

## In vitro study of the effect of antioxidants on enamel bleaching

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### ABSTRACT

**Background:** The objective of this study is to investigate the effects of antioxidants, specifically tannic acid and N-acetyl cysteine (NAC), on enamel bleaching in vitro.

**Materials & Methods:** Enamel samples are obtained from human teeth and standardized for consistency. Ethanolic solution of curcumin are used as a staining agent. Enamel samples are divided into different groups: control (no treatment), bleaching agent only, bleaching agent with tannic acid, and bleaching agent with NAC. Enamel samples are exposed to the respective treatments for a predetermined period, simulating typical clinical conditions. Various methods such as colorimetry and scanning electron microscopy (SEM) are employed to assess enamel changes pre- and post-treatment.

**Results:** Enamel samples treated with antioxidants (tannic acid and NAC) alongside bleaching agents exhibit reduced color changes and less pronounced structural alterations compared to samples treated with bleaching agents alone.

**Conclusion:** The results of this in vitro study suggest that antioxidants such as tannic acid and NAC may have a protective effect on enamel during bleaching procedures. Incorporating antioxidants into bleaching protocols could potentially reduce enamel damage and enhance the safety of dental bleaching treatments.

**Keywords:** Enamel bleaching, tannic Acid, N-Acetyl Cysteine.

### INTRODUCTION

Dental bleaching has become a popular cosmetic procedure for improving the appearance of discolored teeth. However, the use of bleaching agents can often lead to undesirable side effects, including enamel damage and tooth sensitivity.<sup>[1]</sup> These adverse effects are primarily attributed to the oxidative stress induced by the reactive oxygen species (ROS) generated during the bleaching process. In recent years, there has been growing interest in exploring adjunctive therapies aimed at

mitigating the oxidative damage associated with dental bleaching.<sup>[2, 3]</sup>

Antioxidants have emerged as promising candidates for reducing the detrimental effects of oxidative stress on dental tissues. Tannic acid and N-acetyl cysteine (NAC) are two such antioxidants that have shown potential in scavenging ROS and protecting tissues from oxidative damage.<sup>[4]</sup> Tannic acid, a polyphenolic compound found in various plant sources, possesses strong antioxidant properties due to its ability to chelate metal ions and inhibit

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ROS formation. Similarly, NAC, a derivative of the amino acid cysteine, acts as a precursor to glutathione, a key antioxidant enzyme in the body.<sup>[5]</sup> While the potential benefits of antioxidants in mitigating oxidative stress are well-documented in various biological systems, their efficacy in the context of dental bleaching remains relatively unexplored. Understanding the impact of antioxidants on enamel bleaching is crucial for improving the safety and efficacy of this widely practiced cosmetic procedure.<sup>[6, 7]</sup>

This in vitro study aims to investigate the effect of antioxidants, specifically tannic acid and NAC, on enamel bleaching. By subjecting enamel samples to bleaching agents in the presence and absence of antioxidants, we seek to evaluate whether these compounds can attenuate the oxidative damage induced by bleaching agents and preserve the integrity of dental enamel. The findings of this study could have significant implications for the development of adjunctive therapies aimed at enhancing the outcomes of dental bleaching procedures while minimizing the risk of enamel damage and tooth sensitivity.

Through a comprehensive examination of the effects of antioxidants on enamel bleaching, this study endeavors to contribute valuable insights to the field of cosmetic dentistry and promote the adoption of safer and more efficacious bleaching protocols.

## ENAMEL BLEACHING

Enamel bleaching is a cosmetic dental procedure aimed at lightening the color of teeth and removing stains or discoloration. It has gained widespread popularity as a non-invasive method to enhance the aesthetic appearance of the smile (figure 1). The procedure involves the application of bleaching agents to the surface of the teeth, which penetrate the enamel and break down the chromophores responsible for discoloration.<sup>[8, 9]</sup>

There are two primary methods of enamel bleaching:

1. **At-Home Bleaching:** This involves the use of over-the-counter bleaching products such as whitening toothpaste, strips, or trays. These products typically contain lower concentrations of bleaching agents (e.g., hydrogen peroxide or

carbamide peroxide) and are applied by the patient at home over a period of several days or weeks.<sup>[11]</sup>

