Official title- The effects of natural enzyme containing mouthwash in wound healing after surgical removal of impacted mandibular third molar

NCT Number- Not yet assigned

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Study protocol and statistical analysis

Research Questions

1. Does the use of ORAL7® Mouthwash result in a different Landry wound healing index score compared to 0.2% CHX mouthwash after surgical removal of impacted mandibular third molar?

2. Is there a significant difference in pain score between the ORAL7® Mouthwash group and the 0.2% CHX mouthwash group after surgical removal of impacted mandibular third molar?

3. Does the use of ORAL7® Mouthwash result in a difference in mouth opening compared to 0.2% CHX mouthwash after surgical removal of impacted mandibular third molar?

Research Null Hypothesis

1. There is no difference in the Landry wound healing index score between the ORAL 7® Mouthwash group and the 0.2% CHX mouthwash group after surgical removal of impacted mandibular third molar.

2. There is no difference in pain score between the ORAL7® Mouthwash group and the 0.2% CHX mouthwash group after surgical removal of impacted mandibular third molar.

3. There is no difference in mouth opening between the ORAL7® Mouthwash group and the 0.2% CHX mouthwash group after surgical removal of impacted mandibular third molar.

Objectives:

General objective

To explore the effects of natural enzyme-containing mouthwash in comparison to 0.2% chlorhexidine gluconate mouthwash on wound healing after surgical removal of impacted mandibular third molar surgery under local anesthesia.

Specific objectives:

1. To compare the Landry wound healing index score of the natural enzyme-containing mouthwash group in comparison with the 0.2 % CHX mouthwash group following surgical removal of impacted mandibular third molar.

2. To compare the pain score of the natural enzyme-containing mouthwash group with the 0.2 % CHX mouthwash group after surgical removal of impacted mandibular third molar.

3. To compare the mouth opening of the natural enzyme-containing mouthwash group with 0.2 % CHX mouthwash after surgical removal of the impacted mandibular third molar.

Study design

This is a single-blinded randomized controlled trial. The outcome assessor will be blinded to group allocation. It will involve patients presented to Oral and Maxillofacial clinic, Hospital Pakar USM for surgical removal of impacted mandibular third molars under local anesthesia. Participants will be randomized into 2 groups: interventional (ORAL7® Mouthwash) and control (0.2 % CHX mouthwash) groups.

Study period

The study will be conducted from 1st April 2025 to 31st January 2026.

Study area

Oral and Maxillofacial clinic, Hospital Pakar Universiti Sains Malaysia (Hospital Pakar USM)

Study population

All patients planned for surgical removal of impacted mandibular third molar under local anesthesia at Oral and Maxillofacial clinic, Hospital Pakar USM.

Subject criteria

Inclusion criteria:

1. 18 years old and older until required number of samples has been obtained

2. Impacted mandibular third molar required bone removal

3. Absence of acute infection in impacted mandibular third molar

4. Absence of deep caries in the adjacent tooth

5. No known medical illness

6. No known drug allergy

7. Not on medication (prescribed or over the counter)

Exclusion criteria:

1. Mandibular third molar with soft tissue impaction only

2. Distoangulated tooth with deep impaction.

3. Active smoker

4. Pregnant women

5. Patients undergoing removal of impacted mandibular third molar due to trauma justification, such as the impacted tooth is at the fractured site.

6. Mentally challenged patient

Withdrawal criteria:

Participants may be withdrawn from the study under the following circumstances:

1. The participant chooses to withdraw consent at any time during the study period, without any obligation to provide a reason.
2. Failure to follow study instructions (e.g., incorrect use of the assigned mouthwash) despite verbal/written reminders.

3. The participant fails to attend follow-up visits and cannot be contacted after multiple attempts.
4. The participant experiences any adverse effect or allergic reaction to the mouthwash that necessitates discontinuation (e.g., hypersensitivity, burning sensation, swelling).
5. The development of post-operative infection or complications that require systemic antibiotic therapy, which may affect wound healing outcomes.
6. Any new medical condition or intervention that may interfere with study outcomes or participant safety.
7. If a participant becomes pregnant during the study period, they will be withdrawn to eliminate any potential risk to the fetus, even though the mouthwash is low-risk.

