



## Efficacy Evaluation of DENTE91 Anti-Stain Toothpaste: Review of In-Vitro Studies

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### Abstract

**Background:** Tooth stains on the front teeth are a common esthetic issue caused by tooth discoloration. Good oral hygiene habits, such as brushing your teeth twice daily with fluoride toothpaste and after meals, flossing daily, and using a whitening toothpaste, can help reduce staining and prevent cavities. **Material & Methods:** Many in-vitro studies has conducted to develop Dente91 Anti-Stain Toothpaste that can help in resolving the issue of demineralization, hypersensitivity, microbial strains, plaque, dental caries/tooth decay, and dental stain more effectively than other marketed products. Its composition includes ingredients such as Sodium Hexametaphosphate (SHMP), Polyvinylpyrrolidone (PVP), Tetrasodium Pyrophosphate, Covarine Blue, Zinc Citrate, Potassium Citrate, and Xylitol. **Results:** DENTE91 Anti-Stain toothpaste has over 99.9% significant antimicrobial efficacy and effectively reduces demineralization while enhancing the remineralization process. It also provides protection against acid attacks, promotes cell proliferation in NIH/3T3 cells, and shows significant healing activity on dental cavities. Additionally, it has the ability to occlude dentine disc lesions and reduce hypersensitivity. The findings of this study suggest that the toothpaste after using it twice a day for three days removes 63.02 % stains and also shows 80.33% improvement in teeth whitening from baseline. **Conclusion:** These in-vitro studies of dente91 anti-stain toothpaste concluded that toothpaste helps in resolving demineralization, hypersensitivity, plaque, dental caries/tooth decay, and dental stains. It has antimicrobial activity and teeth whitening effect too.

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## INTRODUCTION

We use toothpaste with the help of toothbrush to sustain and upgrade oral health and aesthetics.<sup>[1]</sup> Although, It's not a flashy product, toothpaste is an indispensable necessity for consumers.<sup>[1,2]</sup> If we talk about its origin then several thousand years ago, toothpaste formulations have evolved considerably - from suspensions of crushed egg shells or ashes to complex formulations with often more than 20 ingredients. However, among these can contain

compounds that fight with tooth decay, gum disease, bad breath, tartar, erosion, and dentin sensitivity.<sup>[1]</sup>

Nowadays, teeth are subjected to human personality and impression while modern life style and food habits (high sugar, poor dietetic, fancy and high calorie fast foods etc) creates the necessity of high-quality toothpaste that can resolve multiple dental problem like bacterial infection, Demineralization, hypersensitivity,



dental caries/tooth decay and dental stain etc.<sup>[3,4,5,6,7,8]</sup>

According to WHO Global Oral Health Status Report (2022), oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle-income countries.<sup>[2]</sup> It involves the gums and their supporting tissues, the palate, the lining of the mouth and throat, the tongue, the lips, the salivary glands, the chewing muscles, the nerves, and the bones of the upper and lower jaws. To fight with these dental problems many medications or tooth pastes are available in the market. However, as per the modern-day necessity, a toothpaste is required which can resolve more than one or maximum dental problems.

We have conducted many in-vitro studies to develop Dente Anti-Stain Toothpaste that can help in resolving the issue of demineralization, hypersensitivity, microbial strains, plaque, dental caries/tooth decay, and dental stain more effectively than other marketed products. The composition of the Dente Anti-Stain Toothpaste contains Sodium Hexametaphosphate (SHMP), Polyvinylpyrrolidone (PVP), Tetrasodium Pyrophosphate, Covarine Blue, Zinc Citrate, Potassium Citrate and Xylitol.<sup>[9,10,11]</sup>

Sodium Hexametaphosphate, Polyvinylpyrrolidone and Tetrasodium Pyrophosphate supports in controlling the stain formation, stain removal and reduction in stain formation while Covarine Blue, Zinc Citrate and Xylitol work as an optical modifier, anti-plaque agent and cariostatic agent respectively. Potassium Citrate helps to reduce dentin hypersensitivity.

