 and 4.6±0.5 (12 weeks) in the test group were estimated. For a power of 0.8 and significance level α=0.05, an allocation ratio of 1, a sample size was determined to be n=8 subjects (n=4 in each group). Additional patients were recruited, considering there would be a possible dropout. We assumed the latter to be small, considering the short duration of the study.

To demonstrate the overall surgical difficulty between the groups, statistical analysis was performed using the quantitative measurement of surgical difficulty as an outcome variable. The mean and standard deviation of the surgical difficulty score for each group were calculated.

The data were analyzed by a blinded statistician not involved in patient treatment. GraphPad Prism 5 for Windows (Version 5.03, GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analysis. The normality of distributions of quantitative outcomes was checked using D'Agostino and Pearson's omnibus normality test. When quantitative data was normally distributed, the parametric test was carried out. Otherwise, a non-parametric test was employed in case the data was not normally distributed. A paired Student's *t*-test was used for normally distributed variables and a Wilcoxon signed-rank test was used for variables that were not normally distributed for within-group comparisons. For between-group differences, ANOVA was used for normally distributed variables, and the Kruskal-Wallis test was used for variables that were not normally distributed. The significance level was set at p=0.05.

Results

One hundred and fourteen patients were enrolled (n=54 females and n=60 males). No dropout occurred (Figure 1). Demographic characteristics of participants are shown in Table II.

All variables at T0 were compared to assess among-group differences at baseline. No statistical difference was detected in the use of rescue medication with NSAIDs in the three groups of treatment. There were no significant differences among the three groups for BI, granulation tissue, re-epithelialization, bleeding, and pain on palpation (p≥0.05). Conversely, a significant difference was found in VAS on Day 1 morning and evening, PI, redness, suppuration, swelling, taste, and odor (p≤0.05), as shown in Table III.

Similarly, all variables were compared at the end of the study to assess differences among groups after the use of H_2O_2/HA, placebo, or CHX 0.2%. At time points T1 (end of study) and T0-T1 for VAS assessment (7 consecutive days) (Table IV), statistically significant differences were found in pain (every morning and evening). Also, the odor and taste, redness, granulation tissue, re-epithelialization, bleeding, and pain on palpation were found to be significantly different (p≤0.05), while no significant differences among groups were observed for PI, BI, suppuration, swelling (p≥0.05). **Supplementary Table I** illustrates the presence and absence of signs and symptoms of the healing index among different intervention groups.

The VAS Scores mean, SD, SE, and 95% CI are illustrated in **Supplementary Table II**.

Results indicate that in all groups, a significant decrease in pain day by day was observed, and the use of H_2O_2/HA mouthwash was more effective in pain reduction than placebo and CHX groups (Table V and Figure 2).

Different analyses were performed to evaluate the pain reduction rate in each treatment group, as reported in Figure 2 and **Supplementary Table II**. In particular, a more pronounced reduction in pain was already evident after 3 days of treatment with the H_2O_2/HA group (16.18%) compared to CHX (9.41%) and placebo (-2.99%) underlying the fast action on this parameter. The stronger pain decrease observed in the H_2O_2/HA group was maintained throughout the study period (Table

Table III. Significance of comparison among the three groups for different outcome measures at T0.

Variable	Used test	*p*-value
VAS day 1 morning	ANOVA	**0.033**
VAS day 1 evening	ANOVA	**0.031**
Plaque index	Kruskal-Wallis	**0.000**
Bleeding index	Kruskal-Wallis	0.149
Redness of the area/mucosa	Kruskal-Wallis	**0.000**
Granulation tissue	Kruskal-Wallis	1.00
Suppuration	Kruskal-Wallis	**0.000**
Swelling	Kruskal-Wallis	**0.000**
Re-epithelialization	Kruskal-Wallis	1.00
Bleeding	Kruskal-Wallis	1.00
Pain on palpation	Kruskal-Wallis	1.00
Odor	Kruskal-Wallis	**0.000**
Taste	Kruskal-Wallis	**0.000**

VAS=visual analogue scale; ANOVA=analysis of variance.

V). Regarding odor and taste, patients using CHX reported the highest values, while patients using placebo and the H_2O_2/HA mouthwash reported lower values than CHX at T1.

Table VI summarizes the results of the Wilcoxon signed-rank test for all variables except the VAS score. H_2O_2/HA performed better than placebo and CHX for re-epithelialization and better than CHX (but comparable to placebo) for suppuration and redness. All the other variables shown in Table VI (PI, BI, granulation tissue, swelling, bleeding, pain on palpation, odor and taste) showed comparable results among the three groups. The overall difficulty levels in different groups were 3.59±0.13 (**Supplementary Tables III-VI**). There were no significant variations in different treatment groups in relation to the difficulty level of third molar extractions.

