Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2025**; 30(1): 25859
https://doi.org/10.31083/FBL25859

Review

Winery By-Products *In Vitro* and *In Vivo* Effects on Atherothrombotic Markers: Focus on Platelet-Activating Factor

Maria Choleva[1], Smaragdi Antonopoulou[1], Elizabeth Fragopoulou[1,*]

[1]Department of Nutrition and Dietetics, School of Health Sciences and Education, Harokopio University, 17676 Athens, Greece
*Correspondence: efragop@hua.gr (Elizabeth Fragopoulou)

Academic Editor: Raffaele Serra
Submitted: 30 May 2024 Revised: 21 August 2024 Accepted: 28 August 2024 Published: 21 January 2025

Abstract

Platelet aggregation and inflammation play a crucial role in atherothrombosis. Wine contains micro-constituents of proper quality and quantity that exert cardioprotective actions, partly through inhibiting platelet-activating factor (PAF), a potent inflammatory and thrombotic lipid mediator. However, wine cannot be consumed extensively due to the presence of ethanol. Alternatively, winery by-products are abundant in similar-to-wine micro-constituents that could be used in food fortification and dietary supplements. Also, the vinification process produces millions of tons of by-products worldwide, posing an environmental matter of waste management. Therefore, the purpose of this literature review is to update the existing data concerning the *in vitro* anti-platelet and anti-inflammatory properties of winery by-product extracts and their possible health effects through controlled clinical trials in humans, specifically focused on their effects on PAF's actions. Data from *in vitro* studies report that winery by-product compounds are able to inhibit platelet aggregation against several aggregation factors, as well as to downregulate inflammatory markers. Among their actions, extracts or phenolic compounds present in winery by-products inhibit PAF's actions, a potent inflammatory and thrombotic mediator. Similar conclusions have been drawn from human supplementation studies, which suggest that winery by-product extracts may have beneficial biological effects on the cardiovascular system. Evidence from long-term studies shows that consumption may lower total and low density lipoprotein (LDL) cholesterol, improve insulin sensitivity, decrease lipid and protein oxidative damage, enhance antioxidant capacity, and have mild anti-inflammatory action toward reducing cytokine expression and levels. Data from the limited postprandial studies report that the acute consumption of winery by-product extracts improves glycemic response and reduces platelet reactivity to aggregatory stimuli. Although wine extracts and phenolic compounds have been reported to inhibit PAF's actions and reduce the activity of its biosynthetic enzymes, no data exist concerning the influence of winery by-product extracts. In the future, additional long-term randomized controlled trials or postprandial studies are needed to draw definitive conclusions and establish a viable cardioprotective strategy that incorporates the sustainable use of winery by-products.

Keywords: PAF; winery by-products; platelet aggregation; inflammation; oxidative stress; cardioprotective effects

1. Introduction

1.1 The Involvement of PAF in Atherothrombosis

Atherosclerosis is a chronic arterial disease with clinical manifestations that include coronary heart disease, stroke and peripheral artery disease. Lipid storage in the arterial wall is gradually developed in atheromatous plaques, the rapture of which causes local thrombosis, leading to partial or total vascular blockage [1].

Oxidative, inflammatory and thrombotic mechanisms are crucial for the initiation and the prolongation of atherosclerosis. The oxidative modification of low-density lipoprotein cholesterol (LDL) is an important step in the initiation of the plaque formation. In the first stages, the peroxidation of polyunsaturated fatty acids (PUFAs) in LDL's surface occurs and proceeds to the peroxidation of core lipids, resulting in a various range of biologically active molecules capable of activating endothelial cells, such as oxidized sterols, oxidized fatty acids and oxidized phospholipids [2,3]. Platelet-activating factor (PAF) is a crucial inflammatory and thrombotic mediator, which is thought to play a key role in the initiation and prolongation of atherosclerosis [4] and is a main contributor to the pathogenesis of cardiovascular diseases [5]. Structurally, PAF is a phospholipid and particularly a 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phospho-choline [6]. Several types of cells could synthesize PAF, including platelets and leukocytes upon activation. PAF's levels in organism are balanced through its biosynthetic and catabolic pathways. PAF can be synthesized by two different enzymatic routes, namely the remodeling and the *de novo* pathway. The remodeling pathway is believed to produce PAF under inflammatory conditions and involves a structural modification of ether-linked membrane phosphatidylcholines. More specifically, the action of cytoplasmic phospholipase A_2 yields lyso-PAF which is then acetylated by acetyl-CoA: lyso-PAF acetyltransferases (lyso-PAF AT) leading to the formation of PAF. The *de novo* pathway appears to be responsible for the constitutive production of PAF, which maintains its physiological levels in various tissues and blood. A key step in this pathway is the conversion of 1-*O*-alkyl-2-acetyl-glycerol to PAF by a specific cytidine diphosphate

(CDP)-choline: cholinephosphotransferase (PAF-CPT). As far as PAF's catabolism is concerned, the most important enzyme involved is a PAF-specific acetylhydrolase (PAF-AH), which cleaves the acetyl-group and forms lyso-PAF. The plasma isoform of PAF-AH is known as lipoprotein-associated phospholipase A_2 (Lp-PLA_2) due to its binding to lipoproteins. PAF is also produced non-enzymatically, during the oxidation of LDL, which results in the inactivation of the associated Lp-PLA_2.

Many experimental data revealed that the atherogenic actions of oxidized LDLs (ox-LDL) could be attributed to PAF and PAF-like lipids (oxidized phospholipids), which render these molecules as the initiators of atherosclerosis [4,7]. In early stages of atherogenesis, oxidized phospholipids participate in the activation of the immune system, while in late stages, they contribute to platelet aggregation and plaque disruption [8]. Activated endothelial cells express several types of adhesion molecules that are recognized by monocytes, which are recruited and infiltrated into the subendothelial area. PAF triggers inflammatory response in endothelial cells and at the same time activates blood cells through its specific receptor (PAFR) which is a member of the G-protein-coupled seven-transmembrane receptor superfamily. The interaction of PAF with its receptor leads to increased endothelium permeability which is a crucial event in the initiation of atherosclerosis [9]. Activated endothelial cells rapidly produce and display PAF as well as P-selectin on their cell surface, which act as juxtacrine signals for activation and adhesion of neutrophils and monocytes. Cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), promote the synthesis of E-selectin and PAF [4]. This is a two way interaction since PAF promotes the production of cytokines [10,11], such as IL-6 and IL-8, as well as chemokines such as monocyte chemoattractant protein-1 (MCP-1). Monocytes differentiate into macrophages that uptake ox-LDL, leading to their differentiation into foam cells [12]. Data support that ox-LDL interacts with PAFR in macrophages to increase CD36 expression and ox-LDL uptake, leading to enhanced foam cells formation [13]. Platelets are also important cells in atherothrombosis. Several agonists interact with platelet membrane receptors and facilitate aggregation, such as thrombin, thromboxanes, arachidonic acid (AA), PAF, adenosine diphosphate (ADP), serotonin, fibrinogen, collagen, von Willebrand factor (vWF). Platelet aggregation is mediated through integrin α2bβ3, that has the ability to bind extracellular agonists such as fibrinogen and vWF and is the combined result of reduced cyclic AMP (cAMP) levels and increased intracellular Ca^{2+} levels [14]. PAF's and PAF-like molecules' interaction with its membrane receptor (PAFR) results in AA and metabolites release, thromboxane A_2 (TXA_2) biosynthesis, intracellular Ca^{2+} levelsincrease, substrate phosphorylation, degranulation and platelet aggregation. PAF is synthesized by activated platelets and remains cell associated leading to a tethering and juxtacrine signaling system at the platelet surface which mediates rolling and tight adhesion of neutrophils, through interaction of Mac-1 (CD11b/CD18) with fibrinogen bound to integrin α2bβ3 [15]. As the atheromatous plaque evolves, a fibrous membrane is being developed, in which, dead cells are accumulated. Previous data suggest that local PAF production within the atherosclerotic plaques may contribute to intra-plaque neoangiogenesis [16,17].

Other pathophysiological conditions such as insulin resistance (in the presence or absence of hyperglycemia) promote the risk for cardiovascular disease. Atherosclerosis and plaque progression can be facilitated in an insulin resistant state along with the presence of hyperglycemia, dyslipidemia, hypertension and pro-inflammatory conditions. Impaired insulin signaling may also take place in endothelial cells, macrophages and vascular smooth muscle cells, as these types of cells express the insulin receptor [18]. In diabetic patients, PAFR is up-regulated and hyperglycemia is associated with the downregulation of Sirtuin 1 (SIRT1) that can be abolished by the PAFR antagonist CV3988 [19]. PAF has also been reported to contribute to the progression of non-alcoholic fatty liver disease (NAFLD) through its ability to activate several signaling pathways (5-hydroxytryptamine, inositol triphosphate, AA, diacylglycerol) that eventually promote insulin resistance, inhibition of lipid β-oxidation, accumulation of triglycerides (TG), oxidative stress and inflammation in the hepatocytes as well as its ability to induce platelet activation that is, also, involved in NAFLD progression [20].

1.2 Biological Actions of Wine's Micro-Constituents

The beneficial properties of wine consumption were firstly established in 1979 when an inverse association between red wine consumption and cardiovascular disease mortality was reported [21]. The term "French Paradox" was initially established in 1992, leading to the escalation of scientific research concerning wine consumption. The term describes the epidemiological observation that the French had relatively low incidence of coronary heart disease despite their rich-in saturated fatty acids diet and despite the presence of similar risk factors with other populations [22]. The J-shaped association between moderate wine consumption and stroke incidence as well as cardiovascular mortality has been stated in a meta-analysis. In contrast, the above study found a weaker association concerning beer consumption and cardiovascular disease and no association for other alcoholic beverages [23]. More recently, a meta-analysis has confirmed the protective effects of wine consumption regarding all-cause mortality as opposed to alcohol consumption from non-wine sources [24].

According to scientific literature, the beneficial effects of wine consumption are mainly attributed to its bioactive compounds. In this area, our previous studies revealed that wine contains potent micro-constituents that exert anti-platelet effects and among other actions, reduce PAF-induced platelet aggregation and PAF biosynthetic en-

zymes activities in leukocytes both *in vitro* and *in vivo* [25–28]. Nevertheless, the fact that wine contains ethanol should be taken into consideration since excess ethanol consumption is detrimental for health through various mechanisms. A recent meta-analysis reported that all-cause mortality is highly associated with ethanol consumption and concludes that concerning alcohol consumption, *"the risk of all-cause mortality, and of cancers specifically, rises with increasing levels of consumption, and the level of consumption that minimizes health loss is zero"* [29]. However, the same group 4 years later, based on new statistical models modulated their statement to *"there is strong evidence to support recommendations on alcohol consumption varying by age and location"* [30]. Also, it should be mentioned that they did not take under consideration the type of alcohol consumed or the frequency of drinking during the week. There are data supporting that regular moderate wine consumption is not associated with negative effects and in some cases, especially cardiovascular diseases, it is associated with positive outcomes [31,32].

Considering the above data and the existence of phenolic compounds in wine, several researchers have focused on the health effects of individual phenolic compounds, such as resveratrol, in the form of dietary supplements [33,34]. However, wine is a complex mixture of compounds and its health benefits may be attributed to the coexistence of other micro-constituents apart from phenolic compounds. Due to existing evidence, it can be hypothesized that wine contains a micro-constituent's mixture in proper quality and quantity that provide cardiovascular protection through synergistic action. This hypothesis is supported by the fact that individual phenolic compounds do not or exert weaker biological actions compared to their mixtures or wine extracts [35].

On the other hand, it should be noted that the isolation process of these beneficial wine micro-constituents is non-profitable from an economic aspect. Alternatively, winery by-products are cheap and rich-in similar-to wine constituents sources. Especially, during the vinification process of red grapes, the grape skins and seeds are present in the must, which is fermented for a significant amount of time. Many beneficial micro-constituents are being transferred to wine and yet, the by-products are still an abundant-in bioactive compounds source. The vinification process produces millions of tons of by-products which also poses an environmental matter of waste management. In this direction, their sustainable use is highly considered [36].

1.3 Composition of Winery By-Products

Winery by-products consist of grape pomace (GP) (seeds and skins), stems, leaves and lees. Several environmental factors (such as grape variety, cultivation) as well as grape processing procedures can affect the chemical composition of winery by-products. Grapes are subjected to various invasive processing stages, yet, their chemical composition concerning bioactive compounds is not significantly altered [37].

Phenolic acids contain benzoic and cinnamic acid derivatives, with hydroxycinnamic acids being the largest subcategory in winery by-products. These molecules are usually bound to glycosylated derivatives, shikimic, quinic or tartrate acid esters. Gallic acid is the most frequent hydroxybenzoic acid derivative in stems, skin and seeds, syringic acid in stems and procatechuic acid in grape pomace. Hydroxycinnamic acids are present in grapes and especially in skins. Mainly, caftaric and coutaric acid are found in white grape skins, whereas chlorogenic acid is found in red grape skins [38]. The relative amount of phenolic acids is also dependent on the extraction solvent. In our previous study, the aqueous ethanolic GP extract contained mainly vanillic acid, gallic acid, syringic acid, salicylic acid and p-coumaric acid [39].

