lek. dent. Konrad Michałowski

Ocena kliniczna i mikrobiologiczna wpływu tioglikozydów ekstrahowanych z gorczycy białej na stan higieny jamy ustnej.

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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Pragnę złożyć najserdeczniejsze podziękowania mojemu Promotorowi

Pani dr hab. n. med. Anieli Brodzikowskiej

za życzliwe wsparcie, cierpliwe prowadzenie, inspirujące wskazówki,
a co najważniejsze, ciągłą motywację, bez której ta praca nie mogłaby powstać.

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Spis treści:

Wykaz stosowanych skrótów

Skrót	Nazwa angielska	Nazwa polska
AITC	*Allyl isothiocyanate*	Izotiocyjanian allilu
API	*Approximal Plaque Index (Lange)*	Aproksymalny wskaźnik płytki (wg Lange)
BOP	*Bleeding on Probing index (Ainamo & Bay)*	Wskaźnik krwawienia przy zgłębnikowaniu (wg Ainamo i Bay)
COX-2	*Cyclooxygenase-2*	Cyklooksygenaza-2
CPI	*Community Periodontal Index (WHO)*	Wspólnotowy wskaźnik periodontologiczny (wg WHO)
DMFT	*Decayed Missing Filled Tooth Index*	Wskaźnik PUWZ (próchnica, usunięte, wypełnione)
EFSA	*European Food Safety Authority*	Europejski Urząd ds. Bezpieczeństwa Żywności
IL-6	*Interleukin 6*	Interleukina 6
MIC	*Minimum Inhibitory Concentration*	Minimalne stężenie hamujące
PI	*Plaque Index (Silness & Löe)*	Wskaźnik płytki nazębnej (wg Silnessa i Löe)
ROS	*Reactive oxygen species*	Wolne rodniki tlenowe
SD	*Standard deviation*	Odchylenie standardowe
TNF-α	*Tumour necrosis factor alpha*	Czynnik martwicy nowotworu α
WHO	*World Health Organization*	Światowa Organizacja Zdrowia

Streszczenie

Współczesna stomatologia stale poszukuje rozwiązań opartych na substancjach naturalnego pochodzenia, które mogą stanowić alternatywę lub uzupełnienie profilaktyki i leczenia chorób jamy ustnej. Rośliny rodziny kapustowatych *Brassicaceae* są źródłem bioaktywnych składników takich jak tioglikozydy. Najliczniej występującymi tioglikozydami są sinalbina obecna w gorczycy białej *Sinapis alba* oraz sinigrina, wystepująca w gorczycy czarnej *Brassica nigra* i gorczycy brązowej *Brassica juncea* [1,2]. Produktem ich metabolicznej przemiany, zachodzącej w nasionach przy udziale enzymu myrozyny, jest izotiocyjanian allilu (AITC)[3]. Jest to związek wykazujący działanie przeciwbakteryjne, przeciwzapalne i przeciwgrzybicze.

Dotychczasowe badania dotyczące zastosowania glukozynolanów, a zwłaszcza izotiocyjanianów (AITC), wykonane były głównie na modelach zwierzęcych i w fazie przedklinicznej. Ich potencjał terapeutyczny w stomatologii nie został do tej pory zbadany. Celem niniejszej pracy jest ocena kliniczna możliwości oraz skuteczności zastosowania tioglikozydów jako składników wspomagających w produktach do domowej higieny jamy ustnej.

Poniższa dysertacja składa się z dwóch oryginalnych prac, będących wynikiem randomizowanych badań klinicznych dotyczących klinicznej oceny zastosowania produktów do domowej higieny jamy ustnej wzbogaconych ekstraktem z gorczycy. Zaprezentowano kompleksowe podejście do oceny skuteczności wyciągów z gorczycy białej jako wspomagającej terapii leczenia zapalenia dziąseł, redukcji płytki nazębnej oraz ryzyka próchnicy zarówno na poziomie mikrobiologicznym, jak i klinicznym.

Pierwsza z publikacji *„Clinical Effect of Thioglycosides Extracted from White Mustard on Dental Plaque and Gingivitis: Randomized, Single-Blinded Clinical Trial.”* prezentuje wyniki rocznego badania klinicznego, w którym oceniano wpływ pasty do zębów zawierającej tioglikozydy wyizolowane z nasion gorczycy białej (odmiana „Bamberka”) na wartości wskaźników płytki nazębnej (PI, API) oraz zapalenia dziąseł (BoP i GI). U pacjentów stosujących wzbogaconą pastę odnotowano istotną redukcję wszystkich parametrów periodontologicznych w porównaniu do grupy kontrolnej. Efekty te były najbardziej widoczne po pierwszych 6 miesiącach stosowania i utrzymały się do końca badania. Wyniki te

potwierdzają skuteczność miejscowego stosowania tioglikozydów jako składnika wspomagającego leczenie zapaleń dziąseł.

Druga publikacja *„The Clinical and Antibacterial Effects of a Herbal Toothpaste Containing White Mustard Sinapis alba Extract: A Randomized Clinical Trial."* przedstawia wyniki randomizowanego, podwójnie zaślepionego badania klinicznego, w którym, oprócz obserwacji klinicznej, dodatkowo porównano działanie przeciwbakteryjne eksperymentalnej pasty.
Po 4 tygodniach stosowania zaobserwowano statystycznie istotną redukcję ilości kolonii Streptococcus *mutans* i *Lactobacillus spp.* w ślinie badanej populacji pacjentów, a także obniżenie wartości wskaźników PI i BoP. Wyniki te wskazują, że bioaktywne związki gorczycy mogą efektywnie redukować miano bakterii kariogennych w jamie ustnej i zmniejszać stan zapalny dziąseł, co sugeruje możliwość ich zastosowania w profilaktyce próchnicy i chorób przyzębia.

Przedstawiona praca wypełnia istniejącą lukę badawczą w zakresie stomatologicznego zastosowania gorczycy białej. Jest to pierwsze badanie *in vivo,* w którym implementuje się właściwości tioglikozydów w stomatologii. Wnioski płynące z zaprezentowanych badań mogą stanowić punkt wyjścia do dalszych badań, co przyczynić się może do opracowania nowych produktów do higieny jamy ustnej pochodzenia roślinnego.

Abstract

Title: Clinical and Microbiological Evaluation of the Implact of Thioglycosides Extracted from White Mustard on Oral Hygiene.

Contemporary dentistry explores natural substances as alternative or adjunctive agents for the prevention and treatment of oral diseases. Plants from *Brassicaceae* family are a particularly rich source of bioactive compounds, mostly thioglycosides. The most common thioglycosides are sinalbin in white mustard *(Sinapis alba)* and sinigrin in black mustard *(Brassica nigra)* and brown mustard *(Brassica juncea)*. Their enzymatic conversion by seed myrosinase produces allyl isothiocyanate (AITC), a molecule with pronounced antibacterial, anti-inflammatory and antifungal activities.

To date only in-vitro investigations on glucosinolates especially isothiocyanates was performed on animal models. Their therapeutic potential in dentistry has remained unexplored. The present dissertation aims to provide a clinical evaluation of the ability and efficacy of incorporating thioglycosides into homecare oral hygiene products.

The thesis consists of two original papers, randomised clinical trials that assessed toothpastes augmented with white-mustard extract. Together, they offer a comprehensive evaluation of the extract as an adjunct in gingivitis management, dental-plaque reduction and caries-risk mitigation at both microbiological and clinical levels.

The first publication, "Clinical Effect of Thioglycosides Extracted from White Mustard on Dental Plaque and Gingivitis: Randomized, Single-Blinded Clinical Trial", reports a 12-month study in which a toothpaste containing thioglycosides from the low-erucic cultivar 'Bamberka' was evaluated for its impact on plaque indices (PI, API) and gingival parameters (BoP, GI). Participants using the enriched formulation exhibited a significant reduction in all periodontal parameters versus controls, with the most pronounced benefits observed at six months and persisting throughout the study. These findings substantiate the value of topical thioglycosides as adjuncts in gingivitis therapy.

The second publication, "The Clinical and Antibacterial Effects of a Herbal Toothpaste Containing White Mustard Sinapis alba Extract: A Randomized Clinical Trial", describes

a double-blind, randomised clinical trial that extended the assessment to antibacterial outcomes. After four weeks, statistically significant reductions in salivary *Streptococcus mutans* and *Lactobacillus spp.* counts were recorded in the test group, accompanied by improvements in PI and BoP. These results demonstrate that mustard-derived bioactives can effectively lower cariogenic bacterial loads and decrease gingival inflammation, suggesting their utility in caries and periodontal-disease prevention.

This dissertation addresses a previously unmet research need regarding the dental application of white-mustard thioglycosides. It represents the first in-vivo implementation of these compounds within a dental context. The conclusions drawn lay a foundation for future investigations and the development of plant-based oral-care products with validated clinical efficacy.

Wstęp

Gorczyca biała *(Sinapis spp.)* to jednoroczna roślina należąca do rodziny kapustowatych (*Brassicaceae*, dawniej *Cruciferae*). Uprawiana jest powszechnie w strefach klimatu umiarkowanego Europy, Azji i Ameryki Północnej. Zmiażdżone lub zmacerowane nasiona od wieków stosowane były w tradycyjnej medycynie jako składniki rozgrzewających okładów oraz środków wspomagających trawienie. Ze względu na szeroką dostępność, niski koszt oraz łatwość uprawy, gorczyca stanowi istotny surowiec w przemyśle spożywczym.

Istotnym ograniczeniem w stosowaniu gorczycy jest obecność w oleju z nasion kwasu erukowego. Jest to nienasycony kwas tłuszczowy obecny we wszystkich roślinach z rodziny kapustowatych. Wykazuje on działanie szkodliwe szczególnie dla układu sercowo-naczyniowego oraz toksyczność w dużych stężeniach [4]. EFSA zaleca ograniczenie stężenia kwasu erukowego w żywności do 2% [5,6]. Wprowadzenie odmian niskoerukowowych lub zeroerukowych, takich jak opracowana i uprawiana w Polsce odmiana „Bamberka" o <0.5 % zawartości kwasu erukowego zwiększyło potencjał stosowania gorczycy [7].

W świetle współczesnej wiedzy, nasiona gorczycy stanowią bogate źródło związków bioaktywnych. Profil chemiczny gorczycy obejmuje glukozynolany - tioglikozydy, flawonoidy, kwasy tłuszczowe, białka oraz olejki eteryczne. Hydroliza tioglikozydów przez endogenny enzym mirozynazę prowadzi do powstania izotiocyjanianów, między innymi izotiocyjanianu allilu (AITC). Badania *in vitro* wykazały jego właściwości przeciwbakteryjne oraz przeciwzapalne [8,9]. AITC wykazuje również działanie przeciwgrzybicze hamując wzrost *Candida albicans* w stężeniach MIC 0,125–0,5 mM. Badania *in vitro* dowodzą, że AITC jest odpowiedzialny za uszkodzenie integralności błony komórkowej, zmniejszenie adhezji do nabłonka i hamowanie tworzenia strzępek, ograniczając inwazję tkankową [10]. Działanie przeciwzapalne AITC polega na blokowaniu kluczowych enzymów takich jak cyklooksygenaza-2 (COX-2). W modelach zwierzęcych obserwowana była obniżona ekspresja cytokin prozapalnych TNF-α i IL-6 [11,12].

Obecność flawonoidów oraz tokoferoli stanowi o potencjale antyoksydacyjnym gorczycy. Składniki te neutralizują wolne rodniki tlenowe ROS i uczestniczą w ochronie struktur komórkowych przed uszkodzeniem oksydacyjnym [13]. Mają pozytywny wpływ na układ sercowo-naczyniowy i potencjalnie spowalniają rozwój chorób neurodegeneracyjnych [14].

Z tego względu nasiona gorczycy są cennym surowcem w produkcji olejów funkcjonalnych, suplementów diety, fitofarmaceutyków oraz leków.

Nasiona gorczycy zawierają albuminy 2S (Sin a 1 i Sin a 2), pełniące funkcję białek zapasowych. Zostały zidentyfikowane jako główne alergeny powodując reakcje IgE zależne [15]. Niemniej jednak reakcja alergiczna występuje stosunkowo rzadko, ze względu na zewnętrzny charakter aplikacji, który skutkuje mniejszą ekspozycją. Dostępna literatura opisuje głównie alergie pokarmowe [16].

Mimo wielu wymienionych powyżej właściwości biologicznych, rośliny z rodziny kapustowatych nie znalazły zastosowania w stomatologii. Warto nadmienić, że w tradycyjnej medycynie azjatyckiej od wieków do higieny jamy ustnej stosuje się patyczki z drzewa arakowego *Salvadora Persica (ang.Mustard Tree).* Mimo, iż drzewo arakowe pochodzi z innej rodziny roślin, jest bogate w AITC, co odpowiada za jego przeciwpróchnicowe właściwości.

Brak oceny klinicznej zastosowania pochodnych gorczycy w stomatologii stanowi lukę badawczą, na którą odpowiedzią jest poniższa rozprawa. Składa się ona z dwóch publikacji będących wynikiem przeprowadzonych randomizowanych badań klinicznych. W pierwszym oceniany jest wpływ tioglikozydów ekstrahowanych z gorczycy i ich przeciwzapalnych właściwości na zmianę wartości wskaźników zapalenia dziąseł oraz zalegania płytki bakteryjnej. W drugim dodatkowo badana jest właściwość przeciwbakteryjna poprzez badanie ocenę ilości kolonii bakterii próchnicowych w jamie ustnej oraz związanego z tym ryzyka próchnicy.

Założenia i cel pracy

Stosowanie preparatów roślinnych jako terapii alternatywnych lub uzupełniających stanowi obszar otwartej dyskusji [17]. W związku z obecnością dowodów naukowych w postaci badań *in vitro* dotyczących przeciwzapalnych i przeciwbakteryjnych właściwości glikozydów, zasadnym jest postawienie pytania o możliwości ich zastosowania w stomatologii.

Głównym celem pracy jest kliniczna ocena tioglikozydów obecnych w ekstrakcie z białej gorczycy *(Sinpsis alba)* – odmiana „Bamberka" w kontekście jej możliwości zastosowania w stomatologii.

Cel główny realizowany był w oparciu o cele szczegółowe:

- Ocena wpływu stosowania eksperymentalnej pasty na parametry płytki (API, PI) oraz parametry zapalenia dziąseł (GI i BoP).

- Ocena skuteczności działania przeciwbakteryjnego, w kontekście zmniejszenia miana bakterii próchnicowych *Streptococcus mutans* i *Lactobacillus spp.* w ślinie.

- Ocena dynamiki i trwałości odpowiedzi terapeutycznej na składniki gorczycy w stosowanej paście.

- Analiza sensoryczna dotycząca smaku, zapachu i ewentualnych problemów związanych z codziennym stosowaniem gorczycy w eksperymentalnej paście.

Publikacja # 1

International Journal of
Molecular Sciences

MDPI

Article

Clinical Effect of Thioglycosides Extracted from White Mustard on Dental Plaque and Gingivitis: Randomized, Single-Blinded Clinical Trial

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Abstract: The antibacterial and anti-inflammatory effect of thioglycosides has already been established. This study investigates the effects of thioglycosides extracted from white mustard, specifically the "Bamberka" variety, in the context of oral hygiene. The aim of the study is to clarify an evidence-based link between the documented antibacterial and anti-inflammatory effects attributed to thioglycosides and their practical application in oral care. A randomized, single-blinded (patient-blinded) clinical study was performed on 66 patients using mustard-based toothpaste for oral hygiene. The patients were examined at baseline and after 6 and 12 months. The values of the Approximal Plaque Index (API), the Plaque Index (PI), and Bleeding on probing (BOP) were taken into consideration. The results show a significant reduction in plaque accumulation, especially after 6 months of using mustard-based toothpaste in all examined parameters. This suggests that thioglycosides from mustard contribute to a considerable decrease in dental plaque accumulation, confirming their potential in natural oral care solutions, which is indicated in the main conclusions or interpretations.

Keywords: thioglycosides; white mustard; dental plaque; gingivitis; plant-based products; oral hygiene; toothpaste; dentistry

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1. Introduction

Dental plaque is considered the main etiologic agent in the indication of caries, gingivitis, and its progress to periodontitis. Gingivitis is an inflammatory condition of the gingiva characterized by edema, redness, and bleeding upon probing [1]. Mechanical removal of dental plaque is generally acknowledged as an effective measure for controlling the progression of dental caries and periodontal diseases [2].

Dental plaque biofilm with the composition of specific bacteria species enhances the inflammation process, which is responsible for the development of periodontal diseases. The relationship between the presence of dental plaque and periodontitis was a main figure of periodontal consensuses leading to the new Classification of Periodontal and Peri-Implant Diseases and Conditions (2017) [3].

Periodontitis, a chronic inflammation of tooth-supporting structures, is a multifactorial disease. Severe periodontitis affects 10–15% of the population, and it is the main reason for tooth loss and the sixth most prevalent condition worldwide [4]. Periodontitis is the result of nontreated gingival inflammation connected to bacterial plaque accumulation. Favorable factors causing the disease are smoking [5], immunosuppression [6], diabetes [7], and genetic polymorphism of genes related to the production of inflammatory cytokines and the alteration of leucocytes [6].

The definition of healthy gingiva has also been reconsidered. It now focuses on the absence of visual signs of inflammation and bleeding [8]. The therapeutic approach is mainly focused on removing biofilm, which consists of bacteria, which is mostly covered by home oral care [6,7]. The gold standard in non-surgical periodontal treatment is scaling

and root planning (SRP) [9]. It is mainly focused on the manual removal of biofilm and calculous and smoothening of the root surfaces.

