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Original Article

Assessment of the Effectiveness of Low-Particle-Size Toothpastes in Reducing Extrinsic Pigmentations: A Randomized Controlled Clinical Study

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ABSTRACT

Home-based stain removal strategies primarily target extrinsic dental discolorations using commercially available abrasive toothpastes. This study aimed to compare the effectiveness of two toothpastes formulated with different stain-removing agents—micro-cleaning crystals and activated charcoal—by assessing clinical outcomes. Forty participants with extrinsic dental stains were recruited and randomly assigned to two groups: the Control group, using a micro-cleaning crystal toothpaste (Colgate Sensation White), and the Trial group, using a microparticle-activated charcoal toothpaste (Coswell Blanx Black). Clinical assessments, including the Lobene stain index for intensity and extent, plaque control record (PCR), and bleeding on probing (BoP), were performed at baseline (T0), 10 days (T1), 1 month (T2), and 3 months (T3). Both groups showed statistically significant reductions in extrinsic pigmentation ($p < 0.05$). While the Control group experienced decreases in stain intensity and extent, complete stain removal was achieved only in the Trial group using the activated charcoal toothpaste, although no significant difference was observed between the two groups ($p > 0.05$). No intergroup differences were noted for PCR, BoP, LSI intensity, or LSI extent at any time point. Both toothpastes are therefore suitable for home use in managing extrinsic dental stains.

Keywords: Activated charcoal, Oral hygiene, Dentifrices, Pigments

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Introduction

Discoloration of teeth is among the most frequently reported aesthetic concerns in dental patients. Changes in tooth shade can arise from intrinsic factors within the tooth or extrinsic factors from external sources, such as exposure to chemicals or dietary habits [1, 2]. Extrinsic staining, which alters the natural enamel color, is often associated with consumption of beverages like coffee, tea, infusions, or habits such as tobacco use [3-5]. These stains can appear in a wide range of hues, from light yellow-brown to deep dark brown or black [6-9]. Professional whitening treatments using hydrogen peroxide or carbamide peroxide are standard for intrinsic discoloration, while surface polishing is commonly employed to manage extrinsic stains [10]. Extrinsic stains are closely linked to dental biofilm formation [11] and can be removed during professional oral hygiene through mechanical debridement, polishing with pastes of varying abrasiveness (relative dentin abrasivity—RDA), or air-polishing devices using powders like glycine, bicarbonate, or calcium carbonate [12]. At-home approaches for stain reduction often involve whitening toothpastes containing different abrasive agents and stain-removing molecules [13, 14]. Research has evaluated various ingredients in these formulations, including surfactants, peroxides, enzymes, citrate, pyrophosphates, and

hexametaphosphate, with abrasives being the primary mechanism for stain removal [10, 12].

Activated charcoal has recently gained attention as a tooth-whitening agent. Charcoal toothpastes contain finely powdered, oxidized carbon, typically produced through "slow pyrolysis," which eliminates water and volatile components from natural carbon-rich sources like coconut husks, nutshells, or peat under oxygen-free conditions [10, 15-17]. This study specifically investigates the stain-removing potential of activated charcoal compared with micro-cleaning crystals.

The main objective of this trial was to determine how effectively a toothpaste containing activated charcoal microparticles reduces extrinsic dental stains compared with a toothpaste containing micro-cleaning crystals, a technology frequently used in home oral care products [18]. A secondary goal was to evaluate the impact of these toothpastes on plaque accumulation and gingival bleeding over time. The first null hypothesis was that there would be no difference between the two toothpastes in stain reduction, as measured by the Lobene stain index for intensity and extent (LSI-I and LSI-E). The second null hypothesis assumed no significant effect on plaque control record (PCR), and the third assumed no difference in bleeding on probing outcomes.

Materials and Methods

Study design

The trial was designed as a randomized, double-arm, parallel, active-controlled clinical study with equal allocation (1:1) between the groups. The study followed the ethical principles outlined in the Declaration of Helsinki, adhered to CONSORT guidelines, and received approval from the Unit Internal Review Board (protocol no. 2021-0519). The study protocol was registered at ClinicalTrials.gov (NCT04904978), and all participants signed informed consent forms. The trial was carried out from May 2021 to April 2022.

