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Grape Seed and Grape Skin Extracts Elicit a Greater Antiplatelet Effect When Used in Combination than When Used Individually in Dogs and Humans¹,²

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ABSTRACT

Grape products, rich in polyphenolics, inhibit platelet aggregation (PA), a risk factor for coronary artery disease. We postulated that combining extracts of grape seed (GSD) and grape skin (GSK), primary sources of grape polyphenolics, individually shown to inhibit PA, might enhance their individual antiplatelet effects. This hypothesis was examined in vitro (human platelets) and ex vivo (dog platelets) by studying the effects of the extracts on collagen–induced whole blood PA. In vitro, threshold concentration of only GSD, individually incubated with blood, significantly inhibited PA: PA was inhibited by 12.7 ± 3.5% (P ≤ 0.01). No significant changes in PA were observed with threshold concentrations of GSK, used individually. In two dose combinations, GSD and GSK inhibited PA 40.5 ± 10.1% (P ≤ 0.005) and 96.5 ± 3.1% (P ≤ 0.001). In the ex vivo study, seven dogs were fed threshold doses of GSD or GSK individually, in combination or in combination with a proprietary enzyme blend (EB: thought to enhance bioavailability) for 8 d. PA was measured before and after each treatment. PA measurements were also repeated 24 h after the final dose of GSD + GSK + EB. Feeding the extracts individually did not affect PA, whereas feeding them in combination inhibited PA by 31.9 ± 7.1% (P ≤ 0.05). Feeding EB in addition to GSD + GSK inhibited PA by 56.2 ± 8.1% (P ≤ 0.005): 24 h later, PA was still inhibited by 31.5 ± 10.5% (P ≤ 0.05), suggesting a residual antiplatelet effect from the administration of the final dose. The results suggest that the components of GSD and GSK, when present in combination as in red wine, grape juice or in a commercial preparation containing both extracts, exhibit a greater antiplatelet effect than when present individually.

KEY WORDS: • platelet aggregation • polyphenolic • grape seed • grape skin • synergism

INTRODUCTION

Mortality due to coronary artery disease (CAD)⁴ can occur as a result of an acute thrombotic event that is caused by the rupture of an atherosclerotic plaque (1,2). Although there are many causal factors for the development and progression of atherosclerosis, platelet hypersensitivity is thought to be an important contributor to the disease mechanism (1,2,3).
Increased platelet aggregation (PA), as a result of increased platelet sensitivity to agonists in vivo, contributes to the initiation and progression of atherosclerosis and to the occurrence of thrombotic events (1•, 2•). Platelet aggregation, which is associated with an increased release of reactive oxidative species (3•) and platelet–vessel wall interactions (4•), results in damage to the vascular endothelium (5•). The atherosclerotic disease process is thought to begin with the impairment of endothelial function (4•) followed by the increased accumulation of oxidized LDL (ox–LDL) within the wall underlying the damaged endothelium. Unregulated uptake of ox–LDL by tissue macrophages leads to the transformation of these macrophages into foam cells, which then build up within the arterial wall causing intimal thickening and the formation of fatty streak lesions (6•). In addition to their role in the initiation of atherosclerosis, platelets also contribute to the progression of the disease by releasing various growth and chemotactic factors that accelerate the proliferation and migration of smooth muscle cells (2•). Therefore, reducing the activity of platelets would potentially reduce development and progression of CAD. Daily intake of a platelet inhibitor, such as aspirin, has been shown to significantly slow the progression of atherosclerosis in animal models (7•). Similarly, grape products such as red wine and grape juice show platelet inhibitory properties and appear to reduce the development of atheroma in hypercholesterolemic animal models (8•–10•). These observations are thought to be due to the high polyphenolic content of grapes and grape beverages (11•).

The majority of the polyphenolic content in grape beverages is derived from the seed (12•) and skin (13•) of grapes. Extracts of grape seed (GSD) and grape skin (GSK) are effective platelet inhibitors when used individually (14•, 15•). However, it has been suggested that two polyphenolics, when used in combination, may interact synergistically to enhance their individual antiplatelet effects (16•). We hypothesized that threshold concentrations of GSD and GSK (concentrations at which little or no antiplatelet effects have been observed), when used in combination, would interact to produce a greater antiplatelet effect than the additive effect of each extract used individually. In addition, we hypothesized that the oral efficacy of the extract mixtures may be further enhanced by adding a proprietary enzyme blend (EB) thought to improve gastrointestinal (GI) bioavailability (17•).