To improve outcomes and avoid bacteria recolonization, ancillary therapies like probiotics [10], chlorohexidine, photodynamic treatment [11], and ozone application [12] have been considered. Many plant additions have also been recently evaluated. In the dynamic growth of plant-based oral care products, a diverse array of natural ingredients has played a prominent role [3]. Among these, plants like Neem, Miswak, Aloe Vera, Rheum palmatum, and Rhamnus frangula stand out for their unique salutogenic properties [10].

The expanding realm of plant-based tooth products shows a rich variety of botanical ingredients. Neem, esteemed for its potent antimicrobial properties derived from compounds like nimbin and azadirachtin, acts as a natural shield against oral bacteria [13–20]. Miswak, sourced from the Salvadora persica tree, has been traditionally revered for its natural dental hygiene benefits, attributed to its silica content and alkaloids [20–24]. Aloe Vera, known for its soothing and anti-inflammatory properties, finds application in oral care formulations to alleviate discomfort and promote healing. Rheum palmatum, a herb with roots in traditional medicine, has been associated with potential antioxidant and anti-inflammatory effects [25]. Rhamnus frangula, derived from the buckthorn plant, adds to the holistic approach of plant-based oral care with its potential as a herbal remedy [26–28].

The relationship between a decrease in gingivitis and plant-based resources like postbiotics and *Aloe Barbadensis* leaf juice is constantly being investigated [29]. Nevertheless, a knowledge gap in how to use thioglycosides in dentistry still exists.

Thioglycosides, as presented in Figure 1, are a type of chemical compound characterized by the presence of a sulfur atom within the glycosidic bond. Structurally, they are glycosides in which the oxygen atom in the glycosidic linkage is replaced by a sulfur atom. Glycosides, in general, are compounds in which a sugar molecule (glycone) is bound to a non-sugar molecule (aglycone) via a glycosidic bond. In the case of thioglycosides, the aglycone portion of the molecule is often a sulfur-containing compound. The sulfur atom introduces unique chemical and biological properties to thioglycosides, and these compounds are found in various plants across different families [30,31].

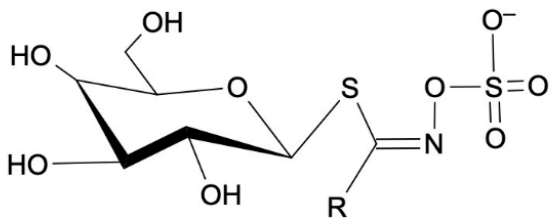

Figure 1. General molecular formula of thioglycosides. R—amino acid group.

Thioglycosides are a group of bioactive compounds renowned for their potential health benefits and are abundantly present in various plants that contribute to their overall health-promoting profile. They are present in various plant sources, mainly Cruciferae and Brassicale including Broccoli, Horseradish, Cauliflower, Brussels Sprout, and Mustard. Scientific investigations into thioglycosides have revealed compelling evidence of their antibacterial and anti-inflammatory effects [32]. These compounds might exhibit the potential to inhibit the growth of oral bacteria, including Streptococcus mutans, and mitigate inflammatory processes in the oral cavity, but there is no evidence in clinical studies. Understanding these effects is crucial as we explore the application of thioglycosides in natural oral care solutions [33,34].

One well-known example of a thioglycoside is allyl isothiocyanate, which is produced by the breakdown of substances and is commonly found in plants like mustard. Allyl isothiocyanate is responsible for the pungent flavor in mustard and exhibits antimicrobial

properties, contributing to its traditional use as a preservative [35,36]. The chemical process of transition of thioglycoside into allyl isothiocyanate is described in Figure 2.

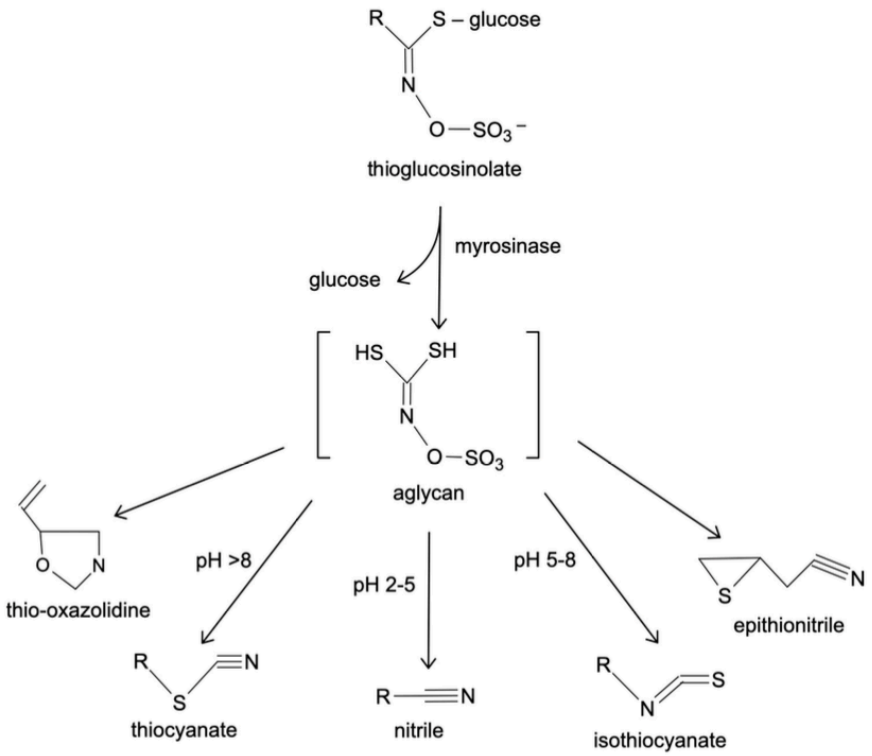

Figure 2. Thioglycosides' transitions.

These bioactive compounds, present in the mustard plant, have sparked scientific interest due to their potential health benefits. It has been known since prehistoric times that they possess a high level of bioactive ingredients. The most common species are white mustard (*Sinapsis alba* L.), black mustard (*Brassica nigra* L.), brown mustard (*Brassica juncea* L.), Ethiopian mustard (Brassica carinata A. Braun), rocket (*Brassica eruca* L.), and wild mustard (*Sinapsis arvensis* L.) [37]. White mustard, a common culinary ingredient and a plant readily available in our European climate, traditionally valued for its distinctive flavor, adds an intriguing dimension to our study [38–41].

In recent years, Piętka et al. presented a new variety of zero-erucic white mustard called "Bamberka" [42]. The major benefit of this new variety is the reduction in the level of erucic acid in mustard oil, which has negative health implications [43]. The European Food Safety Authority (EFSA) states that the tolerable daily intake of erucic acid is 7 mg/kg body weight [43]. The next most important factor is the high concentration of thioglycosides in the 'Bamberka' variety. For a long time, white mustard has been used to produce spices, including mustard, as well as in herbal medicine in the form of an extract from mustard seeds (whole or fragmented) [44]. Aqueous extracts contain slimes and thioglycosides. They are used orally as a protectivum. White mustard seeds contain thioglycosides, mainly sinalbin, as presented in Figure 3.

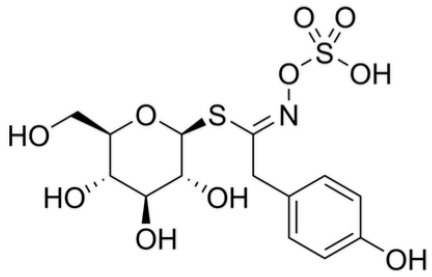

Figure 3. Sinalbin, the main thioglycoside of white mustard.

Seed fragmentation leads to myrosinase enzyme release from other parts of seeds. Myrosinase leads to a cyclic transition of thioglycosides to their derivatives, which are fat-soluble [45]. These compounds are responsible for mustard's pungent taste.

Previous studies have mainly focused on the derivatives of thioglycosides obtained from the mustard plant [46].

There is a knowledge gap in the use of mustard-based products in oral healthcare products. No correlation has been found in the literature. There has been no clinical study disclosing the effect of mustard on periodontal health. Our study is the first clinical trial to fill this gap in science.

The aim of this study was to evaluate the effect of thioglycosides extracted from white mustard "Bamberka" on dental plaque and gingival inflammation. This study aims to establish a concrete and evidence-based link between the documented antibacterial [47–49] and anti-inflammatory effects attributed to thioglycosides and their practical application in oral care. By focusing on patients using mustard-based toothpaste, we aim to elucidate how this natural component, rich in thioglycosides, may play an essential role in promoting oral health. This exploration may develop a bridge between traditional plant-based knowledge and modern oral care practices regarding mustard and its bioactive compounds using evidence-based procedures.

2. Results

Initially, 149 patients were screened, 16 were excluded, 133 were enrolled in the study, 66 patients were allocated to the intervention with toothpaste containing thioglucosides, and 67 patients were allocated to the control group—use of toothpaste without thioglucosides. Four patients were lost during follow-up. A flow chart of the study is shown as a CONSORT flow diagram in Figure 4.

The results for PI, API, and BoP divided into groups and study periods (T_0, T_1, and T_2) are charted and presented in Table 1. Plaque Index and API Index measurements are shown in Figures 5 and 6.

A significant change was observed in each examined group, but the most significant reduction in plaque accumulation was observed after 6 months, especially in the high-DMFT-PD(−) and high-DMFT-PD(+) study groups. The difference between 6 months (T_1) and 12 months (T_2) indicated a progression, but not as rapid. The results of intra and intergroup comparisons using ANOVA one-way analysis of variance with repeated measures showed that there was a significant difference between the variables, $F = 44.34$, $p < 0.05$. Descriptive statistics are presented in Table 1.

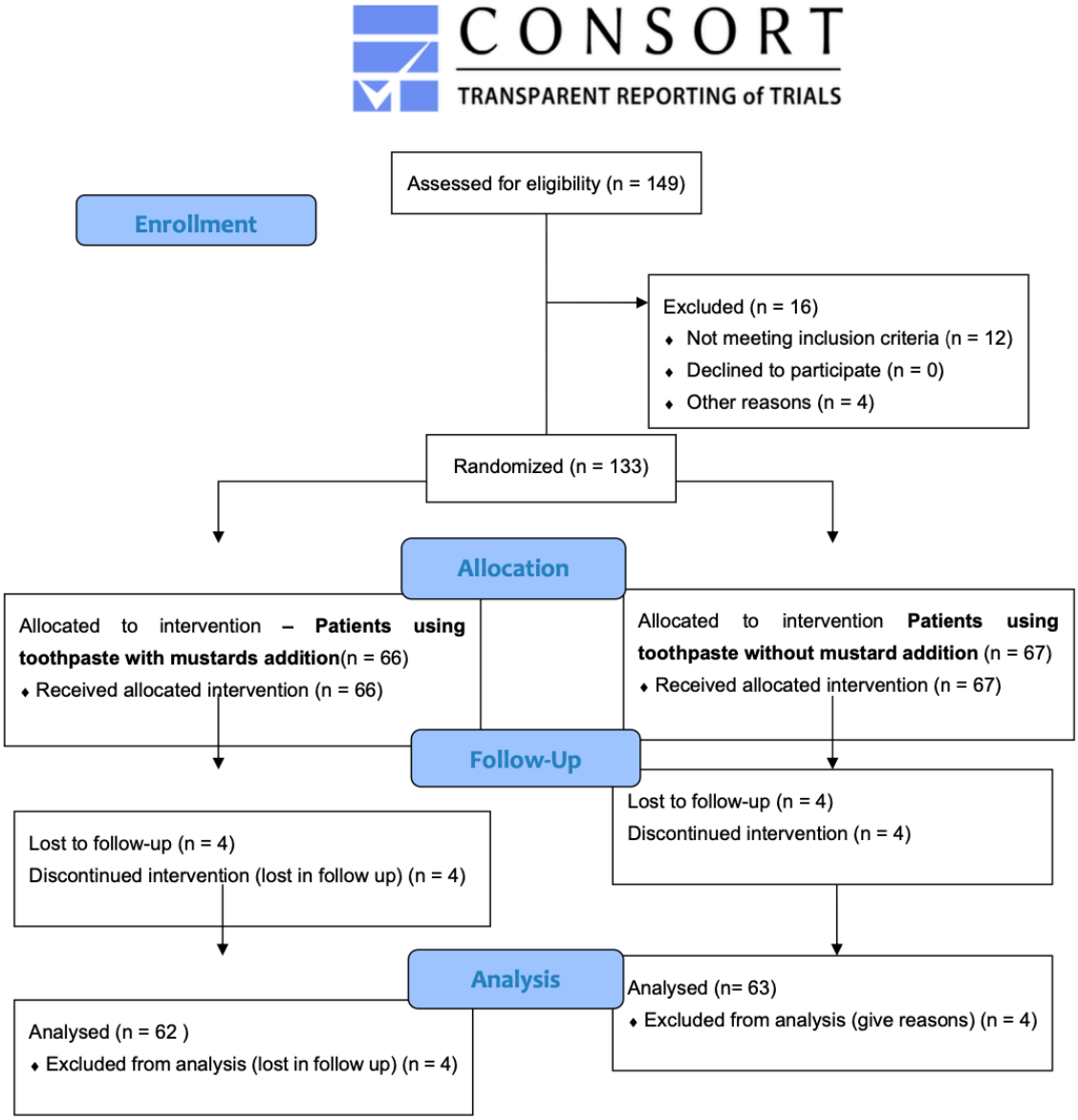

Figure 4. CONSORT diagram showing the study outline.

Table 1. Clinical parameters: PI, API, and BoP at baseline (T_0), 6 months (T_1), and 12 months (T_2).

Group	N	Time	API Mean (SD)	Significance *	PI Mean (SD)	Significance *	BOP Mean (SD)	Significance *
low DMFT-PD (−)	22	T_0	36.4 (19.5)	A	3.38 (1.13)	a	32.9 (10.2)	*A*
	22	T_1	19.8 (15.2)	B	1.83 (0.42)	b	25.0 (6.0)	*B*
	20	T_2	15.2 (13.6)	C	1.48 (0.33)	b	22.0 (5.0)	*C*
high DMFT-PD (−)	19	T_0	39.4 (20.6)	D	3.78 (0.72)	c	46.8 (8.4)	*D*
	19	T1	20.4 (16.6)	B	1.25 (0.52)	d	28.0 (12.0)	*E*
	19	T_2	16.9 (12.3)	C	0.76 (0.48)	e	20.0 (7.0)	*F*
high DMFT-PD (+)	25	T_0	60.2 (35.2)	E	4.35 (0.58)	f	60.1 (12.2)	*G*
	23	T_1	35.5 (19.5)	A	2.15 (0.76)	g	25.0 (6.2)	*B*
	23	T_2	28.5 (16.8)	G	1.42 (0.82)	b	22.0 (5.5)	*C*
Control group low DMFT-PD (−)	22	T_0	37.5 (22.6)	A	3.25 (1.24)	a	31.2 (10.1)	*A*
	20	T_1	31.3 (15.1)	G	2.52 (1.09)	h	28.0 (9.0)	*E*
	20	T_2	25.6 (12.8)	F	2.19 (0.89)	g	26.0 (10.1)	*B*
Control group high DMFT-PD (−)	20	T_0	38.5 (25.9)	D	3.82 (1.13)	c	45.0 (8.1)	*D*
	20	T_1	29.2 (12.1)	G	2.75 (1.15)	h	30.2 (15.2)	*E*
	19	T_2	23.3 (9.5)	F	2.45 (1.04)	h	28.3 (12.6)	*H*
Control group high DMFT-PD (+)	25	T_0	59.5 (19.6)	E	4.55 (0.98)	f	58.0 (15.4)	*G*
	25	T_1	40.2 (12.3)	H	2.84 (1.19)	h	39.2 (20.3)	*I*
	25	T_2	35.5 (20.2)	I	2.25 (1.32)	h	33.5 (18.2)	*J*

DMFT—Decayed, Missing, Filled Tooth index, PD—Periodontal Disease N—Number of patients, $T_{0,1,2}$—Time periods of study, API—Approximal Plaque Index, PI—Plaque Index, BOP—Bleeding on probing, SD—Standard Deviation, * significant intragroup and intergroup differences assessed using Dunn's post hoc test. * The means with the same letters (capital, small, or italics) are not significantly different ($p > 0.05$).

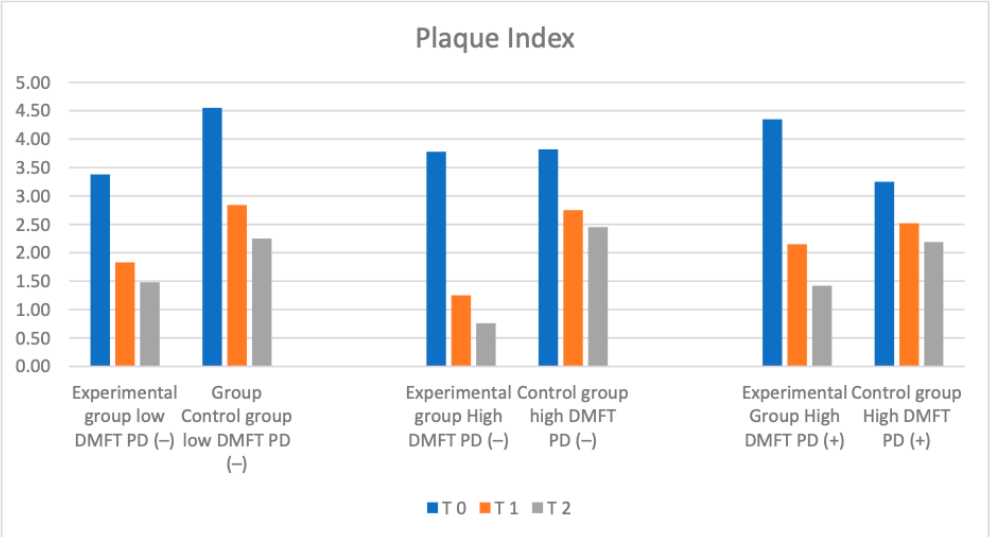

Figure 5. Illustration of changes in PI in each group during the study.