To understand how the product-in-use lowers the pain over a 7-day period, recorded T0-T1 VAS score was compared by paired *t*-test for each patient group. Each day (morning and evening) was compared with the next day (morning or evening) (**Supplementary Table VII**).

Table IV. Differences between the three groups at T1 and T0-T1 for VAS (7 consecutive days).

Variable	Used test	*p*-value
VAS day 2 morning	ANOVA	**0.003**
VAS day 2 evening	ANOVA	**0.001**
VAS day 3 morning	ANOVA	**0.001**
VAS day 3 evening	ANOVA	**0.002**
VAS day 4 morning	ANOVA	**0.009**
VAS day 4 evening	ANOVA	**0.003**
VAS day 5 morning	ANOVA	**0.003**
VAS day 5 evening	ANOVA	**0.003**
VAS day 6 morning	ANOVA	**0.000**
VAS day 6 evening	ANOVA	**0.000**
VAS day 7 morning	ANOVA	**0.002**
VAS day 7 evening	ANOVA	**0.002**
Plaque index T1	Kruskal-Wallis	0.491
Bleeding index T1	Kruskal-Wallis	0.418
Redness of the area/mucosa T1	Kruskal-Wallis	**0.000**
Granulation tissue T1	Kruskal-Wallis	**0.000**
Suppuration T1	Kruskal-Wallis	0.199
Swelling T1	Kruskal-Wallis	0.336
Re-epithelialization T1	Kruskal-Wallis	**0.000**
Bleeding T1	Kruskal-Wallis	**0.000**
Pain on palpation T1	Kruskal-Wallis	**0.000**
Odor T1	Kruskal-Wallis	**0.000**
Taste T1	Kruskal-Wallis	**0.000**

VAS=visual analogue scale; ANOVA=analysis of variance.

Table V. VAS score over a 7-day period in the three groups.

	Day 1 *vs.* 2 morning	Day 1 *vs.* 2 evening	Day 2 *vs.* 3 morning	Day 2 *vs.* 3 evening	Day 3 *vs.* 4 morning	Day 3 *vs.* 4 evening	Day 4 *vs.* 5 morning	Day 4 *vs.* 5 evening	Day 5 *vs.* 6 morning	Day 5 *vs.* 6 evening	Day 6 *vs.* 7 morning	Day 6 *vs.* 7 evening
CHX												
***p*-value**	0.006	0.008	0.00	0.00	0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Placebo												
***p*-value**	0.029	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H_2O_2/HA												
***p*-value**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CHX=chlorhexidine; H_2O_2=hydrogen peroxide; HA=hyaluronic acid.

Table VI. Wilcoxon signed-rank test results (*p*-values) for the evaluated variables.

	Plaque index	Bleeding index	Redness of the area/mucosa	Granulation tissue	Suppuration	Swelling	Re-epithelialization	Bleeding	Pain on palpation	Odor	Taste
CHX	0.00	0.00	0.083	0.00	0.083	0.00	1.00	0.00	0.014	0.119	0.869
Placebo	0.006	0.000	0.001	0.00	0.000	0.00	0.083	0.00	0.002	0.098	0.047
HO_2/HA	0.00	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.644	0.948

CHX=chlorhexidine; H_2O_2=hydrogen peroxide; HA=hyaluronic acid.

