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RESEARCH ARTICLE
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An in vitro analysis of the antioxidant and anti-inflammatory effects of Total-etch dentin adhesive containing Hesperidin

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ABSTRACT

Background: Antioxidants help to protect materials from oxidative damage, which can lead to degradation and discoloration over time. Anti-inflammatory agents can help to reduce inflammation in the oral tissues, which can promote healing and reduce the risk of complications. Hesperidin is a flavonoid compound that is primarily found in citrus fruits, especially in the peels and pulp of oranges and lemons. It has been studied extensively for its potential health benefits, including its antioxidant and anti-inflammatory properties.

Materials And Methods: 2% of Hesperidin was incorporated into total etch adhesive. Anti oxidant property was assessed using DPPH assay and Anti inflammatory property was assessed using BSA assay. Ascorbic acid and Aspirin were used a standard to test anti-oxidant and anti-inflammatory activities respectively. Statistical analysis was performed using independent sample t test.

Results: There was no statistical difference between the groups. In the current investigation, the test groups displayed antioxidant and anti-inflammatory values that were very similar to the tested standards.

Conclusion: In conclusion, Hesperidin-incorporated total etch adhesive offers anti-oxidant and anti-inflammatory properties that may help to increase the stability of the hybrid layer at the tooth restoration interface.

Clinical Significance: Incorporation of hesperidin into bonding agents may offer potential health benefits to patients undergoing restorative dental procedures.

Keywords: *Hesperidin, Collagen-crosslinker, Total etch dentin adhesive, Micro-organisms, Quality of life*

INTRODUCTION

Composite fillings and other adhesive restorations depend on a strong bond forming between the tooth structure and the filling[1]. Over time, inflammatory responses in the dentin might weaken the connection, resulting in tooth microleakage, recurrent caries, and restoration failure. The longevity and clinical performance of restorations can be increased by adding antioxidants and anti-inflammatory compounds to dentin adhesive systems, hence lowering the need for expensive and time-consuming replacements. Antioxidants help to protect materials from oxidative damage, which can lead to degradation and discoloration over time[2]. Anti-inflammatory agents can help to reduce inflammation in the oral tissues, which can promote healing and reduce the risk of complications. Hesperidin is a flavonoid compound that is primarily found in citrus fruits, especially in the peels and pulp of oranges and lemons[3]. It has been studied extensively for its potential health benefits, including its antioxidant and anti-inflammatory properties[4]. Hesperidin has been shown to have antioxidant and anti-inflammatory properties that can potentially improve the durability and esthetics of bonding agents. The antioxidant properties of hesperidin are important because they help to neutralize free radicals, which are highly reactive molecules that can damage cells and tissues. In the mouth, free radicals can be generated by a variety of factors, such as the breakdown of food particles, exposure to tobacco smoke, and even certain dental treatments. By incorporating hesperidin into the bonding agent, it can help to scavenge these free radicals and prevent them from causing damage to the tooth structure especially at tooth-restoration interface[5]. Collagen fibres, which provide dentin its structural support, make up the majority of its complex tissue. Collagen, however, may be vulnerable to breakdown by dentin matrix enzymes like matrix metalloproteinases (MMPs) due to its activation by acid conditioning procedure in total-etch dentin adhesives[6]. Also the bond strength of adhesive restorations may be compromised by excessive MMP activity since it might cause collagen to break down. The anti-inflammatory qualities of dentin adhesives can prevent MMP activation, which lessens collagen breakdown and preserves the structural integrity of dentin. In addition to its antioxidant properties, hesperidin also has anti-inflammatory properties. Inflammation is a natural response to injury or infection, but when it becomes chronic, it can contribute to a range of health problems, including periodontal disease[7]. By reducing inflammation in the mouth, hesperidin can help to promote healthier gums and reduce the risk of gum disease. Incorporating Hesperidin into a bonding agent can enhance the biocompatibility of the material. This can reduce the risk of adverse reactions and improve the overall safety of the bonding agent. Incorporating hesperidin into bonding agents can potentially enhance their antioxidant and anti-inflammatory properties, leading to improved performance and longevity. Additionally, hesperidin has been shown to have antimicrobial properties, which can potentially reduce the risk of bacterial infection and improve oral health outcomes and prevents recurrent caries. The dentin-pulp complex is made up of the dental pulp, which has connective tissue, nerves, and blood vessels, as well as the dentin, which makes up the majority of the tooth structure[8]. The pulp tissue may be harmed by the cytotoxicity of dentin adhesive or inflammatory reaction when it comes into close contact with the pulp during restorative operations. Dental pulp vitality can be preserved by using dentin adhesives with anti-inflammatory characteristics to reduce pulpal inflammation.