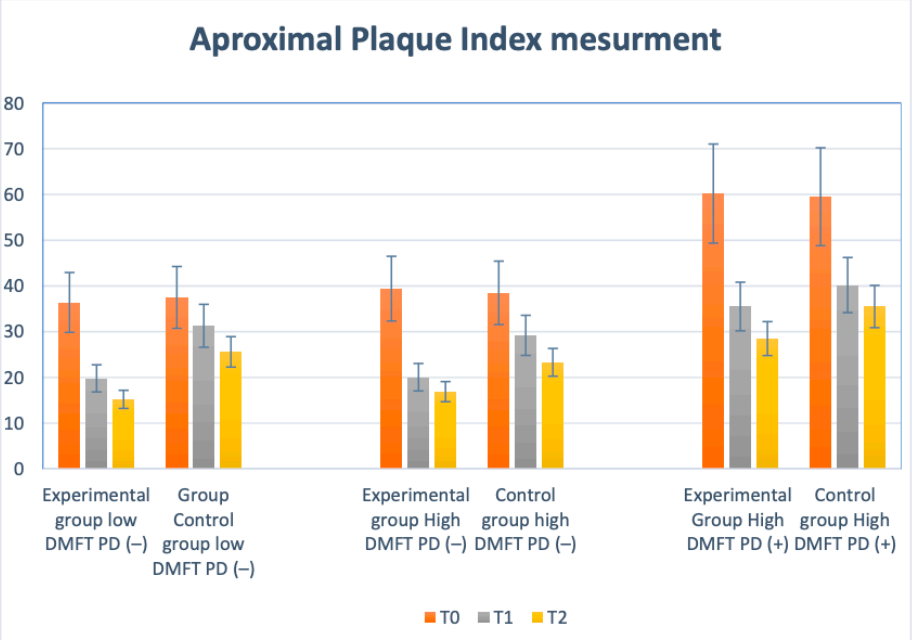

Figure 6. Illustration of changes in API in each group during the study.

2.1. Plaque Index (PI) Measurement Results

On the basis of clinical assessment, no significant differences were found between the groups. Mean PI values were lowest in the low-DMFT-PD(−) group and high-DMFT-PD(−) group, amounting to 3.38 in the low-DMFT-PD(−) group and 3.78 in the high-DMFT-PD(−) group, respectively. The highest mean PI level was observed in the high-DMFT-PD(+) group (PI = 4.35). The mean PI value in the control group in the initial examination was 4.05.

PI levels changed dynamically over 6 and 12 months in the test groups, presenting a downward trend. The observed changes in numerical values of the Plaque Index were statistically significant. The biggest decrease was observed in the high-DMFT-PD(−) and high-DMFT-PD(+) groups. In the final examination, in the high DMFT-PD(+) group, the mean PI value (1.42) reached levels similar to the low-DMFT-PD(−) group. The lowest PI level, amounting to 0.76, was observed in the high-DMFT-PD(−) group. The most dynamic changes were observed after the first six months of the trial. In all groups, a statistically significant decrease in the PI value was observed. Over the next six months, the dynamics of the shift decreased. It was the most prominent in the high-DMFT-PD(+) group. In the control groups, a stable decrease in mean PI values could be observed, compared to the experimental groups. In the final examination, the mean values of the PI index in the control groups were significantly lower than the initial values in these groups.

2.2. Approximal Plaque Index (API) Results

We observed the lowest API initially in the low-DMFT-PD(−) and high-DMFT-PD(−) groups, while the high-DMFT-PD(+) group showed the highest value. Dynamic decreases in mean API values (−16.6; −19.0; −24.7; −10.2) were observed in all groups after 6 months (T_1). Then, after 12 months (T_2), a reduction was still visible but much lower (−4.6; −3.5; −7.0; −4.7). In the high-DMFT-PD(−) and high-DMFT-PD(+) groups, the plaque reduction was the highest. Visualization of the above results is presented in Figure 6.

2.3. Bleeding on Probing Index (BoP) Results

The most distinctive values of the number of bleeding sites during the initial probing were observed in the low-DMFT-PD(−) and high-DMFT-PD(+) groups. In the low-DMFT-PD(−) group, the BoP value was lower than in the rest of the groups—with a mean BoP value of 32.90%. Such a low percentage of sites exhibiting bleeding on probing could result from the fact that patients in this group had low DMFT values and were not diagnosed with periodontal disease. The highest mean BoP level, amounting to 60%, was observed in the high-DMFT-PD(+) group. During the follow-up, after six and twelve months, a constant, dynamic decrease in BoP values was observed in all the tested groups, independently of the toothpaste used. The changes were statistically significant. The rate of this BoP mean change was the lowest in the control group. The most dynamic changes were observed in the high-DMFT-PD(−) and high-DMFT-PD(+) groups. The BoP mean values decreased in these groups very fast. After one year, the lowest value, BoP = 20%, was observed in the high-DMFT-PD(−) group. The analysis of these study results revealed that the most significant drop in the BoP mean value was observed in the first 6 months in all experimental groups, but not in the control groups. The decrease was also present over the next 6 months, but the dynamics of the changes were much lower. The changes in BOP parameters are visualized in Figure 7.

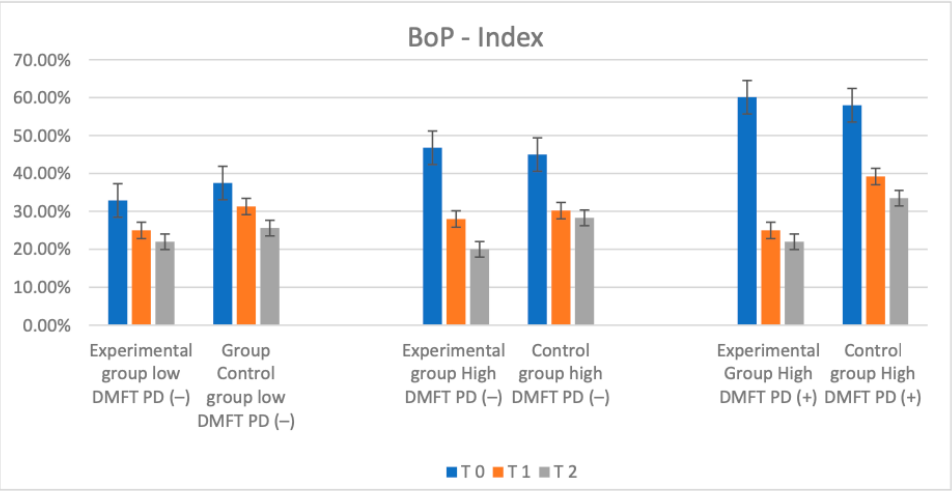

Figure 7. Illustration of changes in BOP index in each group during the study.

3. Discussion

Gingivitis caused by periopatogens present in dental plaque is a serious factor causing the loss of tooth-supporting structures. Non-sufficiently treated gingivitis may lead to loosening of the tooth and edentulism. The fact that SRP plays a major role in the treatment of periodontitis is not questionable. Adjuvant therapies that lead to preventing or slowing down bacteria biofilm recolonization should be taken into consideration [50]. The novel role of postbiotics was investigated as complementary to SRP treatment of gingivitis [51]. The advantages of this type of therapy prompted us to search for other natural ingredients that might be beneficial.

A literature search in databases like PubMed and EMBASE did not show any similar study. The novel concept used, concerning mustard-based compounds in oral hygiene, makes this work unique. This is why we cannot compare it to any other clinical study performed previously. Our randomized, single-blinded clinical trial provides an adequate evidence level to reduce the knowledge gap in this field.

The high concentration of thioglycosides in the "Bamberka" variant plays an essential role in our reasons for picking this plant for our study. The antibacterial and anti-inflammation properties of thioglycosides extracted from other plants, like *Salvadora persica* [20], were described in previous studies. Nevertheless, mustard as a source of an examined substrate had never been considered as a source of oral health products. Only one in vitro study by Echel et al. [52] showed that oral pathogens are susceptible to mustard oil. The novel variant "Bamberka" provides an opportunity to conduct this kind of research. The geographical dispersion of *Brassicaceae* species and mustard in central Europe directly leads to scientific exploration of their properties. There is a clear relationship between the interest and accessibility [53] of plant products and their use for daily care.

Most of the *Brassica* species contain allergens, but the vast majority are related to mustard. According to the Food and Agriculture Organization of The United Nations, mustard is the most commonly used product to manufacture prepacked food like seasoning and flavoring agents and texture control agents [54]. Verhoeckx et al. [55] precisely described this problem and the implications of mustard seed processing in the presence of allergens. Food allergies limit the use of this compound, but the overall prevalence is relatively low [56,57]. Most of the case reports disclosing this problem were related to food allergies. A contact allergy would cause a significantly low level of complications. Labeling toothpaste as a product only for external use will reduce this potential problem.

During the study, patients were divided into six groups. Control groups were treated with toothpaste without thioglycosides. Patients were not informed of their allocation to the test or control group. The increase in periodontal health parameters could be a result of regular oral hygiene kept by the patients while undergoing the study and the Hawthorne Effect [58]. It also supports the statement that non-fluoride oral products, especially plant-based home oral care products that contain Neem or Aloe vera extracts, are appropriate to maintain good periodontal status. The highest results of the measured parameters in the initial examination were in the high-DMFT-PD(+) group. The authors would like to determine whether changes in these parameters occurred in addition to the periodontal status of patients. It is crucial that plaque accumulation, a reduction in gingival bleeding, and an improvement of periodontal parameters were observed in all four groups. Initial relationships between the groups remained and flattened after 12 months, but were nevertheless preserved. That clearly justifies the reason for patient group categories.

Improvement in oral hygiene parameters was significant in all groups, but mostly in the high-DMFT-PD(−) and high-DMFT-PD(+) groups. This relationship was observed in every parameter measured. In each case of comparison, the most relevant reduction was observed in the first 6 months of the trial. In the second period, the trend was still visible but was lower. Hygienic parameter improvement was more significant in periodontally compromised patients in the high-DMFT-PD(+) group than the control group with variable patients and the low-DMFT-PD(−) group containing patients with healthy gingiva. This is a significant indication that thioglycoside-based pastes might be recommended for patients with periodontal diseases. BOP was reduced by 38% in 12 months. All this supports the hypothesis that thioglycosides slow down dental plaque accumulation. Gingiva examination revealed improvements in all groups, with the most significant decrease in mean POB values observed in groups using toothpaste with thioglycosides.

Mustard, as is commonly known, has a pungent smell and taste. It might not have been acceptable to everyone. Due to the low concentration of mustard oil (5%), the taste was mild, so patients did not complain about the taste of toothpaste their during second and third appointments.

Toothpaste composition must be developed in future studies. Stability and stress tests also have to be performed. The authors suggest applying the Accelerate Stability Assessment Program (ASAP) [59] before the commercial use of this product.

This pioneering study involving a small group of patients cannot be compared to any similar study, because there was no similar research available in the literature. The study validates the prophylactic and therapeutic properties of thioglycosides in reducing gingival

inflammation. The promising outcome might be an indication for future research. It is recommended to continue research while taking more parameters into consideration, and longer studies will be necessary to confirm our findings and to better understand how these compounds work. This research contributes to the ongoing exploration of plant-derived ingredients for innovative evidence-based dental and gum care products. It also meets the expectations of the rapidly growing group of patients preferring fluor-free home oral care products. Justification of the use of this kind of product can be found in the improvement of tested parameters in the control group.

4. Materials and Methods

The present study obtained a positive affirmation from the institutional review board (KB/58/2011) and was carried out at the Department of Conservative Dentistry of the Medical University of Warsaw. All clinical procedures were achieved in accordance with the Helsinki Declaration of 1975, as revised in Tokyo in 2013. Participants were examined in the outpatient clinic of the University. All patients were informed about the study's objectives, as well as possible risks and profits of participating in the study.

4.1. Mustard Experimental Paste Preparation

Mustard oil was extracted from white mustard "Bramberka" using the Soxhlet reference method. Using the Soxhlet extractor made from BORO 3.3 glass according to norm DIM 12602 (PHU Chemo-lab, Ruda Śląska, Poland), two formulas were simultaneously assessed: toothpaste made from fragmented entire white mustard seeds (analogously to mustard production) and ethanol extract of fragmented mustard seeds. The concentration of main sinalbin derivatives in the obtained toothpaste was measured by means of a UV absorption test (280 nm). Through the use of high-performance liquid chromatography (HPLS) (Shimazu company, Kyoto, Japan) with a UV diode array detector (DAD) (Knauer, Berlin, Germany), the concentration was expressed in relation to the master alcohol extract of 1 g of mustard seeds.

After the evaporation of alcohol, the remnants (containing mustard oil and thioglycosides transitions' derivatives) were added to the toothpaste base, which had been prepared in advance.

The toothpaste base composition was typical for the cosmetic industry [60,61] and is presented in Table 2. The stability of components in toothpaste was checked and confirmed through the use of HPLS methods.

Table 2. Composition of toothpaste used in the study.

Material	Content (%)
dicalcium phosphate dihydrate	38
demineralized water	32.4
glycerol	25
silica (silicon dioxide)	2.4
carboxymethyl cellulose	1.2
sodium lauryl sulphate	0.6
sodium benzoate	0.2
sodium methyl hydroxybenzoate	0.2

Toothpaste base was enriched with milled mustard seeds—5% of the mass; ethanol extract—the remnants obtained after alcohol evaporation (0.3%).

After enrichment with milled mustard seeds (5%) and alcohol extract (0.3%), a reevaluation of the paste was performed after two weeks with HPLS methods showing the time stability of the experimental paste composition.

The control formula was a base toothpaste without mustard seed preparation. Both pastes were packed into plastic tubes and coded to ensure that participants did not know the content.

4.2. Toxicologic Study

In the experimental study, an extract from a food product was used. It has also been used for many years in herbal medicine. The extract preparation process is typical for the food industry. According to the EFSA, the daily intake of harmful erucic acid is 8 mg/kg [43]. A potential food allergy reaction, which was reported in adults, was correlated to the mean cumulative dose–response circa 125 mg of mustard seed [62]. Mustard seeds are responsible for allergy reactions in approximately 1% of children [63]. The external use of toothpaste decreases the risk of potential allergic incident occurrence. Inclusion criteria (aged older than 18) eliminated the child population, which is more susceptible to a mustard allergy [64]. The concentration of the active ingredients in the prepared formulations was lower than in mustard. For these reasons, no preliminary toxicological studies were performed on laboratory animals.

4.3. Sample Calculation

There has been no similar study conducted before. We made estimations for the proper sample size, ensuring the correlations were statistically significant. The sample size calculation (alpha = 0.05; power = 80%) for two independent research groups and a continuous primary endpoint was calculated. We expected to obtain differences in the mean of approximately 20%. A sample size of 66 participants per group fulfills the statistical criteria. The sample was calculated using the Clinical Sample Calculator (Clin Cal Lcc https://clincalc.com/stats/samplesize.aspx accessed on 12 May 2024).

4.4. Inclusion Criteria

The inclusion criteria were as follows: the presence of teeth in the mouth, aged 18 years old or over, API and BoP values in the initial examination of more than 20%, regular oral hygiene at home, and motivation to take part in the study. Participants were gathered from a pool of patients submitted to the Outpatient Clinic of the Department of Conservative Dentistry, Medical University of Warsaw. Each of them agreed to participate in the study and signed a written consent form.

4.5. Exclusion Criteria

The exclusion criteria were as follows: systemic disease, smoking, diabetes, long-term medication, pregnancy and nursing, or a declared allergy to mustard. Participants with orthodontic appliances were also excluded. Patients with restorative treatment that started during the study were excluded and considered "lost in the follow-up".

4.6. Clinical Study Design

The study was conducted at the Department of Conservative Dentistry, Medical University of Warsaw, involving 149 participants (81 males and 68 females) aged 28 to 62 years. Patients were screened according to the exclusion criteria. Sixteen patients were removed, while 133 were enrolled in the study. Initially, each patient was evaluated through medical history taking while completing a survey related to systematic diseases and allergies. Each patient underwent a preliminary clinical examination according to generally accepted principles, and the required conservative treatment and hygienic protocol were provided prior to the study to avoid outcome disturbances. Baseline appointments consisted of a dental examination involving the calculation of the DMFT index (decayed, missing, and filled index) and the API index (approximal plaque index), CPI (community periodontal index) calculation, and an oral hygiene evaluation including PI (Plaque index), BOP (Bleeding on probing), and gingiva observation [65].

The community periodontal index (CPI) is the result of the development of the Community periodontal index of treatment needs (CPITN) by changes in the World Health Organization oral health survey [66]. The mouth of a patient is divided into sextants, and ten teeth are taken into consideration, i.e., 17, 16, 11, 26, 27, 37, 36, 31, 46, and 47. The examination involves evaluating the presence of sub- and supragingival dental calculus, the occurrence of gingival bleeding, and the measurement of periodontal pockets with

probing depths between 3.5 and 6.0 mm. The examination is performed using a periodontal probe with a 0.5 mm ball tip. The probe has black band markers at 3.5, 5.5, 8.5, and 11.5 mm and is called the WHO probe. Probing is performed with force not exceeding 20 g [67]. The results are marked in each sextant as follows:

0—healthy no bleeding.
1—bleeding visible after probing.
2—calculus present during examination, but all of the black bands are visible on the probe.
3—4–5 mm pocket (gingival margin within the black band on the probe).
4—pocket 6 mm or more (the black band on the probe is not visible).

