

GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 7-8 (376-377) Июль-Август 2026

ТБИЛИСИ - NEW YORK



ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press.
Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებშიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman (Кириллица)**, для текста на грузинском языке следует использовать **AcadNusx**. Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи.** Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - <http://www.spinesurgery.ru/files/publish.pdf> и http://www.nlm.nih.gov/bsd/uniform_requirements.html. В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректур авторам не высылаются, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of 3 centimeters width, and 1.5 spacing between the lines, typeface - **Times New Roman (Cyrillic)**, print size - 12 (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf

In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

**Articles that Fail to Meet the Aforementioned
Requirements are not Assigned to be Reviewed.**

ავტორთა საყურადღებო!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე, დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx**. შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემავსებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრაფიის ფოტოსურათები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგების ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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COMPARATIVE EVALUATION OF TWO HERBAL MOUTHWASHES AND CHLORHEXIDINE ON CALPROTECTIN IN GINGIVITIS PATIENTS: A DOUBLE-BLIND RANDOMIZED CONTROLLED TRIAL

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Abstract.

Background: Gingivitis is a reversible inflammatory condition primarily caused by dental biofilm accumulation. Gingival crevicular fluid (GCF) serves as a non-invasive diagnostic medium reflecting periodontal status. Biomarkers such as Calprotectin (biomarker of neutrophil-mediated inflammatory activity) are associated with immune activation and periodontal inflammation and have also been associated with systemic conditions.

Objective: To evaluate the efficacy of two herbal mouthwashes—GEM (propolis, neem, and chlorophyll) and Propolinse (propolis and green tea)—compared with chlorhexidine on Calprotectin in gingivitis patients.

Materials and Methods: Forty-four male patients with generalized gingivitis were randomly assigned into four groups (n=11). Participants used GEM, Propolinse, 0.12% alcohol-free CHX, or distilled water (control) twice daily for 7 days alongside mechanical oral hygiene. Clinical parameters (Plaque Index (PI), Gingival Index (GI), Bleeding on Probing (BOP)) and GCF biomarkers were assessed at baseline and after 7 days. GCF samples were collected from maxillary anterior teeth by periopaper strips. Calprotectin concentrations were measured via ELISA. SPSS was used for statistical analysis, with a significance threshold of $p < 0.05$.

Results: All groups showed significant improvements in PLI, GI, and BOP ($p \leq 0.001$). And all groups showed significant decreases in calprotectin levels, with no statistically significant intergroup differences observed in mean change values ($p=0.760$). Notably, herbal formulations demonstrated greater reduction in calprotectin (a neutrophil-derived biomarker), suggesting differential modulation of immune pathways.

Conclusion: Both herbal mouthwashes and 0.12% chlorhexidine demonstrated comparable anti-inflammatory effects. Propolis-based formulations may represent effective adjunctive alternatives in the short-term management of gingivitis, potentially exerting differential effects on neutrophil-mediated inflammatory responses, with possible implications for systemic inflammatory conditions.

Key words. Calprotectin, gingival crevicular fluid, gingivitis, propolis, neem, green tea, chlorhexidine.

Introduction.

Periodontal disease involves inflammatory changes affecting the supporting tissues surrounding the teeth [1,2]. The buildup of dental biofilm causes gingivitis, a site-specific inflammatory condition marked by gingival redness, edema, and bleeding without periodontal attachment loss. The most common cause of gingivitis is inadequate dental hygiene. It is reversible with proper plaque control and is indicative of the early stages of periodontal disease [2].

In this regard, Gingival crevicular fluid (GCF) is a non-invasive diagnostic medium capable of reflecting periodontal inflammatory changes [3,4]. GCF is a complex biofluid that is created as a blood ultrafiltrate originating from the periodontal sulcus/pocket. As a result, it comprises a variety of chemicals originating from bacteria or the host that can be utilized as a site-specific biomarker of disease activity and treatment results [5].

Pro-inflammatory cytokines are intricately linked to periodontal inflammation and may indicate the inflammatory load associated with periodontal disease [6].

Calprotectin (CALP) is one of the important inflammatory biomarkers associated with periodontal disease. It is a calcium- and zinc-binding cytosolic protein composed mainly of S100A8 and S100A9 subunits and is predominantly released by neutrophils, monocytes, macrophages, and epithelial cells [7,8]. Calprotectin plays an important role in innate immunity, antimicrobial defense, leukocyte recruitment, and inflammatory regulation [8].

Calprotectin's various roles are mostly associated with ongoing inflammatory processes. These include immune responses mediated by T helper cell type 1 (Th1) and antimicrobial defense mechanisms, such as those observed in autoimmune disorders or allograft rejection [9].

Elevated calprotectin levels have been reported in inflammatory conditions, including periodontal disease, infections, autoimmune disorders, malignancies, and systemic inflammatory diseases [7,8]. In periodontal tissues, increased calprotectin levels in GCF are associated with inflammatory activity and may reflect neutrophil-mediated tissue response [10,11]. Therefore, calprotectin can provide valuable biochemical information regarding the inflammatory burden of gingival tissues and treatment response.

Calprotectin is a sensitive indicator of periodontal inflammation, according to recent research. Patients with gingivitis and periodontitis have been found to have higher calprotectin concentrations than healthy people, which is indicative of enhanced neutrophil activity and host inflammatory response. Additionally, calprotectin contributes to Th1-mediated immune responses and antimicrobial defense, indicating its potential utility as a marker for tracking periodontal disease activity and treatment results [9,12].

