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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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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 ВИНИТИ 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 ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

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. Корректура авторам не высылается, 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 compu-ter-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 საყურადღებოდ!

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 ინგ-ლისურენოვან 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. ექსპერიმენ-ტული 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, შემა-ჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტო-სურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სუ-რათის 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 უცხო-ური 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 ფჩხილებში 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 ხელმძღვანე-ლის 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 შე-ჯერება 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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EFFECT OF CHICKEN EGGSHELL PASTE ON ENAMEL SURFACE MICROHARDNESS AND COLOUR CHANGE OF ARTIFICIAL CARIOUS LESIONS CREATED ON PERMANENTLY EXTRACTED TEETH

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Abstract.

Background: Remineralizing agents such as fluoride and hydroxyapatite (HA) (Ca5 (po4)3OH) are well-known treatment choices for incipient enamel lesions. Hydroxyapatite has been newly presented to return the color of such enamel lesions.

Objective: The purpose of this prospective in vitro study was to compare commercial sodium fluoride paste to hydroxyapatite paste (HA) made from chicken eggshells powder (CESP) in terms of how it affected the microhardness and color of the enamel surface of artificially demineralized permanent teeth. **Materials and Methods:** Fifty healthy maxillary premolars were gathered, decoronated, and the crowns were placed in acrylic moulds with the buccal enamel surfaces exposed. Baseline microhardness evaluation was done for the baseline group, while the colour assessment was done at baseline to three treatment groups. Then specimens were randomly divided into the following five groups (n = 10) based on the treatment of enamel surface: Group 1: Baseline group; Group 2: Acid group demineralized only; Group 3: demineralized followed by the application of hydroxyapatite paste (HA); Group 4: demineralized followed by the application of sodium fluoride toothpaste (Naf); and Group 5: demineralized followed by application of combination treatments (HA paste at morning and Naf paste at evening. The specimens were stored in deionized water at room temperature during treatments, after one week they were subjected to a Vickers microhardness test, and colour assessment to three treatment groups after treatment. One-way ANOVA and Tukey's post hoc multiple comparison tests were used for statistical analysis ($P < 0.05$).

Results: After artificial demineralization, the enamel's surface microhardness was greatly reduced, and after management, it dramatically increased. The combination group had the greatest mean microhardness value when compared to the HA paste group and the Naf paste group. Statistically, there was no significant variance in microhardness values among the Naf paste and HA paste groups. **Conclusion:** Hydroxyapatite sourced from chicken eggshell was as effective as Naf paste in remineralizing and restoring the lost microhardness of artificially demineralized enamel, hydroxyapatite paste changed tooth color, while sodium fluoride paste, a combination group neither changed nor masked color of early caries lesion.

Key words. Chicken eggshells, microhardness, hydroxyapatite paste. Remineralization, colour.

Introduction.

Dental caries is a contagious illness predominantly brought on by the fermentation of carbohydrates by acidogenic bacteria in the mouth, which results in the production of organic acid as a byproduct. As a consequence, the acids penetrate the dental plaque biofilm's hard tissues and break into hydrogen ions. These hydrogen ions have the power to liquefy the tooth's mineral components, particularly calcium and phosphate. Known as demineralization, this procedure. And if the right treatment is provided, this process can be prevented; otherwise, the teeth would progressively deteriorate [1]. Dental caries is preventable, so many approaches focused on many procedures and measures to conserve the development of caries and treat it at its initial stages trying to preserve more tooth structure whenever possible, Reminerlizaing agents are created in a variety of products, including dentifrices, fissure sealants, chewing gum, and mouthwash [2,3]. Therefore, modern dentistry emphasizes prevention and minimal intervention, and one of the principles of minimal intervention dentistry is the replacement of missing tooth substances with biomimetic material (MID) [4,5].

In dentistry, the development of biomimetic materials has led to the development of materials that can entirely replace missing tooth structures and possess qualities resembling those of natural tooth structures. HA is one of these materials and is thought to be the most bioactive and compatible. Both natural and artificial sources can be used to prepare HA. The synthetic version of HA has so far been used to demonstrate the positive benefits of the compound. A natural and inexpensive method of manufacturing HA might use chicken eggshells as a raw material [6]. The null hypothesis of the current study: there is no improvement in the microhardness and color of the artificially demineralized enamel teeth when using Hydroxyapatite (HA) paste synthesis from CESP.

Materials and Methods.

Preparation of Hydroxyapatite powder (HAP): Hydroxyapatite powder (HAP) as a white powder prepared manually from chicken eggshell powder (CESP) at Department of Dental Basic Science/College of Dentistry/University of Mosul, (patent 6987, A61C13/08, A61L27/12).

