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Review

Health aspects of functional grape seed constituents

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Functional ingredients of grape seeds include several flavonoids with a phenolic nature such as monomeric flavanols (catechin and epicatechin), dimeric, trimeric and polymeric procyanidins, and phenolic acids (gallic acid and ellagic acid). These flavonoids have been reported to exhibit antioxidant activity *in vivo* and *in vitro* in a number of studies. The antioxidant activity of flavonoids is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders. In this study, we present a review of functional components of grape seeds or skins with emphasis on health benefits of their use in the human diet.

Introduction

The flavonoids are generally categorized as phenolics or polyphenols because of their chemical structure (Fig. 1). Over 4000 flavonoids have been identified. Flavonoids can be defined as 'a class of plant secondary metabolites derived from the condensation of a cinnamic acid with three malonyl-CoA groups' (Bloor, 2001). Colored flavonoids are responsible for the color of many fruits and vegetables, but numerous colorless flavonoids are also present in nature. Small differences in chemical structure changes the UV spectra of the flavonoids making it easy to differentiate one from the other and quantify the concentration of specific compounds in a sample.

Flavonoids can be divided into 14 classes based on the level of oxidation in the structure of ring C as shown in Fig. 1 (Seigler, 1995). The major dietary flavonoids are classified under six groups (Peterson & Dwyer, 1998) as shown in Fig. 2. These flavonoids are present in nature as glycosides. The sugar moiety attached to the flavonoid structure affects ease of absorption from the intestinal tract and the bioavailability of the compound. For example, glycosides of the flavonoid quercetin in onions are more readily absorbed than aglycons (Hollman & Katan, 1999). Glycosylation lessens the reactivity of flavonoids against free radicals and increases their solubility in water (Rice-Evans, Miller, & Paganga, 1997). Glucose is the main sugar moiety in glycosylated flavonoids, but galactose, rhamnose, xylose and arabinose can also occur.

The most common flavanols found in plants are catechin, epicatechin, gallocatechin and epigallocatechin. Dimeric, trimeric, oligomeric and polymeric catechins are also present in a number of fruits, vegetables, tea and wine besides these catechin monomers. Plant polyphenols were once thought to be undesirable constituents of plants consumed as food due to astringency in the mouth. Astringency has been attributed to complexation of the polyphenols with glycoproteins (Haslam, 1989) in saliva and the lining of the oral cavity. Flavonoids perform diverse functions in nature. They protect plants from UV radiation, insects and mammalian herbivory (Haslam, 1989). In addition, they exhibit antimicrobial and antioxidant activity and they may protect against or cure human diseases (Harborne & Williams, 2000). These compounds also play a role internally in plants as a signaling mechanism to regulate growth (Woo, Kuleck, Hirsch, & Hawes, 2002).

Some of the polyphenolics are known to have antioxidant activity *in vivo*. Many herbal medicines used to treat vascular, viral, gastrointestinal and microbial diseases and inflammatory diseases may contain plant polyphenols (Haslam, 1989). The preventive or therapeutic medicinal properties of flavonoids make it desirable to consume fruits, vegetables and leaf, bark or root infusions that contain these compounds. The average daily intake of flavonoids in the US diet may be only a few hundred milligrams (Hollman & Katan, 1999). Dietary intake of flavonoids is usually underestimated because of the unreliability of data on the amount of all flavonoids present in the foods consumed.

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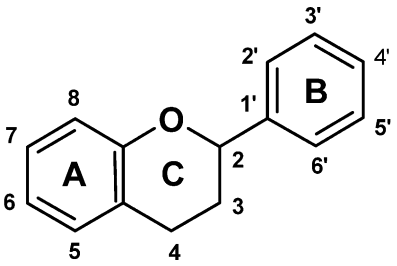

Fig. 1. Basic monomeric structure of flavonoids.

Peterson and Dwyer (1998) reported that dietary intake of flavonoids could reach 1 g/day. Hollman and Katan reported that in the Netherlands, approximately half of the daily flavonol and flavone intake comes from tea.

The biological effects of flavonoids in reducing the risk of cardiovascular diseases are possibly associated with their antioxidant properties. These effects also include protection of tissues against free radical attack and lipid peroxidation. The effects of flavonoids on atherosclerosis, cancer, inflammation, and plasma cholesterol level have been investigated by many researchers. However, conflicting results have been reported in epidemiological studies which seek to establish an inverse relationship between incidence of cancer and dietary flavonoid intake (Hollman & Katan, 1999).

Flavonoids easily scavenge aqueous free radicals (Teissedre & Landrault, 2000) because of their amphipatic characteristics (Riou, Vernhet, Doco, & Moutounet, 2002). Polyvalent phenols in the flavonoid molecular structure allow some flavonoids to chelate metal ions. Phenolic acids, precursors of flavonoids, such as hydroxycinamic (caffeic, coumaric, ferulic, and sinapic acids), hydroxycoumarin (scopoletin), and hydroxybenzoic acids (ellagic, gallic, and vanillic acids) can form complexes