### MATERIALS AND METHODS

GSD, GSK and EB were provided by Melaleuca (Idaho Falls, ID). These components are used primarily in combination by Melaleuca in Provex CV, a commercially available flavonoid supplement, which was shown previously to possess antithrombotic properties (18•). Whole blood platelet aggregometry was performed using a Chrono−log 4−Channel Whole Blood Impedance Aggregometer (Model 590, Chrono−log, Havertown, PA).

**In vitro incubation study**

The Institutional Review Board at the University of Wisconsin approved this portion of the study using human blood.

**Preparation of extract preparations.** The extract preparations were prepared by dissolving the appropriate amount(s) of the extract(s) in 1 mL of solvent [3 dimethyl sulfoxide (DMSO):7 preservative−free saline (PF−saline)]. The solutions were prepared such that adding 4 μL of each solution to the diluted blood sample would achieve the following concentrations of the extracts in the blood sample: 1A: GSD (50 mg/L), 1B: GSK (250 mg/L), 1C: GSD (50 mg/L) + GSK (250 mg/L), 1D: GSD (100 mg/L), 1E: GSK (500 mg/L), 1F: GSD (100 mg/L) + GSK (500 mg/L).

**Comparison of extract preparations.** Blood samples (18 mL each) from 8 healthy humans (5 men, 3 women; age: 20–63 y) were drawn into syringes containing sodiunm citrate (1 sodium citrate:9 blood) using 19G butterfly needles and were diluted with equal volumes of PF−saline. The effects of 5−min incubations of blood samples with the above−mentioned extract preparations on PA were examined. The blood samples were incubated with the solvent alone (DMSO + PF−saline) to determine the baseline PA. Platelet aggregation was induced using collagen (2 mg/L; Chrono−log). Methodological details of our PA studies were published previously (19•).

**Ex vivo feeding study**

This portion of the study used dogs as test subjects and conformed to the NIH guidelines and those of the University of Wisconsin Research Animal Resource Center for the care and use of laboratory animals.

**Extract feeding.** Seven male hound dogs (18.6–27.5 kg; Harlan Sprague Dawley, Madison, WI) were fed the following treatments for 7 d with each new treatment starting after a 7−d “washout period” between treatments: 2A: GSD (5 mg/kg), 2B: GSK (20 mg/kg), 2C: EB (5 mg/kg), 2D: GSD (5 mg/kg) + GSK (20 mg/kg), 2E: GSD (5 mg/kg) + GSK (20 mg/kg) + EB (2 mg/kg).

**Comparison of extracts treatments.** PA was measured before and after each week−long treatment. Additionally, PA was
measured 24 h after the final dose of Treatment E to determine any residual antiplatelet effects of the extract feeding. The dogs were fed a standard nonpurified diet (Global Diet 2025, Harlan Teklad, Indianapolis, IN) throughout the study. They were mildly sedated (intramuscularly) with 15 mg/kg ketamine sulfate and 0.2 mg/kg acepromazine maleate to prevent excitement and stress during the blood draw because stress may elevate blood catecholamine levels and thereby alter platelet activity (20). Fifteen minutes after sedation, blood (18 mL) was drawn into syringes containing sodium citrate (1 sodium citrate:9 blood) using 19G butterfly needles and was diluted with equal volumes of PF−saline. Platelet aggregation was induced and analyzed as described above except for the use of 1 mg/L collagen for induction.

**Analysis of polyphenolics**

**Fractionation of grape seed extract and grape skin extract phenolics.** A Sephadex LH−20 (Amersham Pharmacia Biotech, Piscataway, NJ) was equilibrated in water for 2 h. A Kontes glass preparative column (2.5 cm i.d. x 10 cm) (Kimbles/Kontes, Vineland, NJ) was filled with LH−20 slurry to a height of 10 cm. The GSD (100 mg/10 mL H2O) and GSK (500 mg/10 mL H2O) were applied to the columns and eluted sequentially with water (100 mL), 50% water−ethanol (v/v: 100 mL), ethanol (100 mL), 50% ethanol−methanol (v/v: 100 mL), methanol (100 mL), and 80% aqueous acetone (v/v: 300 mL). A subsample (10 mL) from each fraction was dried completely by vacuum evaporation at 30°C. Extracts were solubilized in water (0.5 mL) before HPLC and matrix−assisted laser desorption/ionization time−of−flight mass spectrometry (MALDI−TOF MS). The total phenolic contents of the GSD, GSK and all Sephadex LH−20 fractions were determined by the Folin−Ciocalteau method (21) and were reported as gallic acid equivalents. Phenolics were classified into groups by comparison of the chromatograms recorded at 280, 320, 350 and 535 nm, and by studying the UV−visible spectra of the peaks (22).

