MegaNatural Gold™

(GRAPE SEED EXTRACT)

TECHNICAL PUBLICATION I

LITERATURE REVIEW OF GRAPE POLYPHENOLS AND THEIR BENEFICIAL HEALTH ASPECTS

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SUMMARY

The purpose of this technical review is to provide information for the benefit of scientists engaged in the formulations of new nutraceutical dietary supplements for human health and well being.

This review highlights the complex relationship between wine polyphenols and their beneficial health aspects which includes decreased LDL oxidation, increased HDL cholesterol content and decreased platelet aggregation. There appears to be a clear association between the epidemiological observation popularly known as the French Paradox and the mounting evidence that polyphenols in wines are very powerful antioxidants.

The effects of extracted grape seed tannins (proanthocyanidins) on *in vivo* inhibition of LDL/VLDL formation, increased level of HDL formation and increased secretion of large amounts of cholesterol in feces, strongly point toward beneficial control of atherosclerosis. The other benefits indicated vasodilation, modulation of inflammatory response and removal of cholesterol from the intestine by absorption.

For a clear understanding on the types of grape phenolics, a section has been devoted to the composition of polyphenols of seeds which includes classification of flavan-3-ol monomers, oligomers and polymers.

A very brief section on the biochemistry/etiology of atherosclerosis and a proposed mode of action of antioxidants is included.

Finally, we provide a brief description of MegaNatural Gold,™ a trade name for Grape Seed Extract developed by Polyphenolics, Inc., and highlight its primary distinctions and marked differentiation from other commercially marketed products. The design and formulations of MegaNatural Gold™ are based upon some of the key observed benefits discussed in this technical review.

We hope that this technical review will be judiciously used by scientists and technologists who are constantly looking for good science. A collection of key important references at the end of this review will provide detailed information on experimental protocols and results.

I. FRENCH PARADOX

Atherosclerosis and coronary heart diseases (CHD) have been linked to excessive consumption of dietary saturated fat and cholesterol.[1] However, examination of World Health Organization epidemiological data showed marked differences in CHD mortality among various countries and regions. An intriguing anomaly existed in a certain French region, where subjects, in spite of high saturated fat consumption, comparable plasma cholesterol and similar risk factors showed considerably lower incidence of death from CHD than Americans did. This apparent discrepancy popularly known as the French Paradox triggered a scientific scrutiny for this advantageous anomaly.[2] ***Table 1*** recreated from Renaud and Lorgeril (1992) shows CHD mortality against plasma cholesterol levels for major world countries.[3]

TABLE 1

MORTALITY FROM CORONARY HEART I (AGE STANDARDIZED MALE, 35-64 YEARS)

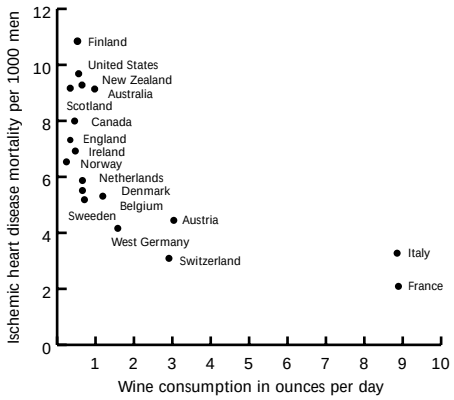
	Mortality per 10^4	Plasma Cholesterol (mg/dl)
Japan	33	—
Toulouse (Fr)	78	224
France (Gen)	102	216
Stanford (USA)	182	209
United Kingdom	380	240

(Renaud and de Logeril, 1992)

Multivariate analysis showed that consumption of wine was the only dietary factor that showed a negative correlation with CHD, suggesting that intake of wine counteracts the effects of saturated fat and reduces the incidence of CHD mortality.[2,3] ***Figure 1*** shows the general correlation between wine consumption and the incidence of mortality from CHD.

The epidemiological data also suggested that alcohol consumption, especially red wine, showed superior protection compared to other beverages such as beer, thus, providing evidence that the beneficial effects of red wine are, at least in part, contributed by components other than alcohol. This was later confirmed by a Danish study which showed that CHD mortality was significantly reduced in populations that consume wine. This study did not find life-prolonging benefits from drinking other forms of alcohol.

