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ENAMEL ABRASION AND STAIN REMOVAL EFFICACY OF CHARCOAL -BASED AND CALCIUM CARBONATE /PERLITE WHITENING TOOTHPASTES (A RANDOMIZED CLINICAL TRIAL AND IN-VITRO TRIAL)

Mohamed osama Elbadry*, Iman Ibrahim Elsayad**,
Mohamed Refaat ibrahim Elbialy*** *and* Engie Safwat Mohmaed ****

ABSTRACT

Objective: The study's objectives are to assess the external stain removal effectiveness of activated charcoal-based tooth paste in comparison to calcium carbonate/perlite tooth paste clinically and to look at enamel surface abrasion for both toothpastes in vitro.

Material and Methods: A total of 32 Patients with extrinsic stains on anterior teeth were divided into 2 groups (n=16); Group 1: patients using activated Charcoal toothpaste (Intervention group), Group 2: patients using calcium carbonate/perlite toothpaste (Control group) 4 weeks brushing (twice daily for 1 min) was performed. Additionally, for the in-vitro test a total of 36 specimens obtained from human permanent premolars were randomly divided into 2 groups (n=18): Group-1: brushed with activated Charcoal toothpaste (intervention group); Group-2: brushed with calcium carbonate/perlite toothpaste (Control group). An atomic force microscope (AFM) was used to quantify the mean roughness value (Ra) of the brushed samples after three minutes of brushing with a powered toothbrush in order to evaluate the surface abrasion of enamel.

Results: The total MLSI score did not alter statistically significantly between the groups at baseline and after brushing ($P > 0.05$). Nonetheless, there were statistically significant variations in each group's overall MLSI score between the pre- and post-brushing periods ($P < 0.05$). However, at baseline, the enamel surface abrasion data did not reveal any significant differences between the groups ($P > 0.05$). Conversely, after brushing, there were notable variations in the amount of surface abrasion on enamel between the groups ($P < 0.0001$).

Conclusions: The tested activated charcoal-based dentifrice showed a similar and clinically perceptible performance in reducing extrinsic stains after 4-weeks of brushing as calcium carbonate/perlite-based dentifrice. As well as activated charcoal-based dentifrice-maintained enamel surface integrity post-brushing.

KEYWORDS: Extrinsic tooth stains, Charcoal dentifrice, calcium carbonate, Abrasion, Atomic force microscope.

* Department of Conservative, Faculty of Dentistry Cairo University, Cairo, Egypt
** Professor, Conservative Dentistry Department, Cairo University, Egypt
*** Instructor, Conservative Dentistry Department, Cairo University, Egypt
**** Associate Professor, Restorative and Dental Materials Department, National Research Centre, Cairo, Egypt.

INTRODUCTION

Oral hygiene treatments and teeth-whitening formulations have become more popular worldwide. Most people would prefer to have white teeth because discolored teeth can have a detrimental effect on one's appearance and quality of life. A variety of whitening options for at-home maintenance are advertised for example, toothpastes, gels, and mouthwashes that have whitening properties.[1] Dentifrice formulations are essential for preventing the formation of extrinsic dental stains, which can be brought on by tobacco use, certain foods, or drugs that attach to proteinaceous molecules in the salivary enamel pellicle.[2] Most tooth-whitening toothpastes use abrasive particles like calcium carbonate, hydrated silica, dicalcium phosphate dihydrate, alumina, perlite, calcium pyrophosphate, and hexametaphosphate to remove extrinsic stains, while some toothpastes use hydrogen peroxide and other oxidizing agents to make teeth whiter and lighter or even change the natural color of enamel. [3-4]

Toothpaste that contains activated charcoal has become more and more popular recently. Activated charcoal has a high porosity, which allows it to absorb some pollutants in an emergency situation. Owing to its ability to bind and extract chemicals that discolor teeth at the nanoscale, its absorption capability could result in ion exchange inside the oral cavity.[5-6]

Enamel, the strongest tissue in the body.[7] Wear of enamel caused by a gradual loss of the tooth's surface is known as abrasion. Some chemicals can change the surface morphology of enamel; however, it can also be produced mechanically.8 In the field of dentistry, it is widely accepted that a dentifrice should have some degree of abrasiveness in order to effectively clean teeth .[9] Concerns over toothpaste's possible abrasive effects on enamel and dentin have unintentionally arisen from the daily tooth brushing habit, which is thought to be crucial for maintaining oral hygiene. [10] The particles and materials included in toothpaste that are intended to remove stains and plaque from teeth are the main source of its abrasive qualities. Toothpaste is made with abrasive ingredients to help in the physical removal of stains, plaque, and food particles. [6] It has been demonstrated that toothpastes with high concentrations of activated charcoal, which are tiny particles, induce tooth wear.

The study's objective was to compare the exterior stain removal abilities of tooth pastes containing activated charcoal to those containing calcium carbonate and perlite from a clinical standpoint. Furthermore, to look at the in vitro surface abrasion of enamel for both toothpastes.