Overall, the protection of the dentin structure, preservation of pulp vitality, and improvement of the long-term success of adhesive restorations depend heavily on the presence of antioxidant and anti-inflammatory qualities in total etch dentin adhesive systems. These attributes contribute to reducing oxidative stress, preventing collagen breakdown, and reducing pulpal inflammation, ultimately enhancing the general wellness and durability of the restored tooth. Numerous studies have looked into the usage of antioxidants in dentin adhesive systems, including quercetin, green tea extract, and vitamin E[9]. These studies have shown that antioxidants can lessen oxidative stress, stop collagen from degrading, and increase the bond strength and longevity of adhesive restorations. Anti-inflammatory substances like curcumin, triamcinolone acetonide, and ibuprofen have been investigated as potential additions to dentin adhesives[10]. These investigations have demonstrated that anti-inflammatory substances can assist in reducing MMP activity, suppressing inflammatory responses, and shielding the tooth

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pulp from inflammation and harm[11]. Studies have concentrated on creating dentin adhesive solutions with anti-inflammatory and antioxidant characteristics. Improved bond strength, marginal integrity, and long-term restoration success are expected as a result of these dual-action systems by its comprehensive protection against oxidative stress, inflammation, and collagen breakdown. Hesperidin-containing total etch dentin adhesive has not been the subject of any additional investigations. This is the first in vitro study to assess the anti-oxidant and anti-inflammatory properties of dentin adhesives with and without hesperidin. Our team has extensive knowledge and research experience that has translate into high quality publications[12–21,22–26]

MATERIALS AND METHODS

Assessment of antioxidant activity

Sigma Chemicals Company in St. Louis, Missouri, the United States, and Sisco Research Laboratories (SRL), in Mumbai, India, provided all the chemicals and reagents used in this experiment.

DPPH free radical scavenging activity

At different concentrations (100, 200, 300, 400, and 500 g/ml), 1.0 ml of test bonding agent solution was mixed with 1.0 ml of DPPH solution. After the mixture remained at ambient temperature for 50 minutes, the activity was detected at 517 nm. The same levels of ascorbic acid were used as a reference. The percentage of inhibition used to measure and express the capacity to scavenge the DPPH radical.

Assessment of Anti inflammatory activity

Protein denaturation assay

A BSA solution (0.4 percent, w/v) in Tris Buffered Saline was created by dissolving one tablet in 15 mL of deionized water to create 0.05 M Tris and 0.15 M sodium chloride, pH 7.6 at 25 C. 5.0 L, 10 L, and 20 L aliquots of bonding agent solutions with concentrations of 0.25 g/mL, 0.50 g/mL, and 1.00 g/mL were introduced to test tubes containing 1 mL of 0.4 percent, w/v BSA buffer solution. Glacial acetic acid was then used to change the pH to 6.4. The same method, aspirin was tested as a positive control. The solutions were heated in a water bath at 72 °C for 10 minutes under laboratory conditions, and then they were allowed to cool for 20 minutes. The turbidity (amount of protein precipitation) of the solutions was determined at 660 nm in a Hach Spectrophotometer using an air blank. The average absorbance values from the two investigations were provided. The percentage blockage of precipitation (protein denaturation) was calculated on a percentage basis.

*% Anti-Denaturation Activity = Absorbance of control – Absorbance of sample x 100 Absorbance of control.

Making the test solution

Hesperidin (HPN) was used in Total-etch dentin adhesive at a concentration of 2% (2 mg of HPN powder in 98 mg of bonding agent). Adper single bond 2 (3m ESPE) was the total etch adhesive employed in this study. For this preparation, a pure version of HPN powder from Sigma-Aldrich with more than 90% purity was used. A little amount of dimethyl sulphoxide was used as a solvent to solubilize the hesperidin. 20 mg of hesperidin (Sigma-Aldrich) powder were dissolved immediately in 0.025 ml of pure dimethyl sulfoxide to achieve a 2% concentration. By adding the Hesperidin/Dimethyl sulfoxide into Adper single bond 2 at the proper ratio (20 mg of HPN(0.0025ml DMSO) in 1 ml of bonding agent), the desired final concentration of 2% hesperidin in the total etch adhesive employed was achieved.

Statistical analysis

Statistical software version 23 was used to analyse every value that was collected using GraphPad Prism. Any significant difference between the groups was determined using a student independent t test.