Preparation of Demineralization solution: Consists of $CaCl_2$ (2.2mM), KH_2PO_4 (2.2 mM) and acetic acid (0.05 M), pH was adjusted with (1M) KOH to (4.4).

Preparation of the specimen: After receiving the ethical approval from the research ethics committee in the University of Mosul/College of Dentistry (UoM. Dent/ H.DM.78/22). Fifty extracted non-carious maxillary first premolars were collected and examined under stereomicroscope (OPTIKA /ITALY) to ensure that they were cavity-free, hypo calcifications, and crack then stored in deionized water containing (0.1% PH.7) Thymol (Flukachemie, Switzerland) in a closed container at room temperature and used within 3 months after extraction [7] to prevent dehydration and microbial growth [8]. The teeth were decoronated at the cervical line with abundant water irrigation using straight diamond bur of a high-speed handpiece (continuous water cooling) to evade damaging the enamel. Then, each tooth crown was positioned such that the buccal surface faced upward and was parallel to the ring's floor inside a self-curing acrylic resin (VERACRIL/Colombia). Filled cylindrical plastic ring (14mm in diameter, 16mm in depth). A circular 6mm piece of adhesive tape was placed in the middle of the buccal surface of each sample. The tape was removed to reveal an enamel window after applying acid-resistant nail polish to the remaining surface [9]. After that, using fine-grit silicon carbide sheets (1200 grit), the exposed enamel surfaces were ground and wet polished one at a time to provide flat, homogeneous surfaces for the surface microhardness test [10], Then polishing the exposed window with the universal polishing machine (Surf-Corder, Japan). Later, all samples were kept in deionized water until they were soaked in a demineralized solution (Figure 1A).

Establishment of the preliminary Carious Lesion: Every tooth in the research, except the baseline group, was submerged in a demineralizing solution for 48 hours (2 days) at 37°C to cause subsurface enamel caries (white spot lesions without cavities) [11], Specimens were washed with deionized water and stored in deionized water.

Synthesis of hydroxyapatite paste from chicken eggshell powder (CESP): The hydroxyapatite paste (HA paste) was prepared according to Natsir et al [12]. which is formulated from Sodium Carboxyl Methyl Cellulose (Na-CMC)(Avonchen, UK.), Distilled water, Glycerol, Methylparaben (NipaginR, Avonchen, UK), Hydroxyapatite powder extract from chicken eggshells by chemical precipitation method. The most preferable concentration of hydroxyapatite in paste formulation to achieve better remineralization of the enamel tooth surface is (10%) WV according to [13]. Therefore, we prepared 5 ml of 10% Hydroxyapatite toothpaste daily for 7 days.

Infrared Spectroscopy Estimation of Important Bands: The prepared HA powder from CESP, and the prepared HA paste components were characterized by using the FTIR-Alfa-Bruker spectrophotometer (Germany) in the region (400-4000 cm^{-1}). This measurement was carried out at the University of Mosul, College of chemistry sciences, Iraq (Figure 1B).

Grouping and surface treatment: In the current research, there were a total of (50) samples of teeth, which were allocated into (5) groups at random with (n=10) samples in each group.

Group A (10 teeth): baseline group without demineralization, (subjected to microhardness test only).

Group B (10teeth): without treatment, demineralized teeth immersed in deionized water only, (subjected to microhardness test only).

Group C (10 teeth): after demineralization, HA paste was applied by a micro brush as in figure (1C) and then brushed by a soft brush, which was applied twice daily on the exposed enamel surface to simulate routine normal hygiene in vivo, (Subjected to microhardness test & colour test).

Group D (10 teeth): The exposed enamel window of each tooth was coated with a thin layer of fluoridated toothpaste that contains (2500 ppm) fluoride ion (Flurokin, Spain) using a fine brush for 2-3 minutes. This procedure was performed twice daily to simulate routine oral hygiene instructions then washed with deionized water and stored in deionized water change after each application until complete one week, (Subjected to microhardness test & colour test) (Figure 1D)

Group E (10 teeth): Combination group (HA toothpaste +fluoridated toothpaste) separately (HA) paste applied once in the morning and (Naf) paste once in the evening until complete one week, (Subjected to microhardness test & colour test).

