



College of Dentistry

Clinical and Immunological Efficacy of *Thymus Vulgaris* Mouthwash in the Management of Biofilm-Induced Gingivitis: A Randomized Double-Blind Clinical Trial

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2025-2026

1. Project details

1.1. Investigator's details

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1.2. Sponsor Details:

College of Dentistry / University of Sulaimani.

1.3. Project Title:

Clinical and Immunological Efficacy of *Thymus Vulgaris* Mouthwash in the Management of Biofilm-Induced Gingivitis: A Randomized Double-Blind Clinical Trial

1.4. Introduction

Biofilm-induced gingivitis is a relatively common and reversible inflammatory disease confined to the gingiva, characterized by signs such as erythema, gingival swelling, and bleeding upon gentle probing, with no loss of attachment or bone (1). The main etiology of this disease is the interaction between microbes in the dental biofilm and the host-immuno-inflammatory response, which results in the release of pro-inflammatory mediators, such as interleukin-1 β (IL-1 β) and matrix metalloproteinase-8 (MMP-8) (2). Overproduction of these mediators contributes to tissue degradation and may lead to periodontal breakdown (3).

Biofilm-induced gingivitis is reversible following proper periodontal therapy. Conversely, persistence of the condition, either due to negligence or improper management, can lead to the development of periodontitis, characterized by the irreversible loss of the periodontal attachment apparatus, which results in tooth loss if left untreated (4).

Scaling and root surface debridement (SRD) is still considered the gold standard in the management of periodontal disease, especially in biofilm-induced gingivitis (5). However, SRD does not always result in the complete removal of dental biofilm, especially in areas with restricted visualization and access, such as deep subgingival periodontal pockets or furcation areas. This resulted in the emergence of adjuncts to aid in the complete eradication of pathogenic bacteria and reverse the disease process to a healthy state (6).

A variety of adjuncts to SRD were proposed for the management of periodontal disease, including mouthwashes, antibiotics, locally delivered antimicrobials, and laser therapy, with mouth rinses being the most commonly used (7–10). Chlorhexidine (CHX) is considered the gold standard for the reduction of microbial load and inflammation (11). However, its use is associated with side effects like external teeth staining, altered taste sensation, dry mouth, discolored or coated tongue, and, on rare occasions, lymphadenopathy, burning sensation, and oral mucosal shedding (12). Consequently, increasing research focuses on mouthwashes made from natural herbal products as safer alternatives with comparable efficacy to CHX (13).

Thymus vulgaris (TV), or thyme as its common name, comes from the *Lamiaceae* family of *Labiatae* of the plant kingdom (14). TV has been mostly used in the culinary industry to enhance the flavor of cheeses and meats. In addition to its culinary uses, TV is also used as a herbal medication for the treatment of alopecia, bronchitis, cough, and gastrointestinal distress (15). The main components of TV, Thymol and carvacrol, have been shown by several studies

to possess antimicrobial, anti-inflammatory, antioxidant, anti-viral, and anti-fungal effects, rendering their efficacy in the eradication of microbes when used in a mouthwash or gel formulation at various concentrations (16–22). Furthermore, herbal mouthwashes in general are highly accepted by patients due to their biocompatibility, safety, pleasant flavor, aroma, availability, and low cost in comparison to chemical agents (23,24).

To the best of our knowledge, no standardized, double blind clinical trial was conducted to assess the clinical and immunological efficacy of TV in a mouthwash formulation for the management of periodontal diseases. Therefore, filling a gap that is present regarding an alternative and safe adjunct to CHX. The study aims to assess whether TV possesses comparable or even superior anti-plaque and anti-inflammatory properties when compared to a placebo and the gold standard adjunct CHX, with the least undesirable side effects. The result of this clinical trial will serve as a sign concerning the therapeutic ability of TV as an innovative adjunct in the treatment of biofilm-induced gingivitis.

1.5. Originality of Research

This study focuses on comparing TV mouthwash with both a placebo and gold-standard CHX mouthwash, providing a detailed comparative assessment of its efficacy in the management of biofilm-induced gingivitis clinically and immunologically. By utilizing a randomized, double-blind design, the study ensures the validity of the findings and reduces the chances of bias. This comparative approach has not been previously investigated in a standardized, double-blind clinical trial frame. By comparing this particular herbal mouthwash with other adjuncts in the management of biofilm-induced gingivitis, a critical gap in the literature will be filled, providing an evidence-based alternative to chemical plaque control agents.

2. Research Questions, Aim(s), and Objectives

2.1. Research Question

Can TV mouthwash have superior or comparable anti-plaque and anti-inflammatory properties when compared to a placebo and the gold standard adjunct CHX with minimal undesirable side effects?

2.2. Aim

This randomized, double-blind, parallel, 3-arm clinical trial aims to evaluate the efficacy of TV mouthwash in the management of biofilm-induced gingivitis by assessing its impact on plaque index (PLI), bleeding on probing (BOP), gingival index (GI), salivary level of pro-inflammatory mediators (IL-1 β and MMP-8) in comparison to a placebo and CHX mouthwash.

2.3. Objectives

1. Registration and obtaining ethical approval for the study.
2. Obtaining a mouthwash containing TV in a 0.156% concentration and evaluating its substantivity.
3. Obtaining informed consent from each participant before data collection.
4. Participant recruitment.
5. Assessing PLI, BOP, and GI before intervention.
6. Saliva collection for the assessment of salivary biomarkers (IL-1 β and MMP-8) before intervention.
7. Re-assessing the same clinical parameters after 2 weeks, and saliva collection.
8. Re-assessing the same clinical parameters after 1 month, and final saliva collection.
9. Comparing the efficacy of TV mouthwash with placebo and CHX mouthwashes by analyzing the data statistically.
10. Evaluating patient acceptance of the assigned mouthwash.

2.4. Hypothesis

The null hypothesis to be tested is:

TV mouthwash has no significant effect in the reduction of clinical parameters such as plaque levels, BOP, and GI, and no major impact in the reduction of immunological markers (IL-1 β and MMP-8) in patients with biofilm-induced gingivitis compared to placebo and CHX mouthwash.

3. Materials and Methods

3.1. Study Design

A randomized, double-blind, parallel, 3-arm controlled clinical trial will be conducted. Participants from the clinics of the Periodontics Department, College of Dentistry/University of Sulaimani will be recruited (Figure 1).