2. **In-Office Bleaching:** Also known as chairside bleaching, this procedure is performed by a dental professional in a clinical setting. It involves the application of higher concentrations of bleaching agents, often in combination with light or heat activation, to achieve more immediate and dramatic results. In-office bleaching typically yields faster results compared to at-home methods but may be associated with increased risk of tooth sensitivity.<sup>[12]</sup>



Figure 1: enamel bleaching<sup>[10]</sup>

Enamel bleaching works by oxidizing the organic molecules responsible for tooth discoloration, thereby breaking them down into smaller, less pigmented fragments. This process effectively lightens the overall color of the teeth, resulting in a brighter and more radiant smile. However, it's important to note that enamel bleaching may not be suitable for all individuals, and its efficacy can vary depending on factors such as the type and severity of staining, the concentration of bleaching agents used, and the condition of the teeth and gums.<sup>[13, 14]</sup>

While enamel bleaching is generally considered safe when performed under the supervision of a dental professional, it can sometimes cause side effects such as tooth sensitivity, gum irritation, or temporary changes in the texture of dental enamel. Additionally, overuse or misuse of bleaching products can lead to damage to the enamel or other oral tissues. Therefore, it's essential to follow the recommendations of dental professionals and use bleaching products judiciously to achieve optimal

results while minimizing the risk of adverse effects.<sup>[15]</sup>

### TANNIC ACID

Tannic acid, also known as gallotannic acid or digallic acid, is a naturally occurring polyphenolic compound found in various plant sources, including fruits, nuts, seeds, and tree barks. It is particularly abundant in plants such as oak, gallnuts, and tea leaves.<sup>[16]</sup> Tannic acid is formed through the esterification of glucose with gallic acid and possesses a complex chemical structure consisting of multiple phenolic hydroxyl groups. Its chemical structure is relatively complex, consisting of multiple phenolic hydroxyl groups attached to a central glucose molecule (figure 2).<sup>[17]</sup>

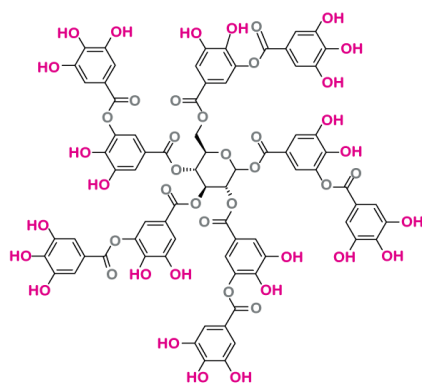


Figure 2: Chemical Structure of tannic acid<sup>[18]</sup>

Tannic acid exhibits potent antioxidant properties, which are attributed to its ability to scavenge free radicals and inhibit oxidative damage to biological molecules such as proteins, lipids, and DNA. As an antioxidant, tannic acid helps protect cells and tissues from oxidative stress and may contribute to overall health and well-being.<sup>[19]</sup>

### N-ACETYL CYSTEINE (NAC)

N-acetyl cysteine (NAC) is a derivative of the amino acid cysteine, with the addition of an acetyl group. Its chemical structure is represented in figure 3.<sup>[20]</sup>

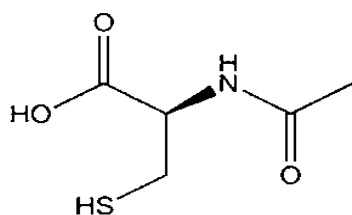


Figure 3: Chemical structure of N-acetyl cysteine<sup>[21]</sup>

NAC is a precursor to cysteine, which is a crucial amino acid involved in the synthesis of glutathione, one of the body's most important antioxidants. Glutathione plays a vital role in protecting cells from oxidative damage and maintaining cellular redox balance. NAC acts as a precursor to glutathione synthesis, thereby enhancing the body's antioxidant defenses and protecting cells from oxidative stress. It scavenges free radicals and reactive oxygen species (ROS), reducing oxidative damage to cellular components.<sup>[22]</sup>

N-acetyl cysteine (NAC) can be obtained from poultry, eggs, dairy products (especially cheese and yogurt), fish, and meat. garlic, onions. NAC is widely available in the form of dietary supplements, including capsules, tablets, and powders.<sup>[23]</sup>

