

## The Protective Role of Alkaline Ionized Water Against Dental Erosion from Acidic Beverages: An In-Vitro Analysis

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### KEYWORDS

Energy drinks, Enamel demineralization, Enamel micro hardness, Beverage-induced erosion, Tooth remineralization.

### ABSTRACT

#### Objectives:

The prevalence of dental erosion has increased due to the regular intake of energy drinks and sports beverages, necessitating the implementation of effective preventive measures. Therefore, this study aimed to evaluate protective role of Alkaline Ionized Water (AIW) against dental erosion induced by energy drinks and sports beverages by assessing enamel microhardness as a quantitative measure of demineralization.

#### Material and Methods:

Sixty healthy mandibular molars were prepared by removing their crowns and divided them into buccal and lingual halves. The buccal sections were polished, affixed to acrylic blocks, and then allocated into six categories: GROUP 1 (distilled water - negative control), GROUP 2 (a popular carbonated beverage - positive control), GROUP 3 (energy drink), GROUP 4 (sports beverage), GROUP 5 (energy drink + AIW), and GROUP 6 (sports beverage + AIW). The samples were exposed to their respective solutions for 10 minutes daily over three days. Changes in Enamel microhardness was investigated by Knoop microhardness test were performed before and after the exposure period. Statistical analysis included one-way ANOVA and Tukey's Posthoc test.

#### Results:

Significant differences in enamel microhardness were observed between groups. AIW-treated groups (Groups 5 and 6) showed higher microhardness compared to those exposed only to acidic beverages. Group 6 demonstrated the best protective outcome, followed closely by Group 5. Groups exposed to acidic drinks without AIW intervention (Groups 2-4) demonstrated significant reductions in microhardness, with Group 2 (carbonated beverage) displaying the most pronounced erosion.

#### Conclusion:

AIW demonstrated efficacy in neutralizing the erosive effects of acidic beverages on enamel, offers a potential preventive measure against beverage induced dental erosion. Further long-term studies are required to explore AIW's long term effects and broader applications in oral health management.

### Introduction

Dental erosion poses a significant challenge in the realm of oral health. It involves the irreversible degradation of dental hard tissue due to chemical processes, independent of bacterial action (1,2). This type of tooth surface damage can impair dental functionality, aesthetics, and overall oral well-being. The prevalence of dental erosion varies widely, ranging from 2% to 100% across different demographic groups and lifestyle choices (3,4). Notably, dietary habits, especially the frequent intake of acidic

beverages, are key contributors to this condition(5). .Consequently, the impact of energy and sports drinks on the development of dental erosion has garnered increasing attention, emphasizing the need for effective preventive measures(6).

Energy beverages, aimed at enhancing energy levels and cognitive alertness, commonly comprise a blend of caffeine, sugars, and additional stimulants, whereas sports drinks are crafted with carbohydrates and electrolytes to restore hydration levels and essential nutrients during physical activities (7). Both varieties of beverages exhibit acidic properties with low pH levels that can lead to enamel erosion. Notably, Red Bull, a prevalent energy drink, boasts a pH level of around 3.24, whereas Gatorade, a widely-consumed sports drink, registers a pH of 2.93 (8). These pH measurements significantly dip below the critical threshold of 5.5 required to dissolve enamel, thereby posing a substantial risk of demineralization to teeth with frequent consumption of these beverages (9). . Furthermore, the sugar contents in these drinks aggravate the issue by fostering acid production through plaque bacteria, and the presence of citric acid compounds contributes to calcium binding, further compromising enamel strength(10). Regular physical activity and sports increase the consumption of these beverages, and although their hydrating benefits are widely recognized, preventing their intake is not a practical solution(8).Hence, it is imperative to develop effective strategies to mitigate the erosive effects induced by these beverages for maintaining optimal oral health.