MATERIALS AND METHODS

The Research Ethics Committee of Cairo University's Faculty of Dentistry upheld the highest ethical standards in all operations carried out during the study's human subjects (Ref.11/8/22). This clinical investigation was conducted in the Conservative Clinic at Cairo University's Faculty of Dentistry in Egypt using a randomized controlled protocol.

All research-related tasks, such as finding volunteers and guiding them through the processes, fell under the purview of the researcher. After being briefed about the study's purpose and methodology, each participant completed an informed consent form. The study's in vitro component was carried out in Cairo, Egypt's Oral and Dental Research Institute National Research Centre in the department of restorative and dental materials.

Participant eligibility requirements for the relevant clinical study At least eight natural anterior teeth (incisors and canines) that are assessable for extrinsic stain and readily available for all study appointments are required of adult male and female patients, aged 18 to 50, who also maintain good general and oral health and oral hygiene. [11] Although the participants' noncooperative behavior met the

exclusion criterion, Individuals having a history of medication side effects, allergies to toothpaste ingredients, or any other significant pertinent issues, ladies who are expecting or nursing, Patients who had undergone dental prophylaxis within eight weeks of screening, patients with fixed orthodontic equipment, patients who had professional teeth whitening within the last six months, or patients with considerable calculus on the canine or incisor facial surfaces.

Vital stained anterior maxillary and mandibular teeth free of cavities or restorations, as well as extrinsic staining less than 40 according to the Macpherson modification of the Lobene stain index [MLSI], were the inclusion criteria for teeth in the clinical investigation. However, there were no cervical lesions, no caries, and no restorations when it came to the in vitro test. Conversely, the clinical trial's exclusion criteria included teeth that were non-vital, restored anterior teeth, parafunctional habits or tooth sensitivity, fluorosis, hypocalcification hyperplasia, endodontic treatment (or requiring endodontic treatment), and extrinsic tooth stain scores greater than 40 according to the Macpherson modified Lobene Stain Index. However, teeth with intrinsic discoloration from fluorosis, tetracycline, hyperplasia, endodontic therapy, and dental restorations were included in the clinical investigation.

Trial description:

Informed consent forms were signed by participants who met the eligibility requirements in accordance with their timetable. Patients seeking dental care in the Conservative Dentistry department were screened continuously until the desired population was reached. Patients underwent comprehensive examinations and diagnoses. Following the identification of the patients who would qualify for this study, the research investigator got in touch with the patients to discuss the study and find out whether they were interested.

Simple randomization was employed, creating numbers from 1:34 into two columns by computerized sequence generation with www.random.org. To make sure there was no tampering with the random list, the randomization list was kept hidden. From a sealed envelope that was opaque, each participant selected a random number. To make sure the patient was placed in the randomized group, the number on the envelope was entered into the patient chart after the participants selected an envelope.

The random allocation sequence was generated by the operator, he enrolled the patients and assigned them to the interventions/comparator group for procedures. The Participants, the outcome assessor and the statistician were all blinded.

Intervention:

32 participants who satisfied both the inclusion and exclusion criteria were split up into two groups of sixteen people each. The toothpastes were given to the study participants under the product codes Toothpaste 1 and Toothpaste 2, with the tube labeled with either No. 1 or No. 2. The whitening toothpaste 1 was given to subjects in group 1 (signal Complete 8 Charcoal toothpaste -Unilever Mashreq - Egypt) and Whitening toothpaste 2 was given to subjects in Group 2 (Signal whitening toothpaste -signal whitening moonlight toothpaste -Unilever Mashreq - Egypt). Moreover, each participant was provided with a soft tooth brush (oral-B pro-expert max clean indicator, Procter & gamble- Egypt) to use it during the whole trial. Showed participants how much toothpaste to use, showed them how to operate the mobile timer, and watched over their first brushing procedure. Afterwards, to ensure standardization, participants were asked to wash their teeth twice a day for one minute at home using the prescribed toothpaste and toothbrush (oral-B pro-expert max clean indication, Procter & Gamble, Egypt). Apart from receiving written and verbal guidance on toothpaste usage, the participants were asked to

maintain a home use diary where they were to record any notable dietary modifications they made throughout the experiment.

After receiving therapy for a month, participants returned to the clinic for a dental stain evaluation. They conducted duplicate exams (outcome assessors). It was desired to score the next subject before the replication exam, with repeatability assessments spaced ten to thirty minutes apart at each assessment session. For repeatability assessments, one person was randomly selected from the attendees for each session (morning or afternoon).

Stain evaluations were carried out throughout the trial in the same room with constant lighting conditions to help with assessment consistency. Participants washed their anterior teeth for thirty seconds with a wetted toothbrush before beginning each stain evaluation to eliminate any external debris. Before and during the evaluation, teeth were allowed to air dry if necessary.

