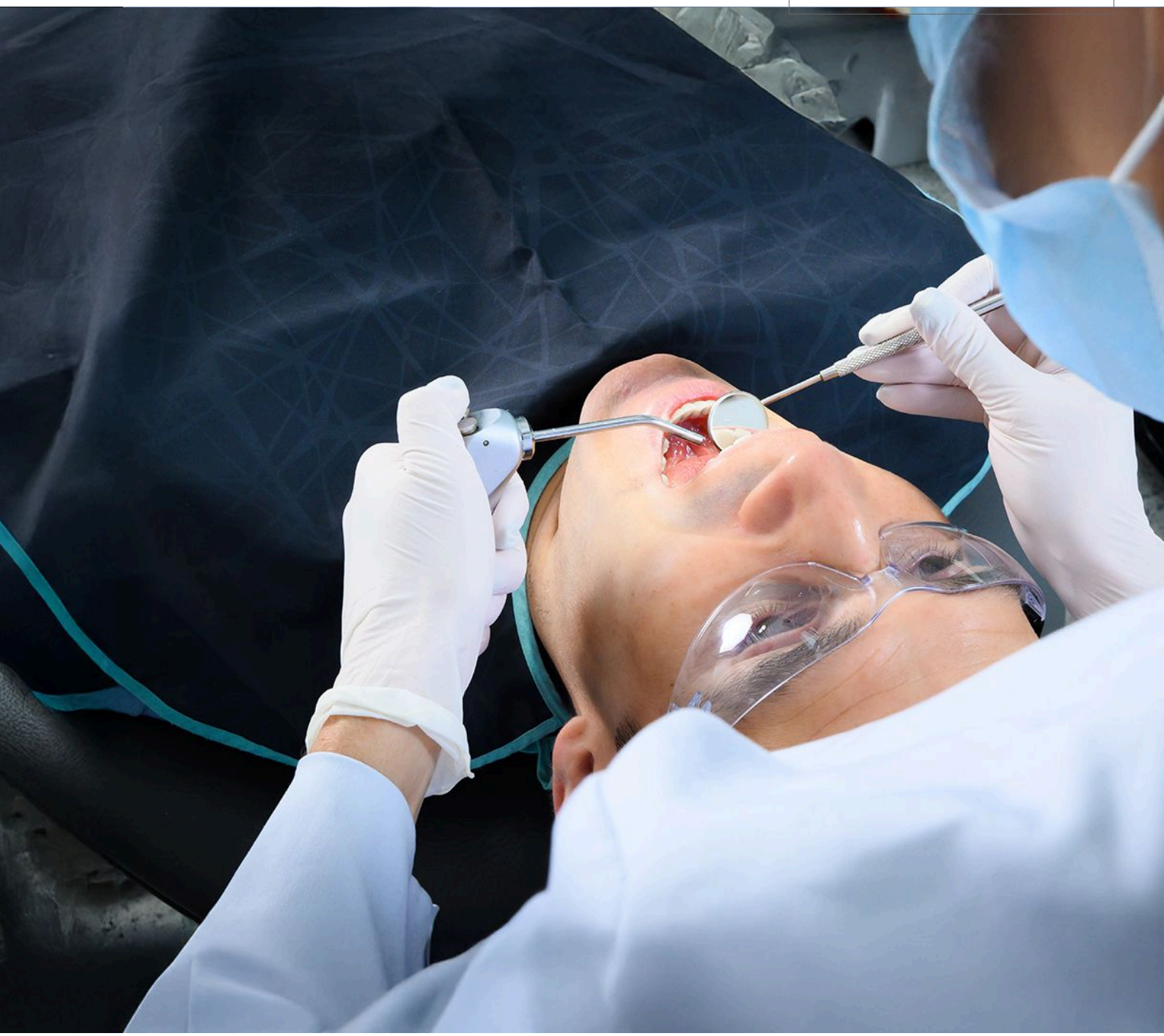
11 MARCH 2025 | REPORT

PATENT INSIGHTS: REVOLUTIONARY ORAL CARE ADVANCEMENTS

Recent oral care innovations emphasise oral microbiome balance, skincare ingredients for gum health and diagnostics for the oral-gut and oral-heart axes.

Neha Srivastava, Senior Patent Analyst - Food & Drink and Beauty & Personal Care



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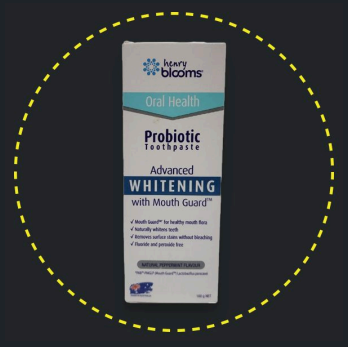


Henry Blooms Oral Health Mouth Guard Probiotic Toothpaste (Malaysia)

What you need to know

What we've seen

- Many consumers take a preventive approach to oral care, with 88% of US oral care product consumers claiming to do so.
- Consumer preference for this approach to oral health is reflected in the popularity of products that help prevent oral health issues like tooth decay.
- Brands are aligning to this preference by innovating in this sector.

Mintel's perspective

- Recent oral care innovations highlight ingredients such as probiotics and prebiotics that support oral microbiome health and help prevent dental or gum issues.
- Patents reveal the use of skincare ingredients like hyaluronic acid in oral care for health benefits such as gum hydration and repair.
- Recent patents emphasise the critical role of the oral cavity in diagnosing and treating gut and heart health concerns.

Why prioritise oral care?

Consumer interest	As science increasingly recognises the role of the oral microbiome in health, maintaining its balance has emerged as a key priority for consumers. For instance, 76% of Canadian consumers agree it is important to have a balanced oral microbiome. Consumers recognise the vital role oral health plays in overall health. In the US, 70% of them agree oral health is important to maintaining overall health. Beyond physical benefits, a healthy oral cavity can boost confidence and foster a sense of wellbeing in personal and professional interactions.
Barriers to entry	For oral care products, solid scientific backing is required for health claims or the ingredients used to support them. Regulations may challenge the use of certain health claims, as they stipulate rigorous testing protocols and approval processes for health claim substantiation. This can be time consuming and costly for companies, ultimately increasing the price of the final product.
Key markets	Asia leads the way in oral care product innovation, with China leading and accounting for 21% of all patent grants. Japan, India and South Korea follow, accounting for 13%, 10% and 9% of all patent grants, respectively.
Future outlook	The oral microbiome is emerging as a key focus area, with growing consumer demand for products that promote both oral and systemic health. Backed by scientific advancements and innovation, the market for such products is expanding, particularly those linked to heart and gut health. Brands that effectively convey these benefits and adapt to regulatory requirements are well positioned to thrive in this evolving landscape.
Why you should care	Using the right oral care products can help prevent the onset of dental problems. Products such as fluoride toothpaste, mouthwash and dental floss play a crucial role in removing plaque, strengthening enamel and preventing cavities and gum disease. This preventive approach to oral care can avoid costly dental treatments. Investing in quality oral care products is also a proactive step towards maintaining overall health and enhancing personal confidence. For instance, 77% of German oral care product users say the appearance of their teeth impacts their confidence.

WHAT CONSUMERS WANT AND WHY

Consumers prioritise effective oral care routines and habits

MOUTHWASH USE

87%

of Chinese consumers use mouthwash in their oral care regimen

TOOTHBRUSH HYGIENE AND CARE

86%

of German oral care product users replace their toothbrush or its head at least once every three months

ORAL CARE ROUTINE

86%

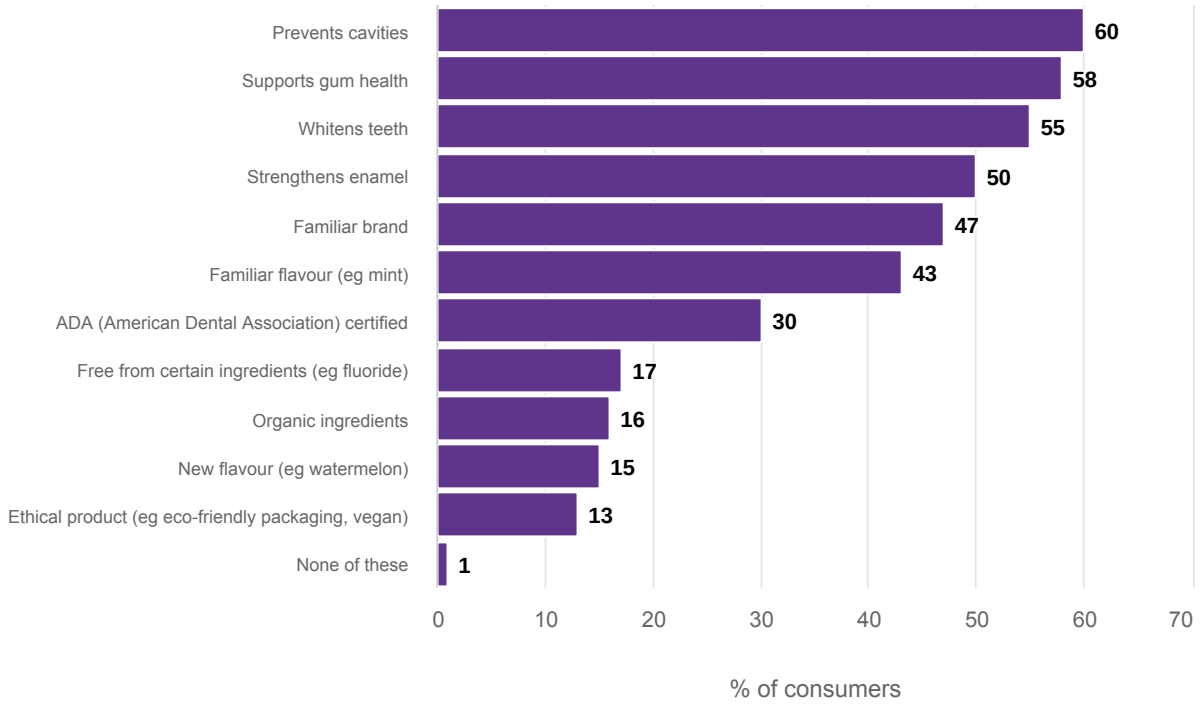
of UK consumers think it's better to stay on top of their oral care routine rather than reacting only when there's a problem

Base: *China: 3,000 internet users aged 18-59; Germany: 1,955 internet users 16+ who have used toothbrushes in the last three months; UK: 2,000 internet users aged 16+*

Source: *KuRunData/Mintel, December 2023; Kantar Profiles/Mintel, March 2024, April 2024*

Consumers prioritise oral health such as cavity prevention over factors like flavour when choosing toothpaste

US: factors considered when purchasing toothpaste (excluding price), 2024



Base: *US: 1,496 internet users aged 18+ who use toothpaste*

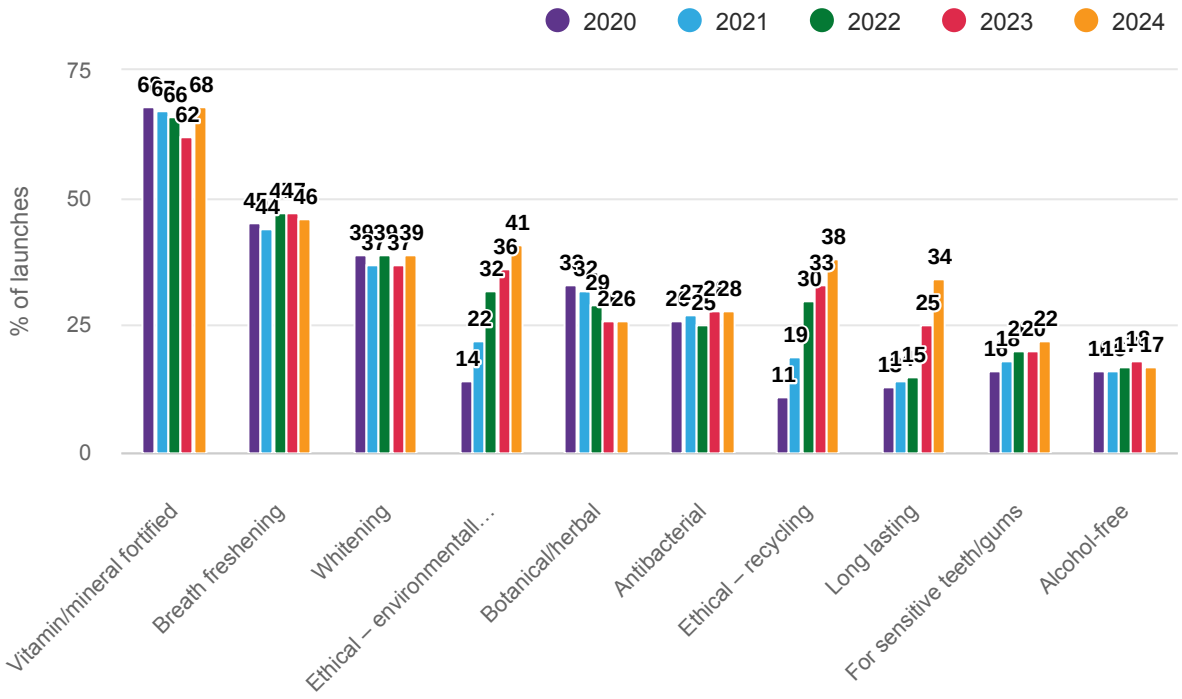
Source: *Kantar Profiles/Mintel, June 2024*

PRODUCT INNOVATION TRENDS

Long-lasting benefit claims have risen over recent years, yet vitamin and mineral fortification claims are on top

Between 2020 and 2024, 72% of toothpaste launches featured a vitamin/mineral fortification claim and 63% of mouthwash launches featured a breath-freshening claim.

Global: top 10 claims in toothpaste and mouthwash launches, 2020-24



Source: *Mintel GNPD, Jan 2020-Dec 2024*

Recent oral care launches provide a diverse array of dental health benefits

Balances the oral microbiome

NYSCPS / Canban Peach Oolong Mouth Wash is formulated with four probiotics (*B. animalis* subsp. *lactis*, *L. paracasei*, *L. casei* and *L. acidophilus*) to support oral health, along with xylitol to help prevent cavities (China).

Teeth remineralisation

Elmex Anticáries Anti-Cavities Daily Use Toothpaste features an amine fluoride formula that binds with the mouth's natural calcium, forming a dual-action shield to efficiently remineralise teeth (Chile).

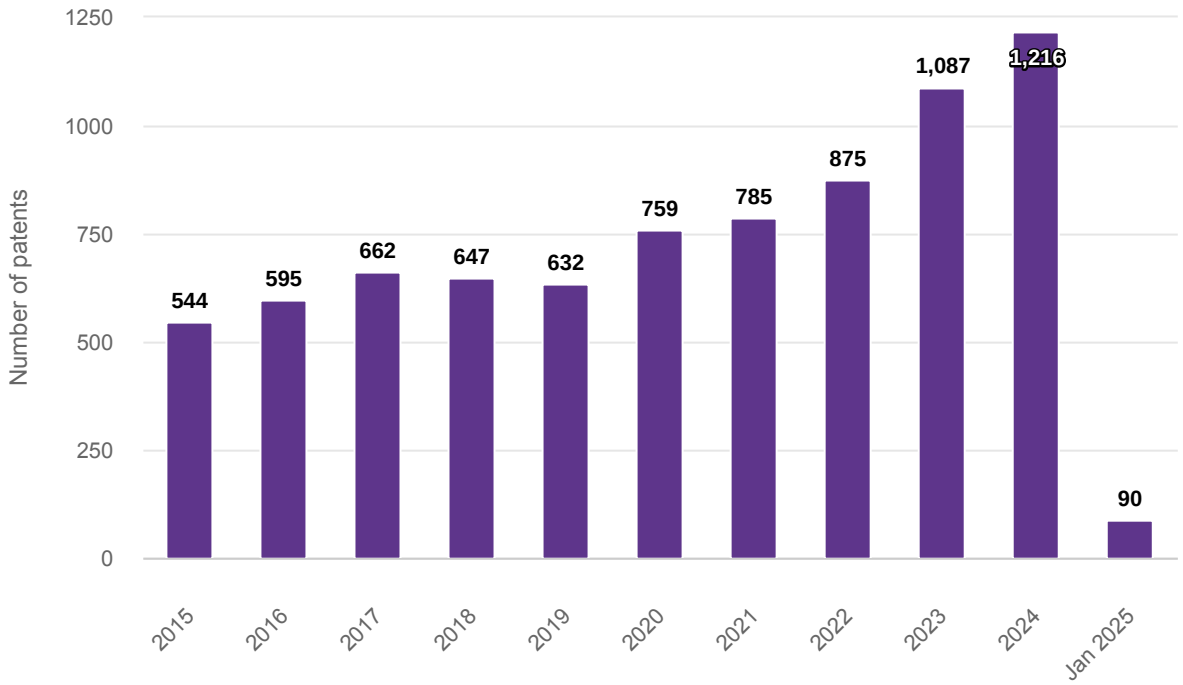
Enamel strength and gum regeneration

Smilepen Pop Mango Jazz Teeth Whitening Strips include nano-hydroxyapatite to strengthen enamel, hyaluronic acid to regenerate gums and antioxidant vitamin E to promote oral health (Czech Republic).

THE PATENT LANDSCAPE

Oral care patent publications have doubled over the past decade

Global (excluding China-only filings*): patents related to oral care products**, published each year, 2015-25



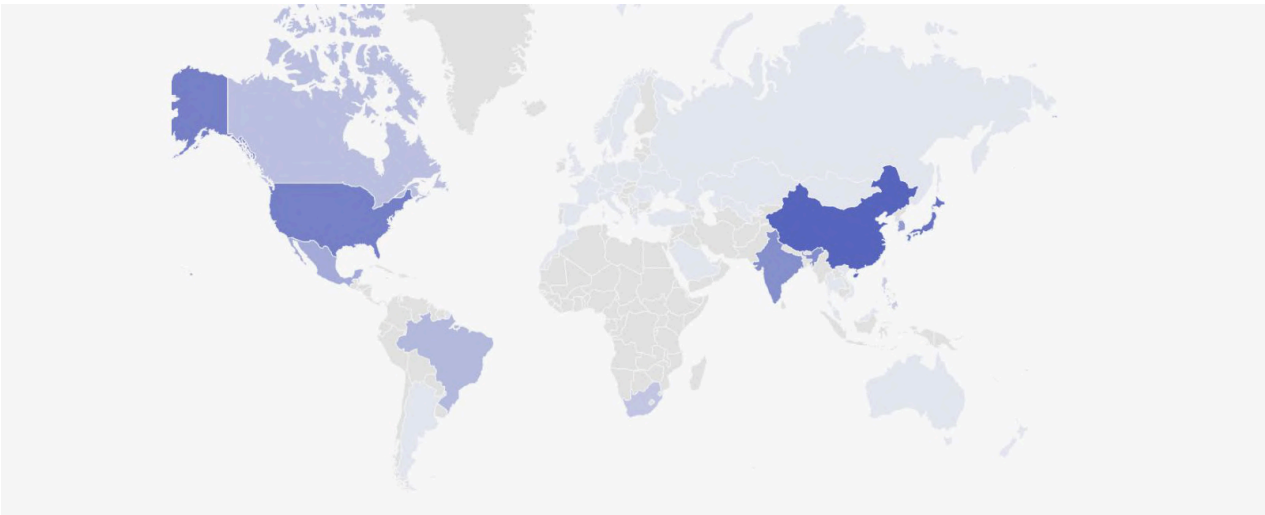
*** refers to patents for oral care products such as toothpaste, mouthwash and oral hygiene spray; excludes toothbrushes and tongue scrapers*

Base: ** for ease of comparison, excludes patents filed only in China given the large number of these (in 2024 alone, 800+ patents for oral care products were published in China); includes published patents that have been granted or are pending*

Source: *Derwent Innovation/Mintel, February 2025*

China leads in patent grants relating to oral care products

The leading countries for granted patents are China (with 21% of all global patent grants), Japan (13%), the US (11%), India (10%) and South Korea (9%).

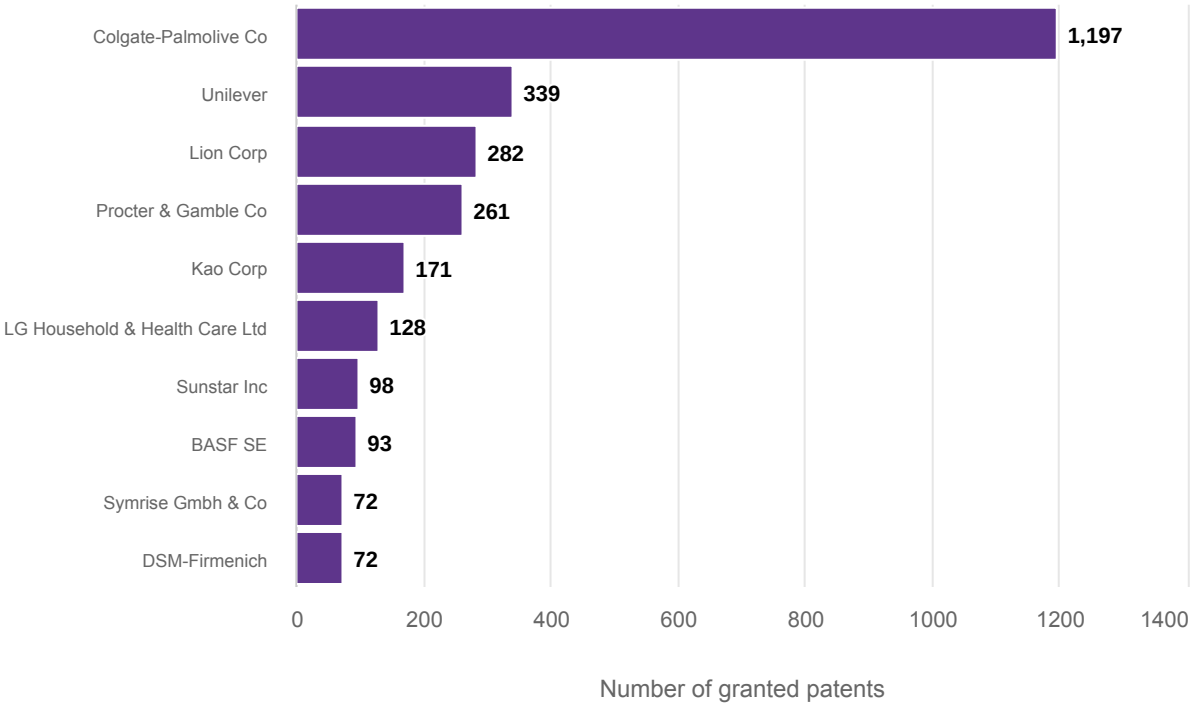


Colour shows the number of active individual patent grants in that country; the darker the colour, the more patents granted

Source: *Derwent Innovation/Mintel, February 2025*

Colgate-Palmolive Co holds the most granted patents in the oral care category

Global: top 10 organisations with granted patents for oral care products, 2025



Source: *Derwent Innovation/Mintel, February 2025*

Nurture a healthy oral microbiome

Promote oral health through a balanced oral microbiome

A balanced oral microbiome is essential for periodontal health, protecting against cavities, gum disease and bad breath. Dysbiosis can cause inflammation that may damage tooth-supporting structures, leading to tooth loss.

Prebiotic and probiotic oral care products naturally support a healthy oral microbiome by balancing beneficial and harmful bacteria. Probiotics are 'beneficial bacteria' that reduce the amount of harmful bacteria, while prebiotics nourish the body's existing 'beneficial bacteria'. Together, they help prevent cavities, gum disease and bad breath. Incorporating them via specialised toothpaste, mouthwash, lozenges or a prebiotic-rich diet enhances oral health and overall wellbeing.

Within global toothpaste and mouthwash launches, the share of probiotic claims grew from less than 1% of launches in 2020 to 3% in 2024, and prebiotic ones grew from almost zero to 1%.