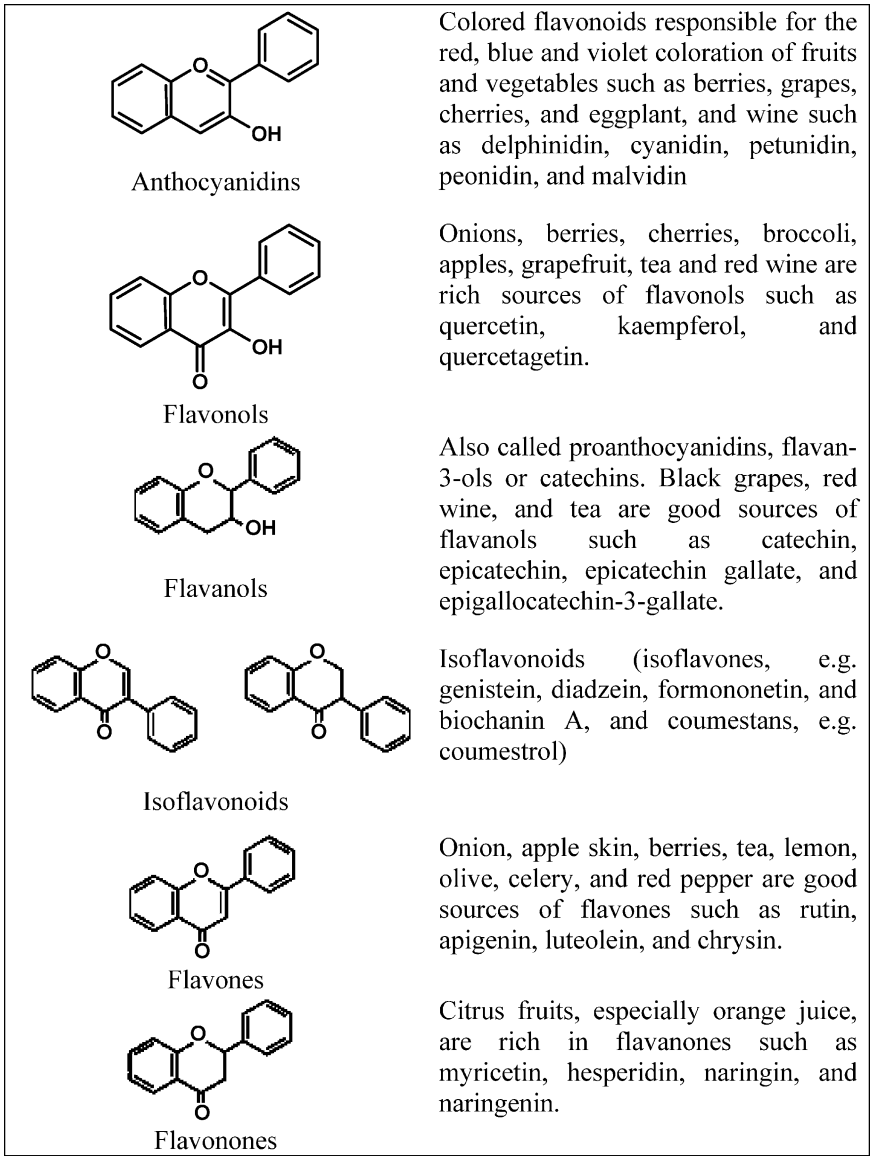

Fig. 2. Molecular structures and sources of major dietary flavonoids.

with metals (Reische, Lillard, & Eitenmiller, 1998). Flavonoids can also scavenge superoxide anions. Quercetin, myricetin and rutin can effectively inhibit either enzymatically or chemically generated superoxide anions (Robak & Gryglewski, 1988).

Cells of living organisms have two major defense mechanisms against damage induced by free radicals: enzymatic (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic (dietary antioxidants such as vitamin E and glutathione). Foods and foodstuffs which contain a variety of antioxidants, are the major agents in the non-enzymatic defense system. Flavonoids are widely present in fruits, vegetables, tea and wine with high antioxidative activities. Among the fruits, grapes and grape products are good sources of dietary flavonoids. Furthermore, the inedible component of the fruit such as the seed and skin contains some compounds that are able to scavenge superoxide radicals (Bouhamidi, Prevost, & Nouvelot, 1998). Grape seed contains high amounts of polyphenol proanthocyanidins, which are the oligomers of flavan-3-ol units, especially catechin and epicatechin. Dimeric proanthocyanidins are the simplest ones, and they have 4→8 linked monomers. B_1, B_2, B_3 and B_4 are the most common proanthocyanidin dimers. These are followed by the less common 4→6 linked isomers such as B_5, B_6, B_7 and B_8 (Fig. 3). Trimers of procyanidins have C_1 isomers. The proanthocyanidin content of grape, apple, hawthorn, elderberry, chokeberry, sour cherry and blackcurrant fruits is between 0.3 and 0.9 g/kg (Wilska-Jeszka, 1996).

Grape polyphenols, anthocyanins and proanthocyanidins play an important role in color, taste (especially astringency) and stability of red wines. Proanthocyanidins are also known as condensed tannins, and are present in grape skins and seeds as procyanidins and prodelphinidins. During