Adverse occurrences (AEs) and any anomalies in the OST examination were recorded beginning with the screening visit and continuing for five days after the toothpastes allocated for the participants were delivered. During this trial, no AE were noticed for any of the participants. Once the participants had been using the assigned toothpastes for a month, data were taken and the stains were re-evaluated. After this period of time, the subjects went back to using their usual toothpaste and discontinued using the research toothpaste.

For each tooth that could be examined, four spots on its facial surface were found to be suitable for assessment: Gingival; Body: the remainder of the tooth, which is separated into sections on the lingual and facial surfaces that are mesial, body, and distal. Only the distal and mesial body regions were included in the interproximal area for analysis Figure (1). For every participant, the MLSI (A × I) score for every tooth location was determined by averaging the scores of the four maxillary and four mandibular anterior teeth's facial surfaces.

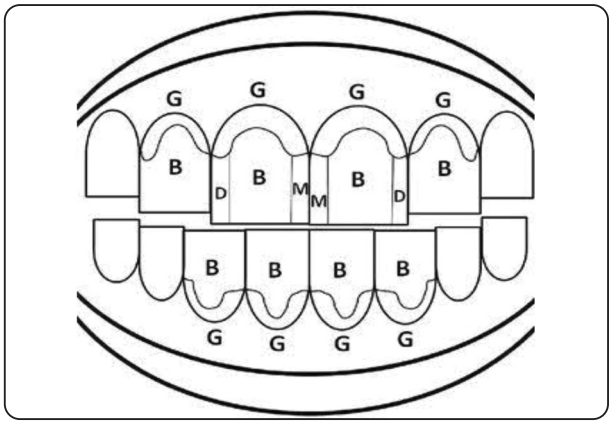

Fig. (1) MacPherson tooth area with MLSl grading sites on the two central upper incisors. G= Gingival; B= Body; M= Mesial; D= Distal

For the in vitro test

Human permanent maxillary premolars were removed from patients in the outpatient clinic of Cairo University's faculty of dentistry who had teeth pulled for orthodontic purposes. The teeth were extracted, cleaned, and disinfected with a 0.1% thymol solution. They were then stored in a 37°C bottle with deionized water and used three months later. The chosen teeth were embedded in acrylic resin, revealing 5 mm of the tooth's buccal surface. For the duration of the investigation, all specimens were kept in deionized water at 37°C.

A powered tooth brush (PTB) was used to brush the teeth. A specially made mimicking brushing apparatus was created to retain the PTB Figure (2) during the trials in order to regulate variables related to the use of PTBs. The custom-made Air-hardening modeling clay was used to secure the specimens in a circular shape. A second custom-made block of Air-hardening modeling clay was used to install the PTB handle at a 90-degree angle, and it was adjusted so that the specimen surfaces lined up with the PTB heads.. The toothpaste/artificial saliva slurries were brushed by both the control and intervention groups. For a month, the brushing method was carried out for three minutes, or twice a day. This was computed using the equation that follows: For every two teeth/brush-head, three surfaces/tooth,

seven teeth/quadrant, and contact time of (2x/d) x (30s/quadrant), the result is 5.7 seconds/surface/day. That means that 2085s/y equals 35m/y. Black (2007). et al.

Using an Atomic Force Microscope AFM (Anton Paar - Tosca™ 200, USA), the surface roughness of the materials was measured in order to examine surface abrasion. A baseline specimen was photographed before to the brushing operation, and four photos were acquired of representative specimens for each treatment group following the brushing procedure. Using an Arrow NCR tapping cantilever, a scanning size of 10 µm × 10 µm and a resolution of 400 × 400 were obtained. The AFM data were processed using the dedicated Tosca analysis software in accordance with ISO25178.[12]

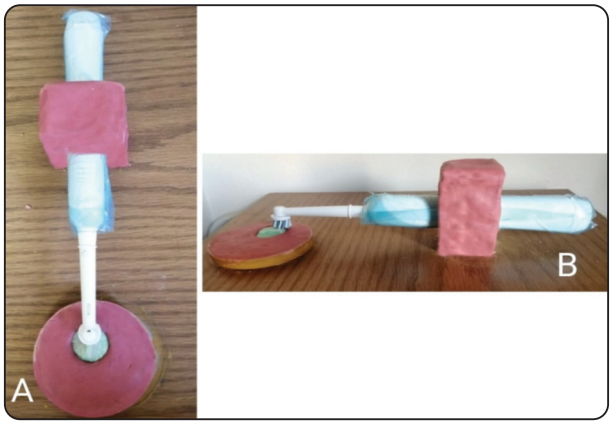

Fig. (2): Custom simulating brushing equipment was designed to hold PTB a: vertical view, b: horizontal view

Data collection, retention, and management:

In the clinical trial, patients were reviewed one month after their baseline examination by the outcome assessors using the Macpherson modified Lobene Stain Index, after one month of brushing.[13] Each tooth was separated into four areas for assessment: the body, distal, gingival, and mesial. For a total of eight anterior teeth (incisors and canines), the stain index identified the amount and strength of extrinsic tooth stain on their facial surfaces.