Periodontal inflammatory indicators may offer significant supplementary insights for evaluating periodontal disease activity alongside conventional clinical markers [13].

Gingival crevicular fluid (GCF) has gained increasing attention as a site-specific biological fluid that reflects local periodontal inflammatory alterations. The utility of GCF biomarkers in detecting active periodontal inflammation and assessing

treatment response more precisely than traditional clinical indicators alone has been highlighted by recent studies [14].

From a diagnostic perspective and over the years, ELISA has undergone several stages of development and is today a widely used diagnostic test in labs [15].

ELISA is an immunological biochemical technique employed to detect and quantify antibodies, antigens, peptides, proteins, glycoproteins, and hormones in biological specimens [15].

The main approaches for preventing periodontal diseases are mechanical and chemical plaque control. In the initial phases of periodontal treatment, non-surgical periodontal therapy is carried out along with oral hygiene instructions to eliminate bacterial deposits and decrease periodontal inflammation [16].

The gold standard antiplaque medication for treating periodontitis and gingivitis is chlorhexidine (CHX) [17,18]. Its adverse effects include burning while washing your mouth, dysgeusia, staining, and irritated mucous membranes.

Therefore, extended and frequent use should be avoided [19].

Propolis, neem, and green tea-based herbal mouthwashes have shown antibacterial, anti-inflammatory, and antioxidant qualities, making them viable substitutes [19-23].

In this context, a number of clinical trials have investigated the efficacy of propolis on plaque and gingivitis, and conflicting results were reported [24]. Generally associated with less side effects than the widely used chlorhexidine mouthwashes. Consequently, propolis has become a substitute for these synthetic mouthwashes for gingivitis management. Propolis mouthwashes are effective and not inferior to other mouthwashes in lowering both the GI and BOPI, according to numerous studies [25].

The use of green tea to treat and prevent periodontal disease is supported by sufficient evidence [20].

Neem has been researched extensively in various fields of medicine due to its antihyperglycemic, immunomodulatory, anti-inflammatory, antimalarial, antioxidant, antiviral, antimutagenic and anticarcinogenic properties [23].

This study aimed to evaluate the effect of herbal mouthwashes compared with chlorhexidine on Calprotectin levels in GCF among gingivitis patients.

Materials and Methods.

The study protocol was reviewed and approved by the MUCOD Human Research Ethics Committee, College of Dentistry, Al-Mustansiriyah University, Iraq (REC Reference No. REC214; Study Number: MUOSU/POST-202607). Written informed consent was obtained from all participants after explaining the nature and purpose of the study prior to enrolment. The study design is illustrated by a flow diagram in (Figure 1).

Study Design:

A double-blind, parallel-group randomized controlled trial design was used (The study followed CONSORT guidelines for randomized clinical trials) (Figure 1) [21,26]. Participants were randomly assigned into four study groups: two herbal mouthwash groups, a chlorhexidine group, and a placebo control group.

At baseline, all participants underwent professional supragingival scaling and polishing and received standardized oral hygiene instructions. Participants were then provided with coded mouthwash bottles to ensure blinding of both the

investigator and participants. All mouthwashes were used twice daily (15 mL) for 7 consecutive days in addition to routine tooth brushing and interdental cleaning using standardized oral hygiene materials.

Gingival crevicular fluid (GCF) samples were collected at baseline and after 7 days for Calprotectin analysis (Figure 2).

All participants were fully informed about the study objectives and procedures and provided written informed consent prior to enrollment. Participation was voluntary, and withdrawal was permitted at any stage without affecting treatment.

Randomization and Allocation Concealment:

Participants were randomly allocated into four study groups in a 1:1:1:1 ratio using a computer-generated randomization sequence prepared in Microsoft Excel. Allocation concealment was achieved using coded opaque containers prepared by an independent assistant who was not involved in clinical examination, laboratory analysis, or statistical evaluation.

Blinding:

The study was conducted using a double-blind design. Participants received mouthwashes in identical coded opaque containers. Both participants and the clinical examiner were unaware of treatment allocation throughout the study period. Allocation codes were maintained by an independent assistant and were disclosed only after completion of data analysis.

Sample Size Calculation:

The sample size was calculated using G*Power software. (Franz Faul, University of Kiel, Germany) with a medium-large effect size of $F = 0.35$, a power of 0.80, and a two-sided alpha error of 0.05 [27]. The minimal necessary sample size in these circumstances was 28 participants. To increase statistical reliability and compensate for possible dropouts, 44 participants were ultimately included.

The total number of measurements was 88 because the Calprotectin biomarker level in each participant's gingival crevicular fluid (GCF) was assessed twice (at baseline and seven days later).

The following effect size conventions are applied: Small = 0.1, Medium = 0.25, and Large = 0.4.

Participants:

Forty-four systemically healthy male patients diagnosed with generalized plaque-induced gingivitis were recruited for this study [28].

Inclusion criteria:

- (1) Male patients between the ages of 18 and 55.
- (2) Patients diagnosed with generalized plaque-induced gingivitis.
- (3) Three months before the experiment began, there was no antibiotic therapy.
- (4) Not taking anti-inflammatory drugs on a regular basis.
- (5) No prior history of oral care product allergies.
- (6) Oral antiseptics should not be used often.

