

EFFECT OF HESPERIDIN ON ANTIBACTERIAL ACTIVITY AND ADHESIVE PROPERTIES OF AN ETCH-AND-RINSE ADHESIVE SYSTEM

Sayed Ghorab* and Ashraf Ibraheim**

ABSTRACT

Objectives: To evaluate the antimicrobial activity and adhesive properties of a simplified total-etch adhesive system containing different proportions of Hesperidin (HPN).

Materials and Methods: Hesperidin was added to dental adhesive in three different ratios producing four experimental adhesive groups (0 [control], 0.2, 0.5, and 1%). The antibacterial activity of the prepared adhesive groups was studied using agar disc-diffusion test against *Streptococcus mutans*. The viscosity of dental adhesives was evaluated using a cone and plate viscometer. Microtensile bond strength was tested immediately and after thermocycling. The fracture patterns were examined using a stereomicroscope. Data were statistically analyzed using ANOVA and Tukey HSD tests ($\alpha = 0.05$).

Results: The antimicrobial activity of HPN-incorporated experimental adhesives exhibited a significant inhibitory effect against *Streptococcus mutans* compared with the control ($P < 0.05$). The viscosity of the experimental adhesives increased with increasing the concentrations of HPN incorporation into the adhesive. The incorporation of 0.2 wt% and 0.5 wt% HPN into the dental adhesive significantly increased the immediate μ TBS ($P < 0.05$). However, experimental adhesives incorporating 1 wt% HPN showed no significant differences in the μ TBS values compared with the unmodified adhesive resin ($P > 0.05$). After thermocycling, all studied adhesive groups revealed significant reduction in μ TBS ($p < 0.001$).

Conclusions: 0.5 wt% HPN incorporated dental adhesives could achieve a promising antibacterial effect without adversely affect the adhesive characteristics; however, thermocycling significantly reduced the μ TBS.

KEYWORDS: antibacterial activity, hesperidin, microtensile bond strength, viscosity

* Lecturer, Dental Biomaterials Department, Faculty of Dentistry, Mansoura University, Egypt

** Lecturer, Department of Operative, Faculty of Dentistry, Mansoura University, Egypt.

INTRODUCTION

One of the most significant benefits of adhesive restoration is to promote an effective durable seal of resin-based restorative materials to hard tooth structures in order to resist postoperative sensitivity and clinical failure.¹ However, currently available dentin bonding systems exhibit some drawbacks that can affect their clinical performance and are the main causes for replacing adhesive restorations.²⁻⁴ These drawbacks include limited stability in vivo and incidence of recurrent caries.^{3,5} As a result, the dentin bonding agents should exhibit multiple therapeutic activities that function in antimicrobial performance whereas maintaining the stability of the materials.⁶

It has been shown that resin-based restorative materials accumulate more biofilm, compare to other tooth-colored restorative materials.⁷ Accordingly, remaining microorganism within the cavity after preparation or penetrating microorganism through restoration/tooth interface could result in the occurrence of recurrent caries and post-operative inflammation. Therefore, antibacterial activity of bonding agents and restorative composites is essential not only to inhibit the growth of residual microorganism but also to avoid colonization of cariogenic microorganism on the tooth/restoration interface.⁸ This activity can be obtained either through adding antimicrobial agents or antibacterial monomers to the bonding agents' composition.^{9,10} However, when these materials were incorporated into the bonding systems, the bonding efficacy and handling property might be affected.^{11,12}

In spite of the latest developments in dentin bonding systems, adhesive interface remains the weakest area of the aesthetic restorations. Hybrid layer' degradation represents a problem that may most likely results in reduced clinical lifetime of applied resin-based restorations.¹³ This degradation can occur via two

mechanisms; hydrolytic degradation of the resin components within the hybrid layer and collagen degradation by host-derived enzymes such as matrix metalloproteinase (MMPs) and/or cysteine cathepsins.¹⁴⁻¹⁶ These enzymes are secreted as inactive proenzymes, but once these enzymes activated, collagen fibril, elastin and extracellular matrix (ECM) components are degraded. It is noted that these enzymes can be activated at a lower pH, as occurs once dentin is conditioned by acid etching during application of the etch-and-rinse adhesive system.¹⁷

Numerous approaches to resist collagen degradation were proposed in order to preserve resin-dentin bond strength. One promising approach is the use of MMP inhibitors either as a primer before adhesive's application¹⁸ or added to the components of dental adhesive systems with the aim of reducing chair time.¹⁹ Another approach includes collagen cross-linking through using chemical agents with cross-linking effect for enhancing the biomechanical characteristics of dentin, and making it less vulnerable to proteolytic attack.²⁰⁻²² Hesperidin (HPN), an extract from citrus fruits, is a natural flavonoid exhibiting multiple benefits such as: anti-oxidant, anti-inflammatory, collagen cross-linker, resistance to caries progression, promotion of the remineralization process and anti-microbial effects.²³⁻²⁶ However, there is a lack of information regarding the incorporation of hesperidin into an etch-and-rinse adhesive system as a type of modification. Accordingly, the rationale of this research was to assess the antimicrobial activity and adhesive properties like viscosity, bonding strength and durability to dentin of a simplified etch-and-rinse adhesive system incorporated with HPN in different proportions. The null hypothesis for this study was that the incorporation of HPN has no effect on the antimicrobial activity and adhesive properties of a dental adhesive.