Base: *Canada: 2,000 internet users aged 18+*

Source: *Kantar Profiles/Mintel, October 2023*

HEALTHY MICROBIOME

76%

of Canadian consumers agree maintaining a balanced oral microbiome is important

Enhance oral health with prebiotics

Sanogyl Biome Protect Gum Care Toothpaste (France)

Sanogyl Biome Protect Gum Care Toothpaste features the prebiotic ingredients alpha-glucan oligosaccharide and yacón root juice, which support the mouth's natural balance and defences to provide daily protection and strengthen teeth and sensitive gums.

Shengan Biotechnology Hefei Co has a pending patent for a prebiotic, antibacterial oral spray. It contains specific amounts of prebiotics, including fructo-oligosaccharide, polydextrose, stachyose and inulin, along with bacteriostatic agents such as tea polyphenol, epsilon-polylysine hydrochloride and lysozyme. The product helps maintain the oral flora balance while inhibiting harmful bacteria.

Source: *Derwent Innovation/Mintel*

Probiotic-powered oral care products deliver advanced dental health solutions

Henry Blooms Oral Health Advanced Whitening with Mouth Guard Probiotic Toothpaste contains PAB-PMGLP *Lactobacillus paracasei* to balance oral microflora, maintain pH, fight harmful bacteria, support gum health and ensure fresh breath.

Protefix Children's Orange Flavour Strengthen Teeth Toothpaste contains patented probiotics LC-86 and LA-85, which help combat harmful oral bacteria, improve the bacterial balance in the mouth and reduce plaque formation.

Henry Blooms Oral Health Probiotic Toothpaste (Malaysia)

Protefix Children's Orange Flavour Strengthen Teeth Toothpaste (China)

Recent advancements highlight the role of beneficial bacteria in oral care

Fujian Mengjiaolan Daily Chemicals Co has a pending patent for a children's toothpaste featuring probiotics (*L. paracasei*, *L. acidophilus* and *L. salivarius*) and prebiotics (like oligofructose, oligoxylose, oligogalactose and inulin), along with mild amino-acid-based surfactants and excipients. This formulation reduces harmful bacteria in the oral cavity, **preventing acid production**, **tooth erosion** and **dental caries**.

Colgate-Palmolive, in collaboration with the University of Ghent and Catholic University Leuven, patented an oral care composition containing N-acetyl-D-mannosamine (a prebiotic) and probiotics selected from *Streptococcus mitis*, *Streptococcus sanguinis*, *Veillonella parvula*, *Streptococcus gordonii* and *Actinomyces naeslundii*. The claimed composition promotes beneficial oral microbiota growth, **supports healthy biofilm formation** and helps **prevent conditions such as gingivitis, periodontitis** and caries.

Source: *Derwent Innovation/Mintel*

The growing 'skinification' trend in oral care

'Skinification' is a trend in which functionalities, ingredients and concepts originating in facial skincare are adopted by other BPC categories. It's now moving into the oral care category as brands start incorporating skincare ingredients like hyaluronic acid into products like toothpaste, not just for beautification but also for health reasons like gum protection.

Haleon Parodontax Daily Mouthwash (Turkey)

Harness the benefits of hyaluronic acid for gum health

Hyaluronic acid is a glycosaminoglycan renowned for its hydrating and anti-ageing benefits in skincare, and it has emerged in dental care. Naturally present in both soft and hard periodontal tissues, hyaluronic acid supports gum health by reducing inflammation and aiding tissue repair, making it effective in managing and preventing gum disease.

Fewer than 1% of global toothpaste and mouthwash launches contain hyaluronic acid, though the number of launches with it are growing.

Gum-related issues are a concern for many consumers. In China, 50% of consumers experience bleeding gums, 36% sensitivity in their teeth or gums and 35% gingivitis or swollen gums. These statistics highlight the growing need for targeted gum-health solutions.

Haleon Parodontax Daily Mouthwash features hyaluronic acid tostrengthen and firm gums for long-term gum protection.

Oral care innovations use hyaluronic acid to fight inflammation and repair gum tissue, redefining the gum health sector

Reduce inflammation

A PCT publication by Unilever details an oral care composition, such as toothpaste or mouthwash, that contains hyaluronic acid or its derivatives, combined with *Panax ginseng* root extract in a weight ratio ranging from 50:1 to 10,000:1. These ingredients in the claimed precise quantity help retain moisture in soft tissues such as gums, alleviate inflammation and promote oral tissue repair.

Repair oral mucosa

Givaudan SA is awaiting approval for a patent on an oral care composition designed to improve mucosal tactile properties. The proposed formulation includes hyaluronic acid with an average molecular weight of 500kDa or less, specifically between 100 and 300kDa, along with thymol α-glycoside as an active component. Hyaluronic acid aids the repair of oral mucosa, while thymol α-glycoside's antibacterial properties help reduce biofilm formation.

Source: *Derwent Innovation/Mintel*

Vitamin K is a promising ingredient for dental care products

Brands like L'Oréal discuss vitamin K's role in skincare, such as its ability to heal, reduce discolouration and improve elasticity. Vitamin K is now gaining attention in dental research. Emerging studies suggest vitamin K has the potential to support oral health by strengthening enamel and promoting bone density. Such research paves the way for vitamin K in innovative dental care applications, highlighting its importance for both oral and skin health.

IDS Research SRL has a pending patent for a teeth-whitening composition featuring a peroxide-based gel and a second gel with vitamin K, vitamin D and hydroxyapatite. This formulation effectively whitens teeth while being gentle on, protective of and safe for enamel and dentin. The formulation creates a regenerative environment through the **synergy of vitamins D and K with peroxides** to **protect and repair the structure of dental tissues** that have been exposed to whitening agents.

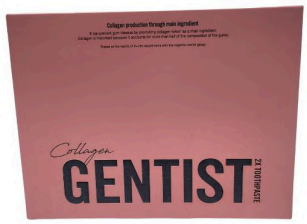
Source: *Derwent Innovation/Mintel*

Address common oral health challenges with collagen-based products

Colway Collagen Toothpaste is enriched with fish skin collagen. The toothpaste supports gum regeneration and helps prevent periodontitis and periodontal disease.

Gentist Collagen Toothpaste Gift Set contains black and pink collagen toothpastes, with charcoal and pomegranate, respectively. The brand claims collagen is important because it accounts for more than half the composition of the gums and it can prevent gum disease.

Colway Collagen Toothpaste - Regenerating & Whitening (UK)



Gentist Collagen Toothpaste Gift Set (South Korea)

Recent innovations use collagen to nourish gums and strengthen the bond between gums and teeth

Nutrabbit Co Ltd holds a patent for an oral hygiene composite that includes films, toothpaste, gum and mouth fresheners. The composite features collagen, *Ginkgo biloba*, vitamin C, tocopherol, lysozyme chloride, zinc and garlic extract to combat periodontal disease and bad breath. Collagen, a key protein, supports metabolism and **strengthens the connection between gums and teeth**.

Yuanhai Biology Dalian Co has a pending patent for toothpaste featuring active collagen derived from jellyfish. Macromolecules from the jellyfish collagen offer benefits such as **stain removal**, teeth whitening, **gum nourishment**, reduced sensitivity and improved tooth resistance. The toothpaste also provides antibacterial, anti-inflammatory and reparative effects for teeth and soft tissues.

Source: *Derwent Innovation/Mintel*

Link oral health to overall health

Consumers associate oral health with multiple facets of overall health and wellness

OVERALL HEALTH

83%

of Spanish consumers agree not looking after one's teeth can negatively impact their general health

GUT HEALTH

50%

of Chinese consumers believe a healthy gut can improve oral health

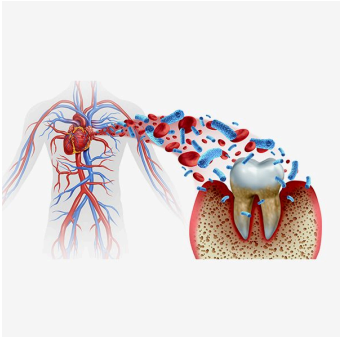
BRAIN HEALTH

55%

of Japanese consumers think maintaining oral health is important for maintaining brain health

Base: *Spain: 2,000 internet users aged 16+; China: 3,000 internet users aged 18-59; Japan: 2,000 internet users aged 18+*

Source: *Kantar Profiles/Mintel, March 2022; KuRunData/Mintel, March 2022; Rakuten Insight/Mintel, September 2023*



Oral hygiene is linked to heart health

Maintaining proper oral hygiene is not just about aesthetics – it's a fundamental aspect of heart health. Research consistently emphasises a strong link between oral and cardiovascular health. Neglecting oral care can lead to gum disease, which may trigger chronic inflammation and release harmful substances into the bloodstream, playing a role in the development of atherosclerosis and other heart-related conditions. Bacteria such as *Porphyromonas gingivalis* can migrate from the mouth to the bloodstream, causing additional inflammation and blood vessel damage, ultimately raising the risk of heart attacks and strokes.

Recent patents reveal the role of oral health in diagnosing and preventing heart disease

Early diagnosis of cardiovascular health

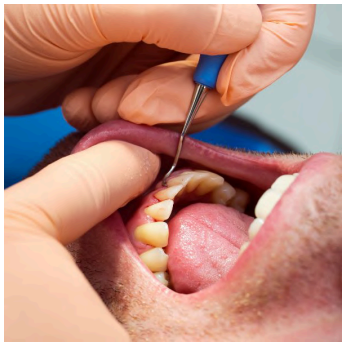
Colgate-Palmolive Co holds a patent for a method to monitor oral health by generating a user health profile. This involves a toothbrush equipped with a pressure sensor that is connected to an interactive display. The toothbrush assesses an individual's oral profile by detecting pathogens in the mouth, measuring body temperature and analysing saliva or breath samples. Additionally, this technique can evaluate oral health indicators linked to broader health risks, such as heart disease risk.

Heart health maintenance

Inventor Reshma N Kheraj has a pending patent for a method to enhance oral health using probiotics in oral care products. The probiotic strains are selected from *B. animalis*, *B. lactis*, *B. longum*, *B. bifidum*, *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. gasseri*, *L. plantarum*, *L. rhamnosus*, *L. salivarius*, *L. lactis* subsp *lactis*, *L. lactis* subsp *Lactis biovardiacetylactis*, *Leuconostoc pseudomesenteroides* and *S. thermophilus*. The composition supports the health, restoration and maintenance of oral health, while also contributing to heart health.

Source: *Derwent Innovation/Mintel*

Unlock the connection between oral and gut health



Oral health is closely linked to gut health through the oral-gut microbiome axis – issues in one can manifest in the other. Problems like gum disease or bad breath may indicate gut bacterial imbalances, such as those seen with conditions like inflammatory bowel disease. Oral bacteria such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis* have been found in the gut, where they may contribute to inflammation and dysbiosis. Emerging tools like saliva-based microbiome analysis test kits show promise as non-invasive systemic disease detectors, reinforcing the vital role oral health plays in overall wellbeing.

Recent patents cover advancements in using the oral microbiome and biomarkers to diagnose sleep and gut issues

Diagnose sleeping disorders and acid reflux

Paragon 28 Inc has a pending patent for an intra-oral device designed to monitor the pH levels of the mouth. The device features pH sensors connected to a microprocessor, a digital signal processor, a module for storing pre-determined pH data and a data interpretation unit. By analysing the oral pH, the device helps identify health conditions, such as sleep-related disorders, particularly those linked to the gastrointestinal tract, and acid reflux.

Evaluate the oral and intestinal flora

Lion Corp has a pending patent for a system that assesses health conditions by analysing the types of bacteria in an individual's mouth. After identifying the oral bacteria, the device provides insights into the intestinal microflora, thereby aiding in the evaluation of health conditions linked to intestinal flora.

Source: *Derwent Innovation/Mintel*

Mintel Spark develops ideas for incorporating heart and gut health benefits into oral care products

Concept 1: Dual Benefit Oral Care Chews

Concept 2: ProBioMint Toothpaste

Drawing on insights from this piece and Mintel's full library, Mintel Spark has generated two concepts with strong market potential that represent future trends.

Dual Benefit Oral Care Chews promote stronger teeth and heart health by combining calcium and fluoride with heart-friendly ingredients like odourless garlic extract and resveratrol.

ProBioMint Toothpaste contains probiotics and prebiotics to support oral and gut health.

These are NOT real products – they have been generated by Mintel's AI-powered concept generator to inspire innovation; generated product images may include example text and as such may contain misspellings or grammatical errors because the image has not been manually manipulated post-generation

Source: *Mintel Spark*

KEY TAKEAWAYS

Key takeaways

Nurture a healthy oral microbiome

Recent oral care innovations focus on the oral microbiome as key for dental and gum health. Probiotics and prebiotics support the oral microflora by promoting beneficial bacteria and suppressing harmful ones, thereby helping prevent tooth decay and gum disease – a fact reinforced by recent patents.

The growing 'skinification' trend in oral care

A fascinating shift in oral care is underway, with patents showcasing innovative uses of skincare ingredients such as hyaluronic acid, vitamin K and collagen for gum and teeth health. This redefining approach to oral care highlights the shared health needs of the skin and mouth, such as hydration, repair and structural support.

Link oral health to overall health

Research highlights a link between oral hygiene and other strands of health, like heart or gut health. Recent patents highlight the potential to diagnose or treat health issues through the analysis and treatment of the oral microbiome. Such advancements may enable preventive, precise and non-invasive diagnostic methods or treatments.

APPENDIX

Research methodology

Patent analysis methodology

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Consumer research methodology

Mintel continuously commissions consumer research around the globe for its syndicated research solutions. Research is carried out by a variety of research providers as detailed in the sources.

Innovation tracking methodology

Via a network of shoppers across 86 countries, Mintel's Global New Products Database tracks product launches in the food, drink, beauty and personal care, health and hygiene, homecare and pet markets.

Shape your future with Mintel bespoke patent analysis. To have a confidential chat or hear more about our custom capabilities in patent analysis, please contact:

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APAC: consultingapac@mintel.com

EMEA: consultingemea@mintel.com

Key patent examples for oral care products

Patent number	Owners	Title
CN118453445A	Shengan Biotechnology Hefei Co	Prebiotic oral care bacteriostatic agent and preparation method thereof
CN118304249A	Fujian Mengjiaolan Daily Chemicals Co	Children's toothpaste containing probiotics and prebiotics components and preparation method thereof
US11419806B2	Colgate-Palmolive; University of Ghent; Catholic University Leuven	Prebiotic oral care methods using a saccharide
WO2024068168A1	Unilever	Oral care composition comprising hyaluronic acid and *Panax ginseng* root extract
JP2023002644A	Givaudan SA	Improvements in or relating to organic compounds
EP4463128A1	IDS Research SRL	Teeth-whitening composition with vitamins

Source: *Derwent Innovation/Mintel*

Key patent examples for oral care products

Patent number	Owners	Title
KR2372384B1	Nutrabbit Co Ltd	Oral hygiene composition for preventing or alleviating periodontal disease and halitosis
CN118743640A	Yuanhai Biology Dalian Co	Toothpaste containing jellyfish active collagen and preparation method thereof
US10398538B2	Colgate-Palmolive Co	Method of monitoring oral health
US20240091135A1	Reshma N Kheraj (Inventor)	Compositions and methods for promoting oral health
US20240032824A1	Paragon 28 Inc	Systems and methods for intraoral pH monitoring
JP2021068239A	Lion Corp	Health state determination device, program, method and intestinal environment determination method

Source: *Derwent Innovation/Mintel*

Meet the expert

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Neha has expertise in patent searching and analysis. In her thirteen years of experience as a patent analyst, she has supported R&D teams at various FMCG, healthcare and biotech companies by providing intelligence on competitor activity and tracking patent technology and emerging innovation trends across the domains.

Read more by this expert | Get in touch

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i-COSMETIC Dentalcare Trends 2025

i-COSMETIC
THE NEW ACTIVE INGREDIENTS FOR DENTAL CARE PRODUCTS 2025

- **NANO CURCUMIN**

anti-inflammation

- **BETA GLUCAN**

enhancing cell proliferation, migration, and mineralization

- **ECTOIN**

oral mucosal coating agents

- **NANO HAp.** (Hydroxyapatite)

The benefits: Restored Natural Whiteness, Cavity Prevention, Enamel Remineralization, Dental Hypersensitivity Prevention, Smooth and Protected Tooth Surface.

Nano curcumin journal

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DOI: 10.1002/cre2.330

ORIGINAL ARTICLE

Clinical and Experimental Dental Research Open Access WILEY

Oral nano-curcumin on gingival inflammation in patients with gingivitis and mild periodontitis

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Abstract

Gingivitis can trigger gingival diseases such as periodontitis. Since the complete removal of microbial plaques by mechanical procedures is not conceivable in some conditions and also chemical mouthwashes have a lot of side effects, finding a new treatment strategy would be useful. In the present study, for the first time, the effects of oral nano-curcumin on gingival inflammation in patients with gingivitis and mild periodontitis were assessed. Forty eight patients with gingivitis and mild periodontitis participated in this clinical trial. In one group the patients were treated with Sina curcumin capsules 80 mg and the other group received a placebo. Clinical parameters, including modified gingival index, papillary bleeding index, and plaque index were determined on days 0, 7, 14, and 28. There were no significant differences in age, sex, papillary bleeding index (PBI), and modified gingival index (MGI) between the two groups at baseline. There was a dropout of two patients (both from the placebo group). The MGI and PBI have a significantly decreasing trend in both case and control groups and the decreases were severe in the case group. The differences between PBI and MGI in the two groups were significant at 14 and 28 days. The plaque index did not significantly change in either group over the study period. The trend of changes in plaque index was not different between the two groups of the study. In the current study, no side effect was found in the patients. Oral nano-curcumin has positive effects on the decrease of inflammation and gingival bleeding in patients with gingivitis and mild periodontitis. Nano-curcumin capsules have a systemic target site with more bioavailability than topical forms.

KEYWORDS

curcumin, gingivitis, nano-biomedicine, periodontitis

1 | INTRODUCTION

Epidemiological studies show that more than 82% of juveniles in the United States have gingivitis and gingival bleeding and a similar or higher prevalence of the disease has been reported in other countries. Gingivitis can trigger gingival diseases such as periodontitis (Farjana, Chandrasekaran, & Gita, 2014).

Periodontal infections can increase the risk of coronary heart diseases, diabetes mellitus, and preterm delivery (Liccardo et al., 2019; Saini, Saini, & Saini, 2010). Gingivitis is the most

common form of gingival diseases caused by dental plaque (Farjana et al., 2014).

The critical role of dental plaque in the progression of dental caries, gingival inflammation, and periodontitis has been addressed in the literatures. Therefore, the control and elimination of dental plaque is an essential step in the prevention of periodontal diseases (Farjana et al., 2014).

The control of dental plaque through mechanical procedures is a necessary step in the control of gingivitis and periodontitis. Mechanical removal of gingival plaque is an efficient method for plaque control and gingival inflammation. Because of irregularity and incorrect toothbrushing and tooth-flossing techniques, a large percentage of the population suffer from periodontal diseases (Christie, Claffey, & Renvert, 1998; Nogueira-Filho, Toledo, & Cury, 2000; Yeturu, Acharya, Urala, & Pentapati, 2016).

Removal of this microbial layer in the advanced stages is doable only by a general dentist, gingival surgeon, or with the aid of special equipment. However, complete elimination of microbial plaque and stimulant agents is not possible solely by employing mechanical procedures (Anuradha et al., 2015; Christie et al., 1998; Nogueira-Filho et al., 2000; Yeturu et al., 2016).

Chemical approaches, such as mouthwashes, can be used as an adjunct to mechanical microbial plaque control. Although chemical mouthwashes, including chlorhexidine, triclosan, and phenolic agents decrease microbial plaque, they have number of side effects such as allergic reactions and change in tooth color and taste sense (Farjana et al., 2014).

Traditionally, herbal drugs have been used in the treatment of various diseases (Nagpal & Sood, 2013). Turmeric is one of the biocompatible plants that is shown to have a therapeutic effect on recurrent aphthous stomatitis, oral lichen planus, gingivitis, and periodontitis (Deshmukh & Bagewadi, 2014; Farjana et al., 2014; Lv, Chen, Wang, Yao, & Yao, 2018; Nagpal & Sood, 2013). Turmeric is a member of the ginger family and it is extracted from the rhizomes of the *Curcuma longa* Linn plant. Turmeric is a yellow flavored spice that can grow approximately up to a height of 1 meter, has spear-shaped leaves, and its orange pulp grows in the rhizomes of the plan (Izui et al., 2016; Kunnumakkara et al., 2017; Samal, 2017; Shah, 2017; Zou, Helson, Maitra, Stern, & McNeil, 2013).

The active component of Turmeric is curcuminoid. Ninety percent of curcuminoid is in the form of curcumin (diferuloylmethane) and the remaining of the 10% is in the form of demethoxy curcumin and bis demethoxy curcumin (Farjana et al., 2014).

Curcumin extracted from the *Curcuma longa* Linn plant is a polyphenolic component and generally is known as turmeric. Curcumin exerts its anti-inflammatory effects by inflammatory pathways regulations and transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB), activator protein1 (AP-1), and mitogen-activated protein kinase (MAP Kinas) (Farjana et al., 2014; Guimarães et al., 2011).

Previous studies showed that the local uses of curcumin gel decreased gingival inflammation and improved the severity of disease. Also, there is evidence that supports curcumin effectively prevents the activation of inflammatory mediators and has therapeutic effects on periodontal diseases (Farjana et al., 2014; Guimaraes-Stabili et al., 2019).

Curcumin in non-nano-formulated products, could stain the teeth and mucosa through local use and have low bioavailability (Jacob, Wu, Zhou, & Wang, 2007; Karabasz et al., 2019; Noorafshan & Ashkani-Esfahani, 2013).

Regarding the lipophilic nature of curcumin, the oral absorption of curcumin in its usual form (powder, tablet, and capsule) is very low. However, in nano-curcumin products, all curcumin is trapped in hydrophobic nanomicelles. These spherical shape nanomicelles are ~10 nm in size and increase the solubility of curcumin in water. After oral consumption, the soft capsules containing curcumin nanomicelles are dissolved in the acidic medium of the stomach in less than 15 minutes. The nanomicelles are stable in the acidic medium of the stomach for at least 6 hours and are transferred to the small intestine without a change in their primary form. In the small intestine, the nanomicelles facilitate the transport of curcumin across the epithelial cells of the small intestine, a barrier against lipophilic substances, and increase the absorption of curcumin when prescribed orally (Muglikar, Patil, Shivswami, & Hegde, 2013; Staff TP, 2004). Curcumin is transferred to other tissues by the bloodstream after absorption in the intestine. Because the inflamed areas have more blood supply (angiogenic response), curcumin can easily reach to the inflamed gingiva tissue (Pober & Sessa, 2015).

Finally, regarding the high prevalence of inflammatory diseases, the side effects of chemical mouthwashes, inflammatory nature of gingivitis and periodontitis, and anti-inflammatory effects of curcumin, in the present study, the effects of nano-curcumin capsules on gingival inflammation were investigated in patients with gingivitis and mild periodontitis. It was hypothesized that the indices of inflammation in the before and after treatment in the nano-curcumin group would be different from those in the placebo group.