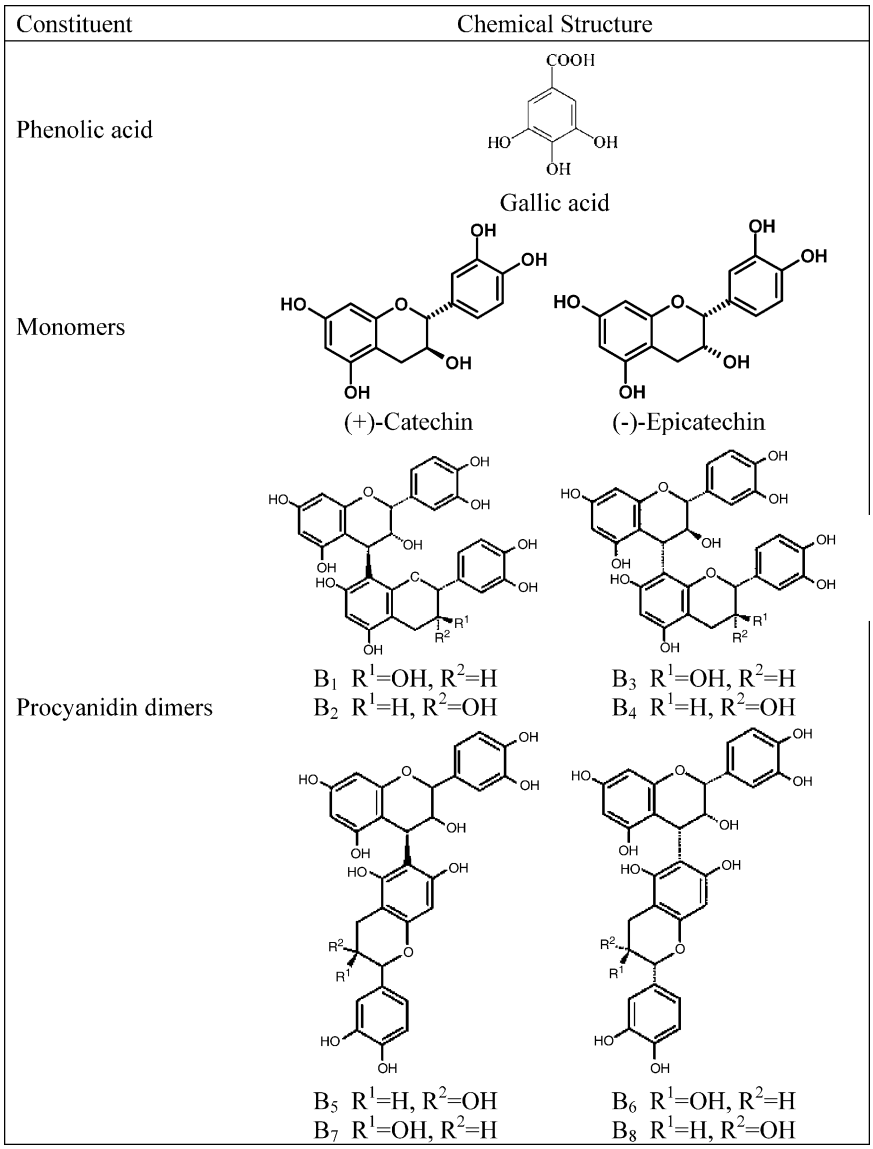

Fig. 3. Structures of phenolic acids, monomeric and dimeric proanthocyanidins present in grape seeds and skins.

Table 1. Safety test results for procyanidin B_2 (Takahashi *et al.*, 1999)

Effect	Medium	Result
Mutagenicity	*S. typhimurium* and *E. coli*	Negative
Acute subcutaneous injection	Rats	Negative
Dermal irritation	Male rabbits	Negative
Skin sensitization	Guinea pigs	Negative
Eye irritation	Rabbits	Slight irritation of conjuctivae, none in cornea or iris

fermentation of grapes these tannins are extracted from the seed and skin into the wine. The process used in wine making has a large influence on the type and level of phenolic compounds in wines.

Takahashi, Yokoo, Inoue, and Ishii (1999) evaluated the safety of procyanidin B_2 obtained from apple juice (Table 1), and found procyanidin B_2 to be non-mutagenic in either bacterial cultures, or in micronucleus tests in mice. The lethal dose 50 in animal models was >2 g/kg while no serious irritation was exhibited on the skin or eye of animals with doses up to 2 g/kg (Takahashi *et al.*). Procyanidin B_2 from apples was also found to induce *in vivo* hair growth (Takahashi, 2001).

Using dogs, monkeys and humans, Folts (2002) found that consumption of red wine (5 ml/kg) and purple grape juice (5–10 ml/kg) resulted in high blood antiplatelet activity. Moreover, consumption of purple grape juice caused further protection in the patients against the oxidation of low-density lipoprotein (LDL) cholesterol. He suggested that the flavonoids present in purple grape juice and red wine 'may inhibit the initiation of atherosclerosis'.

In summary, flavonoids have been reported to play an important role in the inhibition of carcinogenesis, mutagenesis, and cardiovascular diseases. These activities of flavonoids are perhaps related to their *in vivo* and *in vitro* antioxidative activities. Flavonoids in grape seeds have been also reported to exhibit activities against peptic ulcer and several dermal disorders.

Antioxidant activities of flavonoids

Anthocyanidins, phenolic acids and flavanols are major phenolics present in the grape berry particularly in the skin, pulp and seed. Antioxidant activities of these phenolics both *in vivo* and *in vitro* have been investigated by numerous researchers.

In vitro antioxidant activity

Flavonoids present in vegetables, fruits (especially berries) and tea are able to scavenge the radicals of hydroxyl ($^{\cdot}OH$), peroxyl ($ROO^{\cdot}$), superoxide ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$) and DPPH (1,1-diphenyl-2-picrylhydrazyl). Besides the free radical scavenging activities, flavonoids also possess metal chelating properties.

Ricardo da Silva, Darmon, Fernandez, and Mitjavilla (1991) reported that monomeric (catechin and epicatechin) and polymeric grape seed proanthocyanidins (epicatechin-3-*O*-gallate, procyanidin B_2, B_5, B_2-3-*O*-gallate, B_2-3′-*O*-gallate, C_1, and two trimers) scavenge superoxide and hydroxyl radicals but catechin monomers scavenge especially hydroxyl radicals. Another *in vitro* study indicated that epicatechin, gallic acid, and other green tea polyphenols had peroxyl radical scavenging activity and reduced low density lipoprotein peroxidation induced by benzophenone or AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride) (Liu, Ma, Zhou, Yang, & Liu, 2000).

Using the DPPH method, Cuendet, Potterat, Salvi, Testa, and Hostettmann (2000) found that *trans*-piceid, a glucoside of resveratrol, can scavenge free radicals as effectively as BHT. *trans*-Piceid was able to scavenge peroxyl radicals generated by AAPH, but this property was less than that of trolox or chlorogenic acid.