Flavonoids refer to a group of compounds composed by low molecular weight molecules, with a structure of 15 carbon atoms. Winery by-products' flavonoids are integrated by flavonols, flavanols, anthocyanins and proanthocyanidins [40]. Flavonols are characterized by the existence of a double bond between C2 and C3 carbons and a hydroxyl group at C3 carbon. Different sugars can be connected to flavonols resulting in glucosides, galactosides and diglucosides. In red variety stems, quercetin is abundant in the form of quercetin-3-glucoronide, -3-glucoside, -3-galactoside and -3-ramnoside. Red and white varieties express a similar compounds profile and the red varieties have higher concentrations of these molecules [38]. Flavanols have a hydroxyl group in the C3 carbon and no carbonyl group in the C4 carbon and they are the main substances responsible for the astringency of wine. Catechin is the main molecule in both red and white varieties and its concentration appears to be 4-fold higher than epicatechin [40]. Anthocyanins are responsible for the color of red grapes and wine and they possess an aromatic ring (A) bound to a heterocyclic ring (C) that contains oxygen, which is bound to a third aromatic ring (B). The aromatic ring B is bound to sugars or organic acids and is responsible for the different colors of anthocyanins. Malvidin-3-glucoside and peonidin-3-glucoside are the most abundant anthocyanins found in grape skins [37,38]. Condensed tannins or proanthocyanidins consist of flavanol monomer subunits and their structures vary depending on the subunits compounds, the polymerization degree and the coupling sites. Regarding red varieties the most frequent proanthocyanidin is procyanidin B3, whereas in white varieties is procyanidin B1. Procyanidins are mostly present in white varieties compared to the red ones and especially in stems compared to skin and seeds [38]. In accordance with the aforementioned, we have shown that an aqueous ethanolic GP extract contained mainly catechin, epicatechin, quercetin and malvidin-3-glucoside [39].

Stilbenes are phenolic compounds with two aromatic rings attached to an ethylene bridge. They are mostly found

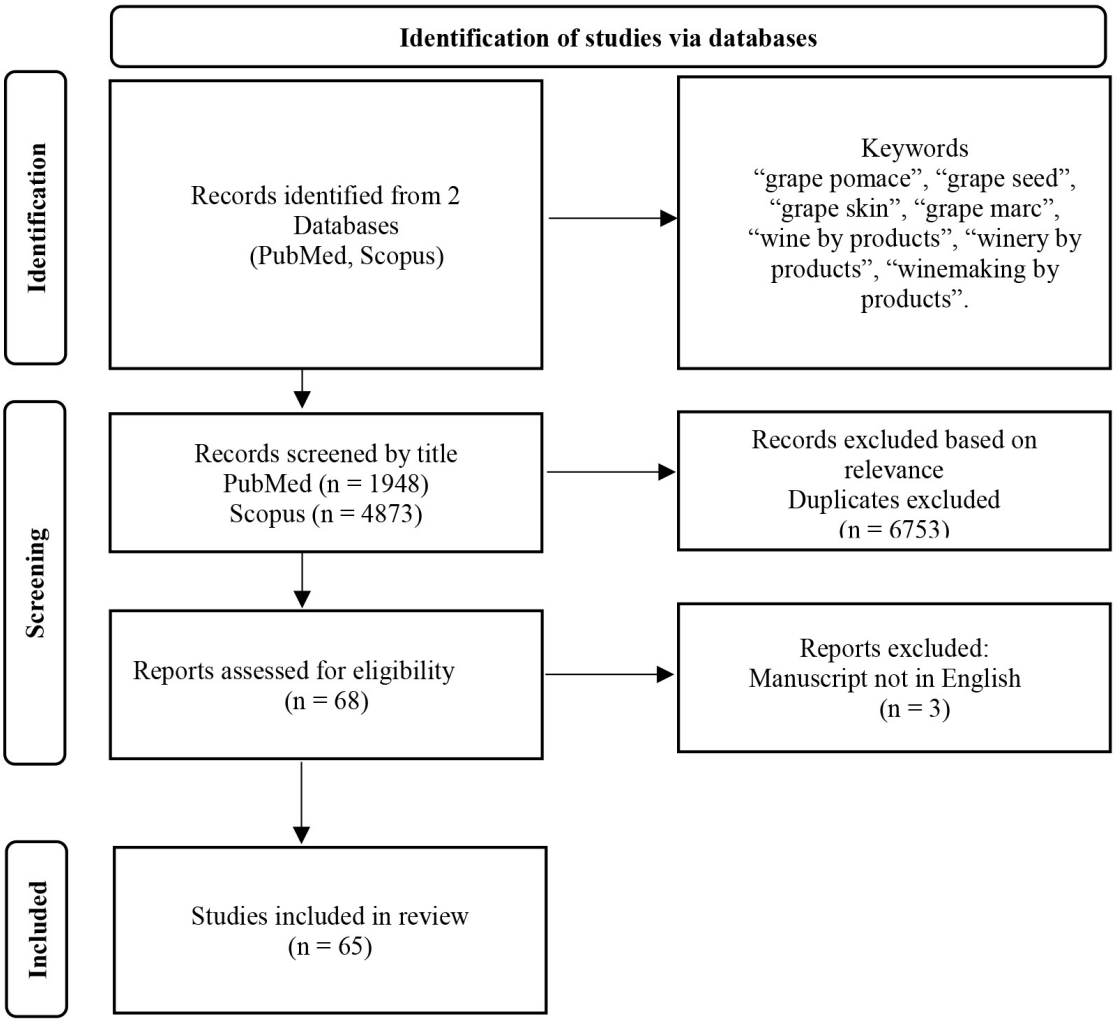

Fig. 1. Flowchart of the literature search.

in grape skins and also in stems and seeds. Grapes produce stilbenes for protection from ultraviolet (UV) radiation [38]. Resveratrol is the most common stilbene and in our previous study we confirmed its existence in an aqueous ethanolic GP extract from red varieties. Tyrosol and hydroxytyrosol that belong to the simple phenolic compounds category were also detected in the above aqueous ethanolic extract [39].

The aim of this review is to update the existing data concerning the *in vitro* anti-platelet and anti-inflammatory properties of winery by-product extracts as well as their possible health effects through controlled clinical trials. Especially, focus is given on the potent inflammatory and thrombotic lipid mediator PAF.

2. Methods

The PubMed and Scopus databases were reviewed from 2000 up to December 2023, using the following keywords: "grape pomace", "grape seed", "grape skin", "grape marc", "wine by products", "winery by products", "winemaking by products". The search yielded 1948 publications from the PubMed database and 4873 items from the Scopus database. The titles of the studies were screened and the appropriate ones were reviewed and included in the manuscript. Additional searches were also carried out using keywords suitable for each individual table of studies. The flowchart of the literature search is presented in Fig. 1.

For the *in vitro* anti-platelet actions, the search focused on human platelet aggregation studies using winery by-product extracts. For the *in vitro* anti-inflammatory actions, studies where human cells (mostly cell lines) were incubated with winery by-product extracts and inflammatory proteins or signal transduction molecules were evaluated, were included. Cell proliferation, cytotoxicity and cell migration assays were out of the scope of this review. Also, controlled clinical studies that administered winery by-product extracts in the form of dietary supplements in human subjects were included in this review. Outcomes including effects in weight management, satiety and hematological markers were not included.

Table 1. Studies investigating the *in vitro* inhibition of platelet aggregation induced by winery by-product extracts.

Type of extract	Platelet sample	Aggregation factors	Experimental procedures	Results	Ref
GS GSK	WB	Collagen	WP pre-incubation (5 min) in the absence of extracts or with: - GS (50–100 µg/mL) - GSK (250–500 µg/mL) - GS (50 µg/mL) + GSK (250 µg/mL) - GS (100 µg/mL) + GSK (500 µg/mL)	12.7% inhibition (100 µg/mL GS) 40.5% inhibition (50 µg/mL GS + 250 µg/mL GSK) 96.5% inhibition (100 µg/mL GS + 500 µg/mL GSK)	[49]
GS GSK	WP	TRAP	WP pre-incubation (30 min) in the absence of extracts or with: - GS (50–100 µg/mL) - GSK (250–500 µg/mL) - GS (50 µg/mL) + GSK (250 µg/mL) - GS (100 µg/mL) + GSK (500 µg/mL)	36% inhibition (100 µg/mL GS) 50% inhibition (250 µg/mL GSK) 64% inhibition (500 µg/mL GSK) 60% inhibition (50 µg/mL GS + 250 µg/mL GSK) 74% inhibition (500 µg/mL GS + 100 µg/mL GSK)	[48]
GS	WP	Thrombin	WP pre-incubation (5 min) in the presence or absence of extracts (5, 7.5, 10, 25 and 50 µg/mL)	IC_{50} = 24 µg/mL	[44]
GS	WP	Thrombin TRAP	WP pre-incubation (1 min) in the presence or absence of extracts (0.5, 5, and 50 mg/mL)	IC_{50} TRAP = 50 µg/mL IC_{50} thrombin = 25 µg/mL	[45]
GS	WP	Thrombin	WP pre-incubation (5 min) in the absence of extracts or with: - GS (2.5–10 µg/mL) - GS (2.5–10 µg/mL) + reduced D, L-homocysteine (100 µM) - GS (2.5–10 µg/mL) +D, L-homocysteine thiolactone (1 µM)	GS 10 µg/mL: 43.6% inhibition (reduced homocysteine assay) 42.1% inhibition (thiolactone assay)	[47]
GS	WP	Thrombin	WP pre-incubation (10 min) in the presence or absence of extracts (0.5, 5, and 50 mg/mL)	IC_{50} = 5 µg/mL	[46]
GS	WP	ADP	HUVECs pre-incubation (24 h) in the presence or absence of extract (1, 2.5, 5, 10 mg/mL) WP pre-incubation with HUVECs (10 min)	GS 1 µg/mL: 15% inhibition in the expression of the activated form of GPIIb/IIIa compared to platelets incubated with untreated HUVECs	[41]
GS	WP	Collagen TRAP	WP pre-incubation (5 min) in the presence or absence of extracts (1, 5, 10, 50 µg/mL)	IC_{50} Collagen = 10 µg/mL IC_{50} TRAP = 10 µg/mL	[51]
GS	WB	ADP	WB pre-incubation (15 min) in the presence or absence of extract (7.5 or 15 µg GAE/mL)	7.5 µg GAE/mL: 19.4% AUC inhibition 15 µg GAE/mL: 38.9% AUC inhibition	[42]

Table 1. Continued.

Type of extract	Platelet sample	Aggregation factors	Experimental procedures	Results	Ref
GP	PRP	PAF ADP TRAP	PRP pre-incubation (5 min) in the presence or absence of extracts (aqueous-ethanolic GP extracts, lipoid GP extracts)	Aqueous-Ethanolic extracts: IC_{50} PAF = 162.1 $\pm$ 66.9 µg extract IC_{50} ADP = 181.2 $\pm$ 82.3 µg extract IC_{50} TRAP = 156.3 $\pm$ 97.5 µg extract Lipoid extracts (Bligh-Dyer method): IC_{50} PAF = 280.9 $\pm$ 115.9 µg extract IC_{50} ADP = 293.2 $\pm$ 102.7 µg extract IC_{50} TRAP = 284.8 $\pm$ 131.8 µg extract	[39]
GP	PRP	ADP TRAP	PRP pre-incubation (3 min) in the presence or absence of extracts (1 mg/mL)	Petit Verdot GP: 67.1% inhibition of ADP-induced and 53.2% inhibition of TRAP-induced platelet aggregation	[43]
GSK	PRP	PAF	PRP pre-incubation in the presence or absence of extracts	Muscat GSK IC_{50} = 19.7 µM GAE Augoustiatis GSK IC_{50} = 26.6 µM GAE	[50]

ADP, adenosine diphosphate; GAE, gallic acid equivalents; GS, grape seed; GSK, grape skin; HUVEC, human umbilical vein endothelial cell; IC_{50}, half maximal inhibitory concentration; PAF, platelet activating factor; PRP, platelet rich plasma; TRAP, thrombin receptor activating peptide; WB, whole blood; WP, washed platelets; GP, grape pomace; AUC, area under the curve.

3. In Vitro Anti-Platelet Actions of Winery By-Products

The studies examining the *in vitro* anti-platelet effects of winery by-products (Table 1, Ref. [39,41–51]) have used human washed platelets, whole blood or platelet-rich plasma (PRP) against several aggregation factors. The winery by-product extracts examined, were mostly dietary supplements from grape skins (GSK) or/and seeds (GS). In this context in our previous study GP extracts from four red grape varieties (Sour Black, Syrah, Cabernet Franc, Cabernet Sauvignon) were prepared using different solvents and were tested for their ability to inhibit platelet aggregation (in PRP) against several agonists. The results indicated that the extracts' anti-platelet effect was not grape-variety dependent, whereas the solvent used for the extraction that determines the extracted micro-constituents was the crucial factor. In specific, the aqueous ethanolic GP extracts exhibited more potent anti-platelet action compared to the lipoid extracts using the Bligh-Dyer method, while the aqueous and the hexanoic extracts exhibited no anti-platelet effect [39]. In our early studies [25,26], potent anti-platelet actions against PAF were reported in the wine lipid extracts using the Bligh and Dyer method. However, this method utilizes chloroform and methanol, which are not suitable solvents for products consumed by humans. Therefore, the discovery of potent inhibitors of platelet aggregation in aqueous ethanolic extracts is promising for the development of dietary supplements and food enrichment.

ADP as an aggregation factor was used in four studies. Luzak *et al*. [41] reported that 1 µg/mL of extract exhibited weak anti-platelet action as it failed to inhibit platelet aggregation above 15%. Bijak *et al*. [42] examined an extract at 15 µg gallic acid equivalents (GAE)/mL which reduced the area under the curve (AUC) by 38.9%. Out of the nine grape varieties examined by another group, only the Petit Verdot GP extract significantly inhibited platelet aggregation against ADP by 67.1% [43]. In our previous study, the aqueous ethanolic GP extracts exhibited more potent action against ADP compared to the lipoid extracts (Bligh-Dyer method), with half maximal inhibitory concentration (IC_{50}) values being 181.2 $\pm$ 82.3 µg and 293.2 $\pm$ 102.7 µg extract respectively [39].