The Decayed, Missing, and Filled Teeth (DMFT) index is the predominant population-based measure of caries experiences worldwide. This index gives the sum of an individual's decayed, missing, and filled permanent teeth or surfaces (DMFS) [68].

PI was measured according to Loe's criteria. It means that during clinical examination, plaque was detected on the gingival margin and scored as follows: 0—if no plaque; 1—a thin layer of plaque at the gingival margin only detected by scraping with a probe; 2—a moderate accumulation of plaque within the gingival pocket, and plaque is visible with the naked eye; 3—plaque presence around the gingival margin with the vast majority of interdental spaces filled with plaque [69].

The Approximal Plaque Index (API) according to Lange et al. [69] is related to a patient's oral hygiene status. The buccal side of the first and third quadrants and the lingual/palatal side of the second and fourth quadrants are examined. Each positive plaque finding is noted, and the sum of the total positive findings is used to calculate API using the following formula: (sum of positive findings/sum of investigated approximal spaces) × 100%. API is a simple numerical grading method of patients' oral hygiene: an API value below 39% represents optimal oral hygiene and a value above 40% indicates insufficient oral hygiene [70,71].

Bleeding on Probing (BoP) during the periodontal examination is directly related to the inflammation process in the gums. The examination was performed using the periodontal probe recommended by the WHO. After probing sockets, the percentage of bleeding sockets will indicate the level of inflammation [72,73].

After baseline data collection, the patient pool was divided into six groups regarding the following key. Participants were categorized based on their DMFT and CPI values into 3 groups, which were then simply randomized using an online randomization tool (http://www.randomization.com accessed on 20 June 2023) into experimental and control groups. This resulted in 3 groups allocated to use the enriched experimental paste and 3 control groups:

Group I: Low DMFT, no periodontal disease (CPI = 0) titled "low DMFT-PD(−)"

Group II: High DMFT, no periodontal disease (CPI = 0) titled "high DMFT-PD(−)"

Group III: High DMFT, periodontal disease present (CPI = 1, 2 or 3) titled "high DMFT-PD(+)"

Group IV: Low DMFT, no periodontal disease (CPI = 0) titled "control group—low DMFT-PD(−)"

Group V: High DMFT, no periodontal disease (CPI = 0) titled "control group—high DMFT-PD(−)"

Group VI: High DMFT, periodontal disease present (CPI = 1, 2 or 3) titled "control group—high DMFT-PD(+)"

Patients in the low-DMFT-PD(−), high-DMFT-PD(−), and high-DMFT-PD(+) groups were allocated to experimental toothpaste containing thioglycosides. Control groups received paste with a control formula—without thioglycosides. The study was simple-blinded (patient-blinded), so all toothpaste samples were delivered in the same non-marked plastic tube so that the patient did not know their allocation. Participants were asked to follow home routine oral hygiene procedures, namely brushing two times a day for 2 min with the experimental product. Participants were instructed not to change any daily routine or hygienic behavior.

The study took 12 months, with assessments conducted before treatment initiation (T_0), at 6 months (T_1), and at 12 months (T_2). Professional hygienization was performed during the preliminary examination to achieve the most relevant study outcome. Dental treatment during the study was provided according to patients' needs but it was an exclusion criterion for the study. Key measurements taken during the study were as follows: the Silness–Loe Plaque Index (PI) [74], the Approximal plaque index (API) according to Lange et al. [69], and Bleeding on Probing (BoP) according to Ainamo and Bay [75]. Each of them was evaluated at each time point: at baseline (T_0) in the preliminary examination, and then after 6 months (T_1) and 12 months (T_2). Additionally, a comprehensive dental and periodontal examination was performed during each time point to eliminate any adversities present.

4.7. Statistical Analysis

The statistical analysis was performed using Statistica v. 13 (TIBCO Software Inc., Palo Alto, Santa Clara, CA, USA). A test of normality was conducted using the Shapiro–Wilk test. Descriptive statistics, including the mean, standard deviation, and the number of subjects, were employed. Parametric t-tests for paired samples to compare each group were used for data analysis. Significance levels were set at $p < 0.05$.

5. Conclusions

Thioglycosydes extracted from white mustard had a significant effect on oral hygiene and periodontal health.

A significant reduction in plaque accumulation and gingivitis was observed, especially after 6 months in the three groups that were allocated mustard-based toothpaste. This suggests that the inclusion of thioglycosides from mustard in oral care formulations contributes to a notable decrease in dental plaque. The findings suggest that mustard-based toothpaste enriched with thioglycosides could be a valuable addition to natural oral care solutions. However, the evidence is not sufficient, so further analyses in randomized clinical trials must be performed.

Author Contributions: Conceptualization, A.B. and K.M.; methodology, A.B.; software, A.B.; validation, K.M. and A.B.; formal analysis, K.M.; investigation, A.B. and K.M.; resources, A.B.; data curation, K.M. and A.B.; writing—original draft preparation, K.M.; writing—review and editing, K.M. and A.B.; visualization, K.M.; supervision, A.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Medical University of Warsaw (KB/58/2011).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

API	Approximal Plaque Index (according to Lange [69])
BOP	Bleeding on Probing index (according to Ainamo and Bay [75])
CPI	Community Periodontal Index (according to WHO)
DMFT	Decayed Missing Filled Tooth Index
N	number of patients
PI	Plaque Index (according to Silness and Loe)
SD	standard deviation
WHO	World Health Organization

References

1. Sanz, M.; Marco del Castillo, A.; Jepsen, S.; Gonzalez-Juanatey, J.R.; D'Aiuto, F.; Bouchard, P.; Chapple, I.; Dietrich, T.; Gotsman, I.; Graziani, F.; et al. Periodontitis and Cardiovascular Diseases. Consensus Report. *Glob. Heart* **2020**, *15*, 1. [CrossRef]
2. Herrera, D.; Sanz, M.; Jepsen, S.; Needleman, I.; Roldán, S. A Systematic Review on the Effect of Systemic Antimicrobials as an Adjunct to Scaling and Root Planing in Periodontitis Patients. *J. Clin. Periodontol.* **2002**, *29*, 136–159. [CrossRef] [PubMed]
3. Chapple, I.L.C.; Mealey, B.L.; Van Dyke, T.E.; Bartold, P.M.; Dommisch, H.; Eickholz, P.; Geisinger, M.L.; Genco, R.J.; Glogauer, M.; Goldstein, M.; et al. Periodontal Health and Gingival Diseases and Conditions on an Intact and a Reduced Periodontium: Consensus Report of Workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J. Periodontol.* **2018**, *89*, 74–84. [CrossRef] [PubMed]
4. Peres, M.A.; Macpherson, L.M.D.; Weyant, R.J.; Daly, B.; Venturelli, R.; Mathur, M.R.; Listl, S.; Celeste, R.K.; Guarnizo-Herreño, C.C.; Kearns, C.; et al. Oral Diseases: A Global Public Health Challenge. *Lancet* **2019**, *394*, 249–260. [CrossRef]
5. Kinane, D.F.; Chestnutt, I.G. Smoking and Periodontal Disease. *Crit. Rev. Oral Biol. Med.* **2000**, *11*, 356–365. [CrossRef]
6. Shapira, L.; Wilensky, A.; Kinane, D.F. Effect of Genetic Variability on the Inflammatory Response to Periodontal Infection. *J. Clin. Periodontol.* **2005**, *32*, 72–862. [CrossRef] [PubMed]
7. Preshaw, P.M.; Alba, A.L.; Herrera, D.; Jepsen, S.; Konstantinidis, A.; Makrilakis, K.; Taylor, R. Periodontitis and Diabetes: A Two-Way Relationship. *Diabetologia* **2012**, *55*, 21–31. [CrossRef]
8. Jepsen, S.; Caton, J.G.; Albandar, J.M.; Bissada, N.F.; Bouchard, P.; Cortellini, P.; Demirel, K.; De Sanctis, M.; Ercoli, C.; Fan, J.; et al. Periodontal Manifestations of Systemic Diseases and Developmental and Acquired Conditions: Consensus Report of Workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J. Periodontol.* **2018**, *89*, S237–S2489. [CrossRef]
9. Haffajee, A.D. The Effect of SRP on the Clinical and Microbiological Parameters of Periodontal Diseases. *J. Clin. Periodontol.* **1997**, *24*, 324–334. [CrossRef]
10. Invernici, M.M.; Salvador, S.L.; Silva, P.H.F.; Soares, M.S.M.; Casarin, R.; Palioto, D.B.; Souza, S.L.S.; Taba, M.; Novaes, A.B.; Furlaneto, F.A.C.; et al. Effects of Bifidobacterium Probiotic on the Treatment of Chronic Periodontitis: A Randomized Clinical Trial. *J. Clin. Periodontol.* **2018**, *45*, 1198–1210. [CrossRef]
11. Chambrone, L.; Wang, H.L.; Romanos, G.E. Antimicrobial Photodynamic Therapy for the Treatment of Periodontitis and Peri-Implantitis: An American Academy of Periodontology Best Evidence Review. *J. Periodontol.* **2018**, *89*, 783–803. [CrossRef]
12. Butera, A.; Gallo, S.; Pascadopoli, M.; Luraghi, G.; Scribante, A. Ozonized Water Administration in Peri-Implant Mucositis Sites: A Randomized Clinical Trial. *Appl. Sci.* **2021**, *11*, 7812. [CrossRef]
13. Chandakavathe, B.N.; Deshpande, D.K.; Swamy, P.V.; Dhadde, S.B. Assessment of Toothpaste Formulations Containing Turmeric and Neem Extract for Prevention of Dental Caries and Periodontal Diseases. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **2018**, *88*, 1523–1529. [CrossRef]
14. Sugiarta, A.P.; Lessang, R.; Natalina. Effect of Herbal Toothpaste Containing Neem Leaves Extract (*Azadirachta indica*) against Gingivitis: A Clinical Study. *Int. J. Appl. Pharm.* **2019**, *11*, 117–119. [CrossRef]
15. Abhishek, K.N.; Supreetha, S.; Sam, G.; Khan, S.N.; Chaithanya, K.H.; Abdul, N. Effect of Neem Containing Toothpaste on Plaque and Gingivitis—A Randomized Double Blind Clinical Trial. *J. Contemp. Dent. Pract.* **2015**, *16*, 880–883. [CrossRef]
16. Lakshmi, T.; Krishnan, V.; Rajendran, R.; Madhusudhanan, N. *Azadirachta indica*: A Herbal Panacea in Dentistry—An Update. *Pharmacogn. Rev.* **2015**, *9*, 41–44. [CrossRef]
17. Khunkar, S.; Linjawi, A. Effect of *Salvadora persica* Extract (Miswak) on the Dentinal Tubules of Sound Root Dentin: Scanning Electron Microscope Study. *J. Microsc. Ultrastruct.* **2021**, *9*, 154–157. [CrossRef]
18. Khunkar, S.; Hariri, I.; Alsayed, E.; Linjawi, A.; Khunkar, S.; Islam, S.; Bakhsh, T.A.; Nakashima, S. Inhibitory Effect of *Salvadora persica* Extract (Miswak) on Collagen Degradation in Demineralized Dentin: In Vitro Study. *J. Dent. Sci.* **2021**, *16*, 208–213. [CrossRef]
19. Kalpavriksha, A.J.; Siddaiah, S.B.; Bilichodmath, S.; Prabhakara, S.; Hanumantha Rao, H.M. Comparative Evaluation of Antibacterial Effect of Gic Containing Chlorhexidine and Miswak on Streptococcus Mutans and Streptococcus Sobrinus in Early Childhood Caries Children: A Pcr Study. *Int. J. Clin. Pediatr. Dent.* **2021**, *14*, 229–234. [CrossRef]
20. Ramli, H.; Nor Aripin, K.N.; Mohd Said, S.; Mohamad Hanafiah, R.; Mohd Dom, T.N. The Effectiveness of Miswak (*Salvadora persica* L. and *Azadirachta indica* A.Juss.) Practices in Reducing Plaque and Gingivitis among Adults: A Systematic Review and Meta-Analysis. *J. Ethnopharmacol.* **2022**, *298*, 115598. [CrossRef]
21. Nordin, A.; Bin Saim, A.; Ramli, R.; Abdul Hamid, A.; Mohd Nasri, N.W.; Bt Hj Idrus, R. Miswak and Oral Health: An Evidence-Based Review. *Saudi J. Biol. Sci.* **2020**, *27*, 1801–1810. [CrossRef] [PubMed]
22. Abhary, M.; Al-Hazmi, A.A. Antibacterial Activity of Miswak (*Salvadora persica* L.) Extracts on Oral Hygiene. *J. Taibah Univ. Sci.* **2016**, *10*, 513–520. [CrossRef]
23. Wassel, M.O.; Khattab, M.A. Antibacterial Activity against Streptococcus Mutans and Inhibition of Bacterial Induced Enamel Demineralization of Propolis, Miswak, and Chitosan Nanoparticles Based Dental Varnishes. *J. Adv. Res.* **2017**, *8*, 387–392. [CrossRef]
24. Azaripour, A.; Mahmoodi, B.; Habibi, E.; Willershausen, I.; Schmidtmann, I.; Willershausen, B. Effectiveness of a Miswak Extract-Containing Toothpaste on Gingival Inflammation: A Randomized Clinical Trial. *Int. J. Dent. Hyg.* **2017**, *15*, 195–202. [CrossRef] [PubMed]