Exclusion criteria:

- (1) Patients who decline to sign an informed consent form.
- (2) Smokers.
- (3) Alcohol consumers

(4) Female participants were excluded to minimize hormonal influences on inflammatory biomarker

(5) Systemic illnesses

Protocol:

Scaling and polishing followed by mouthwash use twice daily for 7 days [17].

Grouping:

1. Group 1: Consisted of 11 male participants with generalized gingivitis ($\geq 30\%$ of the sites are affected) using the GEM natural mouthwash (Propolis, Neem and Chlorophyll), Premium Natural Mouthwash Crisp Mint manufactured in Australia (Figure 3A).

2. Group 2: Consisted of 11 male participants with generalized gingivitis ($\geq 30\%$ of the sites are affected) using the Propolinse mouthwash (Propolis & Green Tea), Natural produced by the Japanese company Pieras (Figure 3B).

3. Group 3: Consisted of 11 male participants with generalized gingivitis ($\geq 30\%$ of the sites are affected) using the Alcohol-free 0.12% Kin Gingival® chlorhexidine mouthwash (Spain) (Figure 3C).

4. Group 4: Consisted of 11 male participants with generalized gingivitis ($\geq 30\%$ of the sites are affected) using the Distilled water (Placebo control).

GCF Collection:

Gingival crevicular fluid (GCF) samples were collected between 10:00 AM and 12:00 PM. The sites were isolated using cotton rolls and cheek retractors to prevent saliva contamination. One disposable 1.5 ml Eppendorf tube (weighed using the scientific scale) were used in each visit for each patient. GCF samples were collected from the labial gingival sulcus of maxillary anterior teeth using PerioPaper® strips. Five PerioPaper strips were used for each participant. The strips were gently inserted into the gingival sulcus using tweezers until minimal resistance was felt and left in place for 30 seconds [28]. And pooled into a single Eppendorf tube containing 250 μ L phosphate-buffered saline (PBS). PerioPaper® strips contaminated with saliva or blood were discarded, and sampling was repeated [29]. Samples were immediately transferred in a cooling box and stored at -80°C until laboratory analysis [30] (Figure 4).

ELISA Analysis:

Calprotectin levels were quantified using enzyme-linked immunosorbent assay (ELISA) ELISA Kits (Calprotectin YH Bio search Laboratory, China) according to the manufacturer's instructions [15]. Information regarding the kit manufacturer, assay sensitivity, detection range, and reagent preparation was provided. Laboratory personnel performing biomarker measurements were blinded to treatment allocation throughout the analysis procedure.

Character:

Item	
Sensitivity	0.94 ng/mL
Detection Range	1.56-100 ng/mL
Specificity	Human CALP is detected in samples using this kit. There was no discernible interference or cross-reactivity between human CALP and its analogues.
Repeatability Test principle	Coefficient of variation is $< 10\%$

Per Protocol Analysis:

A per-protocol analysis was performed. Only participants who completed the 7-day follow-up period and provided complete clinical and biomarker data were included in the final statistical analysis.

Statistical analysis:

The Statistical Package for Social Science (SPSS version 21.0) was used to describe, analyze, and present the data. There are two types of statistical analysis:

1-Descriptive Analysis:

A. Mean and standard deviation (SD) for quantitative variables
B. Clustered bar chart

2- Inferential analysis:

A. One Way Analysis of Variance (ANOVA): used to find differences between K independent means of the quantitative variable using Dunnett's T3 (when the Levene test p value was significant or highly significant) and Tukey Honestly Significant Difference (HSD) (when the Levene test p value was not significant).

B. Levene test: determine whether the variance of the data is homogeneous or heterogeneous for independent groups.

C. Paired t test: compare two related points or two investigators. significance level as follows: highly significant (HS) $P < 0.01$, significant (S) $P < 0.05$, and not significant (NS) $P > 0.05$.

Data normality was assessed using the Shapiro–Wilk test. Homogeneity of variances among groups was evaluated using Levene's test. Statistical significance was set at $p < 0.05$.

Results.

Clinical Periodontal Parameters:

All study groups demonstrated statistically significant improvements in Plaque Index (PLI), Gingival Index (GI), and Bleeding on Probing (BOP) after 7 days of intervention ($p \leq 0.001$). The chlorhexidine group achieved the lowest final plaque and bleeding scores, whereas the Propolinse group demonstrated the greatest numerical reduction in gingival inflammation. However, no clinically meaningful superiority of any intervention over the others was observed.

plaque index:

(Table 1) shows the descriptive and statistical analysis of plaque index (PLI) among the study groups. At baseline, a statistically significant difference was observed between groups ($p = 0.001$). After 7 days, all groups demonstrated a reduction in PLI with no significant difference among them ($p = 0.125$). Within-group comparisons revealed a highly significant decrease over time ($p = 0.001$). The mean change showed the greatest reduction in the Propolinse group, followed by 0.12% CHX, GEM, and placebo. Similarly, the effect size analysis indicated the largest effect in the Propolinse group, with progressively smaller effects observed in 0.12% CHX, GEM, and placebo.

Gingival index:

(Table 2) demonstrates that there were no significant differences in GI values among the groups at baseline ($p = 0.263$), while a significant difference was observed after 7 days ($p = 0.019$). All groups showed statistically significant within-group reductions in GI scores ($p = 0.001$).