Thrombin was used in four studies and the GS extracts' IC_{50} values ranged between 5–25 µg/mL [44–47]. Malinowska *et al*. [47] examined a hyperomocysteine state in healthy subjects' washed platelets and reported inhibition of platelet aggregation by the extracts' compounds. Thrombin receptor activating peptide (TRAP) was also used as an aggregatory factor with IC_{50} values ranging between 10–1000 µg of GS/ GP extract/mL. Also, the aqueous ethanolic GP extracts inhibited platelet aggregation more effectively against TRAP compared to the lipoid extracts (Bligh-Dyer method) as the IC_{50} values revealed: 156.3 $\pm$ 97.5 µg and 284.8 $\pm$ 131.8 µg of extract respectively [39]. Another study that examined GS and GSK extracts concluded that each extract could inhibit platelet aggregation against TRAP separately as well as that their combination led to additive inhibition of platelet aggregation, enhancement of NO release, and prevention of superoxide production [48].

The synergistic action between the GS and GSK constituents is also observed in the study of Shanmuganayagam *et al*. [49] where collagen, as agonist, was used. Specifically, the combination of the two extracts, at concentrations where each one separately did not cause inhibition of platelet aggregation, led to a 40.5% inhibition of platelet aggregation [49].

PAF, apart from its pro-inflammatory actions, is considered a potent platelet agonist. High PAF levels, acting through its specific plasma membrane receptor (PAFR), enhance both inflammation and thrombosis. Indeed, the *ex vivo* platelets response to PAF or the *in vitro* inhibition of PAF-induced platelet aggregation, aside from estimation of the anti-platelet ability, is considered as an indirect method to estimate PAF's actions in organism and bioactive compounds' anti-inflammatory action [4]. We have shown that GP extracts inhibited PAF-induced platelet aggregation, with the aqueous ethanolic extracts being more potent inhibitors compared to the lipoid ones (Bligh-Dyer method) [39]. In another research study, GSK extracts from different grape varieties also inhibited PAF-induced platelet aggregation with IC_{50} values ranging between 19.7–26.6 µM GAE [50].

The fact that the winery by-product extracts are able to inhibit different aggregation factors (PAF, ADP, thrombin/TRAP, collagen), reveals that their anti-platelet action is achieved through different signaling pathways and is probably attributed to the synergistic action of all the extracts' components [49]. The anti-aggregatory actions could be achieved through various mechanisms such as inhibition of agonist binding to its receptor, inhibition of signal transduction enzymes namely phospholipase C (PLC), protein kinase C (PKC) and cyclooxygenase (COX), inhibition of phospholipid oxidation, or through free radicals scavenging. Catechin, the most abundant flavanol detected in GP, has been shown to modulate signal transduction mediated by the PAR (thrombin/TRAP receptor), GPVI (collagen receptor), and P2Y12 (ADP receptor) receptors, integrin α2bβ3 and PLC [52]. Epicatechin is reported to be able to reduce the expression of integrin α2bβ3 induced by ADP and epinephrine [53]. Quercetin has been reported to inhibit cyclooxygenase (COX), thereby reducing TXA_2 formation as well as attenuating signal transduction through the TP receptor (TXA_2 receptor) [52]. It has also been shown that quercetin inhibits signal transduction from other receptors such as P2Y12 [52,54], PAR, GPVI, integrin α2bβ3 as well as pathways mediated by PLC and protein kinase B (PKB)/Akt [52]. Tyrosol and resveratrol have been shown to inhibit PAF-induced platelet aggregation [55]. Resveratrol's anti-platelet actions have also been reported against the PAR [52], P2Y12 and GPVI receptors, PLC action [52,56] as well as the COX- and TXA_2-mediated pathway

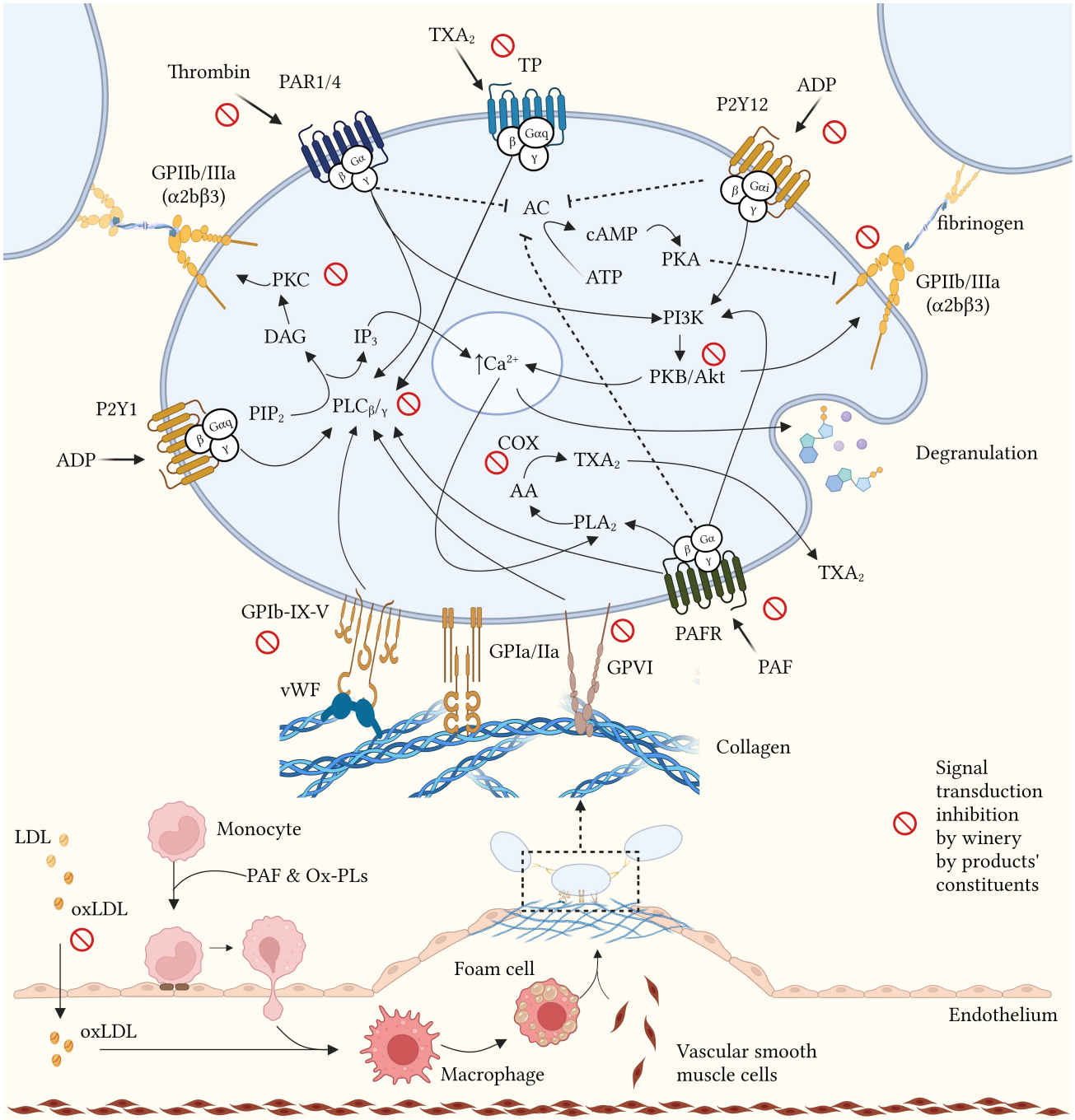

Fig. 2. Potential mechanisms of action of winery by-products in atherothrombosis. Modulation of platelet function mediated by winery by-product constituents through inhibition of aggregation factors binding to their membrane receptors, inhibition of signal transduction enzymes and free radicals scavenging. AA, arachidonic acid; AC, adenylyl cyclase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca, calcium; cAMP, cyclic AMP; COX, cyclooxygenase; DAG, diacylglycerol; GP, glycoprotein; IP3, inositol trisphosphate; LDL, low density lipoprotein; oxLDL, oxidized LDL; Ox-PLs, oxidized phospholipids; PAF, platelet activating factor; PAFR, PAF receptor; PAR, protease-activated receptor; PI3K, phosphoinositide-3 kinase; PIP2, phosphatidylinositol biphosphate; PK, protein kinase; PL, phospholipase; P2Y, platelet receptor for ADP; TP, thromboxane receptor; TXA_2, thromboxane A_2; vWF, von Willebrand factor. Created with BioRender.com (License: OM23Z01REU).

[52]. Taking into consideration the data from the studies that examined winery by-products and individual compounds, the potential mechanisms of anti-platelet action are presented as a whole and summarized in Fig. 2.

4. In Vitro Anti-Inflammatory Actions of Winery By-Products

In studies examining the anti-inflammatory actions of winery by-products in cell lines, the winery by-products

constituents were mostly received by dietary supplements produced by GS, GSK or GP (Table 2, Ref. [57–69]). In the majority of these studies, cells were stimulated by a pro-inflammatory mediator. Lipopolysaccharide (LPS) was mostly used [57–62], as well as ox-LDL [59], hydrogen peroxide (H_2O_2) [63], tumor necrosis factor-α (TNF-α) [61,62,64], *Campylobacter jejuni* [65], advanced glycation end products (AGEs) [66] and high glucose concentration [67].

The cells used in these studies were frequently macrophages, endothelial cells or epithelial cells and several biomarkers of inflammation were measured. In 6 studies, cells incubated with winery by-product extracts were able to modulate the function of transcription factors, mainly nuclear factor kappa B (NF-κB) [61,63,64,67–69], in mRNA and protein levels. The primary mechanism of NF-κB activation is the inducible degradation of IκBα triggered through its site-specific phosphorylation by a multisubunit IκB kinase (IKK) complex. Winery by-product extracts were capable of inhibiting the phosphorylation and the nuclear NF-κB translocation, resulting in reduced pro-inflammatory genes expression and therefore reduced inflammation. Indeed, several cytokines, chemokines and adhesion molecules have been found to be reduced in mRNA expression and protein levels by winery by-product extracts (Table 2). Also, several enzymes involved in the inflammatory process such as COX have been found to be downregulated in mRNA level [60,68,69].

It is worthy to mention that although wine extracts have been reported to inhibit PAF's action in platelets and also to reduce the activity of its biosynthetic enzymes [35], no published data exist concerning the influence of winery by-product extracts. However, apigenin and keampferol, quercetin, naringin hesperidin as well as epicatechin-3-O-gallate and epigallocatechin-3-O-gallate were found to be inhibitors of the lyso-PAF AT and the PAF biosynthesis [70].

5. In Vivo Biological Actions of Winery By-Products Extracts in Humans

Several studies examined the health effects of winery by-products, mostly as dietary supplements, in humans (Table 3, Ref. [71–110]). Concerning the effects of winery by-products on lipid biomarkers, in most studies total cholesterol was reduced in the group receiving treatment compared to control group [71–77] as well as LDL cholesterol [72–74,77]. Winery by-products consumption had mild effect on triglycerides (TG) levels as they were reduced in two studies from which, in one, hyperlipidemic subjects participated in an 8-week intervention [72] and in the second study TG reduced after the consumption of a high-fat lunch meal compared to the placebo treatment [78].

Research has also focused on the effect on glycemic biomarkers, such as insulin levels or sensitivity and glucose levels, although few significant changes regarding the treatment group compared to control were reported. The intake of 600 mg GS extract for 8 weeks managed to improve insulin sensitivity and reduce fasting insulin levels in 40 volleyball athletes [79]. The daily consumption of 20 g bread or cookies made from flour enriched with GP for 16 weeks reduced postprandial insulin levels during an oral glucose tolerance test (OGTT) in subjects with at least one metabolic syndrome (MS) criteria [80]. The acute consumption of a GP extract under postprandial conditions also reduced the insulin and improved insulin sensitivity in 12 healthy men [81]. The intake of GS extract significantly reduced the postprandial glucose 15 and 30 minutes after the meal consumption as well as the glucose response AUC in healthy volunteers [82]. No effect was reported when hemoglobin A1c (HbA1c) levels were measured, possibly due to the long time needed for alterations to be detected in this particular biomarker [76,83,84].

Many studies assessed the extracts' effects on cardiovascular indexes. Flow mediated dilation (FMD) was increased after the 12-week consumption of a yogurt enriched with GS extract, but not after a yogurt enriched with both GS and quercetin, in subjects with cardiovascular risk factors [85]. FMD was also increased after a single dose of GS extract, when hypertensive subjects performed exercise or during resting conditions compared to pre-supplementation [86]. The same study reported reduction in their mean systolic (SBP) and diastolic blood pressure (DBP). Blood pressure was also reduced in subjects with MS receiving either 150 or 300 mg of GS extract compared to the control group [87]. SBP alone was significantly reduced in hypertensive subjects receiving 300 mg of GS extract [83], whereas DBP alone, in women with menopausal symptoms after consumption of 100 or 200 mg of extract for 8 weeks [88].