Using an Atomic Force Microscope AFM (Anton Paar - Tosca™ 200, USA), surface abrasion of the samples was assessed both before and after brushing for the in vitro test. Tosca analysis software was utilized to process the AFM data in accordance with ISO25178, a specific program. [12]

The expected outcome was disclosed to the participant. Professional pictures by digital camera were taken prior to the treatment as baseline data. A record of each patient's contact details was kept in their chart. Every patient was contacted via phone to confirm the time of their scheduled appointment. The next week was set for a follow-up visit in case the patient did not respond for whatever reason. Data entry was done by the research investigator. Every trial's data was encrypted with a password and kept on a computer. To avoid data loss, cloud storage was used for data backups.

This study was observed by the main supervisor. Her responsibility was to check any possibility of bias from participants, operators, or assessors, as well as assessor blinding and safety of patients, any advantages, or hazards. No adverse effect was anticipated or observed. The main investigator and co-supervisors audited the trial to ensure the quality of the research methodology, sampling strategies, and interventions.

Statistical analysis:

Medcalc software, version 22 for Windows (MedCalc Software Ltd, Ostend, Belgium), was used to analyze the data. Continuous data was examined for normality using the Shapiro-Wilk and Kolmogrov-Smirnov tests. The mean, standard deviation, median, minimum, and maximum were used to characterize the stain score data, which did not exhibit a normal distribution. The mean and standard deviation were used to describe the normal distribution of enamel surface roughness data. The Mann Whitney test was used for non-parametric data and the independent t test was used for parametric

data, with a statistical significance level of $P < 0.05$, to compare the two dentifrice groups. The Wilcoxon test was utilized for non-parametric data and the paired t-test for parametric data, with a statistical significance threshold of $P<0.05$, to perform comparisons within each dentifrice group between follow-up periods. All tests were two-tailed, and the confidence limit was set at 95% with 80% power.

RESULTS

1. Demographic data

In the current study's clinical trial, 32 patients with extrinsic stains on their anterior teeth were randomized to receive either the intervention or the comparator arm (n = 16). At the conclusion of the current experiment, 32 individuals had 100% retention rate for the follow-up, and there was no statistically significant difference in gender between the two groups (P = 1.0000). With a mean age of 31.28±5.35 years, the participants in the current trial ranged in age from 23 to 40. The intervention group's age range was 22 to 40 with a mean age of 32.12±6.37 years, while the comparator group's age range was 23 to 37 with a mean age of 30.43±4.14 years. Table 1 shows that there was no statistically significant difference in age between the two groups (P = 0.382).

Table (1): Descriptive statistics for the total stain score MLSI (A*I) of groups per tooth site at baseline and after 4 weeks.

MLSI	Toothpaste	Charcoal				Calcium carbonate/ Perlite				P value
	Time	Median	Min-Max	Mean	SD	Median	Min-Max	Mean	SD	
Gingival	Baseline	5	0.00 to 12.00	5.81	3.43	6	0.00 to 20.00	8.19	6.77	P = 0.5413
	4 weeks	5	0.00 to 12.00	5.25	3.71	5.5	0.00 to 17.00	6.13	5.38	P = 0.8488
	P value	P = 0.0339*				P = 0.0048*				
Mesial	Baseline	7	0.00 to 18.00	8.25	6.05	8	0.00 to 18.00	7.19	5.67	P = 0.6346
	4 weeks	6	0.00 to 16.00	7.56	5.85	3	0.00 to 16.00	5.31	5.8	P = 0.1949
	P value	P = 0.0196*				P = 0.0040*				
Distal	Baseline	5.5	0.00 to 13.00	6.13	3.83	2	0.00 to 18.00	5.44	6.26	P = 0.3226
	4 weeks	4	0.00 to 12.00	5.75	3.7	2	0.00 to 16.00	4.88	5.51	P = 0.2689
	P value	P = 0.0633				P = 0.0952				
Body of facial	Baseline	8.5	0.00 to 20.00	7.69	6.94	8.5	0.00 to 34.00	8.5	8.26	P = 0.8489
	4 weeks	6.5	0.00 to 20.00	7	6.55	5.5	0.00 to 24.00	6.69	6.28	P = 0.8790
	P value	P = 0.0422*				P = 0.0114*				
Total	Baseline	27.5	16.00 to 40.00	27.88	8.66	31	16.00 to 40.00	29.19	8.22	P = 0.7483
	4 weeks	25.5	14.00 to 37.00	25.69	8.22	23	10.00 to 33.00	22.69	7.82	P = 0.3073
	P value	P = 0.0008*				P = 0.0004*				

P-value >0.05 NS; *p-value ≤0.05 S **p-value <0.001 HS * denotes statistically significant difference.