Surface Microhardness Test (SMH): The Surface Microhardness Test (SMH), assesses a material's resistance to plastic deformation under a standard load source [14]. The microhardness of the samples was determined using a Vickers Microhardness tester (Wilson Wolpert Instruments, Aachen, Germany) outfitted with a 500-g load. It took 15 seconds for the exposed enamel surface to sink and settle before the Vickers hardness number (VHN) could be determined. On each specimen, three indentations were made, and the measurements were averaged. This was used as the sample's first Microhardness value (MHV) for the baseline group, control negative (Acid group) after demineralization, and three treatment groups after remineralization then compare between surface Microhardness of all groups and according to [15].

Colour change evaluation: Color evaluation was assessed at two different stages; at baseline before enamel lesion formation, as well as after therapy application consuming a colour colourimeter (NR60CP Precision /3nh/China) as in figure (2). The CIE-lab coordinates were obtained using a colourimeter. L* stands for the light parameter, a* for the green-red chromaticity parameter, and b* for the blue-yellow chromaticity parameter. Each specimen had three readings taken, and the average of those values was recorded. The mean values of colourimeter readings at different stages were compared to evaluate the amount of colour change (ΔE) as follows $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

The colour measurement was performed in a dark room, which could eliminate associated errors [16,17], and the teeth samples

__Figure 1.__ Representative images for the created model in the study. (A) Creation of a window on the buccal surface in order to standardize the area of measurement in the middle third of the tooth. (B) Infrared spectroscopy, Alpha 11 Bruker. Application of (C) Hydroxyapatite paste (D) sodium fluoride paste.

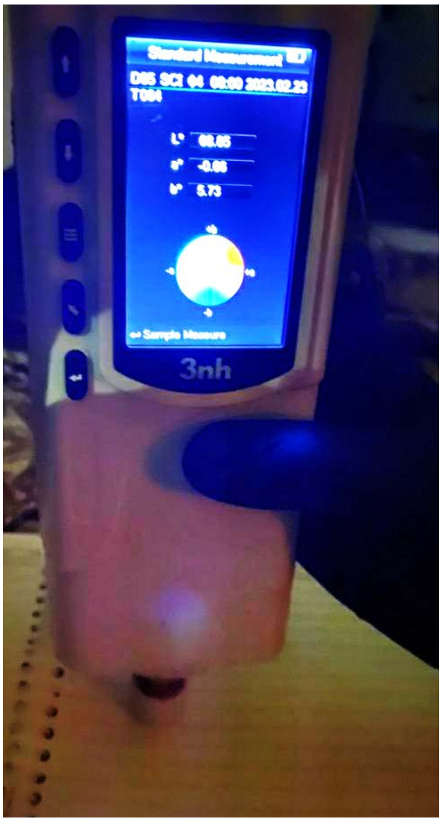

Figure 2. *Display colour measurement of the teeth samples by using a Colorimeter.*

were placed over a white paper during measurements to provide white background [18].

Statistical analysis: Microhardness values of the baseline group, Microhardness values of control negative (acid group) and Microhardness values of three treatments groups and data of the color test were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed parametric (normal) distribution. One-way ANOVA followed by Tukey's post-hoc test was used to match between different clusters of each test. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with SPSS Version 20 for Windows.

Results.

The microhardness test for all groups, including the mean, number, and standard deviation displayed in Table 1. Based on the measurement for tested groups, the Baseline group had the highest surface microhardness mean value and the Combination group had the highest mean between treatment groups, then sodium fluoride group, followed by the HA paste group, and the control negative group.

The contrast of mean values of enamel surface microhardness tested and the results revealed that there were highly statistically significant differences within and in between baseline, Control negative, HA paste, Naf paste, combination groups at $p \leq 0.01$ (Table 2).

The mean microhardness for Baseline, control negative, and treatment groups are highly significantly different from each other at $p \leq 0.01$. The baseline group had surface mean microhardness significantly higher (281.3300) than other groups, the Combination group had a mean microhardness (264.5400), while control negative group had a lesser mean (171.2600) on surface microhardness (Table 3).

We noticed that from mean values of surface microhardness of the enamel teeth samples. The surface microhardness decreased after immersion in demineralizing solution, in comparison to the microhardness of the baseline group.

Color change estimation: To observe the degree of colour change, the mean values of colourimeter readings at two separate phases were calculated, as shown in Table (4).

The hydroxyapatite paste group exhibited an increase in ΔL value more than the other two types of treatment groups associated with a decrease in Δa and Δb, and this reflected that increase in light reflection and decrease in light transmission, through the tooth caused by the HA particles adhering to the enamel (Figure 3).

Discussion.