Torel, Cillard, and Cillard (1986) found that flavonoids including catechin, quercetin and kaempferol inhibited linoleic acid oxidation more efficiently than methyl linolenate oxidation. Moreover, the authors reported that the ability of flavonoids to donate protons terminates the oxidation chain reaction of lipids. Determining the antioxidant activity of resveratrol, quercetin and oak barrel aged red wine extract using human erythrocytes containing free radicals induced by hydrogen peroxide, Tedesco *et al.* (2000) found antioxidant activity of nonalcoholic constituents of red wine. However, oak barrel aged wine had a higher antioxidant activity than resveratrol or quercetin.

Catechin was shown to possess antioxidant activity in human plasma by delaying the degradation of endogenous α-tocopherol and β-carotene and by inhibiting the oxidation of plasma lipids (Lotito & Fraga, 1997).

Ohshima, Yoshie, Auriol, and Gilibert (1998) reported that epigallocatechin gallate, myricetin and quercetagetin induced breaks in DNA single strands in the presence of nitric oxide (NO); but catechin, epicatechin and gallic acid produced fewer breaks on DNA strands than the former compounds. Catechin, epicatechin and gallic acid at a concentration of 0.5 mM inhibited by more than 90% breaks in the DNA strand induced by the presence of 0.5 mM peroxynitrite. These three compounds were also reported to reduce the number of DNA strand breaks in the presence of nitroxyl anion (NO^-). Ohshima *et al.* successfully showed that some flavonoids such as catechin, epicatechin and gallic acid have an *in vitro* antioxidative activity, and these compounds can scavenge nitric oxide, peroxynitrite and nitroxyl anion radicals.

Sanchez-Moreno, Jimenez-Escrig, and Saura-Calixto (2000) found that gallic acid, resveratrol and tannic acid equally inhibited lipid peroxidation while this inhibition was higher than BHA or D-L-α-tocopherol. Using DPPH, they found that gallic acid had the highest free radical

scavenging activity while D-L-α-tocopherol and resveratrol had the lowest. The authors also reported higher antioxidant activities in wines, especially red wine than in grape juices.

The metal ion chelating activity of polyphenols has been demonstrated in the iron-chelating activity of tea polyphenols (Grinberg *et al*., 1997). Catechol units are reported to have the ability to chelate iron (Hider, Liu, & Khodr, 2001). Morel *et al*. (1993) investigated antioxidant and iron-chelating activities of catechin, quercetin and diosmetin (methylated quercetin) using cell cultures. Antioxidant activities were in the order catechin > quercetin > diosmetin on their inhibition of lipid peroxidation. They showed that these three flavonoids could chelate iron *in vitro*, which can explain the reduction in lipid peroxidation.

In vivo antioxidant activity

In vitro studies include assays that use cell cultures to test the reaction of cells to potential antioxidant substances. *In vivo* studies, on the other hand, use animal or human models to determine the antioxidant or prooxidant potential of food materials under live physiological conditions. These studies are considered more valuable in terms of antioxidative potential of phytochemicals in the body.

Free radicals can form as a result of various metabolic activities in the body. The human body has its own defense mechanism for inhibiting tissue damage from free radicals. The enzymatic defense system includes superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase (Das & Maulik, 1994). A majority of the studies indicated that antioxidants from plant origins, for example some flavonoids, are part of the non-enzymatic defense system although Zhang, Yang, Tang, Wong, and Mack (1997) found inhibition of gluthathione reductase by phenolic compounds of plants at various concentrations. Polyphenols in grape seed extracts may reduce the levels of plasma cholesterol in humans with elevated plasma cholesterol levels. Reduction of LDL levels has been reported in humans. An inverse relationship was found between the level of flavonoids in human diet and coronary disease mortality (Palomino, Gomez-Serranillos, Slowing, Carretero, & Villar, 2000).

An *ex vivo* study showed that grape seed procyanidins (GSP) might reduce the oxidation of polyunsaturated fatty acids in mouse liver microsomes (Bouhamidi *et al*., 1998). Addition of 2 mg/l GSP inhibited the oxidation of arachidonic and docosahexaenoic acids significantly upon oxidation induced with UV-C irradiation (200 $\mu W/cm^2$ for 24 h). Monomers of GSP, epigallocatechin and epigallocatechin-gallate were shown to be ineffective in protecting microsomal polyunsaturated fatty acids from oxidation compared to natural GSP which also contains significant amounts of dimers and oligomers of flavanols besides monomers (Bouhamidi *et al*., 1998).

An *in vivo* study with dog, monkey and humans showed that consumption of purple grape juice caused further protection in the patients against the oxidation of LDL cholesterol; therefore, flavonoids present in purple grape juice and red wine may play a role in the inhibition of initiation of atherosclerosis (Folts, 2002).

Sato, Bagchi, Tosaki, and Das (2001) showed that GSP extract acted as an *in vivo* antioxidant after the oral administration of diet supplemented with GSP extract (100 mg/kg per day) in rats. Significant reductions in the appearances of both reactive oxygen species and 'apoptotic cardiomyocytes' in the hearts of ischemic/reperfused rats were reported. Bagchi *et al*. (1998) found that water–ethanol extracts of red grape seeds reduced the production of free radicals including superoxide anions in mouse macrophages. The reducing effect was more than that of vitamin C, β-carotene or vitamin E succinate at the same concentrations. The same study also indicated that grape seed extracts could reduce lipid peroxidation in liver and brain. The incorporation of grape seed procyanidin extract into mouse diet showed protection against DNA fragmentation and this protection was dose-dependent.

