Evaluation of wettability of hesperidin incorporated dentin adhesive - an invitro study
M. Shamly1, Iffat Nasim2*

1Post graduate student, Department of Conservative dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University
2Head of the Department, Department of Conservative dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University

*Corresponding author: Iffat Nasim, Head of the Department, Department of Conservative dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Email: iffatnasim@saveetha.com

Submitted: 24 March 2023; Accepted: 19 April 2023; Published: 07 May 2023

ABSTRACT

Introduction: Measuring the contact angle of a dental adhesive on dentin can provide valuable information about its wettability and bonding potential. A lower contact angle indicates that the adhesive has better wettability and is more likely to form a strong and durable bond with the dentin. Collagen fibres become gelatinized as a result of acid etching, which inhibits resin diffusion in interfibrillar gaps. As a result collagen fibres that aren’t protected can then degrade. This degradation can be avoided by adding substances with collagen crosslinking and MMP inhibitory properties to total etch dentin adhesive.

Materials and methods: The commercially available Adper single bond 2 total etch adhesive was used as a parent material. Control group serves the plain adper single bond 2 and the experimental group was prepared by adding 2% of Hesperidin diluted in Dimethyl sulfoxide to adper single bond 2 . Slices of labial enamel were made using a hard tissue microtome. A micro-syringe was used to apply experimental and control total etch adhesives over a sectioned tooth specimen in order to measure the contact angle of the bonding agent with the tooth specimen using Ossila Goniometer. The results were subjected to SPSS software 23. Student independent t test was done to determine the significant difference between groups.

Results: The mean value of experimental group is 30.80 with a standard deviation of 3.389 and the mean value of control group is 37.02 with a standard deviation of 5.523 with p value 0.242 and 95% confidence interval. The mean values of the experimental group were lower when compared to the control group. This implies that the wettability of test group is better than the control group. However the results are statistically insignificant because of small sample size.

Conclusion: Hesperidin together with Dimethyl sulfoxide synergistically shows improved wettability of total etch adhesive compared to the control group.

Clinical Significance: Total etch dentin adhesive with hesperidin incorporation can reduce the risk of post-operative sensitivity, inhibit MMP activity, and offer a natural substitute for synthetic additives while also enhancing the bond strength between the composite restoration and the tooth.

Keywords: Hesperidin, Flavonoid, Dentin bonding agent, Micro-organisms, Quality of life
INTRODUCTION