## MATERIAL AND METHODS

Total 07 Invitro studies was conducted during the time period from October 2022 to December 2022, which includes antimicrobial effectiveness, Remineralization effects, Effect on healing activity, hypersensitivity against marketed product, Protection against dental caries/tooth decay, effect on dental stain and on tooth whitening. In this review all the study were thoroughly understand and effectiveness of the anti-stain toothpaste were assessed on below mentioned parameters;

### Antimicrobial Effectiveness<sup>[4]</sup>

Antimicrobial Effectiveness of DENTE91 Anti-Stain Toothpaste on four standard microbial strains, namely, *Candida albicans* (Ca), *Streptococcus mutans* (Sm), *Porphyromonas gingivalis* (Pg) & *Pseudomonas* were assessed in microbiological lab inhouse invitro study. % Efficacy or % Germ Killing were calculated using following formula.

$$\% \text{ Survival} = \frac{\text{CFU of sample} \times 100}{\text{CFU of positive control}}$$
$$\% \text{ Efficacy or } \% \text{ Germ Killing} = 100 - \% \text{ Survival}$$

### Following acceptance criteria was followed;

% of survival rate is not more than 0.1% for 2 mins exposure and not more than 0.01% for 5 mins exposure. Efficiency of DENTE91 Anti-Stain Toothpaste for killing organisms shall be not less than 99.9%.

### Remineralization effect<sup>[5]</sup>

It consists of two 2-min toothpaste treatment periods, one 6-h acid challenge, and then storage in remineralizing solution for the rest of the time, including night. Specimens was treated with freshly prepared slurries of



toothpaste two times per day. For treatment, the demineralization and remineralization solutions were stirred, while the toothpaste slurry was static. Specimens were treated individually or collectively as treatment groups.

**Table 1:** Treatment Procedure to assess the remineralization effect:

Time	Treatment
For 96 h	Demineralization
Day 1 is all-day storage in remineralization solution. Then, subsequent days' treatments will be as follows	
2min	Tooth paste treatment
Rinse with deionized distilled water	
6h	Acid challenge(demineralization)
Rinse with deionized distilled water	
2min	Toothpaste treatment
Rinse with deionized distilled water	
16h	Storage in remineralization solution
Repeated for 8 additional days	
Only demineralization and remineralization treatment given to control group.	

In this study, artificial carious lesions were produced using demineralizing solutions. Artificial carious lesions are considered to be more reproducible than natural carious lesions, thus making the experimental model more reliable. We facilitate the testing of multiple areas in any lesions at different time intervals, to assess the remineralizing phenomena.

### Healing Activity<sup>[6]</sup>

In experimental procedure NIH/3T3 cells were culture in alpha-minimum essential medium (MEM) containing 10% FBS at 37°C in humidified CO<sub>2</sub> incubator and confluent culture was used for testing. For cell number measurement, cells were seeded ( $2 \times 10^4$ ) in 48-

well plate and incubated in CO<sub>2</sub> incubator for 3 h. After incubation, initial medium was aspirated and 300  $\mu$ L of statin free medium and 50  $\mu$ L of MTT (1 mg/mL) was added to each well. Plate was further incubated for 2 h. After incubation, 100  $\mu$ L of mixture was transfer to 96-well plate and reaction was stopped by adding 20  $\mu$ L of isopropanol. Optical density was measured at 570 nm in microplate reader. This reading was used as baseline data for further experiment then Cell proliferation assay was performed using this baseline data. Cells in each well were cultured for 12 h under the presence of test sample at concentrations 0.1%, 0.05% and 0.01%.

### Hypersensitivity<sup>[7]</sup>

In sample Preparation teeth was taken and clean it with deionised water then the tooth was cut in longitudinal section and a dentine disc was formed. Discs was divided in 3 different group like Test sample market sample and Control. Treatment was given by immersing the disc in lemon juice for 30 seconds. Toothpaste Dilution is 1:3 (Toothpaste/water) and the procedure for the treatment after the lemon juice treatment, the disc was placed on a plate and treated with toothpaste by brushing. Toothpaste treatment is given for continuously for 2 hrs. A tooth brushing time of six months was simulated (2 hrs of continuous brushing, assuming 28 teeth in an oral cavity and 2 X 3 min tooth brushing per day). After treatment with toothpaste, the Dentine disc was washed by using Deionised water and the control disc was treated with lemon juice and washed with deionized water, no toothpaste treatment was given. Dentine disc was stored in deionized water and observed it under Scanning Electron Microscope. Observation of Sample by



Scanning Electron Microscope (SEM) are following.