Regarding the mean change, Propolinse showed the highest improvement (1.621), followed by GEM (1.561) and 0.12% CHX (1.556), whereas the placebo group exhibited the lowest change (0.543). In terms of effect size, GEM demonstrated the largest effect (5.875), followed by Propolinse (4.910), 0.12% CHX (3.551), and placebo (2.635).

Bleeding on probing: (Table 3) shows BOP mean values across groups at baseline and after 7 days. All groups demonstrated significant reductions over time ($P = 0.001$), with a significant difference between groups at 7 days ($P = 0.019$).

The mean change was highest in 0.12% CHX (68.427), followed by Propolinse (65.502), GEM (57.305), and Placebo (54.382). The effect size was greatest in Propolinse (2.630), then 0.12% CHX (2.496), Placebo (2.464), and GEM (2.226).

Calprotectin ng/ml:

Calprotectin levels significantly decreased in all groups after 7 days. At baseline, no statistically significant differences were observed among the groups ($p=0.498$), and similarly, no significant differences were detected after 7 days ($p=0.802$), indicating comparability among groups.

Within-group analysis demonstrated statistically significant reductions in calprotectin levels in all groups over time ($p=0.001$). The Propolinse group showed the greatest mean reduction, followed by GEM, 0.12% CHX, and placebo. However, comparison of mean change values among groups revealed no statistically significant difference ($p=0.760$).

Calprotectin Levels (ng/mL):

(Table 4) presents the descriptive and statistical analysis of calprotectin mean values among the study groups (GEM, Propolinse, 0.12% CHX, and placebo) at baseline and after 7 days. At baseline, no statistically significant differences were observed among the groups ($p = 0.498$), and similarly, no significant differences were found at day 7 ($p = 0.802$), indicating group comparability. Within-group analysis demonstrated a statistically significant reduction in calprotectin levels across all groups over time ($p = 0.001$). The mean change was greatest in the Propolinse group (14.460 ± 5.831), followed by GEM (14.272 ± 8.152), 0.12% CHX (12.888 ± 6.048), and placebo (11.929 ± 4.936). In terms of effect size, all groups exhibited large effects, with the highest effect size observed in the