MATERIALS AND METHODS

Preparation of experimental adhesives containing hesperidin

The hesperidin powder ($\geq 80\%$ pure, Sigma Chemical, St. Louis, MO, USA) was added in three different ratios (0.2, 0.5 and 1 wt%) to simplified total-etch adhesive system Optibond solo plus (Kerr Italia S.r.l. Scafati, Italy) to form four experimental adhesive groups; Group A: control (0 wt% HPN), Group B: 0.2 wt% HPN, Group C: 0.5 wt% HPN and Group D: 1 wt% HPN. Compositions, manufacturers and batch numbers of the materials used in this study are presented in Table 1. The mixtures were stirred for 1 h using an electric magnetic stirrer ((Thermolyne SP18425, Hi-tech Trader, USA) to ensure complete dispersion of hesperidin in the resin.

Determination of antibacterial activity

The antimicrobial activity was evaluated by the Agar Diffusion Test. This test was conducted in the Medical Diagnostic and Infection Control Unit (MDICU) at the Medical

Microbiology and Immunology Department, Faculty of Medicine, Mansoura University. Five sterile Petri dishes (15 cm diameter and 5 mm thickness) were filled with molten blood agar culture medium and left to cool. When the medium became gel, microbial colonies (*Streptococcus mutans* UA 159) were evenly distribute over the medium surface using a sterile disposable swab and were cultured at 37° C for 24 hours in an aerobic atmosphere.²⁷

Paper disks (6 mm diameter and 1.5 mm thickness) were used to be coated with the tested materials. These disks were prepared as follow: filter paper was punched using a hole punch to make small circular paper disks. These disks were wrapped in aluminum foil and sterilized in the hot air oven at 160° C for 30 min. Twenty specimens were prepared and categorized into four groups (control, 0.2 wt%, 0.5 wt% and 1 wt% HPN incorporated adhesives), each group included five specimens. Twenty μ l of each bonding agent were impregnated into a sterile paper disk and cured for 20 sec using Elipar FreeLight 2 (3M; St Paul, MN, USA, light output: 1226 mW/cm²). Paper disks containing the tested material were seated on the sterile petri dish

TABLE (1) Materials used in the study.

Materials	Composition	Manufacturer/Lot no
Optibond solo plus (Single component total-etch adhesive)	Bis-GMA, HEMA, GDM, GPDM, 2-(ethylhexyl)-4-(dimethylamino) benzoate, butylhydroxytoluene, fillers (barium aluminoborosilicate glass, fumed silica, sodium hexafluorosilicate), ethanol and photoinitiator	Kerr Italia S.r.l. Scafati, Italy/6546327
Herculite™ XRV Ultra (Nanohybrid composite)	Bis-GMA, TEGDMA, Prepolymerized filler, Silica nanofiller (20-50 nm nanoparticles), Barium submicron fillers (0.6 μ m average size), Titanium Dioxide (TiO ₂) and Pigments	Kerr Italia S.r.l. Scafati, Italy/6455231
Hesperidin	Hesperetin- 7-O-rutinoside is a flavonoid extracted from citrus fruits.	Sigma–Aldrich; St. Louis, MO, USA/SLBT3541

Bis-GMA: Bisphenol A glycedyl methacrylate, TEGDMA: Tetra ethylene glycol dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, GDM: glycerol dimethacrylate, GPDM: glycerol phosphate dimethacrylate

at equal distances from each other by applying firm pressure to the disks with a sterile forceps against the medium surface, 4 disks per dish.²⁷ The plates were incubated at 37° C for 24 h. The diameters of inhibition zones were measured at three different points. Sizes of inhibition zones were calculated by subtracting the diameter of specimen from the average of the three measurements of the halo.^{27,28}

Measurement of viscosity

Viscosity of the different adhesives at a given shear rates was measured using a rheometer (DV-III Ultra, Brookfield Engineering Laboratories, INC, USA). The circulating temperature bath for controlling the fluid temperature was adjusted to be within +/- 0.1° C. Cone and plate geometry was used, which consisted of a rotating cone and a stationary plate (the cone angle 5°, cone diameter 40 mm and gap 0.2 mm) where the specimen was filled in the gap between them. Two ml of each adhesive was placed on the plate, then the cone was brought down and viscosities of the samples were measured in centipoise under various shear stresses. Each viscosity measurement took 10 min to complete and all measurements were done at 23° C.^{29,30}

Determination of microtensile bond strength (μ TBS)