### Dental Caries/Tooth Decay<sup>[8]</sup>

It consisted of two 2-min toothpaste treatment periods, one 6-h acid challenge, and then storage in remineralizing solution for the rest of the time, including night. Specimens were treated with freshly prepared slurries of toothpaste two times per day. For treatment, the demineralization and remineralization solutions were magnetically stirred, while the toothpaste slurry was static. Specimens were treated individually or collectively as treatment groups.

**Table 2:** Treatment Procedure to assess the effectiveness of the product over Dental Caries/Tooth Decay:

Time	Treatment
For 96 h	Demineralization
Day 1 is all-day storage in remineralization solution. Then, subsequent days' treatments will be as follows	
2min	Tooth paste treatment
Rinse with deionized distilled water	
6h	Acid challenge(demineralization)
Rinse with deionized distilled water	
2min	Toothpaste treatment
Rinse with deionized distilled water	
16h	Storage in remineralization solution
Repeated for 8 additional days	
Only demineralization and remineralization treatment given to control group.	

### Dental Stain<sup>[9]</sup>

Toothpaste was making slurry with water for stain removal treatment. Slurry was applied to the enamel sample twice a day for 2 minutes with a toothbrush to stain the treated teeth.

After treatment the specimen was washed with water before colour evolution. No toothpaste treatment was given on the stain control teeth, only water was used to wash the teeth. This treatment was given for three consecutive days. The specimen dried with soft tissue before colour measurement. Lux meter was used for the colour measurement.

**% stain removal were calculated with below formula:**

$\% \text{ stain removal} = \% \text{ of stain treated teeth} - \% \text{ of stain control teeth}$

### Tooth Whitening<sup>[10]</sup>

Teeth was taken after visual inspection for retention and stain on it. Toothpaste treatment were given to the initial teeth for twice a day for 2 minutes with a toothbrush. To control teeth only water wash treatment was given. This treatment was given for three consecutive days.

## RESULTS

DENTE91 Anti-Stain toothpaste having significant antimicrobial efficacy against Gram positive (*S.mutans*), Gram negative (*P. aeruginosa*, *Porphyromonas gingivalis*) and Fungi (*C.albicans*). It is also evident that DENTE91 Anti-Stain Toothpaste have antimicrobial effects on microorganisms. It reduces the progress of demineralization, enhancing remineralization process and simultaneously provides protection against the inevitable acid attack. DENTE91 anti-stain toothpaste promoted proliferation of NIH/3T3 cells and there by showed significant healing activity on dental cavity. The study product has the ability to occlude the lesions of dentine disc and reducing hypersensitivity. By analyzing the scanning electron microscopy observation and



mineral profile, DENTE91 Anti-stain toothpaste ensures a mineral regain for demineralized enamel, thereby it protects against dental caries prevent tooth decay. DENTE91 anti-stain study

result suggests that toothpaste after using it twice a day for three days removes 63.02 % stains and shows 80.33% improvement in teeth whitening from baseline.

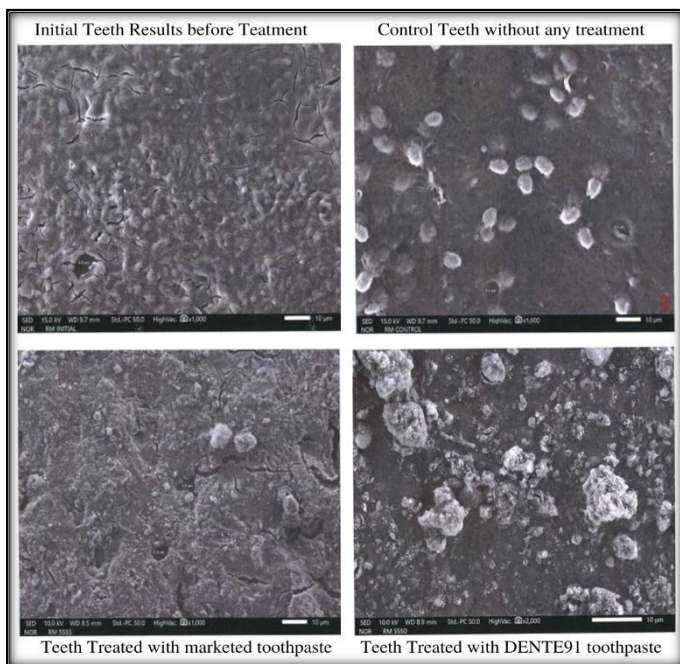
**Table 3:** Survival and Efficacy Results are as follows

