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THE NUTRACEUTICAL PYCNOGENOL: ITS ROLE IN CARDIOVASCULAR HEALTH AND BLOOD GLUCOSE CONTROL

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Pycnogenol® (a registered trademark of Horphag Research Ltd.) is French maritime pine bark extract of the outer bark of Pinus pinaster *Ait. Subsp.* atlantica*. Its specifications are described in the USP 28 - Dietary supplements. Pycnogenol has strong antioxidant profile proven by* in vitro *and* in vivo *studies and further confirmed in clinical trials. Its strong antioxidant profile, vasodilator activity, antithrombotic effect and collagen stabilizing property make it a unique health product. In humans, Pycnogenol has been shown to lower blood pressure in mild to moderate hypertensive individuals and blood glucose levels in diabetics. In this review the diverse biological effects of Pycnogenol are presented and discussed using a target-oriented approach, in health and disease conditions like edema, inflammation, chronic venous insufficiency, deep vein thrombosis, diabetes, diabetic retinopathy, and hypertension. The future trends are the continuous efforts proving its efficacy in conditions which involves oxidative stress and inflammation. Recent preliminary study utilizing "high throughput" methodology and nutrigenomics approach are able to provide a new insight into the molecular mechanism of the biological activity of this botanical sourced nutraceutical.* ***Biomed Rev 2005; 16: 49-57****.*

Key words: diabetes, hypertension, nutraceutical, nutrigenomics, Pyconogenol®

INTRODUCTION

Pycnogenol® (a registered trademark of Horphag Research Ltd.) is French maritime pine bark extract of the outer bark of *Pinus pinaster* Ait. Subsp. *atlantica*. Its specifications are described in the USP 28 - Dietary supplements (1). It is generally recognized as safe (GRAS) in the USA. Pycnogenol has strong antioxidant profile proven by experimental *in vitro* and *in vivo* studies and further confirmed in clinical trials (2). The historical development of Pycnogenol and the utilization of the pine bark as a health promoting botanical have been reviewed (3). The concept of orally administered Pycnogenol, either as "stand alone product" or in combination with other food ingredients, was developed during last two decades. Numerous clinical trials have investigated the efficacy of oral Pycnogenol in individuals with chronic venous insufficiency and diabetic retinopathy (4-9). Most of the clinical data on Pycnogenol are highlighted in different monographs and reviews (10-12).

The objective of present paper is to review the multifaceted biological profile of Pycnogenol using a target-oriented approach, with a special focus on cardiovascular health and glucose control.

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BIOLOGICAL PROFILE OF PYCNOGENOL

Antioxidant and anti-inflammatory activities

The antioxidant and anti-inflammatory effects of Pycnogenol are demonstrated by *in vitro* and *in vivo* models and confirmed in clinical studies. These include: *(i)* antioxidant and free radical scavenging activity (13-22), *(ii)* sparing activity of α-tocopherol and recycling of ascorbate radical (23), *(iii)* inhibition of lipid peroxidation (24), *(iv)* protection of nerve cells against β-amyloid, or glutamate induced toxicity (25), *(v)* protection of erythrocytes in G6PD deficient human (26), *(vi)* stimulation of antioxidant defense system (27-29), *(vii)* improving endogenous antioxidant mechanisms in antioxidant deficient, diabetic rats (30-32), *(viii)* increased antioxidant capacity/activity in human (33-34), *(ix)* antierythema, antioedema, and anti-inflammatory effects (17,35-38), *(x)* inhibition of proinflammatory cytokine actions (39), *(xi)* inhibition of matrix metalloproteases activity (40), *(xii)* inhibition of histamine release from mast cells (41) *(xiii)* wound healing effects (42*),* and *(xiv)* clinical studies in different health and disease conditions (43-55).

Antioxidant profile: **in vitro** *studies*

Several studies have reported that Pycnogenol is a powerful free radical scavenger and/or antioxidant *in vitro.* Oxygen free radical (hydroxyl and superoxide) scavenging activity of Pycnogenol has been assessed by using a highly sensitive electron spin resonance spectrometer and compared to other bioactive free radical scavengers like *Ginkgo biloba* and green tea extract. Analogues of vitamin C and vitamin E were used as reference standards for hydroxyl radical scavenging activity and superoxide dismutase (SOD) was used as the reference standard for superoxide anion scavenging activity (19). In another study, murine macrophages have been activated by the bacterial wall component lipopolysaccharide (LPS) and interferon- γ (IFN-γ) in order to induce the expression of large amounts of the enzyme nitric oxide synthase (NOS). Pycnogenol was found to be a potent free radical scavenger of hydroxyl, superoxide and nitric oxide (NO) radicals (13-14). It increases life span of ascorbate radical and helps in regeneration of vitamin E (23). In addition, it was found to be resistant to the action of heat and ascorbate oxidase (19).