Twenty freshly extracted non-carious human molar teeth were collected from outpatient dental clinic, in Oral Surgery Department of Mansoura University, (age; 40-65 years) and these teeth were extracted due to periodontal disease. Teeth were cleaned by removing remnants of soft tissues and were stored for less than 1 month in 0.5% chloramine-T solution at 4° C. Plastic ring molds were filled with self-cured acrylic resin and teeth were embedded vertically in the resin leaving the crown exposed. Occlusal enamel was removed under water cooling perpendicular to the long axis of each tooth using a trimmer. The exposed dentinal surface was ground flat with 600-grit silicon carbide paper (Soft Flex, 991 A, Germany) under adequate

water cooling to reduce dentin thickness by 1 mm and exposing the mid-dentin.³¹

The specimens were categorized into four equal groups of five teeth each (control, 0.2 wt%, 0.5 wt% and 1 wt% HPN filled adhesives). For each group, phosphoric acid etchant (Gel Etchant, Kerr Corp., USA) was applied for 15 sec on the flat dentin surface and rinsed for 15 sec. Excess water was removed using an absorbent pellet, leaving the dentin surface moist. Two layers of bonding agent were applied using a disposable brush, air dried gently for 2-5 sec and then light-cured for 20 sec using a light curing unit.

After curing of adhesives, a nanohybrid composite (Herculite™ XRV Ultra, Kerr Italia S.r.l. Scafati, Salerno, Italy) was built up over them using a split teflon mold (6 mm diameter and 4 mm height). Composite resin was inserted into the mold by incremental technique of 2 mm thickness per increment. Each increment was compressed using a plastic condenser and light-cured for 40 sec using visible light curing unit. After complete curing of the composite, the mold was removed and specimens were stored in distilled water at 37° C for 24 h in an incubator. Each specimen was sectioned in occlusogingival direction into a series of 1 mm thick beams under water cooling to produce dentin-resin composite sticks. Sectioning was performed using a diamond saw of 0.5 mm thickness on a cutting machine (Ham Co. Machines, Inc, Rocher, USA). They were then rotated 90 degrees and serial sectioning was repeated. Each tooth was removed from the mold and the resulting beams were sectioned free from the root. The beams have cross-sectional areas of 1 mm which were measured using a digital caliper (Iwanson. Martin, Germany). Half of the bonded sticks from each adhesive group were tested immediately (this state was designated as 0 thermocycle), and other half were subjected to thermocycling between 5° C and 55° C for 10 000 cycles with a 30 sec dwell time (Thermocycler THE-1100, SD Mechatronik GmbH, Bayern, Germany). Each of the prepared beams was attached to

a specially designed attachment jig with its ends glued with cyano-acrylate cement (Super Glue Gel, 3M, St. Paul, MN, USA). The testing device was in turn mounted onto the lower fixed compartment of a Universal Testing Machine (LLOYD instruments, LR 5K, England). A tensile load was applied at a crosshead speed of 0.5 mm/min until failure. The data were recorded using computer software (Nexygen-MT Lloyd Instruments). The fractured beams were carefully removed from the testing device and cross-sectional area of each tested specimen at the site of failure was measured using the caliper. The applied tensile force resulted in debonding along the substrate-adhesive interface. The microtensile bond strength was derived by dividing the imposed force at the time of fracture by the bond area in mm² and was expressed in MPa.^{31,32}

After debonding, the fractured sites were observed using a stereomicroscope (Olympus model SZ-PT, Tokyo, Japan) at 20x magnification to identify the mode of failure. Failures can be classified as one of the following: adhesive, if composite resin was fractured at the adhesive-tooth interface; cohesive in dentin, if the restoration was fractured with a large portion of dentin attached; cohesive in composite resin, if the composite resin was fractured inside the composite resin; or mixed failure, a combination of adhesive and cohesive failure in composite or dentin.

Statistical analysis

Using Kolmogorov–Smirnov and Shapiro–Wilk tests, normality of data distribution was tested. According to the Kolmogorov–Smirnov test, the data were normally distributed ($\alpha = 0.05$). Results of antibacterial activity and viscosity were analyzed by one-way analysis of variance (ANOVA). μ TBS results were statistically analyzed by two-way ANOVA with the adhesive types and thermocycling as the independent variables. Tukey test was used to determine any significant differences among the groups. Additionally, the fracture pattern was

analyzed by the Chi-square (χ^2) test. The Statistical Package of Social Sciences (SPSS) version 21 was used at significance level of < 0.05 .

RESULTS

Antibacterial activity

Means and standard deviations of inhibitory zones (mm) for all groups are shown in Table 2. A graphical presentation of these results is shown in Figure 1. All studied groups exhibited inhibition of growth against *Streptococcus mutans* (Figure 2). Comparing the mean inhibitory zones of the tested materials; group D (1 wt% HPN-incorporated group) exhibited the highest mean inhibitory zones (3.37 ± 0.43) while group A (control adhesive) showed the lowest value (1.92 ± 0.57). One-way ANOVA results of inhibitory zones (mm) are presented in Table 3. There was a significant difference in antibacterial activity among the adhesive groups ($P \leq 0.05$). Tukey statistical test showed a significant difference between group C and both groups A and B and between group D and both groups A and B. On the other hand, no significant difference was shown between group A and group B and between group C and group D.