25. Müller-Heupt, L.K.; Vierengel, N.; Groß, J.; Opatz, T.; Deschner, J.; von Loewenich, F.D. Antimicrobial Activity of Eucalyptus Globulus, *Azadirachta indica*, *Glycyrrhiza glabra*, *Rheum palmatum* Extracts and Rhein against *Porphyromonas gingivalis*. *Antibiotics* **2022**, *11*, 186. [CrossRef] [PubMed]
26. Ben Bacha, A.; Jemel, I.; Moubayed, N.M.S.; Abdelmalek, I. Ben Purification and Characterization of a Newly Serine Protease Inhibitor from Rhamnus Frangula with Potential for Use as Therapeutic Drug. *3 Biotech* **2017**, *7*, 148. [CrossRef]
27. Adam, F.A.; Mohd, N.; Rani, H.; Yusof, M.Y.P.M.; Baharin, B. *Salvadora persica* L.: An Effective Anti-Plaque and Anti-Gingivitis Toothpaste: A Systematic Review & Meta-Analysis of Randomized Control Clinical Trials. *J. Herb. Med.* **2023**, *40*, 100677.
28. Akaberi, M.; Sobhani, Z.; Javadi, B.; Sahebkar, A.; Emami, S.A. Therapeutic Effects of *Aloe* Spp. in Traditional and Modern Medicine: A Review. *Biomed. Pharmacother.* **2016**, *84*, 759–772. [CrossRef] [PubMed]
29. Butera, A.; Gallo, S.; Pascadopoli, M.; Taccardi, D.; Scribante, A. Home Oral Care of Periodontal Patients Using Antimicrobial Gel with Postbiotics, Lactoferrin, and Aloe Barbadensis Leaf Juice Powder vs. Conventional Chlorhexidine Gel: A Split-Mouth Randomized Clinical Trial. *Antibiotics* **2022**, *11*, 118. [CrossRef]
30. El-Sayed, W.A.; Abdel Megeid, R.E.; Abbas, H.A.S. Synthesis and Antimicrobial Activity of New 1-[(Tetrazol-5-Yl)Methyl] Indole Derivatives, Their 1,2,4-Triazole Thioglycosides and Acyclic Analogs. *Arch. Pharm. Res.* **2011**, *34*, 1085–1096. [CrossRef]
31. Codée, J.D.C.; Litjens, R.E.J.N.; van den Bos, L.J.; Overkleeft, H.S.; van der Marel, G.A. Thioglycosides in Sequential Glycosylation Strategies. *Chem. Soc. Rev.* **2005**, *34*, 769–782. [CrossRef] [PubMed]
32. Dilmaghani, K.A.; Nasuhi Pur, F.; Pour, M.M.; Nejad, J.M. Novel Oxadiazole Thioglycosides as Potential Anti-Acinetobacter Agents. *Iran. J. Pharm. Res.* **2016**, *15*, 777–782.
33. Springett, M.B.; Adams, J.B. Properties of Brussels Sprouts Thioglucosidase. *Food Chem.* **1989**, *33*, 173–186. [CrossRef]
34. David, J.R.D.; Ekanayake, A.; Singh, I.; Farina, B.; Meyer, M. Effect of White Mustard Essential Oil on Inoculated Salmonella Sp. in a Sauce with Particulates. *J. Food Prot.* **2013**, *76*, 580–587. [CrossRef] [PubMed]
35. Kyung, K.H. Antimicrobial Activity of Volatile Sulfur Compounds in Foods. *ACS Symp. Ser.* **2011**, *1068*, 323–338.
36. Melrose, J. The Glucosinolates: A Sulphur Glucoside Family of Mustard Anti-Tumour and Antimicrobial Phytochemicals of Potential Therapeutic Application. *Biomedicines* **2019**, *7*, 62. [CrossRef] [PubMed]
37. Grygier, A. Mustard Seeds as a Bioactive Component of Food. *Food Rev. Int.* **2023**, *39*, 4088–4101. [CrossRef]
38. Marchioni, I.; Martinelli, M.; Ascrizzi, R.; Gabbrielli, C.; Flamini, G.; Pistelli, L.; Pistelli, L. Small Functional Foods: Comparative Phytochemical and Nutritional Analyses of Five Microgreens of the Brassicaceae Family. *Foods* **2021**, *10*, 427. [CrossRef]
39. Sawicka, B.; Kotiuk, E.; Kiełtyka-Dadasiewicz, A.; Krochmal-Marczak, B. Fatty Acids Composition of Mustard Oil from Two Cultivars and Physico-Chemical Characteristics of the Seeds. *J. Oleo Sci.* **2020**, *69*, 207–217. [CrossRef]
40. Klóska, Ł.; Cegielska-Taras, T.; Pietka, T. Regeneration Capacity of Selected Genotypes of White Mustard (*Sinapis alba* L.). *In Vitro Cell. Dev. Biol.—Plant* **2012**, *48*, 180–188. [CrossRef]
41. Sadowska, U.; Jewiarz, K.; Kopak, M.; Dziadek, K.; Francik, R.; Kopeć, A. Proximate Analysis and Antioxidant Properties of Young Plants of *Sinapis alba* L. Depend on the Time of Harvest and Variety. *Appl. Sci.* **2023**, *13*, 7980. [CrossRef]
42. Piętka, T.; Krzymański, J. Bamberka—Zero Erucic White Mustard. *Rośliny Oleiste—Oilseed Crops*, 2007; XVIII, 511–524.
43. Knutsen, H.K.; Alexander, J.; Barregård, L.; Bignami, M.; Brüschweiler, B.; Ceccatelli, S.; Dinovi, M.; Edler, L.; Grasl-Kraupp, B.; Hogstrand, C.; et al. Erucic Acid in Feed and Food. *EFSA J.* **2016**, *14*, e04593. [CrossRef]
44. Ożarowski, A. *Ziołolecznictwo. Poradnik Dla Lekarzy*; PZWL: Warszawa, Poland, 1979.
45. Chen, S.; Andreasson, E. Update on Glucosinolate Metabolism and Transport. *Plant Physiol. Biochem.* **2001**, *39*, 743–758. [CrossRef]
46. Sofrata, A.H.; Claesson, R.L.K.; Lingström, P.K.; Gustafsson, A.K. Strong Antibacterial Effect of Miswak Against Oral Microorganisms Associated With Periodontitis and Caries. *J. Periodontol.* **2008**, *79*, 1474–1479. [CrossRef]
47. Vitt, A.; Sofrata, A.; Slizen, V.; Sugars, R.V.; Gustafsson, A.; Gudkova, E.I.; Kazeko, L.A.; Ramberg, P.; Buhlin, K. Antimicrobial Activity of Polyhexamethylene Guanidine Phosphate in Comparison to Chlorhexidine Using the Quantitative Suspension Method. *Ann. Clin. Microbiol. Antimicrob.* **2015**, *14*, 36. [CrossRef]
48. Sofrata, A.; Brito, F.; Al-Otaibi, M.; Gustafsson, A. Short Term Clinical Effect of Active and Inactive *Salvadora persica* Miswak on Dental Plaque and Gingivitis. *J. Ethnopharmacol.* **2011**, *137*, 1130–1134. [CrossRef]
49. Sofrata, A.; Santangelo, E.M.; Azeem, M.; Borg-Karlson, A.K.; Gustafsson, A.; Pütsep, K. Benzyl Isothiocyanate, a Major Component from the Roots of *Salvadora Persica* Is Highly Active against Gram-Negative Bacteria. *PLoS ONE* **2011**, *6*, e23045. [CrossRef]
50. Priya, B.M.; Anitha, V.; Shanmugam, M.; Ashwath, B.; Sylva, S.D.; Vigneshwari, S.K. Efficacy of Chlorhexidine and Green Tea Mouthwashes in the Management of Dental Plaque-Induced Gingivitis: A Comparative Clinical Study. *Contemp. Clin. Dent.* **2015**, *6*, 505–509. [CrossRef]
51. Butera, A.; Pascadopoli, M.; Pellegrini, M.; Gallo, S.; Zampetti, P.; Cuggia, G.; Scribante, A. Domiciliary Use of Chlorhexidine vs. Postbiotic Gels in Patients with Peri-Implant Mucositis: A Split-Mouth Randomized Clinical Trial. *Appl. Sci.* **2022**, *12*, 2800. [CrossRef]
52. Eichel, V.; Schüller, A.; Biehler, K.; Al-Ahmad, A.; Frank, U. Antimicrobial Effects of Mustard Oil-Containing Plants against Oral Pathogens: An in Vitro Study. *BMC Complement. Med. Ther.* **2020**, *20*, 156. [CrossRef]
53. Mazur, M.; Ndokaj, A.; Bietolini, S.; Nissi, V.; Duś-Ilnicka, I.; Ottolenghi, L. Green Dentistry: Organic Toothpaste Formulations. A Literature Review. *Dent. Med. Probl.* **2022**, *59*, 461–474. [CrossRef]
54. FAO. Food and Agriculture Organization of United Nations, Rome, Italy. [CrossRef]

55. Verhoeckx, K.C.M.; Vissers, Y.M.; Baumert, J.L.; Faludi, R.; Feys, M.; Flanagan, S.; Herouet-Guicheney, C.; Holzhauser, T.; Shimojo, R.; van der Bolt, N.; et al. Food Processing and Allergenicity. *Food Chem. Toxicol.* **2015**, *80*, 223–240. [CrossRef]
56. Rancé, F. Mustard Allergy as a New Food Allergy. *Allergy Eur. J. Allergy Clin. Immunol.* **2003**, *58*. [CrossRef]
57. Poms, R.E.; Klein, C.L.; Anklam, E. Methods for Allergen Analysis in Food: A Review. *Food Addit. Contam.* **2004**, *21*, 1–31. [CrossRef]
58. McCambridge, J.; Witton, J.; Elbourne, D.R. Systematic Review of the Hawthorne Effect: New Concepts Are Needed to Study Research Participation Effects. *J. Clin. Epidemiol.* **2014**, *67*, 267–277. [CrossRef]
59. Huynh-Ba, K.; Dong, M.W. Stability Studies and Testing of Pharmaceuticals: An Overview. *LCGC N. Am.* **2020**, *38*, 325.
60. Fink, J.K. Toothpaste Compositions. In *Materials, Chemicals and Methods for Dental Applications*; Wiley: Hoboken, NJ, USA, 2018.
61. Martu, M.-A.; Stoleriu, S.; Pasarin, L.; Tudorancea, D.; Sioustis, I.-A.; Taraboanta, I.; Sandu, D.; Solomon, S.-M. Toothpastes Composition and Their Role in Oral Cavity Hygiene. *Rom. J. Med. Dent. Educ.* **2021**, *10*, 179–205.
62. Figueroa, J.; Blanco, C.; Dumpiérrez, A.G.; Almeida, L.; Ortega, N.; Castillo, R.; Navarro, L.; Pérez, E.; Gallego, M.D.; Carrillo, T. Mustard Allergy Confirmed by Double-Blind Placebo-Controlled Food Challenges: Clinical Features and Cross-Reactivity with Mugwort Pollen and Plant-Derived Foods. *Allergy Eur. J. Allergy Clin. Immunol.* **2005**, *60*, 48–55. [CrossRef]
63. Wróblewska, B. Food Allergens. In *Chemical and Functional Properties of Food Components*, 4th ed.; CRC Press: Boca Raton, FL, USA, 2023.
64. Courtois, J.; Bertholet, C.; Cavalier, E.; Gillard, N.; Quinting, B.; Gadisseur, R. Mustard Allergy: Diagnostic and Identification of Specific Allergens by Immunoblotting. *Allergy Eur. J. Allergy Clin. Immunol.* **2017**, *72*. [PubMed]
65. Löe, H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J. Periodontol.* **1967**, *38*, 610–616. [CrossRef]
66. WHO World Health Organization. *Oral Health Surveys Basic Methods*, 5th ed.; World Health Organization: Geneva, Switzerland, 2013.
67. Dhingra, K.; Vandana, K.L. Indices for Measuring Periodontitis: A Literature Review. *Int. Dent. J.* **2011**, *61*, 76–84. [CrossRef]
68. De Abreu Da Silva Bastos, V.; Freitas-Fernandes, L.B.; Da Silva Fidalgo, T.K.; Martins, C.; Mattos, C.T.; De Souza, I.P.R.; Maia, L.C. Mother-to-Child Transmission of Streptococcus Mutans: A Systematic Review and Meta-Analysis. *J. Dent.* **2015**, *43*, 181–191. [CrossRef]
69. Lange, D.E.; Plagmann, H.C.; Eenboom, A.; Promesberger, A. Klinische Bewertungsverahren Zur Objektivierung Der Mundhygiene. *Dtsch. Zahnarztl. Z.* **1977**, *32*, 44–47.
70. Lange, D.E. Neuere Aspekte Der Diangostik Und Therapie von Parodontalerkrankungen Für Den Zahnärztlichen Praktiker. *Quintessenz* **1986**, *37*, 521–532.
71. Cutress, T.W.; Hunter, P.B.V.; Hoskins, D.I.H. Comparison of the Periodontal Index (PI) and Community Periodontal Index of Treatment Needs (CPITN). *Community Dent. Oral Epidemiol.* **1986**, *14*, 39–42. [CrossRef]
72. Hashim, D.; Cionca, N.; Combescure, C.; Mombelli, A. The Diagnosis of Peri-Implantitis: A Systematic Review on the Predictive Value of Bleeding on Probing. *Clin. Oral Implants Res.* **2018**, *29*, 276–293. [CrossRef]
73. Lang, N.P.; Adler, R.; Joss, A.; Nyman, S. Absence of Bleeding on Probing An Indicator of Periodontal Stability. *J. Clin. Periodontol.* **1990**, *17*, 714–721. [CrossRef]
74. Silness, J.; Löe, H. Periodontal Disease in Pregnancy II. Correlation between Oral Hygiene and Periodontal Condition. *Acta Odontol. Scand.* **1964**, *22*, 121–135. [CrossRef]
75. Ainamo, J.; Bay, I. Problems and Proposals for Recording Gingivitis and Plaque. *Int. Dent. J.* **1975**, *25*, 229–235.

Publikacja # 2

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Article

The Clinical and Antibacterial Effects of a Herbal Toothpaste Containing White Mustard *Sinapis alba* Extract: A Randomized Clinical Trial

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Abstract: Objectives: The aim of this double-blind, clinical trial study was to evaluate the clinical and antibacterial effects of a herbal toothpaste containing white mustard *Sinapis alba* extract in comparison to a placebo toothpaste. **Methods:** One hundred and thirteen participants with gingivitis were randomly assigned to the test group (experimental herbal toothpaste) or the control group (placebo toothpaste). The plaque index (PI), approximal plaque index (API), gingival index (GI), and bleeding on probing (BoP) were evaluated, and salivary samples for microbial evaluation of the loads of *Streptococcus mutans* and *Lactobacillus* spp. were collected at baseline (T_0) and after 4 weeks (T_1). Comparisons were performed between and within groups. **Results:** A reduction in all periodontal parameters (PI, API, GI, and BoP) was observed. The experimental toothpaste reduced the PI by 2.43, compared to a 1.95 reduction for the placebo ($p = 0.041$), and BoP by 30.6%, compared to a 26.8% reduction for the placebo ($p = 0.037$). Statistically significant reductions in salivary *S. mutans* and *Lactobacillus* spp. counts were found in the test group. Among patients who used the experimental toothpaste, 19.2% and 9.6% showed counts of *S. mutans* and *Lactobacillus* spp., respectively, below 10^5 CFU/mL, compared to 44.2% and 40.4% in the placebo group. **Conclusions:** Toothpaste enhanced with white mustard extract was more effective in reducing the PI and BoP indices and decreasing *S. mutans* and *Lactobacillus* spp. counts compared to placebo toothpaste.

Keywords: caries; *Lactobacillus*; mustard; preventive dentistry; plant-based products; *Streptococcus mutans*; toothpaste; oral healthcare

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1. Introduction

Dental caries represents a multifactorial pathological condition characterized by the localized destruction of dental hard tissues through acid-mediated demineralization [1]. It is one of the predominant noncommunicable diseases worldwide, ubiquitously affecting diverse demographic cohorts irrespective of socioeconomic status.

The concentration of cariogenic bacteria in saliva, particularly *Streptococcus mutants* and *Lactobacillus* spp., plays a crucial role in the initiation and progression of dental caries. Higher bacteria loads contribute to an increase in acid production, leading to the demineralization of enamel and dentin. Studies have shown that individuals with salivary *Streptococcus mutans* counts exceeding 10^5 CFU/mL have a significantly higher risk of caries development. Bacterial concentrations in saliva are dynamic and can change due to

factors such as dietary intake, oral hygiene, and the use of antimicrobial agents [2]. Various strategies can effectively reduce cariogenic bacterial loads, including mechanical plaque removal by brushing, the use of antibacterial agents such as chlorhexidine or fluoride, and dietary modifications like limiting fermentable carbohydrates. Additionally, natural antimicrobial compounds, such as plant extracts, have been shown to disrupt bacterial adhesion and inhibit biofilm formation.

Bacteria are aggregated in biofilm. A heterogeneous structure comprising mainly microbial cells (10–25%) and a self-produced extracellular polymeric substance (EPS) matrix (75–90%), which helps in nutrient cycling, provides the availability of deoxyribonucleic acid (DNA) for horizontal gene transfer (HGT) and acts as a protective barrier [3]. The biofilm formation is a multi-step process governed by external conditions such as temperature, gravitational forces, hydrodynamic forces, pH, nature of the inhabiting surfaces, quorum sensing, nutrient availability, cell-to-cell communication, signaling cascades, and various secondary messengers [4]. The formation of cariogenic biofilm starts with the attachment of planktonic microbial cells to tooth surfaces, followed by microbial division to create microcolonies, which undergo maturation and dispersion [5]. Dental biofilms are characterized by a high level of spatial matrix organization and up to 700 distinct microbial species or phylotypes [6]. *Streptococcus* spp. is considered a common initial colonizer of dental biofilm due to its virulence and ability to process sugar carbohydrates [7]. Glucosyltransferase enzymes (Gtfs) derived from *Streptococcus mutans* (*S. mutans*) use various sugar carbohydrates to produce glucan polymer, which renders the biofilm recalcitrant to antimicrobials and difficult to remove [8]. This phenomenon leads to the dominance of acidogenic and acid-tolerating species in the biofilm. Apart from *Streptococcus* spp, other species with acidogenic phenotypes, such as *Lactobacillus* spp., play vital roles in prolonging periods of low pH in the biofilm [9]. The presence of specific functional ecotypes with an inclination to saccharification or proteolysis may initiate the demineralization of the apatite structures of enamel and dentin, potentially progressing to cavitation if a biochemical assault persists [10].

Preventative strategies for dental caries necessitate a multipronged approach, integrating both population-level and individual-level interventions. Public health measures involve water fluoridation and structured, community-based educational prevention programs [11,12]. On the individual scale, rigorous adherence to oral hygiene practices—brushing with fluoride toothpaste, flossing, and dietary regulation—are paramount. Herbal toothpaste additives offer promising benefits in the prevention of dental caries due to their natural antibacterial, anti-inflammatory, and remineralizing properties [13]. Integrating herbal additives in toothpaste formulations can substantially enhance oral hygiene practices, providing a natural, holistic approach to caries prevention and overall dental health [14,15].

White mustard *Sinapis alba* extract is known for its potent antimicrobial properties, which are primarily attributed to glucosinolates and their hydrolysis products, particularly isothiocyanates like allyl isothiocyanate [16]. When plant tissues are damaged, glucosinolates are hydrolyzed by the enzyme myrosinase, releasing isothiocyanates [17]. These compounds exhibit strong antibacterial effects by disrupting bacterial cell membranes, increasing permeability and causing the leakage of cellular contents, which ultimately leads to cell death. Isothiocyanates also react with thiol groups in bacterial enzymes, inhibiting essential processes such as energy production and cell wall synthesis [18–20]. Additionally, they induce oxidative stress by generating reactive oxygen species (ROS), damaging cellular components including DNA, proteins, and lipids [21]. Isothiocyanates inhibit biofilm formation, enhancing bacterial susceptibility to antimicrobial agents [22]. These mechanisms collectively contribute to a reduction in microbial populations, making glucosinolates valuable in natural antibacterial strategies.

Thus, the aim of this study was to evaluate the effectiveness of a newly formulated herbal toothpaste containing white mustard extract in reducing salivary *S. mutans* and *Lactobacillus* spp. loads compared to a standard fluoride-free control toothpaste. The working hypothesis was that a toothpaste that incorporates white mustard extract might be more effective in improving periodontal parameters (PI, API, GI, BoP) and would exhibit stronger antibacterial effects in comparison to a control toothpaste.

2. Materials and Methods

2.1. Study Design

This study was designed as a randomized, double-blind clinical trial. Participants in this study were collected from the outpatient clinic of the Department of Conservative Dentistry of Medical University of Warsaw (Warsaw, Poland). This study was carried out at the Department of Conservative Dentistry of the Medical University of Warsaw. The study protocol was approved by the institutional bioethics committee (KB/58/2011). All clinical procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in Tokyo in 2013. Written informed consent was obtained from all participants at the time of being enrolled in the study. This study was registered on ClinicalTrials.gov (NCT06908265, 19 March 2025) "https://clinicaltrials.gov/study/NCT06908265?tab=history (accessed on 19 March 2025)" and was reported in accordance with CONSORT guidelines [23].