2. Stain score:

MLSI (A×I):

According to the Mann Whitney U test (P>0.05), there was no statistically significant difference between activated charcoal and calcium carbonate/perlite dentifrices at baseline and after four weeks of brushing, this was found for gingival, mesial, distal, body of facial and total MLSI (A×I) score while comparing MLSI using Wilcoxon test within activated charcoal-based group or calcium carbonate/perlite-based group has shown statistically significant difference between baseline and 4 weeks (P < 0.05) for all surfaces except distal surface (P > 0.05)

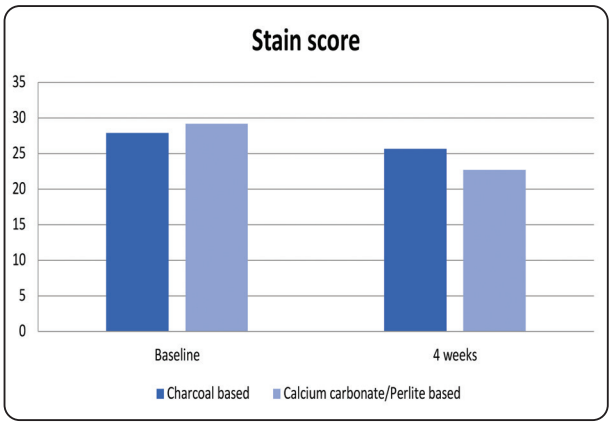

Fig. (3) Bar chart showing total stain score MLSI (A*I) between each group within each time point.

Fig. (4) Patient (1) (before) with extrinsic stain on anterior teeth before brushing with calcium carbonate/perlite toothpaste (comparator group) at baseline (A): frontal view , (B): left lateral view, (C): right lateral view, and after 4 weeks of brushing with calcium carbonate/perlite toothpaste (comparator group) (A): frontal view, (B): left lateral view, (C): right lateral view.

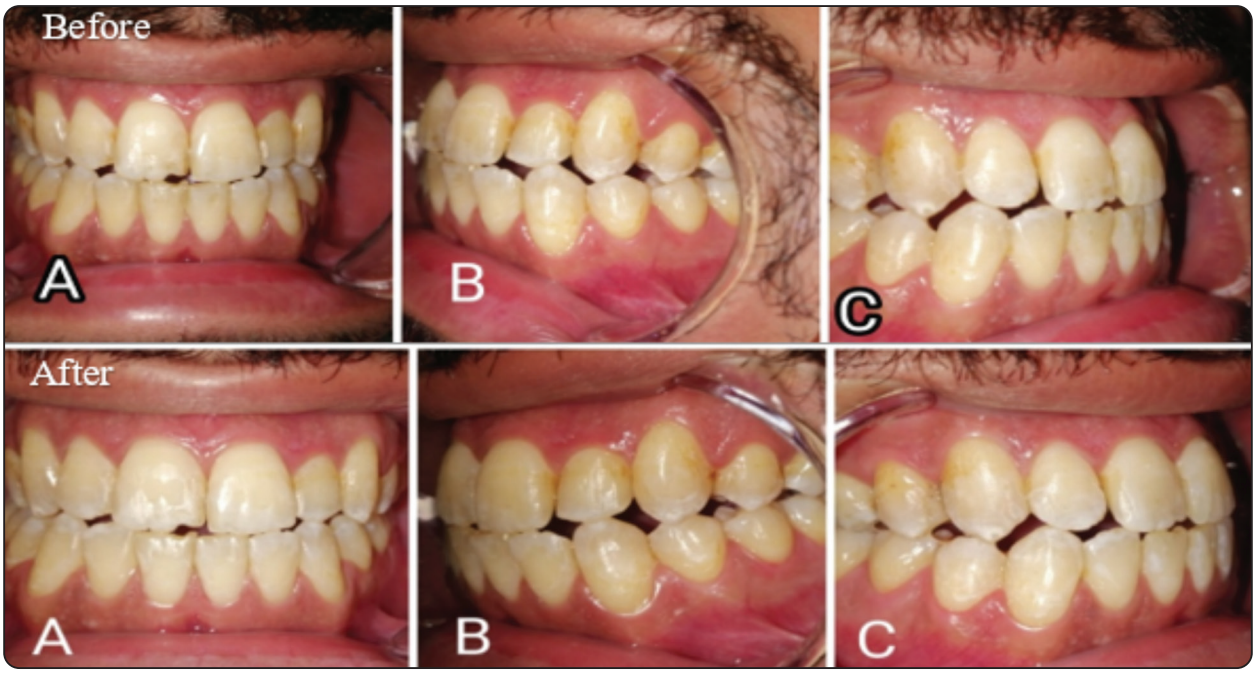

Fig. (5) Patient (2) (before) with extrinsic stain on anterior teeth before brushing with activated charcoal based toothaste (intervention group) at baseline (A): frontal view , (B): left lateral view, (C): right lateral view, and after 4 weeks of brushing with activated charcoal based toothpaste (intervention group) (A): frontal view, (B): left lateral view, (C): right lateral view.