Viscosity

Means and standard deviations of viscosity (centipoise) for all groups are shown in Table 4. A graphical presentation of these results is shown in Figure 3. The highest mean viscosity value was for group D (2.48 ± 0.42 centipoise), while the lowest value was for group A (1.69 ± 0.29 centipoise). One-way ANOVA results of viscosity are presented in Table 5. There was a significant difference in viscosity among the adhesive groups ($P \leq 0.05$). Tukey statistical test showed a significant difference between group D and groups A and B. On the other hand, there was no significant difference between group C and other groups and between group A and group B.

TABLE (2) Means and standard deviations of Tukey HSD test for inhibitory zones (mm) of all adhesive groups against Streptococcus mutans.

	Control	0.2 wt% HPN	0.5 wt% HPN	1 wt% HPN
Inhibitory zones (Mean ± SD)	1.92 ± 0.57 ^a	2.43 ± 0.52 ^a	3.27 ± 0.56 ^b	3.37 ± 0.43 ^b

Mean with the same superscript letter are not significantly different

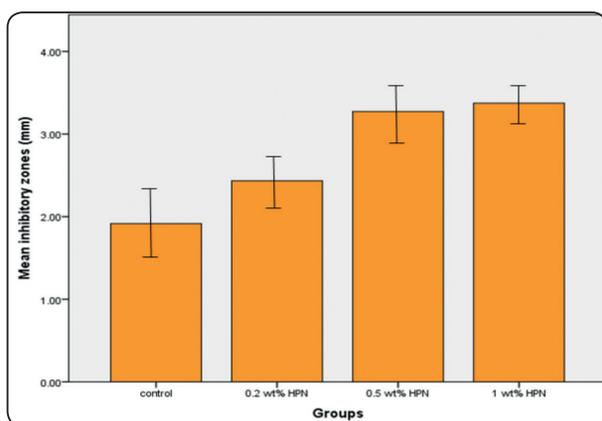


Fig. (1) Histogram showing the means and standard deviations of inhibitory zones in (mm) for the adhesive groups against Streptococcus mutans.

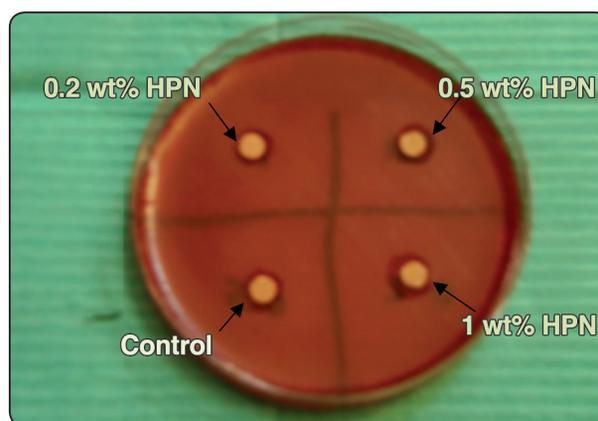


Fig. (2) A photograph showing the inhibitory zones of the tested groups against Streptococcus mutans.

TABLE (3) One-way ANOVA results for inhibitory zones (mm) of adhesive groups against Streptococcus mutans.

Source	df	Sum of Squares	Mean Square	F	P
Between Groups	3	7.286	2.429	8.974	0.001
Within Groups	16	4.330	0.271		
Total	19	11.617			

Statistically significant difference at P < 0.05

TABLE (4) Means and standard deviations of Tukey HSD test for viscosity (centipoise) of different groups.

	Control	0.2 wt% HPN	0.5 wt% HPN	1 wt% HPN
Viscosity (Mean ± SD)	1.69 ± 0.29 ^a	1.79 ± 0.3 ^a	1.98 ± 0.33 ^{a,b}	2.48 ± 0.42 ^b

Mean with the same superscript letter are not significantly different

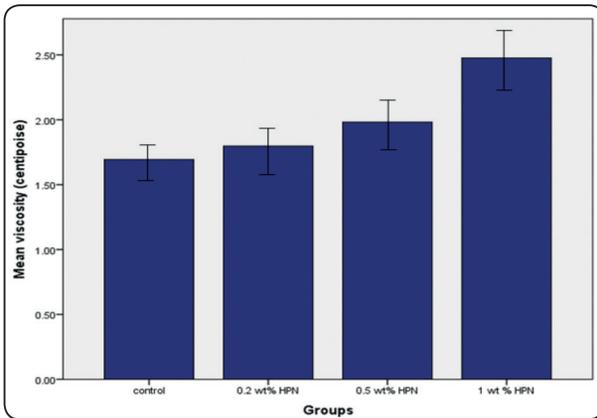


Fig. (3) Histogram showing the means and standard deviations for viscosity (Centipoise) of the tested groups.