The efficacy of hydroxyapatite micro-clusters or nanoparticles in toothpaste and mouthwash in promoting caries remineralization and stopping tooth tissue demineralization has been demonstrated via clinical as well as in situ and in

Table 1. *Descriptive statistics of surface microhardness measurements among tested groups.*

Groups	**N**	**Mean**	**S.D.**
Baseline group	10	281.3300	11.48797
Control negative G	10	171.2600	16.15798
Hydroxyapatite paste G	10	234.7200	12.57120
Sodium fluoride paste G	10	237.4000	10.39177
Combination G	10	264.5400	2.57086
Total	10	237.8500	39.50255

Table 2. *Enamel surface microhardness of the studied groups.*

Microhardness	Sum of Squares	df	Mean Square	F	Sig
Between Groups	70470.940	4	17617.735	132.327	0.000
Within Groups	5991.185	45	133.137		
Total	76462.125	49			
** *p*≤ 0.01, highly statistically significant using one-way analysis of variance (ANOVA) test					

Table 3. *Microhardness value at baseline G, Control negative, Hydroxyapatite toothpaste, Sodium fluoride paste and combination groups.*

Groups	N	Mean	S.D.
Baseline group	10	281.3300 a	11.48797
Control negative G	10	171.2600 d	16.15798
Hydroxyapatite paste G	10	234.7200 c	12.57120
Sodium fluoride paste G	10	237.4000 c	10.39177
Combination G	10	264.5400 b	2.57086
Means in the same column with different letter values denote statistically significant differences using Duncan's Multiple Range Test, significant (p<0.05); and non-significant (p>0.05).			

Table 4. *Mean ΔL*, Δa*, and Δb* values after 1 week of treatment.*

Groups	ΔL *	Δa *	Δb *
HA paste G	4.782(0.294)	-0.328(0.303)	-4.46(2.722)
Naf paste G	1.154(0.457)	-0.274(0.686)	-6.08(2.225)
Combination G	3.340(0.773)	-1.353(0.531)	-6.20(2.996)

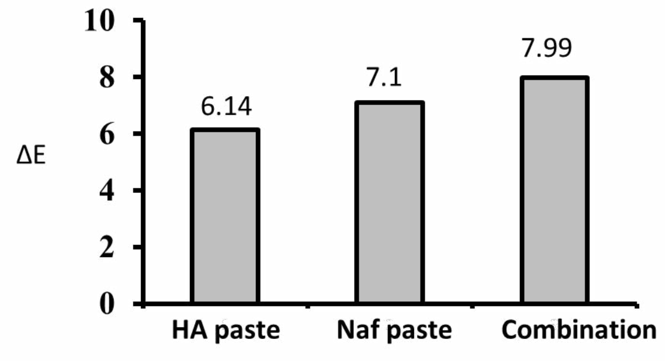

Figure 3. *Mean ΔE values of the Hydroxyapatite paste G, Sodium fluoride paste G, and Combination G. HA paste had the lowest mean value, then Naf paste group, and the combination group.*

