# Remineralization Potential Of Himalayan Rock Salt On Human Extracted Premolar Teeth: An In Vitro Study

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#### Abstract

# Background:

Dental caries remains a prevalent oral disease caused by demineralization of enamel. Novel natural agents like Himalayan rock salt, known for their mineral richness, are emerging as potential remineralizing agents. To evaluate the remineralization potential of Himalayan rock salt on artificially demineralized enamel surfaces of human extracted premolar teeth using an in vitro protocol.

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#### **Materials and Methods**

Forty extracted human premolars were cleaned and sectioned. Samples were divided into 2 groups: Group A (treated with 10% Himalayan rock salt solution) and Group B (control group treated with deionized water). Artificial demineralization was performed using 0.1M lactic acid gel for 72 hours. Samples were treated with respective solutions twice daily for 14 days. Surface microhardness (SMH) was assessed at baseline, after demineralization, and post-treatment using Vickers Hardness Test. Scanning Electron Microscopy (SEM) was used for surface morphology.

#### Results:

Group A showed a statistically significant increase in microhardness values after treatment compared to Group B (p < 0.05). SEM analysis revealed smoother and denser enamel surfaces in the test group.

#### Conclusion:

Himalayan rock salt demonstrated a promising remineralization potential and could be explored as a natural adjunct in preventive dental care.

Keywords: Himalayan rock salt, remineralization, enamel, microhardness, in vitro, SEM

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# I. Introduction

Dental caries is one of the most prevalent chronic diseases globally, affecting individuals across all age groups. It is a multifactorial condition caused by the interaction of host factors, dietary carbohydrates, and cariogenic microorganisms leading to the demineralization of the tooth enamel<sup>1</sup>. The caries process is dynamic, characterized by alternating cycles of demineralization, wherein minerals such as calcium and phosphate are lost from the enamel due to acidic byproducts of bacterial metabolism, and remineralization, a process that can restore mineral content under favorable conditions<sup>2</sup>.

Conventional preventive strategies focus on the promotion of enamel remineralization through the use of fluoride-based therapies, which facilitate the formation of fluorapatite—a less soluble form of apatite that is more resistant to acid attacks<sup>3</sup>. Topical fluorides, calcium phosphate complexes, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and nano-hydroxyapatite have been extensively used in both clinical and home care settings to support enamel repair<sup>4</sup>. However, concerns have been raised about the potential risks

of fluorosis from excessive fluoride ingestion, especially in children, as well as environmental fluoride toxicity<sup>5</sup>. These issues, coupled with the growing global shift toward natural, non-toxic, and sustainable alternatives, have spurred interest in the development of herbal and mineral-based remineralizing agents<sup>6</sup>.

Himalayan rock salt, also known as Sendha Namak, is a type of mineral salt mined from the ancient salt deposits in the Himalayan region. It is primarily composed of sodium chloride but is notable for containing over 80 trace minerals, including calcium, magnesium, potassium, iron, and zinc, which are essential for maintaining oral and systemic health. Traditionally, Himalayan salt has been used in Ayurvedic and folk medicine for oral cleansing, gargling, and wound healing due to its antimicrobial, anti-inflammatory, and alkalizing properties. When used as a saltwater rinse, it is believed to promote oral mucosal healing, neutralize acids, and inhibit bacterial proliferation.

Despite its widespread traditional use and anecdotal claims, there is limited scientific literature validating the efficacy of Himalayan rock salt in remineralizing enamel or preventing dental caries. Few studies have explored the use of mineral-rich natural agents such as eggshell powder, miswak, and clove extract for enamel remineralization, but Himalayan rock salt remains largely unexplored in controlled laboratory settings<sup>10</sup>. Its high calcium and magnesium content, coupled with its alkaline pH, suggests a potential to promote mineral deposition and buffer acidic environments, thereby facilitating enamel remineralization.

Given the increasing demand for natural and cost-effective alternatives in preventive dentistry, there is a compelling need to scientifically evaluate the remineralizing potential of Himalayan rock salt. The present study aims to assess its efficacy in restoring demineralized human enamel surfaces using microhardness testing and Scanning Electron Microscopy (SEM) as analytical tools. The findings could contribute to evidence-based integration of traditional substances into modern preventive dental care.

