

NUTRACEUTICALS

(6) PROANTHOCYANIDINS AND GRAPE SEED EXTRACT

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Proanthocyanidins, particularly grape seed extract, are attracting increasing attention as potential preventives of heart disease and cancer and as antiviral compounds. This article looks at the evidence

A new group of phytochemicals that has been attracting much attention from both the general public and health professionals is the proanthocyanidins (PACs). These are a subgroup of the bioflavonoids (once called vitamin P) that has been called “super antioxidants”.

There is a hypothesis that the ingestion of diets high in fruits and vegetables offers protection against many diseases by the increase of antioxidants in the body. The long list of such illnesses includes cancer, autoimmune disease, cardiovascular disease, trauma, gastrointestinal problems, cataract, Alzheimer's disease, psoriasis, stroke and AIDS.¹⁻³ Grape seed proanthocyanidin extract (GSPE) has been shown to prevent the development of many disease states and it is thought that it exerts these effects by acting as an antioxidant.

PACs are found in the leaves, fruits, bark, seeds, flowers and roots of many plants. They have been used in the leather industry, foods, livestock feeds, beverages (such as wine and tea) and herbal preparations. Some examples are apple (*Malus* spp), grape (*Vitis vinifera*), blackberry, raspberry (*Rubus* spp), bilberry, cranberry (*Vaccinium* spp), hawthorn (*Crataegus* spp) and rosehip (*Rosa* spp).⁴

PAC AS AN ANTI-OXIDANT

Oxidation is a process that occurs naturally in the body when oxygen combines with reduced carbon based molecules (carbohydrates or fats) and provides energy. When there is decreased oxidation or decreased energy production, the cells can no longer function efficiently and disease results. However, this normal process propagates short-lived intermediates known as free radicals, and some free radicals escape and initiate further oxidation setting up a chain reaction. Antioxidants prevent these free radicals from causing excess cellular damage.

“Oxidative stress” is the general phenomenon of oxidant exposure and antioxidant depletion, or oxidant-antioxidant balance.^{1,5} The ideal situation for proper metabolic function is to provide enough pro-oxidants (food) for the generation of sufficient energy but not excess so that many free radicals are released. Many natural (unrefined) foods contain antioxidants, but in processing many of these are lost and oxidation is not limited. In addition, environmental pollutants, radiation, pesticides, medicines, spicy or deep fried foods as well as physical stress can produce free radicals which cause degeneration of body tissues from oxidative stress leading to many disease states.⁶

The products of oxidation reactions can cause much damage including loss of function. This can occur from direct oxidation, such as the oxidation of membrane lipids, and leads to altered membrane permeability or loss of enzyme regulation or activity from the oxidation of proteins. Products of oxida-

tion can also result in inappropriate cell responses.¹ Therefore, the ingestion of certain antioxidant nutrients may be of use to prevent the damage caused by these processes.

Several PACs have been shown to be good antioxidants, including *Crataegus monogyna*,⁷ which has long been used in herbal medicine.

A study⁸ was carried out to determine the concentration response of the *in vitro* free radical scavenging ability (antioxidation) of GSPE, vitamin C and vitamin E succinate. Results showed a good concentration-dependent response for GSPE in the inhibition of superoxide anion and hydroxyl radical production.

Vitamin C and vitamin E succinate also showed free radical scavenging properties, but to a lesser degree.

A similar study was carried out *in vivo* to compare the antioxidant properties of GSPE, vitamin C, vitamin E succinate and β -carotene,⁷ which were administered via a feeding needle to groups of four mice each morning for seven days. Lipid peroxidation (oxidative stress) was induced on the eighth day, two hours after the injection. Two hours later, the mice were killed and their hepatic and brain tissues removed. Results were obtained for reactive oxygen species production, superoxide anion production, lipid peroxidation and DNA fragmentation.

GSPE provided the best protection in all categories at the doses used. It was interesting to note that a combination of vitamin C and vitamin E succinate provided better protection than the individual vitamins alone. These results suggest that GSPE is not only an efficient antioxidant *in vitro*, but

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also *in vivo* where it is absorbed and distributed in target organs and may be useful in preventing damage by oxidation.

GSPE AND ATHEROSCLEROSIS

For many years raised plasma cholesterol levels have been thought to be the cause of the increasing deaths from cardiovascular disease and atherosclerosis. It has long been thought that if levels of low density lipoprotein (LDL) accumulate, the excess cholesterol is deposited in the arteries increasing the risk of cardiovascular disease.