2.2. Eligibility Criteria

The inclusion and exclusion criteria were selected to keep the study population homogenous and minimize confounding factors that could influence the clinical effects of toothpaste. Participants were eligible for inclusion in the study if they met the following criteria: age between 18 and 65 years, presence of at least 20 teeth excluding third molars, a diagnosis of gingivitis according to the 2017 World Workshop [24], and non-smokers. All participants should have had a motivation to take part in the study and maintain proper oral home hygiene. Written informed consent was obtained from all subjects. The exclusion criteria were as follows: (1) patients younger than 18 years; (2) presence of less than 20 teeth; (3) severe systemic diseases and diseases that require regular systemic drugs (diabetes mellitus); (4) use of systemic antibiotics during the last 3 months or local antiseptics that might affect biofilm formation (antibacterial mouth rinses containing chlorhexidine 4 weeks or less prior recruitment); (5) allergy to mustard or any other compound of experimental toothpaste; (6) ongoing orthodontic treatment; and (7) pregnancy or breastfeeding.

2.3. Sample Size Calculation

Assuming a significance level (alpha) of 0.05 and a power of 0.80, the effective size was estimated based on a previous study evaluating a similar intervention [15]. The expected reduction in microbial counts for the experimental group compared to the control group was 30%, and the expected standard deviation was 0.3. Consequently, a sample size of 50 individuals in each of the compared groups was calculated to detect a statistically significant difference between the two groups. In order to account for potential dropouts, 113 subjects were enrolled in this study.

2.4. Experimental Toothpaste Preparation

The experimental toothpaste was based on a standard non-fluoride toothpaste formulation with an addition of an extract from white mustard *Sinapis alba*. Briefly, high-quality "Bamberka" type of white mustard seeds were selected and finely ground. The ground seeds were extracted using a Soxhlet extractor made from BORO 3.3 glass according to the DIM 12602 standard (PHU CHEMO-LAB, Ruda Śląska, Poland). This process involved

soaking the ground seeds in ethanol, followed by filtration and evaporation of the solvent to yield a concentrated extract [18,19,25]. The high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) with UV diode array detector (DAD) (Knauer, Berlin, Germany) methods were used. The extract was then incorporated into the toothpaste base at a concentration of 0.5% by weight. The base formula, a fluoride-free toothpaste without herbal additives, was manufactured by Ziaja LTD (Ziaja ltd Zakład Produkcji Leków sp.z o.o., Gdańsk, Poland). The product was available on the market. The base formula of the toothpaste consisted of common ingredients, such as dicalcium phosphate dihydrate 38%, distilled water 32.4%, glycerol 25%, mustard seeds 5%, hydrated silica 2.4%, carboxymethyl cellulose 1.2%, sodium lauryl sulfate 0.6%, cellulose gum (thickener), essential sodium benzoate 0.2%, and sodium methyl hydroxybenzoate 0.2% [26–28]. This product was also considered as a control toothpaste without mustard additives [29].

The stability and quantity of thioglycosides in both toothpaste samples were evaluated with high-performance liquid chromatography (HPLC). After 4 weeks, both the experimental and the control products were dissolved in ethanol at a proportion of 1:20 and placed in 20 mL tubes [30]. A previously calibrated HPLC column with a detector was used to take measurements of retention.

2.5. Randomization and Blinding

The participants were randomly assigned to one of the two groups (test or control) using the online computer-generated randomization tool (https://www.randomizer.org accessed on 10 May 2023). The toothpastes were identically packaged and labeled with a code to maintain blinding. The researcher gave the corresponding toothpaste to each patient, with both being blinded to the type of toothpaste.

2.6. Study Protocol

The subjects for this study were recruited from among patients of the outpatient clinic by a single investigator (A.B.). Patients who met the inclusion criteria were enrolled in this study (Figure 1). At the first visit (T_0), the clinical evaluation was carried out, and salivary samples for microbial analysis were collected. Subsequently, experimental and control toothpastes were distributed among the patients according to the allocation scheme. The participants were instructed to use a manual toothbrush and brush their teeth using the modified Bass technique. The patients were instructed and trained in this technique at the baseline appointment. All participants were asked to brush their teeth with the assigned toothpaste for 2 min twice a day for 4 weeks.

After 4 weeks (T_1), the patients were recalled and questioned for any inconvenient incidents during the study period. A clinical examination and the collection of saliva samples were conducted.

2.7. Clinical Evaluation

The clinical evaluations were performed by a single masked calibrated examiner (K.M.) at baseline (T_0) and after 4 weeks (T_1). A periodontal probe (PCP UNC 15; Hu-Friedy, Chicago, IL, USA) was used to record the following indices:

1. Plaque index (PI) by Silness and Loe [31] was measured on distal-facial, mesial-facial, facial, and lingual sites of all teeth. Presence of plaque at gingival margin was evaluated and the following scores were given: 0—no plaque; 1—a thin layer of plaque only detected by scraping with a probe; 2—moderate accumulation of plaque within gingival pocket, plaque is visible to the naked eye; 3—plaque presence around the gingival margin with vast majority of interdental spaces filled with plaque;

2. Approximal plaque index (API) by Lange [32] evaluated presence or absence of dental plaque in the approximal sides and was calculated as a percentage of the approximal areas that exhibited plaque.
3. Gingival index (GI) by Loe and Silness [33] was measured on six selected teeth (16, 12, 24, 36, 32, 44) on facial, lingual, mesial, and distal sites after gentle probing. The scores were given as follows: 0—normal gingiva, no inflammation, no erythema, no bleeding; 1—mild inflammation, slight erythema, no bleeding; 2—moderate inflammation, erythema, bleeding on probing; 3—severe inflammation, severe erythema and swelling, tendency to spontaneous bleeding.
4. Bleeding on probing (BOP) by Ainamo and Bay [34] was evaluated on facial, lingual, mesial, and distal sites after gentle probing. BoP was calculated by dividing the sum of bleeding sockets by the sum of all evaluated sockets.

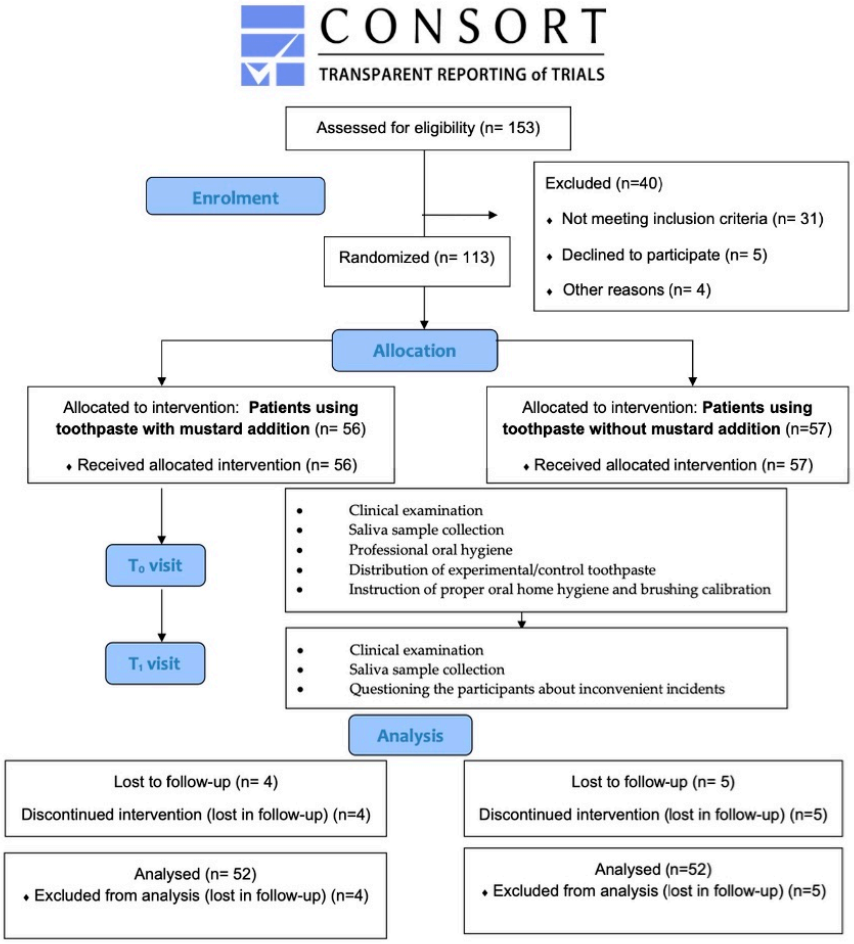

Figure 1. CONSORT diagram showing the study planning.

2.8. Microbiological Analysis

Saliva samples were collected twice from all participants at the beginning of the study (T_0) and after 4 weeks (T_1) of using the assigned toothpaste. The samples were collected at least 2 h after the last meal and tooth brushing. A Caries Risk Test (CRT, Ivoclar Vivadent, Schaan, Lichtenstein) was used to evaluate the loads of *S. mutans* and

Lactobacillus spp. The procedure involved the patient chewing a paraffin pellet to transfer bacteria from tooth surfaces to the saliva, which was then collected in a suitable container. An $NaHCO_3$ tablet was placed in the test vial, and upon contact with moisture, it released CO_2, creating favorable conditions for bacterial growth. After removing the protective foil, the agars were processed quickly to prevent them from standing unprotected for extended periods. Each sample was inoculated using the Ivoclar CRT bacteria test kit, which provides results as either above or below 10^5 CFU/mL (colony-forming unit/milliliter) for *S. mutans* and *Lactobacillus* spp. The plates were incubated anaerobically at 37 °C for 48 h. After incubation, the plates were evaluated for the growth of bacterial colonies characteristic of the targeted species.

2.9. Statistical Analysis

Statistical analysis was carried out using Statistica software, version 13.1 (Statsoft, Kraków, Poland). Descriptive statistics were presented as mean and standard deviation. Any *p*-values of less than 0.05 ($p < 0.05$) were considered statistically significant. A t-test for two independent samples, correlation analysis, and linear regression were employed to thoroughly investigate the relationship between toothpaste use and changes in bacterial loads. The Chi-Square test for independence was conducted to determine if there was a significant association between the type of toothpaste (control vs. experimental) and the loads of *S. mutans* and *Lactobacillus* spp. in saliva.

3. Results

One hundred and thirteen patients (55 women and 58 men, aged 28–62; mean age 44.3 ± 16.9 years) were enrolled in this study. In total, 56 subjects were randomly assigned to the experimental group, and 57 subjects were randomized to the control group. Four participants in the experimental group and five participants in the control group were lost in follow-up, and for these reasons, they were excluded from the final evaluation. No adverse or side effects were noted during the study or later.

Between T_0 and T_1 in the experimental group, a significant reduction was observed in all four compared parameters: PI (3.68 ± 1.13 to 1.83 ± 0.42, $p < 0.001$), API (41.6 ± 20.5 to 18.5 ± 17.3 $p < 0.001$), GI (1.89 ± 0.76 to 1.45 ± 0.81, $p = 0.037$), and BoP (49.3 ± 15.2 to 18.7 ± 13.6, $p = 0.017$). Similarly, significant results were observed in the control group: PI (3.78 ± 0.72 to 1.25 ± 0.52, $p \leq 0.001$), API (39.2 ± 19.8 to 22.2 ± 18.6, $p < 0.001$), GI (1.96 ± 0.83 to 1.55 ± 0.92, $p = 0.043$), and BoP (52.9 ± 13.5 to 26.1 ± 16.3 $p = 0.048$).

A statistical analysis containing a t-test for two independent samples showed statistically significant differences between the experimental and control groups and T_o vs. T_1 in the improvement of the PI, API, GI, and BoP. The *p*-values for both tests were well below the significance level of 0.05. The categorized data related to the periodontal parameters and bacteria were presented in Table 1.

The evaluation of clinical parameters (PI, API, GI, BoP) is illustrated and presented in diagrams, as shown in Figures 2–5.

At the beginning of the study, 55.8% of the control group samples showed *Lactobacillus* count above 10^5 CFU/mL. For *Streptococcus mutans*, the control group had an equal distribution, with 50% of samples below 10^5 CFU/mL and 50% above 10^5 CFU/mL. In the experimental group, 55.8% of samples showed *Lactobacillus* count above 10^5 CFU/mL, while for *Streptococcus mutans*, 51.9% were above the 10^5 CFU/mL level.

After four weeks of using the assigned toothpastes, significant ($p < 0.001$) differences were observed. In the control group, 40.4% of samples showed *Lactobacillus* count above 10^5 CFU/mL. At the same time, 44.2% of *Streptococcus mutans* samples were above 10^5 CFU/mL.

Table 1. Distribution of the results in the groups. Mean values of PI, API, GI, BoP, and bacteria load for number of samples and a paired t-test in the experimental and control groups at T_0 and T_1.

	Time	Experimental	Control	*p*-Value Exp. vs. Contr.
Anamnestic Data Age (mean) Gender		43.2 ± 12.2 years 25 F/31 M	45.9 ± 16.2 years 30 F/27 M	
PI Mean (SD) T_o vs. T_1	T_0 T_1	3.68 (1.13) 1.25 (0.42) $p < 0.001$	3.78 (0.72) 1.83 (0.52) $p < 0.001$	$p = 0.599$ $p = 0.041$
API Mean (SD) T_0 vs. T_1	T_0 T_1	41.6 (20.5) 18.5 (17.3) $p < 0.001$	39.2 (19.8) 22.4 (18.6) $p < 0.001$	$p = 0.692$ $p = 0.846$
GI Mean (SD) T_0 vs. T_1	T_0 T_1	1.89 (0.76) 1.45 (0.81) $p = 0.037$	1.96 (0.83) 1.55 (0.92) $p = 0.043$	$p = 0.661$ $p = 0.565$
BoP Mean (SD) T_0 vs. T_1	T_0 T_1	49.3 (15.2) 18.7 (13.6) $p = 0.017$	52.9 (13.5) 26.1(16.3) $p = 0.048$	$p = 0.675$ $p = 0.037$
***Streptococcus mutans* Bacteria Load** T_0 vs. T_1	T_0 T_1	27/52 ($n > 10^5$ CFU/mL) (51,9%) 10/52 ($n > 10^5$ CFU/mL) (19,2%) $p = 0.012$	26/52 ($n > 10^5$ CFU/mL) (50%) 23/52 ($n > 10^5$ CFU/mL) (44.2%) $p = 0.694$	$p = 0.9876$ $p = 0.011$
***Lactobacillus* spp. Bacteria Load** T_0 vs. T_1	T_0 T_1	29/52 ($n > 10^5$ CFU/mL) (55.8%) 5/52 ($n > 10^5$ CFU/mL) (9.6%) $p < 0.001$	29/52 ($n > 10^5$ CFU/mL) (55.8%) 21/52 ($n > 10^5$ CFU/mL) (40.4%) $p = 0.169$	$p = 1$ $p < 0.001$

T_0,T_1—time frames; M—male; F—female; BoP—bleeding on probing; PI—plaque index; GI—gingival index; API—approximal plaque index; SD—standard deviation N—number of samples; n—number of bacteria colony; CFU—colony-forming unit.

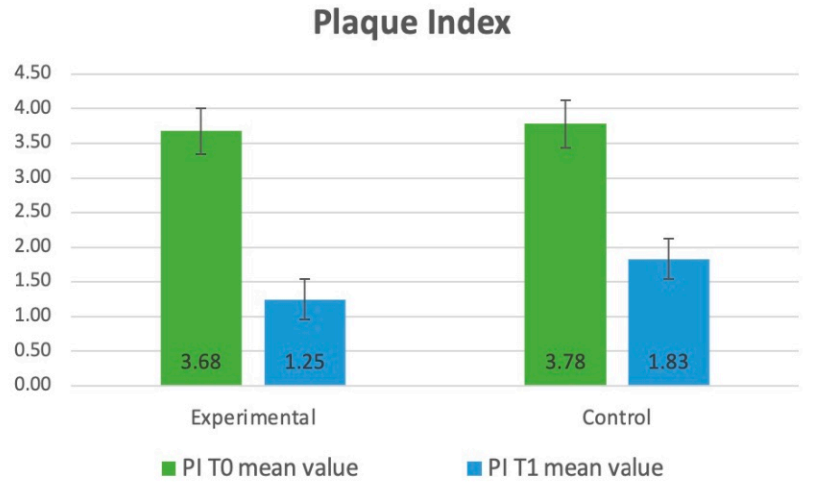

Figure 2. Distribution of the clinical parameters in the groups. Mean values of PI at T_0 and T_1.

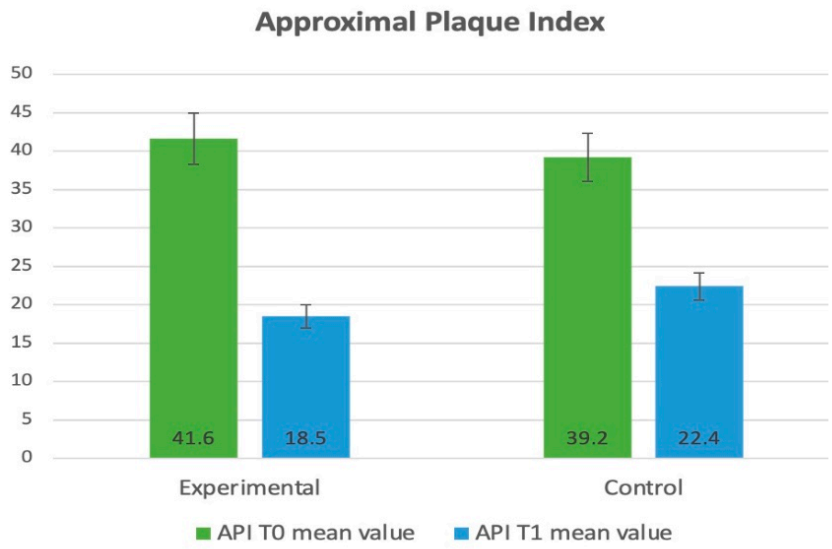

Figure 3. Distribution of the clinical parameters in the groups. Mean values of API at T_0 and T_1.