Regarding the extracts' antioxidant properties, studies examined the oxidative damage on lipids, proteins and DNA as well as their effect on antioxidant enzymes or molecules. The majority of the studies that measured lipid peroxidation products after the consumption of the extract capsules observed reduction in malondialdehyde (MDA) levels [71,73,76,79,89,90], in lipid peroxides [91] as well as in thiobarbituric acid reactive substances (TBARS) levels [92]. Two studies assessed the extracts' effects on protein carbonyls (PC), in which their levels where reduced, compared to the control group [89] and only compared to baseline values [80]. In our study, the acute consumption of a GP extract reduced postprandial PC levels compared to the control group only in overweight/obese women [92]. Few data exist concerning the effects on DNA oxidation, as the two research groups that measured 8-hydroxy-2-deoxyguanosine (8-OHdG) levels did not observe any changes [93,94]. The antioxidant molecule glutathione in its reduced form (GSH) was increased in volleyball players that received 600 mg of GS extract for 8 weeks [79] compared to baseline values and to the control group, whereas

Table 2. Studies investigating the *in vitro* anti-inflammatory properties of by winery by-products extracts.

Type of extract	Type of cells (cell line)	Experimental procedures	Measurements	Significant results	Ref
GS	Human umbilical vein endothe-lial cells (HUVEC)	-Pre-incubation in the absence or presence of extract 5, 15, 25 µg/mL (4 h) -Stimulation with 200 mg/mL AGEs (12 h)	VCAM-1 and ICAM-1 mRNA and protein levels	↓ VCAM-1 mRNA and protein levels dose-dependently compared to control	[66]
GS	-Macro-phages (THP-1) -Adipo-cytes (SGBS)	-Pre-incubation in the absence or presence of extract 50, 100 µg/mL (16 h) -Stimulation with: LPS 2 ng/mL for the THP-1 cells (8 h) TNF-α 1 ng/mL for the SGBS cells (8 h) -Pre-incubation in the absence or presence of extract 100 µg/mL (4 h) -Stimulation with: LPS 2 ng/mL for the THP-1 cells (1 h) TNF-α 1 ng/mL for the SGBS cells (1 h)	CYP mRNA, PPARγ mRNA, leptin mRNA, APM1 mRNA, IL-6 mRNA, MCP-1 mRNA p65 translocation, phospho-IκBα	↓ IL-6 mRNA (TNF-α stimulation) compared to control ↓ MCP-1 mRNA (LPS stimulation) compared to control ↓ p65 translocation, phospho-IκBα (TNF-α and LPS stimulation) compared to control	[61]
GS	Human lens epithelial cell line (HLEB-3)	-Pre-incubation in the absence or presence of extract 20 µg/mL (12 h) -Stimulation with H_2O_2 100 µM (1, 2, 3, 6 h)	nuclear and cytosolic p65 NF-κB (3, 6 h) JNK, phospho-JNK, p38, phospho-p38 (1, 2, 3 h)	↓ nuclear and cytosolic p65 NF-κB at 3 and 6 h incubation with H_2O_2 ↓ p38 and JNK phosphorylation at 1, 2 and 3 h incubation with H_2O_2	[63]
GS	Macro-phages (RAW 264.7)	-Pre-incubation in the absence or presence of extract 5–100 µg/mL (24 h) -Stimulation with LPS (1 µg/mL) (1 h)	mRNA and protein levels: COX-2, TNF-α, IL-1β, IL-6, IL-10, NO, PGE2 ERK1/2, JNK1/2, p38, phospho-ERK1/2, phospho-JNK1/2, phospho-p38, nuclear NF-κB p65, phospho-IKKα/β, IκBα/β	↓ COX-2 mRNA, TNF-α mRNA (50, 100 µg/mL) compared to control ↓ IL-1β mRNA (100 µg/mL) compared to control ↑ IL-10 mRNA and protein levels (50, 100 µg/mL) compared to control ↓ IL-6, IL-1β (25, 50, 100 µg/mL) compared to control ↓ TNF-α (5, 25, 50, 100 µg/mL) compared to control ↓ phospho-ERK, phospho-JNK, phospho-p38, phospho-IKKα/β, IκB, nuclear NF-κB p65 compared to control ↓ NO at 48 h (50, 100 µg/mL) compared to control	[60]

Table 2. Continued.

Type of extract	Type of cells (cell line)	Experimental procedures	Measurements	Significant results	Ref
GS	-Human hepatoma cells (Huh-7) -Human hepatoma cells harboring HCV genotype (Ava5)	Incubation in the absence or presence of extract 2.5–20 µg/mL (3 d)	COX-2 primer's activity, COX-2 mRNA, PGE2, nuclear translocation of p65 NF-κB, IKKα/β, TNF-α mRNA, IL-1 mRNA, iNOS mRNA ERK1/2, p38, JNK, phospho-ERK1/2, phospho-p38, phospho-JNK, IKKα, phospho-IKKα/β, NF-κB, IκBα, phospho-IκBα	↓ of COX-2 primer's activity (10, 20 µg/mL) compared to control ↓ PGE_2 (5, 10, 20 µg/mL) compared to control Inhibition of nuclear transfer of p65 NF-κB (20 µg/mL) compared to control Dose-dependent inhibition of IKKα/β compared to control ↓ TNF-α, IL-1, iNOS mRNA (20 µg/mL) compared to control	[69]
GS	Colon epithelial cells (HT-29)	Stimulation with *C. jejuni* + incubation in the absence or presence of extract 0.02, 0.06, 0.2, 0.5 mg/mL (24 h) -Pre-incubation in the absence or presence of extract 0.02, 0.06, 0.2, 0.5 mg/mL (3 h) -Stimulation with *C. jejuni* (24 h)	IL-6, IL-8, MCP-1 IL-6, IL-8, MCP-1	↓ IL-6, IL-8, MCP-1 (0.02, 0.06, 0.2, 0.5mg/mL) compared to control ↓ IL-6, IL-8, MCP-1 (0.06, 0.2, 0.5 mg/mL) compared to control	[65]
GSt GS	Macro-phages (THP-1)	-Stimulation with ox-LDL 75 µg/mL or LPS 0.05 µg/mL -Simultaneous incubation in the absence or presence of extracts 5–20 µg/mL (24 h)	TNF-α, IL-1β, IL-6, IL-10	ox-LDL stimulation: ↓ TNF-α (GSt 5, 10, 20 µg/mL), (GS 10, 20 µg/mL) compared to control ↓ IL-1β, IL-6 (GSt 5, 10, 20 µg/mL), (GS 5, 10, 20 µg/mL) compared to control LPS stimulation: ↓ IL-6 (GSt 20 µg/mL) compared to control	[59]
GS	Esopha-gus cells (ECA109)	Incubation in the absence or presence of extract 25–80 µg/mL (12, 24, 48 h)	IL-6, COX-2, Bax, Bcl-2 Incubation for 24 h: caspase-3 mRNA and protein, IKK mRNA and protein, p50 NF-κB mRNA and protein, p65 NF-κB mRNA and protein, phospho-IκB, IκB	↓ IL-6, COX-2 (25, 50, 80 µg/mL) at 48 h compared to 12 h ↓ IL-6, COX-2 (50, 80 µg/mL) at 24 h compared to12 h and at 48 h compared to 24 h ↑ Bax and ↓ Bcl-2 time- and dose-dependently ↑ caspase-3 mRNA and protein dose-dependently ↓ IKK mRNA, IκB mRNA and protein levels, phospho-IκB, (25, 50, 80 µg/mL) compared to control ↓ p65 NF-κB mRNA and protein levels, IKK protein, p65 NF-κB mRNA and protein levels, phospho-IκB protein levels (50, 80 µg/mL) compared to control ↓ p65 NF-κB protein levels (80 µg/mL) compared to control	[68]

Table 2. Continued.

Type of extract	Type of cells (cell line)	Experimental procedures	Measurements	Significant results	Ref
GP	Endothe-lial cells (EA.hy926) HG: cells with 25 mM glucose	-Incubation of normoglycemic and HG cells (24 h) -Incubation of HG cells in the absence or presence of wine pomace after gastrointestinal digestion (GI) and after colonic fermentation (PF) 2.5 µg GAE/mL (24 h)	p38-MAPK, phospho-p38-MAPK, Akt, phospho-Akt, Nrf2, phospho-Nrf2, p65 NF-κB, phospho-p65 NF-κB, IKK$\alpha\beta$, phospho-IKK$\alpha\beta$, IκBα, phospho-IκBα, SIRT1 mRNA, COX-2 mRNA, NOX-4 mRNA	↑ phospho-Akt/Akt ratio compared to control HG cells (GI, F) ↑ phospho-p38-MAPK/p38-MAPK ratio, SIRT1 mRNA compared to control HG cells (F) ↓ NF-κB mRNA compared to control HG cells (F) ↓ phospho-IκBα/IκBα ratio, phospho-IKK/IKK ratio, COX-2 mRNA, NOX-4 mRNA compared to control HG cells (GI, F) ↑ phospho-IKK/IKK ratio compared to control normoglycemic cells and F (GI) ↓ phospho-p65 NF-κB/p65 NF-κB ratio compared to control HG cells (F) ↓ COX-2 mRNA compared to normoglycemic cells and GI (F) ↑ NOX-4 mRNA compared to normoglycemic cells and F (GI)	[67]
GS GSK	Colon epithelial cells (Caco-2)	-Stimulation with LPS 5 µg/mL (4 h) -Incubation with *Lactobacillus* strains + extract 50 µg GAE/mL (24 h)	mRNA and protein levels: eotaxin/-2, I-309, IP-10, MIG, MIP-1α/1β/1δ, RANTES, MCP-1/2, IL-1α/1β/2/3/4/6/7/8/10/11/12 p40/12 p70/13/15/16/17/18, IFN-γ, TNF-α/β, TGF-β1, GCSF, GM-CSF, M-CSF, ICAM-1, IL-6 s R, s TNF RI/RII, TGF-β1, PDGF-BB, MMP-2/9, TIMP-1/2, p38α/δ, ERK1/2, JNK1/2, Akt1/2/3, p70S6 K, TOR, CREB, GSK-3$\alpha/\beta/3\beta$, HSP27, MKK3/6, MSK2, p53, RSK1/2 p65 NF-κB protein levels	↓ of mRNA expression: EOTAXIN/-2, I-309, MIG, MIP-1α/1β, MCP-1/2, IL-1α/1β/7/11/12 p40/12 p70/13/17/18, IFN-γ, TNF-α/β, GCSF, GM-CSF, M-CSF, TGF-β1, s TNF RII, TGF-β1, PDGF-BB, MMP-2/9, ERK1/2, JNK2, Akt2/3, p70S6 K, CREB, MKK3/6, MSK2, p53 compared to control ↓ of protein levels: MCP-1, MCP-2, eotaxin, eotaxin-2, MIG, I-309, IL-1α, IFN-γ, IL-7, IL-10, IL-11, IL-12 p40, IL-12 p70, IL-13, TNF-α, TNF-β, GCSF, GM-CSF, M-CSF, s TNF RII, TGF-β1, PDGF-BB, p38γ, p38δ, ERK1, ERK2, JNK2, Akt2, Akt3, p70S6 K, CREB, GSK-3β, MKK3, p53, RSK1, p65 NF-κB compared to control	[58]
GSK	Macro-phages (RAW 264.7)	-Pre-incubation in the absence or presence of extract 250–1000 µg/mL (24 h) -Stimulation with LPS 1 µg/mL (24 h)	NO production	Inhibition of NO production: IC_{50} = 587 µg/mL	[57]
		-Pre-incubation in the absence or presence of extract 250–1000 µg/mL (24 h) -Stimulation with LPS 1 µg/mL + incubation with extract 250–1000 µg/mL (24 h)	NO production	Inhibition of NO production: IC_{50} = 630 µg/mL	

Table 2. Continued.

Type of extract	Type of cells (cell line)	Experimental procedures	Measurements	Significant results	Ref
GP	Colon epithelial cells (Caco-2)	-Pre-incubation in the absence or presence of extract (1, 5, 10 µg/mL GAE) (2 h) -Stimulation with LPS 10 µg/mL and TNF-α (10 ng/mL) (16 h)	IL-6, MCP-1, MMP-9, MMP-2 p65 nuclear translocation mRNA: IL-1β, IL-6 TNF-α, CXCL10, MCP-1, MCSF, COX-2, VCAM-1, ICAM-1, MMP-9, MMP-2, TIMP-1, TIMP-2, GAPDH	↓ of mRNA expression and protein levels: IL-6, MCP-1, MMP-9 (5, 10 µg/mL GAE), MMP-2 (10 µg/mL GAE) compared to control ↑ mRNA TIMP-1, TIMP-2 (10 µg/mL GAE) compared to control ↓ p65 nuclear translocation (5, 10 µg/mL GAE) compared to control ↓ mRNA IL-1β, TNF-α, CXCL10, MCSF, COX-2, VCAM-1, ICAM-1 compared to control	[62]
GP	Colon cancer reporter cells (HT-29-NF-κB-hrGFP)	Simultaneous incubation in the absence or presence of extracts with TNF-α (10 ng/mL) (24 h)	NF-κB activation, IL-8	↓ NF-κB activation (1 Tannat extract), IL-8 (4 Tannat samples) compared to control	[64]

AGEs, advanced glycation end products; Akt, protein kinase B; APM, adiponectin gene; Bax, Bcl-2-associated X protein; Bcl, B-cell lymphoma; COX, cyclooxygenase; CREB, cAMP response element binding; CXCL10, C-X-C motif chemokine ligand 10; CYP, cytochrome P; ERK, extracellular signal-regulated kinase; GAE, gallic acid equivalents; GCSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GP, grape pomace extract; GS, grape seed extract; GSK, grape skin extract; GSt, grape stems extract; H_2O_2, hydrogen peroxide; HSP27, heat shock protein 27; IC_{50}, half maximal inhibitory concentration; ICAM, intercellular adhesion molecule; IFN-γ, interferon-gamma; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IKK, IκB kinase; IL, interleukin; IP-10, interferon gamma-induced protein 10; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MCP, monocyte chemoattractant protein; M-CSF, macrophage colony-stimulating factor; MIG, monokine induced by INF-gamma; MIP, macrophage inflammatory protein; MKK3, mitogen-activated protein kinase kinase 3; MMP, metalloproteinase; NF-κB, nuclear factor kappa B; MSK, mitogen and stress activated protein kinase; NO, nitric oxide; NOX, NADPH oxidase; Nrf2, nuclear factor erythroid 2-related factor 2; p70S6K-1, ribosomal protein S6 kinase beta-1; PDGF, platelet-derived growth factor; PGE, prostaglandin; PPAR, peroxisome proliferator-activated receptor; RANTES, regulated upon activation, normal T cell expressed and secreted; RSK, ribosomal S6 kinase; SIRT, sirtuin; TGF, transforming growth factor; TIMP, tissue inhibitors of metalloproteinase; TNF-α, tumor necrosis factor α; TOR, target of rapamycin; VCAM, vascular cell adhesion molecule. Up arrows: increase; down arrows: reduction.