In two different *in vitro* studies, bovine vascular endothelial cells were treated with Pycnogenol before subjecting them to oxidative stress induced by t-butyl hydroperoxide (t-BHP). LDH release and malondialdehyde (MDA) respectively were utilized as biological markers to assess cell death and lipid peroxidation. Preincubation of endothelial cells with Pycnogenol at concentrations 10-80 μg/ml for 16 hours increased the cell viability after t-BHP treatment and, in addition, caused a dose-dependent decline in MDA induced by t-BHP (18,24).

In another independent *in vitro* study model, bovine retina was used as the tissue substrate to assess the capacity of Pycnogenol in protecting lipids from peroxidation expressed as formation of thiobarbituric acid reactive substances (TBARS). Pycnogenol significantly inhibited lipid peroxidation in a dose dependent manner at a concentration as low as 25 ng/ml and no damage to lipids were detected at a concentration of 250 ng/ml. In the same study Pycnogenol was found relatively more effective than a standardized extract from grape seeds, ascorbate, α-tocopherol, and lipoic acid (21). The antioxidant activity of Pycnogenol was further confirmed in an independent laboratory using three different *in vitro* models addressing the oxidative burst, LDL oxidation and iron/ascorbic acid system as oxidant challenges on different substrates. Pycnogenol exhibited a concentration-dependent inhibition of oxidative burst triggered by zymosan in J774 murine macrophages *in vitro*. Pycnogenol when co-incubated with copper sulphate used to oxidize human plasma LDL (formation of TBARS used as markers) resulted in inhibition of LDL oxidation in a concentration dependent manner. Pycnogenol significantly minimized the strand cleavage (measured by agarose gel electrophoresis) in pBR322 plasmid DNA caused by hydroxyl radicals generated by iron/ascorbic acid (20).

Pretreatment with LPS of murine macrophages (RAW 264.7) leads to increased expression and release of proinflammatory cytokines, such as interleukin-1 β (IL-1β). Incubation with Pycnogenol was associated with a dose-dependent decrease in the production of proinflammatory mediators. According to this report, Pycnogenol has been found to be able to block the activation of two major transcription factors, NF-κB and AP-1, involved in the production of IL-1β (39). Further, the treatment with Pycnogenol induced a concentration-dependent increase in intracellular glutathione (GSH), GSH peroxidase (GSH-Px) and GSH disulphide reductase (GSSG-R), SOD and catalase (CAT) levels expressed as per mg of protein and therefore the enhancement of cellular endogenous antioxidant activity (27-29).

Antioxidant and anti-inflammatory profile: **in vivo** *animal studies*

Pycnogenol was shown to have remarkable free radical scavenging activity *in vitro* and anti-inflammatory activity *in vivo* (17). Anti-inflammatory and wound healing effects were demonstrated subsequently (35-36,42). There is a body of experimental evidence indicating that oxidative

stress is involved in the pathophysiology of diabetes and its complications. In streptozotocin-induced diabetic rats the GSH to GSSG ratio and the activities of endogenous antioxidant enzymes (SOD, CAT, GSH-Px, GSSG-R) were significantly increased after Pycnogenol administration. The activity of γ-glutamyl transpeptidase (γ-GT), and enzyme in the pathway of GSH synthesis, was also increased. These changes were associated with a significant decrease in blood glucose levels in diabetic rats (30). Another study from the same laboratory further showed that Pycnogenol administered alone or in combination with β-carotene, once again increased GSSG-R activity (31).

The experiments were repeated focusing on diabetic retinopathy. Decreased retinal γ-GT activity of diabetic rats was normalized by the administration of Pycnogenol alone or in combination with β-carotene. On the other hand, elevated activity of SOD in diabetic retina, which is considered an indication of a response to a "constitutive" oxidative stress was normalized by Pycnogenol and β-carotene combination (32). The results obtained from the above three studies indicate that Pycnogenol significantly affects intracellular antioxidant defence mechanisms in streptozotocin-induced diabetic rats.

Antioxidant profile: clinical research

Clinical research data on Pycnogenol are provided based on its antioxidant activity in healthy volunteers. The effect of Pycnogenol on plasma antioxidant defenses in human was demonstrated by a significant increase of oxygen radical absorbance capacity (ORAC) in plasma throughout the Pycnogenol supplementation period of three weeks. In addition to its ability to enhance plasma antioxidant capacity, Pycnogenol significantly reduced LDL-cholesterol levels and increased HDL-cholesterol levels in the blood (33). In another independent double-blind study, Pycnogenol significantly improved erectile dysfunction, from moderate to mild stage. The same study reports a simultaneous significant increase of plasma antioxidant activity was observed. The level of total cholesterol decreased from 5.41 to 4.98 mmol/L associated with a decrease LDL cholesterol from 3.33 to 2.78 mmol/L (34).