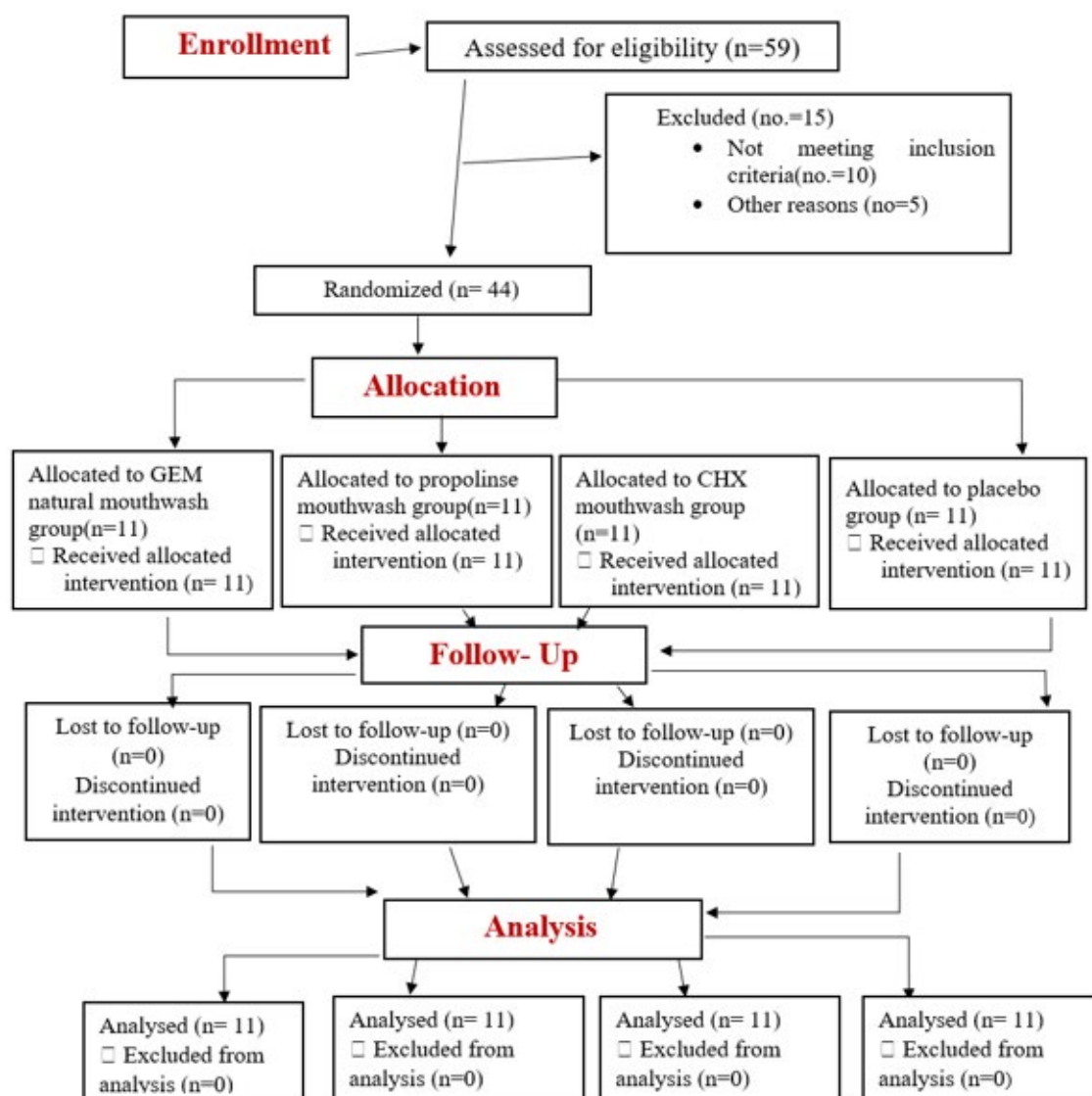


Figure 1. CONSORT 2010 flow diagram of participant enrollment, randomization, allocation, follow-up, and analysis.

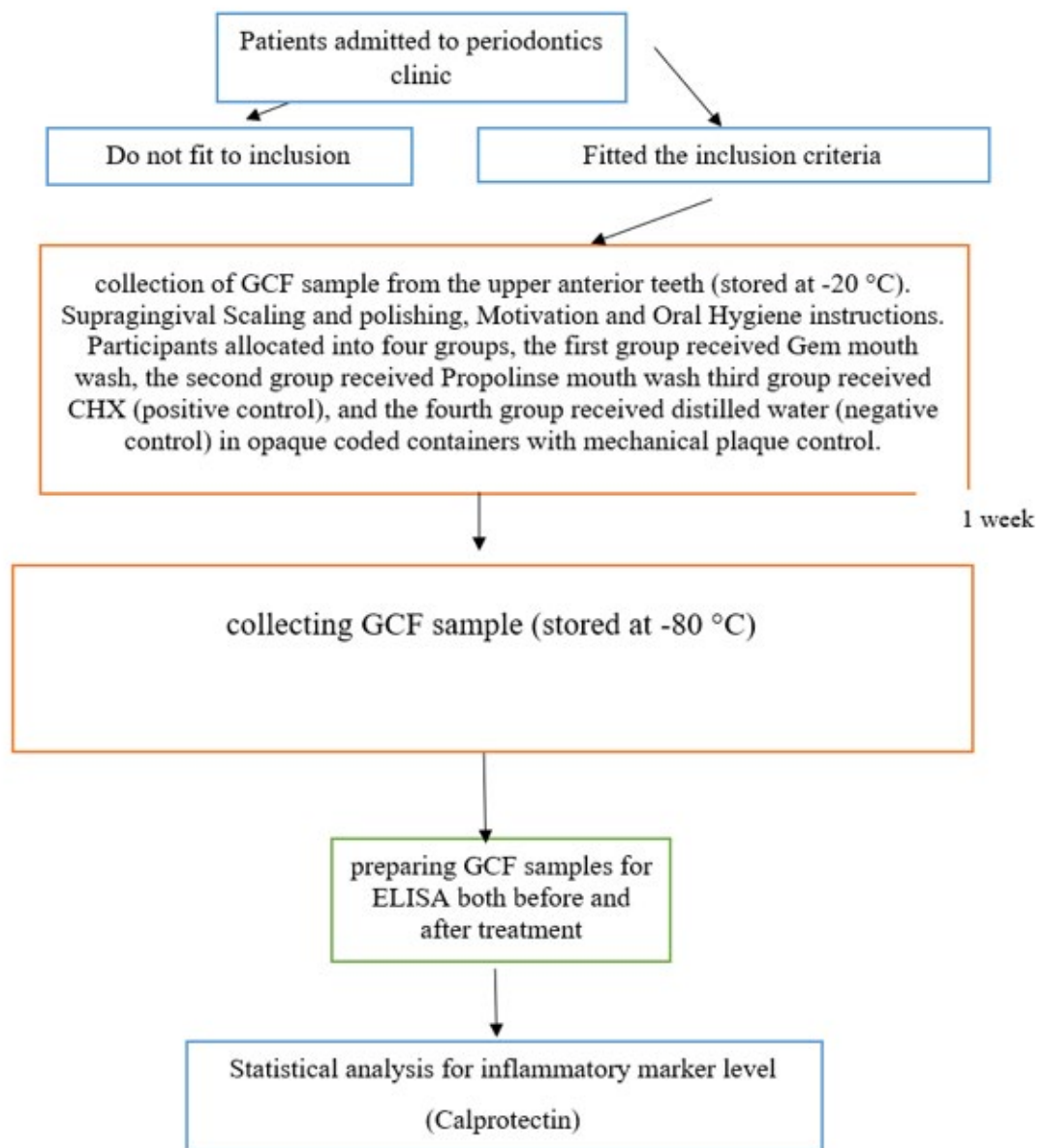


Figure 2. Schematic representation of the study protocol and GCF sampling procedure.



Figure 3(A, B and C). A-GEM natural mouthwash, B-Propolinse mouthwash, C- Kin gingival® 0.12% chlorhexidine mouthwash.



Figure 4. Collection of GCF using periopaper® strip.