Table 3. Supplementation studies of winery by-products extracts in human subjects.

Supplement Dose/day	Study population	Study design and duration	Measurements	Significant results	Ref
-Cr -GSE 100 mg -100 mg GSE + 200 µg Cr	38 hyper-cholestero-lemic	Randomized, double-blind, placebo controlled 2 m	SBP, DBP, TC, TG, HDL, LDL, ox-LDL, homocysteine	GSE + Cr: ↓ TC, LDL compared to baseline and control	[77]
GSE 200 mg and 300 mg (1 week apart)	9 normochole-sterolemic subjects	Single dose with blood collections at 0, 1, 2 and 4 h after ingestion, controlled	FRAP, TEAC, plasma catechins	↑ plasma TEAC at 1 h: 200 and 300 mg compared to control 2 h and 4 h: 200 and 300 mg compared to control, 300 mg compared to 200 mg ↑ plasma FRAP at 2 h and 4 h: 300 mg compared to 200 mg and control ↑ plasma catechins at 1 h, 2 h and 4 h: 300 mg compared to 200 mg	[97]
GSE 400 mg	17 9 normochole-sterolemic, 8 hyperchole-sterolemic	Parallel 3 w	TC, TG, HDL, LDL, TEAC, LDL and VLDL oxidation	Hypercholesterolemic group: ↓ TC, LDL, HDL compared to baseline ↑ TEAC compared to baseline Normocholesterolemic group: ↑ LDL compared to baseline	
GSE 300 mg	8 ♂ healthy	Cross-over, postprandial, controlled (placebo not included)	Cu-induced LDL oxidation, TC, TG, uric acid, ascorbic acid, α-tocopherol, GSH/GSSG, TRAP, lipid peroxides	↑ TRAP (3 h), uric acid (1, 3 h), ascorbic acid (1 h) compared to baseline ↑ ascorbic acid (3 h) compared to control ↓ plasma lipid peroxides (1 h) compared to baseline and control	[91]
GSE 75 mg	24 ♂ smokers	Randomized, double-blind, placebo controlled, cross-over 4 w	TC, TG, HDL, LDL, lipid peroxidation, FRAP, Cu-induced LDL oxidation, plasma and LDL contents of α-tocopherol, vitamin E, β-carotene, and lycopene	↓ TBARS compared to baseline and control ↑ lag phase in serum oxidation, FRAP compared to baseline and control	[90]
-yogurt with GSE -yogurt with GSE + 0.5 g quercetin 1 g	36 hyper-cholestero-lemic, hypertensive, or smokers	Randomized, double-blind, placebo controlled, cross-over 12 w	FMD, GTN-mediated dilation, TC, TG, HDL, LDL, ox-LDL, nitrate/nitrite, urinary F2-isoprostanes, creatinine, vWF, tPA levels and activity, PAI-1 levels and activity, VCAM-1, ICAM-1, SBP, DBP	↑ FMD in the GSE alone intervention compared to control	[85]
-vit C 500 mg -GSE 1 g -500 mg vit C + 1 g GSE	69 hypertensive	Randomized, double-blind, placebo controlled (3-arm) 6 w	SBP, DBP, FMD, GTN-mediated dilation, urinary F2-isoprostanes, urinary 20-HETE, ox-LDL, hs-CRP, plasma α- and γ-tocopherol, plasma vitamin C	↑ SBP, DBP in the vit C+ GSE group compared to placebo, vit C group and GSE group ↓ heart rate in the GSE group compared to vit C group and vit C+ GSE group	[102]

Table 3. Continued.

Supplement Dose/day	Study population	Study design and duration	Measurements	Significant results	Ref
GSE 300 mg	39 ♀ with radiation-induced breast induration	Randomized, double-blind, placebo controlled 6 m	Urinary 8-OH -deoxyguanosine, urinary creatinine, lipid peroxides, TC	NS	[93]
GSE 400 mg	17 ♀ healthy post-menopausal	Randomized, double-blind, placebo controlled, Intervention included the consumption of 2 meals at the day of the measurements 8 w	PFA-100 using ADP and epinephrine	↓ in platelet reactivity against ADP 1, 2, 6 h after ingestion	[101]
-GSE 200 mg -GSE 400 mg (as pro-antho-cyanidins)	53 healthy	Randomized, single-blind 12 w	SBP, DBP, TC, TG, HDL, LDL, Apo-A1, Apo-B, Apo-E, albumin, total bilirubin, AST, ALT, γ-GT, ALP, LDH, CPK, uric acid, blood urea nitrogen, creatinine, glucose, HbA1c, adiponectin, MDA	↓ MDA, MDA/Apo-B in the 400 mg group compared to baseline ↓ MDA in the 400 mg group (6 w) compared to baseline and control ↓ MDA, Apo-B, Apo-B/Apo-A1 in the 200 mg group (12 w) compared to baseline ↑ HDL (12 w) compared to baseline ↓ Apo-B (6 w) in the 400 mg group compared to baseline	[76]
GSE 400 mg	23 ♂ smokers	Randomized, double-blind, placebo controlled, postprandial	PFA-100 using ADP and epinephrine, TRAP, TBARS, TC, TG, HDL, LDL, non-HDL, uric acid	↓ in platelet reactivity to ADP 1, 2, 6 h after ingestion ↓ in platelet reactivity to epinephrine 2 h after ingestion	[100]
GSE 600 mg	32 Type II obese diabetics	Randomized, double-blind, placebo controlled, cross-over 4 w	endothelial function, hs-CRP, GSH/GSSG, fructosamine, total antioxidant status, urine albumin/creatinine, TC, TG, HDL, glucose, insulin	↓ fructosamine, hs-CRP compared to baseline ↑ GSH compared to baseline	[95]
-GSE 150 mg -GSE 300 mg	27 subjects with MS	Double-blind, placebo controlled 4 w	SBP, DBP, glucose, insulin, ox-LDL, TC, HDL, LDL plasma catechins (only 5 subjects in the 300 mg group)	↓ SBP, DPB in both groups compared to control ↓ ox-LDL in the 300 mg group compared to baseline ↑ plasma catechins compared to control	[87]
GSE 3 g -GP 3 g	35 ♂ healthy	Randomized, double-blind, placebo controlled, cross-over (3-arm), Intervention included the consumption of a low-fat breakfast and a high-fat lunch meal at the day of the measurements 2 w	FMD, SBP, DBP, ADP-, collagen- and epinephrine induced platelet aggregation, TC, TG, HDL, LDL	↓ TG in the GP group after the high-fat lunch meal compared to control	[78]
GSE 1300 mg	50 CHD patients or subjects with ≥1 risk factors	Randomized, double-blind, placebo controlled, cross-over 4 w	SBP, DBP, FMD, brachial artery diameter, CRP, IL-6, MDA, 8-isoprostanes, TAC, TC, TG, HDL, LDL, glucose	↑ in brachial artery diameter compared to baseline and control ↓ CRP, MDA compared to baseline	[99]

Table 3. Continued.

Supplement Dose/day	Study population	Study design and duration	Measurements	Significant results	Ref
GSE 200 mg	48 Type II diabetics	Randomized, double-blind, placebo controlled 8 w	FRAP, SOD, GPx, TBARS, SBP, DBP, TC, TG, LDL, fasting glucose, HbA1c	↓ TAC compared to baseline ↑ SOD compared to baseline and control	[84]
GSE 200 mg	28 ♂ smokers	Randomized, double-blind, placebo controlled 8 w	Collagen-induced platelet aggregation, TEAC, 8-isoprostanes, nitrate/nitrite, GSH/GSSG, endothelin-1, FMD, microvascular function, SBP, DBP, arginase activity, TC, TG, HDL, LDL, fibrinogen, TNF-a, IL-10, CRP	↓ TC after 4 w compared to baseline ↓ HDL after 4 and 8 w compared to baseline ↓ %TNF-α concentration after 8 w compared to baseline and control ↑ GSH/GSSG ratio after 8 w compared to baseline ↑ vascular health index after 8 w compared to baseline and control	[96]
-GSE 100 mg -GSE 300 mg	8 healthy	Randomized, double-blind, crossover, controlled (placebo not included), postprandial	glucose	↓ plasma glucose at 15 and 30 min compared to control	[82]
GSE 400 mg	32 obese	Randomized, single-blind, placebo controlled (subjects received both the placebo and the examined supplement) 8 w	TAC, GPx, GSH/GSSG, SOD, lipid peroxides, ox-LDL, anti-ox-LDL, 8-OHdG, gene expression	↑ Antioxidant capacity compared to resveratrol group ↑ GPx compared to resveratrol group and control ↑ Anti-oxLDL compared to control Gene expression: Upregulation of GPX1, GSS, PRDX2, ICAM3, CCL5, HSF4, BCL2, compared to control Downregulation of ICAM1, MMP1, TNIP2, LTA compared to control Upregulation of HSF4, RAC1 compared to resveratrol group Downregulation of ICAM1, TNIP2, CD2, compared to resveratrol group	[94]
GS oil 15% of total energy	39 ♀ overweight/obese	Randomized, double-blind, placebo controlled, Intervention includes weight loss program 8 w	fasting glucose, insulin, hs-CRP, TNF-α	↓ TNF-α, fasting glucose, insulin compared to baseline ↓ hs-CRP compared to baseline and control	[98]
GSE 200 mg	52 (initially recruited) 75 cases at the end of the study (crossover design) mild hyper-lipidemics	Randomized, double-blind, placebo controlled, cross-over 8 w	SBP, DBP, TC, TG, HDL, LDL, VLDL, ox-LDL	↓ TC, ox-LDL compared to baseline and control ↓ LDL compared to baseline	[75]
GSE 300 mg	70 pre- and stage I hypertensive	Randomized, double-blind, placebo controlled 8 w	SBP, DBP, heart rate, HEPES-, ADP-, collagen-, TRAP-induced platelet aggregation, nitrate/nitrite, endothelin-1, ADMA, polyphenol metabolites	↓ SBP, DBP compared to baseline ↑ 4-O-methyl gallic acid, pyrogallol compared to control	[103]

Table 3. Continued.

Supplement Dose/day	Study population	Study design and duration	Measurements	Significant results	Ref
GP 700 mg	60 healthy	Randomized, double-blind, placebo controlled 8 w	TC, HDL, LDL, ORAC, ox-LDL, vit E, ascorbic acid, TNF-α, γ-GT, AST, ALT, fasting glucose	$\downarrow$ TC, LDL compared to baseline and control $\uparrow$ antioxidant capacity (ORAC), vit E compared to baseline and control	[74]
-GSE 100 mg -GSE 200 mg	91 ♀ with at least 1 menopausal symptom	Randomized, double-blind, placebo controlled 8 w	SBP, DBP, heart rate, cardioankle vascular index, physical symptoms, hot flashes, insomnia, depression, anxiety	200 mg group: $\downarrow$ physical symptoms (8 w), hot flashes (8 w), insomnia (8 w), anxiety (4 w, 8 w), SBP (4 w, 8 w), DBP (4 w, 8 w) compared to baseline $\downarrow$ anxiety (8 w), DBP (8 w) compared to control 100 mg group: $\downarrow$ anxiety (4 w, 8 w), SBP (4 w, 8 w), DBP (4 w, 8 w) compared to baseline $\downarrow$ DBP (8 w) compared to control	[88]
GP flour (in the form of bread and cookies) 20 g	38 ♂ with $\geq$1 MS criteria	Randomized, controlled 16 w	SBP, DBP, glucose, insulin, OGTT, TC, TG, HDL, LDL, L-ascorbic acid, tocopherols, PC, DPPH, TRAP	$\downarrow$ SBP, DBP, fasting glucose, average number of MS criteria, postprandial insulin, PC, antioxidant capacity compared to baseline $\downarrow$ postprandial insulin compared to control $\uparrow$ δ-tocopherol, vit C compared to baseline $\uparrow$ γ-tocopherol compared to baseline and control	[80]
GSE 200 mg	30 ESRD patients	Randomized double-blind, placebo controlled 4 w	TC, TG, HDL, LDL, VLDL, hs-CRP, TNF-α, IL-6, parathormone, albumin, calcium, phosphorus, hemoglobin	NS	[104]
GS oil 1 g	34 healthy	Randomized, double-blind, controlled (4 subjects in the control group) 7 d	ADP-induced platelet aggregation (2.34, 1.17, 0.58 µM)	$\downarrow$ in platelet aggregation compared to baseline at 2.34 and 1.17 µM ADP	[105]
GSE 300 mg	29 hypertensive	Randomized, double-blind, placebo controlled 6 w (+4 w follow-up)	SBP, DBP, glucose, insulin, ox-LDL, ICAM-1, HOMA-IR, FMD, plasma phenolic metabolites	$\downarrow$ SBP compared to baseline and control $\downarrow$ DBP compared to baseline $\uparrow$ total plasma phenolic metabolites compared to control	[83]
GSE, vitamins, minerals 1–2 capsules	30 Type II diabetics	Randomized double-blind, placebo controlled 45 d	fasting glucose, postprandial glucose, HbA1c, ALT, AST, TC, TG, HDL, LDL, urea, creatinine	NS	[106]
GS powder 2 g	33 CKD patients	Randomized, double-blind, placebo controlled 6 m	CAT activity, SOD activity, GPx activity, H_2O_2, free iron, CRP, LDH, GFR, plasma urea, plasma creatinine, plasma UA, urine protein, MDA, PC, SBP, DBP, glucose, TG, TC, lipase	$\uparrow$ CAT, SOD compared to control $\uparrow$ CAT, free iron, lipase compared to baseline $\downarrow$ PC compared to baseline $\downarrow$ creatinine, urine protein, MDA, PC compared to control	[89]

Table 3. Continued.