2 | MATERIALS AND METHODS

This double-blind randomized clinical trial was performed on patients who have been referred to the Periodontics Department. To determine the sample size concerning the repetition of the sizes in the two groups for each individual, the effect of each individual was to be taken into account. The formula for duplicate sizes is used to do this. Considering four replications, a correlation of 0.50, the statistical power of 95%, error level of 0.05, and variance obtained from previous studies equal to 0.09 (Anuradha et al., 2015), the minimum sample size was calculated as 24 patients for each group.

$$n = \frac{2\left(z_{\frac{\alpha}{2}} + z_{\beta}\right)^2 \sigma^2 (1 + (1-m)\rho)}{md^2} = \frac{2(1.96 + 1.64)^2 0.09(1 + (3)0.5)}{4(0.25)^2} = 23.33 \cong 24.$$

By taking into consideration the possible loss of some patients, there were 50 patients included in the study. For randomization R software version 3.4.3 was used. The inclusion criteria were as follows: aged between 16–60 years, having at least 20 teeth, a clinical

sign of generalized plaque-induced gingivitis and mild periodontitis (bleeding on probing ≥30%) with CAL 1–2 mm and probing depth ≤ 3, and no history of periodontal treatment in the past 6 months (Trombelli, Farina, Silva, & Tatakis, 2018). Patients who were allergic to turmeric, patients with a history of gallstone and/or biliary obstruction, patients with increased stomach acidity and/or active gastrointestinal ulcer, patients with medication use in the past 3 months, pregnant women, patients with systemic, liver or immunosuppressive diseases, patients with simultaneous use of anticoagulants and antiplatelet, and smokers were excluded from the study. This clinical trial study was approved by the research ethics committee of the university. Registry code in the Primary WHO clinical trial registry center was IRCT 20180416039327N2. Informed consent was obtained from all subjects.

The study participants were randomly classified into two groups as the control and study groups. The demographic data of study subjects including age, sex, medical history, and history of smoking were collected through a prepared questionnaire. All patients were examined on the first day and the plaque index (PI) (Pers et al., 2005), modified gingival index (MGI) (Gomes, Rekhi, Meru, & Efficacy, 2019), and papillary bleeding index (PBI) (Pers et al., 2005) were determined in the subjects. Williams's probe (Hu-Friedy) was used for examinations. Then, one group was treated with nanomicelles curcumin soft gel capsules (Sina curcumin, provided by ExirNanoSina Company, Tehran, Iran) 80 mg, once per day after breakfast for 4 weeks and the other group received placebo. Since the teeth and mucosa can be stained through local use of curcumin gel, due to the very low solubility of curcumin in hydrophilic solvents and for increasing its bioavailability, we used nano-curcumin capsules in the present study. The shape, size, and packing of capsules in both groups were identical. So neither the dentist nor the patients were aware of the type of treatment. The rolling technique was demonstrated to all patients and they were asked not to use other mouthwashes and turmeric in food during the study. MGI, PBI, and PI were assessed for all patients on days 0, 7, 14, and 28 (Mishra et al., 2019; Penmetsa, Vivek, Bhupathi, & Sudha, 2019). At the end of the study, dental scaling was done for all patients to remove dental tartar.

Statistical analysis: SPSS version 24.0 (IBM Corp., Armonk, NY) was used to perform statistical analysis. All results were reported as frequency and mean and standard deviation (mean ± *SD*). Repeated measure analysis of variance was used to assess the time-dependent changes in the MGI, PI, and PBI during the study period in the study groups. To compare the two groups each time, an independent *t*-test was applied. Also, the posthoc Bonferroni test was used to explore the differences between the different times in each group.

3 | RESULTS

Patients included in the study are shown in the CONSORT Flow Diagram (Figure 1). Basic characteristics and clinical indexes at baseline are shown in Table 1. The dropout of two patients (both from the placebo group) was due to the patient's lack of follow-up. Between the two groups there were no significant differences in age, sex, PBI, and

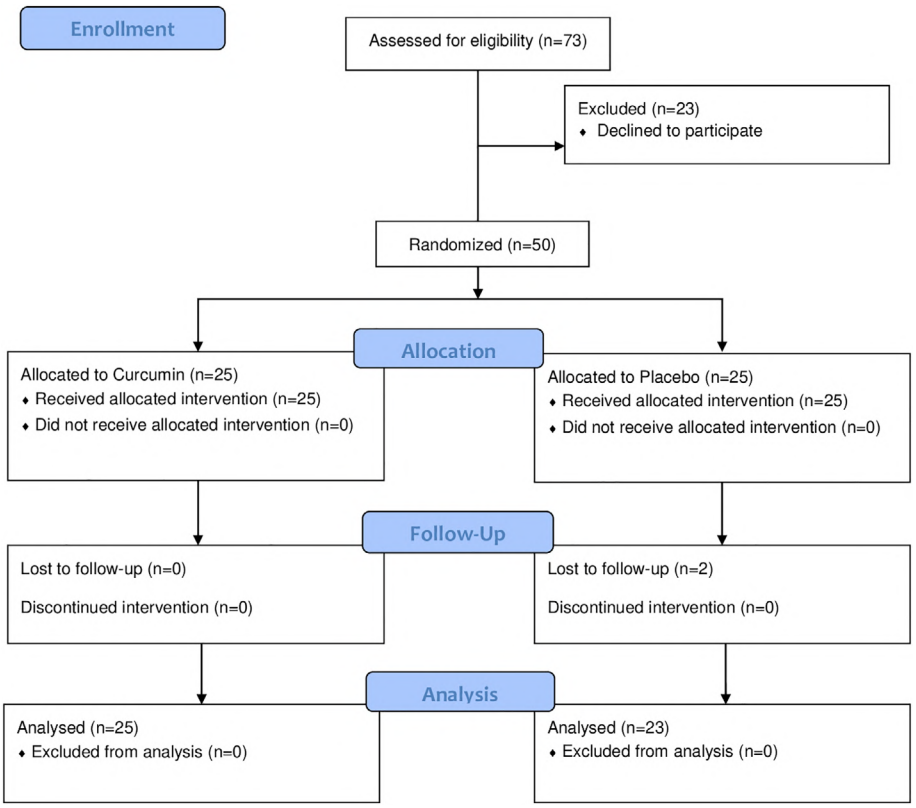

FIGURE 1 CONSORT flow diagram

TABLE 1 Basic characteristics and clinical indexes at baseline

	Curcumin (*N* = 25)	Placebo (*N* = 23)	*p*-value
Age (mean ± *SD*) (minimum-maximum)	30.96 ± 10.10 (16–54)	35.04 ± 11.40 (18–58)	.154
Sex (F/M)	17/8	12/11	.263
Modified gingival index	2.15 ± 0.55	1.95 ± 0.55	.200
Papillary bleeding index	2.10 ± 0.47	1.84 ± 0.58	.089

TABLE 2 The results of PBI in case and control groups during 28 days of follow-up

Time	Group	Mean ± *SD*
Before treatment	Control	1.84 ± 0.58
	Case	2.10 ± 0.47
7th day	Control	1.79 ± 0.57
	Case	1.81 ± 0.47
14th day	Control	1.73 ± 0.56
	Case	1.36 ± 0.43
28th day	Control	1.68 ± 0.57
	Case	1.01 ± 0.38

MGI, so intervention and control groups were matched in these parameters. Statistical analysis showed that the papillary bleeding index has a significant decreasing trend over the study period in the case and control groups ($p < .001$). The trend of changes in PBI was different between case and control so that the rate of reduction in PBI in the case group was greater than the control group ($p < .001$) (Table 2). In Table 3 the comparison of PBI between the two groups is shown at each studied time. The differences were significant at 14 and 28 days.

Also, statistical analysis showed that the MGI has a significant decreasing trend over the study period in the case and control groups ($p < .001$). The trend of changes in MGI was significantly different between the two groups of the study ($p < .001$) (Table 4). In Table 5 the comparison of MGI between the two groups is shown at each studied time. The differences were significant at 14 and 28 days.

Regarding the randomization of the patients into two groups, the PI was significant in both groups at baseline. However, the PI did not significantly change in either group over the study period ($p = .582$). The trend of changes in PI was not different between the two groups of the study ($p = .994$).

4 | DISCUSSION

Gingivitis is a form of periodontal disease that has a high prevalence and usually precedes periodontitis (Farjana et al., 2014). Although the progression of periodontitis is not predictable, its prevention in the earlier stage is still the first step toward preventing periodontitis (Farjana et al., 2014).

TABLE 3 Comparison of PBI between the two groups

Time	0	7	14	28
Time statistics	1.74	0.11	2.56	4.80
p-value	.089	.915	.014	<.001

TABLE 4 The results of MGI in case and control groups during 28 days of follow-up

Time	Group	Mean ± *SD*
Before treatment	Control	1.95 ± 0.55
	Case	2.15 ± 0.55
7th day	Control	1.88 ± 0.55
	Case	1.86 ± 0.53
14th day	Control	1.84 ± 0.54
	Case	1.46 ± 0.45
28th day	Control	1.80 ± 0.53
	Case	1.08 ± 0.45

TABLE 5 Comparison of MGI between the two groups

Time	0	7	14	28
Time statistics	1.30	0.13	2.67	5.13
p-value	.200	.895	.010	<.001

In this study, an attempt was made to clarifying the effects of oral nano-curcumin in gingivitis and mild periodontitis treatment, it was shown that oral nano-curcumin has positive effects on the decrease of inflammation and gingival bleeding in patients with gingivitis and mild periodontitis. In line with our study findings, the anti-inflammatory and antioxidant effects of curcumin have been proven in previous studies (Nagpal & Sood, 2013). Also, in the investigation of the impacts of sex and age (>30 years and <30 years) on the effectiveness of curcumin, it was demonstrated that the age and sex have no significant effects on curcumin efficacy.

Given that the study aimed to investigate the effect of adjuvant therapy, the classifying was based on the type of required treatments. Patients who did not require surgical treatment for periodontal disease were entered in the study. Therefore, patients with gingivitis and mild periodontitis were included in the study.

Turmeric includes protein (6.3%), fat (5.1%), minerals (3.5%), curcuminoid (5%), volatile oil (5%), sesquiterepene (alcohol and ketone) and monoterpene (25%). Rhizomes of the *Curcuma longa* Linn

plant include arabinose (1%), fructose (12%), glucose (2%), and zinciferous starch grains. The rhizomes also contain curcuminoid, demethoxy curcumin, 5′-methoxycurcumin, and dihydro curcumin, which have antioxidant properties (Zou et al., 2013).

Curcumin, a main component of turmeric, is inexpensive and available and its antioxidant, anti-inflammatory, antimicrobial, anti-pain, anti-decay, anti-biofilm, and anti-cancer effects have been shown in previous studies in the literature. Also, curcumin has protective effects on the liver and kidneys, is an inhibitor of blood coagulation, and prevents myocardial infarction. Turmeric, as an anti-inflammatory agent, is traditionally used in the management of several diseases. Curcumin improves the performance of the immune system, increases the conservation of the cardiovascular and nervous systems. Also, turmeric extraction prevents the growth of the different strains of pathogenic bacteria and fungi in the mouth and prevents the formation of microbial plaque on the teeth (Corrêa et al., 2017; Nagpal & Sood, 2013; Samal, 2017; Sambhav, Rohit, Ankit Raj, & Garima, 2014; Shah, 2017; Singhla, Tevatia, Chaudhry, Vaish, & Dodwad, 2017; Vaughn et al., 2017).

In the current study, it was concluded that oral nano-curcumin, due to its anti-inflammatory effects, can be used as a complementary therapy for gingivitis and mild periodontitis. With respect to our study results, Anuradha et al. investigated the effects of turmeric on patients with localized or generalized chronic periodontitis and showed an anti-inflammatory effect of turmeric gel. However, they also showed that turmeric gel significantly decreases PI, which was not compatible with our study results (Izui et al., 2016).

Our results were consistent with the study reported by Farjana et al. However, they used the gel form of the *Curcuma longa* plant and had fewer participants compared to our study. Also, the reduction in the PBI in our study was greater than the study reported by Farjana et al, which may be attributed to the use of a different type of turmeric. In other words, the study utilized a topical form of turmeric and therefore its target site is only a local area, while nano-curcumin capsules have a systemic target site with more bioavailability (Farjana et al., 2014).

Bharat et al. showed that the prevalence of chronic diseases has decreased in people who use curcumin in their daily meals. Although various medications such as steroids, NSAIDs, and chemical mouthwashes are used in the treatment of inflammatory disease, most of them have side effects, especially in long term therapy. Curcumin has a long-established safety record. In a few studies with high dose levels, some side effects have been reported such as diarrhea, headache, rash, and yellow stool. In the current study, no side effect was found in the patients (Aggarwal & Harikumar, 2009; Hewlings & Kalman, 2017).

Also, Chainani et al. showed that curcumin is a safe component with anti-inflammatory, anti-fungi, anti-viral, and antioxidant effects. In the Chainani et al study similar to the Muglikar et al. study; it was shown that curcumin, besides its mechanical therapeutic strategies, can be used as a complementary therapy to reduce inflammation, but not plaque. Also, Muglikar et al. reported that the anti-inflammatory effects of curcumin mouthwashes are similar to that of 0.2% chlorhexidine mouthwashes. However, two studies by Gottumukkala et al. and Jalaluddin et al. have reported that 0.2% chlorhexidine mouthwashes have grater effects on clinical parameters such as PI, gingival index, and bleeding on probing index than 1% curcumin mouthwashes. Also, the effects of 1% curcumin mouthwashes on the clinical parameters were much lower than those found in our study. This may be attributed to the low dosage of curcumin and the use of curcumin in the form of mouthwashes. Therefore, it is suggested that a higher dosage of curcumin be used to achieve better results (Chainani-Wu, 2003; Gottumukkala, Sudarshan, & Mantena, 2014; Jalaluddin et al., 2019; Muglikar et al., 2013). In the current study water solubility, absorption in the gastrointestinal tract, and prolonged plasma half-life were improved by the formulation of curcumin as nano-curcumin capsule form.

Since the possibility of Hawthorne-effect could exist in both intervention and control groups, in the present study, it was not considered as a bias. One of the limitations in our study was the evaluation of solely the clinical outcomes; so, further studies are required to evaluate the therapeutic effects of nano-curcumin on gingivitis and periodontitis by the means of assessing inflammatory mediators such as TNF-α and interleukins. Periodontitis is one of the major biofilm-induced inflammatory diseases. Currently available treatments are not always successful. In the current study the positive effect of curcumin with its anti-inflammatory effect was approved but, extensive research efforts are needed, and additional anti-inflammatory approaches should be investigated to improve treatment efficacy(ref). Also, microbiological analysis of biofilm can be one of the goals of future studies in such treatments. It is also recommended that long term follow-ups be used in future studies to better compare therapeutic effects.

5 | CONCLUSION

Curcumin is traditionally used for the treatment of several diseases. According to the present study, oral nano-curcumin, due to its anti-inflammatory effects, can be used as a complementary therapy for gingivitis and mild periodontitis.

CLINICAL RELEVANCE

The scientific rationale for the study

Gingivitis can trigger gingival diseases such as periodontitis. Complete elimination of microbial plaque and stimulant agents is not possible sloly by mechanical procedures. Nowadays innovated herbal drugs, due to their effectiveness and absence of side effects, are being taken into consideration for the treatment of many disorders.

Principal findings

The rate of reduction in PBI and MGI in the case group was significantly greater than the control group.

Practical implications

Oral nano-curcumin, due to its biological effects, can be used as a complementary therapy for gingivitis and mild periodontitis.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Meisam Malekzadeh and Seyed Javad Kia conceived and designed research. Leila Mashaei and Seyed Javad Kia conducted experiments. Mahdieh-Sadat Moosavi analyzed data and was the main author in writing the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Nanocurcumin in Oral Squamous Cancer Cells and Its Efficacy as a Chemo-Adjuvant

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Abstract

Oral squamous cell carcinoma is the sixth most common cancer worldwide. Despite the available treatment, the survival rate is poor. The addition of agents to make chemotherapeutics safer and more effective is important. Curcumin is a common Indian spice that has shown anticarcinogenic properties. It has been possible to overcome its poor bio-availability using nanotechnology. We aimed to investigate the adjuvant effect of nanocurcumin (NC ~ 200 nm size) treatment on cetuximab (epidermal growth factor receptor inhibitor) in oral squamous cancer cells (KB 3-1 cell). Cancer cells were cultured and treated for 24 hours with cetuximab and NC, in various doses to find the drugs' half-maximal inhibitory concentration (IC_{50}).

Experiments were conducted with a combination dose of both and sensitization treatment with NC before cetuximab with cytotoxicity assessment by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. One-way analysis of variance (ANOVA) was used to compare different treatment groups. We found a concentration-dependent cancer cell death with NC, which was significant compared to cetuximab ($p < 0.001$). The combination treatment group had highly significant cell death ($p < 0.0001$) compared to a single drug, and the NC sensitization caused substantial cell death compared to a single cetuximab treatment ($p<0.01$). Our study findings indicate the potential chemo-adjuvant effect of NC in oral cancer.

Categories: Oncology, Oral Medicine
Keywords: curcumin, cytotoxicity, adjuvant therapy, cetuximab, nanocurcumin, oral cancer

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer. It constitutes 5% of all malignant tumors globally and 45% of all malignant tumors in India, with less than 50% five-year survival [1]. In the Globocan report of 2020, for India, lip and oral cavity cancer incidence is 10.3% [2]. The development of OSCC has been attributed to multiple risk factors like tobacco use, alcohol consumption, infections with agents like Epstein-Barr virus, and Human papillomavirus, which are highly prevalent in India [3]. The treatment for OSCC requires a multi-modal therapeutic approach with surgery, radiotherapy, and with or without chemotherapy [4]. Cetuximab, an epidermal growth factor receptor (EGFR) monoclonal antibody, is a form of targeted therapy, that has increased treatment efficacy and improved overall survival but has shown significant adverse effects and development of drug resistance [5]. Hence use of combination therapy to make chemotherapeutics more effective is the need of the hour.

Curcumin is a phytochemical isolated from the turmeric plant (Curcuma longa). It has been reported to have multiple therapeutic properties such as antiviral, anti-inflammatory, antioxidant, antitumor, antimicrobial, cardioprotective, anti-arthritic, chemopreventive, and anticarcinogenic properties [6]. Studies have shown the therapeutic role of curcumin in various cancers, including oral cancer. Curcumin acts on numerous molecular targets like signal transducer and activator of transcription 3 (STAT3), activator protein 1 (AP-1), protein kinase B (PKB), notch homolog 1 translocation-associated (Drosophila) (Notch1), nuclear factor κβ (NF-κβ), wingless and int1 (Wnt) and mitogen-activated protein kinase (MAPK) [6]. Epidermal growth factor receptor and its downstream signalling pathways play a key role in oral squamous carcinoma pathogenesis and are inhibited by curcumin [7]. Despite its benefits, multiple factors often limit the practical application of curcumin, such as physicochemical instability, rapid metabolism, low pharmacokinetics, and bioavailability. Nevertheless, these barriers are solved by nanotechnology and using nanoformulations of curcumin [8]. Different approaches to curcumin can improve its physicochemical characteristics and enable its efficient use. For that purpose, formulations including liposomes, nanoparticles, micelles, and phospholipid complexes have been described in the reference sources [9]. In epithelial-type cancers, nanocurcumin formulations have shown favorable results adding to the evidence of its therapeutic role in cancer treatment as an adjuvant [10,11]. This study aimed to assess the adjuvant effects of nanoparticle nanocurcumin (NC) on cetuximab treatment in OSCC cells.

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Materials And Methods

This study to evaluate the efficacy of nanocurcumin (NC) as a chemo-adjuvant for oral cancer was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Bhubaneswar, India (approval No. IEC/AIIMS BBSR/PG THESIS/2018-19/15).

Cell culture

Human oral cancer cells (KB 3-1 cell) from National Centre for Cell Science (NCCS), Pune, India, were cultured in T25 flasks. Complete media (CDMEM) used for cell culture was prepared with Dulbecco's Modified Eagle Medium (containing 4.5gms glucose/litre, sodium bicarbonate, and sodium pyruvate with L-glutamine) along with 10% fetal bovine serum and 1% antibiotic antimycotic solution (10,000U penicillin, 10mg streptomycin, 25 µg amphotericin B per ml in 0.9% saline). Cells were incubated at 37°C with 5% CO_2, and 95% humidified air in the CO_2 incubator. All the experiments were done in triplicates, and each independent experiment was repeated thrice.

MTT assay

The live cells with an active metabolism, when treated with 3-(4,5-dimethylthiazol-2-yl-2),5-diphenyltetrazolium bromide (MTT), convert MTT into a purple-colored formazan crystal with an absorbance of 570nm. On dying, the cells lose their ability to convert MTT into formazan, and hence color formation functions as a convenient marker of only the viable cells. The mechanism of MTT reduction into formazan likely involves a reaction with nicotinamide adenine dinucleotide hydride (NADH) or similar, reducing molecules that transfer electrons to MTT [12].

A stock solution of MTT reagent was prepared of 5mg/mL using reagents from the kit. The stock was syringe filtered and kept in a (-)20°C fridge for further use. For cell viability assay, 0.5mg/mL MTT reagent was prepared fresh every time from the stock solution with CDMEM. After treatment, media was changed from all wells, including control wells, and 0.5mg/mL of 100µL MTT reagent was added to all the wells in the 96 well plates. The plate was kept in the dark inside the CO_2 incubator for two hours and then observed under a phase-contrast microscope (10x). The 96 well plates were taken out and placed in a horizontal shaker for about 10 minutes before taking a reading in the i-control microplate reader software (Tecan, Switzerland).

According to the MTT cell assay kit, specific absorbance (Abs) = [Abs (570nm) test - Abs (570nm) blank] - Abs (630nm) test

Cell viability was calculated as: Cell viability (%) = [O.D.(test)−O.D.(blank) / O.D.(control)−O.D.(blank)] × 100

(O.D. = optical density)

Treatment with cetuximab

A 2mg/ml cetuximab stock solution was prepared with 0.9% saline. Sequential doses of cetuximab were prepared from the stock solution (250, 500, 750, and 1000µg/mL) with CDMEM each time before treatment of the cells. Cells were harvested at 70% confluency from the T25 flasks, counted using a hemocytometer, and plated as 100µL of $1x10^4$ cells/well in flat bottomed 96 well plates in triplicates. The plated cells were incubated overnight in the CO_2 incubator. The following day media was removed, and they were treated with serial doses of cetuximab of 100µL volume to each well. In the control wells, only media was changed. After 24 hours, cells were treated with MTT reagent following the manufacturer's guidelines as stated above, and absorbance was measured using an i-control microplate reader machine at wavelength 570nm with reference wavelength 630nm. The half-maximal inhibitory concentration (IC_{50}) of cetuximab was calculated.

Treatment with NC

Nanocurcumin stock solution of 50mM concentration was prepared by dissolving in 0.5M sodium hydroxide and an immediate dilution in phosphate-buffered saline (1X). The stock's serial doses of NC were prepared in CDMEM of 5, 25, 50, 75, and 100µM. Like the cetuximab treatment group, cancer cells were plated, and the following day they were treated with serial doses of NC. After 24 hours of treatment, an MTT assay was performed, following which IC_{50} of NC was calculated.

Combined treatment with cetuximab and NC

The KB 3-1 cells were plated and the following day treated with IC_{50} dose of cetuximab, NC separately, and a combination of NC and cetuximab. After 24 hours of treatment, absorbance was measured following treatment with MTT reagent similarly as above.

Sensitized by NC before cetuximab

The KB 3-1 cells were plated and cultured for the subsequent experiments. One group was pre-treated with NC (at IC_{50} dose) overnight and the following 24 hours with cetuximab (at IC $_{50}$ dose). Another group was treated only with 24 hours of cetuximab (IC_{50} dose). After drug treatment, absorbance was measured in the same way.

Statistical analysis

Categorical variables were summarized as frequencies (percentages). Tukey's post-hoc tests were used to compare categorical variables. One-way ANOVA was used to compare different treatment groups. The results are presented as mean with standard deviation (SD). The IC_{50} was calculated using a non-linear regression model. All tests were two-tailed, and statistical significance was determined if the p-value was <0.05. All statistical analyses were performed with GraphPad Prism 8.4.2 software (GraphPad Software Inc., San Diego, CA, USA).