Current research on preventing dental erosion primarily focuses on remineralization methods. These techniques aim to replenish minerals to the tooth surface by utilizing products containing calcium, phosphate, or fluoride. While effective, these approaches do not directly address the enamel demineralization caused by acidic environments (11). Alternative tactics, such as neutralizing oral pH immediately after consuming acidic beverages, have been under investigation. For instance, substances like sodium bicarbonate, magnesium hydroxide, or antacid tablets can help neutralize oral acids, although their frequent consumption might not be suitable for regular use(12,13).These include electrolytically generated hypochlorite water, ozonated water, and ozone ultrafine bubble water, each showing promise in diverse health contexts, especially in disinfection and oral health.(14). One notable functional water suitable for human consumption that has gained significant attention is Alkaline Ionized Water (AIW). AIW is produced through electrolysis, boasts a water with a high pH and antioxidant properties(15).Research by Tanaka et al. (2019) has confirmed AIW's efficacy in alleviating gastrointestinal symptoms and its safety for human consumption (16). Furthermore, previous research indicates that AIW can neutralize the acidic oral environment post-consumption of acidic beverages. This implies that AIW could be a viable preventive measure for dental erosion, offering a convenient and effective long-term alternative, particularly after the intake of energy or sports drinks (17).

Recently, there has been a growing interest in functional water products, such as Alkaline Ionized Water (AIW), as a potential strategy to prevent dental erosion. AIW produced through electrolysis has a high pH, often ranging from 9 to 12(16).This elevated pH attribute of AIW enables it to neutralize acidic environments in the oral cavity rapidly.(17).Studies have demonstrated that AIW can counteract the acidity induced by beverages like Coca-Cola and sports drinks, swiftly restoring oral pH levels to neutrality in contrast to regular water, which takes significantly longer to achieve the same effect.(8).

Apart from its acid-neutralizing capabilities, AIW has exhibited other promising oral health advantages, including its potential as an anticariogenic agent. Research suggests that AIW could minimize dental plaque formation, presenting a potential alternative to conventional mouthwashes like chlorhexidine(18).Furthermore, its ability to prevent bacterial attachment to tooth surfaces implies a potential role in averting dental caries and secondary caries (8).While AIW shows potential in neutralizing acids and preventing dental erosion, its specific impacts on enamel demineralization caused by energy and sports drinks warrant further investigation. This current study aims to fill this research gap by assessing changes in enamel surface microhardness following exposure to these beverages, followed by AIW treatment. By concentrating on microhardness as a quantitative parameter of demineralization, this study seeks to establish AIW as a practical and effective preventive measure for individuals at heightened risk of dental erosion, particularly athletes and regular consumers of acidic beverages.

**Statement of Clinical relevance:**

Alkaline Ionized Water (AIW) demonstrates significant potential in neutralizing oral acidity and mitigating enamel erosion caused by acidic beverages, offering a practical, non-invasive preventive measure for dental health, particularly for individuals at high risk of beverage-induced dental erosion.

**Materials and methods**

***Sample Preparation***

This study was conducted after obtaining ethical clearance from the Institutional Ethics Committee of Rajarajeshwari Dental College and Hospital. A total of sixty intact mandibular first molars devoid of caries, hypocalcification, erosion, or fractures were chosen to ensure sample uniformity. The teeth were disinfected using 0.1% thymol and kept in distilled water. The selected teeth were sectioned coronally using a water-cooled diamond wafering blade (Allied High-Tech Products). The crowns were sectioned mesiodistally, and the buccal halves were utilized for the study. The buccal surfaces were polished using a 1200-grit silicon carbide paper and diamond polishing paste to ensure consistent surface characteristics. Each specimen was mounted to an acrylic block with the buccal surface exposed. A 5mm x 5mm window was demarcated on the buccal surface and glazed with nail varnish, leaving the window area exposed (20). The specimens were stored in distilled water until the commencement of experimentation.

***Experimental procedure***

The samples prepared were divided into six groups as described in Table 1, each containing 10 samples: Group I (distilled water - negative control), Group II (Coca-Cola - positive control), Group III (Red Bull - energy drink), Group IV (Gatorade - sports drink), Group V (Red Bull + AIW), and Group VI (Gatorade + AIW). Samples in Groups 1 to 4 were exposed to the respective beverage for 10 minutes daily over a period of three days (21). Samples in Groups 5 and 6, the samples were immersed first in Red Bull or Gatorade for 10 minutes, followed by 10 minutes of immersion in Alkaline Ionized Water (AIW), following the same schedule for three consecutive days. Between beverage exposures, all specimens were kept immersed in artificial saliva (Airtech Chemazone Pvt Ltd, India) to simulate natural oral remineralization, with the artificial saliva being replaced after each experimental day.