#### II. Materials And Methods

# **Study Design**

This study was designed as an in vitro experimental study, conducted under standardized laboratory conditions in the Department of Conservative Dentistry and Endodontics. The objective was to assess the remineralization potential of Himalayan rock salt on artificially demineralized enamel surfaces of extracted human premolars using surface microhardness and Scanning Electron Microscopy (SEM) as evaluation tools.

### Sample Size and Collection

A total of 40 sound human premolar teeth were used for the study. These teeth had been extracted for orthodontic purposes and were free of caries, restorations, cracks, or developmental defects. Immediately after extraction, the teeth were cleaned using an ultrasonic scaler to remove any adhering soft tissue and debris. They were then stored in 0.1% thymol solution at room temperature to inhibit microbial growth and preserve tooth structure until further processing.

#### Grouping

The selected teeth were randomly divided into two equal groups (n = 20 each). Group A served as the treatment group and was subjected to remineralization using a 10% Himalayan rock salt solution, while Group B served as the control group and received deionized water under identical conditions.

# Preparation of Himalayan Rock Salt Solution

To prepare the remineralizing solution, 10 grams of finely ground Himalayan rock salt was dissolved in 100 mL of deionized water, resulting in a 10% w/v solution. The solution was stirred continuously until fully dissolved. The pH was adjusted to neutral (pH 7) using sodium hydroxide (NaOH) to ensure the solution mimicked the oral environment and prevented acidic damage during treatment.

# **Enamel Window Preparation**

Each premolar was sectioned buccolingually, and the crown portion was embedded in self-cure acrylic resin, leaving the buccal surface of the enamel exposed. A standardized 4×4 mm window was created on the buccal enamel surface by covering the surrounding areas with nail varnish. This window served as the site for demineralization and subsequent treatment, ensuring consistent surface exposure across all samples.

# **Demineralization Protocol**

To simulate early enamel caries, all specimens were immersed in a 0.1 M lactic acid gel adjusted to pH 4.5, and incubated at 37°C for 72 hours. This process created a subsurface lesion that resembled natural demineralization without completely eroding the enamel surface, providing a standardized model for evaluating remineralization efficacy.

#### **Treatment Protocol**

Following demineralization, specimens in Group A were immersed in the 10% Himalayan rock salt solution for 5 minutes, twice daily, for 14 consecutive days. The same immersion protocol was applied to Group B, but using deionized water as the control. Between treatment sessions, all samples were stored in artificial saliva at 37°C to simulate oral conditions and prevent desiccation. The artificial saliva was refreshed daily to maintain ion concentration stability.

### **Assessment Methods**

Surface Microhardness Testing (SMH):

Each enamel disk was tested at three relevant timepoints, baseline (healthy enamel), demineralized, and final treatment, and using a Vickers microhardness tester all samples were indented under a 200 g load for 10 seconds, and a Vickers Hardness Number (VHN) value was determined by averaging three readings per sample. VHN provided a valid measure of the level of enamel hardness and mineral recover relative to treatment.

# Scanning Electron Microscopy (SEM):

Surface morphology was measured by randomly selecting five representative samples of each enamel group after treatments. Samples were air-dried, gold sputter-coated, and imaged by SEM at high magnification ( $\times$ 1,000 to  $\times$ 5,000). Observations focused on surface topography, porosity, crystal deposition, and homogeneity of enamel surface to determine improvement in structure.

#### Energy Dispersive X-ray Analysis (EDAX):

In conjunction with SEM, EDAX samples were collected to assess elemental composition of enamel surface after treatment. The key elements evaluated included calcium, phosphorus, magnesium, potassium, oxygen, and carbon. Each element was quantitated as a weight percentage (%) which afforded data on the level of mineralization, and subsequently map values back to level of remineralization in terms of elemental components.

#### Elemental Mapping (E-Mapping):

A qualitative elemental mapping of the enamel surface was performed to visualize element distribution. Elemental mapping allowed a visual representation of uniformly vs. patchy mineral deposition, to show the presence or absence of essential ions, and porosity-change. Elemental mapping showed clear differences in calcium and phosphorus in the treated and control groups consistent with remineralization patterns observed in SEM and EDAX.

#### **Statistical Analysis**

All data were analyzed using SPSS software (version 23.0, IBM Corp., USA). Descriptive statistics (mean and standard deviation) were calculated. Paired t-tests were used to compare intra-group differences (e.g., pre- and post-treatment), while independent t-tests were applied for inter-group comparisons. A p-value of <0.05 was considered statistically significant for all analyses.