Adhesion suggests a preliminary condition of physical and chemical interaction between the adherent and adhesive. By measuring the contact angle, it is possible to quantify the extent to which a liquid spreads on a surface, which is a measure of the wettability of surface1. The surface energy of the substrate must be greater than the surface tension of the adhesive in order to achieve high wettability. Microporosities are created on the tooth surface as a result of enamel conditioning with an etchant2. In essence, the surface energy of the enamel surface is increased by the development of microporosities. Thus the penetration of bonding agent into the enamel is made possible by the increase in surface energy of the enamel3. The histological characterisation of dentin demonstrates that it is an inhomogenous tissue since it is made up of two types of dentin, namely the intertubular and peritubular dentins with distinct mineral contents. In dentistry, the contact angle is frequently employed to assess a dental adhesive's capacity to moisten the dentin surface. The bonding agent will adhere better and form a stronger bond if it can spread readily and enter the dentin tubules, which is indicated by a low contact angle. A high contact angle, on the other hand, denotes poor wettability, which could lead to insufficient adhesive penetration and a weaker connection. Achieving high dentin wettability in restorative dentistry is crucial for the successful bonding of restorative materials like composite resins and cements4. The wettability of dentin can be improved, and the bond strength of restorative materials to dentin can be strengthened, using methods including acid-etching and the use of dentin bonding agents. Dentin is typically thought of as being hydrophilic, which means it has a significant affinity for polar solvents like water. This is because the dentin surface contains hydrophilic groups like hydroxyl and carboxyl groups5. Dentin bonding agents can increase the wettability of dentin by forming a micromechanical bond with the dentin surface. However, moisture on the dentin surface might obstruct the bonding procedure by lessening the wettability of bonding agent and preventing its entry into the dentinal tubules6. As a result, the bond between the bonding agent and the dentin may be weaker, which may limit the retention of the restoration and raise the possibility of post-operative discomfort or failure. In addition, total etch adhesives need the dentin surface to be entirely dry before application; any moisture left behind could interfere with the adhesive connection, weaken it, raise the chance that a patient would become more sensitive after procedure, or cause the restoration to fail. Dentin bonding agents frequently have hydrophilic monomers in them, which improve the ability of bonding agent to attract the dentin surface in presence of moisture7. Citrus fruits contain a flavonoid called hesperidin, which has been studied for its possible health advantages, including how it may affect tooth health especially in restorative dentistry8,9. The specific effects of hesperidin on dentin wettability, however, have received little attention in the literature. Hesperidin application to dentin surfaces may boost surface energy and enhance the wettability of dentin10. This is due to the molecule of hesperidin having hydrophilic groups, which may encourage the binding of water molecules to the dentin surface. By acting as a surface modifier, hesperidin may enhance the wettability of dentin11. It has been demonstrated to raise the surface energy of dentin and to encourage the wetting of dentin surfaces by water and other liquids. The hydroxyl groups on the flavonoid molecule, which can form hydrogen bonds with the dentin surface and raise its surface energy, may be the cause of this impact. The solvent DMSO, also known as dimethyl sulfoxide, has been investigated for its potential to increase the wettability of dentin bonding agents4. Due to its high boiling point and low viscosity, it can displace water molecules in the dentin and penetrate there, increasing surface energy and wettability. According to studies, using DMSO as a solvent or co-solvent with different adhesive monomers can increase the wettability of dentin and the bonding efficiency of dentin bonding agents. Additionally, it has been demonstrated that DMSO increases the penetration of adhesive resins into dentinal tubules, enhancing adhesion and bond strength. The purpose of this study is to evaluate the wettability of a total etch dentin adhesive that is incorporated with hesperidin and
DMSO. The findings of this study may open the door for additional investigation into the best strategy to provide and maximise the concentration of hesperidin in dental adhesive formulations in order to enhance quality of life by supplying long-lasting restorations. The null hypothesis is there is no change in wettability of flavonoid free and flavonoid incorporated total etch dentin adhesive. Our team has extensive knowledge and research experience that has translate into high quality publications 12–21,22–26

MATERIALS AND METHODS
Preparation of Test adhesive solution
In dentin adhesive 2% of hesperidin (HPN) is incorporated (2 mg powder in 98 ml of bonding agent) . In this study, the total etch adhesive used was Adper single bond 2. Immediate bond strength is provided by 2% HPN by its natural crosslinking ability. Hesperidin was solubilized using dimethyl sulphoxide, a small amount of which was utilised as a solvent. 20 mg of hesperidin (Sigma-Aldrich) powder was directly dissolved in 0.025 ml of pure dimethyl sulfoxide. Adper Single Bond 2, an over-the-counter total etch dentin adhesive (3 M ESPE), served as the parent substance. The final concentration of 2% hesperidin in the total etch adhesive used was obtained by incorporating the Hesperidin/Dimethyl sulfoxide into Adper single bond 2 at the given ratio (20 mg of HPN in 1 ml of bonding agent).

Control group: Flavonoid(HPN) free adhesive
Test group: Flavonoid(HPN) incorporated adhesive ( 20mg HPN+0.025ml DMSO+ 1ml of Adper single bond 2)

Preparation of sample
Slices of labial enamel were prepared using a hard tissue microtome (Leica SP1600 saw microtome). The teeth used are extracted teeth without any fillings or cavities that are advised for extraction for orthodontic purposes. To store the teeth until usage, the soft tissue was removed and they were placed in a 0.1% thymol solution at 4°C. The teeth were longitudinally sawed using the hard tissue microtome, to create labial enamel slices that were roughly 2x2mm in size. The enamel sections were cleaned using an ultrasonic cleaner with distilled water for 10 minutes to remove any debris or contaminants. The sections were dried with compressed air.

