Introduction

Proanthocyanidins are compounds naturally found in fruits, vegetables, seeds, nuts, flowers and bark. They are a class of phenolic compounds that are either oligomers or polymers of flavan-3-ol units (1). Grape seeds are a particularly rich source of proanthocyanidins, both in quantity and variety (2). Interest in grape seed extract developed in the late 20th Century with explorations into the "French Paradox." The paradox describes how despite a diet high in saturated fats, the French rate of mortality from heart disease is relatively low (3). Investigators were especially interested in the protective role of red wine, a staple of the French diet. Proanthocyanidins, which are present in red wine due because fermentation takes place in the presence of grape seeds and skin, were shown to bestow cardioprotection against cardiac ischemia (4) and had a role in lowering cholesterol levels (5). However, many other benefits of grape seed extract proanthocyanidins have also been uncovered including potent antioxidant activity and cytotoxicity to cancer cells.

The popularity of antioxidants has brought grape seed proanthocyanidin extract (GSPE) into mainstream consumer consciousness. There are many different brands of grape seed extracts on the market in countries such as the United States, Australia, Japan and Korea. One of the mostly prevalent products is a novel IH636 grape seed proanthocyanidin extract available as "ActiVin" from InterHealth Neutraceuticals Incorporated. It is composed of a water-ethanol extract of red grape seeds. The daily recommended dosage is "50 mg for adults ages 30 years to 40 years, 100 mg for adults ages 40 years to 50 years, and 200 mg for adults older than 50 years" (6).

One of the key factors in determining a compound's effectiveness is its bioavailability. Studies have shown that proanthocyanidins that are soluble in water and ethanol are absorbed by the intestine and well distributed throughout tissue and plasma (7).

Cardioprotective Activity of Grape Seed Proanthocyanidin Extract

Stemming from curiosity about the relationship of GSPE to the "French Paradox," studies have been done in both humans and animals to assess their cardioprotective activity. A double-blind, randomized, placebo-controlled study on 40 hypercholesterolemic subjects demonstrated that the combination of chromium polynicotinate, an insulin sensitivizer used for aging-associated diseases, and grape seed proanthocyanidin extract decreased total cholesterol and LDL levels significantly from the control and synergistically (more than either compound alone) (5).

In a 1999 study by Sato et al., researchers administered 100mg/ kg of ActiVin to one group of rats while another group of rats received nothing. After three weeks, the rats were killed and their hearts were mounted to a perfusion apparatus that simulated 30 minutes of global ischemia followed by 2 hours of reperfusion. The ActiVin fed rats had significantly better myocardial function after the period of ischemia than the control group, as determined by measuring contractility (dP/dT of 73.0 ± 6.1mmHg vs. 48.5 ± 7.7 mmHg respectively). Infarct size was also smaller for the GSPE-fed rats compared to the control group (.163 ± .012g vs, .209 ± .017g). Based on fluorescence studies, the researchers demonstrated that proanthocyanidins scavenge both the hydroxyl and peroxyl free radicals formed during ischemic reperfusion, suggesting a mechanism for the cardioprotection (8). A similar study of ischemic reperfusion demonstrated that rats treated with 100 mg /kg of proanthocyanidins showed a reduction in free radicals by 75% ± 7% compared to the control (9). This finding is important because the effects of

reperfusion after ischemia have been linked to oxygen free radical generation, which proanthocyanidins help to control through their antioxidant properties.

Antioxidant Properties of Grape Seed Proanthocyanidin Extract

The ability of GSPE to act as a potent antioxidant is at the heart of its cardioprotective benefits. Free radicals are highly reactive species that are normally produced in our bodies. They damage lipids, proteins, and DNA, and are thought to contribute to the aging process. Proanthocyanidins have been found to have potent antioxidant activities, particularly against oxidation of lipids and low density lipoproteins. Postprandial (after meal) hyperlipidemia increases risk for atherosclerosis, and it has been suggested that oxidation of LDLs (low density lipoproteins) may play a pivotal role in increasing the risk of developing atherosclerosis (10). Eight human volunteers consumed identical lipid-rich meals with or without 300mg of grape seed extract. The control group had a plasma lipid peroxidase level 1.5 times greater than the GSPE group, showing that GSPE enhanced resistance to LDL modification by oxidation (11). One problem with this study is that the number of subjects is low, and a larger study would be useful to confirm this effect.