Name of Organism	Avg. CFU		Exposure Time	DENTE91 anti-stain toothpaste	% Survival	% Efficacy
	Positive Control					
S. mutans	8500000	2 mins	1400	0.0165%	99.98%	
		5 mins	1050	0.0124%	99.99%	
P. aeruginosa	3500000	2 mins	3350	0.0957%	99.90%	
		5 mins	550	0.0157%	99.98%	
C. albicans	6500000	2 mins	800	0.0123%	99.99%	
		5 mins	1250	0.0192%	99.98%	
P. gingivalis	7000000	2 mins	1450	0.0207%	99.98%	
		5 mins	350	0.0050%	100.00%	

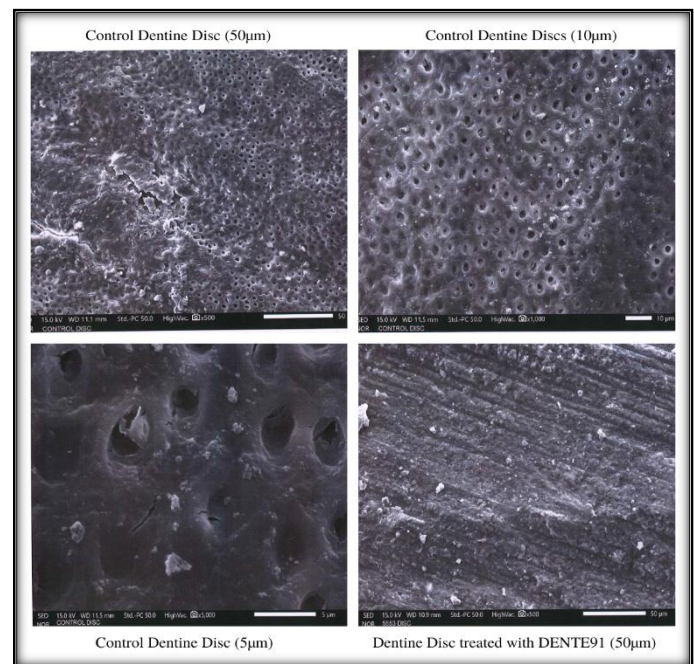
**Table 4:** In-House Study Data of In-Vitro studies

SN	Objective	Comparator	Results
1	Evaluation of Antimicrobial Effectiveness on four standard microbial strains, namely, Candida albicans (Ca), Streptococcus mutans (Sm), Porphyromonas gingivalis (Pg) & Pseudomonas.	None	The survival rate of microbials was less than 0.1% for 2-minute exposure and 0.02% for 5-minute exposure of DENTE91 Anti-Stain Toothpaste. More than 99.9% efficacy for DENTE91 Anti-Stain Toothpaste was achieved.
2	To provide documentary evidence of ‘Remineralization Effects of DENTE91 Anti-Stain Toothpaste on teeth against marketed product’.	Colgate Visible White (Market Sample)	The lesions in DENTE91 Anti-stain toothpaste treated teeth were significantly less as compared to control teeth and teeth treated with marketed toothpaste.
3	Evaluate the effect on Healing Activity of Dental Cavities by “DENTE91 ANTI-STAIN TOOTHPASTE” by performing cell proliferation assay using NIH/3T3 cell line.	None	The cell proliferation of NIH/3T3 was significantly promoted by DENTE91 toothpaste at dosages of 0.01% and comparable with respect to baseline. Study findings indicate that the treatment of toothpaste activated proliferation of cells and subsequent healing of dental cavity.
4	To establish documentary evidence of Reduction in Hypersensitivity effects of DENTE91 Anti-Stain Toothpaste on teeth against marketed product.	Colgate Visible White (Market Sample)	The more coverage of the lesions was observed in DENTE91 Anti-stain toothpaste treated dentine discs with as compared to the dentine discs treated with marketed sample.