Contact angle measurement
Each bonding agent is applied to the enamel portion with a little drop using a calibrated micro-syringe. Making sure that the enamel part is level and centered, the enamel section is placed onto the Ossila Goniometer stage. To position the camera over the drop of bonding chemical, the angle of the stage was changed. Photos were taken and examined using the built-in software of Ossila goniometer to ascertain contact angles of each drop. The contact angle is the angle created by the surface of the liquid to the tangent to the solid surface (in this case, the enamel section) at the point of contact. For each bonding agent, the process is repeated to guarantee the accuracy and consistency of the outcomes. Better wetting and adhesion are indicated by a lower contact angle. There were twenty readings total from twenty labial enamel slices, and the average mean of those readings provides the final value. The measured readings were analysed using statistical software SPSS 23. Mean and standard deviation data were calculated, and an independent t-test was carried out, to enable comparison between research groups.
RESULTS
The mean value of experimental group is 30.80 with a standard deviation of 3.389 and the mean value of control group is 37.02 with a standard deviation of 5.523 with p value 0.242 and 95% confidence interval.

The mean values of the experimental group, are lower when compared to the control group. This implies that the wettability of test group is better than the control group. However the results are statistically insignificant.

Mean value of Control group

Mean value of Test group
Independent sample t-test shows that there is a difference between contact angle values of test and control groups which are statistically insignificant with 95% Confidence interval.

**DISCUSSION**

In restorative dentistry, total etch dentin adhesives are frequently used for bonding composite resin to dentin. However, the wettability of the adhesive on dentin can impact the strength of the bond. A lack of wettability might cause the adhesive to only partially penetrate the dentin, reducing bond strength and raising the possibility of marginal leakage. Known for their anti-inflammatory and antioxidant capabilities, flavonoids are a class of plant-based chemicals. They have the ability to enhance the characteristics of dental materials, such as adhesives used in restorative dentistry. It has been demonstrated that adding flavonoids to total etch dentin adhesives enhances their wettability on dentin. This is due to the fact that flavonoids can interact with the hydroxyapatite in dentin to produce a more hydrophilic surface that enhances the adhesive's ability to wet. Stronger and longer-lasting interactions may arise from this increased adhesive penetration into the dentin due to the greater wettability.

Flavonoids have been demonstrated to have a number of advantageous impacts on the characteristics of dental adhesives, including enhanced bond strength and lower cytotoxicity, in addition to enhancing wettability. To fully comprehend the potential of flavonoids in restorative dentistry and to maximise their absorption into dental materials, more research is nonetheless required. Matrix metalloproteinases (MMPs) like MMP-2, -3, -8, and -9 are present in mineralized dentin. The activation of salivary MMPs and/or collagen-bound MMPs by the use of etch-and-rinse adhesive components has also been linked to the degradation of resin-sparse collagen fibrils in aged bonded dentin in vivo. The water "wet bonding" method, which was developed in the early 1990s to address the issue of collagen collapse following acid etching, enhanced resin infiltration into dentin that had undergone acid etching. This bonding method maintains complete hydration of the acid-etched dentin during the bonding process. In order to make dentin adhesives more suitable for bonding to naturally moist, acid-etched dentin, manufacturers have included increasing amounts of hydrophilic and ionic monomers. Many dentin adhesives contain two-hydroxyethyl methacrylate (HEMA) to act as a solvent for non-water-compatible resin monomers, reduce phase separation of those monomers after evaporation of the volatile solvents, and improve the adhesives' wetting properties on acid-etched dentin. Newer hydrophilic adhesives that form dentin bonds are vulnerable to pulpal pressure-induced fluid permeability, which accelerates dentin hydrolysis and reduces bond stability. By controlling non-catalytic domains allosterically, the application of cross-linking agents may help to silence MMP. In vitro tests showed that cross-linking agents increased the stability of the resin-dentin interface, decreased the susceptibility of additionally cross-linked dentin collagen to enzymatic degradation by collagenases, and improved the short-term mechanical properties of dentin collagen.