An acute exposure to ultraviolet rays (UVR) leads to inflammatory response and skin erythema. The exposure to UVR is to be considered one of the major pro-oxidant challenges, and stimulates, as a tissue response, the expression of many proinflammatory genes such as for tumor necrosis factor-alpha (TNF-α), IL-1α, IL-1β, IL-6, and IL-8. All these cytokines contain NF-κB binding sites in the 5'flanking region of their encoding gene.

The preventive effects of orally supplemented Pycnogenol against UV-induced skin erythema was studied. In addition, the inhibitory effects Pycnogenol on NF-κB -dependent gene expression, chosen as a marker of proinflammatory response induced in spontaneously transformed human keratinocyte cell line (HaCaT) after UV exposure (37). In this study, Pycnogenol produced a significant increase in the dose of UVR necessary to achieve standardized erythema response (minimum erythema dose, MED) in the skin of human subjects. The activation of the proinflammatory and redox-regulated transcription factor (NF-κB) plays a major role in the UVR-induced erythema. Pycnogenol inhibited UVR-induced NF-κB-dependent gene expression in a concentration-dependent manner. This inhibitory mechanism possibly contributes to an antierythema effect (37).

Oxidative stress is also involved in pathogenesis of other clinical conditions like skin ageing, erythema, melasma, abnormal sperm morphology, and gingival bleeding and plaque formation. The effects of Pycnogenol were studied independently in these conditions. Supplementation with Pycnogenol along with other micronutrients in a formulation Evelle improved visible signs of skin aging, increased skin elasticity and decreased skin roughness (53). Pycnogenol supplementation provided relief from erythema and melasma (37,43). The average melasma area and intensity of pigmentation were found to be significantly reduced after supplementation (43). Pycnogenol, possibly by its antioxidant capacity, has also been found to improve abnormal sperm morphology and its functions (44). Supplementation therapy provided relief from pain in dysmenorrhea in an open clinical study (51).

Other beneficial effects of Pycnogenol due at least in part to its anti-inflammatory potential have been demonstrated in asthma (54-55) targeting leukotrienes (55) and histamine release (41). Similarly, the incorporation of Pycnogenol in chewing gum was associated with decreased gingival bleeding and plaque formation. Also this effect has been attributed to its antioxidant and anti-inflammatory activities (45).

Vasodilatory effects and antiplatelet activity

Pycnogenol stimulates the activity of endothelial NOS (e-NOS) *in vitro* and *in vivo* in isolated rat aortic rings (56) as well as human sperms (46). The constitutive form of e-NOS catalyses the synthesis of NO generating a relaxing signal through cyclic GMP to smooth muscles cells underlying endothelial cells. Furthermore, NO reacts with blood platelets

and prevents their aggregation.

Adrenaline, as well as noradrenaline, generally recognized as stress hormones, are very potent vasoconstrictors. In experiments with isolated aortic rings from rats, Pycnogenol inhibited the vasoconstriction induced by these stress hormones. The effect was dose dependent and could not be observed after removal of endothelium suggesting that the observed effect was mediated by the induction of NO generation by endothelial cells (56). Smokers usually present higher levels of stress hormones than non-smokers and therefore have high platelet reactivity. Pycnogenol administration to smokers at a dose of 100 mg significantly decreased platelet reactivity. This effect was similar to that induced by the classical cycloxigenase-2 inhibitor aspirin (at a dose of 500 mg) without the typical increase in bleeding time normally observed after aspirin administration (47). The antiplatelet effect of Pycnogenol was dose-dependent within a dose range between 25 and 200 mg (48). Microvascular blood flow in human nail capillaries has been shown to be improved after administration of Pycnogenol in an independent clinical study (50). In a double blind, placebo-controlled crossover study performed in 11 patients, supplementation with Pycnogenol in a daily dose of 200 mg, normalized the blood pressure with mild hypertension and lowered plasma thromboxane levels (49).

In another double-blind, placebo-controlled trial performed in 58 subjects, supplementation of 100 mg of Pycnogenol per day significantly decreased the consumption of the antihypertensive medicine, the calcium channel blocker nifedipine normally required to control hypertension. Plasma levels of the vasoconstrictor endothelin-1 were reduced in association with a significant increase in plasma concentration of the vasodilator prostacyclin (52).