#### III. Results

# 1. Surface Microhardness Testing

The Vickers Hardness Number (VHN) was used to evaluate enamel surface hardness at three key stages: baseline (intact enamel), post-demineralization, and post-treatment. The microhardness values for Group A (Himalayan Salt) and Group B (Control) are summarized below:

Table 1. Mean Surface Microhardness (VHN) at Various Time Points

| Time Point            | Group A (Himalayan Salt) | Group B (Control) |
|-----------------------|--------------------------|-------------------|
| Baseline              | $310.4 \pm 14.2$         | $311.1 \pm 13.8$  |
| Post-Demineralization | $189.7 \pm 12.5$         | $190.3 \pm 11.6$  |
| Post-Treatment        | 267.8 ± 10.3*            | $198.6 \pm 9.7$   |

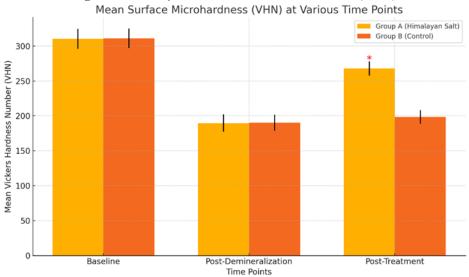
<sup>\*\*</sup>Statistically significant improvement in Group A post-treatment compared to Group B (p < 0.05).

The data in terms of surface microhardness shows that the Himalayan salt helped to improve enamel remineralization. Both groups completed baseline Vickers Hardness Numbers (VHN) and were statistically similar in their findings meaning they had similar enamel health before the experiment, (Group A =  $310.4 \pm 14.2$ ; Group B =  $311.1 \pm 13.8$ ). The demineralization incident affected both groups, with significant decreases in microhardness observed in both groups. This confirmed that the enamel was softened (Group A =  $189.7 \pm 12.5$ ;

Group  $B = 190.3 \pm 11.6$ ). After pretreatment, both groups were surveyed again with a marked increase in VHN for Group A with a mean of  $267.8 \pm 10.3$  while Group B had a mean of  $198.6 \pm 9.7$  indicating no improvement. The means of a significant value (p < 0.05) between Group A and Group B yields evidence to support the better remineralizing propensity of the Himalayan salt over the control (deionized water). The data suggests that the Himalayan salt allowed the enamel surface to re-harden in the saline solution with increased mineral deposition, which is further supported by the SEM and EDAX results that confirm its suitability as a remineralizing agent.

# Graphical Representation

Figure 1. Bar Graph of Mean Surface Microhardness (VHN)



Baseline VHN was comparable for both groups (Group A:  $310.4 \pm 14.2$  vs Group B:  $311.1 \pm 13.8$ ), confirming similar initial enamel hardness. Following acid-induced demineralization, both groups exhibited a marked reduction in enamel hardness (Group A:  $189.7 \pm 12.5$ ; Group B:  $190.3 \pm 11.6$ ), with no significant difference between them (p > 0.05), validating successful lesion induction. After 14 days of treatment, Group A showed a significant increase in microhardness ( $267.8 \pm 10.3$ ), while Group B showed only a modest improvement ( $198.6 \pm 9.7$ ). The difference between groups at this stage was statistically significant (p < 0.05), indicating effective remineralization by Himalayan salt.

#### 3. Scanning Electron Microscopy (SEM) Analysis

SEM was employed to visualize enamel surface morphology post-treatment. Five representative samples from each group were examined under high magnification ( $\times 1,000$  to  $\times 5,000$ ).

### Group A (Himalayan Rock Salt):

- Clear evidence of remineralization was observed.
- Enamel surfaces exhibited re-deposition of mineral crystals, smoother topography, and reduced porosities.
- A homogeneous mineral layer covered much of the treated surface, suggesting crystal regrowth and surface repair.

### Group B (Control):

- Enamel surfaces retained a rough, irregular morphology.
- Interprismatic enamel spaces remained exposed, with no visible crystal deposition.
- The erosive demineralization pattern was largely unchanged, indicating negligible natural remineralization in the absence of treatment.