Resveratrol is a stilbene, which is produced by grapes vines in response to fungal infections like *Botrytis cinerea* and environmental stress. In rats, inhibition of platelet aggregation and LDL oxidation and protection of liver from lipid peroxidation by resveratrol were reported as well as its anticarcinogenic activities (Palomino *et al*., 2000).

Tebib, Rouanet, and Besancon (1997) fed rats with a high cholesterol-vitamin E-deficient diet to study the effect of grape seed tannins on antioxidant enzyme activity, total glutathione and the extent of lipid peroxidation in several tissue samples. A vitamin E deficient diet reduced the levels of enzymatic antioxidants such as catalase, glutathione peroxidase and superoxide dismutase in different tissues such as aortic, hepatic, cardiac, intestinal, muscular and renal tissues. Tebib*et al*. reported that monomeric tannins were ineffective in restoration of the levels of these enzymatic antioxidants. However, in rats fed with polymeric tannins, these enzymes were effectively restored. Vitamin E deficiency reduced the total glutathione level in rat tissues and blood. Polymeric tannin supplementation of rat diet increased the glutathione level back to its original level. Polymeric tannins reduced lipid peroxidation in plasma and tissues as effectively as vitamin E. More interestingly, in rats fed with a diet high in cholesterol and deficient in vitamin E, incorporation of polymeric grape seed tannins in the diet increased total glutathione level in blood approximately four times compared with rats fed the same diet with monomeric grape seed tannins. The authors concluded that polymeric grape seed tannins have *in vivo* antioxidant activity and could be as important as vitamin E in preventing oxidative damage in tissues.

Cancer

Cancer is defined by the American Cancer Society (ACS, 2001) as "a group of diseases characterized by uncontrolled growth and spread of cells". The malfunction of genes, which control cell growth and division, may induce cancer.

Cancer can be induced by many factors such as lifestyle, environmental and genetic factors. According to the ACS, lifestyle factors such as diet and regular exercise contribute to the causes of about one-third of total cancer deaths in 2001. The ACS expects the number of new cancer cases in 2001 to be close to 1.3 million. Diagnosed cancer cases have reached to 15 million since 1990. After heart disease, cancer is the second major cause of death in the US where a quarter of all deaths is from cancer. The National Institutes of Health in the US estimated the cost of cancer to the US economy to be $180.2 billion in the year 2000.

Temple (2000) reported that the dietary antioxidant intake is inversely related to cancer cases according to epidemiological studies. In a review, Kuroda and Hara (1999) indicated that "in Japan, an epidemiological study showed an inverse relationship between habitual green tea drinking and the standardized mortality rates for cancer". Ascorbic acid, β-carotene, lycopene, α-tocopherol and selenium are considered as important dietary micronutrients that could reduce cancer cases or heart disease rates.

Cancer preventing activities of flavonoids have been extensively studied. Components of tea, grape seeds and skins were reported to have anticarcinogenic activities as well as cancer inducing activities. Phenolic compounds present in beans (*Phaseolus vulgaris*) were also shown to be antimutagenic (Mejia, Castano-Tostado, & Loarca-Pina, 1999). Antitumor, antiplatelet, antiallergic, antiischemic, and antiinflammatory activities of flavonoids are mostly associated with the antioxidative properties of plant flavonoids (Shi, Noguchi, & Niki, 2001).

A number of studies investigated the effect of flavonoids on colon cancer. Flavonoids catechin and hesperidin were found to have chemoprotective effect in rats against colon cancer induced with heterocyclic amines (Franke *et al*., 2002). Using rats, Hirose *et al*. (2001) indicated that green tea catechins at a level of 40 mg/kg body weight was ineffective to inhibit carcinogenesis after colon, lung, or thyroid cancer was initiated. However, catechins increased colon carcinogenesis when rats were fed a diet containing 40 mg/kg green tea catechins. On the other hand, Uesato *et al*. (2001) showed that EC (epicatechin), EGC (epigallocatechin), and EGCG (epigallocatechin gallate) showed a stronger inhibition activity against colon cancer cells than hepatic epithelial cells *in vitro*. Moreover, anticancer activity of EC on both types of cancer cells was found weaker than the other two epicatechin derivatives.

Zhao, Whiteman, Spencer, and Halliwell (2001) reported that phenols in the diet such as catechin, EC, EGC, caffeic acid and quercetin can protect the DNA against damage by nitrite (HNO_2) and peroxynitrite ($ONNO^-$). Nie, Wei, Shen, and Zhao (2001) reported that green tea polyphenols EC, EGCG, EGC, and ECG (epicatechin gallate) showed a protective effect on oxidative DNA damage induced by hydroxyl radicals *in vitro*. Weyant, Carothers, and Bertagnolli (2000) reported that catechin showed antitumor activity both *in vivo* and *in vitro*, preventing tumor formation. Antioxidant activity of tea catechins were found to be positively related to their antimutagenic activity (Krul *et al*., 2001).

Tea extracts from green, oolong, and black tea, and tea constituents gallic acid and EGCG showed antimutagenic activity under the Ames test against various chemical mutagens (Hour, Liang, Chu, & Lin, 1999). Hirose *et al*. (1997) found green tea catechins to be ineffective in inhibiting rat mammary cancer progression; however, a diet containing epigallocatechin gallate weakly inhibited early promotion of cancer in rats. A similar research indicated that EGCG, ECG, EGC, EC, and green tea water extract showed anticarcinogenic activity during the initiation stage of chemical carcinogenesis (Han, 1997). Anticarcinogenic effect of green tea extract was found to be higher than any of these polyphenolic substances at that stage of carcinogenesis. However, the anticarcinogenic effect of phenolic substances on cancer cells during promotion stage of cancer was higher than tea water extract (Han, 1997).