Collagen stabilizing properties

Reactive oxygen species (ROS) activate matrix metalloproteinases (57) and thus play a significant role as signaling molecules to contribute to cell injury and collagen proteolysis. A dysfunction in the regulation of the activity of these enzymes contributes to pathogenesis of various chronic inflammatory diseases. Pycnogenol and its metabolites have been shown to produce dose-dependent inhibitory effects on these enzymes and thus contribute to collagen stabilizing properties (58). Moreover, a specific affinity of Pycnogenol for collagen and elastin has been shown to be able to shield and protect these proteins from proteolytic degradation. This effect on structural proteins therefore contributes to the "sealing effect" of Pycnogenol increasing the integrity of small blood vessels (59).

ROLE OF PYCNOGENOL IN CARDIOVASCULAR DISEASE AND GLUCOSE CONTROL

Chronic venous insufficiency and deep vein thrombosis

Pycnogenol has been shown to produce beneficial effects in chronic venous insufficiency. The symptoms improvement score showed a statistically significant reduction in the feeling of heavy legs, swelling and pain in patients treated with Pycnogenol as observed in three independent double blind clinical studies performed in patients with chronic venous insufficiency (CVI). The oedema was the more responsive sign to treatment in these studies (5-7).

Two independent studies have been reported in healthy travellers with risk of deep vein thrombosis (DVT) where beneficial effects of Pycnogenol have been reported when it was either given alone or in combination (60,61). Furthermore, beneficial effects of Pycnogenol supplementation in patients with venous ulcers have been demonstrated (62). Based on the pathophysiology of DVT and motion sickness and biological profiles of Pycnogenol, and ginger extract, a rational of development of a combination product Zinopin was developed by Scurr and Gulati (63), which was followed up by a clinical study in travellers undertaking long-haul flight, beneficial effects of Zinopin in DVT symptoms and incidence on motion sickness have been reported (64).

Hypertension

Supplementation with Pycnogenol over a period of 8 weeks significantly reduced systolic blood pressure; however diastolic component was not changed (49). This observation supports its effect on stress induced component, by virtue of its vasodilatory effect through NO release by endothelial cells. In another placebo-controlled study in hypertensive patients receiving regular antihypertensive medication nifedipine, Pycnogenol supplementation caused decrease in nifedipine consumption to achieve same antihypertensive response. Blood samples analysis showed that Pycnogenol increased the levels of vasodilatory factors NO and prostacyclin and reduced the level of vasoconstrictor factor endothelin-1 (52).

Diabetes and diabetic retinopathy

There is experimental and clinical evidence to support that diabetes is associated with elevated blood levels of lipid peroxidation products (65-67) and role of oxidative stress is well documented (68,69). Since hyperglycemia, elevated blood lipid levels, increased oxidizability of LDL, decreased endogenous antioxidant enzyme system and enhanced platelet aggregation are associated with diabetes and related conditions

such as diabetic retinopathy and other cardiovascular complications, Pycnogenol by working through the above mentioned target parameters produce beneficial effects in diabetes in animal models and in clinical studies (30-32, 70,71)

Pycnogenol lowered fasting and postprandial blood glucose levels in a dose dependent manner and improved endothelial functions by lowering endothelin-1 level and increasing prostacyclin and NO concentration in blood (70). These results were confirmed in a placebo-controlled, double blind study in diabetic patients (71). Pycnogenol has been shown to produce beneficial effects in diabetic retinopathy (8-9). Capillary permeability index was significantly reduced in both eyes after Pycnogenol treatment when measured using fluorescence angiography. In another multicenter study carried out in Germany in 1169 patients with diabetic retinopathy, Pycnogenol administration for six months not only stopped deterioration of visual acuity but visual acuity index was improved in some cases (9). Furthermore, its ability in improving blood lipid profile in diabetic patients has an important systemic role in the clinical management of this condition (8).

In conclusion, Pycnogenol maintains or improves cardiovascular health and glucose control by *(i)* optimizing the release of NO from endothelium, it produces blood vessel relaxation and antiplatelet effects resulting in better circulation, *(ii)* reducing blood pressure in mild to moderate hypertension, *(iii)* improving blood lipid profile, reducing peroxidation of LDL and thus cardiovascular disease risks, *(iv)* reducing blood sugar levels and inhibiting oxidative stress and thus the risk of diabetes, and *(v)* "capillary sealing" effect which is suggested to be the basis for reduction of retinal microbleedings and exudation in diabetic retinopathy.