Sampling method

This study will employ consecutive sampling with simple randomization of all eligible adult patients who present to the Oral and Maxillofacial Surgery (OMFS) clinic at Hospital Pakar USM and are scheduled for surgical removal of impacted mandibular third molars under local anesthesia during the study period (1st April 2025 to 31st January 2026).

Patients meeting the inclusion and exclusion criteria (outlined in Section 3.5) will be invited to participate. Those who provide informed consent will be enrolled, with recruitment proceeding consecutively until the target sample size is achieved (n = 58, accounting for a 10% anticipated dropout rate).

Enrolled participants will be randomized into one of two groups (intervention or control) using a simple randomization method based on a pre-generated random number list (see Section 3.13). This process ensures each participant has an equal chance of allocation to either group, minimizing selection bias.

Sample Size Determination

Objective 1: G*Power software version 3.1.9.7 will be used to calculate the minimum required sample size for determining the Landry wound healing index score of the ORAL7® Mouthwash group compared to the 0.2% chlorhexidine gluconate mouthwash group following the surgical removal of mandibular third molars. The sample size estimation will be conducted using an independent sample t-test with the following parameters: two-tailed analysis, an effect size of 0.8, a significance level (α) of 0.05, a power (1-β) of 0.8, and an allocation ratio of 1. Based on a standardized effect size of 0.8 as defined by Cunningham (1978), the total required sample size is 52, with 26 participants allocated to each group.

Objective 2: G*Power software version 3.1.9.7 will be used to calculate the minimum required sample size to determine the pain scores of the ORAL7® Mouthwash group with the 0.2% chlorhexidine gluconate mouthwash group following surgical removal of mandibular third molars. The calculation will employ ANOVA for repeated measures with parameters including an effect size of 0.4 (as stated by Cunningham, 1978), a level of significance (α) of 0.05, a power (1−β1) of 0.8, two groups, seven measurements, and a correlation of 0.5 among repeated measures. Based on these parameters, the total sample size required is calculated to be 32 participants.

Objective 3: G*Power software version 3.1.9.7 will be used to calculate the minimum required sample size to compare the mouth opening of the ORAL7® Mouthwash group with the 0.2% chlorhexidine gluconate mouthwash group following surgical removal of impacted mandibular third molars. The sample size will be estimated using an independent sample T-test with a two-tailed design, an effect size of 0.8 (as per Cunningham, 1978), a significance level (α\alphaα) of 0.05, a power (1−β1-\beta1−β) of 0.8, and an allocation ratio of 1. Based on these parameters, the total sample size required is 52 patients, with 26 participants in each group

The total sample size calculated based on all 3 objectives will be 52 patients. By anticipating 10% non-compliance and drop out of patients during this study, a final sample size of 58 patients will be included in this study and they will be randomized into 2 groups (interventional and control), with 29 patients in each group.

Research Tools and Variables

Research Tools

a. Oral 7 mouthwash

b. 0.2% chlorhexidine gluconate mouthwash

c. SPSS software

d. Landry wound healing index

e. Visual analogue scale

f. Metal ruler

Research Variables

a. Demographic characteristic such as age, gender

b. Angulation of impacted teeth

c. Duration of surgery

Data collection method

This study is going to be conducted in the Oral and Maxillofacial surgery clinic of Hospital Pakar USM. Patients who require surgical removal of impacted mandibular third molar are enrolled and selected based on inclusion and exclusion criteria. Written informed consent to participate in the study will be taken from the subject without obligation. Informed consent can be withdrawn anytime by the subject throughout the study without penalty and continuation of care and treatment will not be affected.