FIGURE 1



(Renaud and de Logeril, 1992)

II. WINE PHENOLICS AS ANTIOXIDANTS

In vitro studies

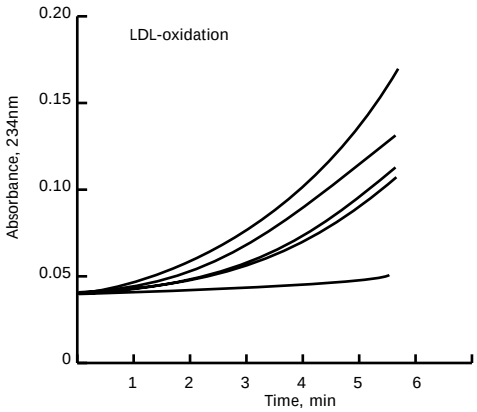
Immediately after the inception of the French Paradox linkage with red wine consumption, a research team at the University of California at Davis (U.C. Davis) has aggressively pursued the effects of wine phenolics in the inhibition of human LDL oxidation through *in vitro* studies. These studies do provide a mechanism for the effectiveness of polyphenols in inhibiting LDL oxidation either through increased wine consumption or direct consumption of polyphenols from nutraceutical preparations. These associations from *in vitro* biological observations, *in vivo* evidence, the origin and nature of wine phenolics and the explicit chemistry of grape seed phenolics tend to provide strong evidence for the beneficial health effects of grape seed extracts rich in phenolics.

This section will provide information on *in vitro* studies. Frankel et al. (1993)[4] from UC Davis, studied the effects of wine phenolics on the oxidative susceptibility of LDL *in vitro,* by measuring the hexenal and conjugated dienes formed by Cu^{2+} catalyzed oxidation of freshly prepared human LDL. The copper catalyzed oxidation of LDL was inhibited by red wine diluted with water and by red wine phenolics

in water. These inhibitions ranged from 50 to 98%. Natural flavonoids can donate hydrogen to and/or react with superoxide anions, hydroxyl radicals and lipid peroxyl radicals, all of which can cause lipid peroxidation *in vivo*, leading to LDL oxidation, implicated in the development of atherosclerosis. Circulating LDL are one of the fundamental targets of deleterious oxidation, resulting in the accumulation of atherogenic lipoproteins *in vivo*.

Kenner et al. (1994)[5] compared the inhibition of LDL oxidation by wine phenolics and α-tocopherol. ***Figure 2*** shows that wine phenolics were twice as effective as a-tocopherol in inhibiting the LDL oxidation in *in vitro* studies. Very effective antioxidants were found in all grape varieties and wines tested, and corresponded to the concentrations of the phenolics tested.

FIGURE 2



Inhibition by wine phenolics (Petite Sirah) and α-tocopherol of low-density lipoprotein peroxidation by copper ions at pH 7.4, 23°C. C, control; 1-T, 1μM α-tocopherol, 2-T, 2μM α-tocopherol; 1w, 1μM wine phenolics; 2w, 2μM wine phenolics. (Kenner et al., 1996).

Frankel et al. (1995)[6] elaborated the antioxidative activity of twenty commercial California wines in inhibiting LDL oxidation *in vitro*. The relative inhibitions varied from 37 to 65% with the red wines and 3 to 7% with white wines. The relative antioxidative activity correlated with total phenol content of wines (r = 0.94), concentration of gallic acid (r = 0.92), catechin (r = 0.76) and malvidin 3,5-diglucoside (r = 0.38). Thus, the capacity to protect LDL from oxidation appears to be distributed widely among a large number of phenolic constituents in wine.

Meyer et al. (1997)[7] compared phenolic extracts from fourteen different types of fresh grapes in inhibiting human LDL oxidation *in vitro*. Relative antioxidant activity correlated with the concentration of total phenols (r = 0.89), anthocyanins (r = 0.56) and flavonols (r = 0.54). When grape seed crushing and longer extraction times were employed, higher amounts of flavan-3-ols and hydroxybenzoates were extracted. With these extracts, relative LDL antioxidative activity correlated highly with the levels of flavan-3-ols (r = 0.86), total phenols (r = 0.79) and hydroxybenzoates (r = 0.77). This clearly demonstrates that seed extractions provided greater concentration of flavan-3-ol flavonoid compounds of increased antioxidative potential in inhibiting LDL oxidation.

Vinson et al. (1995)[8] demonstrated, for the first time, the lipoprotein bound activity of phenols, particularly flavonoids, thus suggesting that phenols can bind to LDL as do tocopherols. Thus, this study showed that polyphenols can bind to lipoprotein as expected *in vivo* before exerting the beneficial effect as antioxidants.