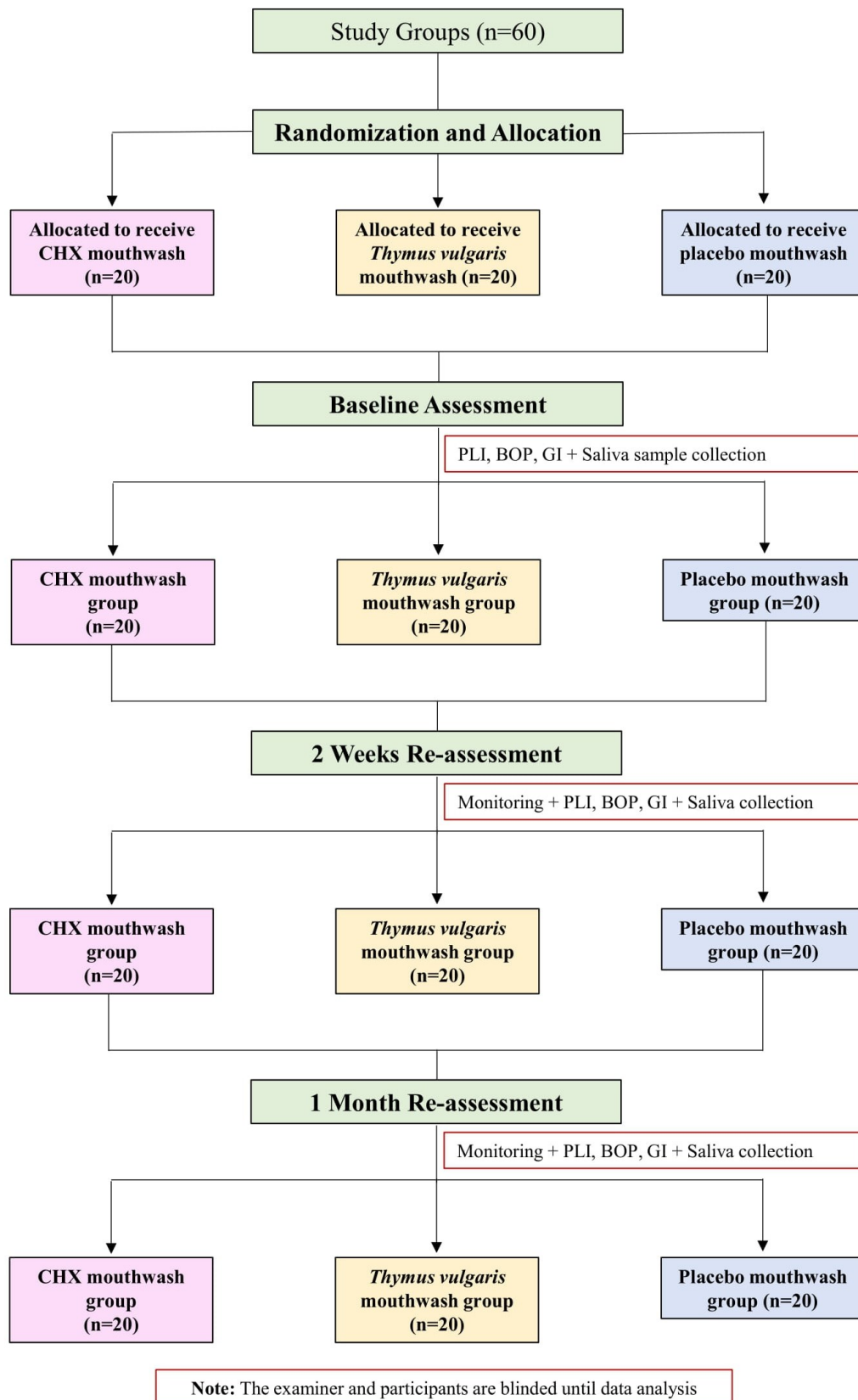


Figure 1: Detailed diagram of the study design

3.2. Study Subjects

Participants will be recruited based on meeting the inclusion and exclusion criteria.

The study's inclusion criteria include individuals who are:

1. 18 – 45 years of age.
2. Diagnosed with biofilm-induced generalized gingivitis (>30% BOP sites, no loss of periodontal attachment (25)).
3. Possessing more than 20 natural teeth.
4. Non-smokers and in good systemic health (ASA I according to the American Society of Anesthesiologists classification (26)).

The study's exclusion criteria consist of individuals who:

1. Have active dental caries requiring immediate treatment.
2. Absence of true periodontal pocket.
3. Have ongoing orthodontic treatment.
4. Are pregnant or intend to become pregnant.
5. Are lactating mothers.
6. Received periodontal treatment and/or antibiotics within the past 3 months.
7. Are allergic to herbal products or mouthwashes.
8. Have any known chronic systemic disease.

Participant information and consent form will be given and signed by all participants before the commencement of the study provided by the examiner (Appendix I and II).

3.3. *Thymus vulgaris* Mouthwash Preparation

TV essential oil will be prepared in a mouthwash formulation in 0.156% concentration provided for another researcher in an ongoing study. The produced mouthwash is used in an *in vitro* study to assess its efficacy against some strains of Streptococci, namely *Streptococcus mitis*, *oralis*, and *sanguinis*. The mentioned study is at its final stages, showing the antibacterial effect of TV mouthwash with all the data collected and analyzed.

3.4. Substantivity Test

The substantivity of TV mouthwash will be evaluated *in vitro* using sound extracted human teeth removed for orthodontic purpose, or due to being periodontally compromised with a hopeless prognosis. These teeth will be used as the substrate, which mimics the clinical environment of the oral cavity. Before testing, the collected teeth will be cleaned of any debris and gently polished. A total of 36 human teeth will be collected and divided into 3 equal groups: test group (TV mouthwash), positive control group (CHX mouthwash), and negative control group (distilled water). The teeth will be stored in a suitable medium (Chloramine-T) to prevent microbial growth, and then air-dried before use (27). Afterwards, the teeth will be incubated at 37°C in filtered human saliva obtained from healthy individuals with no periodontal disease to stimulate an acquired pellicle. The teeth will be divided equally and immersed in a container filled with the designated solution for a standardized period (60 seconds) to simulate clinical rinsing and later inserted back into the filtered saliva at 37°C. At different time intervals (30 minutes, 1 hour, 4 hours, 6 hours, 12 hours, and 24 hours), the samples will be chemically analyzed using High-Performance Liquid Chromatography or Ultraviolet–Visible spectroscopy to measure the mouthwash content on the substrates (28).

3.5. Sample Size Calculation

BOP was set as the primary outcome and used to estimate the sample size using G-Power analysis software (G*Power 3.1.9.7, Franz Faul, Universitat Kiel, Kiel, Germany) (Figure 2). If a mean BOP difference in the change between methods of 0.48 was to be detected at α 0.05 with an 80% power. The appropriate number of participants per group would be 15. To account for any possible dropout during the trial, the sample size was rounded to 20 participants per group (22).