Participants

Participants were recruited from patients attending routine periodontal care at the Unit of Dental Hygiene, Section of Dentistry, Department of Clinical, Surgical, Diagnostic, and Pediatric Sciences, University of Pavia, Italy. Inclusion criteria were:

- Adults
- Presence of extrinsic dental stains

Exclusion criteria included:

- Minors
- Individuals with neurological or psychiatric conditions
- Pregnant women
- Patients undergoing chemotherapy

Interventions and outcomes

At baseline (T0), all participants underwent periodontal assessment using a UNC 15 probe (Hu-Friedy, Chicago, IL, USA), and the following indices were recorded for each tooth:

- Plaque Control Record (PCR) — Assessed according to O'Leary [19], this index evaluates patient oral hygiene compliance by identifying areas of biofilm accumulation. Each tooth was divided into four surfaces: mesial, distal, buccal (vestibular), and palatal/lingual. The presence or absence of plaque on each surface was recorded. The PCR was expressed as a percentage, calculated by dividing the number of plaque-positive surfaces by the total number of surfaces examined and multiplying by 100.
- Bleeding on Probing (BoP) — Measured following the method described by [20], BoP indicates the degree of gingival inflammation. Gentle probing was performed on the three buccal surfaces of each tooth (mesiobuccal, mid-buccal, and distobuccal). Sites were considered positive if bleeding occurred within 20 seconds after probing. The percentage of positive sites was calculated by dividing the number of bleeding sites by the total number of sites examined and multiplying by 100.
- Lobene Stain Index (LSI) — Used to quantify the extent (LSI–E) and intensity (LSI–I) of extrinsic stains on either the buccal or palatal/lingual surfaces of affected teeth [21]. The grading system for LSI allocation is summarized in **Table 1**.

Table 1. Modified Lobene Index

Grade	Intensity	Extension
0	No visible pigmentation	No pigmentation present
1	Light discoloration, yellow to light brown-grey	Pigmentation covering up to one-third of the surface
2	Moderate brown discoloration	Pigmentation present on one-third to two-thirds of the surface
3	Dark brown to very dark brown spot	Pigmentation affecting more than two-thirds of the surface

Participants were randomly allocated into two groups by an independent operator who did not take part in any subsequent clinical evaluations or data analysis:

- Control group: instructed to brush twice daily using Colgate® Sensation White (Colgate-Palmolive, New York, NY, USA) as part of their routine home oral care.
- Trial group: instructed to brush twice daily using Blanx Black® (Coswell S.p.A., Funo di Argelato, BO, Italy) at home.

The detailed formulations of the 2 toothpastes are summarized in **Table 2**.

Table 2. Compositions of the tested toothpastes.

Gel	Manufacturer	Composition
Blanx Black®	Coswell S.p.A., Funo di Argelato, BO, Italy	Limonene, aqua, sodium lauryl sulfate, glycerin, sorbitol, silica, cellulose gum, charcoal powder, sodium monofluorophosphate, hydrated silica, aroma, xylitol, cetraria islandica extract, papain, maltodextrin, benzyl alcohol, sodium saccharin, usnea barbata extract, sodium benzoate, phenoxyethanol.
Colgate Sensation White®	Colgate-Palmolive, New York, NY, USA	Aqua, aroma, hydrated silica, sodium fluoride, sorbitol, sodium saccharin, polyethyleneglycol-12, sodium bicarbonate, sodium lauryl sulfate, cellulose gum, limonene, CI 77891, xanthan gum, CI 74160.

Participants were re-assessed at 10 days (T1), 1 month (T2), and three months (T3) to monitor the effectiveness of their assigned toothpaste. The full clinical assessment protocol is detailed in **Table 3**.

Table 3. Protocol considered for the study.

Appointment	Procedures
	• Participants provided written informed consent to take part in the study
•	• Evaluation of periodontal clinical parameters
•	• Professional cleaning of supragingival and subgingival tooth surfaces
Baseline (T0)	• Supragingival and subgingival debridement using glycine powder • Patient education and reinforcement of oral hygiene practices for home care • Trial Group: instructed to use Colgate® Sensation White toothpaste (Colgate-Palmolive, New York, NY, USA) • Control Group: instructed to use Blanx® Black toothpaste (Coswell S.p.A., Funo di Argelato, BO, Italy)
After 10 days (T1) After 1 month (T2) After three months (T3)	• Re-evaluation of clinical parameters • Reinforcement of oral hygiene instructions and monitoring of the assigned home care regimen

Potential adverse effects were monitored, including enamel discoloration and the presence of mucosal ulcerations or inflammation. Operator calibration was ensured by having the same examiner reassess the same quantitative parameters after a 14-day interval.