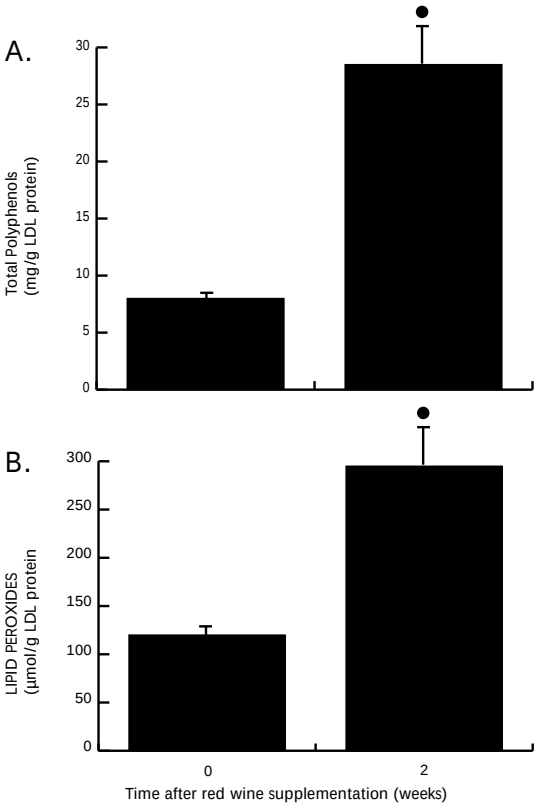
In vivo studies

Various epidemiological studies on the beneficial effects of wine consumption (French Paradox) and a recent Danish study confirm that CHD mortality is significantly reduced in human populations that consume wine along with the diet. The implications of red wine phenolics as well as grape phenolics in inhibiting LDL oxidation have been consistently demonstrated by *in vitro* studies.

A recent clinical study conducted by Fuhrman et. al. (1995)[9] compared the effects of red wine and white wine consumption on the susceptibility of plasma LDL to lipid peroxidation. This clinical trial was based upon seventeen healthy males (ages 25–45 years old). Half the subjects received (400 mL per day) red wine and half received (400 mL per day) white wine with no changes in the habitual diet and life style. This study demonstrated that daily consumption of red wine for two weeks reduced the susceptibility of plasma LDL to lipid peroxidation while white wine showed the opposite effect. This provides important evidence that polyphenols of wines, and not alcohol, per se, inhibited LDL oxidation. This study also showed a dramatic increase in the total phenol concentration in the LDL fraction after red wine consumption. ***Figure 3*** shows the effect of red wine consumption on phenol content and LDL oxidation inhibition. The authors proposed that phenolic substances in red wine with antioxidative properties are absorbed from the gut into circulation, bind

to LDL and thus exert their antioxidation effects. This study also confirmed that antioxidative activity was related to the effects of red wine phenolics and not related to the depletion of carotenoids, vitamin A and α-tocopherol, whose levels remained fairly steady in the plasma during the period of this study.

FIGURE 3

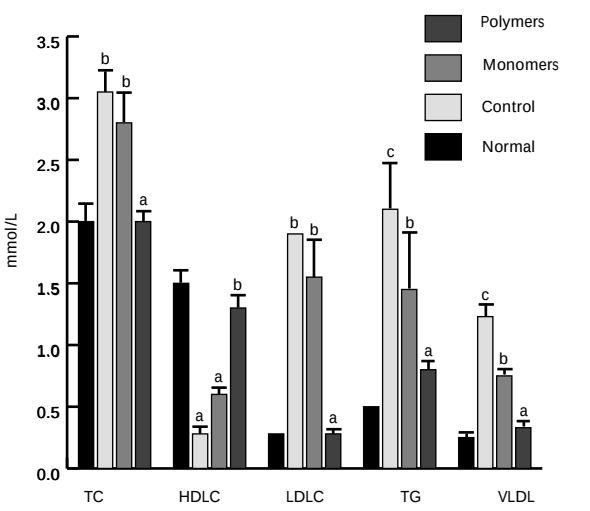


The effect of red wine consumption on LDL associated polyphenols and on LDL susceptibility to lipid peroxidation. Total phenol content of LDL (A) was determined as described in Methods. LDL susceptibility to lipid peroxidation was determined after lipoprotein incubation with copper ions by analysis of the LDL lipid peroxide content. ± SEM; n=8. P <0.01 (vs 0 time). (Furhman et al., 1995).

III. EFFECTS OF GRAPE SEED TANNINS

Tebib et al. (1994)[10] conducted a study of extracted monomeric and polymeric grape seed tannins (procyanidins) on lipoproteins and lipid concentration in rat plasma. The results of this work indicated that dietary polymeric grape seed tannins (2% level in diet) reduced plasma total cholesterol, triglycerol and LDL cholesterol in high cholesterol fed rats. Furthermore, the consumption of grape seed polymers led to significantly lower concentrations of plasma VLDL (very low density lipoproteins) and greater concentrations of plasma HDL cholesterol compared to those in a control group. It was shown that monomeric and polymeric tannin fractions had differential effects; monomers lowered triglycerol and VLDL but not total HDL or LDL concentrations as shown in Figure 4. This study provides direct evidence that grape tannins, also referred to as OPC (oligomeric proanthocyanidins) exert very strong biologic effects in lowering low density lipoprotein concentrations in the plasma of rats.