Apoptosis can be defined as programmed cell death. Apoptosis is necessary for the elimination of damaged or cancerous cells from the body during cancer treatments. Treatment of human Chang liver cells with 25 μg/ml GSP extract reduced apoptosis by reducing the expression of p53 and increasing the expression of cellular Bcl-2 (Joshi, Kuszynski, Bagchi, & Bagchi, 2000). Bcl-2 protein is related to apoptosis expression on cells. It is highly expressed in tumor cells, which are resistant to apoptosis. Bcl-2 gene is an antogonist of apoptosis whereas p53 is a proapoptosis gene. Gallic acid was reported to play a role in the induction of apoptosis, programmed cell death, in the body (Sakaguchi, Inoue, & Ogihara, 1998). Green tea constituents, EC, EGC, EGCG and ECG showed chemoprotective activity against human prostate cancer cells by suppressing their growth and inducing apoptosis (Chung *et al*., 2001).

Ohe, Marutani, and Nakase (2001) showed that tea catechins (catechin, EC, EGC, EGCG and ECG) should not be responsible for the antigenotoxic activity of tea because of the insignificant correlations between catechin contents of tea and antigenotoxicity of tea against nitroarenes. Interestingly, fermented tea, which have low catechin contents, showed a high genotoxicity by suppressing activity of nitroarenes. Apostolides, Balentine, Harbowy, Hara, and Weisburger (1997) previously reported that gallic acid, ungallated tea catechins (catechin, EC and EGC), and methyl gallate did not inhibit *in vitro* mutagenesis from direct mutagens; however, theaflavin, gallated catechins, and tannic acid showed antimutagenic activity. Chen and Chung (2000) also found that tannic acid and its hydrolyzed products including phenolic acids, ellagic acid and gallic acid showed no antimutagenic activity against direct mutagens such as 2-nitrofluorene, 1-nitropyrene, and 2-nitro-*p*-phenylenediamine. Moreover, these compounds were found to be non-mutagenic. Muto, Fujita, Yamazaki, and Kamataki (2001) reported that EC, EGC, EGCG

and ECG inhibited 'metabolic activation of procarcinogens by human cytochrome P450' partially through the inhibition of NADPH-CYP reductase.

Dhawan *et al*. (2002) evaluated the antigenotoxic properties of grape seed procyanidins B_1, B_2, B_3, B_4 and B′2G and of black tea theaflavins and theafluvins *in vitro*. They reported that monomeric and dimeric flavanols of grape seeds did not cause or prevent damage to lymphocyte DNA induced by Trp-P-2 at a concentration up to 100 μM (micromolar). However, black tea theaflavins and theafluvins at a concentration up to 0.5 mg/ml prevented DNA damage in a dose-dependent manner. Dhawan *et al*. concluded that anticarcinogenic potential of black tea could be associated with their theaflavins and theafulvins content.

Cancer preventing activity of chemical drugs may come from their ability to inhibit cyclooxygenase, which can catalyze the conversion reaction of arachidonic acid into tumor cell growth stimulating substances. Jang *et al*. (1997) reported that resveratrol showed anticarcinogenic activities in the initiation, promotion and progression steps of cancer development in several ways, including inhibition of cyclooxygenase and hydroperoxidase activities.

Bomser, Singletary, and Meline (2000) noted that ornithine decarboxylase (ODC), a rate-limiting enzyme in polyamine biosynthesis could have "an essential role in diverse biological processes including cell proliferation and differentiation". High levels of ODC are associated with increased risk for cancer. GSP extract containing mainly oligomeric and polymeric proanthocyanidins was shown to inhibit epidermal ODC activity in mice when it is used before TPA, which is a tumor promoter (Bomser *et al*.).

Breast cancer

ACS estimated new breast cancer cases among US women to be about 192,000 in 2001. 1500 new cases are expected in men. Approximately 40,000 deaths were expected from breast cancer and majority of these deaths was in women. Fat intake was reported to be correlated with breast cancer rates worldwide. 31% of total new cancer incidents in the US among females were expected to be breast cancer cases, while 15% of the expected deaths among the same group were from breast cancer (ACS, 2001).

Nakagawa *et al*. (2001) studied the effect of resveratrol on the inhibition of the growth of cancerous cells obtained from breast cancer patients (Table 2). Suppression in the growth of these cells by resveratrol was reported to come from apoptosis, programmed cell death.

The p53 gene suppresses tumor formation and induces apoptosis. Modulation of p53 expression by red wine and its content of polyphenolic substances was studied in three human breast cancer cell lines and one colon cancer line by Soleas, Goldberg, Grass, Levesque, and Diamandis (2001). Wine polyphenols quercetin, catechin, *trans*-resveratrol and caffeic acid did not affect p53 gene expression in two of the breast cancer lines; however, resveratrol decreased the expression of this gene in breast cancer cells of a wild type (MCF-7). Although catechin and caffeic acid increased the expression of p53 in wild type breast cancer cells, the increase was independent of concentration. Caffeic acid and resveratrol reduced the expression of p53 in colon cancer cell lines, but this reduction again was not dose-responsive. Soleas *et al*. concluded that anticarcinogenic properties of wine should not be attributed to the modulation of p53 gene expression by these wine polyphenolic constituents.

Table 2. The effect of resveratrol concentration on the inhibition of breast cancer cells

Resveratrol concentration (μM)	The effect on breast cancer cells
≤4	Proliferation in MCF-7
≤22	Proliferation in KPL-1
≥44	Suppression in KPL-1, MCF-7 and MKL-F
52–74	Reduction in the effect of a breast cancer stimulator, linoleic acid
	Suppression in KPL-1, MCF-7 and MKL-F

Cell lines: estrogen receptor (ER)-positive=KPL-1 and MCF-7; estrogen receptor (ER)-negative=MKL-F (adapted from Nakagawa *et al*., 2001).