NUTRIGENOMICS

Pycnogenol has been shown to have fundamental role in the antioxidant network recycling the ascorbyl radical and protecting vitamin E against oxidation (23). It has a strong free radical scavenging activity against ROS and reactive nitrogen species (13). In the same study it was demonstrated that pre-treatment of monocyte-macrophage cell line (RAW 264.7) with Pycnogenol for 24 hours before the administration of LPS and IFN-γ was associated to a dose-dependent decrease of nitrite and nitrate generation (13). Although this effect could be the result of a direct free radical scavenging activity against NO, the authors observed a reduction in the expression of the inducible form of NOS, giving a first indication that gene expression modulatory effects play a key role in the protective mechanism exerted by Pycnogenol. Using a dual luciferase reporter gene assay that reveals the NF-κB-dependent gene expression induced by IFN-γ in RAW 264.7 cell line. One hour incubation with procyanidins was associated with different aspects towards gene expression, depending on the degree of polymerization: Pycnogenol and trimeric procyanidin enhanced the NF-κB-dependent gene expression, while monomers and dimers suppressed it (72). Bito *et al* (73) reported that 12 hour pretreatment with Pycnogenol significantly inhibited in a dose-dependent manner IFN-γ induced expression of ICAM-1 in the human immortalized keratinocytes cell line HaCAT. This observations pairs with the reported modulatory effects of Pycnogenol on the adhesion of Jurkat T cells to activated keratinocytes in a cocultutre assay (74). Using gel mobility shift assay, it was identified STAT-1 and IRF-1 as two transcription factors affected by Pycnogenol, critical for IFN-γ dependent gene activation (74).

Cho *et al* (39) confirmed that Pycnogenol pretreatment prevented the activation of both NF-κB and AP-1 in activated RAW 264.7 cell line. They also found that such a pretreatment abolished LPS-induced IκB (an inhibitory protein that associates with NF-kB) degradation and downregulated IL-1β gene expression in RAW 264.7 and IL-2 gene expression in human T-cell line Jurkat E6.1. Pycnogenol ability to inhibit NF-κB activation and VCAM-1 and ICAM-1 expression was also reported in TNF-α-treated human umbilical endothelial cells (25). Pycnogenol treatment of human keratinocytes (HaCaT) inhibited in a concentration dependent manner UVR-induced NF-kB dependent gene expression suggesting a role of Pycnogenol in protecting human skin against erythema (37).

Application of cDNA array techniques

Complementary DNA (cDNA) arrays are global expression analyses tools that have been recently applied also to nutrition science providing information on how nutrients act at molecular level, regulating gene functions, and signal transduction pathways. Application of arrays to nutrigenomics will lead to discoveries in this field and may allow to treat or prevent life style-related diseases. cDNA arrays consist of high density nylon, plastic glass or silica supports bearing immobilized sequences complementary to thousands genes. Supports are hybridized with labeled cDNA sequences obtained by reverse transcription of mRNA prepared from cells and tissues and resulting hybridization signal are compared to controls.

Rihn *et al* (74) reported that 24 hour supplementation of Pycnogenol is associated to significant modulation of 39 out of the 83 genes detected in a commercially available human cDNA array bearing a total of 588 genes in human

cells keratinocyte line HaCaT. Among modulated genes, it is worth mentioning that two genes coding calgranulins A and B, members of the highly conserved S100 family of low weight calcium-binding proteins, which are known to accumulated in psoriasis and various dermatitis conditions.

Targeting to better understanding of the anti-inflammatory and putative anticancer properties of Pycnogenol, we recently applied a genomic analysis method to human tumor monocytic U397 cell line. U397 cells are model for monocyte to macrophage differentiation studies and are currently used in many laboratories in inflammatory and tumorogenicity studies. Preliminary unpublished results suggest that Pycnogenol treatment is associated with a down regulation of gene encoding for monogenic hyperproliferative proteins and an upregulation of genes coding for proliferation check-point proteins. We also found a downregulation of hCDC10 and upregulation of KOX15 genes in Pycnogenol-treated U937 cells (75,76). Gene expression profiling of cancer cell lines challenged by procyanidins provide clues on the molecular pathways that are related to a putative anticancer property of Pycnogenol. It is important to stress that these observations, even though of interest, must be considered preliminary and confirmation is necessary before reaching any final conclusion about the effective role of pine bark procyanidins on gene expression in human cancer cells. The application of array technology to nutrition will help the comprehension of nutrient effects, as well as molecular mechanisms underlying them, on human health and how nutrition influences the normal homeostasis and physiology.