In 1989, the World Health Organisation conducted a world-wide study to establish the mortality rate from cardiovascular disease. It was found that although risk factors such as dietary intakes of saturated fats, blood pressure, obesity and serum cholesterol values in the French were similar to those in populations of other western countries such as Britain and the United States, deaths from cardiovascular disease were much lower. This phenomenon constitutes the "French paradox".⁹

It was claimed that these differences in mortality were due to the high intake of red wine among the French. Moderate intake of red wine (20–30g alcohol/day) was shown to reduce the risk of death from cardiovascular disease by as much as 40 per cent. When the cardiovascular disease risk in Toulouse, France, was compared with that in Belfast and Glasgow the reduction was even greater. However, the consumption of wine, but not of alcoholic drinks, is low in these areas. Therefore, it was assumed that it was the red wine and not alcohol or spirits that led to this unexpected finding.^{10,11}

To determine whether the French paradox was a result of the antioxidant properties of red wine, an *in vitro* study was carried out.¹² Californian red wine was distilled to remove all ethanol to prepare the PAC phenolic component. Samples of the LDL from the blood of normolipidaemic, non-smoking volunteers were incubated for two hours with copper sulphate, which catalyses the oxidation. Gas chromatography was then performed on the products formed. Comparisons with authentic reference samples of products formed in the oxidation of fatty acids were used as a measure of the extent of oxidation.

The polyphenolic samples caused an inhibition of 60 and 98 per cent of the oxidation seen in the controls at concentrations of 3.8mmol/L and 10mmol/L, respectively. When the phenolic samples were diluted 1,000-fold with water, inhibition of oxidation was still seen. When a copper catalyst was used in excess the antioxidant reaction was still effective, indicating that the reaction was not due to metal chelating actions. These results show that the PACs in the red wine were acting as potent antioxidants, preventing the oxidation of the LDL. This could well be the reason behind the reduction of atherosclerosis in populations that consume large amounts of this drink.

A similar experiment was carried out using 10 healthy volunteers.¹³ The non-alcoholic component of red and white wine, or

placebo, was ingested at weekly intervals. The total plasma antioxidant capacity was measured using a plasma sample from the subject in a controlled peroxidation reaction. The antioxidant capacity of the plasma was significantly increased in those subjects who had ingested the non-alcoholic portion of the red wine. This indicated that the non-alcoholic fraction of red wine was indeed offering the antioxidant protection.

It has been claimed that the amount of protection afforded depends on the type of red wine consumed.¹⁴ Research has shown that red wines from Chile have a higher flavonol content than those from France, Italy, Australia and California. This could be explained by the climate in the grape growing regions, the thickness of the grape skins, the time of grape harvesting and the wine-making process, all of which affect the flavonoid content.

An *in vivo* study was carried out using rabbits to determine the effect of PAC extract from grape seeds on atherosclerosis.¹⁵ An extract containing 73.4 per cent PAC was obtained by freeze-drying an aqueous solution of grape seeds. The rabbits were then fed with this extract, cholesterol and probucol (another antioxidant) for eight weeks. Serum lipids were measured as were the lipoprotein components of the serum — very low-density lipoprotein (VLDL) cholesterol, LDL and high-density lipoprotein (HDL). The serum LDL cholesterol and the LDL/HDL ratio decreased at six weeks in the 1 per cent PAC group, and slightly at eight weeks in the 0.1 per cent PAC group.

Oxidation of LDL by the PAC was also determined. The cholesterol content of the aortic arch and the thoracic arch were also measured as well as the percentage of the aortic surface area covered with atherosclerotic lesions. There were similar amounts of aortic plaque in both the probucol and PAC groups, which were lower than the cholesterol group, and no dose-dependent activity was noted for the 0.1 per cent and 1 per cent PAC groups. PAC was not detected in the serum or lipoproteins of the rabbits.

The authors concluded that PAC-rich extract inhibited progression of atherosclerosis in cholesterol-fed rabbits. The activity of the PAC was thought to be related to prevention of LDL oxidation in the arterial cell wall.