The 3 study groups are: Group I: Participants using 0.12% CHX mouthwash. Group II: Participants using the test (TV) mouthwash in 0.156% concentration. Group III: Participants using a placebo mouthwash

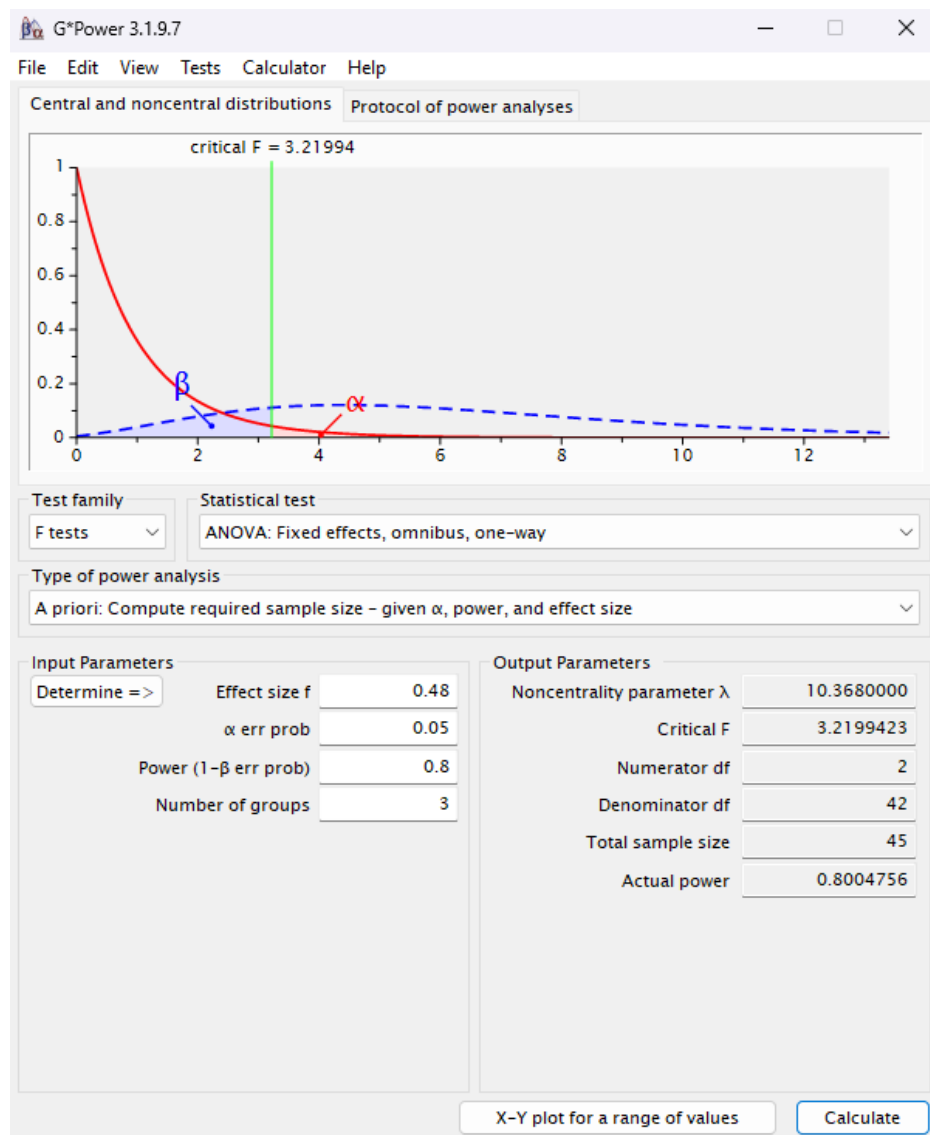


Figure 2: The sample size required for this study was calculated by (G*Power Software).

3.6. Intra- and Inter-Examiner Calibrations

Intra- and inter-examiner calibrations will be performed to check for the validity and repeatability of the clinical periodontal parameters of PLI, BOP, and GI. Cohen's kappa (κ) statistic will be used to assess the inter- and intra-calibration.

3.7. Study Visits and Interventions

Participants from the clinics of the Periodontics Department, College of Dentistry/University of Sulaimani will be recruited on Sunday to Wednesday from 9:00AM to 12:00AM. At the first visit, a case sheet including the participant's name, age, sex, and medical and dental history will be documented (Appendix III). Unstimulated saliva will be collected for the assessment of (IL-1 β and MMP-8) levels. In addition, the clinical parameters (PLI, BOP, and GI) will be assessed. All the participants will receive proper motivation and oral hygiene instruction for daily brushing and flossing, followed by scaling using an ultrasonic scaler, and polishing by rubber cup and pumice. Furthermore, each participant will receive one of 3 different mouthwashes, which include a 0.12% CHX mouthwash (Lacer Peroxidin, Lacer®, Barcelona, Spain) as a positive control, distilled water combined with food additives as a negative control/placebo. The last group, TV mouthwash, will be used as a test intervention.

The participants will be asked to rinse with 10 mL of the assigned mouthwash twice a day for 1 minute for each use, 30 minutes after toothbrushing, and will be instructed to refrain from eating and drinking 30 minutes after mouthwash use. To obtain proper standardization, all participants will be asked to use the same toothbrush with soft bristles (TePe® Select Soft, TePe®, Sweden), and a fluoride-containing toothpaste (Crest® Pro-Health, Crest®, USA) provided by the examiner. Participants will be instructed to follow the same brushing technique (modified Bass technique) and will be instructed to comply with proper flossing technique. A detailed instruction manual will be given to each participant on how to use the mouthwash (Appendix IV & V).

Two weeks after the first visit, participants will be called back to evaluate the compliance with mouthwash usage and assess any potential side effects of the mouthwashes (CHX, TV, Placebo). In addition, the clinical parameters (PLI, BOP, and GI) will be re-assessed, and the saliva sample will be retaken. One month after the first visit, the clinical parameters will be reassessed, and another saliva sample will be collected for immunological and biochemical assessment. Finally, participants will be asked to complete a Visual Analogue Scale (VAS) questionnaire (Appendix VI & VII) in order to obtain feedback for their experience with the assigned mouthwash.

3.8. Randomization and Blindness

Participants will be randomly assigned to 3 intervention groups. Each group will be assigned a distinct letter (A, B, or C), which corresponds to the intervention that will be used. Furthermore, a random number generator will then be used to properly order the groups and participants. Equal randomization (1:1:1 allocation ratio) will be utilized to ensure equal group and participant distribution. This process will be achieved using a Microsoft Excel random table (Microsoft Office 2016, Microsoft Corporation, USA).