Supplement Dose/day	Study population	Study design and duration	Measurements	Significant results	Ref
GSE 600 mg	40 ♀ volleyball athletes	Randomized, double-blind, placebo controlled 8 w	CPK, TAC, FRAP, GSH, TBARS, nitrite/nitrate ratio, fasting glucose, TC, TG, HDL, LDL, VLDL, HOMA-IR, QUICKI, SBP, DBP	↑ GSH, insulin sensitivity compared to baseline and control ↓ MDA, fasting insulin, HOMA-IR compared to baseline and control ↓ TG, VLDL, TC/HDL ratio compared to baseline	[79]
GO: GP + omija fruit (O) extracts -Low GO: GP 342.5 mg + O 57.5 mg -High GO: GP 685 mg + O 115 mg	76 overweight/obese	Randomized, double-blind, placebo controlled 10 w	TC, TG, HDL, LDL, Apo-A1, Apo-B, Lp(a), atherogenic index, TBARS, H_2O_2, IL-1β, TNF-α, GPx activity, SOD activity, GR activity, AST, ALT	High GO: ↓ TC, non-HDL, LDL, atherogenic index, TBARS, H_2O_2 compared to control ↑ apo-A1, GPx activity, GR activity compared to control ↓ Lp(a), TBARS, H_2O_2, IL-1β, TNF-α, compared to baseline ↑ SOD activity compared to baseline Low GO: ↓ IL-1β compared to baseline ↑ SOD activity compared to baseline	[73]
GSE 200 mg	70 mild to moderate hyper-lipidemics	Randomized, double-blind, placebo controlled 8 w	TC, TG, HDL, LDL, Apo-A1, PON	↓ TG, TC, LDL ↑ PON activity, Apo-A1 compared to baseline and control	[72]
GSE 300 mg	9 ♂ hypertensive	Randomized, double-blind, placebo controlled, cross-over, Intervention includes exercise at 40% and 60% VO_{2max} Single dose	SBP, DBP, mean arterial pressure, FMD, cardiac output, stroke volume, total vascular conductance, rate × pressure product	↓ SBP, DBP, mean arterial pressure compared to presupplementation at resting conditions and during exercise ↑ heart rate at 40% VO_{2max} compared to resting condition ↑ cardiac output, stroke volume, total vascular conductance, FMD compared to presupplementation during exercise ↓ rate × pressure product compared to presupplementation at resting conditions and during exercise at 60% VO_{2max}	[86]
GSE 150 mg	27 COPD patients	Randomized, double-blind, placebo controlled 8 w	TC, TG, HDL, LDL, SOD, GPx, CAT, TBARS, lung function	↓ MDA, SOD, TC/HDL ratio compared to baseline and control	[71]
dried GP 8 g	50 subjects with ≥2 MS criteria	Randomized, cross-over 6 w	OGTT, HOMA-IR, QUICKI, TC, HDL, LDL, SBP, DBP, plasma and urine UA, fibrinogen, hs-CRP, AST, ALT, iron status, leptin	↑ in insulin sensitivity compared to baseline	[107]
-GSE 200 mg -GSE 400 mg	30 pre-hypertensive	Randomized, double-blind, placebo controlled 12 w	TC, LDL, HDL, ox-LDL, TG, vascular function (including among others FMD, SBP, DBP)	400 mg group: ↓ SBP compared to baseline at 12 w Improved stiffness, distensibility, PWV compared to baseline at 8 and 12 w ↓ Einc compared to baseline at 12 w	[108]

Table 3. Continued.

Supplement Dose/day	Study population	Study design and duration	Measurements	Significant results	Ref
GSE 300 mg	16 ♂ healthy	Randomized, double-blind, placebo controlled, Intervention includes exercise 3 d	Maximal strength, muscle soreness, CK	↓ CK 96 h after exercise compared to control	[109]
GP 3.7 mmol polyphenols	12 ♂ healthy	Randomized, double-blind, placebo controlled, crossover, postprandial	glucose, insulin, TG	↓ insulin $iAUC_{0-5\ h}$ ↑ insulin sensitivity compared to control	[81]
GP 800 mg	213 subjects (41 healthy, 39 hypertensive, 28 diabetics)	Randomized, double-blind, placebo controlled 8 w	TMAO, ox-LDL, reactive oxygen metabolites	↓ TMAO, ox-LDL, reactive oxygen metabolites at 4 and 8 w compared to control	[110]
GP 3.5 g	18 ♀ healthy	Randomized, double-blind, placebo controlled, crossover, postprandial	glucose, insulin, UA, TC, TG, HDL, LDL, SOD activity, GPx activity, PC, TBARS	↓ UA, TBARS, SOD activity in normal-weight women compared to control ↑ UA, ↓ PC in overweight/obese women compared to control	[92]

ADMA, asymmetric dimethylarginine; ADP, adenosine diphosphate; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Apo, apolipoprotein; AST, aspartate transaminase; CAT, catalase; CHD, coronary heart disease; CK, creatine kinase; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; C(P)K, creatine phosphokinase; Cr, chromium; Cu, copper; DBP, diastolic blood pressure; dGuo, deoxyguanosine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ESRD, eternal stage renal disease; FMD, flow mediated dilation; FRAP, ferric reducing antioxidant power; GFR, glomerular filtration rate; GP, grape pomace; GPx, glutathione peroxidase; GR, glutathione reductase; GS, grape seed; GSE, grape seed extract; GSH, glutathione reduced; GSSG, glutathione oxidized; γ-GT, gamma-glutamyl transpeptidase; GTN, glyceryl trinitrate; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HETE, 20-hydroxyeicosatetraenoic acid; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; H_2O_2, hydrogen peroxide; (hs)-CRP, (high sensitive) C-reactive protein; ICAM, intercellular adhesion molecule; IL, interleukin; LDH, lactate dehydrogenase; LDL, low density lipoprotein; Lp(a), lipoprotein a; MDA, malondialdehyde; MS, metabolic syndrome; OGTT, oral glucose tolerance test; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; OPC, oligomeric proanthocyanidins; ORAC, oxygen radical absorbance capacity; ox-LDL, oxidized LDL; PAI, plasminogen activator inhibitor; PC, protein carbonyls; PFA-100, platelet function assay; PON, paraoxonase; PUFA, polyunsaturated fatty acid; QUICKI, quantitative insulin sensitivity check index; RBC, red blood cells; SBP, systolic blood pressure; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol, TEAC, Trolox equivalent antioxidant capacity; TMAO, Trimethylamine-N-oxide; TNF-α, tumor necrosis factor α; TG, triglycerides; tPA, tissue plasminogen activator; TRAP, total radical trapping antioxidant parameter; TRAP, thrombin receptor activating peptide; UA, uric acid; VCAM, vascular cell adhesion molecule; VLDL, very low density lipoprotein; vWF, von Willebrand factor. Up arrows: increase; down arrows: reduction.

in other studies GSH [95] and GSH/glutathione oxidized (GSSG) ratio [96] were increased compared to baseline values but not to the control group. The effect on antioxidant enzymes can be assessed by the change in their levels or their activity. Superoxide dismutase (SOD) and catalase (CAT) levels were significantly elevated in chronic kidney disease (CKD) patients [89] that received 2 g of GS extract for 6 months. For SOD, the same effect was found in type II diabetic patients receiving 200 mg daily for 8 weeks, whereas, SOD levels were reduced in chronic obstructive pulmonary disease (COPD) patients receiving 150 mg daily for the same period [71]. In our study, postprandial SOD activity was reduced only in normal-weight women compared to the control group after the consumption of a GP extract along with a high-fat meal [92]. SOD activity was increased in one study in overweight or obese subjects [73] but only compared to baseline values. In the same study, glutathione peroxidase (GPx) activity was enhanced only compared to the control group. GPx levels were elevated after the daily administration of 400 mg GS extract for 2 months in obese subjects compared to the group receiving resveratrol and compared to the control group [94]. Paraoxonase (PON) was examined in one study where hyperlipidemic subjects received 200 mg of GS extract for 8 weeks, leading to its activity enhancement compared to baseline values and to the control group [72]. Assays developed for the measurement of the antioxidant capacity in serum and plasma such as total antioxidant capacity (TAC), trolox equivalent antioxidant capacity (TEAC), total radical trapping antioxidant parameter (TRAP), oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP), were performed in several studies examining GS extracts and favorable results were reported [74,84,90,91,94,97].

Concerning the extracts' actions on inflammatory markers, research focused on cytokines, acute phase proteins (C-reactive protein, CRP in particular), adhesion molecules and inflammatory genes expression. Significantly reduced levels of high sensitive CRP (hs-CRP) were reported after 8 weeks of GS oil consumption for 39 overweight/obese subjects, compared to baseline levels and to the control group [98]. Other studies reported reduced CRP levels [95,99] compared to baseline values. The majority of cytokines examined were TNF-α and interleukin-1β (IL-1β). In 28 smokers receiving 200 mg GS extract for 8 weeks TNF-α levels were reduced compared to baseline and to the control group [96]. In the rest of the studies TNF-α [73,98] and IL-1β [73] were reduced only compared to baseline levels. Intercellular adhesion molecule-1 (ICAM-1) protein levels did not change in hyper-cholesterolemic and/or hypertensive subjects [83,85], whereas, downregulation of the ICAM-1 gene expression was reported after the 8-week consumption of 400 mg GS extract in obese subjects compared to the control group, as well as compared to the group receiving resveratrol [94].

The anti-platelet effects of extracts were examined only by three of the studies listed in Table 3. Two of them reported favorable results in the postprandial state by reducing platelet sensitivity to ADP [100,101] and epinephrine [100]. No data exist, yet, concerning PAF as agonist for platelet aggregation.

6. Conclusions

In this review, we provide a brief overview of the atherothrombotic process in order to further investigate the effects of winery by-product extracts. Data from *in vitro* studies report that winery by-product compounds are able to modulate platelet function, as well as to downregulate inflammatory markers. Among their actions, extracts or phenolic compounds present in winery by-products inhibit PAF's actions, a potent inflammatory and thrombotic mediator. In addition, they modulate its levels either through direct inhibition of its metabolic enzymes or through indirect reduction of oxidative stress leading to lower LDL oxidation and PAF or PAF-like molecules production. Similar conclusions have been drawn from the human supplementation studies stating that winery by-product extracts may exert favorable biological actions towards the cardiovascular system. Evidence from long-term studies show that consumption may lower total and LDL cholesterol, improve insulin sensitivity, decrease lipid and protein oxidative damage, enhance antioxidant capacity and a have mild anti-inflammatory action towards reducing cytokine expression and levels. Although data from postprandial studies are limited, the acute consumption of winery by-product extracts seems to improve glycemic response as well as reducing platelet reactivity to aggregatory stimuli. Nutritional supplements or foods fortified with winery by-products could provide a safe and cost-effective complementary treatment towards atherothrombotic complications. Nevertheless, further randomized controlled long-term or postprandial trials need to be conducted in order to draw definitive conclusions for providing a useful cardioprotective approach that includes the sustainable use of winery by-products.

Author Contributions

MC, EF searched, analyzed, interpreted data, drafted the manuscript and SA analyzed data and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

Smaragdi Antonopoulou states that given her role as Guest Editor, she had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Raffaele Serra. The other authors declare no conflict of interest.

References

[1] Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circulation Research. 2016; 118: 535–546.

[2] Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet (London, England). 1994; 344: 793–795.

[3] Tsimikas S, Witztum JL. Oxidized phospholipids in cardiovascular disease. Nature Reviews. Cardiology. 2024; 21: 170–191.

[4] Demopoulos CA, Karantonis HC, Antonopoulou S. Platelet activating factor - a molecular link between atherosclerosis theories. European Journal of Lipid Science and Technology. 2003; 105: 705–716.

[5] Palur Ramakrishnan AVK, Varghese TP, Vanapalli S, Nair NK, Mingate MD. Platelet activating factor: A potential biomarker in acute coronary syndrome? Cardiovascular Therapeutics. 2017; 35: 64–70.

[6] Demopoulos CA, Pinckard RN, Hanahan DJ. Platelet-activating factor. Evidence for 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). The Journal of Biological Chemistry. 1979; 254: 9355–9358.