Skin cancer

New skin cancer cases were expected to be more than a million in 2001 by ACS. Melanoma is the most serious form of skin cancer. Whites were expected to be 10 times more susceptible to skin cancer than blacks (ACS, 2001). Approximately 10,000 deaths were expected to occur from skin cancer in the US and protection could be achieved by limiting outdoor activities between 10 a.m. and 4 p.m., wearing a hat or a long-sleeved shirt and using a sunscreen lotion (ACS, 2001).

Bomser, Singletary, Wallig, and Smith (1999) found that grape seed extract containing mainly oligomeric and polymeric proanthocyanidins showed anti-tumor activity in mouse skin epidermis.

Cardiovascular diseases

Coronary heart disease (CHD) is usually associated with high cholesterol levels in blood. CHD has been a serious health problem affecting populations in many developed part of the world. High levels of LDL cholesterol in plasma may play a role in the initiation of atherosclerotic plaque. Physicochemical and biological properties of LDL can be modified through enzymatic modification such as lipases and oxygenases or non-enzymatic modifications such as glycosylation, proteoglycans and immune complexes (Aviram, 1993). In atherosclerosis, lipid peroxidation plays an important role during the initiation and propagation steps of this disease. Serum cholesterol level may not necessarily be a cause of the problem as manifested in

the French paradox, but oxidation of cholesterol, especially LDL (Aviram, 1993) could be the problem. Piotrowski, Hunter, Eskelson, Dubick, and Bernhard (1990) found high levels of cholesterols and lipid peroxidation in tissues and phospholipids in aortic tissues obtained from people with coronary heart diseases. Atherosclerotic plaque could form as a result of lipid oxidation, especially of LDLs in plasma.

LDL peroxidation can be affected by several factors such as presence of copper ions, antioxidant content of cells (both enzymatic and non-enzymatic), and 'the composition and location of polyunsaturated fatty acids' of LDL (Aviram, 1993). Extrinsic factors like antioxidant concentration in blood and other tissues may play an important role in the reduction of coronary heart disease risks. Epidemiological studies show that risk could be reduced by high dietary intake of fruits and vegetables. Phenolic substances found in grapes, wine and other foods are able to block the oxidation of LDL by acting as antioxidants (Aviram, 1993) and may be responsible for their cardioprotective effect (Manthey, Buslig, & Baker, 2002).

An *in vitro* study indicated that catechin and quercetin have a property to inhibit LDL oxidation (Roland, 1997). Moreover, tannins (tannic acid), flavonols (quercetin and rutin), cinnamic acids (caffeic and ferulic acid), stilbenes (resveratrol), benzoic acids (gallic acid), and anthocyanidins (malvidin) also inhibit the *in vitro* oxidation of LDL in a dose-dependent manner, and this inhibition was better than that of common antioxidants like vitamins E and C (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999).

Dietary phenols were found to be better in inhibiting LDL oxidation *in vitro* than common antioxidants like ascorbic acid and tocopherols (Sanchez-Moreno *et al*., 2000). Red apples and apple juice containing a variety of polyphenolic compounds was found to protect LDL against oxidation induced by *in vitro* copper (Pearson, Tan, German, Davis, & Gershwin, 1999). Cranberry extracts containing about 1.5 g GAE/l reduced the *in vitro* oxidation of LDL induced by cupric sulfate (Wilson, Porcari, & Harbin, 1998). Caldu *et al*. (1997) showed that red and white wine reduced the oxidation of LDL both *in vivo* and *in vitro*. Moreover, higher inhibition of LDL oxidation by red wine compared to white wine was reported.

Meyer, Yi, Pearson, Waterhouse, and Frankel (1997) showed that inhibition of copper induced LDL oxidation in humans by grape extracts vary in degree, depending on the phenolic content of the extracts. Crushing the seeds and extracting for a longer time increased the amount of flavanols and hydroxybenzoates in the extract. The authors found a positive significant correlation between the relative inhibition rate of LDL oxidation and the levels of flavanols, total phenols and hydroxybenzoates.

Studies were also done to determine whether alcohol in wine is responsible for the reduced levels of LDL in blood of moderate wine drinkers. Frankel, Kanner, German, Parks, and Kinsella (1993) diluted red wine in order to get 10 μmol/l total phenolics (1000 times dilution) to study the effect of wine phenolics on the *in vitro* inhibition of human LDL oxidation. Diluted wine and 10 μmol/l quercetin equally inhibited the human LDL oxidation induced by copper, and this inhibition was from non-alcoholic constituents of red wine. They also indicated that the inhibition was independent of the copper concentration; thus, antioxidant activity was not from the metal chelating activity of wine phenolics. The inhibition was significantly higher than that induced by α-tocopherol.

Platelets are small cells that are able to adhere to damaged arteries and capillaries. Platelets circulate in the blood. By sticking to the damaged area they can prevent bleeding and promote healing. Under normal conditions, platelets do not stick to healthy endothelium because of the release of NO, a platelet inhibitor, by endothelial cells (Rabbani & Loscalzo, 1994). When endothelial cells are damaged, platelets are able to stick and aggregate on the wall of arteries (Folts, 2002). This could lead to CHD.

De-alcoholized red wines showed antiplatelet activity of wine phenolics (Pace-Asciak, Hahn, Diamandis, Soleas, & Goldberg, 1995). Although *trans*-resveratrol and quercetin inhibited platelet aggregation induced with both thrombin and ADP in a dose-dependent manner, ethanol inhibited only aggregation induced with thrombin. (Pace-Asciak *et al*., 1995).