Data collection form will be prepared for each patient, to record:

a. Subject identification number (ID)

b. Age

c. Gender

d. Tooth angulation

e. Duration of surgery

f. Pain score (day 1 to 7)

g. Numbers of analgesic consumed (day 1 to 7)

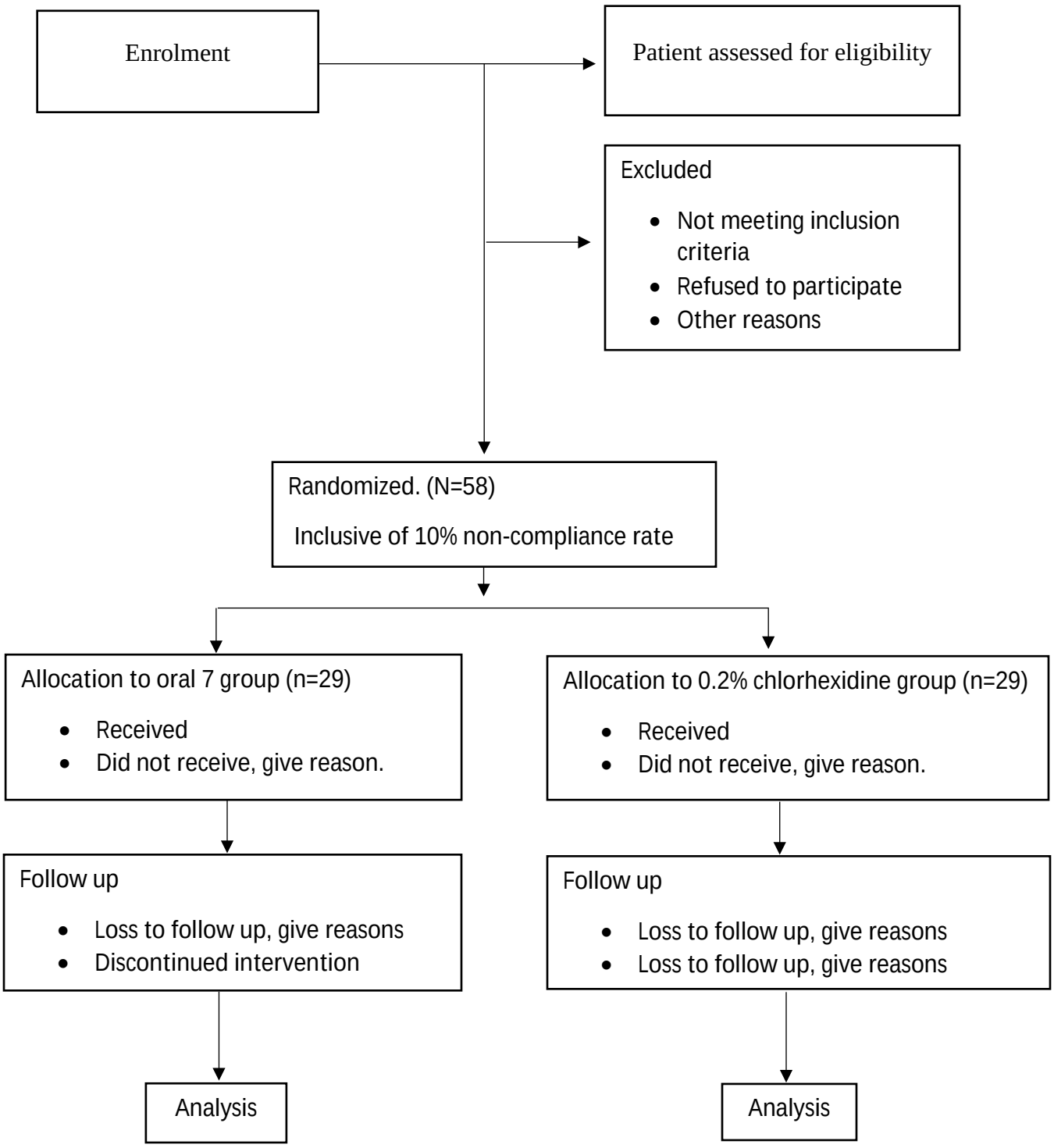
h. Landry wound healing score on day 7

Randomization process- Following confirmation of eligibility and acquisition of informed consent, participants will be assigned a unique subject identification number and subsequently randomized into one of two study arms: the intervention group (ORAL7® Mouthwash) or the control group (0.2% chlorhexidine gluconate mouthwash). Randomization will be conducted using a computer-generated list of random numbers (generated via random.org) to ensure equal probability of assignment and to minimize selection bias. Allocation concealment will be maintained by preparing 58 sealed, opaque envelopes, each containing a number corresponding to one of the two groups. Participants will be asked to draw one envelope prior to surgery, which will determine their group assignment. The designated mouthwash, pre-packaged and unlabeled by a designated study assistant, will then be provided post-operatively.

Data Analysis

Data will be entered and analyzed using SPSS version 29.0.2.0. Descriptive statistics will be used to summarize the socio-demographic characteristics of subjects. Numerical data will be presented as mean (SD) or median (IQR) based on their normality distribution. Categorical data will be presented as frequency (percentage). G*Power software version 3.1.9.7 will be used for repeated measures ANOVA and independent t-test will then be used to determine the P-value. P-value 0.05 as non-significant.

Study flowchart



Sample recruitment

Patients planned for surgical removal of impacted mandibular third molars under local anesthesia will be selected based on inclusion and exclusion criteria. Only those agree to participate in this study will be recruited and written informed consent will be obtained from participants.

Randomization

All participants recruited in the study will be randomly assigned into 2 groups. One group will receive ORAL7® Mouthwash as postoperative mouth rinse and the other group is control, in which 0.2 % chlorhexidine gluconate mouthwash will be given. Randomization of participants will be conducted using random numbers generator (random.org).

A list of random numbers will be generated for each group (total 58 numbers for 2 groups). Each group will have a set of 29 numbers. A container containing 58 papers with one written number on each will be prepared. Each participant will be instructed to draw a paper from the container before the surgical procedure commences. The number written in the paper will indicate the allocated group for the participant and as subject identification number.