To ensure the blindness of the study, all the mouthwashes will be dispensed in identical, opaque containers, each of which will be labelled with a random letter (A, B, or C) assigned by a third party not involved in the clinical trial. This strategy will maintain a double-blind design, where both the examiner and participants remain unaware of the intervention given throughout the study. Finally, group codes will be revealed only after the completion of the trial, during the data analysis step.

3.9. Saliva Sample Collection and Preparation

Saliva will be collected by the passive drool method, which is a common and efficient saliva collection technique in which participants are seated comfortably in an upright position and asked to tilt their heads forward to allow saliva to pool passively in the floor of the mouth, which facilitates the collection of whole, unstimulated mixed saliva. Saliva will then be allowed to passively drip into a sterile, graduated plastic tube for 5 minutes, without any stimulation or active spitting. 2-5 mL of saliva is aimed to be collected per participant using this technique. Before saliva collection, participants will be instructed to refrain from food intake, beverages, and oral hygiene practices for a minimum of 2 hours (29).

The collected saliva will then be aspirated using a micropipette and transferred to a labelled plastic test tube. Samples will then be stored immediately in a portable cooling container. In addition, samples will be centrifuged at 4000 rpm for 15 minutes to separate salivary supernatants from cellular components. After centrifugation, the saliva samples will be aspirated into Eppendorf tubes and stored at -80 °C until analysis (30).

3.10. Clinical Measurements

3.10.1. Plaque Index

Modified Quigley-Hein Plaque Index (MQHI) will be utilized to check for the amount of accumulated dental biofilm on all the surfaces of all teeth except the third molars. Six surfaces per tooth will be examined (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). The presence of the biofilm will be visualized by using a disclosing agent (GC Tri Plaque ID Gel, GCTM, Japan), which will stain the teeth and allow for the identification of the available dental biofilm. For the assessment, the examiner will apply the disclosing agent on the participant's teeth with a brush applicator and leave it on the teeth for approximately one minute. Following the gel application, the participant will be instructed to lightly rinse their mouth with water. Afterwards, the stained dental plaque will be scored on a 0-5 scale based on the MQHI, which will indicate the amount of accumulated plaque. The measurement will be done using a UNC-15 probe from the gingival margin to the edge of the stained plaque. The mean plaque index score will be determined by dividing the sum of the scores by the total number of surfaces assessed (31).

3.10.2. Bleeding on Probing

This index will be assessed by gently inserting a periodontal probe into the gingival sulcus or pocket parallel to the long axis of the tooth and using a light force of 20-25 grams. The probe is advanced until slight resistance is felt. Six surfaces of each tooth will be examined (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). Scores will be assigned based on the absence or presence of bleeding after 15-30 seconds of probing at a site (0= no bleeding, 1= bleeding) (32).

3.10.3. Gingival Index

GI will be recorded on the mesial, distal, buccal, and lingual surfaces of all teeth except the third molars. Each surface will be carefully dried and examined under adequate illumination, and a UNC-15 periodontal probe will be used to assess the presence of bleeding following gentle probing at the gingival margin. Each surface will then be assigned a score ranging from 0 to 3. The scores of the four surfaces of each tooth will be summed and divided by four to

yield the mean gingival index score for that tooth. Subsequently, the individual tooth scores will be averaged to obtain the participant's overall gingival index (33).

3.11. Data Analysis

The statistical analysis will be conducted with IBM SPSS Statistics® (version 26, IBM, Chicago, USA). A p-value ≤ 0.05 will be considered statistically significant. The Shapiro-Wilk test will be utilized to check if the results are normally distributed. If the results are normally distributed, parametric tests (Mean $\pm$ Standard Deviation, ANOVA and paired t-test) will be conducted. However, if the results are not normally distributed, non-parametric tests (Median/Mean rank, Mann-Whitney, and Kruskal-Wallis) will be used.

3.12. Outcome Measures

- Primary outcome measure: Reduction in BOP
- Secondary outcome measures: Reduction in PLI, GI, and (IL-1 β , MMP-8) levels

3.13. Setting

Periodontics Department – College of Dentistry – University of Sulaimani

3.14. Ethical Considerations

Approval to conduct a clinical trial will be sought, and participants will receive an official consent form before participating in the clinical trial. Furthermore, the study will follow the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)(Appendix VIII). Registration and approval of conducting a randomized clinical trial online will be applied to <https://clinicaltrials.gov>. The data collected will be used for publication in a scientific or academic journal.

3.15. Intervention and Quality Control

The intervention treatment in this study will be scaling and polishing with the application of 3 groups of therapeutic agents (CHX mouthwash in 0.12% concentration, mouthwash containing TV, and a placebo mouthwash consisting of distilled water and food additives).

4. Project Management and Expertise

Dr. Aram Mohammed Sha, BDS, MSc, PhD, Periodontics Department, College of Dentistry, University of Sulaimani, Kurdistan Region, Iraq.

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5. Ethical issues

Ethical approval will be obtained from the College of Dentistry / University of Sulaimani.

6. Dissemination

The findings of this research will form the basis of the MSc, and there is a plan to publish the findings in a scientific journal.

7. Intellectual Property

The College of Dentistry/ University of Sulaimani will hold all rights for intellectual property.

8. Funding, Costing Schedule, and Funding Arrangements

This research will be entirely self-funded. The estimated total expenditure of this research study is approximately 7,000,000 IQD

367 **9. Conflict of Interest**

368 Upon receiving the ethical approval, the authors declare no conflict of interest in this research.

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370 **10.Acknowledgment**

371 The resources provided by the staff and faculty of College of Pharmacy in University of
372 Sulaimani are highly appreciated.

373

374 **11.Author's Contribution**

- 375 - **Yousif OA:** Methodology, Software, Validation, Formal Analysis, Resources, Data
376 Curation, Writing Original Draft, Investigation, and Visualization
- 377 - **Sha AM:** Conceptualization, Supervision, and Project administration, review of draft, and
378 dissemination.

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APPENDICES

APPENDIX I



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PARTICIPATION INFORMATION & CONSENT

Title of the Study:

Clinical and Immunological Efficacy of Thymus Vulgaris Mouthwash in the Management of Biofilm-Induced Gingivitis: A Randomized Double-Blind Clinical Trial

Principal Investigator:

Dr. Omer Abdalla Yousif. Periodontics Department, College of Dentistry, University of Sulaimani.
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Introduction:

You are being invited to participate in a clinical research study. Please take the time to carefully read the information provided. You have the opportunity to ask any question about the research study. You will be asked to sign this form if you choose to agree to participate.