### MATERIAL AND METHODS

**Selection of enamel samples:** Enamel samples are obtained from human teeth and standardized for consistency.

**Preparation of staining agents:** Obtain high-quality curcumin powder from a reputable supplier. Ensure that the curcumin is pure and free from contaminants. Ethanol or dimethyl sulfoxide (DMSO) are commonly used solvents for curcumin due to their ability to dissolve the compound effectively. 10 gram accurately weigh the amount of curcumin powder using a laboratory balance. The amount of curcumin will depend on the desired concentration of the solution and the volume needed for the experiment.

Add the weighed curcumin powder to a clean, dry container. Slowly add the chosen solvent (e.g., ethanol) to the container while stirring gently to dissolve the curcumin. The concentration of the curcumin solution can be adjusted by varying the ratio of curcumin to solvent. If necessary, filter the curcumin solution through a filter paper or membrane to remove any undissolved particles or impurities. This step may not be required if the curcumin powder is of high purity and readily dissolves in the chosen solvent. Transfer the prepared curcumin solution to a clean, tightly sealed container for storage. Store the solution in a cool, dark place away from direct sunlight and heat sources to prevent degradation of the compound.

Exercise caution when applying the curcumin solution to enamel for experimental purposes. Use a small amount of the solution and apply it to the surface of the enamel using a clean applicator brush or cotton swab. Avoid contact with the gums and soft tissues of the mouth.

Allow the curcumin solution to remain in contact with the enamel for the desired duration, typically 5-6 weeks, depending on the experiment. Observe any changes in the color of the enamel caused by the curcumin staining.

**Preparation of solutions:** 10 gram of tannic acid was dissolved in 100ml of distilled water to make 10% tannic acid solution, and 10 gram of N-acetyl cysteine was dissolved in 100ml of distilled water to make 10% N-acetyl cysteine solution

**Experimental groups:** Enamel samples are divided into different groups: control (no treatment), bleaching agent only, bleaching agent with tannic acid, and bleaching agent with NAC.

**Application of treatments:** Enamel samples are exposed to the respective treatments for a predetermined period, simulating typical clinical conditions.

**Evaluation of enamel changes:** Two methods such as colorimetry (Pharmacia Biotech - Ultrospec 3000) and scanning electron microscopy (Schaefer SEE Srl) are employed to assess enamel changes pre- and post-treatment.

## RESULT

The in vitro study investigated the effect of antioxidants, specifically tannic acid and N-acetyl cysteine (NAC), on enamel bleaching. Enamel samples were exposed to bleaching agents with and without the addition of antioxidants, and various parameters were assessed to evaluate the effects on enamel color and morphology.

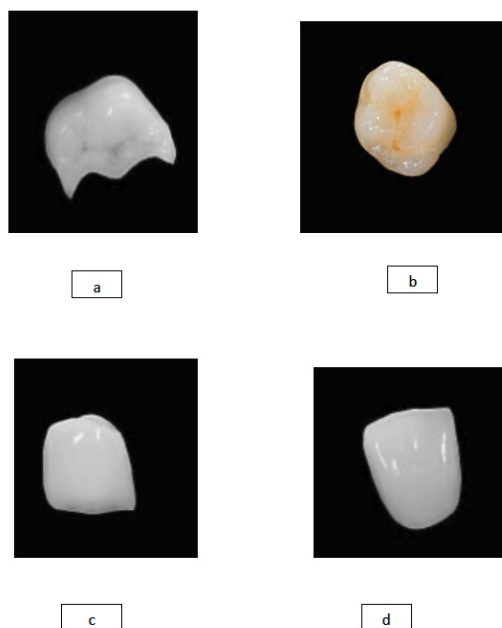
Table 1 representing a effect on enamel samples with or without treatment of antioxidants treatment