Allocation concealment

The Dental Surgery Assistant (DSA) will identify the participants as control or interventional group, and mouthwash without label will be given to participants after completion of surgery.

Calibration

Intra and inter examiner calibration of Landry healing index will be conducted before study commences. It will consist of 2 sessions: alignment followed by assessment session. In this study, 2 examiners will be involved for assessment of Landry healing index score of surgical wounds after removal of impacted mandibular third molar.

Sample size for the calibration process will be calculated based on intraclass correlation, with 5 observations per subject, level of significance: 0.05, power: 0.8, minimum acceptable reliability: 0.6, expected reliability: 0.8, drop out 10% (Walter et al., 1998).

The sample size will be 28 for this study.

Calibration process will be repeated until full agreement is achieved between 2 examiners.

1) Alignment session

In this session, the Landry healing index will be explained. The clinical photographs of surgical site 1-week post-op with their respective Landry healing index scores are shown and explained. Agreement between 1st and 2nd examiner on the scoring will be obtained.

2) Assessment session

This session will begin after the alignment session. Subjects for assessment exercise will be recruited with entry criteria comparable to study subjects. Informed consent is obtained. Examiners will rotate among subjects to assess the surgical site of each subject and respective Landry healing index score is recorded.

Total 2 rounds of examination are completed. Pairs of repeated scores are used to assess intra examiner reproducibility. Results will be analyzed using SPSS, intraclass correlation coefficient will be obtained and classified. Values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability (Koo & Li, 2016). Assessment session will end when good or excellent reliability is obtained.

Clinical Procedures

Orthopantomogram (OPG) will be taken for routine pre-operative assessment. Cone beam computed tomography (CBCT) will be ordered if indicated. Written consent will be taken from patients. Mouth opening of the patients will be measured by using a metal ruler in millimeters from mesioincisal edge of upper right central incisor to mesioincisal edge of lower right central

incisor prior to surgery. Different relevant reference points can be taken if central incisor(s) is missing, and alternative points will be recorded in data collection form.