A 2000 study by Bagchi et al. compared the concentration-dependent and dose-dependent scavenging ability of popular antioxidants such as vitamins C and E with GSPE in the form of ActiVin. In vitro, superoxide anions and hydroxyl radicals were generated and their concentrations were assessed in combination with varying concentrations of the antioxidants through chemiluminescence and cytochrome c reduction assays, respectively. At 50mg/L, GSPE had an 84% better free radical scavenging ability (RSA) against superoxide anions and a 98% greater RSA against hydroxyl radicals as compared to vitamin E. At 100mg/l, GSPE showed 439% greater RSA against superoxide anions and a 575% greater RSA in comparison to vitamin C (12). The abilities of GSPE, vitamins E, C, and beta-carotene to protect against lipid peroxidation and DNA fragmentation caused by 12-o-tetradeca-noylphorbol-13-acetate (TPA) in the liver and brain tissues of mice have also been examined. Treating mice with 100 mg/kg GSPE, vitamin C, and beta-carotene decreased the production of reactive oxygen species measured by chemiluminescence by 70%, 18%, and 16%, respectively, and cytochrome c reduction by approximately 65%, 15%, and 19%, when compared to controls. TPA-induced DNA fragmentation in both hepatic and brain tissues was reduced by 47% and 50% respectively, which was a 3 to 4 fold better reduction than either vitamin C or beta-carotene (13). This data provides support for the use of GSPE as a powerful antioxidant.

Grape Seed Proanthocyanidin Extract Inhibits Cancer Cell Growth But Promotes Growth in Normal Cells

Another therapeutic effect of grape seed proanthocyanidin extract concerns its activities against cancer. Ye et al. described how GSPEs showed varying levels of cytotoxicity to malignant and normal cells. MFC breast cancer cells, A-427 human lung cancer cells, CRL-1739 human gastric adenocarcinoma cells, and K562 chronic myelogenous leukemia cells were incubated with 25 and 50mg/L concentrations of GSPE for 0 to 72 hours. The effect of GSPE on normal human gastric mucosal cells and normal murine macrophage cells were also examined. Concentration and time dependant cytotoxic effects were discovered in the breast cancer cells, with a 6.5% inhibition of growth at 24 hrs, 30% inhibition at 48 hours, and a 43% inhibition at 72 hours with treatment of 25 mg/l of GSPE. At 50 mg/l, inhibition of growth for breast cancer cells was 11%, 35%, and 47% at 24, 48 and 72 hours respectively. GSPE showed similar effects on the other

cancer cells. However, the growth of the normal human cells was accelerated by GSPE, undergoing increases in growth of 32%, 26%, and 18%, at 24, 48, and 72 hours respectively, with treatment of 50mg/L of GSPE. The possible mechanisms for these effects include the upregulation of the bcl-Xs gene as a death promoter and the downregulation of bcl-Xl gene as a death inhibitor in the cancer cells, but not in regular cells (14). Further investigations are necessary to elucidate the true mechanism.

Chemotherapeutic agents are effective in inhibiting growth in cancer cells, but their toxicity to normal cells is problematic. 25 ug/ml of GSPE was shown to lessen the toxic and growth-inhibitory effects of 30nM Idarubicin and 1ug/ml 4-hyrdoxyperoxycyclophosphamide on normal human liver cells in culture. The apoptosis rate was also decreased by 50% in normal liver cells when compared to the control (15). This study suggests the possibility of using GSPE to help protect normal cells against the effects of chemotherapeutic drugs.

Studies of the outcomes of GSPE treatment in human prostate cancer cells have suggested other mechanisms of GSPE activity. Similar to its effects on other cancer cells, GSPE was found to inhibit advanced and androgen-independent human prostate cancer DU145 cells in culture (16). Another study further showed that incubation with 50 ng/ml of GSPE inhibited mitogenic signaling in prostate cancer DU145 cells and activated JNK, a protein kinase associated with the apoptosis response (17). 40-200ul/ml of GSPE was also found to cause mitochondrial damage in the prostate cancer cells which, leading to cytochrome c release into the cytosol and activation of caspases that cause apoptosis (18).

Testing the Safety of Grape Seed Proanthocyanidins

Because of the widespread use of grape seed proanthocyanidin extract as a dietary supplement, studies have been performed to test the safety of its use. This is important in particular because reaping the benefits of GSPE, particularly for ischemic cardioprotection, requires taking grape seed proanthocyanidin extract beforehand. The toxicity of commercially available ActiVin was examined in a series of studies by Bagchi et al in 2002. An acute oral toxicity study demonstrated no gross findings in rats that had been fed to a level of 5g/kg of body weight, and studies of dermal toxicity on rats at a level of 2g/kg yielded very slight to slight erythema and desquamation that subsided within 12 days (12). A study in Japan of a locally produced proanthocyanidin grape seed extract (Gravinol Super, Kikkoman Co. Japan) showed that administering 0, 2, and 4g/kg doses to groups of mice did not induce any abnormalities for the length of the trial. The GSPE was also found to be non-mutagenic through the reverse mutation test using Salmonella tymphimurium and the chromosomal aberration test using CHL cells (19). Studies done on rats that were fed the Meganatural brand of grape seed and grape skin extract have also shown no histological findings that indicate toxicity (20). However, long term studies in humans are needed to fully assure the safety of grape seed proanthocyanidin extract use.