**Table 1. Composition and pH levels of tested beverages utilized in the study**

| <b>Beverages</b>       | <b>Composition</b>  | <b>pH Level (0-14)</b> |
|------------------------|---|------------------------|
| Distilled water        | Water without minerals  | 7                      |
| Coca-cola              | Carbonated water, High fructose corn syrup, Caramel color, Phosphoric acid, Natural flavors, Caffeine   | 2.49                   |
| Red bull               | Water, Sucrose syrup, Glucose-fructose syrup, Citric acid Natural lemon/lime Flavors, Natural flavors, Salt, Sodium Citrate, Monopotassium phosphate, Ester gum, yellow dye #5    | 3.24                   |
| Gatorade               | Water, Sucrose, Glucose, Sodium citrate, Taurine Glucuronolactone, Caffeine, Inositol, Niacinamide, Calcium-Pantothenate, Pyridoxine HCL, Vitamin B12, Artificial flavors, Colors | 2.93                   |
| Alkaline ionized water | Water with minerals and ions  | 9                      |

**Enamel Surface Microhardness testing**

After three days, the enamel surface microhardness was measured using the Knoop microhardness test (Shimadzu HMV-G31DT) with a quadrangular pyramidal diamond indenter. Each sample was subjected to a 50 g load with a 30-second dwell time to quantify the changes in surface hardness. Three indentations were placed on the flat surface of each sample, separated by 0.4 mm apart, and the average value was recorded as the surface microhardness.

**Statistical Analysis**

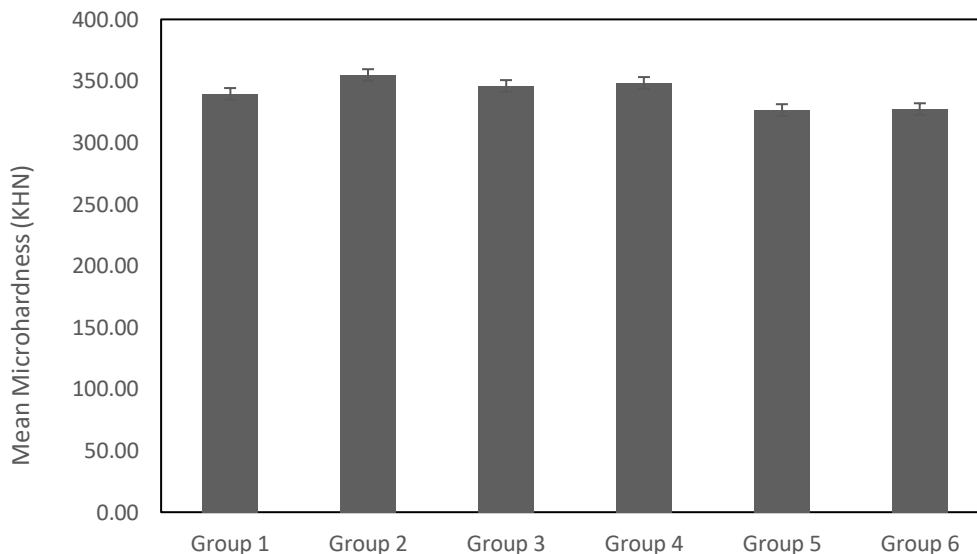
Statistical Package for Social Sciences (SPSS) for Windows Version 22.0, released in 2013 by IBM Corp. in Armonk, NY, was utilized to conduct statistical analyses. Mean and standard deviation were employed for quantitative variables, while frequency and proportions were used for categorical variables during the descriptive analysis of all explanatory and outcome parameters. To compare the Knoop Micro hardness values among six groups, a one-way ANOVA test followed by Tukey’s Post hoc test was conducted. The significance level was set at  $P < 0.05$ .

**Results**

**Pre-treatment Microhardness Values:**

Prior to treatment, analysis revealed no significant discrepancies in mean microhardness values among the six groups. This indicates that all groups commenced with relatively similar baseline enamel conditions, establishing a uniform basis for evaluating the effects of the distinct treatments (Figure 1 and Supplementary Table 1).