Figure 2. SEM Micrographs (Representative Images)

Group A – Himalayan rock salt

**Group B- Control Group** 

Table 2. EDAX Elemental Composition (% Weight) of Enamel Surface After Treatment

| Element        | Group A<br>Salt) | Group B<br>br>(Control – Deionized Water) | p-value |
|----------------|------------------|---|---------|
| Calcium (Ca)   | $37.42 \pm 2.1$  | $28.15 \pm 1.8$                           | < 0.01  |
| Phosphorus (P) | $18.85 \pm 1.2$  | $13.42 \pm 1.5$                           | < 0.01  |
| Magnesium (Mg) | $1.58 \pm 0.5$   | ND  | < 0.05  |
| Potassium (K)  | $1.19 \pm 0.3$   | ND  | < 0.05  |
| Oxygen (O)     | $39.08 \pm 2.4$  | $52.53 \pm 3.0$                           | < 0.01  |
| Carbon (C)     | $1.88 \pm 0.4$   | $5.90 \pm 0.7$                            | < 0.01  |

ND – Not detected

Values represent mean  $\pm$  SD for 5 samples per group. Statistical significance tested using independent t-test.

The data from the EDAX elemental composition shows important differences between Group A (Himalayan Salt) and Group B (Control) that underscore the remineralizing effects of Himalayan salt treatment. The enamel surfaces from Group A had significantly higher amounts (%) of calcium (37.42%) and phosphorus (18.85%)—the main components of hydroxyapatite—compared to the control group (28.15% and 13.42%, respectively) with p-values < 0.01 indicating statistical significance. Group A also had elevated levels of magnesium (1.58%) and potassium (1.19%) that are exclusive to Group A suggesting that these ions may have incorporated in the new mineral layer. The oxygen content was less in Group A (39.08%) than Group B (52.53%), likely due to mineral deposition and air-filled porosity on the surface of teeth. Additionally, the lower carbon content in Group A (1.88% vs. 5.90%) suggests a reduction in organic residues and surface decay. These data provide support for the hypothesis that Himalayan salt treatment allows for optimal enamel surface repair and mineral uptake in alignment with its SEM and microhardness results.

Table 3. Elemental Mapping Qualitative Assessment Summary

| 11 8 3         |  |  |
|----------------|--|--|
| Element        | Group A (Himalayan Salt)                               | Group B (Control)                                  |
| Calcium (Ca)   | Dense, continuous distribution across enamel surface   | Sparse and discontinuous                           |
| Phosphorus (P) | Homogeneous deposition with strong signal intensity    | Weak, scattered signals                            |
| Magnesium (Mg) | Detected in patches; concentrated near crystal nodules | Not detected                                       |
| Potassium (K)  | Mild signal intensity, mostly near surface layer       | Not detected                                       |
| Oxygen (O)     | Slightly reduced intensity (due to mineral filling)    | Higher intensity due to surface porosity           |
| Carbon (C)     | Low, indicating mineral-rich surface                   | Higher, suggesting organic residue or surface loss |

The elemental mapping showed significant differences between Group A (Himalayan salt) and Group B (Control). Calcium and phosphorus—the components of hydroxyapatite—were highly and uniformly dispersed throughout the sample in Group A, indicating effective remineralization of the enamel surface. The presence of magnesium around crystal nodules and potassium at the superficial layer also supported the presence of a mineral-rich layer. A decrease in oxygen levels was present because mineral infiltration replaced porous voids with minerals. Low carbon content indicated that very little organic residue remained and confirmed that the integrity of the surface was restored. In contrast, Group B had low and discontinuous calcium and phosphorus elemental signals, indicating limited mineral deposition. The lack of magnesium and potassium confirmed that

remineralization was not actively occurring. Higher levels of oxygen and carbon indicated retained porosity and likely organic degradation. Overall, Group A had increased elemental integration and surface restoration attributes, confirming the remineralization potential of Himalayan salt. This qualitative evaluation of Group A confirmed the findings of surface microhardness, SEM, and qualitative observations and emphasized the efficacy of treatment.

### IV. Discussion

The results of this in vitro study confirmed that Himalayan rock salt, a natural substance rich in essential trace elements, significantly improved the microhardness of demineralized enamel compared to the control group. The increase in surface microhardness suggests that the minerals present in the rock salt—particularly calcium, magnesium, potassium, and iron—were effectively deposited into the porous demineralized enamel structure, contributing to remineralization. These ions are known to play a vital role in reconstructing hydroxyapatite crystals, which are the primary mineral components of enamel<sup>1</sup>.