**Table 1:** Enamel samples with or without treatment of antioxidants treatment

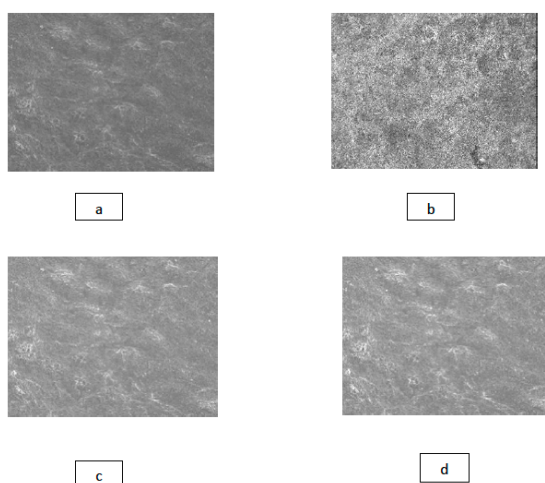
| Enamel Sample | Staining Agent                  | Antioxidants treatment | Effect           |
|---------------|---------------------------------|------------------------|------------------|
| 1             | None                            | None                   | Whiten Enamel    |
| 2             | Ethanollic solution of curcumin | None                   | Yellowish Enamel |
| 3             | Ethanollic solution of curcumin | Tannic acid            | Whiten Enamel    |
| 4             | Ethanollic solution of curcumin | N-acetyl cysteine      | Whiten Enamel    |

Colorimetric analysis revealed significant changes in enamel color following treatment with bleaching agents alone (figure 4). Samples treated solely with bleaching agents exhibited noticeable color lightening, indicative of the bleaching effect. However, these color changes were accompanied by alterations in enamel morphology, as observed through scanning electron microscopy (SEM) shown in figure 5. Enamel surfaces showed signs of irregularities and microstructural changes, suggesting potential damage induced by the bleaching agents.

In contrast, enamel samples treated with bleaching agents in combination with antioxidants (tannic acid and NAC) exhibited reduced color changes and less pronounced structural alterations. The addition of antioxidants appeared to mitigate the adverse effects of bleaching agents on enamel, preserving enamel integrity to a greater extent compared to samples treated with bleaching agents alone.



**Figure 4:** Colorimetric analysis revealed significant changes in enamel color; (a) sample 1 not treated with staining agents, (b) sample 2 treated with staining agents, (c) sample 3 treated with Tannic acid, (d) sample 4 N-acetyl cysteine



**Figure 5:** Scanning Electron Microscopy (SEM) analysis revealed significant changes in enamel color; (a) sample 1 not treated with staining agents, (b) sample 2 treated with staining agents, (c) sample 3 treated with Tannic acid, (d) sample 4 N-acetyl cysteine

## DISCUSSIONS

The results of this in vitro study suggest that antioxidants, such as tannic acid and N-acetyl cysteine, have a protective effect on enamel during bleaching procedures. By scavenging free radicals

and inhibiting oxidative damage, antioxidants may help mitigate the adverse effects of bleaching agents on enamel color and morphology.

The observed reduction in color changes and structural alterations in enamel samples treated with antioxidants highlights the potential of these compounds to improve the safety and efficacy of dental bleaching procedures. Preservation of enamel integrity is crucial for maintaining dental health and preventing complications such as tooth sensitivity and enamel erosion.

Overall, the results of this study contribute valuable insights to the field of cosmetic dentistry and provide a foundation for the development of safer and more effective bleaching protocols.

## CONCLUSION

The results of this in vitro study suggest that antioxidants such as tannic acid and NAC may have a protective effect on enamel during bleaching procedures. Incorporating antioxidants into bleaching protocols could potentially reduce enamel damage and enhance the safety of dental bleaching treatments.

## REFERENCES

1. Attin, T., & Albrecht, K. (2003). Effect of bleaching on restorative materials and restorations a systematic review. *Dental Materials*, 19(8), 737-754.
2. Basting, R. T., Rodrigues, A. L., Serra, M. C., & Dibb, R. G. (2001). The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness. *Operative Dentistry*, 26(6), 531-539.
3. Carey, C. M. (2014). Tooth whitening: what we now know. *Journal of Evidence-Based Dental Practice*, 14, 70-76.
4. Costa, R., Perote, L. C., Alonso, H., & De Goes, M. F. (2010). Effect of 10% carbamide peroxide bleaching on the microhardness of sound and demineralized enamel. *Operative Dentistry*, 35(2), 205-210.
5. Da Silva, T. M., Dos Santos, P. H., Tonholo, J., & Basting, R. T. (2017). Microhardness and color change assessment of enamel exposed to a