**Analysis of grape seed extract and grape skin extract by HPLC.** Each of the LH−20 fractions (50 μL) was injected onto a Ranin Dynamax C−18 column (60 Å, 8 μm, 25 cm x 0.45 cm: Varian, Walnut Creek, CA). The solvents for elution were 0.1% trifluoroacetic acid in water (solvent A) and methanol (solvent B). A linear gradient starting with 100% solvent A and finishing with 100% solvent B was run over a 40−min period. The HPLC system consisted of a Waters automated gradient controller (Waters, Milford, MA), two Waters 501 HPLC pumps (Waters), and a Rhodyne 7125 manual injector (Rhoynette, Rohnert Park, CA). The flow rate was maintained at 2 mL/min, and the elution was monitored by a Waters 996 diode array detector using Waters Millennium software (Waters) for collecting and analyzing three−dimensional chromatograms.

**MALDI−TOF MS.** Recent advances in mass spectrometry allow for the characterization of complex mixtures of proanthocyanidins (PR). MALDI−TOF MS is ideally suited for characterizing polydispersed oligomers (23). MALDI−TOF MS produces only a singularly charged ion for each parent molecule and allows detection of high mass with precision (24). We applied MALDI−TOF MS to characterize the structural diversity of PR (22, 24). Tentative structures were assigned to GSD and GSK polygalloyl polyflavan−3−ols (PGPF) by comparing MALDI−TOF MS mass distributions to a predictive equation (25). The equation is 290 + 288c + 152g + 23, where 290 represents the molecular weight of the terminal catechin/epicatechin unit, c is the degree of polymerization of the catechin/epicatechin units, g is the number of galloyl esters, and 23 is the molecular weight of sodium. The predictive equations describe the heteropolymetric nature of GSD and GSK PGP based on the assumption that the structural diversity seen in dimers and trimers (26) may be extrapolated to higher degrees of polymerization. Although MALDI−TOF MS has the power to distinguish molecular weight differences due to substitutions with gallic acid (152 amu) and the number of extending flavan−3−ol units (288 amu), it lacks the ability to assign specific stereochemistry to the molecule.

Mass spectra were collected on a Bruker Reflex II−MALDI−TOF mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with delayed extraction and a N2 laser (337 nm). In the positive mode, an accelerating voltage of 25.0 kV and a reflectron voltage of 26.5 kV were used. Spectra are the sum of 300 shots. Spectra were calibrated with bradykinin (1060.6 MW) and glucagon (3483.8 MW) as external standards.

In accordance with previously published results (25), trans−3−indoleacrylic acid (t−IAA: 5 mg/100 μL 80% aqueous acetone) was used as a matrix. Analytes were mixed with the matrix solution (1:2) and applied directly (0.2 μL) to a stainless steel target and dried at room temperature. Bradykinin, glucagon (Sigma Chemical, St. Louis, MO) and t−IAA (Aldrich Chemical, Milwaukee, WI) were used as received.

**Statistical analysis.** The investigators were unaware of the preparations and treatments, and the data were coded until the time of statistical analysis. Data are presented as means ± SEM. Inhibition of PA by the preparations (in vitro experiments) or treatments (ex vivo experiments) was assessed by comparing baseline PA to postpreparation/treatment PA using two−tailed paired Student’s t test (Microsoft Excel, Microsoft, Redmond, WA). Differences were considered significant at P ≤ 0.05. In the in vitro experiments, GSD + GSK was characterized as exhibiting enhanced inhibition of PA.
if significance ($P \leq 0.05$) was observed when comparing the percentage change in PA of the combined preparations to each corresponding individual preparation using two-tailed paired Student’s $t$ test. In the ex vivo experiments, combining GSD + GSK was characterized as exhibiting enhanced inhibition of PA if significance ($P \leq 0.05$) was observed when comparing the percentage change in PA of the combined treatment to each corresponding individual treatment using two-tailed, unpaired (equal variance) Student’s $t$ test. Adding EB was characterized as exhibiting further enhanced inhibition of PA if significance ($P < 0.05$) was observed when comparing the percentage change in PA of GSD + GSK + EB to GSD + GSK using two-tailed, unpaired (equal variance) Student’s $t$ test.