**Figure 1.**



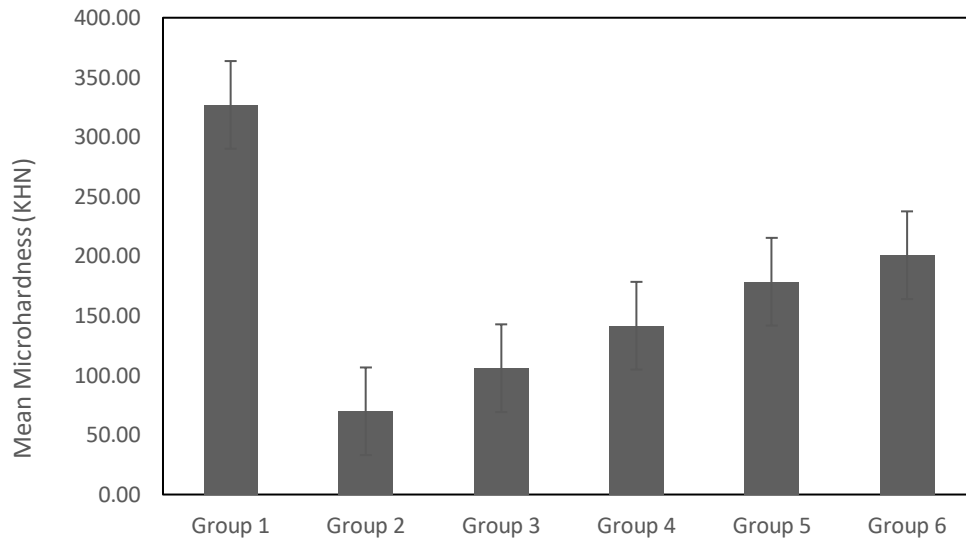
**Figure 1:** Mean Microhardness (KHN) between groups during pre-treatment period. The mean microhardness of enamel samples from six groups measured before exposure to various beverages. Note: Group 1: Distilled Water [Negative Control], Group 2: Coco-Cola [Positive Control], Group 3: Red Bull [Energy Drink], Group 4: Gatorade [Sports Drink], Group 5: Red Bull + Alkaline Ionized Water, Group 6: Gatorade + Alkaline Ionized Water.

**Post-treatment Microhardness Values:**

Following treatment, a notable disparity in the mean microhardness values was observed across the various groups, signifying that the treatments had diverse impacts on enamel microhardness. The statistical significance highlights that the alterations in enamel hardness were not random but rather a consequence of the specific treatments administered to each group (Figure 2 and Supplementary Table 2).

| Groups  | N  | Mean   | SD    | Min | Max | p-value |
|---------|----|--------|-------|-----|-----|---------|
| Group 1 | 10 | 339.53 | 43.81 | 263 | 411 | 0.77    |
| Group 2 | 10 | 354.82 | 32.26 | 300 | 408 |         |
| Group 3 | 10 | 345.98 | 65.30 | 254 | 456 |         |
| Group 4 | 10 | 348.45 | 50.46 | 281 | 428 |         |
| Group 5 | 10 | 326.43 | 76.23 | 242 | 468 |         |
| Group 6 | 10 | 327.19 | 16.29 | 290 | 350 |         |

Figure 2.



**Figure 2.** Mean Microhardness (KHN) between groups during post-treatment period. The mean microhardness values of the enamel samples after exposure to different beverages over three days. The results indicate a significant reduction in microhardness in all groups except Group 1 (Distilled Water).

**Supplementary Information:**

**Supplementary Table 1.** Comparison of mean Micro hardness (KHN) between 6 groups during pre-treatment period using One-way ANOVA Test **Note:** Group 1: Distilled Water [Negative Control], Group 2: Coco-Cola [Positive Control], Group 3: Red Bull [Energy Drink], Group 4: Gatorade [Sports Drink], Group 5: Red Bull + Alkaline Ionized Water, Group 6: Gatorade + Alkaline Ionized Water.