FIGURE 4



Concentration of plasma total cholesterol TC, high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC), triacylglycerol (TG) and very low density lipoproteins (VLDL) in rats receiving a low fat diet (normal) or hypercholesterolemic diets without added tannins (control) or with added monomeric or polymeric grape seed tannins. (Tebib et al., 1994)

This study also supports the view that the antihypercholesterolemic effect of grape seed polymeric tannins in rats can be attributed to the inhibition of intestinal cholesterol absorption, since cholesterol was excreted in large amounts in the feces. It was also demonstrated that bile acid excretion was two fold higher in the group fed polymeric tannins than in the one receiving monomers. These findings suggest that tannins impair the intestinal absorption of bile acids and that the level of bile acid excretion is dependent on the degree of polymerization of tannins. The results suggest that the binding of the bile salts and of cholesterol to tannins may be hydrophobic in nature.

IV. CHEMISTRY OF PHENOLIC SUBSTANCES OF GRAPE SEEDS

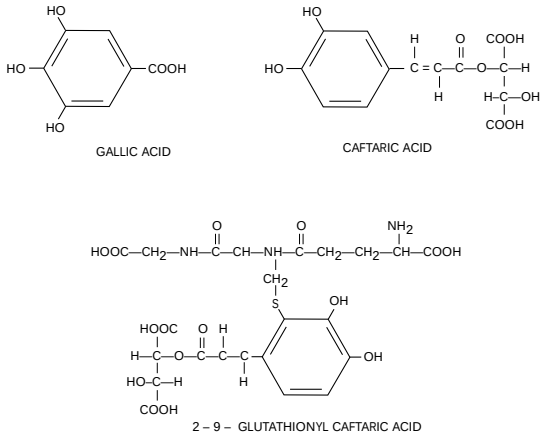
A general composition of the grape consists of 2–6% stems, 5–12% skins, 80–90% juice and 0–5% seeds. Considering several varieties with differing composition, the best estimates for total seeds are 4.4% of total weight.[11]

The phenolic substances are frequently called polyphenols which embrace many classes of compounds ranging from phenolic acids, colored anthocyanins, simple flavonoids and complex flavonoids. A major difference between white and red grapes is that anthocyanins are the major coloring matter of red grapes. The pigment is confined to the skin tissue of the berry and little appears in the must or juice unless appreciable maceration releases them from the skins. Grape seeds, if present, also contain large amounts of flavonoid derivatives and may contribute to juice or wine depending upon the extent of contact time, severity of processing and/or fermentation on the skins and seeds.

In white wine and juice production, the juice is normally separated from skins and seeds. In essentially all red wine production, the berries are crushed and fermented. Therefore, the total phenol content of red wine could be substantially higher than white wine and rich in flavonoids (both anthocyanins and other flavonoids). The rich red wines prior to aging may contain 3000–6000 ppm total phenols, which on stabilization, may provide a wine with approximately 2000 ppm total phenols or less depending upon style, etc, Singleton (1988).[12]

The major nonflavonoid phenols in juices made without appreciable contact with skins and seeds are referred to as phenolic acids and cinnamic acid derivatives. The cinnamic acid derivatives have been further elaborated into coutaric acid, caftaric acid (3-deoxycaftaric acid); fertaric acid (3-methoxy caftaric acid). The simplest phenolic acid is gallic acid, which also is implicated in flavonoid chemistry. ***Figure 5*** shows chemical structures of gallic acid and caftaric acid and oxidation product of caftaric acid found in white wine, Singleton (1988).[12]

FIGURE 5



Examples of important nonflavonoid phenols of wine. Coutaric acid = 3-deoxycaftaric acid; fertaric = 3-methoxycaftaric acid.

Anthocyanins, the red pigments associated with red grape varieties, belong to the flavonoid class and are a fairly complex group of compounds in red wine or red juice. These anthocyanin pigments are glycosylated and some of them acylated. Nagel and Wulf (1979)[13] were able to separate 16 different anthocyanin pigments from red Cabernet Sauvignon wine.