Patients will be disinfected and draped under standard procedure. All the surgeries will be performed by qualified operators under aseptic techniques. Local anesthesia will be achieved by administering inferior alveolar, long buccal, and lingual nerve blocks using 2 cartridges 2.2 ml of 2% Scandonest special (2% mepivacaine in 1:100,000 adrenaline).

Copious irrigation of the surgical site will be done using normal saline solution (40 ml) before the incision is made using no. 15 blade. A full mucoperiosteal envelope flap will be reflected. Buccal and distal bone guttering and the impacted mandibular third molar will be sectioned, when necessary, using surgical drill and normal saline irrigation, the tooth will be removed completely. Socket curettage and smoothening of sharp bony edges will be undertaken, flap and the extraction socket will be thoroughly irrigated with normal saline. Closure of the surgical wound will be done using 4-0 Vicryl suture. The duration of the surgery will be calculated from the time of incision till the last suture is given.

Post-operative instructions will be given to the patient. The clinical research assistant will explain the instructions to the patients in Bahasa Malaysia. Patients will be instructed to start using the respective mouthwash (blinded from the principal investigator and patient) given on day 1 post op, in which one cup of about 10ml solution to be gargled for at least 30 seconds and then spit out. This is to be done 3 times per day for 1 week. Non-steroidal anti-inflammatory analgesic (capsule Celecoxib 200mg BD for 1 week) will be given to the patient. Rescue analgesic (tablet paracetamol 1000mg QID for 1 week) will be prescribed. No systemic antibiotics will be prescribed before or after the procedure. All patients will be requested to record their pain score and numbers of analgesics consumed, at 8am each day, for 7 days post operatively in each notebook.

Pain assessments are standardized at 08:00 hours each day to enhance methodological rigor and ensure temporal consistency across all participants. This fixed time point allows for standardization, thereby minimizing diurnal variation in pain perception and controlling for confounding influences related to circadian rhythms, physical activity, and analgesic pharmacodynamics. Recording pain at a uniform time facilitates comparability between subjects and across postoperative days, strengthening the internal validity of the dataset.

The choice of 08:00 hours specifically is informed by clinical and physiological considerations. It reflects a likely peak in discomfort following the waning effects of local anesthesia and overnight analgesia, capturing a more accurate baseline of spontaneous postoperative pain. Moreover, this time aligns with the routine clinical workflow, enabling synchronized follow-up assessments and promoting adherence among participants. Thus, the fixed 08:00 a.m. time point is not only methodologically sound but also pragmatically appropriate within the clinical setting of this study.

A review appointment 1 week after surgery for suture removal and examination for the assessment of pain score, mouth opening, and wound healing will be given to patients. These assessments will be performed by a blinded examiner after calibration with another expert.

Pain will be measured by using visual analogue scale from 0 to 10. Mouth opening will be measured by metal ruler using the same reference points as preoperatively. Soft tissue healing of the surgical site will be investigated using the Landry Healing Index of Landry, Turnbull and Howley. This index is intended to assess healing of extraction sockets one week after intervention based on the following parameters: tissue color, bleeding response to palpation, presence of granulation tissue, characteristics of the incision margins and the presence of suppuration. Each wound can be scored from 1 (very poor healing) to 5 (excellent healing) (Marini et al., 2019).

The total duration of involvement of each patient will be 8 days starting from the day of the surgery till the 7^{th} post-operative day that is the day for follow-up visit. The duration of the first visit that is the day on which the sugery will take place will depend entirely on factors like type and position of impaction, surgeon's experience, tooth morphology and root anatomy, patient factors like mouth opening, anxiety level, cooperation etc. and will usually take around 45 minutes for completion. The duration of the follow-up visit will be around 30 minutes. This is the standard time for each patient. In case of breakthrough pain which cannot be controlled by celecoxib and paracetamol, the patient will be instructed to contact one of the investigators as soon as possible as per the contact information given in the patient consent form which will be given to each patient participating.