5	To establish documentary evidence of Protection Against Dental Caries/Tooth Decay by DENTE91 Anti-Stain Toothpaste and Compared with Marketed Product.	Colgate Visible White (Market Sample)	In treated teeth, pores were not observed and crystalline structure was found which indicated the regain of mineral content. Improvement of minerals in teeth treated with DENTE91 Anti-Stain Toothpaste is more than marketed product.
6	To establish documentary evidence of effectiveness on dental stain of DENTE91 Anti-Stain Toothpaste.	None	By using DENTE91 Anti-Stain Toothpaste twice a day for three days removes 63.02 % stains from baseline.
7	The objective of this study was to establish documentary evidence of effectiveness on tooth whitening of DENTE91 Anti-Stain Toothpaste.	None	By using DENTE91 Anti-Stain Toothpaste twice a day for three days shows 80.33% improvement in teeth whitening from baseline.



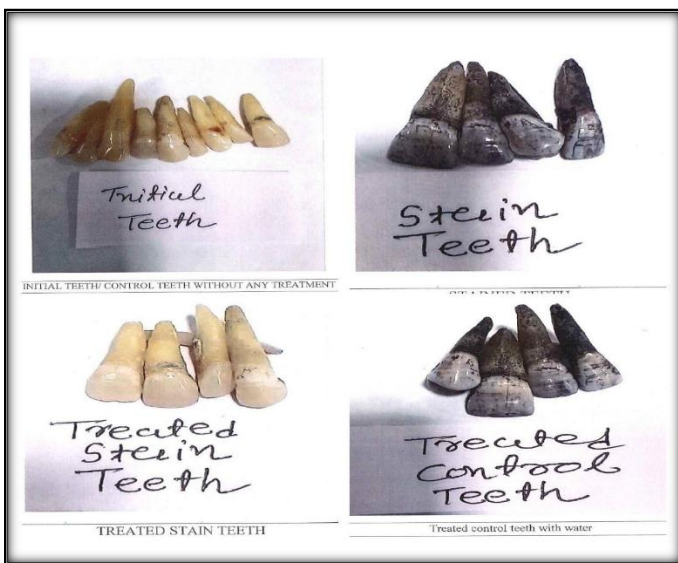
**Figure 1:** Remineralization effect



**Figure 2:** Hypersensitivity



**Figure 3:** Dental Caries/Tooth Decay



**Figure 4:** Dental Stain



**Figure 5 (a):** Tooth Whitening: Control teeth After Water Treatment



**Figure 5 (b):** Tooth Whitening: Teeth After Teeth Whitening Treatment

### DISCUSSION & CONCLUSIONS

Toothpaste is a key part of our daily oral hygiene routine. Oral health is an integral part of overall health, and each influences the



other.<sup>[18,19,20,21]</sup> Improper diet, smoking, alcohol intake, and poor oral hygiene practices are the most significant factors influencing the occurrence of various oral diseases.<sup>[16]</sup> Some of the most common oral diseases that impact the oral health includes cavities (tooth decay), gum (periodontal) disease, teeth stains, dentine hypersensitivity and oral cancer.<sup>[12,13,14,15,16,17,18,19,20]</sup>

Though there are many toothpastes available in market but owing to differences in composition and fluoride content, not all toothpastes are equally effective in maintaining oral health. Total 07 in vitro studies were conducted to evaluate the effectiveness of dente91 anti stain toothpaste.

Based on antimicrobial efficacy of DENTE91 Anti-Stain Toothpaste, it can be claimed that DENTE91 Anti-Stain Toothpaste can be effective in Candidiasis (Fungal Infection), Diabetic halitosis (Bad Breath), Gingivitis (Mild Gum Disease) and Periodontitis (Serious Gum

Infection / Advanced stage of Gingivitis). It is also prevents the caries development (net demineralization) or net remineralization of early caries lesions. Proposed toothpaste showed significant healing activity on dental cavity. It has the ability to occlude the lesions of dentine disc and reducing hypersensitivity. Through scanning electron microscopy observation and mineral profile, it ensures a mineral regain for demineralized enamel, thereby it protects against dental caries prevent tooth decay. The study result suggests that toothpaste after using it twice a day for three days removes 63.02 % stains and shows 80.33% improvement in teeth whitening from baseline. Further, clinical usage study is suggested to evaluate the clinical efficacy.<sup>[21]</sup> These in-vitro studies of dente91 anti-stain toothpaste concluded that toothpaste helps in resolving demineralization, hypersensitivity, plaque, dental caries/tooth decay, and dental stains. It has antimicrobial activity and teeth whitening effect too.

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