2. Enamel abrasion:

As can be seen in Figure (6), there was no statistically significant difference in the enamel abrasion findings between the activated charcoal and calcium carbonate/perlite groups (p=0.4255). This indicates that at the start of the trial, there were no differences in the enamel surfaces between the two groups. However, an independent t-test revealed a statistically significant change after brushing (P<0.0001). In contrast to the calcium carbonate/perlite group, which did not significantly alter enamel abrasion following brushing, the activated charcoal-based group produced a statistically significant decrease in enamel abrasion (Ra values) post-brushing.

According to the paired t test P = 0.1641, which indicates that there was no statistically significant difference in enamel abrasion between baseline and post brushing, there was no difference in enamel abrasion for the activated charcoal-based group. There was no statistically significant difference in enamel abrasion between baseline and post-brushing for the calcium carbonate/perlite-based group (P = 0.8557).

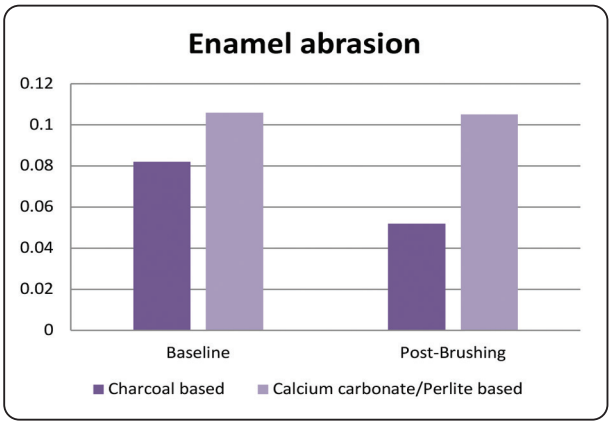

Fig. (6) Bar chart showing mean enamel abrasion between groups at baseline and post brushing

DISCUSSION

A more radiant smile is highly valued by patients and is a very fulfilling dental treatment that the dentist can provide. This has an advantageous psychological impact on patients and frequently helps them have a better sense of themselves and more self-esteem [14]. These ambitions encouraged dentists to try a wide range of whitening treatment options. Whitening toothpastes are considered a conservative option for the reduction or elimination of superficial discoloration with low cost and few risks

For the purpose of evaluating stains in this study, the modified Lobene stain index was employed. Using the MSLI, extrinsic dental stain was graded in each tooth area.

In this work, the abrasivity of toothpaste based on charcoal and toothpaste based on calcium carbonate/perlite was measured both quantitatively and qualitatively using AFM. The resulting mean roughness values (Ra) were used for statistical analysis. AFM is widely used to get quantitative (roughness value) and qualitative (3D photos) oral tissue evaluation at a very small scale concurrently; these details can provide important information about the abrasion process.

They show reduction in the overall stain score (MLSI) (A×I) (combined measure of maxillary and mandibular staining), the overall strength of tooth stain and the overall zone of enamel covered by stain for both groups after 4 weeks of brushing. Activated charcoal based paste reduced the overall stain score by 8% when the different tooth positions were looked at separately, activated charcoal based toothpaste removed 10% of gingival stain, 10% of mesial stain, 10% of distal stain and 10% of body of facial stain. This finding came in accordance with **Butera et al. (2023)** who claimed that extrinsic pigmentation was completely eliminated with the use of micro particle-activated charcoal toothpaste, which is why it is advised for patients with extrinsic pigmentations to practice proper home hygiene.[18] The impact of activated charcoal toothpaste on teeth treated with coloring additives was examined by **Borges et al. (2023)** the results of the study indicated that charcoal toothpastes, were effective in decreasing the color changes caused by staining solutions.[19] Moreover **Marchan et al. (2022)** assessed the charcoal-containing toothpaste's cleaning and whitening abilities. They discovered that the charcoal-containing toothpastes significantly reduced stains after six months.[20] Furthermore, **Aydin et al. (2022**) found that, after 28 days of usage, toothpaste with activated charcoal had the best whitening impact when compared to hydrogen peroxide, blue covarine, and conventional toothpaste. [21]

On the other hand, other researchers came in contradiction of the results of this clinical trial **Ribeiro et al. (2024)** conducted a trial to assess the whitening effectiveness of products containing activated charcoal.[22] The trial concluded that activated charcoal toothpastes products have no significant whitening effect in comparison to CP whitening products. As well as a recent systematic review conducted by **Tomás et al. (2023**) found that toothpastes containing activated charcoal are less safe because of their high risk for abrasion and have a lesser whitening impact than other products.[23] Furthermore **ElAziz et al. (2022)** investigated the stain-removal efficacy of activated charcoal toothpaste on stained bovine incisors and came to the conclusion that it does not provide complete stain removal or whitening effect.[24] **Franco et al. (2020)** examined the effectiveness of dentifrices containing activated charcoal in a laboratory's randomized study, and concluded that the charcoal-based powder had no bleaching impact.[25] Moreover, **Palandi et al. (2020)** investigated the effects of carbamide peroxide versus activated charcoal powder in combination with regular toothpaste or whitening toothpaste on the color of the enamel.[26] They discovered that using products containing

activated charcoal as a natural whitener prior to brushing with toothpaste does not change the color of teeth; additionally whitening with CP was more effective over the same length of treatment, making it a better option for teeth whitening.