RESULTS

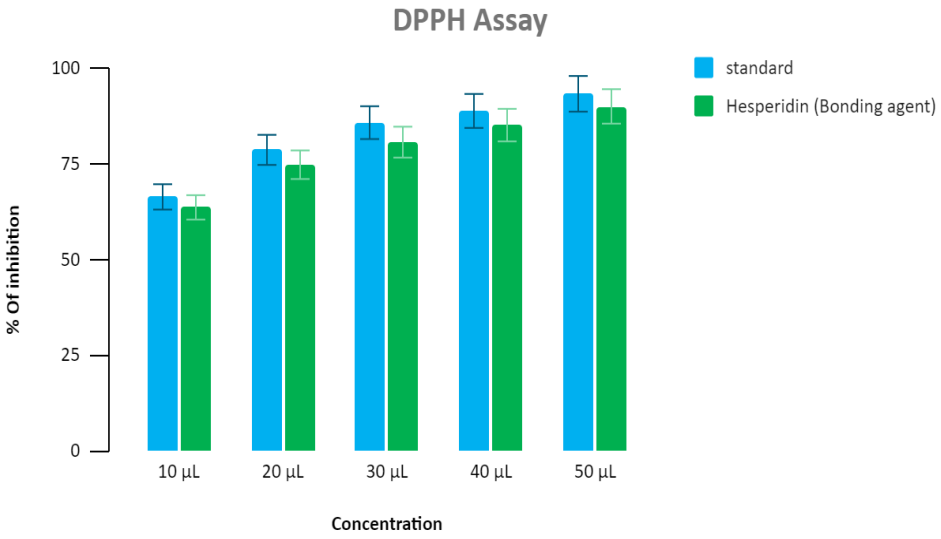

FIGURE 1: Assessment of Anti-oxidant activity of HPN incorporated total-etch adhesive. Each bar represents the mean ± SD of 6 observations. Significance at the levels of $P < 0.05$.

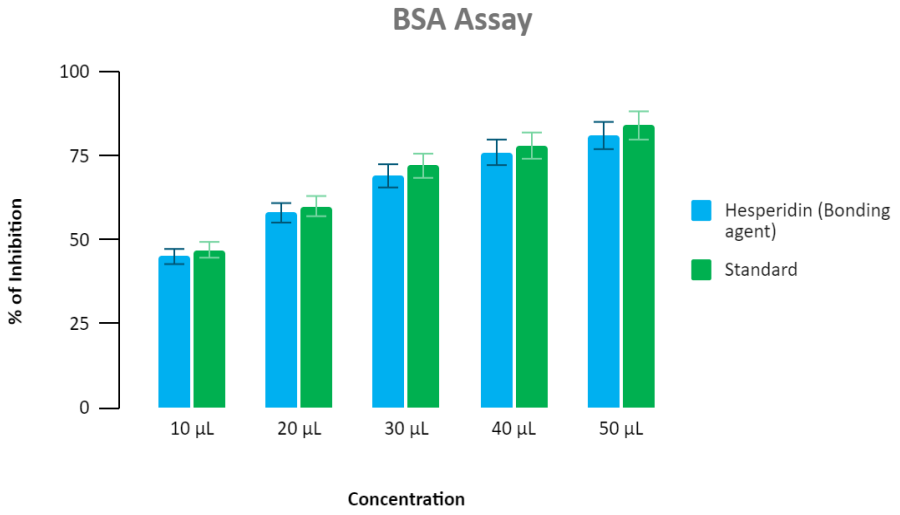

FIGURE 2: Assessment of Anti-inflammatory activity of HPN incorporated total-etch adhesive. Each bar represents the mean ± SD of 6 observations. Significance at the levels of $P < 0.05$.

DISCUSSION

Hesperidin is a natural compound found in citrus fruits and is considered safe for human consumption. The incorporation of hesperidin into the bonding agent has been shown to improve the bond strength between the tooth and the restoration[27]. Additionally, hesperidin-incorporated bonding agents may improve the longevity of restorations by reducing the risk of secondary caries. This is likely due to the antioxidant properties of hesperidin, which can reduce the oxidative stress that can weaken the bond between the two structures[28]. Hesperidin has been shown to possess potent antioxidant properties, which can scavenge free radicals and reduce oxidative stress. The incorporation of hesperidin into the bonding agent can therefore provide additional protection against oxidative stress and improve the longevity of the restoration. Hesperidin has also been shown to possess anti-inflammatory properties, which can reduce the inflammatory response of the tooth to the restoration[7]. This can reduce post-operative sensitivity and improve the overall success of the