[7] Ninio E. Phospholipid mediators in the vessel wall: involvement in atherosclerosis. Current Opinion in Clinical Nutrition and Metabolic Care. 2005; 8: 123–131.

[8] Pantazi D, Tellis C, Tselepis AD. Oxidized phospholipids and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) in atherosclerotic cardiovascular disease: An update. BioFactors (Oxford, England). 2022; 48: 1257–1270.

[9] Victorino GP, Newton CR, Curran B. Modulation of microvascular hydraulic permeability by platelet-activating factor. The Journal of Trauma. 2004; 56: 379–384.

[10] Hamel-Côté G, Lapointe F, Gendron D, Rola-Pleszczynski M, Stankova J. Regulation of platelet-activating factor-induced interleukin-8 expression by protein tyrosine phosphatase 1B. Cell Communication and Signaling: CCS. 2019; 17: 21.

[11] Hamel-Côté G, Lapointe F, Véronneau S, Mayhue M, Rola-Pleszczynski M, Stankova J. Regulation of platelet-activating factor-mediated interleukin-6 promoter activation by the 48 kDa but not the 45 kDa isoform of protein tyrosine phosphatase non-receptor type 2. Cell & Bioscience. 2019; 9: 51.

[12] Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. The New England Journal of Medicine. 2005; 352: 1685–1695.

[13] Rios FJO, Gidlund M, Jancar S. Pivotal role for platelet-activating factor receptor in CD36 expression and oxLDL uptake by human monocytes/macrophages. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2011; 27: 363–372.

[14] Davì G, Patrono C. Platelet activation and atherothrombosis. The New England Journal of Medicine. 2007; 357: 2482–2494.

[15] Zimmerman GA, McIntyre TM, Prescott SM, Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Critical Care Medicine. 2002; 30: S294–301.

[16] Jobling L, Eyre L. Haemostasis, blood platelets and coagulation. Anaesthesia & Intensive Care Medicine. 2013; 14: 51–53.

[17] Lupia E, Pucci A, Peasso P, Merlo M, Baron P, Zanini C, *et al*. Intra-plaque production of platelet-activating factor correlates with neoangiogenesis in human carotid atherosclerotic lesions. International Journal of Molecular Medicine. 2003; 12: 327–334.

[18] Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell Metabolism. 2011; 14: 575–585.

[19] Balestrieri ML, Servillo L, Esposito A, D'Onofrio N, Giovane A, Casale R, *et al*. Poor glycaemic control in type 2 diabetes patients reduces endothelial progenitor cell number by influencing SIRT1 signalling via platelet-activating factor receptor activation. Diabetologia. 2013; 56: 162–172.

[20] Yin H, Shi A, Wu J. Platelet-Activating Factor Promotes the Development of Non-Alcoholic Fatty Liver Disease. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2022; 15: 2003–2030.

[21] St Leger AS, Cochrane AL, Moore F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. Lancet (London, England). 1979; 1: 1017–1020.

[22] Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet (London, Eng-land). 1992; 339: 1523–1526.

[23] Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis. European Journal of Epidemiology. 2011; 26: 833–850.

[24] Schaefer SM, Kaiser A, Behrendt I, Eichner G, Fasshauer M. Association of alcohol types, coffee and tea intake with mortality: prospective cohort study of UK Biobank participants. The British Journal of Nutrition. 2023; 129: 115–125.

[25] Fragopoulou E, Nomikos T, Tsantila N, Mitropoulou A, Zabetakis I, Demopoulos CA. Biological activity of total lipids from red and white wine/must. Journal of Agricultural and Food Chemistry. 2001; 49: 5186–5193.

[26] Fragopoulou E, Antonopoulou S, Nomikos T, Demopoulos CA. Structure elucidation of phenolic compounds from red/white wine with antiatherogenic properties. Biochimica et Biophysica Acta. 2003; 1632: 90–99.

[27] Argyrou C, Vlachogianni I, Stamatakis G, Demopoulos CA, Antonopoulou S, Fragopoulou E. Postprandial effects of wine consumption on Platelet Activating Factor metabolic enzymes. Prostaglandins & other Lipid Mediators. 2017; 130: 23–29.

[28] Xanthopoulou MN, Kalathara K, Melachroinou S, Arampatzi-Menenakou K, Antonopoulou S, Yannakoulia M, *et al*. Wine consumption reduced postprandial platelet sensitivity against platelet activating factor in healthy men. European Journal of Nutri-tion. 2017; 56: 1485–1492.

[29] GBD 2016 Alcohol Collaborators. Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet (London, England). 2018; 392: 1015–1035.

[30] GBD 2020 Alcohol Collaborators. Population-level risks of alcohol consumption by amount, geography, age, sex, and year: a systematic analysis for the Global Burden of Disease Study 2020. Lancet (London, England). 2022; 400: 185–235.

[31] Wojtowicz JS. Long-Term Health Outcomes of Regular, Moderate Red Wine Consumption. Cureus. 2023; 15: e46786.

[32] Lucerón-Lucas-Torres M, Saz-Lara A, Díez-Fernández A, Martínez-García I, Martínez-Vizcaíno V, Cavero-Redondo I, *et al*. Association between Wine Consumption with Cardiovascular Disease and Cardiovascular Mortality: A Systematic Review and Meta-Analysis. Nutrients. 2023; 15: 2785.

[33] Molani-Gol R, Rafraf M. Effects of resveratrol on the anthropometric indices and inflammatory markers: an umbrella meta-

analysis. European Journal of Nutrition. 2024; 63: 1023–1040.
[34] Yadegar S, Mohammadi F, Yadegar A, Mohammadi Naeini A, Ayati A, Milan N, *et al*. Effects and safety of resvera-trol supplementation in older adults: A comprehensive systematic review. Phytotherapy Research: PTR. 2024; 38: 2448–2461.
[35] Fragopoulou E, Choleva M, Antonopoulou S, Demopoulos CA. Wine and its metabolic effects. A comprehensive review of clinical trials. Metabolism: Clinical and Experimental. 2018; 83: 102–119.
[36] Fontana AR, Antoniolli A, Bottini R. Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. Journal of Agricultural and Food Chemistry. 2013; 61: 8987–9003.
[37] Bordiga M, Travaglia F, Locatelli M. Valorisation of grape pomace: an approach that is increasingly reaching its maturity - a review. International Journal of Food Science & Technology. 2019; 54: 933–942.
[38] Teixeira A, Baenas N, Dominguez-Perles R, Barros A, Rosa E, Moreno DA, *et al*. Natural bioactive compounds from winery by-products as health promoters: a review. International Journal of Molecular Sciences. 2014; 15: 15638–15678.
[39] Choleva M, Boulougouri V, Panara A, Panagopoulou E, Chiou A, Thomaidis NS, *et al*. Evaluation of anti-platelet activity of grape pomace extracts. Food & Function. 2019; 10: 8069–8080.
[40] Machado NFL, Domínguez-Perles R. Addressing Facts and Gaps in the Phenolics Chemistry of Winery By-Products. Molecules (Basel, Switzerland). 2017; 22: 286.
[41] Luzak B, Kosiorek A, Syska K, Rozalski M, Bijak M, Podsedek A, *et al*. Does grape seed extract potentiate the inhi-bition of platelet reactivity in the presence of endothelial cells? Advances in Medical Sciences. 2014; 59: 178–182.
[42] Bijak M, Sut A, Kosiorek A, Saluk-Bijak J, Golanski J. Dual Anticoagulant/Antiplatelet Activity of Polyphenolic Grape Seeds Extract. Nutrients. 2019; 11: 93.
[43] Muñoz-Bernal ÓA, De La Rosa LA, Rodrigo-García J, Martínez-Ruiz NR, Sáyago-Ayerdi S, Rodriguez L, *et al*. Phytochemical Characterization and Antiplatelet Activity of Mexican Red Wines and Their By-products. South African Journal of Enology and Viticulture. 2021; 42: 77–90.
[44] Olas B, Wachowicz B, Tomczak A, Erler J, Stochmal A, Oleszek W. Comparative anti-platelet and antioxidant properties of polyphenol-rich extracts from: berries of Aronia melanocarpa, seeds of grape and bark of Yucca schidigera in vitro. Platelets. 2008; 19: 70–77.
[45] Olas B, Wachowicz B, Stochmal A, Oleszek W. The polyphenol-rich extract from grape seeds inhibits platelet signaling pathways triggered by both proteolytic and non-proteolytic agonists. Platelets. 2012; 23: 282–289.
[46] Bijak M, Saluk J, Ponczek MB, Nowak P. Antithrombin effect of polyphenol-rich extracts from black chokeberry and grape seeds. Phytotherapy Research: PTR. 2013; 27: 71–76.
[47] Malinowska J, Oleszek W, Stochmal A, Olas B. The polyphenol-rich extracts from black chokeberry and grape seeds impair changes in the platelet adhesion and aggregation induced by a model of hyperhomocysteinemia. European Journal of Nutrition. 2013; 52: 1049–1057.
[48] Vitseva O, Varghese S, Chakrabarti S, Folts JD, Freedman JE. Grape seed and skin extracts inhibit platelet function and release of reactive oxygen intermediates. Journal of Cardiovascular Pharmacology. 2005; 46: 445–451.
[49] Shanmuganayagam D, Beahm MR, Osman HE, Krueger CG, Reed JD, Folts JD. Grape seed and grape skin extracts elicit a greater antiplatelet effect when used in combination than when used individually in dogs and humans. The Journal of Nutrition. 2002; 132: 3592–3598.
[50] Michalaki A, Iliopoulou EN, Douvika A, Nasopoulou C, Skalkos D, Karantonis HC. Bioactivity of Grape Skin from Small-Berry Muscat and Augustiatis of Samos: A Circular Economy Perspective for Sustainability. Sustainability. 2022; 14: 14576.
[51] Jin JW, Inoue O, Suzuki-Inoue K, Nishikawa G, Kawakami Y, Hisamoto M, *et al*. Grape seed extracts inhibit platelet aggregation by inhibiting protein tyrosine phosphatase. Clinical and Applied Thrombosis/hemostasis: Official Journal of the Interna-tional Academy of Clinical and Applied Thrombosis/Hemostasis. 2014; 20: 278–284.
[52] Tamer F, Tullemans BME, Kuijpers MJE, Claushuis TAM, Heemskerk JWM. Nutrition Phytochemicals Affecting Platelet Signaling and Responsiveness: Implications for Thrombosis and Hemostasis. Thrombosis and Haemostasis. 2022; 122: 879–894.
[53] Faggio C, Sureda A, Morabito S, Sanches-Silva A, Mocan A, Nabavi SF, *et al*. Flavonoids and platelet aggregation: A brief review. European Journal of Pharmacology. 2017; 807: 91–101.
[54] Bojić M, Debeljak Z, Tomičić M, Medić-Šarić M, Tomić S. Evaluation of antiaggregatory activity of flavonoid aglycone series. Nutrition Journal. 2011; 10: 73.
[55] Fragopoulou E, Nomikos T, Karantonis HC, Apostolakis C, Pliakis E, Samiotaki M, *et al*. Biological activity of acety-lated phenolic compounds. Journal of Agricultural and Food Chemistry. 2007; 55: 80–89.
[56] Vilahur G, Badimon L. Antiplatelet properties of natural products. Vascular Pharmacology. 2013; 59: 67–75.
[57] Fernández-Fernández AM, Iriondo-DeHond A, Dellacassa E, Medrano-Fernandez A, del Castillo MD. Assessment of antioxidant, antidiabetic, antiobesity, and anti-inflammatory properties of a Tannat winemaking by-product. European Food Research and Technology. 2019; 245: 1539–1551.
[58] Pistol GC, Marin DE, Dragomir C, Taranu I. Synbiotic combination of prebiotic grape pomace extract and probi-otic *Lactobacillus* sp. reduced important intestinal inflammatory markers and in-depth signalling mediators in lipopolysaccha-ride-treated Caco-2 cells. The British Journal of Nutrition. 2019; 121: 291–305.
[59] Nieto JA, Jaime L, Arranz E, Reglero G, Santoyo S. Winemaking by-products as anti-inflammatory food ingredients. Food and Agricultural Immunology. 2017; 28: 1507–1518.
[60] Chu H, Tang Q, Huang H, Hao W, Wei X. Grape-seed proanthocyanidins inhibit the lipopolysaccharide-induced inflammato-ry mediator expression in RAW264.7 macrophages by suppressing MAPK and NF-κb signal pathways. Environmental Toxicology and Pharmacology. 2016; 41: 159–166.
[61] Chacón MR, Ceperuelo-Mallafré V, Maymó-Masip E, Mateo-Sanz JM, Arola L, Guitiérrez C, *et al*. Grape-seed pro-cyanidins modulate inflammation on human differentiated adipocytes in vitro. Cytokine. 2009; 47: 137–142.
[62] Calabriso N, Massaro M, Scoditti E, Verri T, Barca A, Gerardi C, *et al*. Grape Pomace Extract Attenuates Inflamma-tory Response in Intestinal Epithelial and Endothelial Cells: Potential Health-Promoting Properties in Bowel Inflammation. Nutrients. 2022; 14: 1175.
[63] Jia Z, Song Z, Zhao Y, Wang X, Liu P. Grape seed proanthocyanidin extract protects human lens epithelial cells from oxidative stress via reducing NF-κB and MAPK protein expression. Molecular Vision. 2011; 17: 210–217.
[64] Fariña E, Daghero H, Bollati-Fogolín M, Boido E, Cantero J, Moncada-Basualto M, *et al*. Antioxidant Capacity and NF-kB-Mediated Anti-Inflammatory Activity of Six Red Uruguayan Grape Pomaces. Molecules (Basel, Switzerland). 2023; 28: 3909.
[65] Silvan JM, Mingo E, Martinez-Rodriguez AJ. Grape seed extract (GSE) modulates campylobacter pro-inflammatory response in human intestinal epithelial cell lines. Food and Agricultural Immunology. 2017; 28: 739–753.
[66] Zhang FL, Gao HQ, Wu JM, Ma YB, You BA, Li BY, *et al*.