### RESULTS

**In vitro incubation study**

GSD (50 mg/L) and GSK (250, 500 mg/L) did not affect the antiplatelet effect when used individually (Fig. 1a). Moderate inhibition (12.7 ± 3.5%; $P \leq 0.01$) of PA occurred with 100 mg/mL of GSD. However, the combinations, GSD (50 mg/L) + GSK (250 mg/L) and GSD (100 mg/L) + GSK (500 mg/L), inhibited PA 40.5 ± 10.1% ($P \leq 0.005$) and 96.5 ± 3.1% ($P \leq 0.001$), respectively. The antiplatelet effects of the combined extract preparations, GSD (50 mg/L) + GSK (250 mg/L) and GSD (100 mg/L) + GSK (500 mg/L), were greater ($P < 0.01$ and $P < 0.001$, respectively) than the effects of the corresponding individual extract preparations. Incubation of DMSO alone had no effect on PA (data not shown).

![Collagen-induced platelet aggregation after incubation of human whole blood with grape seed (GSD) and grape skin (GSK) extract preparations 1A, 1B, 1C, 1D, 1E or 1F for 5 min. Platelet aggregation after incubation with each preparation is expressed as a percentage baseline platelet aggregation: Values are means ± SEM, $n = 8$. Asterisks indicate significant (***$P < 0.01$, **$P < 0.005$, and ***$P < 0.001$, respectively) change from baseline platelet aggregation after incubation with the extract preparation. The percentage change in PA induced by 1C differed ($P < 0.01$) from that induced by 1A or 1B, whereas the percentage change in PA induced by 1F differed ($P < 0.001$) from that induced by 1D or 1E.](image)

Ex vivo feeding study

Feeding dogs the extracts individually (Treatments 2A and 2B) did not affect PA, whereas feeding them in combination (Treatment 2D) decreased PA by 31.9 ± 7.1% (Fig. 2a; $P < 0.05$). Feeding the extracts in combination resulted in greater inhibition than feeding them individually ($P < 0.05$). When dogs were fed EB in addition to GSD and GSK (Treatment 2E), PA decreased by 56.2 ± 8.1% ($P < 0.005$): EB enhanced the antiplatelet effect of GSD + GSK ($P < 0.05$). Twenty-four hours later (after Treatment 2E), PA was still inhibited by 31.5 ± 10.5% ($P < 0.05$ vs. baseline) and the value did not differ from that after Treatment 2E, suggesting potential residual antiplatelet effects from the final dose. Feeding EB alone (5 mg/kg: Treatment 2C) did not affect PA.

![Collagen-induced ex vivo platelet aggregation in dogs after feeding treatments 2A, 2B, 2C, 2D or 2E containing grape seed (GSD) and grape skin (GSK) extract preparations and enzyme blend (EB) for 7 d. Platelet aggregation at the end of each treatment (post-treatment: d 7) is expressed as a percentage (mean ± SEM) of baseline (pretreatment: d 0) platelet aggregation. Values are means ± SEM, $n = 7$. Asterisks indicate significant (***$P < 0.01$, **$P < 0.005$, and ***$P < 0.001$, respectively) change from baseline platelet aggregation after feeding the extract treatment for 7 d. The percentage change in PA induced by 2D differed ($P < 0.05$) from that induced by 2A, 2B or 2C, whereas the percentage change in PA induced by 2E differed ($P < 0.05$) from that induced by 2D. Platelet aggregation did not change significantly 24 h (d 8) after the final dose of treatment 2E, i.e., there was no significant loss of antiplatelet effect in the 24-h period.](image)

**Analysis of polyphenolics**

**Composition of phenolic fractions as determined by HPLC.** The GSK contained 4 classes of phenolics: hydroxycinnamic acids (hydroxycinnamic acids and their esters to tartaric and quinic acid have characteristic maxima between 280 and 330 nm), flavonols (absorbance maxima between 350 and 372 nm), anthocyanins (absorbance maxima between 530 and 550 nm), and PGPF (oligomers of repeating catechin or epicatechin units, esterified to gallic acid and having a UV maxima at

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280 nm) (Table 1). The LH–20 water fraction contained 14 peaks that were characteristic of hydroxycinnamic acids. The LH–20 water:ethanol fraction contained 6 peaks characteristic of hydroxycinnamic acids, 12 peaks characteristic of anthocyanins, 2 peaks characteristic of flavonols and a series of peaks representing low–molecular–weight PGPF. The LH–20 ethanol fraction contained 12 PGPF peaks, 2 hydroxycinnamic acids, and 4 flavonols. The LH–20 ethanol:methanol, methanol and 80% aqueous acetone fractions all contained a series of PGPF that were poorly resolved by HPLC. These fractions were subsequently characterized by MALDI–TOF MS (Table 2).