Results

Our study found that treatment with sequential doses of cetuximab for 24 hours had cytotoxic effects on KB 3-1 cells in a dose-dependent manner. The cytotoxicity for the doses 250µg/mL, 500µg/mL, 750µg/mL and 1000µg/mL was 38.2%, 67.5%, 74.8% and 80.7%, respectively (Figure *1*).

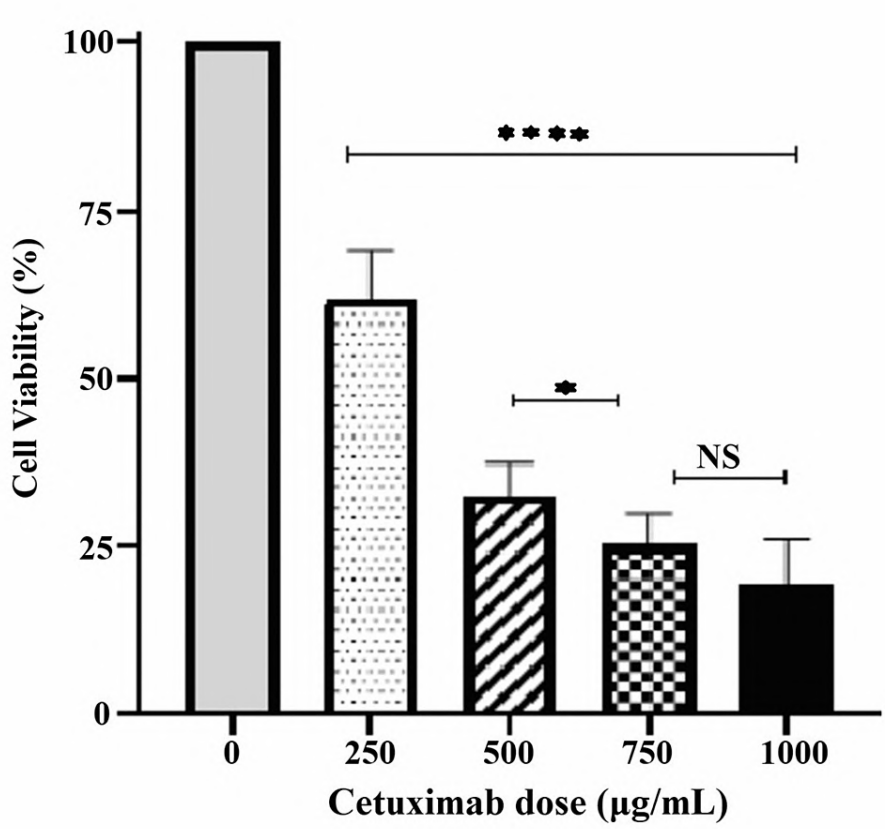

FIGURE 1: Cytotoxic effect of 24-hour cetuximab treatment

The graph shows the cytotoxic effect of cetuximab on KB cell lines after 24 hours of treatment. All doses were significant compared to the control (p<0.0001). From the dose of 750µg/mL onwards the drug cytotoxicity plateaued as there was no significant cell death between the doses of 750µg/mL and 1000µg/mL.

NS: Not significant

The cytotoxicity observed was significant at all the sequential doses compared to control (p <0.0001), with a plateauing effect after 750µg/mL. The IC_{50} of cetuximab was calculated to be 335.1µg/mL (Figure *2*). For further experiments we used 330µg/mL as cetuximab IC_{50}.

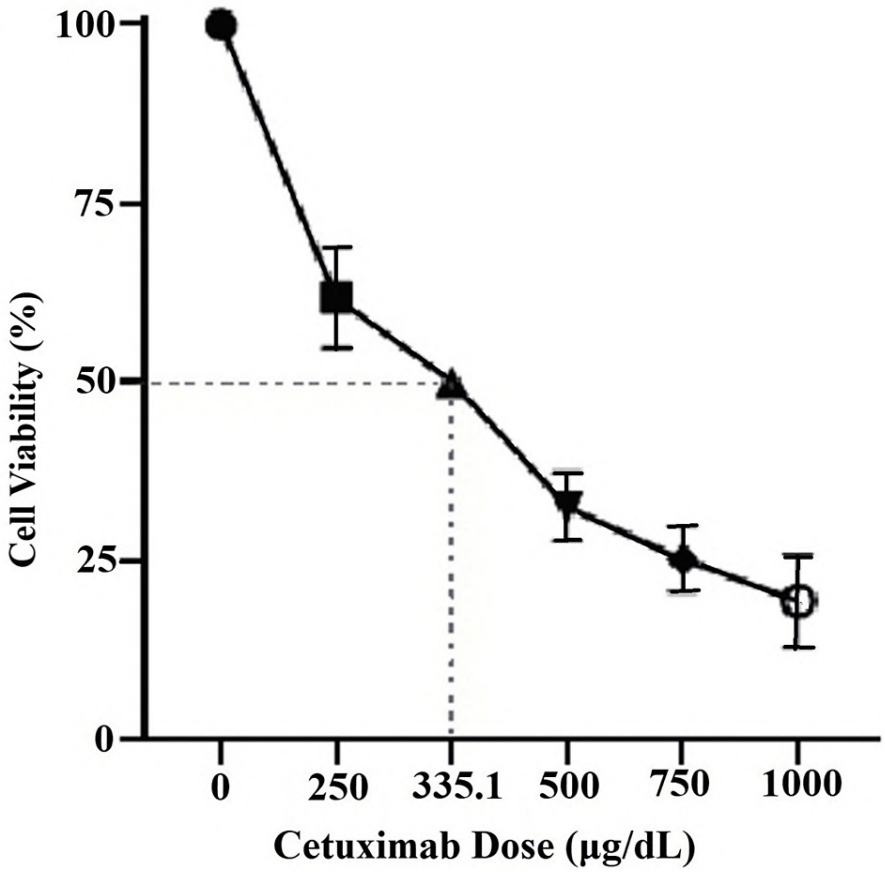

FIGURE 2: Dose-response curve of 24-hour cetuximab treatment

The graph shows the cell viability (%) of the KB cells treated with cetuximab for 24 hours. There was a decline in cell viability observed in a dose-dependent manner. Drug's IC_{50} was calculated using GraphPad Prism software and plotted on the dose-response curve to be 335.1µg/mL.

Similar dose-dependent cytotoxic effects were seen with sequential doses of NC. The cytotoxicity for the doses 5µM, 25µM, 50µM, 75µM and 100µM was 24.0%, 76.8%, 85.9% and 90.7%, respectively (Figure *3*).

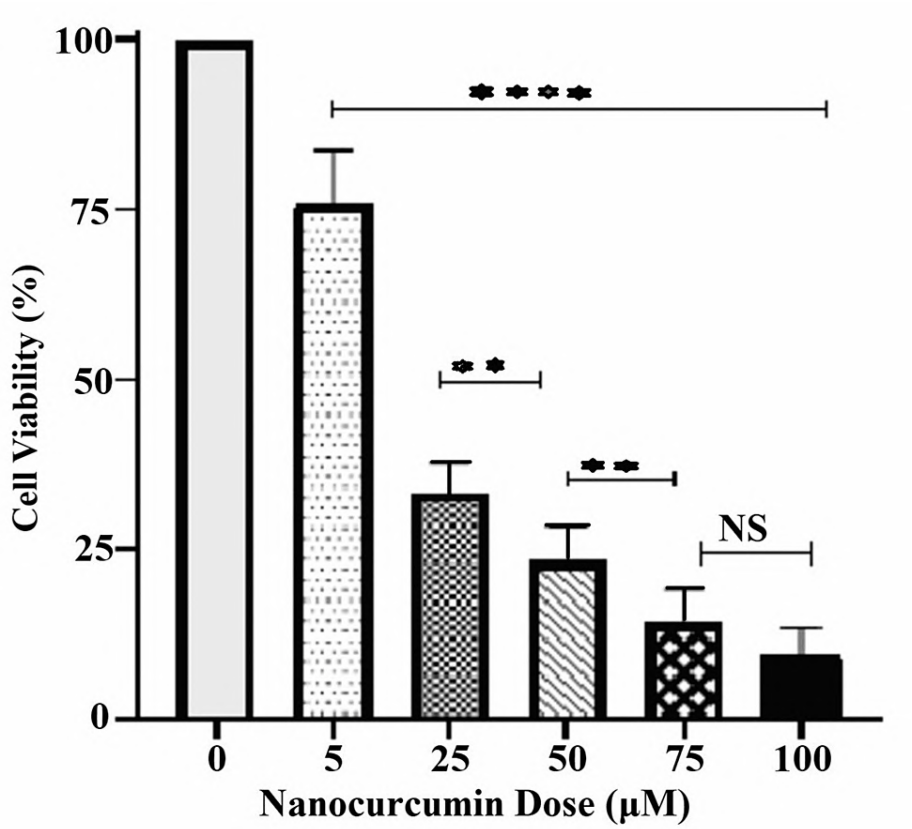

FIGURE 3: Cytotoxic effect of 24-hour nanocurcumin treatment

The graph shows the cytotoxic effect of nanocurcumin (NC) on KB cells lines with 24 hours treatment. All doses were significant compared to control ($p<0.0001$). From the dose 75µM onwards the drug cytotoxicity plateaued as there was no significant cell death between the doses 75µM and 100µM.

NS: Not significant

The cytotoxicity was significant at all doses. The IC_{50} of NC was calculated to be 14.14µM (Figure *4*). For further
experiments we used 15µM as NC IC_{50}."

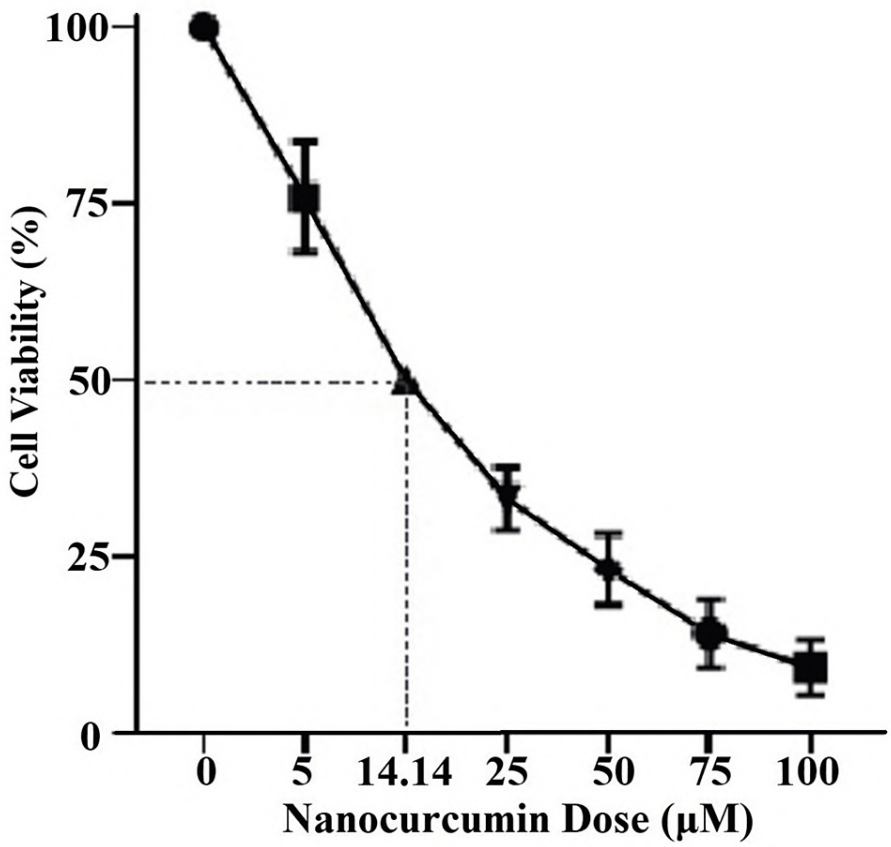

FIGURE 4: Dose-response curve of 24-hour nanocurcumin treatment

The graph shows the cell viability (%) of the KB cells treated with nanocurcumin for 24 hours. There was a decline in cell viability in a dose-dependent manner. The IC_{50} of nanocurcumin was calculated using GraphPad Prism software and plotted on the dose-response curve to be14.14µM.

In the combination treatment experiments, we found the cytotoxicity of the NC alone, cetuximab alone, and their combination treatment to be 25.4%, 15.5%, and 46.0%, respectively (Figure *5*).

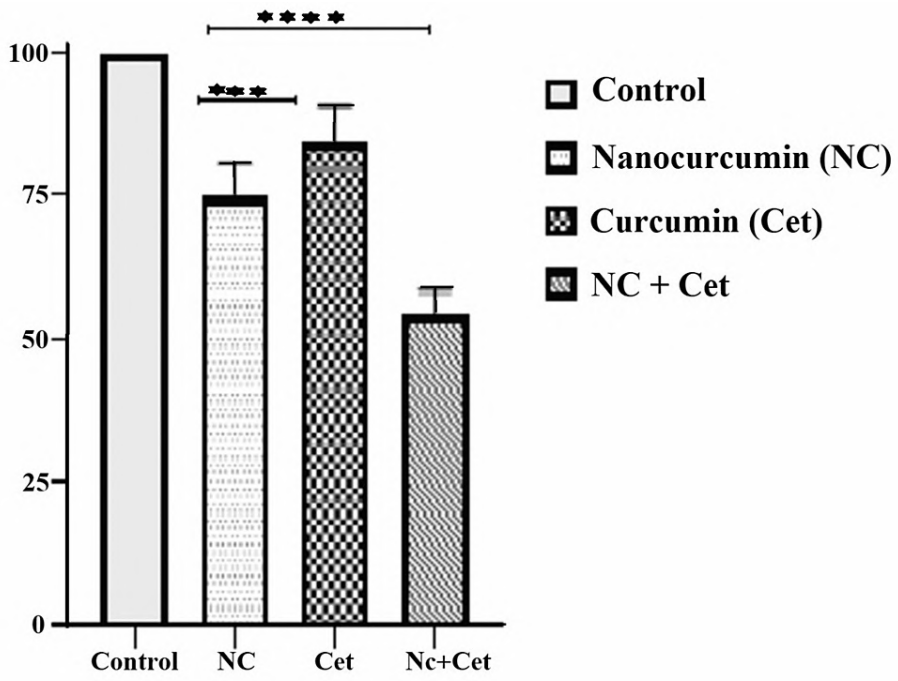

FIGURE 5: Cytotoxic effect of combined treatment with nanocurcumin (NC) and cetuximab

The graph shows the cell viability (%) of the KB cells treated with the combination of IC_{50} doses of NC and cetuximab against a single treatment with NC and cetuximab for 24 hours. There was highly significant cell inhibition in the combined treatment group compared to the single treatment groups ($p<0.0001$). The NC caused more cell death compared to cetuximab alone ($p<0.001$).

The combination group had highly significant cell death ($p <0.0001$) compared to the single-drug treatments. Cell death by NC treatment was significant ($p <0.001$) against cetuximab treatment.

Oral cancer cells sensitized with NC before cetuximab treatment showed a cytotoxic effect of 30.9%. The results of this experiment were compared to the single treatment effect of cetuximab and the combined therapy of NC-cetuximab. Sensitization to NC caused significant cell death compared to cetuximab treatment alone (Figure *6*).

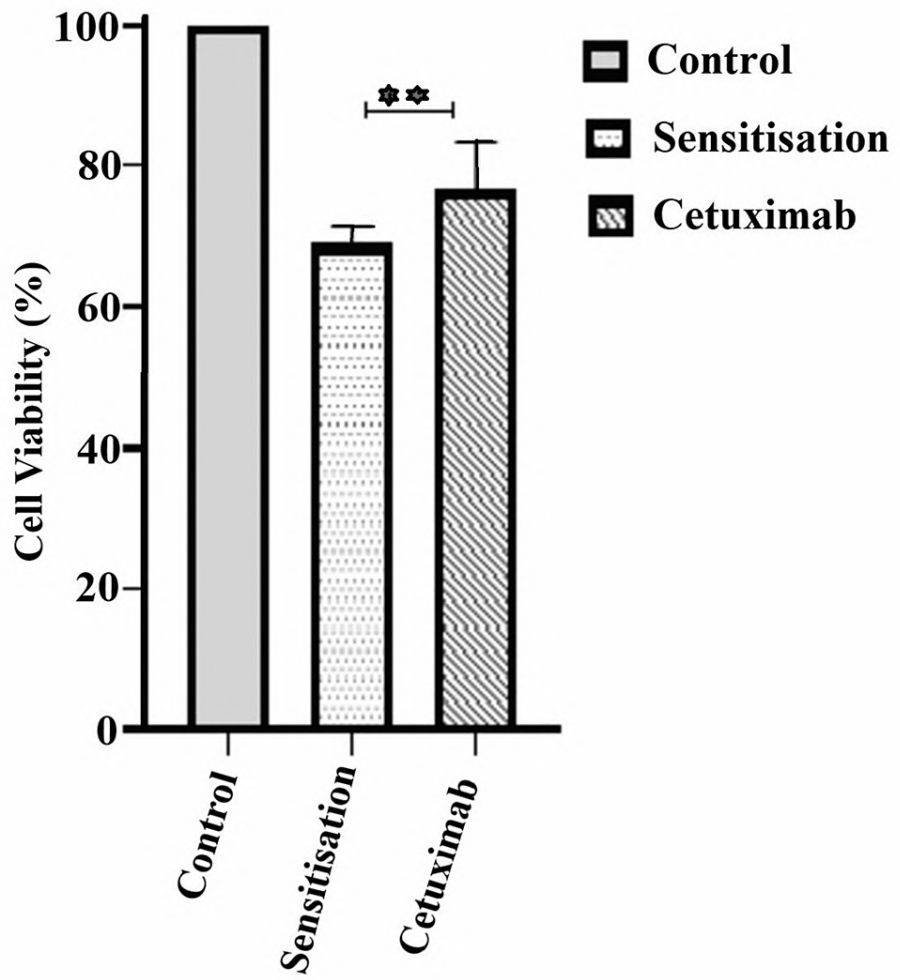

FIGURE 6: Cytotoxic effect of nanocurcumin sensitised cetuximab against cetuximab alone treatment

The graph shows the cell viability (%) of the KB cells sensitised with nanocurcumin prior to 24 hours of cetuximab treatment. There was significant cell growth inhibition in the sensitised treatment group compared to the cetuximab single treatment group ($p<0.01$).

Prior sensitization with NC treatment had less cytotoxic effect than the combination therapy (Figure 7).

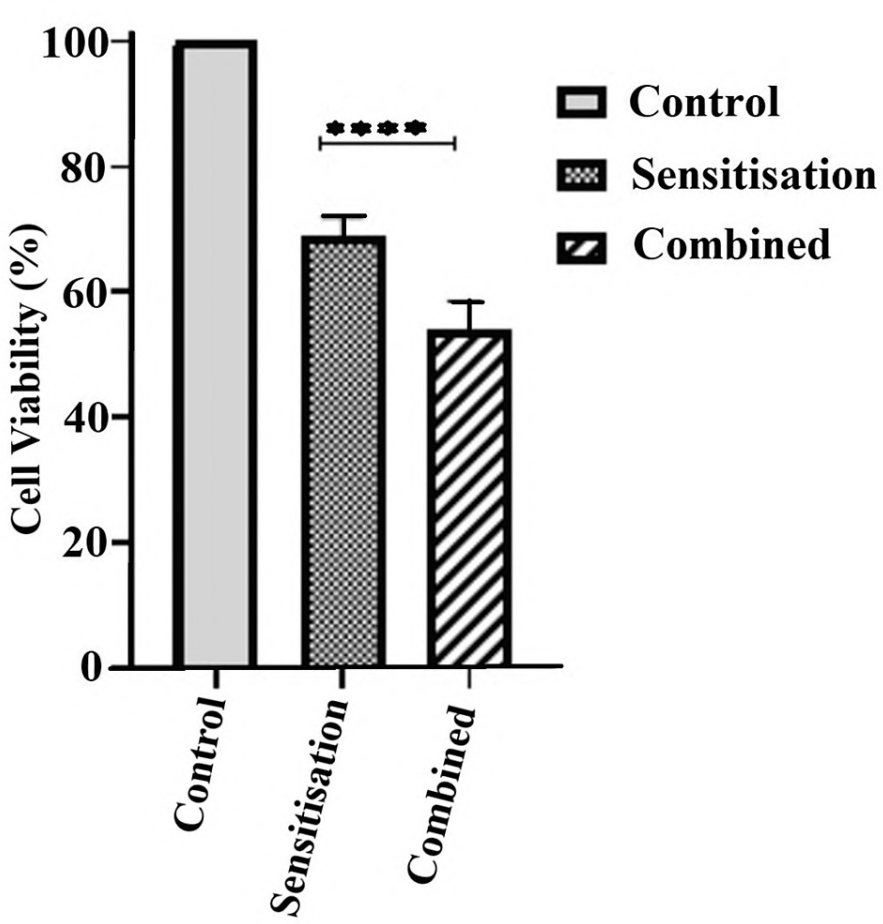

FIGURE 7: Cytotoxic effect of sensitisation treatment compared to combined treatment

The graph shows the cell viability (%) of the KB cells being sensitised with nanocurcumin prior to 24 hours of cetuximab treatment against their combination treatment. Cell viability was higher in the sensitised group compared to the combination treatment group ($p<0.0001$).

Discussion

We studied the effect of sequential doses of cetuximab and NC treatment individually on oral cancer cells for 24 hours. Oral cancer cells underwent a concentration-dependent cell death with both NC and cetuximab. Cell death by NC treatment (25.4%) was significant ($p <0.001$) against cetuximab treatment (15.5%). We observed highly significant cell death ($p <0.0001$) in the NC and cetuximab combined treatment group (46.0%) as compared to each NC (25.4%) and cetuximab (15.5%) monotherapy. The group treated with cetuximab post-NC sensitization (30.9%) showed more significant cell death ($p <0.01$) than the group with only cetuximab treatment (15.5%).

Our results with cetuximab treatment follow the study findings by Park et al., where significant cytotoxic effects of cetuximab on KB cells were observed at doses starting from 200µg/mL [13]. Cetuximab, a recombinant human-murine chimeric monoclonal antibody, binds explicitly to EGFR on the cell surface, internalizes it, and blocks the downstream signal transduction leading to inhibition of tumor cell proliferation, invasion, metastasis, angiogenesis, and promoting tumor cell apoptosis. The KB cells are known to overexpress the EGFR receptor, which explains the cytotoxic effect seen with cetuximab treatment [14].

The results of NC treatment in our study are similar to the observations made by Srivastava et al., where NC (~200nm size) used in oral cancer cells showed dose-dependent cytotoxicity [15]. Our findings corroborate with the results observed in another study using nanoparticle curcumin (in the range of 34-359.4nm) by Adahoun et al. on prostate cancer cells, where overnight treatment showed a significant cytotoxic effect [16]. In recent years various types of NC in oral cancer cell lines have been studied, and their results were promising cancer cell growth inhibitory and cytotoxic effects [17,18]. A study by Wichitnithad et al. on oral

cavity cancer cell line (KB cells) using mono-PEGylated curcumin conjugates (mPEG2000-succinyl-Curcumin, mPEG2000-glutaryl-Curcumin, and mPEG2000- methylcarboxyl-Curcumin) showed cytotoxic results with IC_{50} in the range of 1-3µM, a much lower dose range than our study findings [19].

We observed that the combined treatment with NC and cetuximab was more cytotoxic than the single treatment of each agent, which signifies the adjuvant effect of NC. Duarte et al., in their study, treated two head and neck squamous cell carcinoma (HNSCC) cell lines with the combination of a liposomal form of nanocurcumin and the anticancer drug cisplatin [20]. Our findings are similar to their observation of the combination treatment being more cytotoxic than cisplatin monotherapy. We also found NC treatment to cause more cell death than cetuximab treatment, and a similar effect is observed by Manohar et al. and Chen et al. [21,22]. In our study, the NC-sensitized cetuximab-treated group had highly significant cell death against the only-cetuximab-treated group, highlighting the chemo-sensitizing effect of NC. Similar studies using curcumin molecules on different cancer cell lines support our findings [23,24].