Microtensile bond strength

Means and standard deviations of the μ TBS values (MPa) are presented in Table 6. A graphical presentation of these results is shown in Figure 4. Comparing the mean μ TBS of the adhesives to dentin showed that group C (0.5 wt% HPN-incorporated group) exhibited the highest value (immediate: 37.45 ± 3.09 MPa and after thermocycling: 28.66 ± 2.34 MPa), while group A (control group) showed the lowest value (immediate: 29.61 ± 5.28 MPa and after thermocycling: 22.87 ± 5.31 MPa). Two-way ANOVA of the μ TBS (MPa) testing data (adhesive type and thermocycling) revealed that the bond strength was significantly affected by the type of adhesive and by thermocycling ($P < 0.001$). There was no significant interaction between type of adhesive and thermocycling ($P = 0.406$) as presented in Table 7. Tukey statistical test showed that 0.2 wt% and 0.5 wt% HES-filled groups exhibited a significantly higher immediate μ TBS, when compared to the control group ($p < 0.001$). After thermocycling, there was no significant difference between the

TABLE (6) Means and standard deviations of Tukey HSD test for μ TBS (MPa) of all groups.

	Control	0.2 wt% HPN	0.5 wt% HPN	1 wt% HPN
Immediate	29.61 ± 5.28^{a1}	35.49 ± 5.32^{bc1}	37.45 ± 3.09^{b1}	31.41 ± 2.34^{ac1}
Thermocycling	22.87 ± 5.31^{ab2}	24.34 ± 3.19^{acd2}	28.66 ± 2.34^{c2}	23.13 ± 4.56^{bd2}

Means with the same superscript lowercase letter (row) are not significantly different

Means with different superscript numbers (column) are significantly different

TABLE (5) One-way ANOVA results for viscosity of different groups.

Source	df	Sum of Squares	Mean Square	F	P
Between Groups	3	1.804	0.601	5.174	0.011
Within Groups	16	1.859	0.116		
Total	19	3.663			

Statistically significant difference at $P < 0.05$

control group and 0.2% HPN-filled group ($P \geq 0.05$). On contrary, the 0.5% HPN-filled group showed significantly higher μ TBS when compared with the control ($P < 0.001$). The 1 wt% HPN-filled group did not show any significant difference in μ TBS both immediate and after thermocycling when compared with the control ($P > 0.05$). Significant reduction in μ TBS was observed for all studied groups after thermocycling ($P < 0.001$).

Stereomicroscopic examination at the debonding sites showed two modes of failure; adhesive failure (at the interface between adhesive and dentin surface) and mixed failure (parts of the composite and adhesive remained attached to dentin surface). These modes of failure were varied between the 4 adhesive groups as shown in Figure 5, where control group and 1 wt% HPN incorporated group showed more adhesive failure, while 0.2 wt% and 0.5 wt% HPN incorporated groups showed more mixed one ($\chi^2=5.91$, $P=0.12$). Moreover, all studied groups showed more adhesive failure after thermocycling ($\chi^2=6.7$, $P=0.08$).

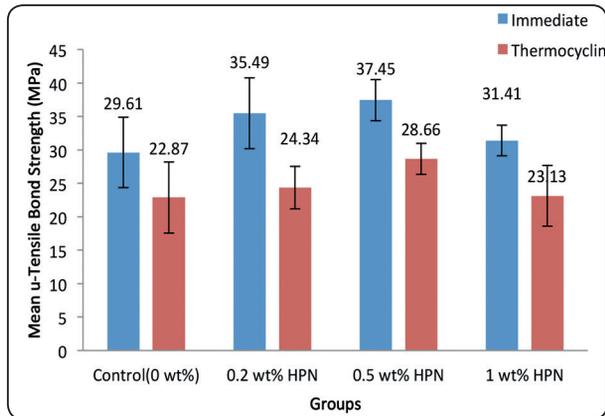


Fig. (4) Histogram showing the means and standard deviations of microtensile bond strength (μ TBS) in MPa of the tested groups.

TABLE (7) Two-way ANOVA results for μ TBS (MPa) of all tested groups.

Source of variation	Sum of Squares	df	Mean Square	F	P
Adhesive type	556.275	3	185.425	10.916	<0.001*
Thermocyclin	1527.402	1	1527.402	89.917	<0.001*
Adhesive type* Thermocyclin	50.032	3	16.677	.982	0.406
Errors	1223.050	72	16.987		
Total	71200.535	80			

Statistically significant difference at $P < 0.05$

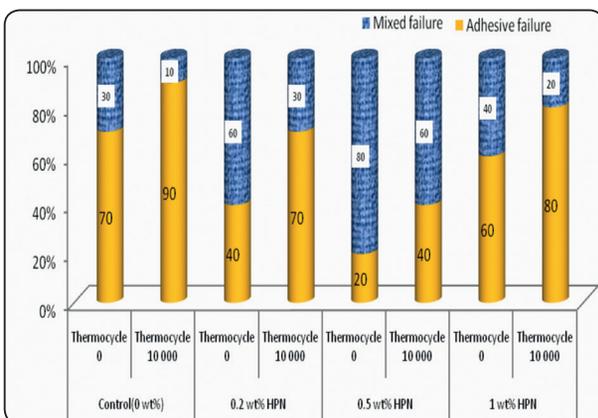


Fig. (5) Histogram showing the failure pattern distribution of different groups tested (adhesive failure at the interface between adhesive and dentin surface and mixed failure).