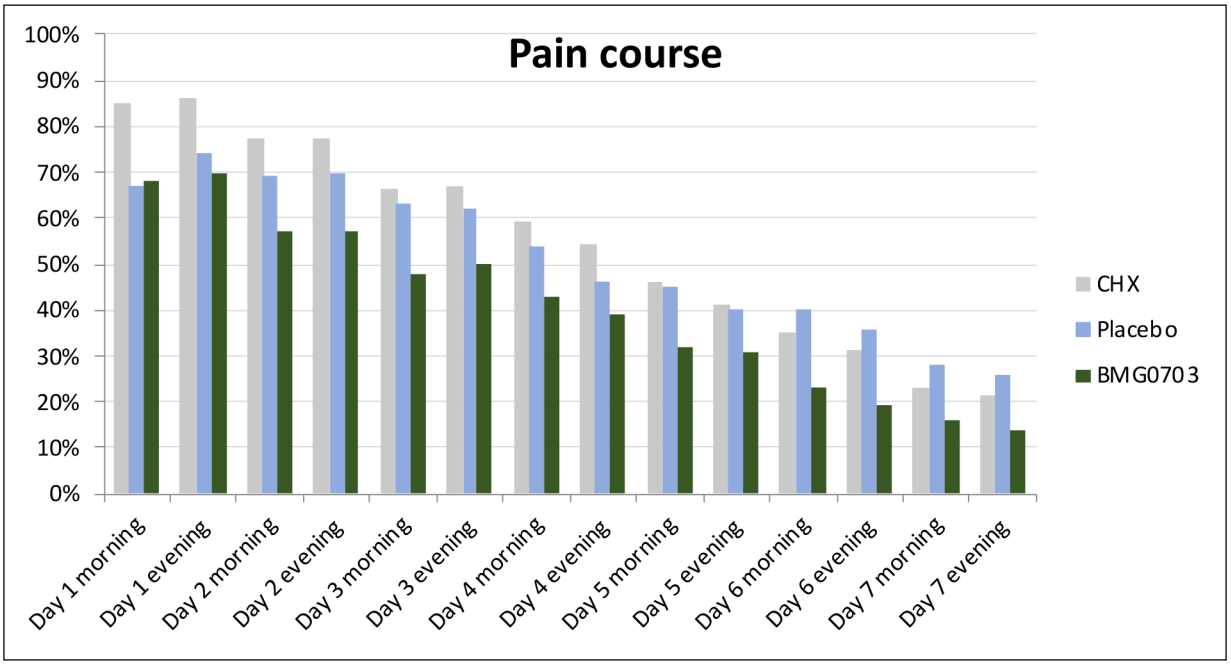

Figure 2. Pain scores for different treatment groups at different times and intervals. CHX=chlorhexidine.

Discussion

Third molar extraction is a common surgical procedure, with an overall percentage of complications ranging from 4.6% to 30.9%[26]. The most frequent postoperative complications include pain, trismus, edema, bleeding, inflammation, infection, alveolar osteitis, and iatrogenic damage to the inferior alveolar nerve or the adjacent tooth. Sinus involvement, mandibular or tuberosity fracture, and emphysema may occur, but they are less common[27,28]. In order to minimize such complications, a new formulation enriched with hyaluronic acid and hydrogen peroxide was developed. To date, no other study has tested an H_2O_2/HA rinse formulation, but a few clinical trials[8,28,29] have focused on the efficacy of HA after third molar extraction, both as a spray or a gel.

The pain was one of the main outcomes of the present study, as it is a common side effect of third molar extraction. It usually peaks about 3 hours after the surgery and then starts to decrease[30]. Many studies[31,32] on humans in laboratories have discovered sex differences in sensitivity to unpleasant stimuli, implying that biological mechanisms are at work. Sex hormones regulate pain sensitivity; pain threshold and pain tolerance in women vary depending on the time of the menstrual cycle. Men and women react differently to acute pain in terms of spatial pattern and severity, according to brain imaging studies[31]. However, Lövgren et al[32] found that in healthy individuals, the gender of the examiner impacts the pressure pain threshold (PPT), pressure pain tolerance (PTol), and pain intensity evaluated across the masseter muscles.

Guazzo et al[28] evaluated the effectiveness of a single intra-socket administration of sodium hyaluronate in order to promote healing after the third mandibular extraction. They found a non-significant difference in postoperative pain perception between the test and the control group, which became significant only on day 7 (p=0.02). These results are in contrast with ours, but it is difficult to make a comparison since they used a gel formulation, which was administered in a single post-operative application, thus with short retention time on the wounded tissues. A different clinical indication, i.e., gingivitis in regard to the effectiveness of H_2O_2/HA mouthwash, was reported previously by Boccalari et al 2022[33].

Other outcomes of the present study were the seven parameters of the Laundry-Turnbull and Howley index assessing healing: the