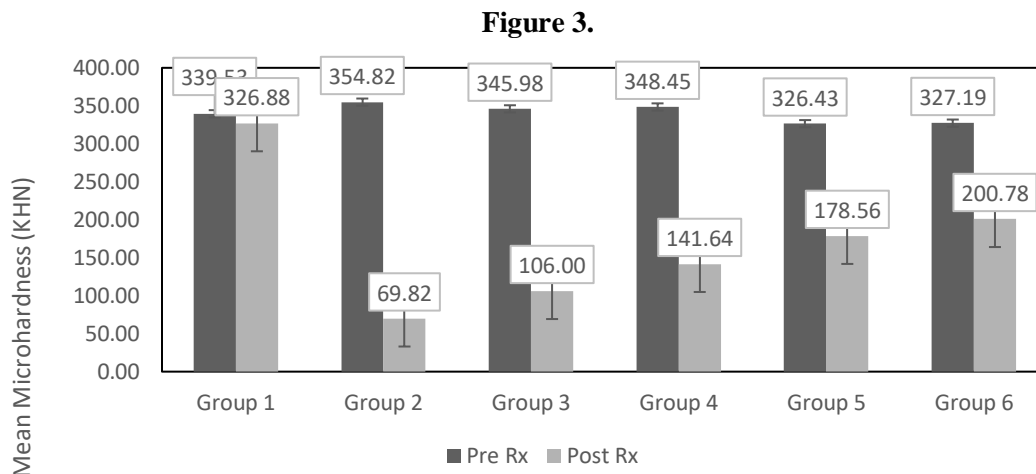
| Groups  | N  | Mean   | SD    | Min   | Max   | p-value |
|---------|----|--------|-------|-------|-------|---------|
| Group 1 | 10 | 326.88 | 36.60 | 259.1 | 394.7 | <0.001* |
| Group 2 | 10 | 69.82  | 12.78 | 48.3  | 87.2  |         |
| Group 3 | 10 | 106.00 | 8.80  | 90.7  | 118.3 |         |
| Group 4 | 10 | 141.64 | 7.40  | 132.6 | 151.8 |         |
| Group 5 | 10 | 178.56 | 11.48 | 164.5 | 194.4 |         |
| Group 6 | 10 | 200.78 | 11.53 | 186   | 215.7 |         |

**Supplementary Table 2.** Comparison of mean Micro hardness (KHN) between 6 groups during post-treatment period using One-way ANOVA Test.

**Note:** Group 1: Distilled Water [Negative Control], Group 2: Coco-Cola [Positive Control], Group 3: Red Bull [Energy Drink], Group 4: Gatorade [Sports Drink], Group 5: Red Bull + Alkaline Ionized Water, Group 6: Gatorade + Alkaline Ionized Water.

**Inter-group Comparison:**

Multiple comparisons showed distinct differences in the microhardness values. Group 1 (Distilled Water) maintained the highest microhardness, acting as a negative control and demonstrating minimal impact from acidic or alkaline exposure. Groups 6 (Gatorade + AIW) and 5 (Red Bull + AIW) exhibited significantly higher microhardness values compared to groups exposed only to acidic drinks, indicating a protective effect of AIW. Group 4 (Gatorade) and Group 3 (Red Bull) showed intermediate values, while Group 2 (Coca-Cola) displayed the lowest microhardness, suggesting the highest degree of enamel demineralization due to its high acidity (Figure 3 and Supplementary Table 3).



**Figure 3. Mean Microhardness (KHN) before and after treatment in each group.** Comparing the mean microhardness of enamel samples before and after treatment with different beverages across six experimental groups. The pre-treatment and post-treatment values are presented for each group, showing the extent of enamel demineralization caused by the beverages.

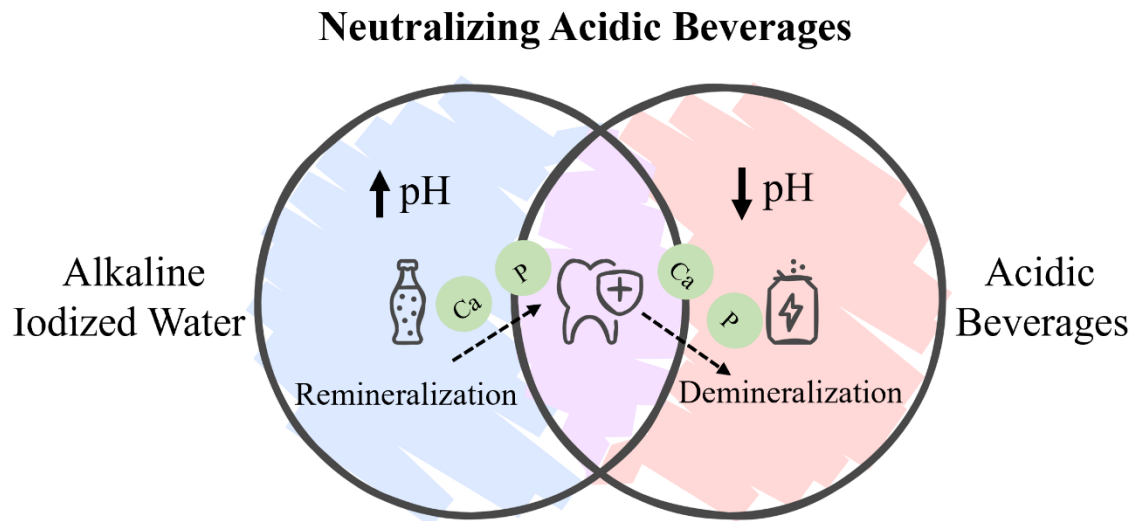
**Supplementary Table 3.** Multiple comparison of mean diff. in mean Micro Hardness b/w 6 groups using Tukey's Post hoc Test. **Note:** Group 1: Distilled Water [Negative Control], Group 2: Coco-Cola [Positive Control], Group 3: Red Bull [Energy Drink], Group 4: Gatorade [Sports Drink], Group 5: Red Bull + Alkaline Ionized Water, Group 6: Gatorade + Alkaline Ionized Water.