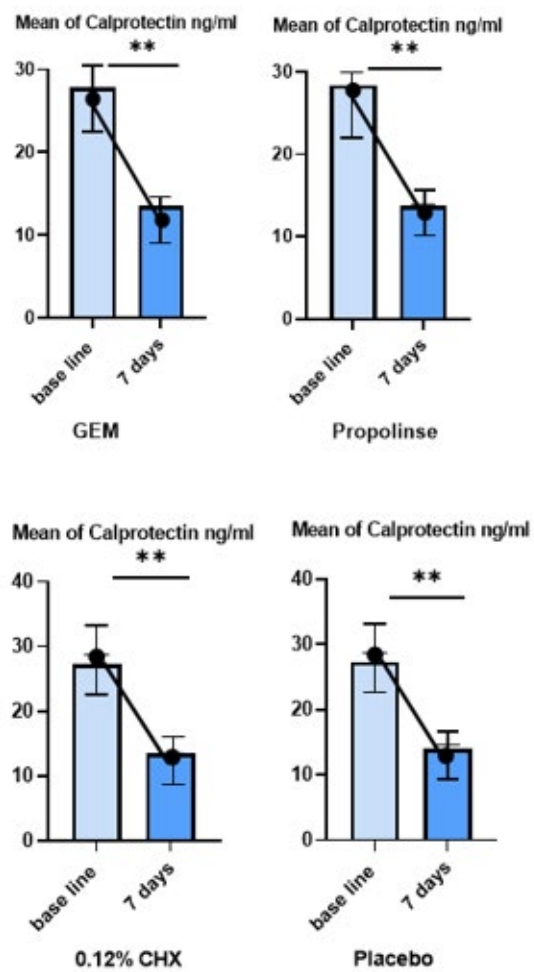


Figure 5. Mean Calprotectin levels among study groups at baseline and after 7 days.

Table 1. Descriptive and statistical tests of PLI mean values among groups and intervals.

	Groups				Statistical Analysis
	GEM	Propolines	0.12% CHX	Placebo	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	P value*
Baseline	1.319 $\pm$ 0.365	1.785 $\pm$ 0.307	1.416 $\pm$ 0.201	1.164 $\pm$ 0.395	0.001 sig
7 days	0.137 $\pm$ 0.295	0.066 $\pm$ 0.016	0.022 $\pm$ 0.019	0.164 $\pm$ 0.059	0.125 NS
P value**	0.001	0.001	0.001	0.001	
Effect size	3.522	5.743	7.252	2.716	
Mean change	1.182 $\pm$ 0.443	1.718 $\pm$ 0.312	1.394 $\pm$ 0.198	1.052 $\pm$ 0.356	0.001 sig

GEM: GEM natural mouthwash (Propolis, Neem and Chlorophyll), CHX: chlorhexidine, * comparison performed by one-way ANOVA test; ** comparison performed by Paired T-test; Sig.=significant at $p<0.05$, SD: standard deviation.

Table 2. Descriptive and statistical tests of GI mean values among groups and intervals.

	Groups				Statistical Analysis
	GEM	Propolines	0.12% CHX	Placebo	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	P value*
Baseline	1.618 $\pm$ 0.279	1.709 $\pm$ 0.358	1.674 $\pm$ 0.505	1.395 $\pm$ 0.416	0.263
7 days	0.057 $\pm$ 0.029	0.087 $\pm$ 0.065	0.118 $\pm$ 0.227	0.343 $\pm$ 0.380	0.019 Sig
P value**	0.001 sig	0.001 sig	0.001 sig	0.001 sig	
Effect size	5.875	4.910	3.551	2.635	
Mean change	1.561 $\pm$ 0.264	1.621 $\pm$ 0.383	1.556 $\pm$ 0.473	0.543 $\pm$ 0.207	0.001 Sig

GEM: GEM natural mouthwash (Propolis, Neem and Chlorophyll), CHX: chlorhexidine, * comparison performed by one-way ANOVA test; ** comparison performed by Paired T-test; Sig.=significant at $p<0.05$, SD: standard deviation.

Table 3. Descriptive and statistical tests of BOP mean values among groups and intervals.

	Groups				Statistical Analysis
	GEM	Propolines	0.12% CHX	Placebo	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	P value*
Baseline	60.710 $\pm$ 26.656	69.479 $\pm$ 26.061	70.777 $\pm$ 28.286	61.605 $\pm$ 23.803	0.729
7 days	3.404 $\pm$ 1.948	3.977 $\pm$ 2.504	2.350 $\pm$ 1.840	7.222 $\pm$ 4.040	0.019 sig
P value**	0.001sig	0.001sig	0.001sig	0.001sig	
Effect size	2.226	2.630	2.496	2.464	
Mean change	57.305 $\pm$ 25.327	65.502 $\pm$ 26.256	68.427 $\pm$ 27.307	54.382 $\pm$ 20.705	0.515

GEM: GEM natural mouthwash (Propolis, Neem and Chlorophyll), CHX: chlorhexidine, * comparison performed by one-way ANOVA test; ** comparison performed by Paired T-test; Sig.: significant at $p<0.05$, ns=nonsignificant at $p>0.05$, SD: standard deviation.

Table 4. Descriptive and inferential statistics of GCF calprotectin levels (ng/mL) among study groups.