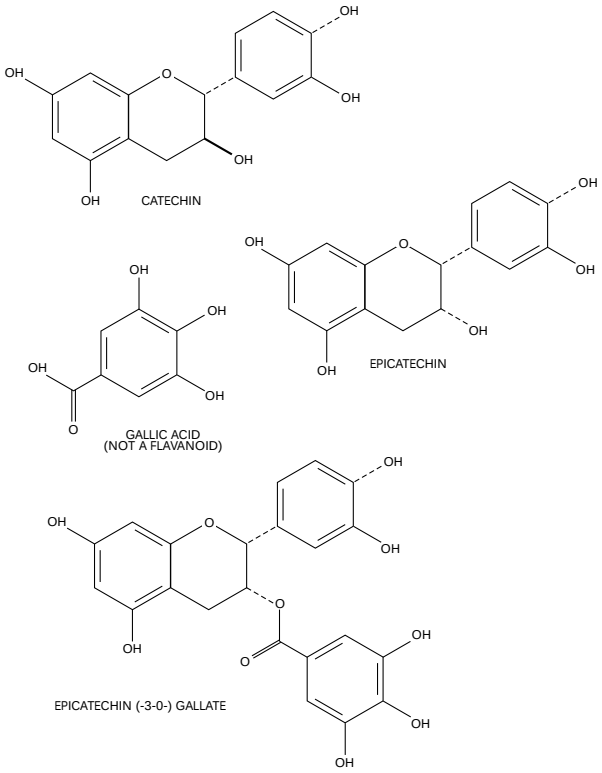
Grape Seed Phenols

The phenolic substances have considerable importance as food constituents. The berry's total extractable phenols are present in only about 10% or less in pulp with remaining two-thirds in the seeds and one-third in the skin. The seeds are an important source of phenols in grape products, particularly red wines.[14] The seeds are highest in phenol content and may contain from 5–8% phenol by weight.[11]

The phenol composition of grape seeds is essentially all flavonoids. The terminology of flavonoids in grape seeds is referred to as monomeric flavan-3-ols. These are monomeric compounds and have been identified as (+) - catechin; (-) -epicatechin and (-) - epicatechin-3-gallate; Su and Singleton (1969).[14] ***Figure 6*** shows the structure of these compounds and gallic acid; a nonflavonoid which is attached to epicatechin in epicatechin-3-*0*-gallate.

FIGURE 6

CATECHINS (MONOMERS, SUBUNITS OF PROCYANDINIS)



The flavan-3-ol repeated molecules joined together are extensively distributed in grape seeds and are frequently referred to as procyanidins. In a classical chemistry sense, it means that they produce cyanidin (an anthocyanidin) on acid hydrolysis, as contrasted with flavan-3-o1 molecules which do not.

The proanthocyanidins as a class of natural products have been intensively studied over the past two decades by groups led by Weinges[15] and Haslam.[16, 17, 18] The old terminology of leucocyanidin or leucoanthocyanidin is reserved for monomeric flavan-3, 4-diols, which are not major phenolic components in fruits. The proanthocyanidins are also condensed tannins.

Andrew et al.[19], were able to separate the oligomaric proanthocyanidins from wines made with extensive contact with skins and seeds and identified five different dimers (B_1, B_2, B_3, B_4, B_5) and two stereoisomeric trimers. These dimers and trimers are made of catechins and epicatechins.

With the advent of HPLC, a rapid progress was made in the separation of procyanidins. Thorngate and Singleton (1994)[20] extracted whole seeds of two grape varieties as well as the outer coat and endosperm of seeds. The monomeric and polymeric flavan-3-ols of grape seeds are found to be localized in the outer parenchymous cell (soft seed coat) layer with endosperm being much lower in flavan-3-o1 (monomeric) material.

Fuleki and Ricardo-da-Silva (1997)[21] compared the catechin (monomeric) and procyanidin (oligomeric) composition of seeds of 17 vinifera, hybrid and labrusca type red and white grape cultivars. A total of 11 monomers, dimers, and trimers were separated and identified by reverse phase HPLC. The data suggested that the quantities of catechins and procyanidins were influenced by genetic factors. In general the seeds of red grape cultivars contained higher quantities of these compounds than the whites.

Although the presence of flavan-3-o1 monomers, dimers and trimers has been extensively reported, there exists considerable evidence that the degree of polymerization can reach much higher numbers. Prieur et al. (1994)[22] employing gel permeation chromatography and normal phase HPLC were able to predict degrees of polymerization of 16 units.

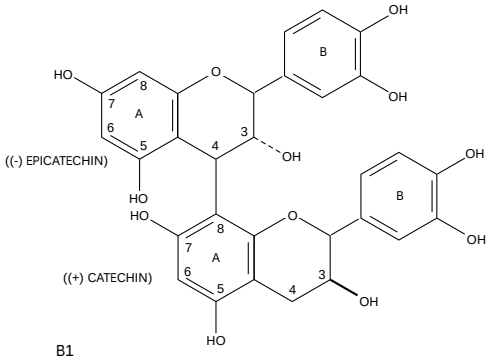
Shirley and Waterhouse (1997)[23] using normal phase HPLC could quantitatively separate monomeric, oligomeric and polymeric flavonoids of grape seeds and indicated that the procedure provides group separation based on the degree of polymerization.

There is, however, a great deal of discrepancy in the literature. It is surprising that Fulcki and Ricardo-da-Silva[21] did not report these higher molecular weight polymers. It is possible that higher polymers observed by other workers may be due to oxidative polymerization after extraction from seeds.