The results of our trial can be explained that the activated charcoal works by removing stains from the teeth by means of mechanical or abrasive activity and the capacity to absorb pigments, chromophores, and stains.[27] The process of activation eliminates impurities and produces tiny, fine particles that increase internal pours and provide a large surface area, leading to a dentition that is effectively and gradually cleaned. The majority of activated charcoal toothpaste manufacturers market their products as teeth-whitening pastes, despite the fact that the substance's potential to whiten was not totally verified. [6]

A paste made of calcium carbonate and perlite decreased the stain score overall by 23 percent. When the various tooth sites were examined independently, toothpaste containing calcium carbonate and perlite eliminated 25% of the stain from the gingiva, 26% from the mesial, 11% from the distal, and 12% from the body of the face stain. The outcome aligned with the findings of **Matheson et al. (2004)**, who evaluated the efficacy of a natural calcium carbonate toothpaste that included microgranules and Perlite in removing extrinsic teeth stains. Their findings indicated that the whitening toothpaste was significantly effective in eliminating stains.[28] A study by **Collins et al. (2005)** showed that, in the comparatively short period of two weeks, the calcium carbonate/perlite abrasive combination effectively decreases discoloration.[11] In an additional study, **Chandu et al. (2009)** evaluated the effectiveness of whitening toothpaste with perlite as an abrasive for two weeks in order to remove stains and prevent new ones.[29] They discovered that the toothpaste with perlite showed a notable decrease in extrinsic stain over that time**.** More recently, **Patil et al. (2015**) investigated the efficacy of whitening toothpaste that used an abrasive activity (perlite/calcium carbonate) with toothpaste that used an enzymatic action (papain and bromelain) to remove extrinsic stains. Based on Macpherson's modification of the Lobene Stain Index, the study found that extrinsic stains may be effectively treated with both kinds of toothpaste.[30]

Thus, it is conceivable that the reason for the change in the color with calcium carbonate/perlite toothpaste could originate from the various abrasive agents' ability to gradually eliminate external stains. The number of abrasives in dentifrices has been linked to the whitening effect [31] and additionally their type. The amount of abrasive is closely related to dentifrice abrasiveness as stated by **Lima et al. (2008)**.[32]

It was observed that the MLSI within activated charcoal-based group and in calcium carbonate/ perlite-based group has shown statistically significant difference between baseline and after 4 weeks of brushing for all surfaces except distal surface. After four weeks, there was no statistically significant difference in the overall distal MLSI for either the charcoal-based paste or the calcium carbonate/perlite-based paste. This is noteworthy because the distal surfaces are thought to be hard to reach areas and are notoriously difficult to clean **Milleman et al. (2018)**.[33]

One of the main causes of dental problems in the general population is reportedly dental abrasion from toothpaste abrasives. This means that measuring and standardizing toothpaste abrasives is crucial for preventing tooth abrasion. This study's in-vitro component aims to evaluate the abrasiveness of toothpastes with a base of activated charcoal and toothpastes containing calcium carbonate/perlite on enamel surfaces. Toothpaste abrasive products are known to cause some damage on dental tissue. The method of surface roughness can be used to evaluate how various erosive or

abrasive treatments have changed the enamel or dentine's surface texture. [34-35] The relative enamel abrasivity (REA) and/or relative dentin abrasivity (RDA) is a recognized test for assessing the abrasiveness of desensitizing paste, approved by the International Organization for Standardization (ISO 11609). [15] However, the process involves the use of a radiation-based technology for which regulatory authorization is needed before using isotope. 16 a result, ISO 11609 recommended using a different method, such as profilometry analysis, to determine the abrasivity of toothpastes. Similar to profilometry, the atomic force microscope (AFM) is another helpful tool that is becoming more and more popular in dental hard tissue research. 17 The roughness measurement data is represented in Ra values, which stand for roughness average – μm. The average measurements made in the samples prior to and following the treatment are the baseline and final roughness, respectively. By deducting the baseline measurements from the post-treatment values, the roughness is determined. [36]

Surface roughness has critical relevance when viewed from a clinical standpoint. Because surface roughness greatly affects bacterial adherence, it may lead to an increase in biofilm development or build-up, which might permanently harm the hard tissues and restoration surfaces of teeth. [37] Additionally, surface roughness can lead to dentin hypersensitivity, gingival recession, and the build-up of oral pigments, all of which can affect how restoration margins and enamel seem from an optical aspect. [38]

Based on the study results, the null hypothesis for the in-vitro part of the study was rejected as the study results revealed that activated charcoal toothpaste group had lower Ra values (0.052 μm) after tooth brushing compared with calcium carbonate/ perlite groups (0.105 μm). Both toothpastes' Ra levels, however, were below the 0.2 μm criterion for enamel. The intraoral hard surface surface roughness threshold Ra is the value at which in vivo supra-plaque development is greatly increased. [39] Ra of 0.2 μm or less is predicted to have no effect on bacterial retention. The first signs of plaque development on the uneven surface of enamel arise from the attachment and growth of microorganisms in that region. [40] Periodontal inflammation and cavities develop as a result of plaque formation. The data within the activated charcoal group indicates that Ra values did not differ statistically significantly between baseline (0.082 μm) and post-brushing (0.052 μm). This indicates that activated charcoal preserved the surface integrity of enamel and did not erode tooth enamel specimens.