DISCUSSION

Adhesive restorative materials were used widely due to their tooth-colored appearance. Nevertheless, there were several drawbacks that could affect the performance of dental adhesive. These include poor antimicrobial activity and reduced bond stability.⁸ Therefore, numerous modifications of dental adhesive have been introduced to obtain new materials with improved characteristics. These modifications can only improve one purpose, either enhancing the antibacterial effect of the dental adhesives via incorporation of fluorides³³ or improving the bond stability through application of MMPs inhibitor.¹⁸ Therefore, searching for achieving dental adhesives with multifunction effect have been done for obtaining dental adhesives with improved various performances simultaneously.⁶

Hesperidin (HPN) has been reported to exhibit several benefits, however the application of HES in total-etch adhesive systems as a type of modification has not been investigated. Previous studies verified that HPN exhibited antibacterial activity, collagen cross-linking effect and prevention of proteolytic degradation of dentin collagen in vitro.^{25,26} The incorporation of hesperidin significantly altered the antibacterial activity and adhesive properties evaluated in this study. Thus, the null hypothesis was rejected.

In the present study, for antimicrobial activity evaluation, agar-disc diffusion test method was used because it is a simple direct inhibition test and it has been most frequently used.²⁷ Moreover, *Streptococcus mutans* strains were tested because they are essential in initiation of dental caries due to their capability of colonization on the tooth surfaces, synthesization of insoluble polysaccharides (glucans), and fermentation of sucrose to form lactic acid that demineralized the tooth structure.³⁴ The results of the present study showed that the total etch adhesive system used in this study (Optibond solo plus) has demonstrated

antibacterial action against streptococci but to lesser degrees. These results may be attributed to fluoride releasing nature and low pH value (pH < 5.0) of this adhesive system (presence of fluoride and decrease pH values of these systems kill or inactivate the bacteria).³⁵ The results of the present study also showed that addition of HPN produced a significant improvement in antibacterial activity among the different groups. This can be explained on the basis that HPN is a phenolic flavonoid, possessing antibacterial activity against a plenty of microorganisms similar to natural flavonoids.³⁶ This result goes with the finding obtained by Dua et al. (2012)³⁷ that investigated the antibacterial potential of a dental adhesive incorporated with a natural flavonoid, Epigallocatechin-3-gallate (EGCG), in different concentration. Results revealed that antimicrobial activity of a dental adhesive against *Streptococcus mutans* was a dose dependent which increased with increasing the concentrations of EGCG incorporation into the adhesive.

Adhesive resin must present proper fluidity to permit its infiltration and be able to fully penetrate into the etched substrate and polymerize in situ. Too high viscous adhesive is less likely to spread out on a surface due to its high resistance to flow. On the other hand, too low viscous adhesive is difficult to be controlled during its placement on the surface.³⁸ Because the commercial adhesives have their unique formulation, other substances cannot be incorporated into them without considering the possible negative effects on handling property. Therefore, viscosity of dental adhesives incorporated with HPN was assessed. In this study, the viscosity of different adhesive groups was measured by cone and plate viscometer. This cone-and-plate geometry was widely used for measuring viscosity of resin materials because it works by sandwiching a small amount of fluid in between a rotating cone and a fixed plate.³⁰ The results of the present study showed that the viscosity of dental adhesive was not increased significantly after HPN incorporated

(0.2 wt% and 0.5 wt% HPN). However, at higher proportion (1 wt% HPN), higher viscosity was observed. This can be attributed to the fact that at a low HPN concentration, particles could be trapped within monomers, improving dispersion and stability. However, at high concentration, particles were closer to each other and the probability of collision was higher under shear and caused aggregates.³⁹

Stability of resin-dentin bonded interface is a prerequisite in order to achieve an adequate functional outcome and long-term durability. In the oral cavity, daily routine of eating, drinking and breathing can result in temperature changes that produce functional thermal stress affecting the stability of the bonded interface over time. In this study, a thermocycling test machine with 10,000 times in vitro thermal cycles, representing 1 year of in vivo function, was used to examine the durability of restorations. It is an appropriate method that simulates in vitro aging of specimens because it produces standardized thermal stresses at the interface.^{40,41} Furthermore, the microtensile bond strength (μ TBS) test was used because it is a reliable bond strength test since it provides a more uniform and homogeneous stress distribution during loading, and failure mainly occurs at the adhesive interface because of small bonded interfaces (approximately 1 mm²) of the specimens used in this test.³²