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REFERENCES

1. Maritime Pine Bark Extract – USP 28. United States Pharamacopeial Convention, Inc. *The United States Pharmcopeia* official from January 1, 2004; 2024-2025.
2. Rohdewald PJ. Pycnogenol®, French maritime pine bark extract. *Encyclopedia of Dietary Supplements* 2005; 545-552.
3. Drehsen G. From ancient pine bark uses to Pycnogenol. In: L. Packer, M. Hiramatsu, T. Yoshikawa, editors. *Antioxidant Food Supplements in Human Health*. Academic Press, 1999; 20: 311-322.
4. Gulati OP. Pycnogenol® in venous disorders: A review. *Eur Bull Drug Res* 1999; 7: 8-13.
5. Arcangeli P. Pycnogenol® in chronic venous insufficiency. *Fitoterapia* 2000; 71: 236-244.
6. Petrassi C, Mastromarino A, Spartera C. Pycnogenol® in chronic venous insufficiency. *Phytomedicine* 2000; 7: 383-388.
7. Koch R. Comparative study of Venostasin® and Pycnogenol® in chronic venous insufficiency. *Phytother Res* 2002; 16: S1-S5.
8. Spadea L, Balestrazzi E. Treatment of vascular retinopathies with Pycnogenol. *Pythother Res* 2001; 15: 1-5.
9. Schönlau F, Rohdewald P. Pycnogenol® for diabetic retinopathy: A review. *Int Ophthalmol* 2002; 24: 161-171.
10. Rohdewald, P. A review of the French maritime pine bark extract (Pycnogenol®), a herbal medication with a diverse clinical pharmacology. *Int J Clin Pharmacol Ther* 2002; 40: 158-168.
11. Watson RR. Review: Pycnogenol® and cardiovascular health. *Evidence Based Integr Med* 2003; 1: 27-32.
12. Blumenthal, M. Pycnogenol (French Maritime Pine Bark Extract) *Pinus Pinaster* Aiton subsp. atlantica. *The American Botanical Council Guide to Herbs* 2003 ; 369-373.
13. Virgili F, Kim D, Packer L. Procyanidins extracted from pine bark protect α-tocopherol in ECV 304 endothelial cells challenged by activated RAW 264.7 macrophages: role of nitric oxide and peroxynitrite. *FEBS Letters* 1998; 431: 315-318.
14. Virgili F, Kobuchi H, Noda Y, Cossins E, Packer L. Procyanidins from *Pinus maritima* bark: Antioxidant activity, effects on the immune system and modulation of nitrogen monoxide metabolism. In: L. Packer, M. Hiramatsu, T. Yoshikawa, editors. *Antioxidant Food Supplements in Human Health*. Academic Press 1999; 21: 323-342.
15. Packer L, Rimbach G, Virgili F. Antioxidant activity and biologic properties of a procyanidin-rich extract from the pine (*Pinus maritima*) bark, Pycnogenol. *Free Rad Biol Med* 1999; 27: 704-724.
16. Rohdewald P. Pycnogenol®. In: Catherine A, Rice E, Packer L, editors. *Flavonoids in Health and Disease*. Marcel Dekker Inc., NY. 1998; 17: 405-419.
17. Blazso G, Gabor M, Sibbel R, Rohdewald P. An anti-inflammatory and superoxide radical scavenging activities of a procyanidins containing extract from the bark of *Pinus pinaster* sol. and its fractions. *Pharm Parmacol Lett* 1994; 3: 217-220.
18. Rong Y, Li L, Shah V, Lau BHS. Pycnogenol protects

vascular endothelial cells from t-butyl hydroperoxide induced oxidant injury. *Biotechnol Ther* 1995; 5: 117-126.