vitro research, HA particles that have been adsorbed on tooth surfaces induce remineralization of initial caries lesions by directly filling micropores in demineralized tooth tissue, where it acts as a crystal nucleus, and by continuously attracting large amounts of calcium and phosphate ions from the surrounding remineralization material, which promotes crystal deposition and growth [19]. The microhardness of the enamel surface is a good indicator of the mineral makeup of the enamel, and measuring microhardness is sensitive enough to ascertain how the enamel is resistant to demineralization. Additionally, a strong connection was discovered between mineral loss in carious lesions and the enamel's surface microhardness [20]. According to the findings of the current study, the null hypothesis was not accepted. The enamel bio-mineralization of hydroxyapatite in combination with fluoride following treatment on artificially demineralized enamel of them exhibited promising remineralizing capability in the results. Therefore, based on the results, we observed that enamel microhardness decreased after being exposed to the demineralizing solution, which pointed to the mineral loss triggered by the acid, and the difference in mean surface microhardness values(SMH) between the groups following treatment, which reflected the various remineralization potentials for each treatment procedure, was highly statistically significant. The remineralization system needs to supply constant, bioavailable calcium, phosphate, and fluoride ions since these minerals are lost during demineralization [21]. In the current study and depending on the means values, the remineralization effect of the combination group (HA paste +Naf paste) was better than hydroxyapatite paste alone, and sodium fluoride paste alone but both of them were valuable to protect the microhardness of permanent teeth enamel when compared to with the control negative group where the deionized water was used for storing teeth after demineralization. The combination group exhibited the highest mean surface enamel microhardness than the Naf paste alone or HA paste alone, and this agrees with Ebadifar et al. [21-23]. The obtained results of the SMH values found in the combined group denote that the presence of copious amounts of calcium, phosphate from hydroxyapatite paste and fluoride ions increased hydroxyapatite crystallization utilizing the high level of ions available so enhancement remineralization effect due to the synergistic effect of two remineralizing agents. Additionally, fluoride toothpaste is well known for enhancing the remineralization of human enamel following acid assault and is well-regarded by the general population [23].The results of our experiment on the remineralization of sodium fluoride paste agreed well with those of Oliveira et al [24]. Whose noted that after a week of treatment, toothpaste containing just fluoride significantly increased the microhardness of demineralized specimens. While HA pastes alone group exhibited a minimum mean value than the combination paste group, and Naf paste alone group, this might be due to the paste used in this study being locally synthesis not commercially supplied, the short duration of treatment, and the size of hydroxyapatite used in the toothpaste preparation is micro-sized instead of nanosized, resulting in less entering the lesion and caused HA paste group exhibiting least mean for surface microhardness [10,25]. However statistically speaking, there was no visible difference in the mean SMH value between the HA paste group and the Naf paste group, and this was in good agreement with the study conducted by [26,27]. While regarding color change results, The colourimetric analysis in this study was carried out to describe the ability of hydroxyapatite paste, sodium fluoride toothpaste and a combination of both of them together to induce a color change (ΔE, ΔL, Δa, Δb) in the artificially induced white spot lesion or capability of these remineralizing agents to cover the visible white color of early enamel caries. The attachment of HA particles to the tooth surface may be used to explain why the HA-oral care product can whiten teeth. Fabritius-Vilpoux et al. (2019) concluded that HA particles from a mouthwash adhere to enamel surfaces an in vitro research; this is supported by the scan electron microscope (SEM) examination carried out in the study performed by Sarembe et al. (2020). So, the result of the colour analysis of hydroxyapatite paste of the current study was in good agreement with [28,29]. However, studies on teeth whitening products have shown that remineralization causes a considerable improvement in the yellowness [Δb] and lightness [ΔL] of the teeth. Additionally, the human eye is more sensitive to changes in brightness [ΔL] than the other colour characteristics [Δa, Δb] [30]. According to studies [30,31], the ΔL colour parameter is the most important factor in tooth colour assessments. In the current study, we found that HA paste significantly increased brightness (L) compared to other treatment groups while significantly reducing (b, a), making teeth appear whiter, less red, and less yellow. These optical changes can be attributed to the HA particles sticking to the enamel, which increases light reflection and decreases light transmission through the tooth [16,17,32], While sodium fluoride paste cannot conceal the whiteness of early caries lesions, this is because a high fluoride concentration can accelerate mineral precipitation on the enamel surface and obturate the enamel surface pores. This procedure may further restrict the remineralization of the subsurface demineralized enamel [33]. The esthetic and structural qualities of the deeper lesions are not significantly enhanced by this surface-only remineralization [21]. Although the high mean ΔE value of the combination group point to the worst result and this can be explained by that, remineralization by using the synergistic effect of HA toothpaste and Naf toothpaste leads to the form of a new product on the tooth surface caused to change in the colour negatively through irregular remineralization making like a bridge on the enamel surface, so further studies we needed to observe the outer layer of the demineralized enamel tooth surface treated by this type of synergistic treatment such as evaluation by using scanning electron microscope. However mean ΔE value of HA paste is the lowest mean between the three remineralizing agents and this indicated the best result for colour restoration, and according to the study performed by Abdel-Hakim et al. (2016) [15], who said that the lowest mean of ΔE indicated the best result for changing the colour of the white spot lesion (WSL). Attempt should be made to provide protection of the whole teeth keeping it safe from porosity, enamel loss, and opalescent tooth colour [34].

Conclusion.

The microhardness of artificially demineralized enamel surface is improved by hydroxyapatite paste made from chicken eggshells and sodium fluoride toothpaste (Flurokin), which together have remineralizing properties. Statistically speaking, the ability of sodium fluoride paste, and hydroxyapatite paste to remineralize and restore the enamel's lost microhardness was comparable; however, the combination paste group (HA+ Naf) was thought to be the most successful in remineralizing and regaining the enamel lost microhardness, followed by Naf paste and finally HA paste. Toothpaste containing sodium fluoride had no impact in restoring the colour of lesions with white spots. While hydroxyapatite paste can improve but not mask the white colour of white spot lesions, when it is combined with sodium fluoride as in the combination paste group it is neither improved nor masks the colour of white spot lesions.

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