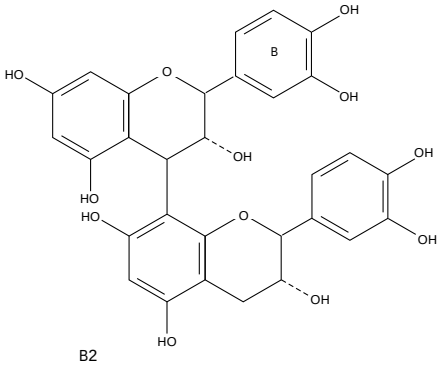
Figure 7 shows the general chemical structures of dimers, trimers, tetramers based upon repeated molecules of flavan-3-ols (monomers). Shirley and Waterhouse (1997)[23] predicted 16 dimers, 64 trimers, 256 tetramers and 1,024 pentamers as possible configurations of flavan-3-ols present in grape seed extracts.

FIGURE 7

STRUCTURES OF PROCYANIDINS FROM GRAPE SEEDS

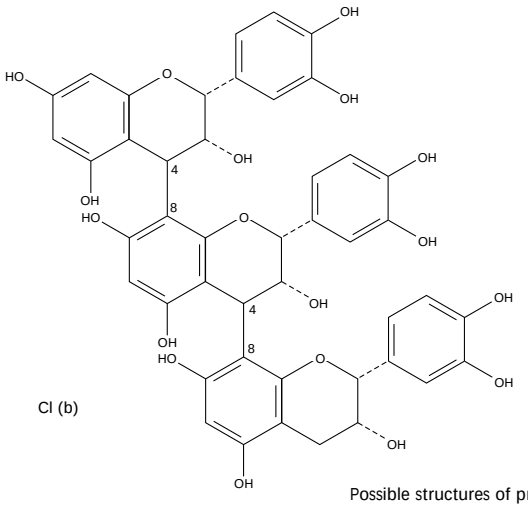


B1

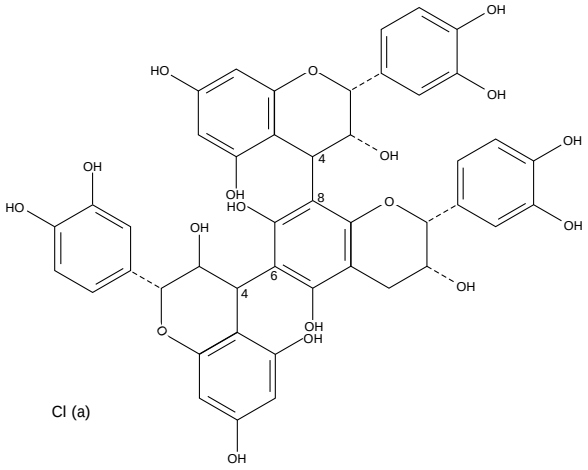


B2

Dimer structures frequently reported in grape seeds

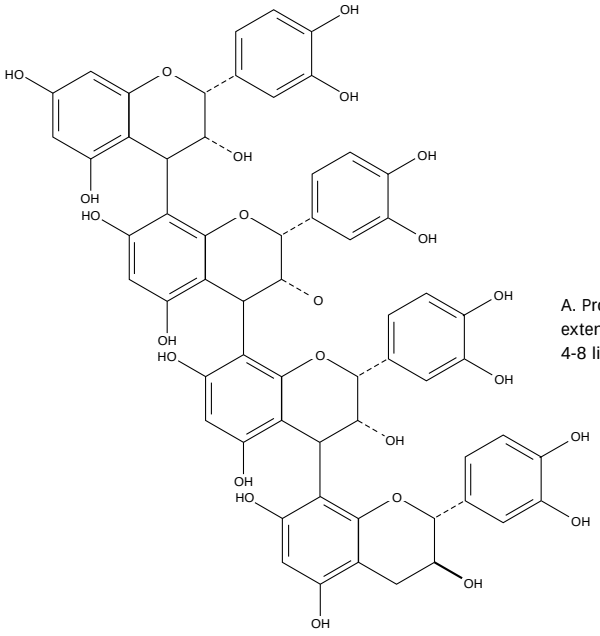


Cl (b)



Cl (a)

Possible structures of procyanidin trimers in grape seeds.
1. Epicatechin extensions units with 4-8 linkage.
2. 4-8 and 4-6 linkages.