**Pre- and Post-treatment Comparison:**

The comparison of pre- and post-treatment microhardness values revealed significant reductions in all groups except Group 1 (Figure 3). This suggests that exposure to acidic beverages led to considerable enamel demineralization. However, Groups 5 and 6, treated with AIW, showed a lessened reduction in microhardness, indicating a potential protective role of AIW against the demineralizing effects of energy and sports drinks. The statistical significance of these findings underscores the potential benefits of AIW in managing dental health.

**Discussion:**

**Figure 4.**



**Fig.4. Neutralizing Effect of Alkaline Ionized Water on Acidic Beverages.** Acidic beverages lower oral pH, leading to enamel demineralization and a significant reduction in enamel microhardness due to the loss of calcium (Ca) and phosphate (P). Alkaline iodized water neutralizes the acidity, increases pH, and promotes remineralization by facilitating the redeposition of Ca and P ions, effectively restoring enamel microhardness and protecting its structure.

The current study demonstrated that Alkaline Ionized Water (AIW) significantly reduces enamel demineralization caused by acidic beverages such as energy and sports drinks, providing new insights into effective preventive strategies for dental erosion (Figure 4). The study's key finding, that AIW mitigates the loss of enamel microhardness after exposure to acidic drinks, supports its potential role in oral health management. This aligns with previous research on the erosive effects of acidic beverages, where pH levels below 5.5 have been shown to weaken enamel integrity(23). The results reinforce the hypothesis that neutralizing oral pH immediately after exposure to acidic beverages can be an effective preventive measure. However, further analysis is required to understand the broader implications of AIW's protective properties, particularly regarding long-term use.

Significant enhancement in enamel surface microhardness was noted in the cohorts subjected to AIW treatment subsequent to exposure to Red Bull and Gatorade, indicating the efficacy of AIW in neutralizing the acidic conditions induced by these beverages. Notably, Group 6 (Gatorade + AIW) showcased the highest levels of microhardness post-treatment among the experimental groups, closely trailed by Group 5 (Red Bull + AIW). This suggests that AIW's capacity to neutralize acidity can

counteract the erosive potential of both sports and energy drinks, renowned for their low pH levels that prompt rapid demineralization of tooth enamel(24). These findings are consistent with prior studies revealing the erosive nature of such beverages owing to their acidic nature and sugar content(11,25). . For instance, research has highlighted how energy drinks like Red Bull, with a pH around 3.24, markedly decrease oral pH, rendering enamel more vulnerable to demineralization Similarly, despite being an electrolyte-replenishing sports beverage, Gatorade with a pH of 2.93 poses a substantial risk of enamel erosion. The restoration of enamel microhardness observed with AIW in both scenarios underscores its potential as a preventive intervention, swiftly elevating oral pH levels and reducing demineralization. (17). Notably, although both AIW-treated groups displayed enhanced microhardness, Group 6 (Gatorade + AIW) exhibited marginally superior values compared to Group 5 (Red Bull + AIW), possibly indicating that Gatorade's composition, devoid of carbonation and slightly higher in pH than Red Bull, may render it less aggressive in its erosive effects (8). The slightly lower acidity of Gatorade relative to Red Bull might elucidate why AIW manifested a more pronounced protective influence in the Gatorade group. These outcomes imply that AIW could be particularly advantageous when consumed subsequent to beverages with mild acidity, offering a robust protective response and more effectively mitigating erosive effects.