Several British doctors have suggested that the "French paradox" is not due to the higher consumption of red wine at all and is rather due to a "time lag".¹⁶ They suggest that although animal fat consumption in Britain and in France has been similar for the past 15 years or so, before that the French consumed far less animal fat. It is this fact, so they say, that has protected the French from high levels of coronary heart disease and not the consumption of red wine. The researchers claim that many studies of red wine drinkers have shown no difference in risk of heart disease. Clearly, more work is needed on this controversial issue before conclusive statements about the consumption of red wine and heart disease can be reached.

GSPE AND CANCER

As the 21st century begins, many degenerative diseases are becoming more common. Cancer claims many lives and ways of reducing the risk of developing the disease are continually being sought. Although the best way of eliminating cancer is by elimination of causative agents, such as cigarette smoke, in many cases the cause is unknown. In other cases, neoplasia may have been initiated and the aim is to suppress the process. A large number of chemical compounds have been shown to prevent cancer by different mechanisms and appear to work at different stages in the neoplastic process.¹⁷

It has been suggested that a diet rich in fruits and vegetables provides protection against tumour development. Geographical studies have shown that increased cancer risk is associated with high fat/low fibre diets, whereas diets based more on fruits and vegetables lead to a much lower risk.¹

Phytochemicals such as PACs, which are widespread in fruits and vegetables, are an example of bioactive compounds that have been shown to reduce risk of many types of cancer. Three PAC dimers, and also a trimer, were investigated for anticancer activity and compared to the monomers and a control.¹⁸

PACs were applied 15 minutes before a potent tumour promoter, 12-O-tetradecanoylphorbol 13-acetate (TPA), to the shaved dorsal region in groups of four or six mice and results compared with those from controls where PACs were not applied. TPA tumour promotion is linked with biochemical events, which were measured, and the epicatechin trimer showed the most inhibition (90 per cent) of tumour activity, compared with the dimers (56 per cent) and monomers (35 per cent).

Hydroperoxide production was not inhibited by either monomer but the dimers and trimer showed a 35 to 40 per cent inhibition. In the inhibition of DNA synthesis all structures showed inhibition of 35 to 40 per cent.

The exact mechanism of tumour inhibition is unknown, but it could be connected to complex formation with the metal ions and cofactors required for the enzyme and non-enzyme generation of reactive oxygen species in multi-stage carcinogenesis. The authors concluded that these PACs may prove to be effective anti-tumour agents.

In another study, extracts of the fruits of *Vaccinium* spp, were tested *in vitro* for anticancer compounds.¹⁹ Again, the greatest activity against induced carcinogenesis was found in the polymeric PAC fractions.

Another *in vitro* study was carried out to determine the effect of GSPE against human breast cancer cells, human lung cancer cells, human gastric adenocarcinoma cells and leukaemic cells.²⁰ The action of GSPE on normal human gastric mucosal cells was also assessed. When the cells were incubated with GSPE, concentration- and time-dependent inhibition was seen with the cancer cell lines. However, the normal cells showed enhanced growth.

All of these studies suggest that PACs

and GSPE have a place in the chemoprotection of cancer. More extensive work with larger sample sizes is required to provide conclusive evidence. It is certainly worth investigating what place these plant products have in the prevention of cancer, and so far the research seems promising.

The mutagenicity of several purified PACs, which included several dimers, a trimer and a polymer, was tested on *Salmonella typhimurium* strains.²¹ The authors quote previous work claiming that PAC extracts can induce liver tumours and oesophageal cancers. None of the samples examined showed any mutagenic activity except one. It was further found, using the techniques of high performance liquid chromatography and thin layer chromatography, that this sample was contaminated with a mutagen — rutin — which was identified using ultraviolet spectroscopy. This highlights the importance of using purified products and obtaining them from reliable sources.

As the medical profession, as well as the public, becomes more aware of the availability and usefulness of nutraceuticals it is of vital importance that the products offered by the industry are of the highest quality. A contaminant can turn a phytochemical being promoted for its anti-tumour properties into a mutagen.

OTHER USES FOR PACS

Hair growth Many extracts from the roots, fruits, plants and seeds of different plants have been examined in the hope of finding a natural cure for male baldness.²² PACs from grape seeds were found to be effective in promoting hair follicle cell growth. An *in vitro* study was carried out using isolated and cultured mouse hair follicle cells. GSPE was found to increase hair growth in the cell culture by about 230 per cent, compared with controls after five days, and 160 per cent compared with minoxidil, a medicine for treating male baldness.