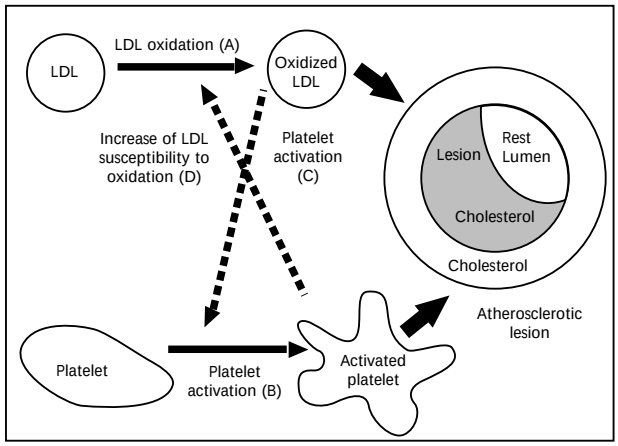


A. Procyanidin tetramer composed of 3 epicatechin extension units with a catechin terminal unit joined by 4-8 linkages

V. MECHANISM OF ANTIOXIDATION OF GRAPE POLYPHENOLS

Process of Atherosclerosis: Aviram[24] has presented an excellent review of the events leading to atherosclerosis. Atherosclerosis is associated with endothelial dysfunction, platelet activation, lipoprotein aggregation, macrophage foam cell formation, inflammation and thrombosis. These events are closely related and interact among themselves, finally leading to the formation of atherosclerotic lesions. Atherosclerosis involves both LDL oxidation and platelet formation (***Figure 8A & B***) and both of these processes can affect each other. Platelet activation is increased by oxidative stress, and oxidized LDL, in turn, were shown to enhance platelet activation (***Figure 8C***). Activated platelets in turn can increase LDL oxidizability.

FIGURE 8

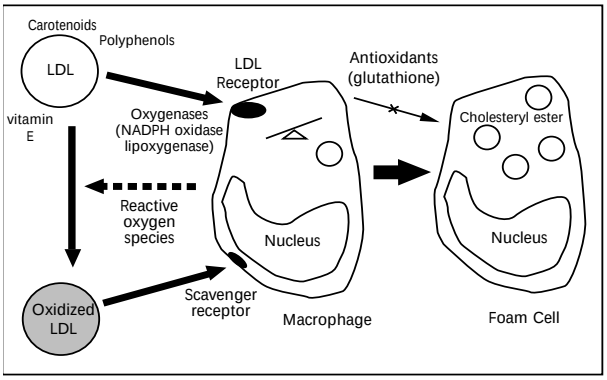


Oxidized LDL, platelet activation and atherosclerosis. LDL oxidation (A) and platelet activation (B) are two key events in atherogenesis, which lead to the formation of the atherosclerotic lesion. These processes are interrelated, in that oxidized LDL can activate platelets (C), and activated platelets increase the susceptibility of LDL to oxidation (D). (Aviram, 1996).

Antioxidant Intervention of LDL Oxidation

Any means of intervention that favorably affects the balance between the activity of the macrophage oxygenases and cellular antioxidants, as well as elevation of LDL associated antioxidants or extrinsic antioxidants in plasma or extracellular space, can possibly contribute to the inhibition of the atherosclerolic process (***Figure 9***). It was recently demonstrated that polyphenols in olive oil, red wine and licorice root extracts act as antioxidants of LDL oxidation *in vivo* (Fuhrman et al., (1995)[9]. Strategies to reduce LDL oxidation thus involves the use of various antioxidants which synergistically act on the cells and/or on the LDL molecules. (Aviram, 1996).[24]

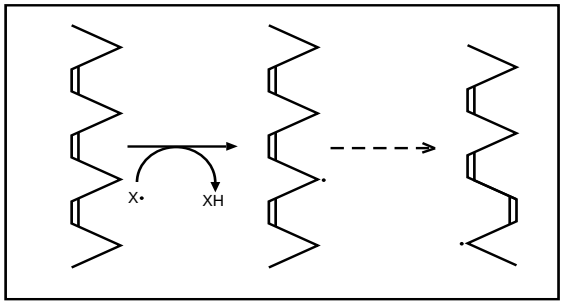
FIGURE 9



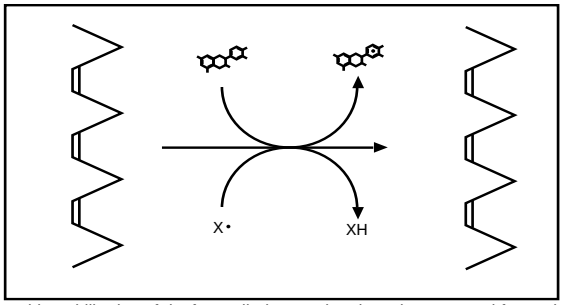
Lipoproteins and antioxidants. LDL oxidation by macrophages is affected by both the LDL-associated antioxidants (vitamin E, carotenoids, polyphenols), and by the balance between cellular antioxidants [such as glutathione (GSH)] and cellular oxygenases [such as NADPH oxidase and lipoxygenases]. Oxidized LDL but not native LDL lead to the conversion of macrophages to cholesterol [cholesteryl ester and unesterified cholesterol]-laden foam cells (Aviram, 1996).

In very simple terms, flavonoids inhibit the oxidation of polyunsaturated fatty acid groups in LDL by intercepting the free radical propagation of LDL oxidation (***Figure 10***).