19. Noda Y, Anzai K, Mori A, Kohno M, Shinmei M, Packer L. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized, JES-FR30 ESR spectrometer system. *Biochem Mol Biol Int* 1997; 42: 35-44.
20. Nelson AB, Lau BHS, Ide N, Rong Y. Pycnogenol inhibits macrophage oxidative burst, lipoprotein oxidation and hydroxyl radical-induced DNA damage. *Drug Dev Indust Pharm* 1998; 24: 139-144.
21. Chida M, Suzuki K, Nakanishi-Ueda T, Ueda T, Yasuhara H, Koide R, Armstrong D. *In vitro* testing of antioxidants and biochemical end-points in bovine retinal tissue. *Ophthalmol Res* 1999; 31: 407-415.
22. Feng WH, Wei HL, Liu GT. Effect of Pycnogenol® on the toxicity of heart, bone marrow and immune organs as induced by antitumor drugs. *Phytomedicine* 2002; 9: 414-418.
23. Cossins E, Lee R, Packer L. ESR studies of vitamin C regeneration, order of reactivity of natural source phytochemical preparations.*Biochem Mol Biol Int* 1998; 45: 583-597.
24. Kim J, Chehade J, Pinnas JL, Mooradian AD. Effect of selected antioxidants on malondialdehyde modification of proteins. *Nutrition* 2000; 16: 1079-1081.
25. Peng QL, Buz'Zard AR, Lau BHS. Research report: Pycnogenol® protects neurones from amyloid β peptide-induced apoptosis. *Mol Brain Res* 2002; 104: 55-65.
26. Sharma SC, Sharma S, Gulati O. Pycnogenol® prevents haemolytic injury in G6PD deficient human erythrocytes. *Phytother Res* 2003; 17: 671-674.
27. Bayeta E, Bejamin MS, Lau HS. Pycnogenol inhibits generation of inflammatory mediators in macrophages. *Nutr Res* 2000; 20: 249-259.
28. Wei ZH, Peng QL, Lau BHS. Pycnogenol® enhances endothelial cell antioxidant defenses. *Redox Rep* 1997; 3: 219-224.
29. Janisch K, Hippeli S, Dornisch K, Kern S, Elstner EF. Determination of the antioxidative potential of human plasma after supplementation with Pycnogenol® and whey. *Food Res Int* 2002; 35: 257-266.
30. Maritim A, Dene BA, Sanders RA, Watkins JB. Effect of Pycnogenol® treatment on oxidative stress in streptozoitin-induced diabetic rats. *J Biochem Mol Toxicol* 2003; 17: 193-199.
31. Berryman AM, Maritim AC, Sanders RA, Watkins JB. Influence of treatment of diabetic rats with combinations of Pycnogenol, β carotene, and α-lipoic acid on parameters of oxidative stress. *J Biochem Mol Toxicol* 2004; 18: 345-352.
32. Dene BA, Maritim AC, Sanders RA, Watkins JB. Effects of antioxidant treatment on normal and diabetic rat retinal enzyme activities. *J Ocular Pharmacol Ther* 2005; 21: 28-35.
33. Devaraj S, Kaul N, Schönlau F, Rohdewald P, Jialal I. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters plasma lipoprotein profile. *Lipids* 2002; 37: 931-934.
34. Durackova Z, Trebaticky B, Novotny V, Zitnanova I, Breza J. Lipid metabolism and erectile function improvement by Pycnogenol®, extract from the bark of *Pinus pinaster* in patients suffering from erectile dysfunction, a pilot study. *Nutr Res* 2003; 23: 1189-1198.
35. Blazso G, Rohdewald P, Sibbel R, Gabor M. Anti-inflammatory activities of procyanidin-containing extracts from *Pinus pinaster* sol. Proceedings of the International Bioflavonoid Symposium, Vienna, Austria, Antus S, Gabor M, Vetschera K, editors. July 16-19, 1995; 231-238.
36. Blazso G, Gabor M, Rohdewald P. Anti-inflammatory activities of procyanidin containing extracts from *Pinus pinaster* Ait. after oral and cutaneous application. *Pharmazie* 1997; 52: 380-382.
37. Saliou C, Rimbach G, Moini H, McLaughlin L, Hosseini S, Lee J, *et al*. Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B-dependent gene expression in keratinocytes are modified by French maritime pine bark extract. *Free Radical Biol Med* 2001; 30: 154-160.
38. Sime S, Reeve V E. Protection from inflammation, immunosuppression and carcinogenesis induced by UV radiation in mice by topical Pycnogenol®. *Photochem Photobiol* 2004; 79: 193-198.
39. Cho K-J, Yun C-H, Yoon D-Y, Cho Y-S, Rimbach G, Packer L, *et al*. Effect of bioflavonoids extracted from the bark of *Pinus maritime* on proinflammatory cytokine interleukin-1 production in lipopolysaccharide-stimulated raw 264.7. *Toxicol Appl Pharmacol* 2000; 1687: 64-71.
40. Grimm T, Schäfer A, Högger P. Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (Pycnogenol). *Free Radical Biol Med* 2004; 36: 811-822.
41. Sharma SC, Sharma S, Gulati OP. Pycnogenol® inhibits