This result was consistent with research conducted by **Jamwal et al. (2023)** on the impact of whitening toothpastes on the tooth surface roughness of humans. They came to the conclusion that the activated charcoal-containing whitening toothpaste greatly decreased the surface roughness. The outcome of this investigation aligns with our findings.[41] **Ghajari et al. (2021)** assessed the whitening and abrasiveness of different charcoal toothpastes on permanent teeth. Abrasion analysis showed no significant differences among the toothpaste brands. They concluded that all toothpastes demonstrated both abrasive and whitening effects with comparable outcomes among the tested brands.[42] **Franco et al. (2020**) found no discernible variation in surface roughness following 14 days between bovine enamel surfaces manually brushed with ordinary toothpaste (1450ppm F) or tooth powder containing activated charcoal.[25] **Palandi et al. (2020**) noticed modest changes to the enamel surfaces following brushing with ordinary toothpaste and powdered activated charcoal.[43] **Vural et al. (2021)** examined the impact of several whitening toothpastes containing charcoal on surface roughness of enamel they found that the surface roughness of all the groups, with the exception of one brand paste, increased after using whitening toothpastes containing charcoal.[44] The researchers

deduced that the reason for this variation in one of the toothpastes' abrasive contents could be that it contains a low amount of abrasive material.

On the other hand, the findings of this study came in contrast with **Pertiwi et al. (2017)** who found that using whitening toothpaste containing charcoal for one or three months increased the roughness of the surface, this comes into contrast with our findings.[45] This was believed to be caused by the abrasive material that toothpaste contains, which includes hydrated silica and silica in addition to silica. Additionally, it was anticipated that after brushing with toothpaste containing charcoal, greater particle sizes would raise the value of Ra on the enamel surface.

The reason why there was a decrease in Ra values for charcoal-based toothpaste group may be originated from its abrasive content as this toothpaste has "low abrasive" ingredient. Another explanation might be that the other research' results differed due to distinct implementation protocols. In the current study, the samples were teeth and were brushed by a powered toothbrush held in custom simulating equipment. Furthermore, a number of studies have demonstrated alterations in the morphology of enamel linked to whitening toothpastes. These studies have also indicated regions of depressions, with craters forming, reduced micro hardness, rugosity, and surface wear, as well as prism exposure in the most impacted region. [46] We assumed that all these processes provided smoothness on tooth surface with the help of other abrasives of toothpaste as well in the study conducted by **Alpan et al. (2020**) two of the six whitening toothpastes examined Colgate Optic White and Splat Special Blackwood reduced the roughness of enamel.[47] This is consistent with what we found. There hasn't been much research done on charcoal toothpastes, so it's difficult to compare the results of this study to those of other studies.

Results within the calcium carbonate/perlite group indicated that Ra values between baseline (0.106 μm) and post-brushing (0.105 μm) did not alter statistically significantly, indicating that calcium carbonate/perlite did not significantly erode enamel. This finding came into accordance with **Pickles et al. (2005**) who found that when whitening toothpaste with calcium carbonate was compared to similar commercially available products, it did not cause significant abrasion to enamel or dentine.[48] Other studies by **Camargo et al. (2001**) and **Moore et al. (2005**) had noticed that the abrasivity of toothpaste containing calcium carbonate was lower than that of toothpaste containing hydrated silica.[49-50] Furthermore, a study by **Tandon et al. (2023**) discovered that non-herbal tooth gel including abrasives such as calcium carbonate, perlite, calcium pyrophosphate, dicalcium phosphate dihydrate (DCPD), hydrated silica, and sodium bicarbonate generated the least degree of enamel abrasion as compared to herbal toothpaste. [51]

Longer-term randomized clinical trials are required to determine the best oral hygiene and teeth-whitening regimen. It would also be interesting to compare the toothpastes tested in this study to other toothpastes that are sold and have different chemical bases (such as silica, sodium phytate, sodium polyaspartate, and sodium pyrophosphate).

CONCLUSION

Under the limitation of the trial, it can be concluded that:

1. The tested activated charcoal-based dentifrice has a similar and clinically perceptible performance in reducing extrinsic stains after 4-weeks of brushing when compared to calcium carbonate/perlite-based dentifrice
2. Activated charcoal-based dentifrice-maintained enamel surface integrity post-brushing
3. Calcium carbonate/perlite-based dentifrice caused substantial degree of abrasion to the surface of enamel.

4. Although toothpastes with abrasives caused enamel abrasion, the amount of roughening was below the clinically significant threshold of 0.2 μm for enamel roughness.

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