	Groups				Statistical Analysis
	GEM	Propolines	0.12% CHX	Placebo	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	P value*
Baseline	27.781 $\pm$ 6.101	28.325 $\pm$ 4.638	26.270 $\pm$ 4.111	26.356 $\pm$ 2.375	0.498
7 days	13.508 $\pm$ 2.739	13.864 $\pm$ 2.198	13.382 $\pm$ 2.929	14.427 $\pm$ 2.846	0.802
P value**	0.001 sig	0.001 sig	0.001 sig	0.001sig	
Effect size	2.696	3.598	3.515	4.514	
Mean change	14.272 $\pm$ 8.152	14.460 $\pm$ 5.831	12.888 $\pm$ 6.048	11.929 $\pm$ 4.936	0.760

GEM: GEM natural mouthwash (Propolis, Neem and Chlorophyll), CHX: chlorhexidine, * comparison performed by one-way ANOVA test; ** comparison performed by Paired T-test; Sig.=significant at $p<0.05$, ns=non-significant at $p>0.05$, SD: standard deviation.

placebo group (4.514), followed by Propolinse (3.598), 0.12% CHX (3.515), and GEM (2.696). Despite these variations, the differences in mean change among groups were not statistically significant ($p = 0.760$).

(Figure 5) illustrates the mean calprotectin levels for each study group at baseline and after 7 days. A consistent downward trend is observed across all groups, reflecting a reduction in calprotectin levels over time. The reductions appear relatively similar among the groups, which aligns with the non-significant differences observed in the between-group analysis. The

presence of statistically significant differences between baseline and day 7 within each group (indicated by ** symbols) confirms the effectiveness of all interventions in reducing calprotectin levels.

Discussion.

The present study demonstrated significant reductions in gingival crevicular fluid calprotectin levels in all study groups after 7 days of treatment. This finding indicates a reduction in neutrophil-mediated inflammatory activity following

professional periodontal therapy, oral hygiene instruction, and adjunctive mouthwash use.

The significant improvement observed in all study groups, including the placebo group, can be attributed not only to the intervention but also to professional supragingival scaling and standardized oral hygiene instructions provided at baseline. These measures are fundamental components of gingivitis management and likely contributed substantially to the observed clinical improvements. Therefore, the tested mouthwashes should be considered adjuncts to mechanical plaque control rather than the sole determinant of treatment outcomes.

Although some treatment groups demonstrated numerically greater improvements in certain clinical and biochemical parameters, no statistically significant intergroup differences were observed. Therefore, these findings should be interpreted as numerical trends rather than evidence of the superiority of any specific mouthwash.

The current results align with recent studies showing that oral products containing propolis have positive effects on gingival inflammation [25], reported that propolis-based formulations were effective in reducing gingival inflammation and clinical periodontal parameters while exhibiting fewer adverse effects than chlorhexidine-containing products.

The present study demonstrated a highly significant reduction in all clinical periodontal parameters (PLI, GI, and BOP) after 7 days of treatment ($p \leq 0.001$), indicating the effectiveness of both mechanical plaque control and adjunctive chemical agents.

For plaque index (PLI), mean values decreased substantially in all groups. GEM showed a reduction from 1.319 to 0.137, Propolinse from 1.785 to 0.066, CHX from 1.416 to 0.022, and placebo from 1.164 to 0.164. These findings clearly indicate that CHX achieved the lowest plaque accumulation, followed by Propolinse and GEM.

For gingival index (GI), all groups demonstrated significant improvement. GEM decreased from 1.618 to 0.057, Propolinse from 1.709 to 0.087, CHX from 1.674 to 0.118, and placebo from 1.395 to 0.343. Interestingly, herbal mouthwashes showed comparable reductions in gingival inflammation to CHX, which may be related to their anti-inflammatory properties rather than only antimicrobial effects. Bleeding on probing (BOP) values also showed marked reduction. GEM decreased from 60.71 to 3.40, Propolinse from 69.47 to 3.97, CHX from 70.77 to 2.35, and placebo from 61.60 to 7.22. CHX showed the greatest reduction.

Calprotectin is mainly released from neutrophils and monocytes and is considered an important biomarker of innate immune activation [7,8]. Since gingival inflammation is characterized by neutrophil migration and activation in response to dental biofilm, the reduction in GCF calprotectin levels may reflect improvement in local inflammatory status.

Plaque-induced gingivitis is primarily initiated by biofilm accumulation, which stimulates a localized host immune response characterized by gingival redness, swelling, and bleeding without attachment loss [1,2]. GCF provides a site-specific diagnostic medium that reflects both clinical periodontal status and biological inflammatory changes [3,4]. Therefore, the assessment of calprotectin in GCF adds biochemical value

to the evaluation of treatment response beyond conventional clinical parameters.

In the present study, calprotectin levels decreased from 27.781 ± 6.101 to 13.508 ± 2.739 ng/mL in the GEM group, from 28.325 ± 4.638 to 13.864 ± 2.198 ng/mL in the Propolinse group, from 26.270 ± 4.111 to 13.382 ± 2.929 ng/mL in the 0.12% CHX group, and from 26.356 ± 2.375 to 14.427 ± 2.846 ng/mL in the placebo group. The greatest numerical reduction was observed in the Propolinse group, followed by GEM, chlorhexidine, and placebo. However, no statistically significant intergroup difference was detected, suggesting comparable short-term effects among the tested mouthwashes.