Purpose of the Study:

The purpose of the study is to evaluate the clinical, immunological, and biochemical effects of an herbal mouthwash containing Thymus vulgaris (Thyme) in the treatment of biofilm-induced gingivitis. The efficacy of the mentioned mouthwash will be compared with 2 other mouthwashes (Chlorhexidine and placebo). The aim is to publish this research in a scientific journal.

Study Design:

The frame of the study is a randomized double-blind clinical trial, which means neither you nor the researcher will know which mouthwash is given (Test mouthwash / Chlorhexidine / Placebo) to maintain an unbiased result.

Procedure:

If you agree to participate:

1. You will undergo an initial oral examination, including plaque and gingival index assessment.
2. A small amount of your saliva will be collected for biomarker analysis.

3. You will be randomly assigned to use one of three mouthwashes (Thymus vulgaris, Chlorhexidine, or placebo).
4. You will be instructed to rinse with the assigned mouthwash twice daily for 1 month
5. You will return for a follow-up visit after 1 month to assess clinical outcomes and provide further saliva samples.

Duration of Participation:

Your participation in the study will last 4 weeks, including follow-up visits.

Benefits:

Although you may not directly benefit from participation, your oral health may improve due to regular care. Additionally, the study will help advance scientific understanding of herbal treatments in periodontology.

Risks and Discomforts:

- Mild and temporary burning sensation or taste alteration may occur.
- Allergic reactions to herbal or chemical components are rare but possible.
- Collection of saliva is non-invasive and painless.

Voluntary Participation and Freedom to Withdraw:

Your participation is entirely voluntary. You may refuse to participate or withdraw at any time without penalty or loss of care. Your decision will not affect your relationship with the university clinic.

Confidentiality:

All data collected from you will be kept strictly confidential. Your name will not appear in any publication. Data will be stored securely and only accessible to the research team.

Participation Cost

There is no cost to you for participating. All materials (mouthwash, toothpaste, and toothbrush) will be provided free of charge.

Right to Ask Questions:

You have the right to ask questions about the study at any time during your participation period. If you have any questions, you can contact the principal investigator with either the phone number or email provided in the first page.



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Please select the appropriate field	Yes	No
I understand that my participation is entirely voluntary.		
I confirm that I have been informed about this research project, and I have been given the opportunity to ask questions about the project.		
I agree to participate in this project, and I understand that participating in the project includes collecting samples of saliva for use in this study.		
I understand that I will not benefit directly from participating in this research.		
I agree that the investigator(s) may use any information obtained from me for the purpose of this research project.		
I affirm that I am 18 years of age or older.		

Participant's Name:

Date:

Signature:

APPENDIX II



College of Dentistry

زانپاری و پرهمانهندی به‌شداربوون

ناونیشانی تویرینهوه:

کاریگهري به کار هیئانی دهمشوری (جائره) بو چار مسیری هه و کردنی پروک: توژی نه مهیه کی کلینیکی.

لِيَكُوْلَهُ رِي سَاهِر هِي:

تویژەر: دکتور عمر عبدالله یوسف. بهشی نهشتهرگهري پووک، کولیزی پزشکی ددان، زانکوی سلیمانی.

ژماره‌ی موبایل: +9647702221140

ئيمهیل: omer.yousif@univsul.edu.iq

پیشہ کی:

بهریزت بانگمهیشت کراویت بو بهشداریکردن له تووژینهومیکی کلینیکی. تکایه به وردی ئهو زانیاریانه بخوڤنهوه که نوسراوه دهوانیت ههر پرسیاریک بکهیت دهرباره ی تووژینهومه. له ئهگهری رازیبوونت به تووژینهومه، داوات لیدهکرتیت ئهم فورمه واژوو بکهیت.

ئامانجى تويژىنەۋە:

ئامانجى توۋىنەمكە ھەلسەنگەندىكى كارىگەر يېمەكلىكى گىياھىيە كەلە (جائىرە) دروسىتىراۋە بۇ چارەسىمىرى كەندى ھەمىرى كەندى پۈۈك. كارىگەرى دىمىشۋىرى باسكاراۋ لەگەل ۲ دىمىشۋىرى دىكە (كلور ھىكسىدىن ۋە تىكەلەلى ناۋى پالىۋىراۋ ۋە رەنگى خۇراكى) بىراۋرد دىمىرەت. ئامانجى بىلاۋرىنەمەلى ئىمە توۋىنەمەمەن ھەمە لە گۇقارىكى زانستىدا.

ش یوازی تو یژینه وه:

تویژینه مه که تاقیکردنه‌وه‌یه‌کی کلینیکیه و به شیوه‌یه‌کی ههرمه‌کی ۳ دهمشور مه که دابهش دمکریته، واته نه به‌ریزه و نه تویژه ده‌زان کام دهمشوره دهریته به به‌داربوو (دهمشوری جائزه/ کلور هیکسیدین / تیکه‌له‌ی ناوی پالئوراو و به‌هنگی خوراکه) به مه‌به‌ستی ده‌ستکه‌وتنه نه‌جام‌یکه بنالاین.

ریکاری تویرینهوه:

ئەگەر بەرپۆزەت رەزەمەند بېيت بە بەشدار بوون:

۱. پشکینینی سهرهتایی ناو دهمت بۆ نهجام دهمدریت به مههستی ههسهنگاندنی ریژهی ههوکردنی پووکت.
۲. بریکی کهم له لیکی بهریزت کۆدهکریتهوه بۆ شیکارکردن.
۳. به شیوهیهکی ههرمهکی بهکێک له سێ دهمشۆرت پیدهمدریت (دهمشۆری جاتره، کلۆر هیکسیدین، یان تیکهلهی ئاوی پالیوراو ورنگی خۆراکی).

۴. رېنماييت دمکريت که رۆژى دوو جار بۆ ماوهى ۱ مانگ دهمشورى پندراو بهکاربهئيت

۵. دواى ۱ مانگ له بهرپزىت دواو دمکريت سەردانمان بکەينەوه بۆ هەلسەنگاندنى دەرمانجامه کلينيکيهکان و پندانى برىکى تر له ليک بۆ پشکين.

ماوهى بهشداريکردن:

بهشداريکردنت له تويزينهوهکدا ۴ ههفته دهخايهئيت، به سەردانى سەرمەتابى و بهدواداچوونهوه.