- combination of at-home whitening and enamel microabrasion techniques. *Clinical Oral Investigations*, 21(3), 947-953.
6. Demarco, F. F., Meireles, S. S., Masotti, A. S., & Overbeck, V. B. (2009). Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources. *Operative Dentistry*, 34(6), 628-635.
7. Feiz, A., Barekatin, M., Maleknejad, F., & Ghaffari, N. (2014). Microhardness of enamel surface after home bleaching and laser-assisted in office bleaching. *Journal of Dentistry*, 15(2), 61-66.
8. Joiner, A. (2006). The bleaching of teeth: a review of the literature. *Journal of Dentistry*, 34(7), 412-419.
9. Kwon, S. R., & Wertz, P. W. (2007). Review of the Mechanism of Tooth Whitening. *Journal of Esthetic and Restorative Dentistry*, 19(5), 309-317.
10. [https://gardensdental.com.au/wp-content/uploads/2020/01/shutterstock\\_1578258496.jpg](https://gardensdental.com.au/wp-content/uploads/2020/01/shutterstock_1578258496.jpg)
11. Mueller, S., & Senekowitsch, C. (2014). Professional bleaching with 38% hydrogen peroxide in different protocols: influence on tooth color and risk of pulp damage. *Journal of Endodontics*, 40(4), 481-484.
12. Nawareg, M. A., & Elkateb, M. A. (2015). Influence of cavity preparation technique on enamel bond strength of different bonding agents. *Journal of Conservative Dentistry*, 18(2), 116-119.
13. Parreiras, S. O., Vianna, P., Kossatz, S., Loguercio, A. D., Reis, A., & Farago, P. V. (2014). Effect of enamel pretreatment with fluoride on the penetration and bonding durability of universal adhesive systems. *Journal of Dentistry*, 42(8), 1001-1007.
14. Sulieman, M. A. (2005). An overview of tooth-bleaching techniques: chemistry, safety and efficacy. *Periodontology 2000*, 48(1), 148-169.
15. Swift Jr, E. J. (2017). Critical Appraisal: Clinical Study Designs, Part II. *Inside Dentistry*, 13(5), 70-73.
16. Torneck, C. D., Titley, K. C., & Smith, D. C. (1988). Adhesion of composite resin to bleached and unbleached bovine enamel. *Journal of Oral Rehabilitation*, 15(5), 489-495.
17. Ünlü, N., Güler, E., Altay, N., & Ozer, F. (2007). Effect of different adhesive systems and laser treatment on the shear bond strength of bleached enamel. *Operative Dentistry*, 32(6), 588-595.
18. <https://cdn1.byjus.com/wp-content/uploads/2019/04/structure-of-tannic-acid.png>
19. Wiegand, A., Krieger, C., Drebenstedt, S., Magalhães, A. C., & Attin, T. (2006). Abrasion of eroded dentin caused by toothpaste slurries of different abrasivity and toothbrushes of different filament diameter. *Journal of Dentistry*, 34(7), 480-485.
20. Yalcin, F., & Korkmaz, Y. (2002). Evaluation of different amounts of sodium ascorbate in the prevention of bleaching-induced tooth sensitivity: a randomized, double-blind clinical trial. *Journal of Endodontics*, 28(11), 810-813.
21. <https://www.researchgate.net/publication/259808967/figure/fig1/AS:341271500607498@1458376874904/Molecular-structure-of-N-acetylcysteine.png>
22. Zanini, M., Szesz, A. L., Bonatto, L. R., & Bresolin, C. R. (2010). In vitro evaluation of the microhardness of human enamel exposed to acidic drink and submitted to brushing abrasion. *European Journal of Dentistry*, 4(4), 398-402.
23. Zantner, C., Derdilopoulou, F., Martus, P., Kielbassa, A. M., & Raab, W. H. (2007). In vitro evaluation of toothbrushing abrasion of differently bleached enamel surfaces. *Clinical Oral Investigations*, 11(3), 231-236.