The potential of several flavonoids to enhance the wettability of dentin bonding agents has been investigated. The following are some flavonoids that have demonstrated potential in this regard:

Green tea contains a flavonoid called...
Evaluation of wettability of hesperidin incorporated dentin adhesive - an invitro study

epigallocatechin-3-gallate (EGCG), which has been demonstrated to increase the wettability and binding strength of dentin bonding agents37. Research has revealed that the flavonoid quercetin, which is present in many fruits and vegetables, increases the wettability of dentin bonding agents31,38. The flavonoid kaempferol, which is present in tea, broccoli, and other plants, may increase the wettability and binding strength of dentin bonding agents39. Studies have demonstrated that the flavonoid fisetin, which is present in strawberries, apples, and other fruits, increases the wettability of dentin bonding agents. Overall, these flavonoids have demonstrated potential for enhancing the wettability and bond strength of dentin bonding agents, and they may have application in restorative dentistry3138. To fully realise their potential and maximise their inclusion into dental materials, more research is necessary.

Oranges, lemons, and grapefruits are among the citrus fruits that contain the most hesperidin, a flavonoid40. Hesperidin's potential to enhance the wettability of dentin bonding agents has not received much research, although some studies have suggested that it may possess special qualities that make it a suitable choice for usage in dental materials. Hesperidin is employed in this investigation to assess the bonding agent's wettability. Hesperidin's capacity to increase the bonding agent's wettability may have been aided by its special chemical structure, which includes a methoxy group and a rhamnose sugar moiety11,41. Hesperidin may play a dual role in enhancing the wettability of dentin bonding agents and suppressing MMP activity to increase their longevity42. Hesperidin may have special qualities that make it a good choice for enhancing the wettability and durability of dentin bonding agents, while more research is required to fully grasp its potential in dental materials. In this present study, Hesperidin incorporated total etch dentin adhesive shows improved wettability compared to control group. This is because , Hesperidin has the capacity to interact with the mineral hydroxyapatite, which makes up a portion of dentin, and this interaction may help bonding agents become more wettable. The methoxy group and rhamnose sugar moiety in hesperidin interact with the hydroxyapatite surface to provide a more hydrophilic surface, which improves the bonding agent's ability to wet surfaces. Stronger and longer-lasting bonding may arise from the adhesive penetrating the dentin more effectively as a result of the greater wettability. Hesperidin has also been demonstrated to block the activity of matrix metalloproteinases (MMPs), which can break down the collagen in dentin and weaken the bonds between dentin bonding agents. Hesperidin can assist maintain the integrity of the dentin-collagen matrix by decreasing MMP activity, which can strengthen and lengthen the bonding agent's durability. Overall, hesperidin may have the potential to enhance the wettability and durability of dentin bonding agents due to its capacity to interact with hydroxyapatite and decrease MMP activity43. Ghorab et al. (2018) examined the antimicrobial effectiveness and adhesive characteristics of a total-etch adhesive system containing varying concentrations of hesperidin (HPN), coming to the conclusion that doing so significantly increased the immediate Tensile Bond Strength (P 0.05). Thermocycling significantly reduced the TBS of dental adhesives with 0.5 wt % HPN incorporated, and the adhesive properties remained unaffected while the antibacterial impact was promising. In this study, 2% HPN is used as it has ability to improve immediate bond strength without causing marginal staining. Hesperidin also has many properties like Antiinflammatory, analgesic, antibiotic, antioxidant, bone loss prevention, prevents demineralization and promotes remineralization apart from anticaries effect like preventing caries progression because of its collagen cross linking property, MMP inhibition and collagen cross linking property. In the present study there is no significant difference in wettability between control and test groups. This may be because of small sample size. However wettability of test group is better compared to the control group. The wettability of the bonding agent has also been increased by the dimethyl sulfoxide utilised as a solvent in this investigation.Thus Hesperidin together with Dimethyl sulfoxide synergistically shows improved wettability of total etch adhesive. To completely comprehend the mechanisms by which hesperidin regulates the wettability of
bonding agents and to optimise its incorporation into dental materials, more study is nonetheless required.

CONCLUSION
In conclusion, the incorporation of hesperidin into dentin adhesive has been shown to improve its wettability. This is likely due to hesperidin's unique chemical properties, which enable it to interact with hydroxyapatite and create a more hydrophilic surface. These findings suggest that hesperidin has potential as a natural additive to enhance the performance of dentin adhesive, and further research is warranted to explore its full potential in dental applications.

REFERENCES
21. Subashri A, Maheshwari TNU. Knowledge and attitude of oral hygiene practice among dental