An *in vivo* test was then carried out using eight-week-old mice. The test substance was applied for 19 days to a shaven area on the backs of the mice. A 1 per cent minoxidil solution resulted in 90–100 per cent hair growth, 3 per cent GSPE gave 80–90 per cent growth and the control (vehicle only) resulted in 30–40 per cent growth. No side effects were seen in any group. The authors suggest that the PAC extract caused prevention of cell differentiation and retention of the growing phase. These results indicate the possible use of GSPE in the treatment of baldness, but more extensive research is required in this area.

Natural sweetener Sucrose, which is used extensively as a sweetener in foods, drinks and medicines, is known to be the cause of dental caries. Artificial sweeteners, including saccharin and aspartame, have been used for many years, but claims exist for possible toxicity. There is increasing demand for natural food ingredients, and plant compounds are already used as natural sweeteners in many countries, but the search for non-cari-

ogenic, non-calorific sweeteners, which can be used in diabetic preparations continues.

A novel sweetener has been isolated from the rhizomes of the fern *Selligues feei*. The structure of this constituent was shown to be similar to the typical PAC structure and showed lack of toxicity when fed to mice. PACs are usually astringent when tasted but this finding necessitates more research into different PACs for their possible role as natural sweeteners.

Anti-viral agents Viruses are responsible for many widespread diseases. An example is the herpes simplex virus types 1 and 2, which is the cause of stomatitis, meningitis and viral genital disease. A study was carried out to determine the structure-activity relationships of the anti-viral activity of various tannins.²⁴ It was found that the more condensed the chemical structure the greater the antiviral effect, and it was suggested that the active groups interact with the viral proteins and the host cell surface, causing decreased viral action and infection.

In another study,²⁵ PACs were shown to have anti herpes simplex activity in which the 50 per cent effective doses (needed to reduce herpes simplex plaque formation to 50 per cent of controls) were two to three orders of magnitude less than the 50 per cent cytotoxic dose (needed to reduce thymidine uptake to 50 per cent of controls). Radioactive viruses were used to show that the effect was due to the inhibition of virus adsorption to the host cells.

Pilostigma thonningii is an African plant used in folk medicine to treat gum inflammation. The purified root bark contains several PAC structures. The purified polyphenolic fraction was used to test for anti herpes simplex type 1 activity in African green monkey cells. The 50 per cent effective dose was 17.5 µg/ml and the 50 per cent cytotoxic dose was 44.8 µg/ml.²⁶ These data agree with those reported above and show that, at doses required for antiviral activity, cytotoxicity is negligible.

An *in vitro* experiment was carried out using the PAC from *Cupressus sempervirens* (CPAC), to determine antiviral activity against the HIV and HTLV retroviruses.²⁷ These retroviruses are responsible for an attack on the immune system of the host organism, leading to increased susceptibility to infections. T lymphoblast and H9 cells were cultured and infected with HIV and HTLV, respectively, at different concentrations. Each concentration was incubated with either the purified CPAC fraction or AZT (3-azido-3-deoxythymidine).

The results showed that CPAC was more active against HIV than HTLV. Concentrations of AZT necessary for the same amount of viral inhibition were much lower. However, AZT was found to be highly toxic even at low concentrations, whereas CPAC showed no toxicity at the 50 per cent effective dose and could be used with no risk. Further studies *in vivo* are required to see if this is a possible new use for PACs.

Cough suppressant *Pilostigma thonningii* has

been used in Africa for many years to treat coughs. Isolated compounds including epicatechin, PAC B2, catechin trimers and oligomers, all found in PACs, were extracted from the plant. Antitussive activity was tested in guinea pigs using codeine as a reference drug. In coughs induced by ammonia aerosol, the extract was slightly less effective than codeine, but the effect increased as the time between cough induction and extract delivery increased. With intravenous treatment the extract had a stronger effect than the codeine.²⁸

CONCLUSION

The proanthocyanidins, and more specifically grape seed proanthocyanidin extract, are showing some interesting results in important health issues. More research is still required, particularly in the area of absorption and metabolism in human studies.

In vitro and *in vivo* work in many areas shows that this non-essential nutrient could have a promising place in the treatment of diseases such as atherosclerosis, cancer and viral infections. Little information is available concerning side effects or contraindications with other medicines. However, the study and use of antioxidants is increasing and it is important to keep abreast of these new findings.

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