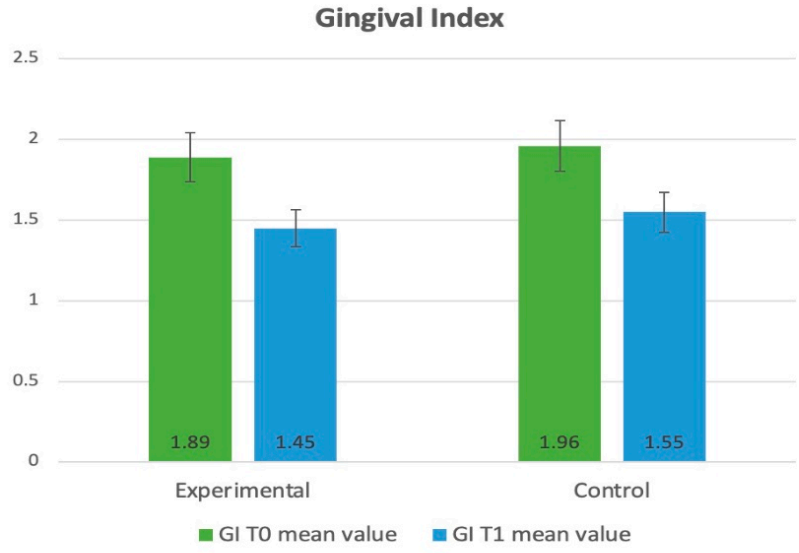

Figure 4. Distribution of the clinical parameters in the groups. Mean values of GI at T_0 and T_1.

In contrast, the experimental group showed a more pronounced reduction in bacterial load. For *Lactobacillus*, only 9.6% was above 10^5 CFU/mL. For *Streptococcus mutans*, 19.2% samples were above 10^5 CFU/mL.

Bacteria counts are graphically visualized in Figure 6.

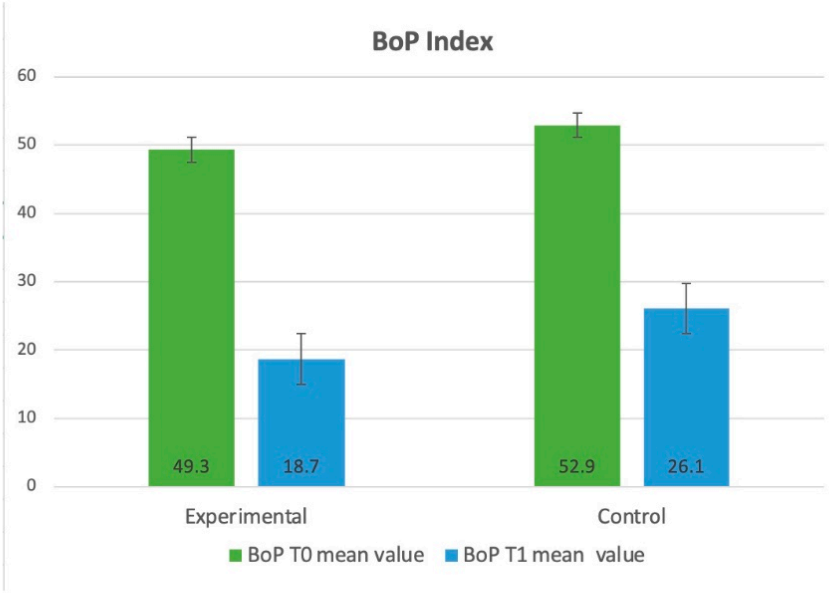

Figure 5. Distribution of the clinical parameters in the groups. Mean values of BoP at T_0 and T_1.

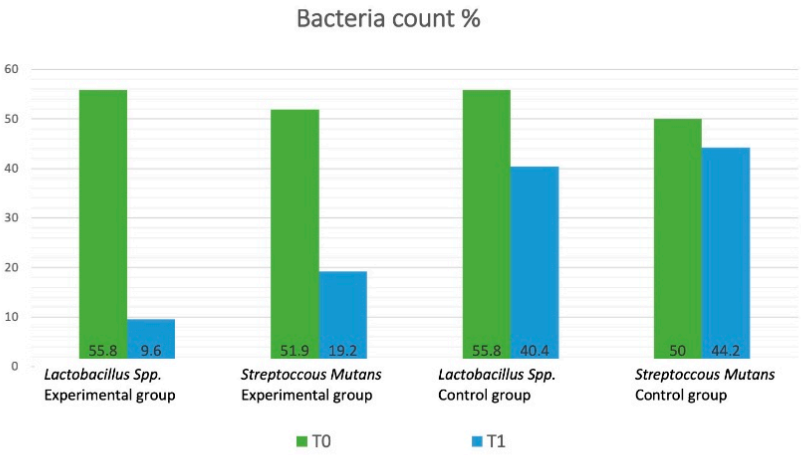

Figure 6. Distribution of the bacteria count in the groups. Percent values of positive ($n > 10^5$ CFU/mL) test at T_0 and T_1.

The Chi-Square test for independence was conducted to determine if there was a significant association between the type of toothpaste (control vs. experimental) and the load of cariogenic bacteria *Streptococcus mutans* and *Lactobacillus* spp. The results for *Streptococcus mutans* showed a Chi-Square statistic T_0—T_1 (χ^2) of 10.74 ($p = 0.000615$) and 1 degree of freedom, and for *Lactobacillus* spp., the results showed (χ^2) 23.12 $p = 0.000015$. These *p*-values are significantly lower than $p < 0.05$, so we assume that the reduction in bacteria count is statistically significant.

4. Discussion

The presented study aimed to evaluate the effectiveness of an experimental toothpaste containing white mustard (*Sinapsis alba*) extract in reducing cariogenic bacteria in the oral

cavity compared to a standard fluoride-free toothpaste. White mustard was selected for this study due to its well-documented antimicrobial properties, primally attributed to glucosinolates and their hydrolysis products, such as isothiocyanates. These compounds exhibit strong antibacterial effects by disrupting bacterial cell membranes, inhibiting biofilm formation. The findings presented in this study indicate a significant association between the type of toothpaste used and the reduction in bacterial levels, as evidenced by the Chi-Square test results. The experimental toothpaste, enhanced with white mustard extract, showed a marked improvement in reducing both *Streptococcus mutans* and *Lactobacillus* spp. compared to the control toothpaste. This reduction was significant for two key bacterial species responsible for the development of dental caries. The results suggest that the antimicrobial properties of white mustard extract are effective in significantly lowering the levels of cariogenic bacteria. This leads to the conclusion that herbal additives like white mustard extract can be potent agents in oral healthcare. The ability of the experimental toothpaste to eliminate bacteria in a considerable proportion of participants highlights its potential as a preventive measure against dental caries. In addition, this study also revealed significant improvements in clinical parameters related to oral hygiene and periodontal health. These improvements in the PI, API, GI, and BoP suggest that the toothpaste has a positive impact on oral hygiene and decreases the risk of periodontal disease.

The effectiveness of various herbal additives in oral care has been explored, but there is still limited research on the use of mustard-based compounds in toothpaste. However, several studies have investigated the benefits of other plant-based additives, such as tea tree oil, clove oil, and aloe vera, which similarly exhibit antibacterial, anti-inflammatory, and anti-caries properties [28,35]. For example, a study by Arweiler et al. [15] found that toothpaste containing *Scutellaria baicalensis* extract significantly reduced plaque and gingivitis, supporting the potential of herbal additives in promoting oral health. Mustard seed oil properties were also examined by Eichel et al. [19]. This study showed antibacterial effectiveness in an in vitro examination. A recently published study by Michałowski et al. [36] showed an association between using mustard oil extract-based toothpaste and a reduction in periodontal parameters. Literature searches in databases like PubMed and EMBASE did not show any studies linking cariogenic bacteria and mustard oil extract. The concept of incorporating mustard-based compounds in oral hygiene makes our study novel. This is why we cannot compare it to any other clinical study performed previously. This randomized, single-blinded clinical trial provides an adequate evidence level to reduce the knowledge gap in this field.

Fluoride toothpastes are widely recognized as the gold standard in caries prevention due to their ability to enhance enamel remineralization and inhibit demineralization. Numerous studies have demonstrated the efficacy of fluoride in significantly reducing dental caries [37,38]. However, some individuals seek fluoride-free alternatives due to concerns about overexposure or personal health preferences [39]. The current study did not include a direct comparison with fluoride-containing toothpastes, but the significant reduction in bacterial levels observed with the experimental toothpaste suggests that white mustard extract could serve as a potent natural antimicrobial agent. It is worth mentioning that the addition of mustard did not have a significant impact on the taste and odor. None of the participants complained about it.

The literature has not disclosed a clear relation between oral bacteria and mustard oil products. The use of mustard as an additive to oral health products may lead to beneficial results. We would like to address this question in this study. The seed extract preparation process is also typical for the food industry. It proves that it does not lead to a chemical modification of natural contents. The concentration of active ingredients in the prepared formulations was lower than in food mustard [40].

This study had several limitations. The short duration of the study—four weeks—does not allow for a comprehensive evaluation of the long-term effects on oral health. However, the four-week study duration was chosen because bacterial levels in saliva change rapidly, with measurable reduction occurring within days of antimicrobial intervention. This period allows for assessing both short-term antibacterial effects and clinical improvements while ensuring participant compliance. A longer duration increases the number of dropouts and additional variables. This period provides a practical and controlled evaluation. Previously conducted studies on herbal additives in toothpaste like chitosan lasted four weeks [41].

The sample size, while sufficient for initial findings, could be expanded in future studies to increase statistical power and generalizability. The lack of comparison with fluoride-containing toothpastes is another limitation, as fluoride remains the most well-researched and effective agent in caries prevention. A direct comparison of mustard extract with fluoride would provide valuable insights into the relative efficacy of natural alternatives. Another potential limitation is the allergenic potential of mustard seeds. Although no allergic reactions were reported during this study, mustard seed is known for its high allergic potential [42,43]. Only external use was applied in this study. For these reasons, no preliminary toxicological studies were performed.

Future studies should explore the mechanisms by which white mustard extract demonstrates its antibacterial effects. Further research should investigate the optimal concentration of white mustard extract and its combination with other herbal additives to maximize its antimicrobial properties. Researchers should focus on long-term studies that assess the sustained effectiveness of white mustard toothpaste in reducing cariogenic bacteria and preventing caries. Additionally, studies that directly compare mustard-based toothpaste with fluoride-containing formulations would provide a more comprehensive understanding of its efficacy. A comparison of mustard-based toothpaste to other plant-based would be beneficial. Investigating the optimal concentration of mustard extract and its potential synergistic effects with other natural compounds, such as xylitol or tea tree oil, could further enhance its antibacterial properties. Moreover, exploring the broader impact of mustard extract on the oral microbiome, including its effects on non-cariogenic species and overall microbial diversity, would provide valuable insights into its role in promoting oral health.

The results suggest that regular use of herbal toothpaste with mustard extract could contribute to caries prevention, offering a natural alternative for individuals seeking non-fluoride solutions.

5. Conclusions

This study demonstrates that toothpaste enhanced with white mustard extract is significantly more effective in reducing cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus* spp., compared to a fluoride-free control toothpaste. Continued research in this area could lead to the development of more effective and natural oral hygiene products, ultimately expanding the options available for maintaining oral health in diverse populations. Further research is recommended to confirm long-term benefits, assess additional health effects, and analyze the potential of these toothpastes to prevent dental caries.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical University of Warsaw (protocol code: KB/58/2011, date of approval: 12 April 2011).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PI	Plaque Index
GI	Gingival Index
API	Approximal Plaque Index
BoP	Bleeding on probing

References

1. Machiulskiene, V.; Campus, G.; Carvalho, J.C.; Dige, I.; Ekstrand, K.R.; Jablonski-Momeni, A.; Maltz, M.; Manton, D.J.; Martignon, S.; Martinez-Mier, E.A.; et al. Terminology of Dental Caries and Dental Caries Management: Consensus Report of a Workshop Organized by ORCA and Cariology Research Group of IADR. *Caries Res.* **2020**, *54*, 7–14. [CrossRef] [PubMed]
2. Chen, X.; Daliri, E.B.-M.; Tyagi, A.; Oh, D.-H. Cariogenic Biofilm: Pathology-Related Phenotypes and Targeted Therapy. *Microorganisms* **2021**, *9*, 1311. [CrossRef]
3. Rather, M.A.; Gupta, K.; Mandal, M. Microbial Biofilm: Formation, Architecture, Antibiotic Resistance, and Control Strategies. *Braz. J. Microbiol.* **2021**, *52*, 1701–1718. [CrossRef]
4. Muhsin, J.; Ufaq, T.; Tahir, H.; Saadia, A. Bacterial Biofilm: Its Composition, Formation and Role in Human Infections. *Res. Rev. J. Microbiol. Biotechnol.* **2015**, *4*, 317.
5. Chandki, R.; Banthia, P.; Banthia, R. Biofilms: A Microbial Home. *Proc. J. Indian Soc. Periodontol.* **2011**, *15*, 111–114.
6. Borisy, G.G.; Valm, A.M. Spatial Scale in Analysis of the Dental Plaque Microbiome. *Periodontology 2000* **2021**, *86*, 97–112. [CrossRef]
7. Hajishengallis, E.; Parsaei, Y.; Klein, M.I.; Koo, H. Advances in the Microbial Etiology and Pathogenesis of Early Childhood Caries. *Mol. Oral Microbiol.* **2017**, *32*, 24–34. [CrossRef]
8. Hoshino, T.; Fujiwara, T. The Findings of Glucosyltransferase Enzymes Derived from Oral Streptococci. *Jpn. Dent. Sci. Rev.* **2022**, *58*, 328–335. [CrossRef]
9. Bowen, W.H.; Burne, R.A.; Wu, H.; Koo, H. Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments. *Trends Microbiol.* **2018**, *26*, 229–242. [CrossRef]
10. Johansson, I.; Witkowska, E.; Kaveh, B.; Lif Holgerson, P.; Tanner, A.C.R. The Microbiome in Populations with a Low and High Prevalence of Caries. *J. Dent. Res.* **2016**, *95*, 80–86. [CrossRef]
11. James, P.; Harding, M.; Beecher, T.; Browne, D.; Cronin, M.; Guiney, H.; O'mullane, D.; Whelton, H. Impact of Reducing Water Fluoride on Dental Caries and Fluorosis. *J. Dent. Res.* **2021**, *100*, 507–514. [CrossRef]
12. Ruff, R.R.; Niederman, R. Comparative Effectiveness of School-Based Caries Prevention: A Prospective Cohort Study. *BMC Oral Health* **2018**, *18*, 53. [CrossRef] [PubMed]
13. Kanouté, A.; Dieng, S.N.; Diop, M.; Dieng, A.; Sene, A.K.; Diouf, M.; Lo, C.M.; Faye, D.; Carrouel, F. Chemical vs. Natural Toothpaste: Which Formulas for Which Properties? A Scoping Review. *J. Public Health Afr.* **2022**, *13*, 13. [CrossRef] [PubMed]
14. Biria, M.; Rezvani, Y.; Roodgarian, R.; Rabbani, A.; Iranparvar, P. Antibacterial Effect of an Herbal Toothpaste Containing Bamboo Salt: A Randomized Double-Blinded Controlled Clinical Trial. *BMC Oral Health* **2022**, *22*, 193. [CrossRef]
15. Arweiler, N.B.; Pergola, G.; Kuenz, J.; Hellwig, E.; Sculean, A.; Auschill, T.M. Clinical and Antibacterial Effect of an Anti-Inflammatory Toothpaste Formulation with *Scutellaria baicalensis* Extract on Experimental Gingivitis. *Clin. Oral Investig.* **2011**, *15*, 909–913. [CrossRef] [PubMed]
16. Melrose, J. The Glucosinolates: A Sulphur Glucoside Family of Mustard Anti-Tumour and Antimicrobial Phytochemicals of Potential Therapeutic Application. *Biomedicines* **2019**, *7*, 62. [CrossRef] [PubMed]
17. Marcinkowska, M.; Jeleń, H.H. Inactivation of Thioglucosidase from *Sinapis alba* (White Mustard) Seed by Metal Salts. *Molecules* **2020**, *25*, 4363. [CrossRef] [PubMed]