Conclusion

Grape seed proanthocyanidin extract has numerous benefits including protection against damage brought on by cardiac ischemia, potent antioxidant activity, and cytotoxicity to cancer cells. GSPE has been shown to help to lower cholesterol levels and to protect against heart damage from ischemia and reperfusion. It has extensive antioxidant properties surpassing other popular antioxidants such as vitamin C and E. GSPE has cytotoxic activity against human cancer cells,

while preserving and promoting growth in normal human cells. Current research needs to be done to fully elucidate the mechanisms of GSPE activity. A shortcoming of the current body of research is the lack of large, long-term, human clinical trials to test the effectiveness of GSPE in treating for example, human prostate cancer. Overall, based upon the potential benefits and lack of toxicity or mutational propensity discovered through research, grape seed proanthocyanidin extract use can be supported.

REFERENCES

1. Santos-Buelga, C; Scalber, A. Proanthocyanidins and tannin-like compounds in human nutrition. J. Food Sci. Agric. 2000;80:1094-1117.
2. Santos-Buelga, C, Francia-Aricha, E.M., Escribano-M.T. Comparative flavan-3-ol composition of seeds from different grape varieties. Food Chemistry. 1995; 53:197-201.
3. A.S. St Leger, F. Moore and A.L. Cochrane Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. Lancet. 1979;1:1017-20.
4. Natella F, Belelli F, Gentili V, Ursini F, Scaccini C. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. J Agric Food Chem. 2002;50:7720-5.
5. Preuss HG, Bagchi D, Bagchi M. Protective effects of a novel niacin-bound chromium complex and a grape seed proanthocyanidin extract on advancing age and various aspects of syndrome X. Ann N Y Acad Sci. 2002;957:250-9.
6. Anonymous. Grape Seed FAQs. Dry Creek Nutrition Website. 2001.
7. Jimenez-Ramsey, L.M., Rogler, J.C., Housley, T.L., Butler, L.G., Elkin, R.G., Absorption and distribution of 14C-labeled condensed tannins and related sorghum phenolics in chickens. J. Agric. Food. Chem. 1994;42:963-967.
8. Sato M, Maulik G, Ray PS, Bagchi D, Das DK. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. J Mol Cell Cardiol. 1999;31:1289-97.
9. Pataki T, Bak I, Kovacs P, Bagchi D, Das DK, Tosaki A. Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. Am J Clin Nutr. 2002;75:894-9.
10. Frei B. Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. Crit Rev Food Sci Nutr. 1995;35:83-98.
11. Natella F, Belelli F, Gentili V, Ursini F, Scaccini C. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. J Agric Food Chem. 2002; 50:7720-5.
12. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology. 2000;148:187-97.
13. Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J, Stohs SJ. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. Gen Pharmacol. 1998;30:771-6.
14. Ye X, Krohn RL, Liu W, Joshi SS, Kuszynski CA, McGinn TR, Bagchi M, Preuss HG, Stohs SJ, Bagchi D. The cytotoxic effects of a novel IH636 grape seed proanthocyanidin

extract on cultured human cancer cells. Mol Cell Biochem. 1999;196:99-108.
15. Joshi SS, Kuszynski CA, Benner EJ, Bagchi M, Bagchi D. Amelioration of the cytotoxic effects of chemotherapeutic agents by grape seed proanthocyanidin extract. Antioxid Redox Signal. 1999;1:563-70.
16. Agarwal, C., Sharma, Y. and Agarwal, R. Anticarcinogenic effect of a polyphenolic fraction isolated from grape seeds in human prostate carcinoma DU145 cells; modulation of mitogenic signaling and cell cycle regulators and inductin of G1 arrest and apoptosis. Mol. Carcinog. 2000;28:129-138.
17. Tyagi, A., Agarwal, R., Agarwal, C., Grape seed extract inhibits EGF-induced and consitutively active mitogenic signaling, but activates JNK in human prostate carcinoma DU145 cells: possible role in antiproliferation and apoptosis. Oncogene. 2003;22:1302-1316.
18. Agarwal C, Singh RP, Agarwal R. Grape seed extract induces apoptotic death of human prostate carcinoma DU145 cells via caspases activation accompanied by dissipation of mitochondrial membrane potential and cytochrome c release. Carcinogenesis. 2002;23:1869-76.
19. Yamakoshi J, Saito M, Kataoka S, Kikuchi M. Safety evaluation of proanthocyanidin-rich extract from grape seeds. Food Chem Toxicol. 2002;40:599-607.
20. Bentivegna SS, Whitney KM. Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. Food Chem Toxicol. 2002;40:1731-43.