Sample size

The sample size was calculated for two independent groups, assuming a type I error (α) of 0.05 and a statistical power (1–β) of 80%, with a continuous primary outcome. The primary endpoint selected was the Lobene stain index intensity, and the two-tailed sample size was determined using the following formula:

$$\text{Sample size} = \frac{z^2_{\left(1-\frac{\alpha}{2}\right)} p(1-p)}{d^2} \quad (1)$$

The standard normal deviate, $z_{\left(1-\frac{\alpha}{2}\right)}$, corresponding to a 5% type I error is 1.96. In the sample size formula, p represents the expected proportion in the population, expressed as a decimal based on prior studies, and d reflects the desired confidence level, also expressed as a decimal. According to the results reported by Yin *et al.* [22], an anticipated mean value of 1.10 was assumed. The expected difference between group means was set at 0.41 with a standard deviation of 0.46, which indicated that 20 participants per group were necessary to achieve adequate statistical power.

Randomization and blinding

Patients were assigned to study groups using block randomization, with the allocation sequence generated by a statistician for a total of 40 participants. Enrollment, professional dental procedures, and outcome collection were performed by an operator who was not involved in the randomization. Group assignments were concealed using sequentially numbered, opaque, sealed envelopes (SNOSE), and an assistant provided each participant with the appropriate toothpaste without revealing the product identity. During the home-care phase, both toothpastes were masked so that neither the participants nor the operators knew which product was used. The data analyst also remained blinded to all allocations and outcomes.

Statistical analysis

Statistical analyses were conducted using R software (version 3.1.3, R Foundation for Statistical Computing, Vienna, Austria). For each variable and group, descriptive statistics were calculated, including mean, standard deviation, median, minimum, maximum, and interquartile range. Normality of the data was tested using the Kolmogorov–Smirnov method (PCR: $p < 0.0285$; LSI-E: $p < 0.0013$; LSI-I: $p < 0.0012$; BoP: $p < 0.0423$). Since the data did not follow a normal distribution, Friedman's test for repeated measures was applied, followed by Dunn's post hoc test for pairwise comparisons. Intra-operator reliability was evaluated using the intraclass correlation coefficient (ICC) calculated with the K statistic based on repeated measurements performed two weeks apart. A p-value < 0.05 was considered statistically significant.

Results and Discussion

Figure 1 illustrates the CONSORT flow of the trial. Following screening, 40 participants who met the inclusion criteria were enrolled, and all completed the study through the 3-month follow-up (T3).