Sato, Maulik, Ray, Bagchi, and Das (1999) investigated cardioprotective properties of grape seed proanthocyanidins in ischemic/reperfused rats. Myocardial infection rate was found lower in animals fed with GSPE than the control group. They showed that GSP extract could protect the heart against ischemic/reperfusion injury. The authors indicated that this protection may come from the ability of GSP extract to scavenge peroxyl and hydroxyl radicals generated during ischemia and reperfusion.

In another study, Sato *et al*. (2001) showed that 100 mg GSP extract/kg per day can reduce the number of apoptotic cells in rats with ischemic/reperfused hearts. There was about 50 and 75% reduction in the production of free radicals in rats fed with 50 and 100 mg/kg per day GSP extract, respectively. The cardioprotective effect of GSP extract was shown to come from the reduced expression of JNK-1 factor and c-Jun gene, which are proapoptotic factors in the ischemic/reperfused myocardium. GSP extract inhibits the expression of proapoptotic transcription factor and gene, JNK-1 and c-Jun (Sato *et al*.).

Yamakoshi, Kataoka, Koga, and Ariga (1999) found no effect of 1% GSP extract (w/w) in diet on serum lipid profiles in rabbits. However, it reduced atherosclerosis in the aorta. Grape seed proanthocyanidins can trap free radicals, especially aqueous peroxyl radicals in plasma and 'interstitial fluid of the arterial wall'. GSP extract reduces atherosclerotic activity of LDL by inhibiting its oxidation. Yamakoshi *et al*. also found that a diet with 1% (w/w) of catechin showed very weak antiatherosclerotic activity in cholesterol-fed rabbits compared with procyanidin rich extract. They concluded that preventive activity of grape

seed extracts against atherosclerosis comes mostly from proanthocyanidins.

Ulcer

Flavonoids and phenolic acids present in various foods may exhibit anti-ulcer activity (Bae, Han, & Kim, 1999). For example, several phenolic constituents of licorice extract were able to inhibit *Helicobacter pylori*, an ulcer-causing bacterium in the stomach and duodenum (Fukai *et al*., 2002).

Grape seed extracts of *Vitis vinifera*L. grapes containing either high or low flavanol contents showed antiulcer activity in rats (Saito, Hosoyama, Ariga, Kataoka, & Yamaji, 1998). Protection against stomach injury for grape seed extract with high flavanol content was higher than the one with low flavanol content. Saito *et al*. also showed that catechin, procyanidin B_3 and dimeric and trimeric procyanidins did not have any protective activity against ulcer. On the other hand, tetramers, pentamers and hexamers of grape seed proanthocyanidins were shown to have antiulcer activities in animal models. The authors speculated that this activity of longer oligomeric procyanidins might come from their ability to bind proteins on the surface of the stomach.

Estrogenic activity of flavonoids

Flavonoids have been shown to possess estrogenic and/or antiestrogenic activities both *in vivo* and *in vitro* (Breinholt, Hossaini, Svendsen, Brouwer, & Nielsen, 2000; Collins-Burow, Burow, Duong, & McLachlan, 2000; Kuruto-Niwa, Inoue, Ogawa, Muramatsu, & Nozawa, 2000; Schmitt & Stopper, 2001).

Basly, Marre-Fournier, Le Bail, Habrioux, and Chulia (2000) studied the estrogenic/antiestrogenic effects of resveratrol isomers on the *in vitro* human breast cancer cells (Table 3). *cis* Form was less effective than *trans* in both cell lines. DPPH assay and Fe^{+3} reduction assay showed that these isomers can be both antioxidant and prooxidant, depending on their concentrations (Basly *et al*.).

Table 3. The effect of resveratrol isomers on human breast cancer cells (adapted from Basly *et al.*, 2000)

	Effect
Concentration (μM)	
10–25	Increased *in vitro* growth of MCF-7 cell lines
0.1–1	No effect
>50	Cytotoxic, reduced cell growth
Concentration (mM)	
25 *trans*	Reduced proliferation induced by estradieol
10 *cis*	No interference with estrogen receptor
10–25 *trans* and 25 *cis*	'Supergonists of estradieol'

Other biological activities of flavonoids

The effect of plant flavonoids on intestinal microflora has been investigated. Tebib, Besancon, and Rouanet (1996) reported that monomeric proanthocyanidins of grape seeds did not cause any change in the activities of fecal bacterial enzymes on rats. However, polymeric proanthocyanidins showed 'a beneficial cecal metabolic and colonic protective effect' by reducing colonic enzymatic activity of β-glucosidase, β-glucuronidase, mucinase and nitroreductase 'due to a dilution effect'. Feeding rats with polymeric tannins increased the formation of volatile fatty acids, an indicator of bacterial activity, thus reducing the pH in cecum. Polymeric tannin supplementation was shown to stimulate fermentative activities without increasing the activity of harmful enzymes on animal models. Tannins may also inhibit the growth of some bacteria in human intestines. Chung, Lu, and Chou (1998) reported that tannic acid inhibited the growth of intestinal bacteria such as *Clostridium perfringens*, *Entrobacter cloacae*, *E. coli* and *S. typhimurium* while showing no inhibitory effect on *B. infantis* or lactic acid bacteria *L. acidophilus*. A fluorescence assay was developed to determine the bacterial degradation of flavonoids by Schoefer, Braune, and Blaut (2001). This assay could differentiate the abilities of colonic bacteria to degrade various flavonoids. However, the bacterial degradation of catechin could not be determined due to the lack of its quenching effect of the fluorescing compound. Future studies may reveal details on the mechanisms of flavonoid degradation by colonic microflora.

Khanna, Roy, Bagchi, Bagchi, and Ken (2001) showed that grape seed proanthocyanidin extract containing 5 mg/g *trans*-resveratrol induces the expression of vascular endothelial growth factor (VEGF) in keratinocytes. Therefore, GSP extract containing resveratrol can be used to treat dermal wounds and other dermal disorders.

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