The results of the present study showed that incorporation of HPN into the dental adhesive results in a significant enhancement of the immediate μ TBS and it was a dose dependant effect. This can be attributed to the fact that HPN is a natural flavonoid, exhibiting a chroman ring that can interact with proline-rich proteins such as collagen resulting in cross-linking action and improving the mechanical properties of dentine.⁴² This result goes with the finding obtained by previous studies^{25,26,42-44} that investigated the impact of natural cross-linkers such as tannic acid, proanthocyanidins (PA) and

hesperidin on μ TBS. Results showed that natural cross-linkers improved the resin-dentin bonding due to their collagen cross-linking properties. The results were also showed that thermocycling significantly decreased the μ TBS of all tested experimental groups when compared to their immediate values. This can be explained on basis that the coefficients of thermal expansion of tooth structure and bonding system change differently during thermal changes. This induces stress at the bonding interface that could affect the long-term bonding durability of dental adhesives.^{40,41}

The fracture analysis for all adhesive groups revealed that mixed failures occurred more commonly in 0.2 wt% and 0.5 wt% HPN-filled adhesives, while unfilled and 1 wt% HPN-filled adhesives showed more adhesive failures. This result is comparable to the finding of the previous report that the percentage of adhesive failure is inversely proportional to the value of μ TBS.⁴⁵ The results were also showed that a higher percentage of adhesive failure was obtained after thermocycling and this evidenced the degradation of resin-dentin interfaces.

CONCLUSION

Within the limitations of this study, it can be concluded that the incorporation of 0.5 wt% HPN into a simplified total-etch adhesive system could accomplish therapeutic goals that play in antibacterial performance without compromising the adhesive properties of dental adhesives.

ACKNOWLEDGMENT

Authors would like to express their sincere gratitude to all members in Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University for their help, continuous support and encouragement during making the microbiological activity test.

REFERENCES

1. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent* 2003;28:215-35.
2. Malhotra N, Mala K, Acharya S. Resin-based composite as a direct esthetic restorative material. *Compend Contin Educ Dent* 2011;32:14-23.
3. Brackett MG, Li N, Brackett WW, Sword RJ, Qi YP, Niu LN, et al. The critical barrier to progress in dentine bonding with the etch-and-rinse technique. *J Dent* 2011;39:238-48.
4. Spagnuolo G, Annunziata M, Rengo S. Cytotoxicity and oxidative stress caused by dental adhesive systems cured with halogen and LED lights. *Clin Oral Invest* 2004; 8:81-5.
5. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Leonarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater* 2008;24:90-101.
6. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater* 2011;27:1-16.
7. Svanberg M, Mjor IA, Orstavik D. Mutans streptococci in plaque from margins of amalgam, composite, and glass ionomer restorations. *J Dent Res* 1990;69:861-4.
8. Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. *Dent Mater J* 2009;28:11-9.
9. Walter R, Duarte WR, Pereira PN, Heymann HO, Swift Jr EJ, Arnold RR. In vitro inhibition of bacterial growth using different dental adhesive systems. *Oper Dent* 2007; 32:388-93.
10. de Carvalho FG, Puppim-Rontani RM, Soares LE, Santo AM, Martin AA, Nociti-Junior FH. Mineral distribution and CLSM analysis of secondary caries inhibition by fluoride/MDPB containing adhesive system after cariogenic challenges. *J Dent* 2009;37:307-14.
11. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 2003;19:449-57.
12. Li F, Chen J, Chai Z, Zhang L, Xiao Y, Fang M, et al. Effects of a dental adhesive incorporating antibacterial monomer on the growth, adherence and membrane integrity of *Streptococcus mutans*. *J Dent* 2009;37:289-96.

13. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Leonardo R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater* 2008;24:90-101.
14. Sanabe ME, Costa CA, Hebling J. Exposed collagen in aged resin-dentin bonds produced on sound and caries-affected dentin in the presence of chlorhexidine. *J Adhes Dent* 2011;13:117-24.
15. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. *J Dent Res* 2004;83:216-21.
16. Vidal CM, Tjäderhane L, Scaffa PM, Tersariol IL, Pashley D, Nader HB, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. *J Dent Res* 2014;93:269-74.
17. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. *Biomaterials* 2006;27:4470-76.
18. Takce N, Tunser S, Demirci M, Balci S. Do matrix metalloproteinase inhibitors improve the bond durability of universal dental adhesives? *Scanning* 2016;38:535-44.
19. Almahdy A, Koller G, Sauro S, Bartsch JW, Sherriff M, Watson TF, et al. Effects of MMP inhibitors incorporated within dental adhesives. *J Dent Res* 2012;91:605-11.
20. Viviane Hassa, Issis Virginia Luque-Martinez, Mario Felipe Gutierrez, Camila Guimarães Moreirad, Valéria Bisinoto Gottie, Victor Pinheiro Feitosa. Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition. *Dent Mater* 2016;32:732-41.
21. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. *Dent Mater* 2018;34:78-96.
22. Chen C, Mao C, Sun J, Chen Y, Wang W, Pan H, et al. Glutaraldehyde-induced remineralization improves the mechanical properties and biostability of dentin collagen. *Mater Sci Eng C Mater Biol Appl* 2016;67:657-65.
23. Trzeciakiewicz A, Habauzit V, Mercier S, Barron D, Urpi-Sarda M, Manach C, et al. Molecular mechanism of hesperetin-7-O-glucuronide, the main circulating metabolite of hesperidin, involved in osteoblast differentiation. *J Agric Food Chem* 2010;58:668-75.
24. Corciova A, Ciobanu C, Poiata A, Mircea C, Nicolescu A, Drobotu M, et al. Antibacterial and antioxidant properties of hesperidin: β -cyclodextrin complexes obtained by different techniques. *J Incl Phenom and Macrocycl Chem* 2015;81:71-84.
25. Islam S, Hiraishi N, Nassar M, Yiu C, Otsuki M, Tagami J. Effect of natural cross-linkers incorporation in a self-etching primer on dentine bond strength. *J Dent* 2012;40:1052-9.
26. Islam S, Hiraishi N, Nassar M, Yiu C, Otsuki M, Tagami J. Effect of hesperidin incorporation into a self-etching primer on durability of dentin bond. *Dent Mater* 2014;30:1205-12.
27. Ozer F, Karakaya S, Unlu N, Erganis O, Kav K, Imazato S. Comparison of antibacterial activity of two dentin bonding systems using agar well technique and tooth cavity model. *J Dent* 2003;31:111-6.
28. Penmetsa RK, Rekha SA, Poppuri KC, Prashanth SP, Garapati S. An invitro evaluation of antibacterial properties of self etching dental adhesive systems. *J Clin Diagn Res* 2014;8:1-5
29. Silikas N, Watts DC. Rheology of urethane dimethacrylate and diluents formulations. *Dent Mater* 1999;15:257-61.
30. Tavas M, Watts DC. The use of a cone and plate viscometer for determination of flow properties of unfilled resins and etching gels. *J Oral Rehabil* 1989;16:185-92.
31. Kasraei SH, Atai M, Khamverdi Z, Khalegh S. Effect of nanofiller addition to an experimental dentin adhesive on microtensile bond strength to human dentin. *Dent Mater* 2009;6:91-6.
32. Cortiano FM, Rached RN, Mazur RF, Vieira S, Freire A, de Souza EM. Effect of desensitizing agents on the microtensile bond strength of two-step etch-and-rinse adhesives to dentin. *Eur J Oral Sci* 2016;124:309-15.
33. Hara AT, Queiroz CS, Freitas PM, Giannini M, Serra MC, Cury JA. Fluoride release and secondary caries inhibition by adhesive systems on root dentine. *Eur J Oral Sci* 2005;113:245-50.
34. Vokus RP, Cisneros GJ, Levi M. Antibacterial properties of current orthodontic band cements. *Pediatr Dent* 1998;20:43-8.
35. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 2003;19:449-57.

36. Taleb-Contini SH, Salvador MJ, Watanabe E, Ito IY, Rodrigues de Oliveira DC. Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. *Braz J of Pharm Sci* 2003;39:403-8.
37. Dua X, Huang X, Huang C, Wanga Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. *J Dent* 2012;40:485-92.
38. Faria-e-Silva AL, Piva E, Moraes RR. Time-dependent effect of refrigeration on viscosity and conversion kinetics of dental adhesive resins. *Eur J Dent* 2010;4:150-5.
39. Luo J, Liang J, Hu AY, Liu XH. Effect of surface modified nano-diamond on viscosity of dental adhesives. *Shanghai Kou Qiang Yi Xue* 2012;21:274-8.
40. Xie C, Han Y, Zhao XY, et al. Microtensile bond strength of one- and two-step self-etching adhesives on sclerotic dentin: the effects of thermocycling. *Oper Dent* 2010;35:547-55.
41. Nikaido T, Kunzelmann KH, Chen H, et al. Evaluation of thermal cycling and mechanical loading on bond strength of a self-etching primer system to dentin. *Dent Mater* 2002;18:269-75.
42. Ebrahim M, Sarraf Shirazi A, Abdolhoseinpour F, Abdollahi M. Effect of Tannic Acid on Bond Strength of Etch and Rinse and Self-etch Adhesive Systems in Dentin of Primary Teeth. *J Contemp Dent Pract* 2017;18:34-8.
43. Alandia-Roman CC, Vencenti SA, Silami FD, Silveira FE, Pento GC, Pires-de-Souza FC. The effect of proanthocyanidins and chlorhexidine on the bond strength of glass fiber posts to root dentin. *Gen Dent* 2017;65:23-27.
44. Jain K, Beri L, Kunjir K, Borse N, Neekhara N, Kadam A. Comparative evaluation of the effect of 10% sodium ascorbate, 10% hesperidin, 1% riboflavin 5 phosphate, collagen cross-linkers, on the push-out bond strength of fiber postluted to radicular dentin: In vitro study. *J Conserv Dent* 2018;21:95-9.
45. Hipólito VD; Alonso RC, Carrilho MR, Anauate Netto C, Sinhoreti MA, Goes MF. Microtensile bond strength test and failure analysis to assess bonding characteristics of different adhesion approaches to ground versus unground enamel. *Braz Dent J* 2011;22:122-8.