restoration. By giving reactive oxygen species (ROS) or free radicals hydrogen atoms or electrons, hesperidin functions as an antioxidant by counteracting their negative effects. Hesperidin's antioxidant action is highly dependent on the presence of numerous hydroxyl (-OH) groups in its structural makeup. Free radicals can get hydrogen atoms from these hydroxyl groups, which stops their chain reactions and averts oxidative damage[29]. The 3',5',7-trihydroxy-4'-methoxyflavanone moiety, which is present in hesperidin's structure, is thought to be responsible for the compound's capacity to scavenge free radicals and suppress lipid peroxidation. This moiety can stop lipid peroxidation and subsequent cell damage by giving hydrogen atoms to lipid radicals[30]. Hesperidin can also bind metal ions like iron and copper that are involved in the production of reactive oxygen species, which increases the antioxidant activity of the compound. Hesperidin has anti-inflammatory properties via a number of different pathways[31]. It can stop the creation and release of inflammatory mediators such prostaglandins and cytokines, which are essential for the inflammatory response (e.g., interleukin-1, tumour necrosis factor-). Hesperidin can reduce inflammation by, among other things, regulating the activity of nuclear factor-kappa B (NF-B). A transcription factor called NF-B controls the expression of genes related to inflammation. The generation of pro-inflammatory mediators can be decreased by hesperidin's ability to prevent NF-B from becoming activated. The suppression of enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), which are responsible for the creation of prostaglandins and leukotrienes, respectively, is another approach. Hesperidin reduces the generation of these inflammatory mediators by blocking these enzymes. Hesperidin can also reduce the activation of immune cells like macrophages by preventing these cells from releasing pro-inflammatory cytokines and other inflammatory chemicals. By doing so, the total inflammatory reaction is lessened.

Hesperidin has both anti-inflammatory and antioxidant qualities, which make it a good complement to a total etch dentin adhesive. In addition it also acts as a natural collagen cross linker and MMP inhibitor. The host-derived pro-enzymes cysteine cathepsins and MMPs are inactive. Since acid conditioning can degrade collagen, elastin, and extracellular matrix (ECM), they become active at lower pH levels[6]. Due to this, the bond stability of composite resin is reduced in the hybrid layer. Additionally, collagen fibres become gelatinized as a result of etching, which also inhibits resin transport in interfibrillar gaps. Collagen fibres that aren't protected can degrade as a result. Total etch can assist avoid this breakdown by including substances with MMP inhibitory and collagen crosslinking properties. The antioxidant properties of hesperidin can aid in protecting the dentin from oxidative stress brought on by reactive oxygen species (ROS). The deterioration of dentin components brought on by oxidative stress might weaken the binding between the adhesive and the tooth structure[32]. Hesperidin can assist in preserving the integrity of the dentin and enhancing the long-term stability of the adhesive connection by scavenging free radicals and preventing lipid peroxidation[33]. Dental procedures like bonding and etching can cause the dentin to become inflamed. The success of the adhesive restoration may be jeopardised by this inflammatory response, which might cause post-operative discomfort. The anti-inflammatory effects of hesperidin can lessen this reaction by preventing the generation of pro-inflammatory mediators and lowering immune cell activation. Hesperidin can help patients feel more comfortable and produce better clinical results by lowering inflammation. In close contact to the dental pulp, dentin adhesives are used. The pulp tissue may suffer from the inflammatory response that adhesive techniques set off. The pulp can be protected by hesperidin's anti-inflammatory capabilities, which inhibit the release of inflammatory cytokines and other mediators. The vitality of the pulp may be preserved and potential difficulties may be reduced with the help of this protection. Thus Hesperidin's anti-inflammatory and antioxidant properties may aid in the long-term strength of the adhesive bond. Hesperidin can assist in preserving the dentin's structural integrity and preventing the gradual deterioration of the adhesive contact by lowering oxidative stress and inflammation. As a result, the restoration may hold together better and last longer. In the current investigation, the test groups displayed antioxidant and anti-inflammatory values that were very similar to the tested standards. Ascorbic acid and Aspirin were used a standard to test anti-oxidant and anti-inflammatory

activities respectively. The antioxidant and anti-inflammatory qualities of ascorbic acid and aspirin are well recognised, and their inclusion in the adhesive can help reduce any negative effects on the dental pulp and surrounding tissues. The analogous activity of hesperidin would offer a natural substitute that would possibly provide similar advantages. There is no statistically significant difference between the test groups and standard, according to the results of the student t test. Mean anti oxidant effect with 50 micro liter of test group was approximately 85% and anti inflammatory effect was approximately 80%. But the investigation found that adding hesperidin to total etch adhesive preserves the substance's anti-oxidant and anti-inflammatory properties and still has a positive therapeutic benefit. However more investigations are still needed to evaluate the findings invivo.

CONCLUSION

In summary, Hesperidin-incorporated total etch adhesive offers anti-oxidant and anti-inflammatory properties that may help to increase the stability of the hybrid layer at the tooth restoration interface.

Clinical Significance

Due to its antioxidant capabilities, hesperidin can aid in preventing oxidative stress and dentin deterioration. As a result, the link between the adhesive and the tooth structure may last longer.

By reducing the inflammatory response in the dentin and surrounding tissues, hesperidin's anti-inflammatory effect can help minimise post-operative discomfort. This may result in greater patient satisfaction and comfort after adhesive restorations.

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