Curcumin has been researched to exert its actions through various pathways exhibiting its anticancer properties as a therapeutic agent. A study by Zhen et al. showed that curcumin inhibited the proliferation of oral cancer cells in a dose-dependent manner along with inhibition of cancer cell invasion and inhibition of activation of both EGFR and EGFR downstream signalling molecules PKB, mitogen-activated protein kinase 1 (MAPK1/2), and signal transducer and activator of transcription 3 (STAT3) [7]. The HNSCC in vitro study models show that curcumin suppresses the activation of transcription factor nuclear factor-kappa B (NF-κB) via the inactivation of inhibitor of nuclear factor-κB (IκKB) activity. The NF-κB inactivation leads to the suppression of many NF-κB-regulated genes involved in cancer development like tumor necrosis factor α (TNF-α), cyclin D1, cellular myelocytomatosis oncogene (c-myc), matrix metalloproteinases (MMP-9), cyclooxygenases (COX-2), and various other interleukins (IL-6,8) [25]. Curcumin has been shown to induce cancer cell autophagy by inhibiting the PKB/mammalian target of rapamycin (mTOR)/p70S6 pathway and MAPK1/2 pathway and inhibits signalling protein STAT3 by suppressing the IL6-mediated phosphorylation of STAT3 [25]. The cytosine-cytosine-adenosine-adenosine-thymidine (CCAAT)/enhancer-binding protein α and insulin-like growth factor binding protein-5, known suppressors of head and neck cancers, are upregulated by curcumin by activating p38 which leads to suppression of oral carcinogenesis [26]. In addition, curcumin modulates the cell cycle by downregulating anti-apoptotic proteins such as B-cell lymphoma 2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xL) and upregulating the apoptotic ones (p53, Bax, Bad, Bim) causing cell cycle arrest at the G2/M phase [25].

Apoptosis induction, inhibition of cell cycle, expression of anti-apoptotic proteins and angiogenesis, blocking of multiple cell survival signalling pathways, modulation of immune responses, induction of p53 dependent and p53 independent G2/M phase cell cycle arrest are the effects of curcumin. Together, all these factors are likely to be the reason for higher cancer cell death observed with NC treatment than with cetuximab monotherapy (EGFR inhibitor). We also found NC sensitized group to have more cell death than the combined treatment group. Our findings conflicted with the study by Yallapu et al., where pre-treatment of curcumin caused more cell death than the combined treatment (curcumin and cisplatin) [23]. The reason for such observation is to be explored by further studies, including the effect of NC treatment on the downstream molecular pathways, which is a limitation of this study. Future experiments targetting chemotherapeutic dose-lowering effects would further strengthen the outcomes of this study.

Conclusions

In this lab-controlled cell culture study, we observed the concentration-dependent cytotoxic effect of the NC on the oral squamous cancer cells. There was significant cancer cell death by NC treatment compared to cetuximab monotherapy. The combination treatment had a more substantial effect than single-drug treatment. On sensitization with NC, cetuximab caused more cell death than only cetuximab. All these findings indicate the potential chemo-adjuvant impact of NC. Further studies are needed to determine the molecular mechanisms of NC and its effect on other cancer types with future scope in clinical application.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. All India Institute of Medical Sciences (AIIMS) issued approval IEC/AIIMS BBSR/PG THESIS/2018-19/15. This study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Bhubaneswar, India (Reference number: IEC/AIIMS BBSR/PG THESIS/2018-19/15). . **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Data are available on reasonable request to Diptasree Mukherjee (dr.diptasree@gmail.com).

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RESEARCH ARTICLE Open Access

Comparison of oral Nano-Curcumin with oral prednisolone on oral lichen planus: a randomized double-blinded clinical trial

Seyed Javad Kia[1], Maryam Basirat[1], Tahereh Mortezaie[1] and Mahdieh-Sadat Moosavi[2*]

Abstract

Background: Oral lichen planus (OLP) is a mucocutaneous autoimmune disease with T-cell mediation. Corticosteroids are considered as a first choice in OLP and should be used for a long period with a subsequent increase in dose since the disease has a chronic and recalcitrant nature. There have been efforts to use alternative therapies due to the Corticosteroid's side effects. Curcumin is a non-toxic natural product with different effects on various oral diseases. It demonstrates antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities. It seems that Curcumin can be used as a proper alternative for Corticosteroid treatments. To overcome limitations in the bioavailability of Curcumin, the therapeutic effect of oral Nano-Curcumin was evaluated for the first time.

Methods: Sixty OLP patients were included in this double-blinded randomized clinical trial. The patients were randomly divided into two groups and received either 'Nano-Curcumin 80 mg' or 'Prednisolone 10 mg' treatments for 1 month. The patients should take one capsule after having their breakfast. The VAS scale was used to analyze pain severity and burning sensation. To assess lesion size the Thongprasom scale was employed. Repeated measures and independent t-tests, as well as LSD paired-test, were used to analyze the data.

Results: Data from 57 patients were analyzed. The level of pain, burning sensation, and OLP lesions decreased in both groups of Curcumin and Prednisolone and no statistically significant difference was observed between the two groups.

Conclusion: Despite many studies conducted to find an effective approach for managing OLP, the results have often been unsatisfactory. In comparison with previous studies, current results clarify the importance of Nano-Curcumin bioavailability in therapeutic effects. Pain VAS and lesion size were decreased with oral Curcumin. The results have shown that oral Curcumin can be used as an alternative therapy for OLP in patients with the contraindicated Corticosteroids or should be used with caution. Oral Curcumin can be used in preventing the recurrence of OLP lesions after the treatment and initial control. Moreover, the amount of Curcumin dose is more important than its use duration in improving OLP.

Trial registration: IRCT, IRCT20100101002950N5. Registered 9 February 2019, https://www.irct.ir/trial/36704.

Keywords: Curcumin, Nanoparticles, Oral lichen Planus

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Background

Oral lichen planus (OLP) is a kind of chronic mucosal disease identified as an immune disorder [1]. There are several forms of OLP lesions, namely reticular, papular, plaque-like, bullous, erythematous (atrophic), and ulcerative [2]. There is a range of epidemiological researches. It has been estimated that the frequency of OLP would range from 0.55 to 2% [3, 4].

The commonest involvement site for OLP is the buccal mucosa. However, other oral cavity sites, such as labial mucosa, tongue, and gingiva, might be influenced as well [5]. OLP presentation is primarily connected with symptoms, ranging from a burning sensation to severe pain. This presentation barely remits extemporaneously [3, 5, 6]. Most patients suffering from OLP have periods of relapses and remissions. There is a growth in detectable clinical signs and symptoms within periods of exacerbation, which can be related to psychological disorders or stress [5, 7, 8].

The treatment options presently available focus on alleviating the symptoms and monitoring any possible dysplastic changes [9]. Given the lesion's severity, different therapeutic modalities have been employed- either on their own or in combination- topically, intralesionally, or systemically. Using corticosteroids is the current acceptable mode of treatment [10]. These drugs should be used for a long period with a subsequent increase in dose since the disease has a chronic and recalcitrant nature. Nonetheless, the topical drugs bring about different side effects including thinning of the oral mucosa, secondary candidiasis, stomatopyrosis, and altered taste sensation. On the other hand, systemic steroids can lead to other side effects, such as suppression of the hypothalamic-pituitary axis, diarrhea, fluid retention, osteoporosis, hypertension, diabetes mellitus, and increased susceptibility to infection [11].

As a result, researchers have been continuously looking for a substitute natural or herbal drug to be taken as monotherapy or in combination with the first choice drugs [12]. These experts have been searching for drugs that could be taken in the treatment of lichen planus on a long-term basis with minimal side effects. This can help the specialists to control the disease and prevent the recurrence of the lesion. Curcumin is a non-toxic natural product with therapeutic effects on various oral diseases such as oral submucous fibrosis, leukoplakia, and Chemoradiotherapy-induced oral mucosal lesion [13, 14]. Scientists have categorized Curcumin as a natural phytochemical and active principle in turmeric, the ground powder of the rhizomes of *Curcuma longa*. Curcumin demonstrates antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities [15].

Furthermore, high doses of Curcumin would not be dangerous. The down-regulation of inflammatory transcription factors (e.g., nuclear factor-kappa B), enzymes (e.g., cyclooxygenase 2 and 5, lipoxygenase), and cytokines (e.g., TNF-α, IL-1, IL-6, IL-8) helps Curcumin to mediate its anti-inflammatory effects. Besides, the inhibition of free radicals and nitric oxide makes Curcumin produce its antioxidant effect [16]. Some previous studies have examined the effect of Curcumin on OLP. However, these studies have been accompanied by some limitations. In some studies, it has been used topically or systemically and with non-Nano-Curcumin form, which reduces its therapeutic effects due to Curcumin low bioavailability [2, 17, 18]. Another study has evaluated the effect of Curcumin in combination with Corticosteroids, which can continue to be limited in people with a contraindication on corticosteroid use [19].

Thus in the current study, the therapeutic effects of oral Nano-Curcumin in oral lichen planus are investigated for the first time. Curcumin nanoparticles have a greater absorption dose and bioavailability than Curcumin.

Methods

The current RCT report followed the standard checklist of CONSORT. This was a phase 3 parallel clinical trial study. The patients with erosive and atrophic forms of OLP referred to the Oral Medicine Department *in 2018–2019 were considered as the study population.* The written informed consent was completed by the patients, and the study protocol was approved by the Ethics Committee of the University (IR.Gums.Rec.1397.295). The registration number of the clinical trial in a Primary Registry in the WHO Registry Network was IRCT20100101002950N5.

To determine the sample size, according to the repetition of sizes in 2 groups for each person, the effect of each person was considered. To do this, the formula was used to fit the repetitive sizes. Considering 4 repetitions, a correlation of 0.50, a statistical power of 95%, an error level of 0.05, and a standard deviation of 2.98 were all obtained from previous studies; the minimum sample size was 25 people for each group [20].

$$n = \frac{2\left(z_{\frac{\alpha}{2}} + z_{\beta}\right)^2 \sigma^2 (1 + (m-1)\rho)}{md^2} = \frac{2(1.96 + 1.64)^2 2.98^2 (1 + (3)0.5)}{4(2.4)^2} = 24.98 \cong 25$$

The patient recruitment was shown in a CONSORT flow diagram. Sixty patients were examined, and patient characteristics containing personal and clinical data, such as age, sex, medical background, smoking habit, type and site of oral lesions, disease duration, type of treatment received earlier, as well as their pain or lesion severity, were recorded. The patients were then randomly (generated random numbers in Excel) divided into two groups.

OLP diagnosis was made by the modified WHO criteria (clinically the presence of bilateral lesions with white, reticular/popular components, and histologically the presence of liquefaction degeneration of the basal layer, and band-like infiltration of mononuclear inflammatory cells in the superficial connective tissue) [21]. Exclusion criteria were as follows: pregnancy; lactation; patients taking Corticosteroids; patients with elevated liver enzymes taking anticoagulants or anti-fungal drugs such as Warfarin (Curcumin has an inhibitory effect on platelet aggregation), orthodontic treatment, gastric ulcer, duodenal ulcer, and gallstone (Curcumin may cause an upset stomach and gallbladder contraction); the presence of any malignant or viral infection in the mouth; the presence of dysplasia in histopathology, receiving topical treatment for OLP within the last 2 weeks or systemic treatment for OLP within the last 4 weeks; taking azathioprine, cyclosporine, PUVA, UVA, or UVB within the last month; having allergies to Corticosteroids or herbal compounds, such as turmeric.

Study groups

Group A

The 80 mg Curcumin capsule was prescribed. Nano-Curcumin capsules were provided by ExirNanoSina (a knowledge-based company). Its trade name is SinaCurcumin that contains 80 mg Curcumin in the Nano-Micellar Soft gel capsule. Oral nano-curcumin is available with two different dosages; 40 mg and 80 mg. According to the pilot study on OLP patients, 40 mg is not sufficient enough for symptoms relief, and based on the safety of 80 mg without any side effects, the 80 was chosen. The patients were told to take one capsule after having their breakfast.

Group B

The 10 mg Prednisolone was provided in capsules as group A. The patients should take one capsule after having their breakfast.

10–20 mg Prednisone and Prednisolone were taken daily for rather serious cases, and 35 mg Prednisone and Prednisolone were used daily for more serious cases within 2 weeks in order to treat OLP. Patients were treated with this standard protocol or/and topical steroids if they dropout.

In the present research, the 10 mg dose was consumed daily within 4 weeks. Since a dose of less than 7.5 mg Prednisone is physiologic (which is as strong as Prednisolone), tapering was done by taking a daily dose of 5 mg Prednisolone within 10 days.

Data collection

This was a double-blind study. The random selection information was only available to a person who was not involved in the study and the required number of drugs for the two groups were counted and placed in an envelope for each patient; patients were unaware of the type of intervention. The necessary explanations for taking the medication for each patient were provided privately. The patient's clinical examination, besides the measurement of their lesions and pain severity, was performed by an oral medicine specialist without knowing the grouping of patients. The examinations were done at the beginning of treatment and after its onset under the unit's light within 1, 2, 4 weeks intervals and recorded in the patient's datasheets. The patients' pain severity was measured by VAS (Visual Analogue Scale). At each visit, the patient was asked for a degree of pain that ranged between 0 and 10. Grade 0 indicated painlessness and grade 10 represented the most severe kind of perceptible pain. Sterile calipers were employed to measure the lesions. Thongprasom scale was also employed. This scale was graded as follows:

0) There is no lesion and the person is not sick.
1) Mild white stretch marks, with no erythematous site.
2) White stretch marks with the atrophic site, with a size smaller than 1 cm^2.
3) White stretch marks with the atrophic site, with a size bigger than 1 cm^2.
4) White stretch marks with the erosive site, with a size smaller than 1 cm^2.
5) White stretch marks with the erosive site, with a size bigger than 1 cm^2.

Statistical analysis

Due to the normal distribution of data, ANOVA with repeated measure test was used for statistical analysis. Friedman's test was employed if the data was not normally distributed. All the tests were carried out at a 5% level using SPSS24.

Results

Data from 57 patients (Group A, $n = 29$; Group B, $n = 28$) with oral lichen planus were analyzed in this study due to the lack of referral of 3 patients. There were no significant differences between the two groups for age and gender (p-value > 0.05). The basic characteristics of the studied groups were shown in Table 1.

Table 1 Basic characteristics in the studied groups

	Curcumin	Prednisolone	*P*-value
Female/Male ratio	25/4	23/5	0.274
Age (mean (SD))	51.86 (9.94)	53.67 (8.90)	0.533
Baseline VAS (mean (SD))	4.65 (3.39)	4.89 (3.34)	0.818
Baseline Lesion Size (mean (SD))	3.83 (1.17)	3.61 (0.98)	0.515

Table 2 A two-by-two inter-time VAS comparisons in Curcumin group

	Before the study	Week 1	Week 2	Week 4
Before the study	–	–	–	–
Week 1	0.348	–	–	–
Week 2	0.001	0.002	–	–
Week 4	< 0.001	< 0.001	< 0.001	–

Table 2 presents a two-by-two inter-time comparison between the two groups. Based on the results, the mean VAS did not differ significantly only before and 1 week after the study ($p = 0.348$). At other times, the difference was significant, and the average VAS score decreased with increasing time. Table 3 provides the inter-time comparison in the two examined groups. According to the results, the VAS mean had a significant difference at all the examined times, and the VAS mean dropped with an increase in time. Table 4 shows a comparison of VAS means in the two groups at each of the examined times. According to the results, there was no significant difference at any of the examined times. The results of the test demonstrated that the VAS score followed a falling and meaningful change trend during the examined time ($p < 0.001$). However, this changing trend did not have a significant difference in the two groups ($p = 0.428$).

Table 5 shows inter-time comparisons of lesion size in the Curcumin group. According to the results, there were significant differences between all the examined times. Lesion size changes followed a falling trend. Table 6 presents inter-time comparisons of lesion size in the Prednisolone group. According to the results, there were significant differences between all the examined times. Lesion size changes followed a falling trend. Table 7 demonstrates a comparison summary of the lesion size means in the two groups at each of the examined times. Base on the results, there was no significant difference at any examined time.

According to the results, lesion size changes followed a falling trend and were significant ($p < 0.001$). However, this changing trend did not have a significant difference in the two groups ($p = 0.568$).

Tables 8 and 9, showed inter-group comparisons between two groups. Based on the results, the level of pain, burning sensation, and OLP lesion decreased in both Curcumin and Prednisolone groups and there was no significant difference between them.

Table 3 A two-by-two inter-time VAS comparisons in Prednisolone group

	Before the study	Week 1	Week 2	Week 4
Before the study	–	–	–	–
Week 1	0.042	–	–	–
Week 2	< 0.001	0.002	–	–
Week 4	< 0.001	< 0.001	0.001	–

Table 4 A comparison of VAS mean in the two groups at each of the examined times

Time	Before the study	Week 1	Week 2	Week 4
Test statistics	0.23	0.30	0.16	0.46
Significance	0.818	0.766	0.869	0.65

Discussion

Based on the results, the level of pain, burning sensation, and OLP lesion decreased in both Curcumin and Prednisolone groups and there was no significant difference between them.

Lichen planus is T cell-mediated autoimmune and an inflammatory disease that affects mucocutaneous. Antigen presentation to CD4+ helper T cells results in the production of cytokines and the activation of CD8+ cytotoxic lymphocytes.

Different amounts of free radicals and reactive oxygen species (ROS) are induced during this process. These reactions can damage lipids, proteins, and nucleic acids in cells [12, 22]. OLP (as a potentially malignant disorder) symptoms, along with its classification, mandates effective management and regular monitoring [22].

As severe pain exists in OLP erosive and atrophic forms with greatly intolerance to hot or spicy food, and these forms may be associated with higher malignancy risk than other forms of OLP [23], they are included in the current study.

Despite many studies conducted to find an effective approach for managing OLP, the results have mostly been unsatisfactory [23]. Although topical Corticosteroid is the first choice in OLP management, it is associated with several adverse effects, such as atrophy of oral mucosa, candidiasis, and tachyphylaxis [23]. In prolonged treatment with local corticosteroids, systemic adverse effects of ocular, endocrine, and metabolic may occur [24].

Many studies have been performed to find an alternative treatment [12].

Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities [25]. Given the numerous benefits of Curcumin in treating of lichen planus over steroids, several studies have been done in the past.

Table 5 Inter-time Lesion Size comparisons in Curcumin group

	Before the study	Week 1	Week 2	Week 4
Before the study	–	–	–	–
Week 1	0.005	–	–	–
Week 2	< 0.001	< 0.001	–	–
Week 4	< 0.001	< 0.001	0.003	–

Table 6 Inter-time Lesion Size comparisons in Prednisolone group

	Before the study	Week 1	Week 2	Week 4
Before the study	–	–	–	–
Week 1	0.015	–	–	–
Week 2	< 0.001	< 0.001	–	–
Week 4	< 0.001	< 0.001	0.003	–

The results of case-control studies demonstrated that topical treatment with Curcumin would improve lesions and reduced pain severity similar to triamcinolone cream [2, 17].

The results of other studies demonstrated that oral lesion recovery rate in the OLP patients treated with Prednisone and Curcumin was higher than those received Prednisone alone [19, 26]. Based on the further research results, the group with Curcumin in three doses of 2000 mg per day and for 14 days demonstrated a noticeable recovery in clinical signs and symptoms in comparison with the control group [27]. Chainani et al. conducted another research to evaluate the effect of the treatment duration increase with Curcumin on improving OLP symptoms. The patients were treated with an average dose of 2134.5 Curcumin per day and for 30 months. Sixty percent of the patients showed symptom relief, 35% of them did not trust the results, and 5% of the patients reported that Curcumin did not cause any symptom relief [18]. Patil et al. reported that doses higher than Curcumin 6000 mg perfectly controlled the OLP clinical symptoms, and diarrhea was recognized as one of the side effects of Curcumin dosage [28]. These results are in line with those of the present study.

Curcumin has limits in water solubility and bioavailability due to its hydrophobic nature, challenging Curcumin's clinical translation into a practical therapeutic agent. Nanoparticles increase the dissolution rate of the hydrophobic agents by supplying a large surface-to-volume ratio [29].

Since, in the current study, Curcumin was Nano-Curcumin, a dose of 80 mg was used, which was significantly less than the dose in other studies using non-nano silic forms. In vivo study showed that low-dose (20 mg/kg) Nano-Curcumin has an equivalent therapeutic effect as high-dose (400 mg/kg) pure Curcumin [29].

Table 7 A summary of lesion size mean comparison in the two groups at each of the examined times

Time	Before the study	Week 1	Week 2	Week 4
Test statistics	0.66	0.74	0.74	1.60
Significance	0.515	0.465	0.462	0.117

Table 8 Pain VAS means in the two groups at the examined times

Time	Group	Mean (Standard deviation)	Significance group's time test statistics
Before the study	Curcumin	4.65 (3.39)	0.85
	Prednisolone	4.89 (3.34)	0.428
Week 1	Curcumin	4.38 (3.03)	
	Prednisolone	4.67 (3.45)	
Week 2	Curcumin	3.41 (2.74)	
	Prednisolone	3.28 (2.74)	
Week 4	Curcumin	2.69 (2.89)	
	Prednisolone	2.33 (2.03)	
Time test statistics		40.02	
Significance		< 0.001	

In the research conducted by Thomas et al., Curcumin 1% gel 3 times a day and Curcumin 1% gel 6 times a day was compared to Triamcinolone cream. All the groups were treated for 3 months; they showed a decrease in burning sensation, redness, and ulcer. However, the triamcinolone group experienced the highest reduction in burning sensation, redness, and an ulcer [9]. The results of this study are not in line with ours. This dissimilarity can be justified by referring to the different types of Curcumin employed. In the present study, Curcumin was used as an oral-systemic capsule (80 mg), while Thomas et al. employed its 1% gel. Also, the Curcumin treatment duration was shorter than ours. It can be concluded that the amount of Curcumin dose is more important than its use duration in improving OLP. Chainani et al. referred to the same point [26].

Another comparison that clarifies the importance of Nano-Curcumin bioavailability in therapeutic effects in the current study is with the research conducted by

Table 9 Lesion size means comparison in the two groups

Time	Group	Mean (Standard deviation)	Significance group's time test statistics
Before the study	Curcumin	3.83 (1.17)	0.61
	Prednisolone	3.61 (0.98)	0.568
Week 1	Curcumin	3.48 (1.27)	
	Prednisolone	3.22 (1)	
Week 2	Curcumin	2.79 (1.15)	
	Prednisolone	2.56 (0.92)	
Week 4	Curcumin	2.34 (1.14)	
	Prednisolone	1.83 (0.92)	
Time test statistics		67.60	
Significance		< 0.001	

Amirchaghmaghi et al. OLP patients were randomly treated with oral Curcumin (2000 mg per day) and placebo for 4 weeks. According to the results, no therapeutic effect was considered for Curcumin in the treatment of OLP [20]. Although in his study all the patients were treated by routine OLP treatments, using Dexamethasone 0.5 mg mouthwash and Nystatin 100,000 unit/ml oral suspension. Using routine OLP treatments can improve the patients' clinical symptoms to the maximum extent achievable by drug therapy and using other therapies can no longer increase the recovery rate.

Further studies with more follow-ups with recurrence rate estimations are recommended to introduce Nano-Curcumin as a new therapeutic agent in OLP.

Conclusion

The present research results revealed that oral Nano-Curcumin could be used as an alternative treatment for OLP lesions in those who should not take oral Corticosteroids or in the patients who should take Corticosteroids cautiously. Moreover, oral Curcumin could be used for preventing the recurrence of OLP lesions after the treatment and initial control. Further studies are recommended concerning the latter issue.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-020-03128-7.