سوودهکانى بهشداربوون:

هەرچەندە راستهوخۆ سوود له بهشداريکردن وەرناگریت، بهلام تەندروستى دەم و ددانى باشتەر دەبێت بههوى چاودێرى بهردهوام. و ههروهها بهشداريکردنت يارمەتيدەر دەبێت له پشخستنى تيگهيشتنى زانستى له چارەسەرە گياهيەکان له پزىشکى ددان.

مەترسە و نارهەتییەکان:

- ئەگەرى نام گۆرین یاخود سووتانیکی کەم و کاتى ناو دەم هەیه بهلام کەمە.
- هەستىارى به پیکهاته گيايى بان کيميايیهکان دەگمەنن بهلام ئەگەرى روودانى هەیه
- کۆکردنەهوى ليک بى نازارە ونايئته هوى نارەحەتى.

بهشداريکردنى خۆبهخشانه و نازادى له کشانهوه:

بهشداريکردنى بهرپزىت به تهواوى خۆبهخشانهیه، و دەتوانیت له هەر کاتێکدا له تويزينهوهکە بکشینینەوه بهیى هیچ سزا و لایرسينهوهیهکی ياسایى. بریارهکەت کارىگەرى لەسەر پەيوەندیت لەگەڵ کلينيکى زانکۆ نابێت.

پاراستنى زانیارى:

هەموو ئەو زانیاریانەى له بهرپزىت کۆدەمکريتەوه به نهينى دهئێلرینهوه. ناوت له هیچ گۆفاریکى زانستیدا دەرناکهوێت. زانیارییهکان تەنها تيمى تويزينهوهکە بهکارى دههینن و به شێوهیهکی پارێزراو هەڵدهگیرین.

تیچووی بهشداريکردن:

هیچ تیچرویهک له بهرپزىت وەرناگیریت بۆ بهشداريکردن. هەموو کەمرستهکان (دهمشۆر و هەمیری ددان و فلچەى ددان) بهخۆرایى دەدریت به بهشداربوو.

مافى پرسىارکردن:

مافى ئەوت هەیه له هەر کاتێکى ماوهى بهشداريکردنت پرسىار لەبارەى تويزينهوهکە بکەیت. له ئەگەرى هەبوونى هەر پرسىاریک، دەتوانیت پەيوەندى به لێکۆلەرى سەرمەکی بکەیت له رێگەى تەلهفون بان ئيمەیل که له لاپەرەى يەکهەدا نووسراوه.



College of Dentistry

نهخير	بهلى	تکایه خانەى گونجاو هەلژیره
		ناگدارم که بهژداریکردنم به تهواوی خوبهخشانهیه.
		دوو پاتی دهکهمهوه که زانیاری تهواوم لهسەر ئەم توژینهوهیه پێدراوه، دهرفتهشتم پێدراوه که پرسیار لهبارهی پرۆژمهکهوه بکهه.
		من رازیم به بهشداریکردن لهم پرۆژهیه، وه تیدهگهه که بهشداریکردن له پرۆژمهکهه بریتیه له کوکردنهوهی نمونه له لیک بۆ بهکارهێنان لهم توژینهوهیههه.
		تیدهگهه که سوودی راستهوخۆ وه ناگرم به بهشداریکردنم لهم لیکۆلینهوهیهه.
		من رازیم که لیکۆلەر (هکان) دهتوانن ههر زانیارییهک که له منهوه بهدهست هاتوه بۆ مهههستی ئەم توژینهوهیه بهکار بهێنن.
		دوو پاتی دهکهمهوه که تهمهنم ۱۸ سال یان زیاتره.

واژوو:

بهروار:

ناوی بهژداربوو:

APPENDIX III

Participant Case Sheet



College of
Dentistry

Department of
Periodontics

Participant Case Sheet

Study Title: *Clinical and Immunological Efficacy of Thymus Vulgaris Mouthwash in the Management of Biofilm-Induced Gingivitis: A Randomized Double-Blind Clinical Trial*

Section 1: Patient Information

Name: _____ Age: _____ Sex: _____ Study ID: _____
Occupation: _____ Address: _____ Telephone No: _____

Section 2: Medical and Dental History

Systemic diseases: ☐ None ☐ Diabetes ☐ Hypertension ☐ Others:

Medications in last 3 months:

Allergies:

Smoking status: ☐ Current ☐ Former ☐ Never

Oral hygiene habits:

- Toothbrushing frequency: ☐ Once ☐ Twice ☐ >2/day
- Technique of tooth brushing: ☐ Horizontal ☐ Vertical ☐ Circular ☐ Rotary
- Mouthwash use: ☐ Yes ☐ No (if yes, specify type: _____)

Section 3. Eligibility Screening

- Inclusion criteria met: ☐ Yes ☐ No
- Exclusion criteria present: ☐ Yes ☐ No (If yes, specify: _____)

Section 4. Examiner Signature

Name:

Signature:

Date:

Plaque Index, Bleeding Index, and Probing Pocket Depth
(Baseline Visit)

GI																																
BI																																
PI																																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38																
PI																																
BI																																
GI																																

Average of: PI= BI= % GI=

Diagnosis:

Immunological and Biochemical Data:

- Saliva collection date:
- IL-1 β = _____ pg/mL MMP-8= _____ pg/mL

Plaque Index, Bleeding Index, and Probing Pocket Depth
(Follow-up Visit)

GI																																
BI																																
PI																																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38																
PI																																
BI																																
GI																																

Average of: PI= BI= % GI=

Immunological and Biochemical Data:

- Saliva collection date:
- IL-1 β = _____ pg/mL MMP-8= _____ pg/mL

APPENDIX IV

Mouthwash Use Instructions

Dear participant, thank you for being part of this study. Please read the instructions carefully and follow them exactly as written. This will ensure that your oral health is protected and the achieved results are accurate.

How to use the mouthwash:

- Brush your teeth with the provided toothbrush and toothpaste 30 minutes before using the mouthwash
- 30 minutes after brushing, use 10mL (about 2 teaspoons) of the mouthwash during each application
- Do not dilute with water
- Rinse well for 1 minute, and try not to swallow the mouthwash
- After rinsing for 1 minute, spit out the mouthwash completely
- Do not rinse with water afterwards, and refrain from eating or drinking for at least 30 minutes after spitting the mouthwash
- Use the mouthwash twice daily (morning and evening), 30 minutes after brushing your teeth, for 1 month

Notes:

- Before rinsing with the mouthwash, only use the provided toothbrush and toothpaste during the study period
- Do not use any other mouthwash during this period, use only the one provided
- If you notice any burning, allergy, or unusual taste, stop using the mouthwash and report to the study dentist
- Store the mouthwash at room temperature and away from direct sunlight and heat
- Keep away from children

Contact Information

Study Dentist: Dr. Omer Abdalla Yousif.