In Group 5 (Red Bull + AIW), the AIW treatment exhibited slightly reduced effectiveness in restoring microhardness compared to Group 6 (Gatorade + AIW), despite both beverages having acidic properties. This difference could be attributed to various factors, with one significant factor being the carbonation found in Red Bull. Carbonation has the potential to exacerbate the acidity and erosive properties of drinks. Studies have shown that carbonation enhances the demineralizing effects of acidic beverages by promoting the dissolution of carbon dioxide in the drink, leading to the formation of carbonic acid and a further decrease in pH (26). This phenomenon likely contributed to the heightened enamel demineralization caused by Red Bull compared to Gatorade, as well as the slightly diminished neutralizing impact of AIW on enamel exposed to Red Bull. Moreover, the presence of caffeine in Red Bull might also play a role in reducing salivary flow, thereby compromising the mouth's natural buffering capacity. Reduced salivary flow can delay the neutralization of acids, prolonging the exposure of enamel to acidic conditions. (27). In contrast, Gatorade, lacking carbonation and having lower caffeine levels, potentially allows AIW to more effectively neutralize the acidity in samples exposed to Gatorade. These findings underscore the importance of considering beverage composition, not just pH, in understanding enamel erosion and the efficacy of neutralizing agents like AIW. The varying protective effects of AIW against different beverages suggest that energy and sports drink formulations, incorporating factors such as carbonation, caffeine levels, and sugar content, can significantly impact erosion outcomes and preventive measures. Future research should explore how these individual beverage components interact with AIW and their prolonged effects on enamel when used alongside neutralizing agents.

The results of this study indicate that AIW may offer significant benefits in protecting against enamel erosion, particularly for individuals at high risk of dental erosion due to frequent consumption of acidic beverages. Red Bull and Gatorade both contain high amounts of refined carbohydrates like glucose and sucrose. These sugars cause a drop in plaque pH, leading to acid production by plaque microorganisms, which in turn erodes enamel. Additionally, the citric acid in these beverages further lowers the pH and chelates calcium, causing demineralization(28). Implementing AIW as a routine oral rinse after consuming energy or sports drinks could reduce the incidence of dental erosion, thus improving overall dental health. This finding is particularly relevant for athletes and individuals with high consumption of such drinks, as it provides a simple and accessible preventive measure(8). Incorporating AIW into daily oral hygiene routines could offer an easy and effective strategy to maintain enamel integrity in the face of modern dietary challenges.

Several limitations must be acknowledged when interpreting the study outcomes. The sample size was relatively small, and the experimental duration was restricted to three days, which might not capture long-

term effects. Moreover, the study was carried out in an in-vitro environment, which fails to fully replicate the intricate dynamics of the oral milieu, including plaque and other oral biofilms. Additionally, although microhardness testing offers a dependable gauge of enamel demineralization, it does not consider potential remineralization processes that could occur in vivo (29). Subsequent studies should strive to include larger sample sizes, longer observation periods, and in-vivo evaluations to substantiate these results and investigate the broader potential of AIW in preventive dentistry.

### **Conclusion:**

In conclusion, this study has demonstrated that AIW significantly reduces the erosive effects of acidic beverages on enamel, suggesting its potential as an effective preventive measure in oral health. These findings contribute to the growing body of evidence supporting the use of functional water products, such as AIW, in dental care. Building on these results, future research could explore the long-term impact of AIW on enamel integrity and investigate its effects on other aspects of oral health, such as bacterial adhesion and plaque formation. Ultimately, this research offers a promising foundation for improving dental health by mitigating the risks posed by modern dietary habits, paving the way for more comprehensive and effective oral health interventions.

### **Declaration:**

#### **Author contribution:**

Conceptualization: K.D, S.N.; Data curation:., K.D.S.N, A.K; Formal analysis: K.D, S.N, V.S.V, V.C.R; Investigation: K.D. S.N, M.I.K, V.C.R; Methodology: K.D; S.N; V.S.V: Project administration: S.N, M.I.K, V.C.R.; Resources: S.N.,V.S.V.; Supervision: S.N., M.I.K, A.K; Validation: V.S.V, A.K, M.I.K.; Visualization: K.D, S.N.; Writing - original draft: K.D.SN; Writing - review & editing: V.V.S, M.I.K.,

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### **Conflict of Interest statement:**

The authors declare no Conflict of Interest.

### **Data Availability Statement:**

Data are available on request from the authors.

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