Additional file 1. CONSORT 2010 Flow Diagram.

Additional file 2. Table (*)-Pain VAS mean in the intervention and control groups at the examined times. Table (**): A summary of lesion size mean in the two groups at the examined times.

Abbreviations

OLP: Oral lichen planus; PUVA: Psoralen and ultraviolet A; VAS: Visual Analogue Scale; UVA: Ultraviolet A; UVB: Ultraviolet B; WHO: World Health Organization

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None.

Authors' contributions

SYK, MB and MSM contributed to conceptualization, methodology, review, and editing in writing. TM collected the samples and drafted the manuscript. MSM was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The written informed consent was completed by the patients and study protocol was approved in the Ethics Committee of Guilan University of Medical Sciences (IR.Gums.Rec.1397.295).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Original Article

Beta-glucan promotes dental pulp healing by enhancing cell proliferation, migration, and mineralization

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Wound healing

Abstract *Background/purpose:* Effective dental pulp healing is essential for preserving tooth vitality. Although beta-glucan has shown promise in wound healing in the medical fields, its potential effects on human dental pulp cells (HDPCs) remain unexplored. This study aimed to assess beta-glucan's effects on HDPC proliferation, migration, collagen synthesis, mineralization, and differentiation.
Materials and methods: Primary HDPCs were cultured and assigned into five groups: control, vehicle, and beta-glucan at concentrations of 5, 7.5, and 10 mg/mL. Cell proliferation was quantified using the alamarBlue® assay at 24, 48, and 72 h. Cell migration was assessed at 12 and 24 h via the scratch wound healing assay. Flow cytometry was employed to detect integrin beta 1 (CD29) expression during wound healing. Mineralization and differentiation at day 14 were evaluated through alizarin red S staining and quantitative real-time polymerase chain reaction (qRT-PCR), measuring Dentin Sialophosphoprotein (DSPP), Interleukin-10 (IL-10), and Collagen type I (COL1) gene expression. Statistical significance was established at $P < 0.05$.
Results: At 24 and 72 h, all concentrations of beta-glucan significantly induced cell proliferation. In the wound healing assay, beta-glucan improved cell migration and increased the expression of integrin beta 1 after 24 h. Mineralized matrix formation and the expression of IL-10 and COL1 were significantly observed at 14 days. The upregulation of DSPP was detected in groups supplemented with 5 and 7.5 mg/mL beta-glucan.
Conclusion: Beta-glucan enhanced cell proliferation, cell migration potential, integrin beta 1 expression, mineralized matrix formation, and DSPP, IL-10, and COL1 gene expression in HDPCs.

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Introduction

Vital pulp therapy (VPT) aims to maintain the vitality of teeth affected by deep caries, trauma, or iatrogenic errors.[1] Preserving pulp vitality is beneficial as it provides a defense against external harms and supports the self-protective and reparative processes when injured.[2,3] Modern VPT involves removing infected pulp tissue and applying a bioactive capping material to the exposed pulp.[4–6] The potential for pulpal wound healing is crucial post-VPT due to the uncertain condition of the pulp after infected tissue removal. Dental pulp stem cells at the injury site are believed to contribute to the initial repair process.[7,8] However, information on the reparative potential of pulp tissue healing, particularly wound closure after VPT, is limited. Therefore, supplements, techniques, and innovations that promote initial wound healing could significantly benefit VPT.

Beta-glucans, natural polysaccharides found in plants, bacteria, fungi, and algae, have gained attention for their therapeutic potential in wound healing.[9] These compounds exhibit remarkable biological properties, including the ability to enhance critical cellular processes such as cell proliferation, migration, reepithelization, angiogenesis, and collagen synthesis.[10–12] As a result, beta-glucans are emerging as promising candidates for the development of natural wound-healing agents in the medical field. Clinical studies have shown that beta-glucan applications can accelerate wound closure and reduce treatment costs, particularly for chronic wounds.[13,14] Additionally, beta-glucans show promise in bone regeneration due to their ability to promote bone growth, inhibit osteoclastogenesis, enhance mesenchymal stem cell adhesion, and support osteoblast differentiation, thereby facilitating bone formation.[15] While research on beta-glucans in dentistry is still limited, one study demonstrated that beta-glucans could reduce inflammation and alleviate alveolar bone loss in diabetic animals with periodontitis.[16] These findings suggest that beta-glucan-containing wound dressings may represent promising therapeutic potential for improving wound healing in both medical and dental applications.

This study aimed to evaluate the effects of beta-glucan on pulpal wound healing by examining cell proliferation, cell migration, collagen synthesis, and mineralization in isolated human dental pulp cells.

Materials and methods

Isolation and culture of HDPCs

Human dental pulp cells (HDPCs) were obtained from healthy, non-carious third molars with no pulp disease, extracted for orthodontic purposes from patients aged 19–21 years (n = 3) following approval from the Human Experimental Committee, Faculty of Dentistry, Chiang Mai University, Thailand (No.7/2022). Prior to tooth extraction, patients performed an oral rinse with chlorhexidine mouthwash to minimize the microbial load in the oral cavity. Following extraction, the teeth were immediately rinsed with sterile saline. Strict aseptic protocols were adhered to throughout the tissue isolation process, including the preparation of guide grooves on the buccal aspects of the crowns of the extracted teeth using diamond burs (FG D8; Intensiv, Zurich, Switzerland). These grooves facilitated division of the teeth into two pieces using a chisel and mallet. The pulp tissue was then carefully collected using sterile instruments. Pulp tissues were digested with Collagenase I (Gibco, Gaithersburg, MD, USA) and Dispase II (Sigma–Aldrich, St Louis, MO, USA) for 45 min at 37 °C. Cells were cultured in complete alpha-minimum essential medium (Sigma–Aldrich) with 10 % fetal bovine serum (Sigma–Aldrich), 1 % penicillin-streptomycin (Sigma–Aldrich), and 100 mol/L L-ascorbic acid (Sigma–Aldrich) at 37 °C and 5 % CO2. Cells from the second to fourth passages were used. All experiments were performed in triplicate.

Beta-glucan preparation

Beta-glucan from Euglena gracillis (Sigma–Aldrich) was used. A stock solution was prepared and stored at 4 °C using 0.1 % dimethyl sulfoxide (DMSO) (Sigma–Aldrich) as the vehicle. The stock solution was diluted with culture or differentiation medium, as required, and filter sterilized with 0.2-μm microfilters (Corning, Oneonta, NY, USA).

Cell proliferation assay

HDPCs were seeded into 96-well plates at 5000 cells/well. After attachment, media was replaced with beta-glucan at 1, 2.5, 5, 7.5, and 10 mg/mL, with regular complete media as the negative control. A vehicle group examined DMSO's effect. To evaluate proliferation, 15 μL of alamarBlue® (Bio-Rad Laboratories, Hercules, CA, USA) were added to each well. Fluorescence was monitored at 24, 48, and 72 h using a plate reader (Tecan Trading AG, Männedorf, Switzerland) at 530 nm excitation and 590 nm emission. Percentage differences between control and treated groups were calculated. The three concentrations with the most pronounced proliferative effect were used in subsequent experiments.

After selecting the appropriate concentration of beta-glucan from the previous part, the following investigations were set into 5 experiment groups:

1. Control: HDPCs cultured in regular complete media
2. Vehicle: HDPCs cultured in regular complete media containing 0.1 % DMSO
3. BG 5: HDPCs cultured in regular complete media containing 5 mg/mL beta-glucan
4. BG 7.5: HDPCs cultured in regular complete media containing 7.5 mg/mL beta-glucan
5. BG 10: HDPCs cultured in regular complete media containing 10 mg/mL beta-glucan

Wound healing assay

HDPCs were seeded in a 24-well plate at 30,000 cells/well. At 80 % confluence, a scratch was made using a sterile 100 μL pipette tip. Culture media with or without beta-glucan was added. The plates were incubated for 12 and

24 h under an automated live-cell imaging microscope (DMi8 microscope) (Leica Microsystems, Buffalo Grove, IL, USA) for live monitoring. Cell migration was assessed, and quantitative analysis was performed using Image J software.

Investigation of integrin beta 1 expression using flow cytometry

To examine the role of integrin in cell migration, HDPCs were cultured in a 6-well plate at 300,000 cells/well. At 80 % confluence, scratches were made, and cells were treated with beta-glucan or complete media for 24 h. Cells were harvested, centrifuged, resuspended in FACs buffer, and incubated with FITC-conjugated integrin beta 1 antibody (Invitrogen, Carlsbad, CA, USA) for 30 min at 4 °C. Flow cytometry analysis was conducted using a CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA, USA). Data were analyzed based on mean fluorescence intensity (MFI).

Evaluation of mineralization production using alizarin red S staining

HDPCs were seeded in a 24-well plate at 20,000 cells/well. At 50 % confluence, scratches were made. Differentiation medium with beta-glucan (BG + diff) was used to stimulate mineralization for 14 days with medium changes every 3 days. Cells were fixed with 4 % paraformaldehyde and stained with pH 4.2 alizarin red S solution (Sigma—Aldrich). After incubation and washing, calcium deposits were measured by destaining with 10 % cetylpyridinium chloride monohydrate. The stained solution was measured using a spectrophotometer (Tecan Trading AG) at 550 nm.

Gene expression using quantitative real-time PCR (qRT-PCR)

To evaluate DSPP, IL-10, and COL1, HDPCs at 200,000 cells/well were seeded in a 6-well plate and induced as previously described. Cells were collected on day 14. RNA extraction was performed with TRIzol (Invitrogen), followed by cDNA synthesis using the ReverTra Ace™ qPCR RT Kit (TOYOBO, Osaka, Japan). Gene expression was measured on the LightCycler® 480 II system (LifeScience, Roche, Indianapolis, IN, USA) using SYBR Green PCR master mix (SensiFAST™ SYBR® No-ROX Kit) (Bioline, Memphis, TN, USA). Relative expression levels were calculated by the $2^{-\Delta\Delta CT}$ method, employing GAPDH as the internal control. Primer sequences are provided in Table 1.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS Statistics 21.0 (IBM, Chicago, IL, USA), with Tukey's or Dunnett's T3 test applied for post-hoc analysis. Statistical significance was set at $P < 0.05$.

Table 1 Primer sequences of genes used in the study. DSPP: dentin sialophosphoprotein. IL-10: interleukin 10. COL1: collagen type I. GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Gene	Primer sequences (5′-3′)
DSPP	Forward primer: TGG CGA TGC AGG TCA CAAT
	Reverse primer: CCA TTC CCA CTA GGA CTC CCA
IL-10	Forward primer: CCC AGA AAT CAA GGA GCA TT
	Reverse primer: CTC TTC ACC TGC TCC ACT GC
COL1	Forward primer: GAT GAT GCC AAT GTG GTT CGT G
	Reverse primer: CAG GCT CCG GTG TGA CTC GT
GAPDH	Forward primer: ACC ACA GTC CAT GCC ATC AC
	Reverse primer: TCC ACC ACC CTG TTG CTG TA

Results

Cell proliferation assay

All concentrations (1, 2.5, 5, 7.5, and 10 mg/mL) of beta-glucan significantly increased cell proliferation relative to the control and vehicle groups at 24 h ($P < 0.05$) (Fig. 1A). At 48 h, cell proliferation trends increased in all beta-glucan groups, with the 10 mg/mL concentration showing significant stimulation compared to the control and vehicle groups ($P < 0.05$) (Fig. 1B). At 72 h, beta-glucan concentrations of 5, 7.5, and 10 mg/mL significantly increased cell proliferation relative to the control and vehicle groups ($P < 0.05$) (Fig. 1C). The 10 mg/mL concentration notably enhanced cell proliferation compared to the 1, 2.5, and 5 mg/mL concentrations ($P < 0.05$).

Based on the proliferation assay results, 5, 7.5, and 10 mg/mL of beta-glucan demonstrated the greatest cell proliferation. These concentrations were selected for the subsequent part of the experiment.

Wound healing assay

The results showed no significant difference was observed in wound closure between the groups at 24 h. However, at 24 h, the concentrations of 5, 7.5, and 10 mg/mL of beta-glucan significantly enhanced wound closure when compared to the control and vehicle groups ($P < 0.05$) (Fig. 2A). The 10 mg/mL concentration of beta-glucan showed the most extensive wound closure ($P > 0.05$). Similar repairing potentials were observed among the beta-glucan-treated groups.

Integrin beta 1 expression

The findings indicated that beta-glucan at 5 and 7.5 mg/mL significantly increased the expression of integrin beta 1 when compared to the control group ($P < 0.05$). The concentrations of 5, 7.5, and 10 mg/mL of beta-glucan significantly induced the expression of integrin beta 1 when compared to the vehicle group ($P < 0.05$). Similar expressions were observed among the beta-glucan groups (Fig. 2D).

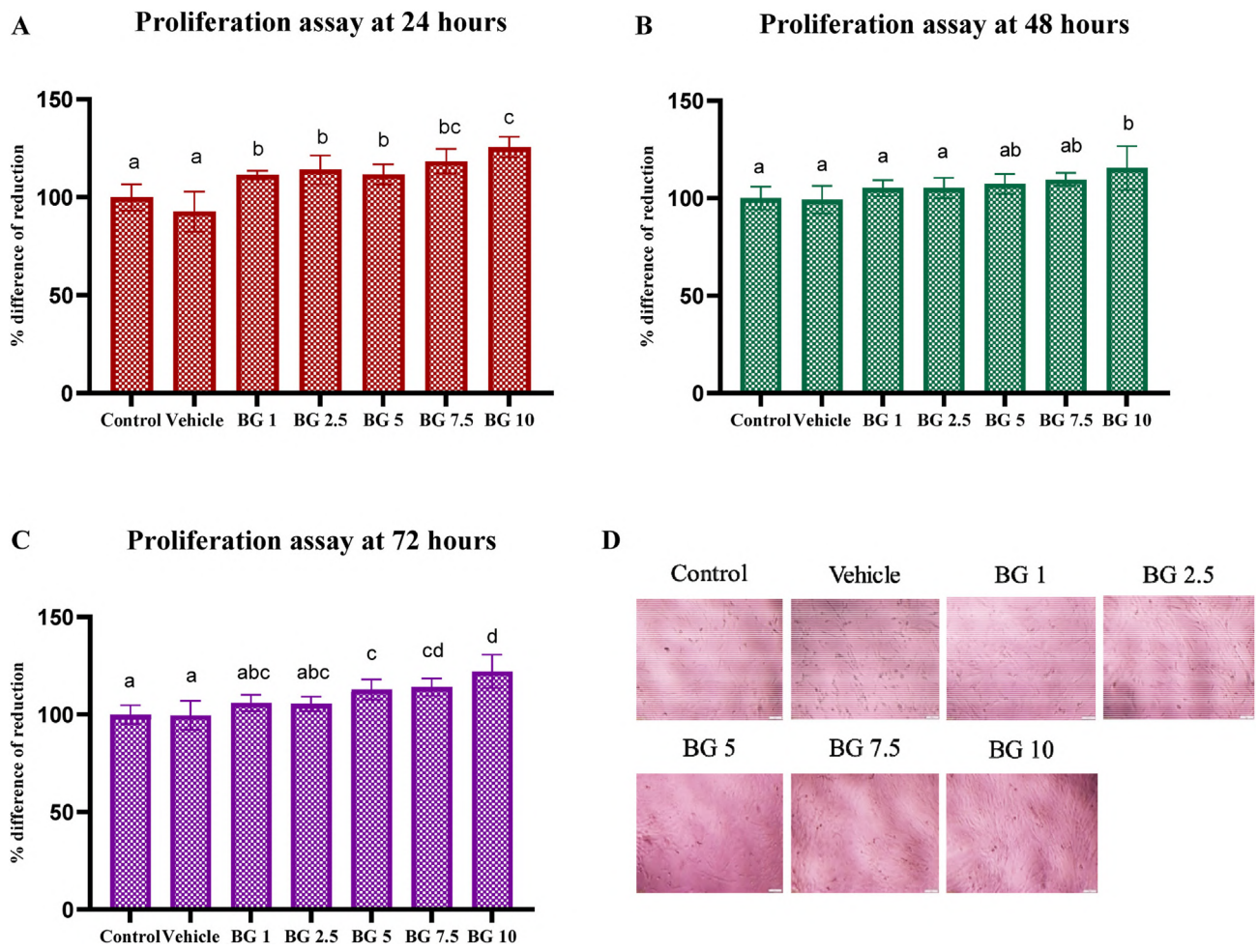

Figure 1 The proliferative effect of beta-glucan on HDPCs. (A) Cell proliferation at 24 h. (B) Cell proliferation at 48 h. (C) Cell proliferation at 72 h. (D) Cells at 72 h under an inverted-light microscope at 5× magnification Scale bar = 50 μm. Different letters in the graph represent significant differences between groups. BG: beta-glucan.

Alizarin red S staining

A significant increase in mineralized matrix formation was observed when compared to control and vehicle groups ($P < 0.05$). The addition of 5, 7.5, and 10 mg/mL of beta-glucan to differentiation media resulted in a significantly higher level of mineralized matrix formation when compared to control and vehicle groups under differentiating conditions ($P < 0.05$). Similar results were observed among the beta-glucan groups (Fig. 3A).

The expression of mineralization-related and collagen synthesis-related genes

The expression of the DSPP gene significantly increased in the control, BG5, BG7.5, and BG10 groups under differentiating conditions compared to the control group ($P < 0.05$). Similar levels of DSPP expression were observed between the beta-glucan-treated groups and the control group under differentiating conditions. However, both the BG5 and BG7.5 groups showed a significant increase in DSPP expression compared to the vehicle group ($P < 0.05$) (Fig. 3C). Regarding IL-10 expression, all the BG5, BG7.5, and BG10 groups exhibited a significant upregulation of IL-10 expression compared to the control group ($P < 0.05$). Compared to the control group under differentiating conditions, the BG5 and BG10 groups displayed a significant elevation in IL-10 expression ($P < 0.05$). Additionally, all the BG5, BG7.5, and BG10 groups induced a significant increase in IL-10 expression compared to the vehicle group ($P < 0.05$) (Fig. 3D).

In terms of collagen gene expression, significant upregulation of COL1 was observed in the BG5 and BG10 groups under differentiating conditions compared to the control group ($P < 0.05$). When compared to the control with differentiation media group, only the BG5 group exhibited a significant increase in COL1 expression ($P < 0.05$). However, all the BG5, BG7.5, and BG10 groups displayed significant upregulation of COL1 when compared to the vehicle group in differentiation media ($P < 0.05$). There was also a significant difference in COL1 expression between the BG5 group and both the BG7.5 and BG10 groups ($P < 0.05$) (Fig. 3E).

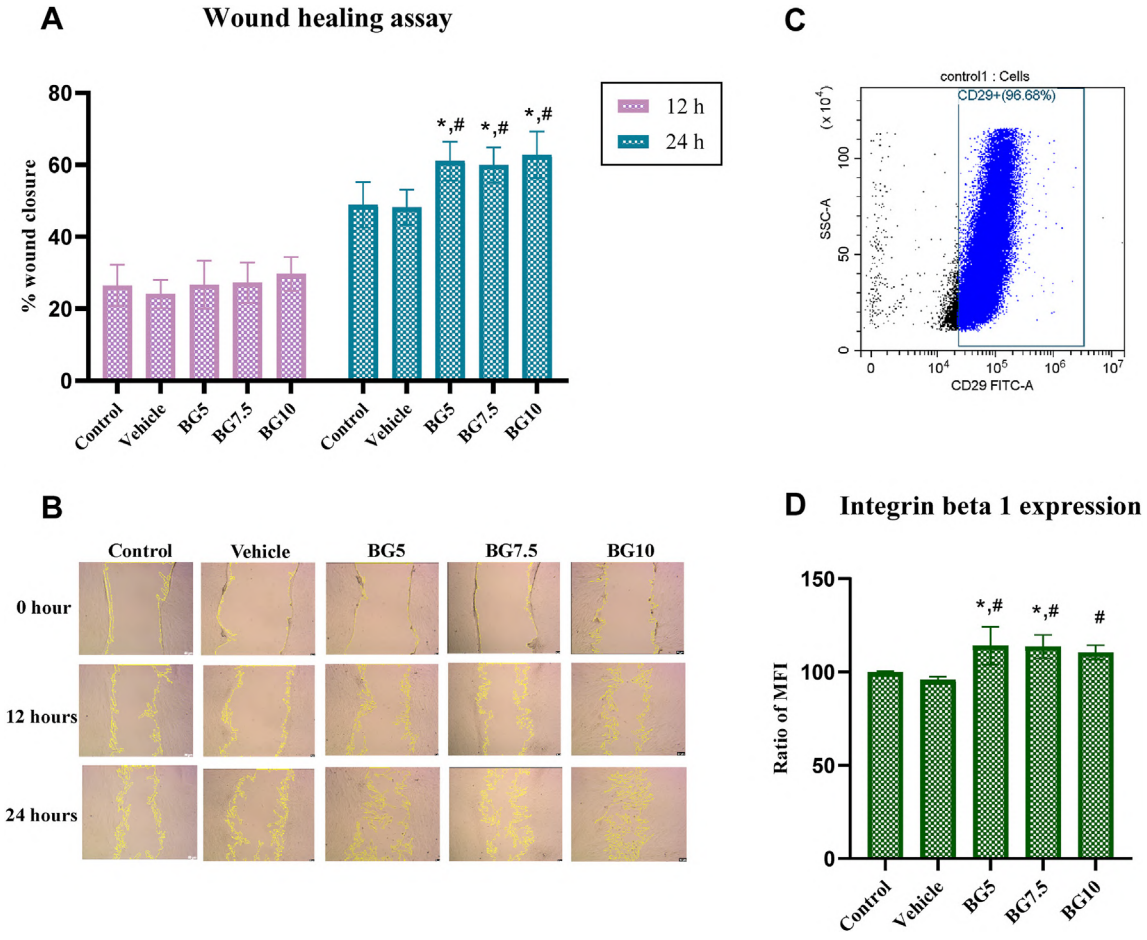

Figure 2 The migration potential of HDPCs after exposure to beta-glucan. (A) The migration potential results presented at the 12-h and 24-h time points. (B) Representative images of the migrated cells from the wound healing assay at 0, 12, and 24 h. The scale bar represents 50 μm. (C) The percentage of integrin beta 1 positive cells in HDPCs. (D) After 24-h exposure to beta-glucan, the expression of integrin beta 1 was assessed using MFI and presented as a percentage difference compared to the control. * indicates significance compared to the control group while # indicates significance compared to the vehicle group. BG: beta-glucan. MFI: mean fluorescence intensity.

Discussion

The potential use of beta-glucan for pulpal wound healing was examined in isolated human dental pulp cells, focusing on cell proliferation, migration, collagen synthesis, and mineralization. Significant positive effects were observed at concentrations of 5—10 mg/mL, suggesting beta-glucan could promote healing in both soft tissue and mineralization, indicating its promise for regenerative endodontics.

Beta-glucans are complex polysaccharides found in various natural sources, known for numerous health benefits, including wound healing.[17—20] They mediate healing through several receptors, particularly dectin-1, found on immune and non-immune cells such as keratinocytes, fibroblasts, and dental pulp tissues.[19,21,22] Despite extensive research on their wound-healing effects, no studies have specifically examined their impact on dental pulp cells until now.

Current dental treatments, especially VPT, emphasize regenerative trends where dental pulp cells are crucial. VPT aims to maintain pulp vitality and promote healing post-infection removal.[1] Dental pulp cells initiate and coordinate healing responses, including proliferation, migration, differentiation into odontoblasts, and reparative dentin formation.[7] Enhancing dental pulp cell potential is key to successful VPT outcomes.