Phone: 07702221140.

Email: omer.yousif@univsul.edu.iq.

APPENDIX V

رینمایی بهکارهینانی دهمشور

بهشداربووی بهریز، سوپاس بۆ بهشداریکردنت لهم توژیڤنهوهیه. تکایه رینماییهکان به وردی بخوینهرهوه و بهو شیوهیهی که نووسراوه جییهجیان بکه. بهم جۆره دَلنیا دهبین لهوهی تهنروستی دهم و ددانت پاریزراو دهبیت و ئهنجامیکی راست و درووستمان دهست دهکهویت.

چۆنیهتی بهکارهینانی دهمشورکه:

- ۳۰ خولهک پێش بهکارهینانی دهمشورکه، ددانت بشو به بهکارهینانی ئهو فلچه و ههویری ددانهی بۆت دابینکراوه
- ۳۰ خولهک دواى ددان شتن، ۱۰ مل (نزیکی ۲ کهوچکی چا) له دهمشورکه بهکاربهینه
- دهمشورکه به ئاو شل مهکهرهوه

- بۆ ماوهی ۱ خولهک جوان دهمشورکه له دهمت وهرده، و ههول بده دهمشورکه قووت نهدهیت

- دواى ئهوه، دهمشورکه به تهواوی تف بکهرهوه

- دواى تف کردنهوهی دهمشورکه، ئاو له دهمت وهرمهده، و هیچ شتیک مهخۆ و مهخۆرهوه ههتا ماوهی ۳۰ خولهک

- رۆژانه ۲ جار (بهیانیان و شهوان) ۳۰ خولهک دواى شوشتنی ددانهکان دهمشورکه بهکاربهینه بۆ ماوهی ۱ مانگ

تێبینی:

- لهم ماوهی توژیڤنهوه و پێش بهکارهینانی دهمشورکه، تهنها ئهو فلچه و ههویری ددانه بهکاربهینه که بۆت دابین کراوه

- هیچ دهمشوریکی تر بهکارمههینه له ماوهی توژیڤنهوهکه، تهنها ئهو دهمشوره بهکاربهینه که دابینکراوه.

- ئهگهر ههستت به ههر سووتانهوهیهک، حساسیهتیک، یاخود تامیکی نااسایی کرد، واز له بهکارهینانی دهمشورکه بینه و پهوهندی به پزشکی ددانی توژهر بکه.

- پێویسته دهمشورکه له پلهی گهرمی ژووردا ههلبگیریت و دوور بیت له تیشکی راستهوخۆی خۆر و گهرما

- پێویسته دهمشورکه دوور بیت له دهستی منداڵ

زانایاری پزشکی ددانی توژهر:

ناو: د. عمر عبدالله يوسف

تلهفون: ۰۷۷۰۲۲۲۱۱۴۰

ئیمیل: omer.yousif@univsul.edu.iq

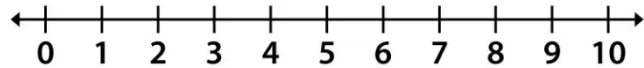
APPENDIX VI

Visual Analogue Scale (VAS)

1. Gingival Pain/Discomfort

Please mark on the line the level of gingival pain or discomfort you are currently experiencing.

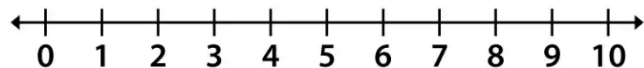
(0=No pain, 10=Worst pain imaginable)



2. Burning Sensation

Please indicate the intensity of any burning sensation in your mouth after using the mouthwash.

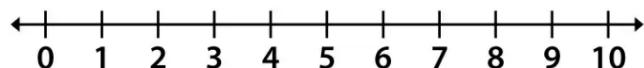
(0=No burning, 10=Extremely intense burning)



3. Mouthwash Taste Acceptability

How pleasant or unpleasant was the taste of the mouthwash?

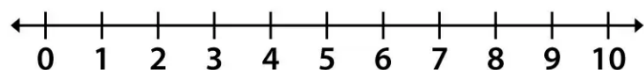
(0=Extremely unpleasant, 10=Very pleasant)



4. Teeth staining

Please indicate the level of staining on teeth and tongue if present

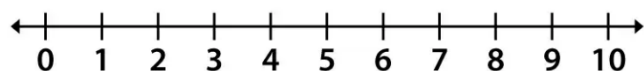
(0= No staining, 10=Severe staining)



5. Overall Satisfaction with the Mouthwash

How satisfied are you overall with using the mouthwash?

(0=Not satisfied at all, 10=Extremely satisfied)

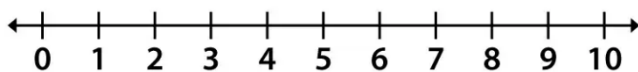


APPENDIX VII

هه‌لسه‌نگاندنی نه‌زموونی به‌کاره‌ینانی دهمشور

۱. نازار/ناره‌حتی پووک:

تکایه له‌سەر هه‌لکه‌که ئاستی نازار یان ناره‌حتی پووک نیشانه بکه که ئیستا هه‌ته

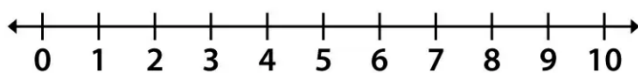


بی
نازار

نازاریکی زور
بیزارکەر

۲. هه‌ستی سووتانه‌وه:

تکایه نامازه به‌ریژه‌ی هه‌ر هه‌ستیکی سووتانه‌وه له‌دمتدا بکه دوا‌ی به‌کاره‌ینانی دهمشورکه

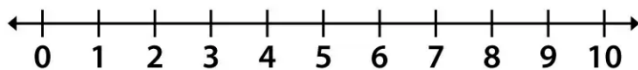


بی
سووتانه‌وه

سووتانه‌وه‌یه‌کی زور
بیزارکەر

۳. تامی دهمشورکه:

تامی دهمشورکه تا چه‌ند خۆش بوو یان ناخۆش بوو؟

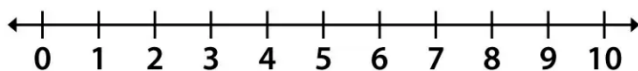


زور
ناخۆش

زور
خۆش

۴. ره‌نگ گرتنی ددانه‌کان:

تکایه نامازه به‌ریژه‌ی ره‌نگ گرتنی ددان یا خود زمان بکه نه‌گه‌ر هه‌بیت

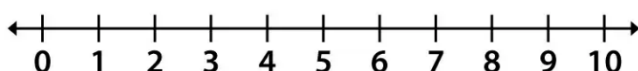


بی
ره‌نگ

ره‌نگی
زور

۴. رازی‌بوونی گشتی له‌دهمشورکه:

به‌گشتی تا چه‌ند رازیت له‌به‌کاره‌ینانی دهمشورکه؟



هیچ لئی
رازی نه‌بووم

زور لئی
رازی بووم

APPENDIX VIII

SPIRIT 2025 checklist of items to address in a randomized trial protocol*

Section / Topic	No	SPIRIT 2025 checklist item description	Reported on page no.
Administrative information			
Title and structured summary	1a	Title stating the trial design, population, and interventions, with identification as a protocol	5-7
	1b	Structured summary of trial design and methods, including items from the World Health Organization Trial Registration Data Set	Study design: 146-149
Protocol version	2	Version date and identifier	23-37
Roles and responsibilities	3a	Names, affiliations, and roles of protocol contributors	Author's contrib.: 377-381
	3b	Name and contact information for the trial sponsor	Funding: 366-368??
	3c	Role of trial sponsor and funders in design, conduct, analysis, and reporting of trial; including any authority over these activities	39-40
	3d	Composition, roles, and responsibilities of the coordinating site, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable	Author's Contribution: 379-383
Open science			
Trial registration	4	Name of trial registry, identifying number (with URL), and date of registration. If not yet registered, name of intended registry	Ethical consid.: 334-340
Protocol and statistical analysis plan	5	Where the trial protocol and statistical analysis plan can be accessed	N/A
Data sharing	6	Where and how the individual de-identified participant data (including data dictionary), statistical code, and any other materials will be accessible	N/A

Funding and conflicts of interest	7a	Sources of funding and other support (e.g., supply of drugs)	Funding: 368-370
	7b	Financial and other conflicts of interest for principal investigators and steering committee members	Conflict of Interest: 372-373
Dissemination policy	8	Plans to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., reporting in trial registry, plain language summary, publication)	Ethical consid.: 334-340
Introduction			
Background and rationale	9a	Scientific background and rationale, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Introduction: 49-92
	9b	Explanation for choice of comparator	Introduction: 85-92
Objectives	10	Specific objectives related to benefits and harms	Objectives: 121-134
Methods: Patient and public involvement, trial design			
Patient and public involvement	11	Details of, or plans for, patient or public involvement in the design, conduct, and reporting of the trial	Study design: 146-149
Trial design	12	Description of trial design including type of trial (e.g., parallel group, crossover), allocation ratio, and framework (e.g., superiority, equivalence, non-inferiority, exploratory)	Study design: 146-149 Randomization: 256-268
Methods: Participants, interventions, and outcomes			
Trial setting	13	Settings (e.g., community, hospital) and locations (e.g., countries, sites) where the trial will be conducted	Study visits: 226-227
Eligibility criteria	14a	Eligibility criteria for participants	Study subjects: 159-177
	14b	If applicable, eligibility criteria for sites and for individuals who will deliver the interventions (e.g., surgeons, physiotherapists)	N/A

Intervention and comparator	15a	Intervention and comparator with sufficient details to allow replication including how, when, and by whom they will be administered. If relevant, where additional materials describing the intervention and comparator (e.g., intervention manual) can be accessed	Study visits: 225-253
	15b	Criteria for discontinuing or modifying allocated intervention/comparator for a trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	Appendix IV: Notes
	15c	Strategies to improve adherence to intervention/comparator protocols, if applicable, and any procedures for monitoring adherence (e.g., drug tablet return, sessions attended)	Study visits: 257-260
	15d	Concomitant care that is permitted or prohibited during the trial	N/A
Outcomes	16	Primary and secondary outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome	Outcome measures: 329-331
Harms	17	How harms are defined and will be assessed (e.g., systematically, non-systematically)	Appendix IV
Participant timeline	18	Time schedule of enrollment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Study visits: 225-253 Diagram of study design: 157
Sample size	19	How sample size was determined, including all assumptions supporting the sample size calculation	Sample size calc.: 205-215
Recruitment	20	Strategies for achieving adequate participant enrollment to reach target sample size	Study visits: 225-227
Methods: Assignment of interventions			
Randomization:			
Sequence generation	21a	Who will generate the random allocation sequence and the method used	Randomization: 257-262
	21b	Type of randomization (simple or restricted) and details of any factors for stratification. To reduce predictability of a random sequence, other details of any planned restriction (e.g., blocking) should be	Randomization: 256-268

		provided in a separate document that is unavailable to those who enroll participants or assign interventions	
Allocation concealment mechanism	22	Mechanism used to implement the random allocation sequence (e.g., central computer/telephone; sequentially numbered, opaque, sealed containers), describing any steps to conceal the sequence until interventions are assigned	Randomization: 260-261
Implementation	23	Whether the personnel who will enroll and those who will assign participants to the interventions will have access to the random allocation sequence	Randomization: 265-268
Blinding	24a	Who will be blinded after assignment to interventions (e.g., participants, care providers, outcome assessors, data analysts)	Randomization: 265-268
	24b	If blinded, how blinding will be achieved and description of the similarity of interventions	Randomization: 256-268
	24c	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Randomization: 267-268
Methods: Data collection, management, and analysis			
Data collection methods	25a	Plans for assessment and collection of trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of trial instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be accessed, if not in the protocol	Intra- and Inter-Examiner Calib.: 217-220
	25b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Study visits: 225-253
Data management	26	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be accessed, if not in the protocol	N/A
Statistical methods	27a	Statistical methods used to compare groups for primary and secondary outcomes, including harms	Data Analysis: 319-325

	27b	Definition of who will be included in each analysis (e.g., all randomized participants), and in which group	Intervention and Quality Control: 343-346
	27c	How missing data will be handled in the analysis	N/A
	27d	Methods for any additional analyses (e.g., subgroup and sensitivity analyses)	Substantivity Test: 188-203
Methods: Monitoring			
Data monitoring committee	28a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and funder; conflicts of interest and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	28b	Explanation of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Trial monitoring	29	Frequency and procedures for monitoring trial conduct. If there is no monitoring, give explanation	Study visits: 246-253
Ethics			
Research ethics approval	30	Plans for seeking research ethics committee/institutional review board approval	Ethical issues: 356-357
Protocol amendments	31	Plans for communicating important protocol modifications to relevant parties	N/A
Consent or assent	32a	Who will obtain informed consent or assent from potential trial participants or authorized proxies, and how	Study subjects: 159-177
	32b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	33	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Dissemination: 361-363

Ancillary and post-trial care	34	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
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