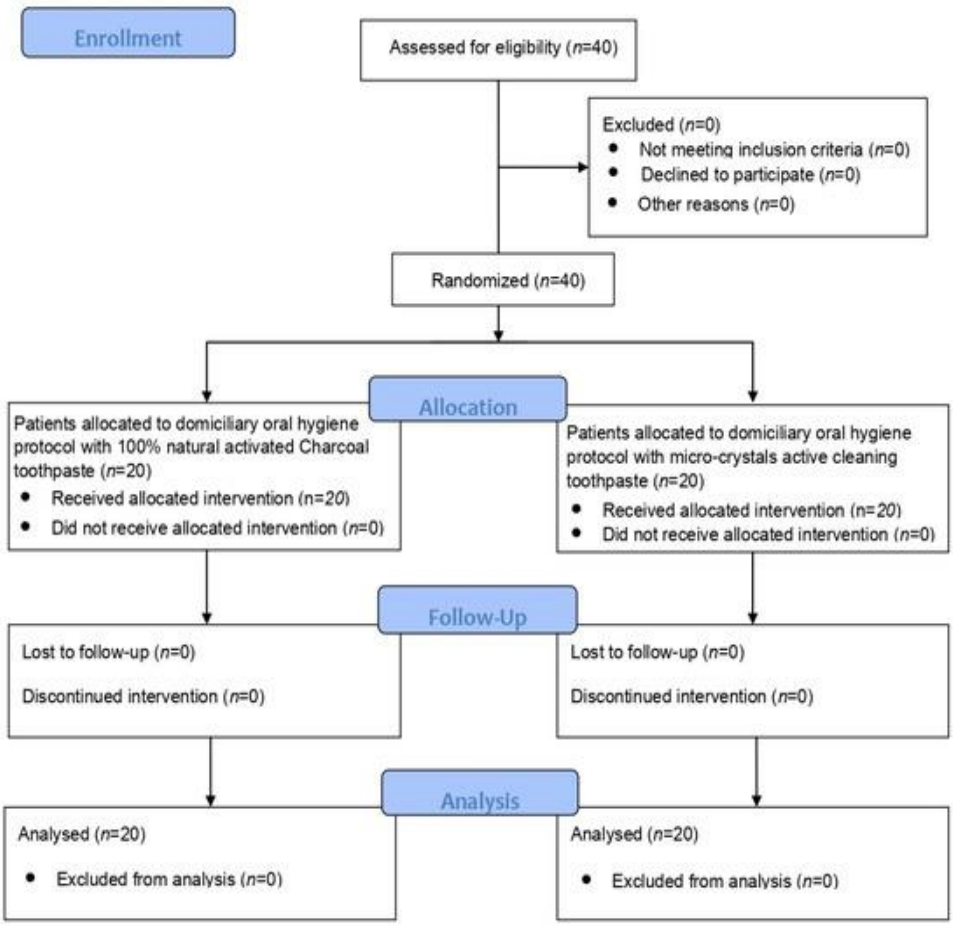

Figure 1. CONSORT flow chart.

At the beginning of the study, the average age of participants was 34.7 years (±10.6), comprising 17 men and 23 women. Each group included 20 participants, with the Trial group having a mean age of 31.7 ± 10.0 years and the Control group 37.7 ± 10.5 years. Detailed descriptive and inferential statistics for all measured

variables are provided below, with letter codes indicating significant differences both within and between groups. Reliability testing using the K statistic yielded an intraclass correlation coefficient (ICC) of 0.83, demonstrating strong consistency for repeated measurements by the same operator.

Plaque control record (PCR)

Table 4 displays the PCR outcomes. Both groups showed a decline in plaque scores from baseline (T0) through the final assessment at 3 months (T3). In the Control group, statistically significant reductions occurred between T0–T2 and T0–T3 ($p < 0.05$). In the Trial group, significant changes were detected between T0–T2, T0–T3, and T1–T3 ($p < 0.05$). No significant differences were observed when comparing the two groups at any individual time point ($p > 0.05$).

Table 4. Descriptive statistics of PCR, and significant intragroup and intergroup differences assessed by the Dunn's post hoc test. * The means with the same letters are not significantly different ($p > 0.05$).

Group	Time	Mean	St. Dev.	Min.	Max.	Median	Interquartile Range	Significance *
Control (crystals)	T0	78.75	25.07	25.00	100.00	80.00	20.00	A
	T1	63.50	31.71	15.00	100.00	65.00	70.00	A, B, C
	T2	49.75	24.14	10.00	80.00	50.00	42.50	B, C, D
	T3	44.50	27.76	10.00	80.00	50.00	51.25	B, C, D
Trial (charcoal)	T0	72.75	29.67	20.00	100.00	80.00	46.25	A, B
	T1	43.25	20.98	10.00	80.00	50.00	36.25	B, C
	T2	31.75	17.49	10.00	80.00	25.00	21.25	C, D
	T3	17.75	10.19	5.00	50.00	20.00	10.00	D

Bleeding on probing (BoP)

The findings for BoP are summarized in **Table 5**. Both the Control and Trial groups exhibited a downward trend in gingival bleeding from baseline (T0) to the 3-month follow-up (T3). In the Control group, a statistically significant reduction was observed specifically between T0 and T2 ($p < 0.05$). In the Trial group, notable decreases were seen between T0–T3 and T1–T3 ($p < 0.05$). Across all follow-up points, comparisons between the two groups revealed no significant differences ($p > 0.05$).

Table 5. Descriptive statistics of BoP, and significant intragroup and intergroup differences assessed by the Dunn's post hoc test. * The means with the same letters are not significantly different ($p > 0.05$).