In this study, beta-glucan enhanced dental pulp cell proliferation, migration, mineralization, and differentiation, with higher concentrations (5—10 mg/mL) being more effective. These findings are consistent with previous studies on beta-glucan's effects on other cell types. *In vitro* studies have shown that beta-glucan promotes cell proliferation across various cell types. For instance, 0.2 mg/mL mushroom-derived beta-glucan stimulated keratinocyte proliferation within 48—72 h.[23] High concentrations (5 mg/mL) also showed greater efficacy in promoting fibroblast cell growth compared to lower doses.[24]

Cell migration is also crucial for wound healing, and previous studies have shown that beta-glucan enhances keratinocyte and fibroblast migration.[10,11,25] Similarly, our study found that beta-glucan promoted HDPC migration in wound healing assays. Migration involves complex processes

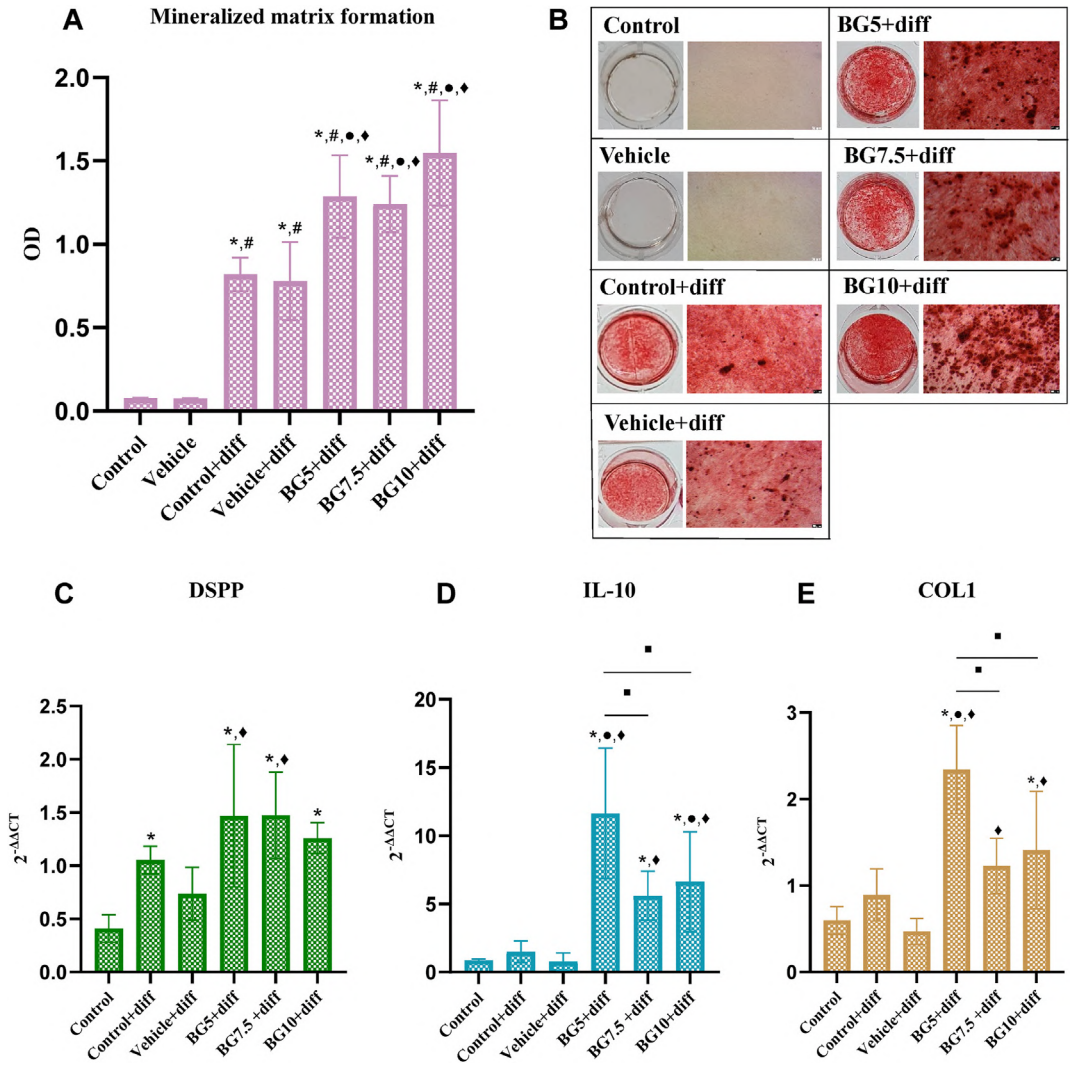

Figure 3 The effect of beta-glucan on mineralized matrix formation and collagen synthesis of HDPCs. (A) The quantitative analysis of mineralized matrix formation at 14 days. (B) Alizarin red staining of the experimental groups under a light microscope at a magnification of 5×. Scale bar = 50 μm. (C) The expression of the mineralization-related gene: DSPP (D) The expression of IL-10, and (E) The expression of collagen synthesis-related gene: COL1. * indicates significance compared to the control group. # indicates significance compared to the vehicle group. ● indicates significance compared to the control + diff group. ◆ indicates significance compared to the vehicle + diff groups. ■ indicates significance between two different groups. OD: optical density. BG: beta-glucan. Diff: differentiation medium.

where integrins, acting as cell surface receptors, connect the extracellular matrix (ECM) to the cell's cytoskeleton and activate the FAK-Src pathway to facilitate movement.[26–28] Previous research has associated increased cell migration with the expression of specific integrin subunits, including integrin alpha 3, alpha 5, and beta 1.[29–31] In this study, the expression of integrin beta 1 correlated with improved HDPC migration following beta-glucan treatment, suggesting an association between these factors.

Collagen is essential for wound healing, providing structural support and maintaining tissue integrity within the ECM.[32] IL-10 also plays a significant role in tissue formation and maturation, facilitating organized ECM deposition without compromising strength.[33,34] Our study reveals that beta-glucan stimulates collagen synthesis gene (COL1) expression in HDPCs, aligning with previous research on human dermal fibroblasts.[35] A consistent pattern of IL-10 and COL1 expression across beta-glucan-treated groups suggests IL-10's role in maintaining balance during wound healing.

For pulpal healing, a key goal is cell differentiation into mineral-producing cells. Various genes, including DSPP, DMP-1, and BMP, are involved in mineralization, with DSPP recognized as a marker of odontoblastic differentiation.[36,37] Our study showed upregulation of DSPP expression and observed mineralization after beta-glucan treatment. While limited studies have examined beta-glucans' direct influence on mineralization, some reported mineralized matrix formation and bone deposition in beta-glucan-incorporated scaffolds.[15,38,39] Further research is needed to explore beta-glucan's direct role in mineralization, including dentinogenic differentiation.

These finding suggest that beta-glucan may have a supportive role in processes relevant to dental pulp healing. The healing process following VPT involves complex cellular and molecular mechanisms that support pulp tissue regeneration and repair.[40] Stimulating early cell proliferation, migration, and differentiation is critical for the success of VPT, and beta-glucan shows promising potential in these aspects. Its ability to enhance these processes highlights its possible application in improving the outcomes of VPT. However, further research is needed to fully understand the mechanisms behind beta-glucan's effects on HDPCs and to assess its long-term impact on pulp tissue regeneration and repair *in vivo*.

In conclusion, our study found that beta-glucan at concentrations of 5, 7.5, and 10 mg/mL demonstrated the ability to stimulate cell proliferation, cell migration, integrin beta 1 expression, and mineralization in HDPCs. The expression of IL-10, COL1, and DSPP genes was upregulated. This study highlights the potential of beta-glucan as a valuable enhancer of processes critical to dental pulp healing, paving the way for innovative approaches in regenerative endodontics.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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ORIGINAL RESEARCH

Effectiveness, Tolerability, and Safety of Ectoine-Containing Mouthwash Versus Those of a Calcium Phosphate Mouthwash for the Treatment of Chemotherapy-Induced Oral Mucositis: A Prospective, Active-Controlled, Non-interventional Study

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ABSTRACT

Introduction: Oral mucositis is a frequent complication of cancer chemotherapy and radiotherapy. Ectoine is a natural extremolyte that can stabilize biological membranes and counteract inflammatory reactions. This study investigated ectoine-containing mouthwash for the prophylaxis and the treatment of oral mucositis. Its effectiveness, tolerability, and safety were compared to those of the local standard-of-care calcium phosphate mouthwash.

Methods: This prospective, active-controlled, observational study was conducted in two study centers in Hungary from January 2016 to October 2017. Sixty patients undergoing chemotherapy were to be recruited and allocated to one of three treatment arms: prophylactic treatment with ectoine (20 patients), active treatment with ectoine (20 patients), or calcium phosphate (20 patients). The study lasted 21 days, comprising four visits on day 0, day 7, day 14, and day 21.

Results: In all, 45 patients were included in the study (prophylactic ectoine, 10 patients; active ectoine, 20 patients; calcium phosphate, 15 patients). In the prophylactic ectoine group, few mucositis symptoms of mild or moderate severity occurred throughout the study. In the active ectoine and the calcium phosphate groups, symptoms of mild and moderate severity at inclusion were reduced significantly after 14 days of treatment and were mostly resolved at the end of the study. The difference between the active ectoine and the calcium phosphate groups was not significant. According to patients' assessments, ectoine mouthwash was

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more effective and tolerable than calcium phosphate mouthwash.
Conclusions: Ectoine mouthwash is safe, well tolerated, and effective for the active treatment of oral mucositis following chemotherapy. Its effectiveness is comparable to that of calcium phosphate. Patients prefer ectoine mouthwash to calcium phosphate mouthwash.
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PLAIN LANGUAGE SUMMARY

Oral mucositis is the inflammation of the mucosa of the oral cavity. It is a frequent complication of cancer chemotherapy and radiotherapy. Approximately 20–40% of patients undergoing chemotherapy suffer from oral mucositis. It is very painful, impairs eating, drinking, and quality of life. One of the most effective yet simple measures to prevent and treat oral mucositis is oral care with mouthwash. Ectoine is a natural substance that was discovered in halophilic (salt-loving) bacteria. Ectoine can protect these bacteria against dehydration because it can attract water molecules and strengthen biological membranes. Ectoine is used to treat many diseases caused by allergens, UV light, air pollution, heat, and dryness. Ectoine (Ectoin®) mouthwash is produced by bitop AG (Dortmund, Germany) to treat dry mouth and other symptoms of inflamed oral mucosa.

This study investigated ectoine mouthwash for the treatment of oral mucositis following chemotherapy. It was compared to the local standard-of-care calcium phosphate mouthwash. One group of patients was treated with ectoine mouthwash and the other with calcium phosphate mouthwash. After 14 days, mucositis symptoms were substantially reduced in both groups. After 21 days, all patients were almost cured of oral mucositis. Additionally, after the treatment, patients rated how effective and tolerable the treatment was. Here, more patients treated with ectoine rated their treatment as effective and tolerable than those treated with calcium phosphate.

This study shows that ectoine mouthwash is tolerable and effective for the treatment of mucositis. Patients preferred ectoine mouthwash to calcium phosphate mouthwash.

Keywords: Chemotherapy; Ectoine; Mouthwash; Mucositis; Oral care

INTRODUCTION

Oral mucositis, clinically defined as inflammation of the mucosal lining of the oral cavity, is a frequent complication of cancer chemotherapy and radiotherapy. It occurs in 20–40% of patients undergoing standard chemotherapy [1] and in almost every patient receiving bone marrow transplantation [2]. Risk factors for the development of mucositis include age, nutritional status, type of malignancy, and oral care during cancer treatment [3]. Mucositis is very painful, often requires analgesics, and impairs eating, drinking, and quality of life. Severe mucositis can even necessitate reducing or discontinuing cancer therapy. Medical costs for mucositis are considerable because of symptom management, nutrition support, and hospitalization [4, 5].

There are numerous interventions for mucositis, such as basic oral care, anti-inflammatory agents, anti-radical scavengers, antimicrobials, coating agents, laser therapy, and cryotherapy. Basic oral care, despite being the least invasive treatment option, is vital in preventing infections. The Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) Clinical Practice Guidelines for Mucositis recommend basic oral care for preventing oral mucositis following all cancer treatments in all age groups. However, at present, there is no standard treatment guideline for oral mucositis [4]. A systematic review on currently available oral care protocols, dental care as well as mouthwash containing saline, sodium bicarbonate, chlorhexidine, mixed medication, and calcium phosphate has yielded insufficient and conflicting evidence [6].

The development of mucositis is divided into five phases, each of which is characterized by inflammatory and apoptosis-triggering factors, such as reactive oxygen species (initiation phase), TNF-α, IL-1β, IL-6, the mitogen-activated protein kinase pathway, the ceramide signaling pathway (damage response and signal amplification phase) as well as bacterial and fungal colonization (ulceration phase) [7–10].

Ectoine is a natural extremolyte which has been shown to counteract inflammatory reactions involving IL-6, IL-8, TNF-α, IL-1β [11–13], the ceramide signaling pathway [14], and the mitogen-activated protein kinase signaling pathway [15]. It has also been shown to rescue cells from apoptosis [16, 17]. Furthermore, ectoine can stabilize biological membranes and rehydrate dry, irritated mucosa [18–20], which is essential for the body's defense against oral infections. These membrane-stabilizing and inflammation-reducing effects of ectoine-containing products have been demonstrated in several clinical studies [21–23]. Taken together, ectoine can be expected to thwart the development of oral mucositis.

This study investigated ectoine-containing mouthwash for the prophylaxis and the treatment of oral mucositis. Its effectiveness, tolerability, and safety were compared to those of the local standard-of-care calcium phosphate mouthwash.

MATERIALS AND METHODS

Study Design

This prospective, active-controlled, observational study was conducted in two study centers in Hungary from January 2016 to October 2017. Patients were included in the trial after the decision about the treatment option had been made on the basis of the patients' preference. The study complied with laws and regulations effective in Hungary, namely §17 para (1), point c) of Government Regulation 235/2009. (X.20.). It was carried out in accordance with legal statutes and regulations for the protection of human subjects. This study was approved by the Department of Medical Devices at the Healthcare Registration and Training Center (ENKK), Budapest, Hungary (Reference number: 001654/2016). It is listed at clinicaltrials.gov under the number NCT02816515.

We aimed to recruit 60 male and female adult patients who had an inoperable/metastatic small cell lung cancer (SCLC) tumor, non-small cell lung cancer (NSCLC) tumor, gastrointestinal stromal tumor, renal cell carcinoma, or pancreatic neuroendocrine tumor. Patients receiving targeted tyrosine kinase inhibitor anticancer therapy were treated with sunitinib (Sutent®, Pfizer Pharma GmbH). Patients could be chemotherapy-naïve or have had chemotherapy before the study.

Patients were allocated to one of three treatment arms: prophylactic treatment with ectoine (20 patients), active treatment with ectoine (20 patients), or calcium phosphate (20 patients). In the prophylactic ectoine arm, patients received ectoine mouthwash on the first day of chemotherapy. In the active ectoine arm and the calcium phosphate arm, treatment was initiated when mucositis occurred.

The study lasted 21 days, comprising an initial visit (V1) on day 0, visit 2 (V2) on day 7, visit 3 (V3) on day 14, and visit 4 (V4) on day 21.

Study Medications

Ectoine (Ectoin®) mouthwash is a registered medical device manufactured by bitop AG (Dortmund, Germany). An ampoule (single dose unit) contains 5 ml preservative-free solution of ectoine (2%), hydroxyethyl cellulose (for better viscosity), and xylitol (for sweetness) in phosphate buffer and water. It was administered at least three times daily, each time for 30 s with 1–2 ampoules.

Caphosol® mouthwash (EUSA Pharma, UK) is a supersaturated calcium phosphate solution. It was used at least four times daily according to the manufacturer's instructions.

Clinical Assessments

Effectiveness was assessed on the basis of symptom scores and mucositis grading results

(World Health Organization (WHO) classification) [24]. The symptoms of dry mucosa, coated tongue, mucosal irritation, unpleasant breath, decreased saliva production, pain, swelling, ulcer, difficulties speaking, and difficulties eating/drinking were assessed by the physicians together with the patients at each visit on a 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe). The grade of mucositis was determined for each patient at each visit: grade 0 = none, grade I = mild (oral soreness, erythema), grade II = moderate (oral erythema, ulcers, solid diet tolerated), grade III = severe (oral ulcers, liquid diet only), and grade IV = life-threatening (oral alimentation impossible). Changes in symptom scores and mucositis grades from V1 to V2, V3, and V4 were compared between groups.

At the last visit, patients rated the overall effectiveness and tolerability of the treatment as well as its effectiveness against the individual symptoms of redness, burning sensation, swelling, pain, mucosa irritation, and dry mucosa (0 = very poor, 1 = poor, 2 = neither poor nor good, 3 = good, and 4 = very good). Additionally, patients were asked to rate their likelihood of buying or recommending the product after the study (0 = no, 1 = maybe, and 2 = yes).

All adverse events and serious adverse events were to be documented. Their possible relations to the treatment were evaluated by the investigators.

Statistical Analyses

Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). As this was an exploratory study, no sample size calculation was performed. There was a 95% probability that side effects occurring at an incidence of 5.9% or higher would have been detected (Clopper–Pearson).

Symptom scores, general effectiveness, and tolerability that had been measured on a standard scale were analyzed descriptively, and the Wilcoxon signed-rank test was used to detect significant differences between the baseline scores and the final scores during and after treatment. Frequencies and percentages of mucositis grades were presented by visit and treatment group. Frequencies and percentages were determined for the variables from the patient questionnaires at the end of the study. Comparisons between treatment groups were performed using the two-sided Fisher's exact test with an α-level of 5%.

Compliance with Ethics Guidelines

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study

RESULTS

Study Population

In all, 45 patients were recruited: 10 patients were allocated to the prophylactic ectoine arm, 20 to the active ectoine arm, and 15 to the calcium phosphate arm. The distribution of female and male patients in these groups was 1 and 9, 7 and 13, and 6 and 9, respectively. The mean age of patients was similar across all groups (prophylactic ectoine, 61.10 ± 7.11 years; active ectoine, 61.55 ± 7.52 years, and calcium phosphate, 63.87 ± 6.45 years). In total, 29 patients had lung cancer (SCLC/NSCLC) and 16 patients had metastatic renal cell carcinoma (mRCC). These 16 patients were treated with sunitinib.

Prophylactic Treatment with Ectoine

During prophylactic treatment with ectoine, 3 out of 10 (30%) patients were completely free of symptoms (WHO grade 0). Symptoms of mild intensity (WHO grade I) were reported in 2 patients at V2 and V3. In 6 patients (60%), mild symptoms (WHO grade I) occurred at V4 (Tables 1, 2, Fig. 1).

Table 1 Changes in mucositis grades (WHO classification) over time

	Grade 0	Grade I	Grade II	Grade III	Grade IV
Prophylactic ectoine					
V1					
N	10	–	–	–	–
%	100.0				
V2					
N	9	1	–	–	–
%	90.0	10.0			
V3					
N	8	1	1	–	–
%	80.0	10.0	10.0		
V4					
N	3	6	1	–	–
%	30.0	60.0	10.0		
Active ectoine					
V1					
N	–	8	9	3	–
%		40.0	45.0	15.0	
V2					
N	1	6	11	2	–
%	5.0	30.0	55.0	10.0	
V3					
N	2	15	2	1	–
%	10.0	75.0	10.0	5.0	
V4					
N	16	2	1	1	–
%	80.0	10.0	5.0	5.0	
Calcium phosphate					
V1					
N	–	3	11	–	1
%		20.0	73.3		6.7
V2					
N	–	3	11	–	1
%		20.0	73.3		6.7

Table 1 continued

	Grade 0	Grade I	Grade II	Grade III	Grade IV
V3					
N	–	9	5	–	–
%		64.3	35.7		
V4					
N	6	7	1	–	–
%	42.9	50.0	7.1		

Table 2 Symptom scores

Symptom	Treatment	V1 (mean ± SD)	V2 (mean ± SD)	V3 (mean ± SD)	V4 (mean ± SD)
Dry mucosa	Prophylactic ectoine	0.0 ± 0.0	0.2 ± 0.6	0.5 ± 0.8	0.5 ± 0.8
	Active ectoine	1.7 ± 0.6	1.4 ± 0.6	1.0 ± 0.7	0.4 ± 0.9
	Calcium phosphate	1.9 ± 0.5	1.7 ± 0.6	1.3 ± 0.5	0.6 ± 0.5
Mucosa irritation	Prophylactic ectoine	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.8	0.5 ± 0.8
	Active ectoine	1.7 ± 1.7	1.4 ± 0.7	1.1 ± 0.9	0.3 ± 0.6
	Calcium phosphate	1.9 ± 0.5	1.6 ± 0.6	1.1 ± 0.4	0.6 ± 0.5
Coated tongue	Prophylactic ectoine	0.0 ± 0.0	0.2 ± 0.6	0.2 ± 0.6	0.0 ± 0.0
	Active ectoine	1.4 ± 0.9	1.1 ± 0.8	0.4 ± 0.9	0.3 ± 0.9
	Calcium phosphate	1.8 ± 0.6	1.6 ± 0.6	0.9 ± 0.5	0.4 ± 0.5
Unpleasant breath	Prophylactic ectoine	0.2 ± 0.4	0.0 ± 0.0	0.3 ± 0.9	0.2 ± 0.6
	Active ectoine	1.3 ± 0.7	0.9 ± 0.8	0.4 ± 0.6	0.1 ± 0.4
	Calcium phosphate	1.7 ± 0.6	1.5 ± 0.6	0.9 ± 0.8	0.4 ± 0.5
Decreased saliva production	Prophylactic ectoine	0.0 ± 0.0	0.2 ± 0.6	0.2 ± 0.6	0.3 ± 0.7
	Active ectoine	1.2 ± 0.7	0.8 ± 0.5	0.4 ± 0.9	0.3 ± 0.9
	Calcium phosphate	1.6 ± 0.6	1.5 ± 0.6	0.9 ± 0.6	0.3 ± 0.5