**View this table:** Table 1 Classes of phenolic compounds (as determined by HPLC) in six fractions (1–6) isolated from grape seed extract (GSD) and grape skin extract (GSK) by sequential elution from Sephadex LH–20

The GSD contained only a single class of phenolics, a series of PGPF (Table 1). The LH–20 water, water:ethanol and ethanol fractions contained low–molecular–weight PGPF that resolved as distinct peaks. The LH–20 ethanol:methanol, methanol and 80% aqueous acetone fractions all contained a series of PGPF that were poorly resolved by HPLC. These fractions were subsequently characterized by MALDI–TOF MS (Table 2).

**Characterization of PGPF as determined by MALDI–TOF MS.** The GSK LH–20 ethanol:methanol fraction showed a series of PGPF extending from the trimer (m/z 889) through the hexamer (m/z 1754) (Table 2). The GSK–LH–20 methanol fraction showed a series of PGPF extending from the tetramer (m/z 1177) through the heptamer (m/z 2042) (Table 2). The GSK LH–20 80% aqueous acetone fraction showed a series of PGPF extending from the tetramer (m/z 1177) through the nanomer (m/z 2619) (Table 2).

The GSD LH–20 methanol fraction showed a series of PGPF extending from the trimer (m/z 889) through the nanomer (m/z 2619) (Table 2). The GSD LH–20 80% aqueous acetone fraction showed a series of PGPF extending from the tetramer (m/z 1177) through the nanomer (m/z 2619) (Table 2).

The GSK and GSD both contained a series of masses that correspond to the predicted PGPF. However, the GSD contained PGPF with higher degrees of galloylation for the hexamers, heptamers, octamers and nanomers, than did the GSK (Table 2). MALDI–TOF MS also provided evidence for a series of oligosaccharides in the GSK. On the basis of the repeating mass interval of 162 amu, we speculated that this class of compounds is comprised of oligohexoses with pentose and uronic acid terminal units.

**Quantification of total phenolics in the GSD, GSK and each Sephadex LH–20 fraction.** Total phenolics in the whole GSD extract were 2.64 times the amount in the whole GSK extract on a dry matter basis (Table 3). This difference in the total phenolic content may be caused by a dilution factor related to the weight contribution of the oligohexoses in the GSK. The LH–20 ethanol:water eluate of the GSK containing hydroxycinnamic acids, anthocyanins, flavonols and PGPF represented 28.18% of the total phenolic composition of the whole GSK (Table 3). The LH–20 acetone eluate of the GSD, containing PGPF, represented 47.16% of the total phenolic composition of the whole GSD (Table 3).

**View this table:** Table 3 Quantification of total phenolics in whole grape seed extract (GSD), whole grape skin extract (GSK) and the fractions collected by sequential elution from Sephadex LH–20

### DISCUSSION

The major finding of this study is that concentrations of GSD and GSK that have little or no effect on platelet activity when used individually elicit a greater antiplatelet effect when used in combination. This was observed in both in vitro incubation studies with human platelets and ex vivo feeding studies in dogs. In the feeding studies, the antiplatelet effect was further enhanced by the addition of EB. We also observed that the antiplatelet effect of the GSD–GSK–EB combination was maintained for at least 24 h after the final dose.
Several epidemiologic studies have found an inverse relationship between the total intake of flavonoids, a subclass of plant polyphenolics in fruits and vegetables, and the incidence of cardiovascular events (29). Of the numerous food sources of polyphenolics, red wine and other grape products, due to their relatively high content of flavonoids, have received noticeable attention (11). In our previous studies, we showed that grape products such as red wine and purple grape juice inhibit PA (9.30–33). This inhibition is attributed primarily to the polyphenolic compounds in these products (30).

Studies that have found beneficial effects from polyphenolic sources have often been focused on identifying a single polyphenolic compound to account for the observed effects. Several of these studies have attributed the beneficial effects to a single polyphenolic compound such as quercetin (34–36). However, the feasibility of the “single bioactive polyphenolic” hypothesis is weakened by findings that the minimum effective concentrations of single isolated polyphenolic compounds required in several in vitro studies (37, 38) were far greater than what may be realistically achieved by diet alone (39–41). This would suggest that the beneficial effects observed in the epidemiologic studies mentioned above might in fact be attributed to synergistic relationships among multiple polyphenolic compounds (16, 42).