18. Sawicka, B.; Kotiuk, E.; Kiełtyka-Dadasiewicz, A.; Krochmal-Marczak, B. Fatty Acids Composition of Mustard Oil from Two Cultivars and Physico-chemical Characteristics of the Seeds. *J. Oleo Sci.* **2020**, *69*, 207–217. [CrossRef]
19. Eichel, V.; Schüller, A.; Biehler, K.; Al-Ahmad, A.; Frank, U. Antimicrobial Effects of Mustard Oil-Containing Plants Against Oral Pathogens: An in Vitro Study. *BMC Complement. Med. Ther.* **2020**, *20*, 156. [CrossRef]
20. David, J.R.D.; Ekanayake, A.; Singh, I.; Farina, B.; Meyer, M. Effect of White Mustard Essential Oil on Inoculated Salmonella sp. in a Sauce with Particulates. *J. Food Prot.* **2013**, *76*, 580–587. [CrossRef] [PubMed]
21. Jensen, L.M.; Halkier, B.A.; Burow, M. How to Discover a Metabolic Pathway? An Update on Gene Identification in Aliphaticglucosinolate Biosynthesis, Regulation and Transport. *Biol. Chem.* **2014**, *395*, 529–543. [CrossRef] [PubMed]
22. Yang, Y.; Yu, H.; Zhou, X. Covalent Immobilization of Thioglucosidase from Radish Seeds for Continuous Preparation of Sulforaphene. *Chem. Eng. Res. Des.* **2020**, *155*, 146–155. [CrossRef]
23. Lee, J.S.; Ahn, S.; Lee, K.H.; Kim, J.H.; Schulz, K.F.; Altman, D.G.; Moher, D. CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomised Trials. *Epidemiol. Health* **2010**, *36*, e2014029. [CrossRef] [PubMed]
24. Chapple, I.L.C.; Mealey, B.L.; Van Dyke, T.E.; Bartold, P.M.; Dommisch, H.; Eickholz, P.; Geisinger, M.L.; Genco, R.J.; Glogauer, M.; Goldstein, M.; et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J. Periodontol.* **2018**, *89* (Suppl. S1), S74–S84. [CrossRef]
25. Grygier, A. Mustard Seeds as a Bioactive Component of Food. *Food Rev. Int.* **2023**, *39*, 4088–4101. [CrossRef]
26. Martu, M.-A.; Stoleriu, S.; Pasarin, L.; Tudorancea, D.; Sioustis, I.-A.; Taraboanta, I.; Sandu, D.; Solomon, S.-M. Toothpastes Composition and Their Role in Oral Cavity Hygiene. *Rom. J. Med. Dent. Educ.* **2021**, *10*, 179–205.
27. Fink, J.K. Toothpaste Compositions. In *Materials, Chemicals and Methods for Dental Applications*; Wiley: Hoboken, NJ, USA, 2018.
28. Oluwasina, O.O.; Idris, S.O.; Ogidi, C.O.; Igbe, F.O. Production of Herbal Toothpaste: Physical, Organoleptic, Phyto-Compound, and Antimicrobial Properties. *Heliyon* **2023**, *9*, e13892. [CrossRef] [PubMed]
29. Herbal toothpaste formulation and assessment: A comprehensive review. *Int. Res. J. Mod. Eng. Technol. Sci.* **2023**, *5*, 11. [CrossRef]
30. Herzallah, S.; Holley, R. Determination of Sinigrin, Sinalbin, Allyl- and Benzyl Isothiocyanates by RP-HPLC in Mustard Powder Extracts. *LWT* **2012**, *47*, 293–299. [CrossRef]
31. Silness, J.; Löe, H. Periodontal Disease in Pregnancy II. Correlation Between Oral Hygiene and Periodontal Condition. *Acta Odontol. Scand.* **1964**, *22*, 121–135. [CrossRef]
32. Lange, D.E.; Plagmann, H.C.; Eenboom, A.; Promesberger, A. Clinical Methods for the Objective Evaluation of Oral Hygiene. *Dtsch. Zahnarztl. Z* **1977**, *32*, 44–47. [PubMed]
33. Löe, H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J. Periodontol.* **1967**, *38*, 610–616. [CrossRef]
34. Ainamo, J.; Bay, I. Problems and Proposals for Recording Gingivitis and Plaque. *Int. Dent. J.* **1975**, *25*, 229–235. [PubMed]
35. Nagansurkar, S.B.; Bais, S.K.; Deokate, S. Preparation and Evaluation of Herbal Toothpaste. *Int. J. Adv. Res. Sci. Commun. Technol.* **2023**, *3*, 222–233. [CrossRef]
36. Michałowski, K.; Brodzikowska, A. Clinical Effect of Thioglycosides Extracted from White Mustard on Dental Plaque and Gingivitis: Randomized, Single-Blinded Clinical Trial. *Int. J. Mol. Sci.* **2024**, *25*, 5290. [CrossRef]
37. Mazzoleni, S.; Gargani, A.; Parcianello, R.G.; Pezzato, L.; Bertolini, R.; Zuccon, A.; Stellini, E.; Ludovichetti, F.S. Protection against Dental Erosion and the Remineralization Capacity of Non-Fluoride Toothpaste, Fluoride Toothpaste and Fluoride Varnish. *Appl. Sci.* **2023**, *13*, 1849. [CrossRef]
38. Walsh, T.; Worthington, H.V.; Glenny, A.-M.; Marinho, V.C.C.; Jeroncic, A. Fluoride Toothpastes of Different Concentrations for Preventing Dental Caries. *Cochrane Database Syst. Rev.* **2019**, *3*, CD007868. [CrossRef]
39. Baik, A.; Alamoudi, N.; El-Housseiny, A.; Altuwirqi, A. Fluoride Varnishes for Preventing Occlusal Dental Caries: A Review. *Dent. J.* **2021**, *9*, 64. [CrossRef]
40. Singla, N.; Acharya, S.; Martena, S.; Singla, R. Effect of oil gum massage therapy on common pathogenic oral microorganisms - A randomized controlled trial. *J. Indian Soc. Periodontol.* **2014**, *18*, 441–446. [CrossRef]
41. Mohire, N.; Yadav, A. Chitosan-based polyherbal toothpaste: As novel oral hygiene product. *Indian J. Dent. Res.* **2010**, *21*, 380–384. [CrossRef]
42. Pałgan, K.; Żbikowska-Gotz, M.; Bartuzi, Z. Dangerous Anaphylactic Reaction to Mustard. *Arch. Med. Sci.* **2018**, *14*, 477–479. [CrossRef] [PubMed]
43. Rancé, F. Mustard Allergy as a New Food Allergy. *Allergy Eur. J. Allergy Clin. Immunol.* **2003**, *58*, 287. [CrossRef] [PubMed]

Podsumowanie wyników

Przeprowadzone badania potwierdzają kliniczną skuteczność zastosowania ekstraktu z gorczycy białej (*Sinapis alba*) z odmiany „Bamberka" zawierającej tioglikozydy jako środek wspomagający w profilaktyce chorób jamy ustnej. Wyraźny efekt zaobserwowano w zakresie redukcji agregacji płytki i wskaźników stanu zapalnego dziąseł oraz obniżenia ryzyka próchnicy.

W pierwszym badaniu zaobserwowano statystycznie istotne obniżenie wartości wskaźników akumulacji płytki nazębnej (PI i API) oraz redukcję wartości wskaźnika krwawienia przy zgłębnikowaniu (BoP). Największe zmiany odnotowano po pierwszych 6 miesiącach stosowania badanej pasty. W kolejnych miesiącach tempo poprawy parametrów uległo spowolnieniu, jednak efekty oraz trend wzrostowy utrzymał się przez cały okres trwania badania. Warto zauważyć, że najbardziej wyraźna poprawa parametrów klinicznych obserwowana była w grupie badanych z wysokimi wartościami wskaźnika DMFT oraz stwierdzonym stanem zapalnym przyzębia, co może świadczyć o istotnej skuteczności preparatu u pacjentów dotkniętych chorobą przyzębia.

W drugim badaniu również zaobserwowano znaczne obniżenie wartości wskaźników płytki nazębnej (PI), zapalenia dziąseł (GI) oraz krwawienia przy zgłębnikowaniu (BoP). Ponadto, w ślinie grupy pacjentów, którzy stosowali pastę z ekstraktem znacząco spadła liczebność kolonii bakterii *Streptococcus mutans* oraz *Lactobacillus spp*., co potwierdza potencjał przeciwbakteryjny tego preparatu.

W obu badaniach klinicznych nie zaobserwowano żadnych działań niepożądanych, co świadczy o dobrej tolerancji przez pacjentów pasty z ekstraktem z gorczycy. Wyniki te dają możliwość potencjalnego zastosowania ekstraktów tioglikozydowych jako bezpiecznej alternatywy dla past zawierających fluor, szczególnie w grupie pacjentów preferujących środki higieniczne naturalnego pochodzenia.

Mimo obiecujących rezultatów, badania miały pewne ograniczenia, takie jak stosunkowo krótki okres obserwacji w drugim badaniu oraz brak porównania z pastami zawierającymi fluor. Dalsze badania, uwzględniające większe grupy pacjentów i dłuższy okres obserwacji, są konieczne dla szerszego potwierdzenia uzyskanych rezultatów i lepszego określenia długoterminowej skuteczności i bezpieczeństwa stosowania ekstraktu z gorczycy białej w produktach do higieny jamy ustnej.

Wnioski

Przeprowadzone randomizowane badania kliniczne były pierwszą próbą zastosowania ekstraktu z gorczycy jako środka wspomagającego w domowej higienie jamy ustnej. W obu badaniach zastosowanie eksperymentalnego, wzbogacanego o dodatek ekstraktu z gorczycy produktu, dało obiecujące rezultaty.

Szczegółowe wnioski można podzielić w następujący sposób:

1. Redukcja akumulacji płytki bakteryjnej oraz wartości wskaźników klinicznych stanu zapalnego dziąseł.

   - Pierwsze badanie wykazało istotne statystycznie zmniejszenie wartości wskaźników PI, API i BoP po 6 miesiącach używania pasty zawierającej ekstrakt z gorczycy „Bamberka". Trend spadkowy widoczny był do końca badanego okresu, choć z mniejszą dynamiką w drugim półroczu.

   - Druga próba potwierdziła statystycznie istotną redukcję wartości wskaźników PI, API, GI i BoP w grupie badanej po miesiącu stosowania.

2. Widoczne działanie przeciwbakteryjne wobec drobnoustrojów kariogennych.

   - W drugim badaniu, po zastosowaniu pasty z wyciągiem gorczycy, zaobserwowano spadek liczebności kolonii bakterii *Streptococcus mutans* > 10^5 CFU/mL z 51,9 % do 19,2 %, natomiast bakterii *Lactobacillus* spp. z 55,8 % do 9,6 %. Redukcji powyższych parametrów nie zaobserwowano w grupie kontrolnej. Pozwala to sformułować wniosek, że stosowanie wzbogaconej pasty redukuje miano bakterii próchnicowych w jamie ustnej, a tym samym zmniejsza ryzyko próchnicy.

   - Zastosowane płytkowe testy oceny ryzyka próchnicy *Caries Risk Test* (CRT, Ivoclar Vivadent, Lichtenstein) nie są swoiste wobec całego spektrum bakterii. Dlatego też testy typu PCR pozwalające na dokładną, jakościową i ilościową analizę mikrobiologiczną powinny zostać wykonane w kolejnych badaniach.

3. Poprawa badanych parametrów u pacjentów z wysokim ryzykiem choroby próchnicowej oraz aktywnym stanem zapalnym dziąseł.

- W badaniu nr 1 u badanych z grupy z wysokimi wartościami wskaźnika DMFT i obecnym zapaleniem dziąseł zaobserwowano największy spadek wartości wskaźników PI, API oraz BoP. Wskazuje to, że preparaty z ekstraktem z gorczycy mogą być przydatne w terapii wspomagającej u pacjentów z grupy wysokiego ryzyka choroby przyzębia.

4.Brak negatywnych wrażeń sensorycznych ze strony pacjentów biorących udział w badaniu.

- Tioglikozydy i glukozylany są odpowiedzialne między innymi za ostry i gorzki smak musztardy. Pacjenci biorący udział w badaniu nie zgłaszali zastrzeżeń dotyczących smaku czy zapachu pasty. Oczywiście istotne jest stężenie składników czynnych, aczkolwiek
w przypadku badanego preparatu nie wpłynęły one negatywnie na odbiór produktu.

5. Konieczne jest prowadzenie dalszych badań mających na celu ustalenie właściwego terapeutycznego stężenia tioglikozydów w produktach do użytku w jamie ustnej.

6. Potwierdzony w badaniach potencjał alergiczny gorczycy wymaga właściwego oznakowania produktu przed szerszym jego zastosowaniem.

Piśmiennictwo:

1. Fahey, J.W. Brassica: Characteristics and Properties. In *Encyclopedia of Food and Health*; 2015.
2. Fahey, J.W.; Zalcmann, A.T.; Talalay, P. The Chemical Diversity and Distribution of Glucosinolates and Isothiocyanates among Plants. *Phytochemistry* 2001, *56*.
3. S, S.B.; B, V.; M, L.N. Allyl Isothiocyanate from Crucifers Potentiates β-Lactam Activity against Methicillin-Resistant Staphylococcus Aureus. *Journal of Medical and Scientific Research* **2014**, *2*, doi:10.17727/jmsr.2014/2-033.
4. Galanty, A.; Grudzińska, M.; Paździora, W.; Paśko, P. Erucic Acid—Both Sides of the Story: A Concise Review on Its Beneficial and Toxic Properties. *Molecules* 2023, *28*.
5. Knutsen, H.K.; Alexander, J.; Barregård, L.; Bignami, M.; Brüschweiler, B.; Ceccatelli, S.; Dinovi, M.; Edler, L.; Grasl-Kraupp, B.; Hogstrand, C.; et al. Erucic Acid in Feed and Food. *EFSA Journal* **2016**, *14*, doi:10.2903/j.efsa.2016.4593.
6. Schwarzinger, B.; Feichtinger, M.; Blank-Landeshammer, B.; Weghuber, J.; Schwarzinger, C. Quick Determination of Erucic Acid in Mustard Oils and Seeds. *J Anal Appl Pyrolysis* **2022**, *164*, doi:10.1016/j.jaap.2022.105523.
7. Piętka T.; Krzymański J. Bamberka – Zero Erucic White Mustard. *ROŚLINY OLEISTE – OILSEED CROPS* **2007**, *Tom XXVIII*.
8. Kamii, E.; Isshiki, K. Antimicrobial Efficacy of Benzyl Isothiocyanate. *Journal of the Food Hygienic Society of Japan* **2009**, *50*, doi:10.3358/shokueishi.50.311.
9. Lin, C.M.; Preston, J.F.; Wei, C.I. Antibacterial Mechanism of Allyl Isothiocyanate. *J Food Prot* **2000**, *63*, doi:10.4315/0362-028X-63.6.727.
10. Pereira, C.; Calado, A.M.; Sampaio, A.C. The Effect of Benzyl Isothiocyanate on Candida Albicans Growth, Cell Size, Morphogenesis, and Ultrastructure. *World J Microbiol Biotechnol* **2020**, *36*, doi:10.1007/s11274-020-02929-9.
11. Bajpai, D.; Malaiappan, S.; S, R. Evaluation of Anti-Inflammatory and Antimicrobial Properties of Mustard Seed Extract-Based Hydrogel: An In Vitro Study. *Cureus* **2023**, doi:10.7759/cureus.45146.
12. Mazumder, A.; Dwivedi, A.; Plessis, J. Du Sinigrin and Its Therapeutic Benefits. *Molecules* 2016, *21*.
13. Zhu, H.; Jia, Z.; Zhang, L.; Yamamoto, M.; Misra, H.P.; Trush, M.A.; Li, Y. Antioxidants and Phase 2 Enzymes in Macrophages: Regulation by Nrf2 Signaling and Protection against Oxidative and Electrophilic Stress. *Exp Biol Med* **2008**, *233*, doi:10.3181/0711-RM-304.
14. Kaur, C.; Kapoor, H.C. Antioxidants in Fruits and Vegetables - The Millennium's Health. *Int J Food Sci Technol* 2001, *36*.

15. Rancé, F. Mustard Allergy as a New Food Allergy. *Allergy: European Journal of Allergy and Clinical Immunology* 2003, *58*.

16. Poms, R.E.; Klein, C.L.; Anklam, E. Methods for Allergen Analysis in Food: A Review. *Food Addit Contam* 2004, *21*.

17. Mazur, M.; Ndokaj, A.; Bietolini, S.; Nissi, V.; Duś-Ilnicka, I.; Ottolenghi, L. Green Dentistry: Organic Toothpaste Formulations. A Literature Review. *Dent Med Probl* 2022, *59*, 461–474.

Warszawa, 10.06.2025 r.

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OŚWIADCZENIE

Jako współautor pracy pt. „**The Clinical and Antibacterial Effects of a Herbal Toothpaste Containing White Mustard Sinapis alba Extract: A Randomized Clinical Trial**" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji oceniam na **10%** stanowi on:
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OŚWIADCZENIE

Jako współautor pracy pt. „**Clinical Effect of Thioglycosides Extracted from White Mustard on Dental Plaque and Gingivitis: Randomized, Single-Blinded Clinical Trial**" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi **20 %** obejmował on:

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Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. dent. Konrada Michałowskiego

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po zapoznaniu się z wnioskiem /wymienić wnioskodawcę/ - **w dniu 12 kwietnia 2011r.**
Dr n.med.Aniela Brodzikowska, Zakład Stomatologii Zachowawczej,
ul.Miodowa 18, 00-246 Warszawa,

dotyczącym: wyrażenia opinii w sprawie badania pt.: " Przeciwbakteryjne i przeciwgrzybiczne działanie tioglikozydów ekstrahowanych z gorczycy białej-bamberka w higienie jamy ustnej."

Uwagi Komisji-verte

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- stwierdza, że są one dopuszczalne i zgodne z zasadami naukowo-etycznymi*.
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Pouczenie-w ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje Prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym.

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) Zarządzenie nr 56/2007 z dnia 15 października 2007 r.w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
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W załączeniu- skład Komisji oraz lista obecności.

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Komisji Bioetycznej

Prof. nadzw.dr hab. n .med. Bożena Tarchalska-Kryńska

• niepotrzebne skreślić