Group	Time	Mean	St. Dev.	Min.	Max.	Median	Interquartile Range	Significance *
Control (crystals)	T0	13.50	9.33	0.00	30.00	10.00	10.00	A, C
	T1	8.50	7.96	0.00	20.00	5.00	16.25	A, B, D
	T2	5.00	5.13	0.00	15.00	5.00	10.00	B, D
	T3	6.75	3.73	0.00	10.00	7.50	5.00	A, D
Trial (charcoal)	T0	28.25	27.92	0.00	80.00	20.00	26.25	C
	T1	11.50	10.14	0.00	30.00	10.00	12.50	A, B, C
	T2	6.75	6.34	0.00	20.00	7.50	10.00	A, B, D
	T3	3.25	4.38	0.00	10.00	0.00	6.25	D

Lobene stain index – intensity (LSI-I)

The intensity scores for extrinsic stains, as measured by LSI-I, are summarized in **Table 6**. Both the Control and Trial groups demonstrated a reduction in stain intensity from baseline (T0) to the 3-month follow-up (T3). In the Control group, significant decreases were observed between T0–T3 and T1–T3 ($p < 0.05$). In the Trial group, statistically significant reductions were found across multiple intervals: T0–T2, T0–T3, T1–T3, and T2–T3 ($p < 0.05$). No significant differences were

identified between the two groups at any of the evaluated time points ($p > 0.05$).

Table 6. Descriptive statistics of LSI-I, and significant intragroup and intergroup differences assessed by the Dunn's post hoc test. * The means with the same letters are not significantly different ($p > 0.05$).

Group	Time	Mean	St. Dev.	Min.	Median	Max.	Significance *
Control (crystals)	T0	1.85	0.67	1.00	2.00	3.00	A, B
	T1	1.70	0.66	1.00	2.00	3.00	A, B
	T2	1.15	0.75	0.00	1.00	2.00	B, C, D, E
	T3	0.85	0.49	0.00	1.00	2.00	D, E
Trial (charcoal)	T0	2.25	0.64	1.00	2.00	3.00	A
	T1	1.85	0.81	0.00	2.00	3.00	A, B, C
	T2	1.30	0.66	0.00	1.00	3.00	B, C, D
	T3	0.35	0.49	0.00	0.00	1.00	E

Lobene stain index – extension (LSI-E)

Table 7 presents the LSI-E scores, reflecting the spread of extrinsic stains. Both the Control and Trial groups showed reductions in stain coverage from baseline (T0) to the 3-month follow-up (T3). In the Control group, significant within-group changes were observed between T0–T3 and T1–T3 ($p < 0.05$). The Trial group demonstrated statistically significant decreases across several intervals: T0–T2, T0–T3, T1–T3, and T2–T3 ($p < 0.05$). No significant differences were found between the two groups at any follow-up time points ($p > 0.05$).

Table 7. Descriptive statistics of LSI-E, and significant intragroup and intergroup differences assessed by the Dunn's post hoc test. * The means with the same letters are not significantly different ($p > 0.05$).

Group	Time	Mean	St. Dev.	Min.	Median	Max.	Significance *
Control (crystals)	T0	1.90	0.72	1.00	2.00	3.00	A, B
	T1	1.60	0.68	1.00	1.50	3.00	A, B
	T2	1.15	0.49	0.00	1.00	2.00	B, C, D
	T3	0.80	0.52	0.00	1.00	2.00	D, E
Trial (charcoal)	T0	2.05	0.60	1.00	2.00	3.00	A
	T1	1.60	0.68	1.00	1.50	3.00	A, B, C
	T2	1.25	0.44	1.00	1.00	2.00	B, C, D
	T3	0.25	0.44	0.00	0.00	1.00	E

Harms and adverse effects

No adverse events or harmful effects were observed throughout the study period.

Discussion

While professional in-office procedures, including polishing and air-polishing techniques, remain the gold standard for managing extrinsic dental stains [3], the use of whitening toothpastes at home can effectively support stain removal. Numerous commercially available products claim to reduce discoloration and whiten teeth [1].

Some of these toothpastes are also formulated with anti-plaque and anti-gingivitis agents, such as lactoferrin, rendering them multifunctional in combination with fluoride [23, 24]. Developing such formulations is challenging because interactions between ingredients must be carefully managed, and active compounds need to retain their efficacy throughout the product's shelf life [25]. Previous research has investigated the home-use effects of activated charcoal on extrinsic stains [10, 15].