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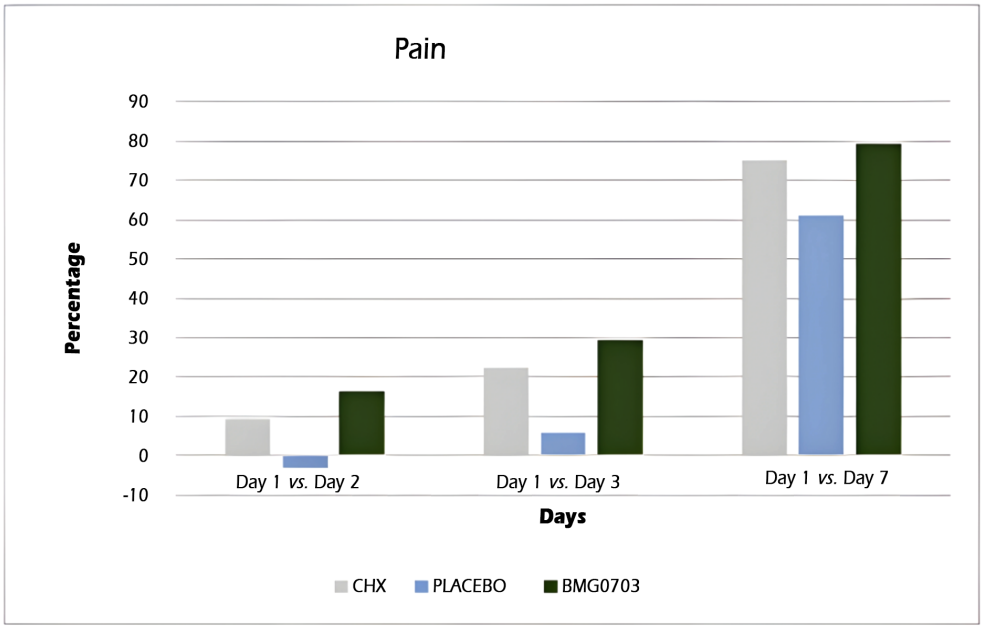

Figure 3. Percentage of reduction in VAS.

H_2O_2/HA mouthwash outperformed both the CHX and placebo ones regarding re-epithelialization. As shown by Voigt and Driver[34], HA improves the healing of chronic wounds of different etiologies, including surgical wounds and it also plays an important role in the tissue healing process[34-35]. As already said, the effect of H_2O_2 during wound healing has been observed since the very early stages of the healing process. H_2O_2 facilitates hemostasis through several mechanisms, including the activation of various tissue growth factors, the promotion of platelet aggregation, and the regulation of contractility and barrier function of endothelial cells. H_2O_2 has a strong oxidative and pro-inflammatory activity; it is also useful for removing cellular and pathogenic debris and stimulates the secretion of cytokines that promote tissue regeneration[12,13]. At low concentrations (250 μM), H_2O_2 promotes re-epithelialization through keratinocyte migration in the healing site[13].

The mechanism of action of the H_2O_2/HA mouth rinse is based on the synergy of these two ingredients, which together generate highly protective and filmogenic action; we believe the combined action of these two factors accelerates the healing process.

This study has a few limitations. Firstly, the use of NSAIDs on the day of surgery could have influenced the pain perception. Also, pain is a subjective assessment. Secondly, the sample size was relatively low. This was a proof-of-concept study, and a larger sample size with a multicenter clinical trial is needed. Lastly, the confounders were handled by randomization and statistical analysis[36]. However, it's important that the protocol should be similar so that the results can be replicated and be valid.

Conclusions

Under the limitations of the study, the results demonstrated that H_2O_2/HA mouthwash was more effective than CHX and placebo in decreasing pain after wisdom tooth extraction. Similarly, it was the most effective in re-epithelialization, decreasing redness and suppuration. H_2O_2/HA mouthwash does not have side effects typical of CHX, like tooth staining. The H_2O_2/HA-based mouth rinse can be considered a valid alternative to other popular mouthwashes, such as CHX, in the management and prevention of complications after third molar extractions.

Conflict of Interest

None to declare.

Ethics Approval
This research involving human participants and human data was performed in accordance with the Declaration of Helsinki. Ethics approval was granted by the Institutional Ethics Committee of the University of Milan, Milan, Italy (Prot. No. 22/19).

Informed Consent
Written informed consent was obtained from the patients (or their parents/legal guardians) to receive treatment and participate in the trial and to allow the use of their data for publication in this study.

Availability of Data and Materials
The datasets generated and data analyzed during the current study are not publicly available due to privacy but are available from the corresponding author upon reasonable request.

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BMG Pharma (Milan, Italy) provided the HA and H_2O_2 mouthwash used in the current study. The funding organization did not have any role in data collection and presentation of the results. Publication costs were covered by BMG Pharma.

Authors' Contributions
All authors have contributed equally to the conception and design, acquisition of data, analysis, and interpretation of data, as well as to drafting the article or revising it critically for important intellectual content, and final approval of the draft submitted for publication. All authors meet ICMJE criteria. No further changes to authorship were made after this point.

Trial Registration
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ORCID ID
Elisa Boccalari: 0000-0001-5078-7284
Shahnawaz Khijmatgar: 0000-0002-9177-3505
Chiara Occhipinti: 0009-0001-0008-5302
Massimo Del Fabbro: 0000-0001-7144-0984
Francesco Inchingolo: 0000-0003-3797-5883
Gianluca Martino Tartaglia: 0000-0001-7062-5143

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