FIGURE 10



Free radical attack of a polyunsaturated fatty acid group present in LDL



Catechin stabilization of the free radical protecting the polyunsaturated fatty acid

VI. OTHER HEALTH BENEFITS OF GRAPE POLYPHENOLS

Intestinal Cholesterol Absorption

Tebib, et al. (1994)[10] observed that fecal cholesterol excretion by rats fed the polymeric tannins (procyanidins) was approximately twice as high as in the control rats and was significantly higher for animals fed polymers than for those fed monomers. The effect of grape seed polymeric tannins in rats can be attributed to a reduction or an inhibition of intestinal cholesterol absorption, since cholesterol is excreted in large amounts in the feces. The binding of cholesterol to tannins may be of a hydrophobic nature. This mechanism of cholesterol removal in the intestinal lumen may be similar to that of dietary fiber.

Anti-Inflammation

Tixier, et al. (1994)[25] provided conclusive evidence that procyanidins bind to thin elastin fibers when injected intradermally into young rabbits. This resulted in a resistance of the fibers to the hydrolytic action of porcine pancreatic elastase when injected at the same site. These *in vivo* studies further emphasized the potential beneficial effect of these compounds in preventing elastin degradation by elastases as occurs in inflammatory processes.

Vasodilation

Cishek, et al. (1997)[26] recently indicated that red wine causes an endothelium dependent relaxation (EDR) in aortic rings of New Zealand white rabbits. Alcohol treatment alone did not show EDR. A second series of experiments indicated that flavonoid monomers such as catechin, epicatechin and quercetin had no effect upon aortic rings. However, oligomeric procyandins found in MegaNatural Gold™ produced a dose dependent EDR.

Dr. Tissa Kappagoda and colleagues[27,28] of the division of cardiovascular medicine, at the University of California, Davis, have demonstrated that constituents present in MegaNatural Gold™ grape seed extracts relax isolated blood vessels from rabbits, thereby documenting the potential benefit of the extract in increasing blood flow in blood vessels. Compounds present in MegaNatural Gold™ relax the isolated blood vessels by a pathway in which nitric oxide production is implicated. Oligomeric proanthocyanidins found in MegaNatural Gold™ appeared to be the key active substances. The novel findings revealed that only the oligomers possessed blood vessel relaxing activity. MegaNatural Gold™ possesses highly bioactive special components of these flavonoids in a unique composition with consistent proportions of the different chemical constituents.

The above research findings have far reaching implications in light of the nitric oxide studies of the Nobel Prize winners, Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad. The three pharmacologists won the 1998 Nobel Prize for medicine, for discovering that nitric oxide produced by the body acts as a signal, influencing blood vessels to dilate which, in turn, lowers blood pressure. This property is deemed significant for the prevention and treatment of heart diseases.

The identification of naturally occurring blood vessel relaxing flavonoids, such as those found in MegaNatural Gold ™ that can modulate the production of nitric oxide in body cells is an exciting prospect. The UC Davis scientists are conducting further studies on MegaNatural Gold™ to delineate clearly its mechanism of action and to identify the most active poliphenol fractions responsible for this action.

VII. MegaNatural Gold™—A PRODUCT OF DISTINCTION

Canandaigua Brands, Inc., the parent company of Polyphenolics Inc., entered the grape seed extract market because it had distinct advantages over its European competitors.

- A diversified business of wine and grape juice concentrate with enormous availability of grape seeds. European producers rely on collection of seeds from small wine operations.
- A distinct advantage of seeds obtained from juice operations, where polyphenols essentially remain intact as opposed to red wine seeds in which a majority of polyphenols are partitioned into wine.
- A proprietary blend of white and red grape seeds allows for consistency of MegaNatural Gold ™ products.
- The seeds are immediately separated from pomace and frozen to maintain the quality of the seeds and the polyphenols.
- A proprietary process developed to produce a product with a consistent composition of

monomers, oligomers and polymers in order to provide several key biological benefits mentioned in the published literature.

- The scientists associated with our Research & Development have developed a distinct approach and use four different analytical procedures based upon intricate chemistry to maximize molecular and quantitative information on MegaNatural Gold™. Such procedures provide reliable estimates of percent catechin equivalents, percent flavonoids in extract, average degree of polymerization as well as qualitative profiles of extracts by HPLC.
- These particular aspects of analytical procedures and the composition of polyphenols in MegaNatural Gold™ are a subject of a second technical communication.
- Polyphenolics, Inc. has established research contracts with reputed scientists at the University of California at Davis, for the development of analytical procedures for MegaNatural Gold™ as well as for the evaluation of the efficacy of MegaNatural Gold™ in relaxing blood vessels in experimental animals, and inhibiting LDL oxidation in human subjects. The key findings from this research will be published as separate technical communications.

VIII. LITERATURE CITED

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