Selective inhibition by grape seed proanthocyanidin extracts of cell adhesion molecule expression induced by advanced glycation end products in endothelial cells. Journal of Cardiovascu-lar Pharmacology. 2006; 48: 47–53.
[67] Gerardi G, Cavia-Saiz M, Rivero-Pérez MD, González-SanJosé ML, Muñiz P. Modulation of Akt-p38-MAPK/Nrf2/SIRT1 and NF-κB pathways by wine pomace product in hyperglycemic endothelial cell line. Journal of Functional Foods. 2019; 58: 255–265.
[68] Guo F, Hu Y, Niu Q, Li Y, Ding Y, Ma R, *et al*. Grape Seed Proanthocyanidin Extract Inhibits Human Esophageal Squamous Cancerous Cell Line ECA109 via the NF-*κ*B Signaling Pathway. Mediators of Inflammation. 2018; 2018: 3403972.
[69] Chen WC, Tseng CK, Chen BH, Lin CK, Lee JC. Grape Seed Extract Attenuates Hepatitis C Virus Replication and Virus-Induced Inflammation. Frontiers in Pharmacology. 2016; 7: 490.
[70] Nomikos T, Fragopoulou E, Antonopoulou S. Food Ingredients and Lipid Mediators. Current Nutrition & Food Science. 2007; 3: 255–276.
[71] Lu MC, Yang MD, Li PC, Fang HY, Huang HY, Chan YC, *et al*. Effect of Oligomeric Proanthocyanidin on the An-tioxidant Status and Lung Function of Patients with Chronic Obstructive Pulmonary Disease. In Vivo (Athens, Greece). 2018; 32: 753–758.
[72] Argani H, Ghorbanihaghjo A, Vatankhahan H, Rashtchizadeh N, Raeisi S, Ilghami H. The effect of red grape seed extract on serum paraoxonase activity in patients with mild to moderate hyperlipidemia. Sao Paulo Medical Journal = Revista Paulista De Medic-ina. 2016; 134: 234–239.
[73] Han HJ, Jung UJ, Kim HJ, Cho SJ, Kim AH, Han Y, *et al*. Combined Supplementation with Grape Pomace and Omi-ja Fruit Ethanol Extracts Dose-Dependently Improves Body Composition, Plasma Lipid Profiles, Inflammatory Status, and Antioxidant Capacity in Overweight and Obese Subjects. Journal of Medicinal Food. 2016; 19: 170–180.
[74] Yubero N, Sanz-Buenhombre M, Guadarrama A, Villanueva S, Carrión JM, Larrarte E, *et al*. LDL cholesterol-lowering effects of grape extract used as a dietary supplement on healthy volunteers. International Journal of Food Sciences and Nutri-tion. 2013; 64: 400–406.
[75] Razavi SM, Gholamin S, Eskandari A, Mohsenian N, Ghorbanihaghjo A, Delazar A, *et al*. Red grape seed extract improves lipid profiles and decreases oxidized low-density lipoprotein in patients with mild hyperlipidemia. Journal of Medicinal Food. 2013; 16: 255–258.
[76] Sano A, Uchida R, Saito M, Shioya N, Komori Y, Tho Y, *et al*. Beneficial effects of grape seed extract on malondial-dehyde-modified LDL. Journal of Nutritional Science and Vitaminology. 2007; 53: 174–182.
[77] Preuss HG, Wallerstedt D, Talpur N, Tutuncuoglu SO, Echard B, Myers A, *et al*. Effects of niacin-bound chromium and grape seed proanthocyanidin extract on the lipid profile of hypercholesterolemic subjects: a pilot study. Journal of Medicine. 2000; 31: 227–246.
[78] van Mierlo LAJ, Zock PL, van der Knaap HCM, Draijer R. Grape polyphenols do not affect vascular function in healthy men. The Journal of Nutrition. 2010; 140: 1769–1773.
[79] Taghizadeh M, Malekian E, Memarzadeh MR, Mohammadi AA, Asemi Z. Grape Seed Extract Supplementation and the Ef-fects on the Biomarkers of Oxidative Stress and Metabolic Profiles in Female Volleyball Players: A Randomized, Double-Blind, Pla-cebo-Controlled Clinical Trial. Iranian Red Crescent Medical Journal. 2016; 18: e31314.
[80] Urquiaga I, D'Acuña S, Pérez D, Dicenta S, Echeverría G, Rigotti A, *et al*. Wine grape pomace flour improves blood pressure, fasting glucose and protein damage in humans: a randomized controlled trial. Biological Research. 2015; 48: 49.
[81] Costabile G, Vitale M, Luongo D, Naviglio D, Vetrani C, Ciciola P, *et al*. Grape pomace polyphenols improve insulin response to a standard meal in healthy individuals: A pilot study. Clinical Nutrition (Edinburgh, Scotland). 2019; 38: 2727–2734.
[82] Sapwarobol S, Adisakwattana S, Changpeng S, Ratanawachirin W, Tanruttanawong K, Boonyarit W. Postprandial blood glucose response to grape seed extract in healthy participants: A pilot study. Pharmacognosy Magazine. 2012; 8: 192–196.
[83] Park E, Edirisinghe I, Choy YY, Waterhouse A, Burton-Freeman B. Effects of grape seed extract beverage on blood pressure and metabolic indices in individuals with pre-hypertension: a randomised, double-blinded, two-arm, parallel, placebo-controlled trial. The British Journal of Nutrition. 2016; 115: 226–238.
[84] Pourghassem-Gargari B, Abedini S, Babaei H, Aliasgarzadeh A, Pourabdollahi P. Effect of supplementation with grape seed (Vitis vinifera) extract on antioxidant status and lipid peroxidation in patient with type II diabetes. Journal of Medicinal Plants Research. 2011; 5: 2029–2034.
[85] Clifton PM. Effect of Grape Seed Extract and Quercetin on Cardiovascular and Endothelial Parameters in High-Risk Subjects. Journal of Biomedicine & Biotechnology. 2004; 2004: 272–278.
[86] Kim JK, Kim KA, Choi HM, Park SK, Stebbins CL. Grape Seed Extract Supplementation Attenuates the Blood Pressure Response to Exercise in Prehypertensive Men. Journal of Medicinal Food. 2018; 21: 445–453.
[87] Sivaprakasapillai B, Edirisinghe I, Randolph J, Steinberg F, Kappagoda T. Effect of grape seed extract on blood pressure in subjects with the metabolic syndrome. Metabolism: Clinical and Experimental. 2009; 58: 1743–1746.
[88] Terauchi M, Horiguchi N, Kajiyama A, Akiyoshi M, Owa Y, Kato K, *et al*. Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular parameters in middle-aged women: a randomized, double-blind, placebo-controlled pilot study. Menopause (New York, N.Y.). 2014; 21: 990–996.
[89] Turki K, Charradi K, Boukhalfa H, Belhaj M, Limam F, Aouani E. Grape seed powder improves renal failure of chronic kid-ney disease patients. EXCLI Journal. 2016; 15: 424–433.
[90] Vigna GB, Costantini F, Aldini G, Carini M, Catapano A, Schena F, *et al*. Effect of a standardized grape seed extract on low-density lipoprotein susceptibility to oxidation in heavy smokers. Metabolism: Clinical and Experimental. 2003; 52: 1250–1257.
[91] Natella F, Belelli F, Gentili V, Ursini F, Scaccini C. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. Journal of Agricultural and Food Chemistry. 2002; 50: 7720–7725.
[92] Choleva M, Matalliotaki E, Antoniou S, Asimomyti E, Drouka A, Stefani M, *et al*. Postprandial Metabolic and Oxida-tive Stress Responses to Grape Pomace Extract in Healthy Normal and Overweight/Obese Women: A Randomized, Double-Blind, Placebo-Controlled Crossover Study. Nutrients. 2022; 15: 156.
[93] Brooker S, Martin S, Pearson A, Bagchi D, Earl J, Gothard L, *et al*. Double-blind, placebo-controlled, randomised phase II trial of IH636 grape seed proanthocyanidin extract (GSPE) in patients with radiation-induced breast induration. Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology. 2006; 79: 45–51.
[94] De Groote D, Van Belleghem K, Devière J, Van Brussel W, Mukaneza A, Amininejad L. Effect of the intake of resveratrol, resveratrol phosphate, and catechin-rich grape seed extract on markers of oxidative stress and gene expression in adult obese subjects. Annals of Nutrition & Metabolism. 2012; 61: 15–24.
[95] Kar P, Laight D, Rooprai HK, Shaw KM, Cummings M. Effects of grape seed extract in Type 2 diabetic subjects at high car-

diovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxida-tive stress and insulin sensitivity. Diabetic Medicine: a Journal of the British Diabetic Association. 2009; 26: 526–531.
[96] Weseler AR, Ruijters EJB, Drittij-Reijnders MJ, Reesink KD, Haenen GRMM, Bast A. Pleiotropic benefit of monomeric and oligomeric flavanols on vascular health–a randomized controlled clinical pilot study. PloS One. 2011; 6: e28460.
[97] Vinson JA, Proch J, Bose P. MegaNatural((R)) Gold Grapeseed Extract: In Vitro Antioxidant and In Vivo Human Supplementation Studies. Journal of Medicinal Food. 2001; 4: 17–26.
[98] Irandoost P, Ebrahimi-Mameghani M, Pirouzpanah S. Does grape seed oil improve inflammation and insulin resistance in overweight or obese women? International Journal of Food Sciences and Nutrition. 2013; 64: 706–710.
[99] Mellen PB, Daniel KR, Brosnihan KB, Hansen KJ, Herrington DM. Effect of muscadine grape seed supplementation on vascular function in subjects with or at risk for cardiovascular disease: a randomized crossover trial. Journal of the American College of Nutrition. 2010; 29: 469–475.
[100] Polagruto JA, Gross HB, Kamangar F, Kosuna KI, Sun B, Fujii H, *et al*. Platelet reactivity in male smokers follow-ing the acute consumption of a flavanol-rich grapeseed extract. Journal of Medicinal Food. 2007; 10: 725–730.
[101] Shenoy SF, Keen CL, Kalgaonkar S, Polagruto JA. Effects of grape seed extract consumption on platelet function in postmenopausal women. Thrombosis Research. 2007; 121: 431–432.
[102] Ward NC, Hodgson JM, Croft KD, Burke V, Beilin LJ, Puddey IB. The combination of vitamin C and grape-seed polyphe-nols increases blood pressure: a randomized, double-blind, placebo-controlled trial. Journal of Hypertension. 2005; 23: 427–434.
[103] Ras RT, Zock PL, Zebregs YEMP, Johnston NR, Webb DJ, Draijer R. Effect of polyphenol-rich grape seed extract on ambulatory blood pressure in subjects with pre- and stage I hypertension. The British Journal of Nutrition. 2013; 110: 2234–2241.
[104] Alirezaei AH, Barough AS, Azizi T, Shirzadeh Barough S, Ghorbanihaghjo A, Rashtchizadeh N, *et al*. Anti-inflammatory effects of grape seed extract in hemodialysis patients; a pilot study. Journal of Renal Injury Prevention. 2016; 6: 184–187.
[105] Bazán-Salinas IL, Matías-Pérez D, Pérez-Campos E, Pérez-Campos Mayoral L, García-Montalvo IA. Reduction of Platelet Aggregation From Ingestion of Oleic and Linoleic Acids Found in Vitis vinifera and Arachis hypogaea Oils. American Journal of Therapeutics. 2016; 23: e1315–e1319.
[106] Rao Y, Vidyasagar S, Bairy KL. A 45-day randomized, open-label, comparator study to evaluate the safety and efficacy of zincovit tablets with grape seed extract (Nutritional food supplement) in patients with type 2 diabetes mellitus. Asian Journal of Phar-maceutical and Clinical Research. 2016; 9: 220–222.
[107] Martínez-Maqueda D, Zapatera B, Gallego-Narbón A, Vaquero MP, Saura-Calixto F, Pérez-Jiménez J. A 6-week supplementation with grape pomace to subjects at cardiometabolic risk ameliorates insulin sensitivity, without affecting other metabolic syn-drome markers. Food & Function. 2018; 9: 6010–6019.
[108] Odai T, Terauchi M, Kato K, Hirose A, Miyasaka N. Effects of Grape Seed Proanthocyanidin Extract on Vascular Endothe-lial Function in Participants with Prehypertension: A Randomized, Double-Blind, Placebo-Controlled Study. Nutrients. 2019; 11: 2844.
[109] Kim J, So WY. Effects of acute grape seed extract supplementation on muscle damage after eccentric exercise: A randomized, controlled clinical trial. Journal of Exercise Science and Fitness. 2019; 17: 77–79.
[110] Annunziata G, Ciampaglia R, Maisto M, D'Avino M, Caruso D, Tenore GC, *et al*. Taurisolo®, a Grape Pomace Polyphenol Nutraceutical Reducing the Levels of Serum Biomarkers Associated With Atherosclerosis. Frontiers in Cardiovascular Medicine. 2021; 8: 697272.