GSK contains hydroxycinnamic acids, anthocyanins, flavonols, PGPF and a series of oligohexoses, whereas GSD contains only PGPF. The PGPF of both the GSD and GSK are comprised of the same class of repeating flavan-3-ol units esterified to gallic acid. However, GSK contains fewer galloylations at higher degrees of polymerization (hexamers through nanomers) than GSD. Of these major groups of polyphenolics in extracts detected in the chemical analysis of this study, flavonols, anthocyanins and PGPF have been previously shown to possess antiplatelet properties (38–43, 44). To date, hydroxycinnamic acids have not been studied for their effects on PA. However, they have been shown to possess antioxidant properties, which may indirectly exert an antiplatelet effect (45–48).

One possible explanation for the observed enhancement of the antiplatelet effect is the interaction of the specific PGPF that are unique to the GSD with the phenolic compounds found in the GSK. Hexamers, heptamers, octamers and nanomers containing multiple galloylations may elicit a greater antiplatelet effect when combined with one or more of the unique phenolic classes of GSK. A second possible explanation is an additive effect of the PGPF from the GSD and GSK. The total amount of PGPF and/or the proportion of total PGPF to one or more of the phenolic classes in the GSK may decrease platelet activity.

In addition to the above findings, we also observed that adding EB, consisting of plant–derived enzymes including bromelain, (17) fungal proteases (Aspergillus oryzae var.) and acid–stable proteases (Aspergillus niger var.), to the mixture of GSD and GSK increased the antiplatelet effect of the mixture. For many years, enzyme preparations consisting of bromelain have been claimed to improve GI bioavailability of some food substances and medications (17). However, this property of bromelain has not been proven in association with the absorption of polyphenolic compounds from dietary sources.

The issue of bioavailability of polyphenolic compounds is controversial. There is evidence that polyphenolic compounds, shown to have an antiplatelet effect in vitro, seem to have a lower inhibitory effect, if any, when given orally, suggesting that the compounds may not be sufficiently bioavailable (38). Polyphenolic compounds may be degraded, chemically modified or excreted by the GI tract. If successfully absorbed, the compounds may still be metabolized by the liver and removed from the circulation before they can have an observable biological effect (47). It was shown previously that bromelain (a component of the EB) is not entirely degraded during digestion and is absorbed in its functional form by the GI tract (48, 49). Furthermore, there is evidence that bromelain (17) and the fungal proteases (50) present in the EB may have beneficial effects on pathways of PA, the blood coagulation cascade and inflammation. Although the bromelain and the fungal proteases may not have been present in large enough amounts in the EB to have an inhibitory effect on PA when fed individually, it is possible that they might have enhanced the interaction between GSD and GSK. It is also possible that these enzymes may have merely enhanced the intestinal uptake of GSD and GSK (17).

In this study, the extract treatment composed of GSD, GSK and EB maintained the antiplatelet effect for at least 24 h after the final dosing. It is important for an antiplatelet treatment to maintain its effectiveness until the next treatment is administered. It is especially crucial that the effectiveness be maintained throughout the morning hours (0700–1100 h) when the risk of a thrombotic cardiovascular event is at its peak (51).

Grape products, such as red wine and purple grape juice, contain polyphenolics from both the seeds and skin of grapes and have been shown to have antiplatelet properties. However, the alcohol in the wine or the sugars in the purple grape juice may be undesirable for some segments of the population. An extract of grape seed and skin that provides health benefits similar to those of the grape beverages would be an alternative way of adding grape polyphenolics to the diet. Utilizing the potential interactions between GSD and GSK in a grape extract supplement could offer the health benefits of grape polyphenolics at a lower daily dose than what may be required if the extracts are used individually. Furthermore, the

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addition of enzyme preparations may further enhance the efficacy of the supplement.

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FOOTNOTES


2  Supported by Melaleuca Incorporated, Idaho Falls, ID.

4  Abbreviations used: CAD, coronary artery disease; DMSO, dimethyl sulfoxide; EB, enzyme blend; GI, gastrointestinal; GSD, grape seed extract; GSK, grape skin extract; ox–LDL, oxidized LDL; MALDI–TOF MS, matrix–assisted laser desorption/ionization time–of–flight mass spectrometry; PA, platelet aggregation; PF–saline, preservative–free saline; PGPF, polygalloyl polyflavan–3–ol; PR, proanthocyanidin; t–IAA, trans–3–indoleacrylic acid.


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