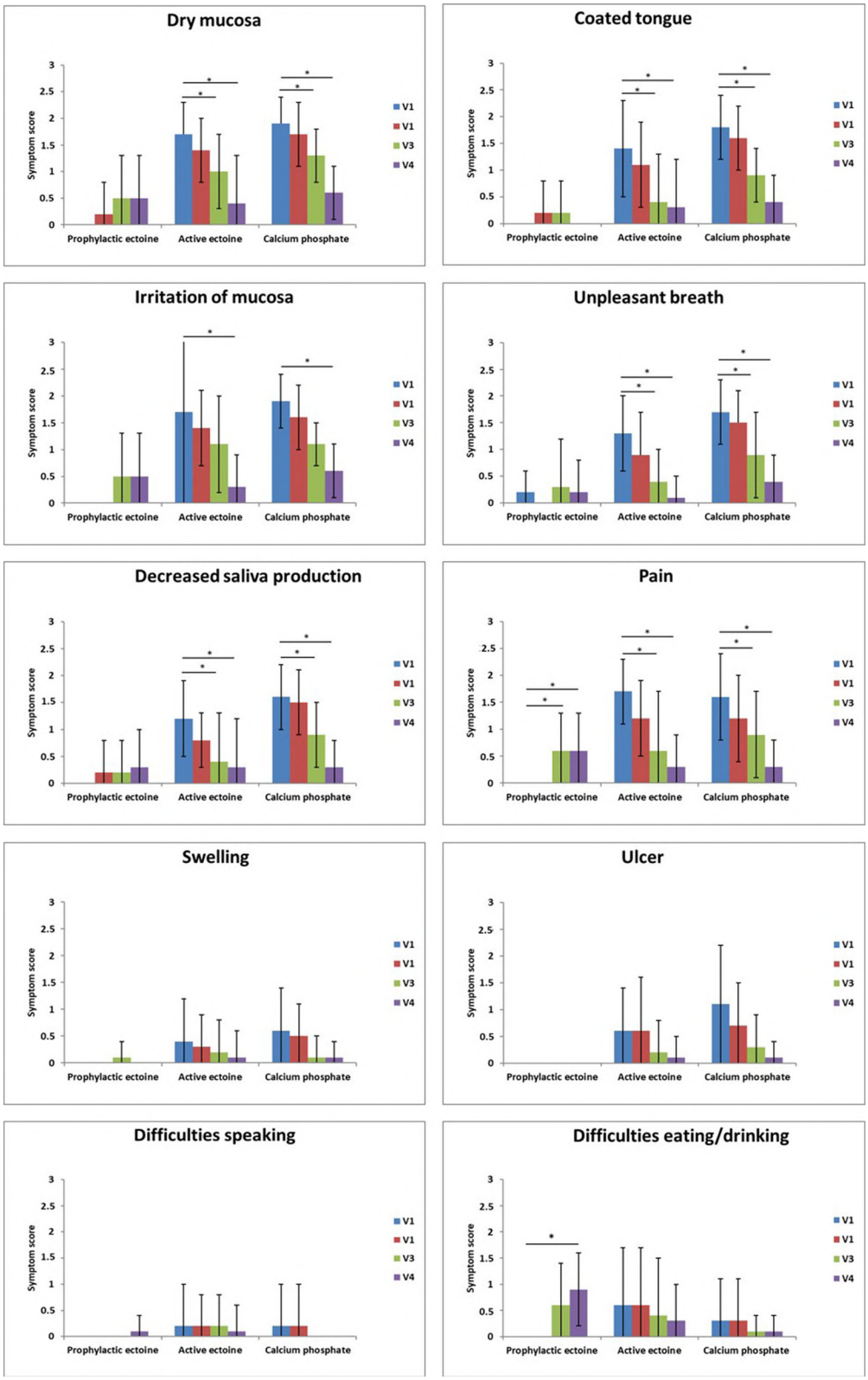
Table 2 continued

Symptom	Treatment	V1 (mean ± SD)	V2 (mean ± SD)	V3 (mean ± SD)	V4 (mean ± SD)
Pain	Prophylactic ectoine	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.7	0.6 ± 0.7
	Active ectoine	1.7 ± 0.6	1.2 ± 0.7	0.6 ± 1.1	0.3 ± 0.6
	Calcium phosphate	1.6 ± 0.8	1.2 ± 0.8	0.9 ± 0.8	0.3 ± 0.5
Swelling	Prophylactic ectoine	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
	Active ectoine	0.4 ± 0.8	0.3 ± 0.6	0.2 ± 0.6	0.1 ± 0.5
	Calcium phosphate	0.6 ± 0.8	0.5 ± 0.6	0.1 ± 0.4	0.1 ± 0.3
Ulcer	Prophylactic ectoine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Active ectoine	0.6 ± 0.8	0.6 ± 1.0	0.2 ± 0.6	0.1 ± 0.4
	Calcium phosphate	1.1 ± 1.1	0.7 ± 0.8	0.3 ± 0.6	0.1 ± 0.3
Difficulties speaking	Prophylactic ectoine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
	Active ectoine	0.2 ± 0.8	0.2 ± 0.6	0.2 ± 0.6	0.1 ± 0.5
	Calcium phosphate	0.2 ± 0.8	0.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
Difficulties eating/drinking	Prophylactic ectoine	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.8	0.9 ± 0.7
	Active ectoine	0.6 ± 1.1	0.6 ± 1.1	0.4 ± 1.1	0.3 ± 0.7
	Calcium phosphate	0.3 ± 0.8	0.3 ± 0.8	0.1 ± 0.3	0.1 ± 0.3

Active Treatment with Ectoine vs. Calcium Phosphate

The distribution of mucositis grades in the active ectoine group at inclusion was 8 patients (40%) with grade I, 9 patients (45%) with grade II, and 3 patients (15%) with grade III. After 21 days of treatment, 16 patients (80%) were completely cured, 2 patients (10%) had grade I, 1 patient (5%) had grade II, and 1 patient had grade III. At V1, the calcium phosphate group consisted of 3 patients (20%) with grade I, 11 patients (73.3%) with grade II, and 1 patient (6.7%) with grade IV. At V4, 6 patients (42.9%) were completely cured, 7 patients (50.0%) had grade I, and 1 patient (7.1%) had grade II (Table 1).

Analyses of the symptom scores show that patients in the two groups had comparable symptoms at V1. Symptoms such as dry mucosa, mucosa irritation, coated tongue,


Dry mucosa
Coated tongue
Irritation of mucosa
Unpleasant breath
Decreased saliva production
Pain
Swelling
Ulcer
Difficulties speaking
Difficulties eating/drinking
Symptom score
Prophylactic ectoine
Active ectoine
Calcium phosphate
V1
V1
V3
V4

◀**Fig. 1** Symptom scores assessed by the physicians together with the patients at V1, V2, V3, and V4 (0 = none, 1 = mild, 2 = moderate, and 3 = severe). Values plotted are mean ± standard deviation (SD). Significant differences between visits were determined using the Wilcoxon signed-rank test. *Indicates $P < 0.05$

unpleasant breath, decreased saliva production, and pain were mild or moderate at V1 and reduced to very mild or mild at V4. Significant reductions in all symptoms, except for irritated mucosa, were recorded at V3 in both groups ($P < 0.05$). Symptoms such as swelling, ulcer, difficulties speaking, and difficulties eating/drinking were very mild or mild at V1 and mostly resolved at V4 (Table 2, Fig. 1). The reductions in symptom scores were not significantly different between groups.

Patients' Assessments

The overall effectiveness and tolerability of the treatment as well as its effectiveness against individual symptoms were assessed as "good" and "very good" by patients in both ectoine groups and as "good" by calcium phosphate patients. The superiority of the active ectoine group over the calcium phosphate group was significant ($P < 0.05$). The difference between the two ectoine groups was not significant (Fig. 2).

In the prophylactic ectoine arm, 90% of patients would buy the product (mean score = 1.9 ± 0.3), and 100% would recommend the product (2.0 ± 0.0). In the active ectoine group, 80% of patients would buy the product (1.8 ± 0.6), and 87.6% would recommend the product (1.8 ± 0.6). It should be understood that the term "this product" referred to the product and the specific treatment regimen (i.e., prophylactic or active treatment with ectoine), not only the product itself. In contrast, 40% of calcium phosphate patients would buy the product (1.3 ± 0.6), and 53.3% would recommend the product (1.5 ± 0.5). The difference between the active ectoine group and the calcium phosphate group was significant ($P < 0.05$) (Fig. 3).

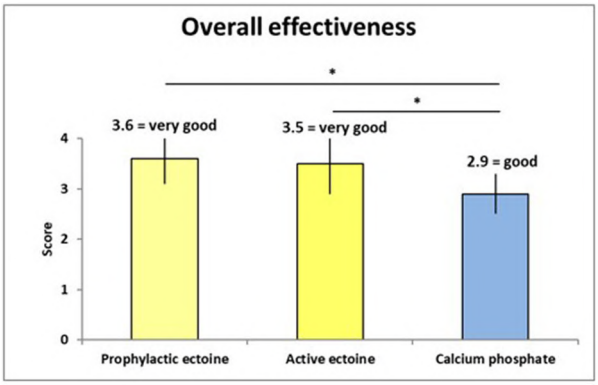
Safety

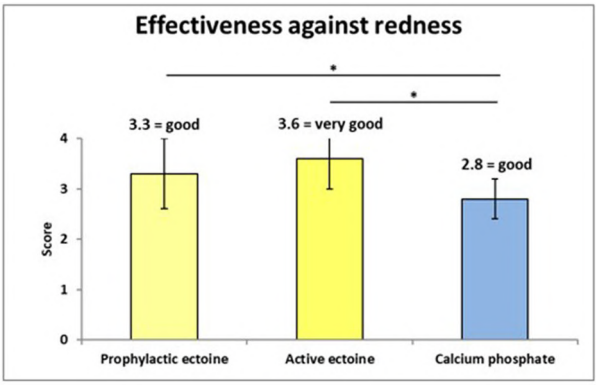
One patient suffered from exsiccosis and fever 2 months after the completion of the study and died shortly after that. These events were classified as unrelated to the study treatment.

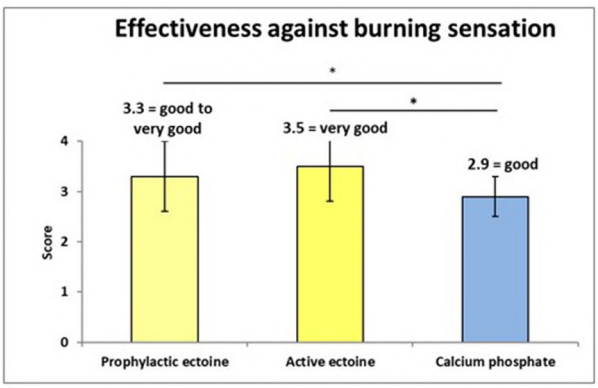
DISCUSSION

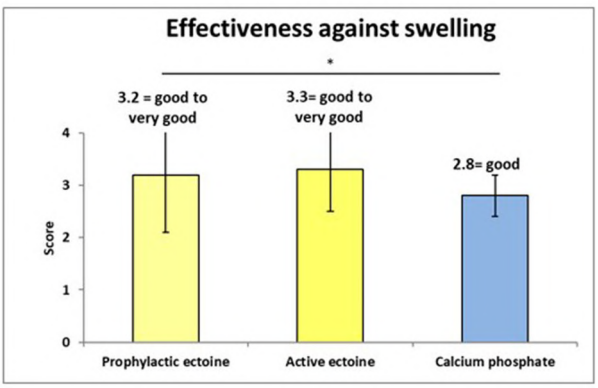
Patients applying ectoine prophylactically show few mild or moderate symptoms throughout the study. As a result of the lack of placebo, it is not possible to extrapolate the actual percentage of patients who would not develop mucositis at all, even without being treated prophylactically. In the literature, the incidence of mucositis following chemotherapy with sunitinib ranges from 12% to 29% [25–27]. In general, 20–40% of patients receiving conventional chemotherapy develop mucositis [4]. Considering that the majority of patients did not necessarily need to be treated, prophylactic treatment with ectoine may nevertheless be beneficial for patients in whom symptoms of dry mucosa, coated tongue, unpleasant breath, and decreased saliva production occur. However, one should not disregard the study design and patients' medical history when making cross-study comparisons. Therefore, we cannot draw robust conclusions about the prophylactic effects of ectoine on the basis of the results of this study.

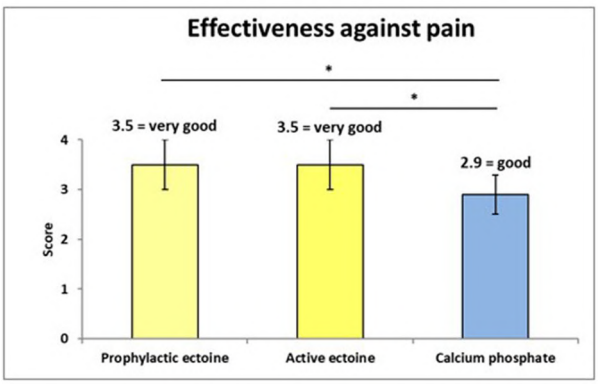
Ectoine mouthwash seemed to be better than calcium phosphate mouthwash for the active treatment of oral mucositis. The reductions in symptom scores were comparable; however, patients rated the effectiveness and tolerability of ectoine more favorably. We found conflicting evidence for the effectiveness and tolerability of calcium phosphate mouthwash in the literature. In a systemic review of 30 studies in patients undergoing chemotherapy and/or radiotherapy, 24 studies reported that calcium phosphate mouthwash reduced symptoms and analgesics needed as well as the incidence and mean days of the disease [28]. However, in many studies the results were not statistically significant. Recent data suggests that calcium phosphate mouthwash is not more beneficial

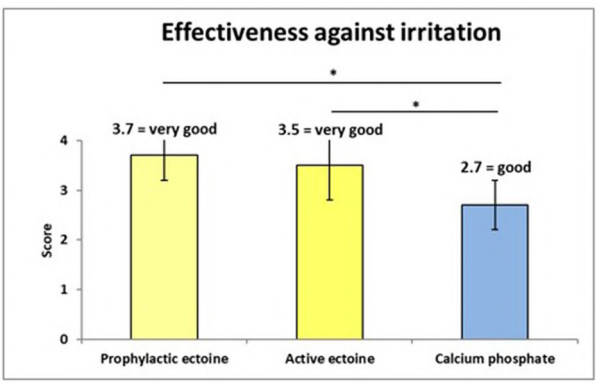

Overall effectiveness
*
*
3.6 = very good
3.5 = very good
2.9 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate

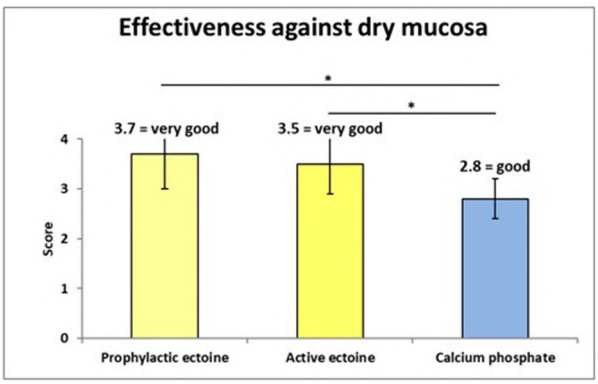

Effectiveness against redness
*
*
3.3 = good
3.6 = very good
2.8 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate

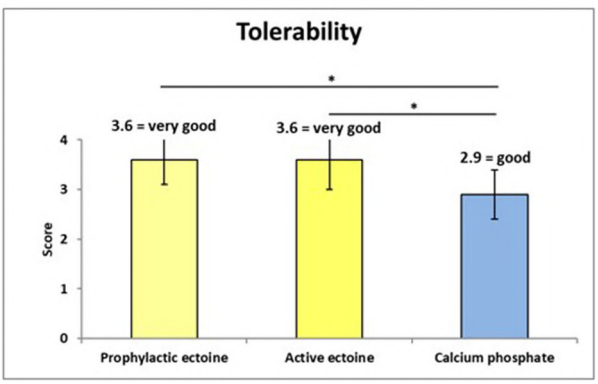

Effectiveness against burning sensation
*
*
3.3 = good to very good
3.5 = very good
2.9 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate


Effectiveness against swelling
*
3.2 = good to very good
3.3= good to very good
2.8= good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate


Effectiveness against pain
*
*
3.5 = very good
3.5 = very good
2.9 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate


Effectiveness against irritation
*
*
3.7 = very good
3.5 = very good
2.7 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate


Effectiveness against dry mucosa
*
*
3.7 = very good
3.5 = very good
2.8 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate


Tolerability
*
*
3.6 = very good
3.6 = very good
2.9 = good
Score
0
1
2
3
4
Prophylactic ectoine
Active ectoine
Calcium phosphate

◀**Fig. 2** Effectiveness and tolerability of investigational products assessed by the patients at V4 (0 = very poor, 1 = poor, 2 = neither poor nor good, 3 = good, and 4 = very good). Data plotted are mean ± SD. Differences between groups were analyzed using the two-sided Fisher's exact test. *Indicates $P < 0.05$

than saline/aspirin mouthwash, cryotherapy, or even placebo [29–31]. Another study showed that calcium phosphate mouthwash did not reduce the incidence of WHO mucositis grade II below historic rates [32]. Taken together, while the evidence-based efficacy of calcium phosphate mouthwash is still disputable, ectoine mouthwash can be a viable treatment option for oral mucositis.

Recently, two studies have shown that ectoine reduced DNA damage caused by ionizing radiation. The authors described ectoine as a hydroxyl radical scavenger and suggested its use as a protective agent in radiotherapy [33, 34]. Hence, one might extrapolate that ectoine mouthwash can be beneficial not only for chemotherapy-related but also for radiotherapy- and radiochemotherapy-related oral mucositis.

This study was conducted as a non-interventional study under routine clinical practice. Though placebo control and randomization were not permitted in this study design according to §17 para (1), point c) of Government Regulation 235/2009 (X.20.) effective in Hungary, this study compared ectoine mouthwash to an active control—calcium phosphate mouthwash. This study design allows for comparison of the effectiveness between these two treatments under real-life conditions. The relatively low number of patients was a limitation in our study. However, the similar baseline characteristics allowed us to compare the effectiveness of the treatment between groups.

Given that ectoine is a natural substance that has remarkably few side effects, we recommend ectoine mouthwash for the active treatment of oral mucositis. Follow-up, placebo-controlled studies are needed to confirm its prophylactic effects.

CONCLUSIONS

Mucositis following chemotherapy can be safely and effectively treated with ectoine-containing mouthwash. Significant reductions of symptoms were detected on day 14. After 21 days of treatment, symptoms were almost completely resolved. Physicians' assessments deduce that ectoine is as effective as calcium phosphate. According to patients' assessments, ectoine is more effective and tolerable than calcium phosphate. Further studies are needed to confirm the effects of ectoine for the prophylaxis of mucositis.

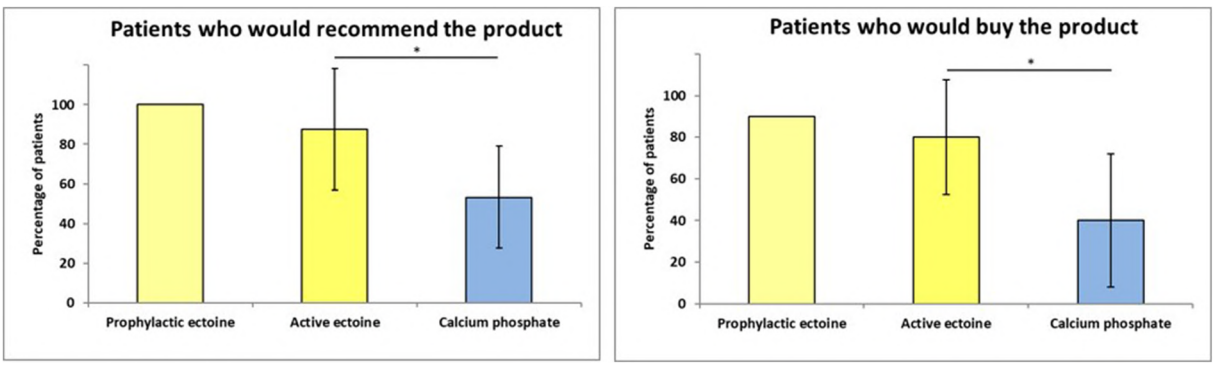


Fig. 3 Patients' likelihood of buying or recommending the product after the study, which confirms their satisfaction with the treatment results. Significant differences between treatment groups were determined using the two-sided Fisher's exact test. *Indicates $P < 0.05$

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Compliance with Ethics Guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Data Availability. All data generated or analyzed during this study are included in this published article/as supplementary information files.

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Ectoin
journal

FLUIDINOVA
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CARE PASTE

WITH NANO-HYDROXYAPATITE

THE FUTURE OF ORAL CARE: AN ALTERNATIVE TO FLUORIDE

- To enhance enamel remineralization
- To prevent dental hypersensitivity
- To create a natural white effect

INNOVATIVE ORAL CARE

FLUIDINOVA

THE NANO-HYDROXYAPATITE SPECIALIST

ABOUT US

FLUIDINOVA was founded in 2005 in Portugal as a specialized manufacturer of synthetic nano-hydroxyapatite and tricalcium phosphate materials, which are commercialized worldwide for different applications (e.g., oral care, biomaterials, 3D printing, food-supplements, biotech, catalysts, etc), under the brand name nanoXIM®.

nanoXIM
CARE PASTE

Can I effectively develop a fluoride-free oral care product?

YES, you can!

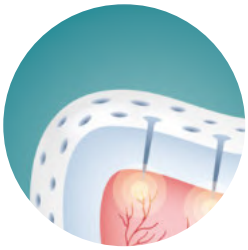
An alternative to fluoride is Hydroxyapatite – a 100% safe and non-toxic ingredient to oral care products and routine.

The nanoXIM•CarePaste is a nano-hydroxyapatite (nHAp) ingredient produced and marketed by FLUIDINOVA.

Its excellent performance is related to its nanometer size, being very similar to natural teeth.

BENEFITS

- Pain reduction
- Smooth and protected tooth surface
- Dental hypersensitivity prevention
- Enamel remineralization
- Cavity prevention
- Natural white effect



1.

Dental hypersensitivity, a short and sharp pain, prevents us from drinking hot coffee, ice cream, or even an orange juice without feeling pain. The action of certain food and drinks (hot, cold, acidic) are considered aggressions to our teeth, resulting in the exposure of dentin tubules and the underlying nerves to the external environment (the dentin loses its protective covering).

2.

nHAp has a great potential in the treatment of dental hypersensitivity, as its nanosized particles can be incorporated inside the dentin tubules. Consequently, these become sealed and pain is reduced.

3.

As a result, a new layer is formed, remineralizing the tooth enamel and protecting the tooth surface, preventing the appearance of new cavities and making it resistant to acid attacks of our favourite meals.

4.

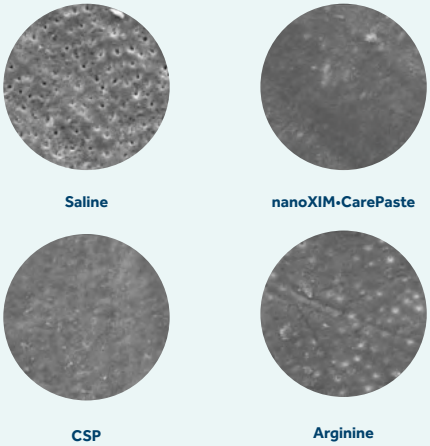
The deposition of nHAp on the enamel surface improves its smoothness for better light reflection, and consequently brighter and whiter teeth.

What is Hydroxyapatite?

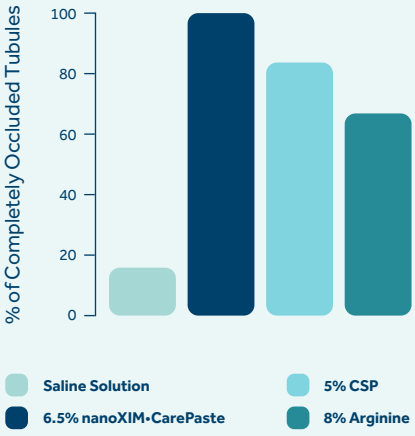
Hydroxyapatite (HAp) is a form of calcium phosphate that composes 97% of tooth enamel and 70% of the dentin. Since it is a key component of your teeth, it is biocompatible. Our body recognizes HAp as a familiar compound.

Thus, products that incorporate HAp simulate the natural composition and structure of teeth, which is why they work effectively.

The effectiveness of nanoXIM•CarePaste has been confirmed in numerous studies:



Scanning Electron Microscopy images of dentin discs treated daily with saline solution (negative control), 6.5% **nanoXIM•CarePaste**, 5% Calcium Sodium Phosphosilicate (CSP) and 8% Arginine for 2 minutes during 7 days (real examples).



Percentage of completely occluded dentin tubules after 7 days of treatment (2 minutes daily treatment) with the different desensitizing agents.

This study demonstrated that nanoXIM•CarePaste was the most effective desensitizing agent, showing almost complete tubule occlusion during the treatment period.

FEATURES

- Synthetic water-based suspension ingredient
- Nanoparticles: size < 100 nm and rod-shaped
- High purity
- Biocompatible
- Paraben-free
- Vegan
- Safe if accidentally swallowed

nanoXIM•CarePaste is the recommended ingredient for aqueous formulations.

It is incorporated in toothpastes, gels, mouthwashes, dental floss and other oral care products (personal and professional use).

Toothpastes | Gels | Mouthwashes | Dental Floss

We aim to innovate and improve the Oral Care industry.

If you want to be part of this journey, contact us! ↓

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