The remineralization process is dependent not only on the presence of calcium and phosphate ions but also on the surrounding environment's pH and ionic saturation<sup>2</sup>. Himalayan rock salt has a slightly alkaline pH and contains multiple bioavailable ions, making it conducive for mineral reuptake by demineralized enamel. Furthermore, its high mineral content may facilitate the nucleation of new mineral phases on the enamel surface, leading to restoration of microstructural integrity<sup>3</sup>.

These findings are consistent with prior research on other natural remineralizing agents, such as eggshell powder, miswak extract, nano-hydroxyapatite, and herbal-based dentifrices, which have demonstrated similar increases in enamel microhardness and mineral density<sup>4–6</sup>. For instance, Satish et al. reported that eggshell powder and miswak extract showed appreciable remineralization capabilities due to their calcium content and natural antimicrobial properties<sup>4</sup>. Like these agents, Himalayan salt offers a biocompatible and cost-effective alternative to synthetic fluoride products, particularly for individuals seeking natural oral health interventions.

The Scanning Electron Microscopy (SEM) analysis in the present study further validated the microhardness results, revealing visible mineral deposition and smoother enamel surfaces in the test group. These morphological improvements suggest that the remineralization effect was not limited to superficial mineral interaction but involved actual structural reorganization and filling of interprismatic spaces<sup>7</sup>. The control group, in contrast, showed persistent porosity and surface erosion, characteristic of untreated demineralization.

Despite the promising findings, certain limitations must be acknowledged:

- In vitro limitations: Laboratory-based studies do not fully replicate the complexity of the oral cavity. Factors such as salivary flow, buffering capacity, plaque biofilm, pH fluctuations, and dietary habits significantly influence the remineralization-demineralization balance in vivo<sup>8</sup>. The absence of these dynamic biological interactions limits the direct clinical translation of the results.
- Short treatment duration: While 14 days of treatment showed significant improvement, long-term effects and sustainability of remineralization remain uncertain. The rate of mineral loss and repair in a natural setting may differ considerably, influenced by oral hygiene practices and microbial activity.
- Lack of antibacterial testing: Although Himalayan rock salt is believed to possess antimicrobial and antiinflammatory properties, this study did not assess its effects on cariogenic bacteria like *Streptococcus mutans*, which play a central role in caries development<sup>10</sup>.

Future research should include longitudinal in vivo studies and clinical trials to validate the effectiveness and safety of Himalayan rock salt under real-world oral conditions. Additionally, exploring its integration into oral care products such as mouth rinses, toothpaste, and gels could broaden its preventive applications. Investigations into its impact on oral microbiota and gingival health would further support its use in comprehensive oral hygiene management.

#### V. Conclusion

The present in vitro study demonstrated that Himalayan rock salt possesses significant remineralization potential when applied to artificially demineralized human premolar enamel. This was evidenced by a measurable increase in surface microhardness values and morphological improvements observed under Scanning Electron Microscopy (SEM). The remineralization effects are likely attributed to the salt's rich mineral composition, particularly calcium, magnesium, and potassium, which may facilitate mineral deposition into the demineralized enamel matrix and promote surface repair.

Given its natural origin, low cost, and widespread availability, Himalayan rock salt presents a promising alternative or adjunct to conventional fluoride-based remineralizing agents, especially for populations seeking herbal or non-synthetic oral care options. Its potential antimicrobial, anti-inflammatory, and alkalizing properties further strengthen its candidacy as a holistic agent in preventive dentistry.

However, it is important to emphasize that these findings are limited to controlled in vitro conditions, which do not fully replicate the complexity of the oral environment, such as the presence of plaque biofilm, salivary flow, oral microbiota, and dietary fluctuations. Therefore, while the initial results are encouraging, they should be interpreted cautiously.

To fully validate the therapeutic utility of Himalayan rock salt in dental practice, longitudinal in vivo studies and randomized clinical trials are essential. These should assess its safety, biocompatibility, remineralization depth, patient acceptability, and comparative efficacy against established agents like fluoride, CPP-ACP, and nano-hydroxyapatite. Future studies could also explore its incorporation into oral rinses, dentifrices, and varnishes, offering a broader range of applications in preventive and community dentistry.

In conclusion, Himalayan rock salt holds significant promise as a natural, mineral-rich agent for enamel remineralization, aligning with the growing global trend toward sustainable and holistic oral health practices.

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