Several studies have assessed the stain-removal capabilities of both crystal- [26, 27] and charcoal-based toothpastes [28, 29], reporting positive outcomes for each approach. The present study aimed to directly compare these two agents in the same clinical context.

The first null hypothesis was confirmed, as no statistically significant differences were observed between the two groups at any evaluated time point.

Both LSI-E and LSI-I scores decreased significantly within the Control group, indicating effective stain reduction. After three months (T3), the Trial group achieved slightly greater stain removal, although this difference was not statistically significant.

The secondary aim focused on the evaluation of PCR and BoP for the two toothpastes. The second and third null hypotheses were partially rejected, as no significant intergroup differences were observed. In the Trial group, PCR decreased by over 50%, which may be attributed to the microporous structure of charcoal particles (approximately 80% cavities), hypothesized to trap plaque and other debris [30]. Both groups exhibited reductions in BoP over time; however, in the Control group, the T0–T3 comparison did not reach statistical significance, whereas the Trial group demonstrated a clearer downward trend. Although BoP and PCR values at T3 were lower in the Trial group compared to the Control group, the differences did not achieve statistical significance.

Recent research on anti-stain toothpastes has expanded considerably, with numerous new ingredients tested, including sodium polyaspartate, silica, sodium phytate, and sodium pyrophosphate [31, 32]. The effectiveness varied depending on the specific component, but comparisons are limited due to methodological heterogeneity among studies.

Regarding crystal-based dentifrices, Putt *et al*. [33] evaluated a sodium bicarbonate, dual-phase toothpaste containing calcium and phosphate (Trial Dentifrice) against a commercially available hydrated silica toothpaste (Control Dentifrice) over six weeks of routine use. The trial toothpaste demonstrated superior efficacy in removing naturally acquired extrinsic stains and was significantly more effective than the control dentifrice.

Several studies have investigated the whitening potential of charcoal-based dentifrices. Dursun *et al*. [34] compared the effects of abrasives, polyphosphates, activated charcoal, and hydrogen peroxide, finding similar whitening outcomes among these agents; however, as their study was conducted in vitro, direct comparisons with the current clinical findings are not possible. Vertuan *et al*. [35] reported that charcoal-containing toothpastes combined with pyrophosphate may exert a strong abrasive effect on eroded enamel, suggesting that patients should exercise caution when using such products. Conversely, another in vitro study indicated that the abrasiveness of charcoal dentifrices falls within ISO-defined safety limits [36]. A recent systematic review of in vitro research concluded that while activated charcoal toothpastes exhibit a lower whitening capacity than alternative agents, their high abrasiveness raises safety concerns [37]; however, these findings cannot be directly applied to in vivo conditions. The scarcity of clinical trials on this topic underscores the relevance of the present study.

The results from the current trial indicate that both tested agents—activated charcoal and conventional micro-cleaning particles—are effective in reducing extrinsic dental stains at home, as well as in lowering plaque and bleeding indices, without significant differences between groups. These findings suggest that both types of toothpaste function effectively as oral hygiene products and external whitening agents. Further research could explore the combination of these molecules with other formulations to evaluate potential synergistic effects.

Limitations of this study include possible bias in patients' home-care practices, the relatively short three-month follow-up period for assessing long-term whitening, and the fact that operator calibration was performed only for quantitative measures, while qualitative variables could not be calibrated.

Future randomized clinical trials with extended follow-up periods and standardized home-care protocols are warranted. Comparing the tested toothpastes with other commercially available formulations containing different active compounds—such as sodium polyaspartate, silica, sodium phytate, or sodium pyrophosphate—would help determine the most effective domiciliary approach for both oral hygiene and tooth whitening. Additionally, future studies could investigate the combination of charcoal with probiotic-based products, including para-probiotics (heat-inactivated bacteria) [38], lysates (bacterial fragments) [39], and postbiotics (bacterial metabolites) [40], all of which have shown promising clinical results in dentistry, to further enhance treatment outcomes.

Conclusion

The findings of this study demonstrate that both toothpaste containing activated charcoal and toothpaste with conventional micro-cleaning particles are effective in significantly reducing extrinsic dental stains when used as part of a home-care routine. Both products also contributed to reductions in plaque and bleeding indices, with no significant differences observed between the two groups.

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Ethics Statement: None

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