The greater numerical reduction observed in the Propolinse group may be attributed to the combined biological effects of propolis and green tea. Propolis contains flavonoids and phenolic compounds with antimicrobial, antioxidant, and anti-inflammatory effects [19,24]. Green tea contains catechins, particularly epigallocatechin gallate, which may suppress oxidative stress, inhibit inflammatory mediators, and reduce periodontal pathogens [20,22]. These mechanisms may explain the stronger numerical influence of Propolinse on calprotectin reduction.

The GEM mouthwash also demonstrated a considerable reduction in calprotectin levels. This effect may be related to its combination of propolis, neem, and chlorophyll. Neem has antibacterial and anti-inflammatory properties and has been reported to reduce plaque and gingival inflammation [21]. Chlorophyll may also contribute to oral freshness and supportive anti-inflammatory effects. Therefore, GEM may provide a combined herbal effect that supports gingival inflammatory control.

Although chlorhexidine remains the gold standard antiplaque agent due to its broad-spectrum antimicrobial activity and substantivity, its prolonged use is limited by adverse effects such as staining, taste disturbance, burning sensation, and mucosal irritation [17,18]. Therefore, herbal mouthwashes may be useful as safer alternatives for long-term daily use, particularly when prolonged chlorhexidine use is not desirable.

The significant reduction observed in the placebo group may be explained by the professional scaling and polishing and standardized oral hygiene instructions provided to all participants. Mechanical plaque control remains the primary factor in gingivitis improvement and may have contributed strongly to the biomarker reduction across all groups [1,2]. The short duration of the intervention may also explain the absence of statistically significant differences between mouthwashes.

The reduction in calprotectin levels supports the concept that improvement in gingival inflammation is associated not only with clinical changes but also with measurable biochemical changes in GCF. Since calprotectin reflects neutrophil-mediated inflammatory activity, its reduction may indicate suppression of local acute inflammatory response within gingival tissues [10]. From a clinical perspective, the findings suggest that herbal mouthwashes may serve as promising adjunctive agents in the management of plaque-induced gingivitis. Herbal formulations showed similar biochemical anti-inflammatory effects and may be more appropriate for long-term daily usage because of their

favorable safety profile, even if chlorhexidine is still quite efficient for controlling plaque.

Significantly, a number of systemic inflammatory disorders have been connected to periodontal disease. Calprotectin is becoming more widely acknowledged as a biomarker of the burden of systemic inflammation in many disorders [17]. Therefore, although further long-term research is needed, regulation of GCF calprotectin by periodontal treatment and herbal mouthwashes may have potential ramifications beyond local periodontal healing.

These results should be viewed cautiously, though. The investigated mouthwashes' long-term anti-inflammatory and immunomodulatory benefits might not be completely reflected in the brief 7-day follow-up period. Furthermore, small differences in sample may have an impact on biomarker quantification since GCF collection is technique-sensitive [28]. It is advised that future research with longer follow-up times and more inflammatory indicators be conducted to elucidate the long-term function of herbal mouthwashes in reducing gingival inflammation and halting the development of periodontitis from gingivitis.

To the best of the authors' knowledge, this is among the first randomized clinical trials evaluating the effect of GEM mouthwash and Propolinse mouthwash on GCF calprotectin levels in gingivitis patients.

The present study was limited by the short intervention period of seven days, which allowed assessment of only the short-term effects of the tested mouthwashes. Consequently, the present findings should be interpreted as short-term outcomes and should not be extrapolated to long-term clinical effectiveness.

Study Limitations.

1. The short duration of the study (7 days) limits the evaluation of long-term effects. The timing of GCF collection may influence biomarker levels due to circadian variation.
2. GCF volume is influenced primarily by time and inflammatory status. In some cases, repeated sampling was required due to blood contamination, which may affect biomarker concentration.
3. Individual differences in mechanical plaque control may still influence results.
4. Only male participants were included to minimize the influence of hormonal fluctuations on periodontal inflammatory biomarkers. Consequently, the findings may not be fully generalizable to female populations. Future studies should include both sexes to improve the external validity of the findings.

Conclusion.

Within the limitations of this 7-day randomized clinical trial, both herbal mouthwashes and 0.12% chlorhexidine demonstrated favorable clinical and biochemical outcomes. No statistically significant intergroup differences were observed. Although Propolinse showed the highest numerical decrease in calprotectin levels, it was not proven to be superior to chlorhexidine or placebo. To verify the long-term efficacy and safety of these herbal formulations, additional long-term randomized controlled trials are needed.

Conflict of interest: The authors declare no conflict of interest.

Ethical Approval: The study protocol was reviewed and approved by the MUCOD Human Research Ethics Committee, College of Dentistry, Al-Mustansiriyah University, Iraq (REC Reference No. REC214; Study Number: MUOSU/POST-202607).

Informed Consent: Written informed consent was obtained from all participants after explaining the nature and purpose of the study prior to enrollment.

Source of Funding: This research received no external funding.

Authors' contributions: H.F.A. contributed to the study conception and design, clinical data collection, laboratory procedures, statistical analysis, data interpretation, and manuscript drafting. R.A.B supervised the research project, critically revised the manuscript, and approved the final version for publication.

Abbreviations: CHX: Chlorhexidine; ELISA: Enzyme-Linked Immunosorbent Assay; GCF: Gingival Crevicular Fluid; GEM: GEM natural mouthwash (Propolis, Neem and Chlorophyll); SPSS: Statistical Package for the Social Sciences; SD: Standard Deviation; ANOVA: Analysis of Variance; HSD: Honestly Significant Difference.

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