the release of histamine from mast cells. *Phytother Res* 2003; 17: 66-69.
42. Blazso G, Gabor M, Schönlau F, Rohdewald P. Pycnogenol® accelerates wound healing and reduces scar formation. *Phytother Res* 2005; 18: 579-581.
43. Ni Z, Mu Y, Gulati O. Treatment of melasma with Pycnogenol®. *Pytother Res* 2002; 16: 567-571.
44. Roseff SJ. Improvement in sperm quality and function with French maritime pine tree bark extract. *J Reprod Med* 2002; 47: 821-824.
45. Kimbrough C, Chun M, Dela Roca G, Lau BHS. Pycnogenol® chewing gum minimizes gingival bleeding and plaque formation. *Phytomedicine* 2002; 9: 410-413.
46. Stanislavov R, Nikolova V. Treatment of erectile dysfunction with Pycnogenol® and L-arginine. *J Sex Marital Ther* 2003; 29:207-213.
47. Pütter M, Grotemeyer KHM, Würthwein G, Araghi-Niknam M, Watson RR, Hosseini S, *et al*. Inhibition of smoking-induced platelet aggregation by aspirin and Pycnogenol. *Thromb Res* 1999; 95: 155-161.
48. Araghi-Niknam M, Hosseini S, Larson D, Rohdewald P, Watson RR. Pine bark extract reduces platelet aggregation. *Integr Med* 1999; 2: 73-77.
49. Hosseini S, Lee J, Sepulveda R T, Rohdewald P, Watson RR. A randomized, double-blind, placebo-controlled, prospective, 16 week crossover study to determine the role of Pycnogenol in modifying blood pressure in mildly hypertensive patients. *Nutr Res* 2001; 21: 1251-1260.
50. Wang S, Tan D, Zhao Y, Gao G, Gao X, Hu L. The effect of Pycnogenol® on the microcirculation, platelet function and ischemic myocardium in patients with coronary artery diseases. *Eur Bull Drug Res* 1999; 7: 19-25.
51. Kohama T, Suzuki N, Ohno S, Inoue M. Analgesic efficacy of French maritime pine bark extract in dysmenorrhea – An open clinical trial. *J Reprod Med* 2004; 49: 828-832.
52. Liu X, Wei J, Tan F, Zhou S, Würthwein G, Rohdewald P. Pycnogenol® French maritime pine bark extract improves endothelial function of hypertensive patients. *Life Sci* 2004; 74: 855-862.
53 Segger D, Schönlau F. Supplementation with Evelle® improves skin smoothness and elasticity in a double blind, placebo-controlled study with 62 women. *J Dermatol Treat* 2004; 15: 222-226.
54 Hosseini S, Pishnamazi S, Sadrzadeh MH, Farid F, Farid R. Watson RR. Pycnogenol® in the management of asthma. *J Med Food 2000;* 4: 201-209.
55 Lau BHS, Riesen SK, Truong KP, Lau EW, Rohdewald P, Barreta RA. Pycnogenol® as an adjunct in the management of childhood asthma. *J Asthma* 2004; 41: 825-832.
56. Fitzpatrick DF, Bing B, Rohdewald P. Endothelium-dependent vascular effects of Pycnogenol. *J Cardiovasc Pharmacol* 1998; 32: 509-515.
57. Belkhiri A, Richards C, Whaley M, McQueen SA, Orr FW. Increased expression of activated matrix metalloproteinase-2 by human endothelial cells after sublethal H_2O_2 exposure. *Lab Invest* 2004; 77: 533-539.
58. Grimm T, Schäfer A, Högger P. Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (Pycnogenol). *Free Rad Biol Med* 2004; 36: 811-822.
59. Schönlau F. The cosmeceutical Pycnogenol®. *J Appl Cosmetol* 2002; 20: 241-247.
60. Cesarone MR, Belcaro G, Nicolaides AN, Ricci A, Geroulakos G, *et al*. Prevention of venous thrombosis in long-haul flights with Flite tabs: The Lonflit-Flite randomized, controlled trial. *Angiology* 2003; 54: 531-539.
61. Belcaro G, Cesarone MR, Rohdewald P, Ricci A, Ippolito E, Dugall M, *et al*. Prevention of venous thrombosis and thrombophlebitis in long haul flights with Pycnogenol®. *Clin Appl Thromb Hemost* 2004; 10: 373-377.
62. Belcaro G, Cesarone MR, Errichi BM, Ledda A, Di Renzo A, Stuard S, *et al.* Venous ulcers: Microcirculatory improvement and faster healing with local use of Pycnogenol®. *Angiology 2005;* 56: 699-705.
63. Scurr JH, Gulati OP. Zinopin® - the rationale of its use as a food supplement in traveller's thrombosis and motion sickness *Phytother Res* 2004; 18: 687-695.
64. Scurr JH, Gulati OP. Zinopin® - its use as a food supplement in traveller's thrombosis, oedema and motion sickness. *Eur Bull Drug Res* 2005; 13, 77-81.
65. Garg MC, Singh KP, Bansal DD. Effect of viatamin E supplementation on antioxidant status of diabetic rats. *Med Sci Res* 1996; 24:325-326.
66. Valazquez E, Winocour PH, Kesteven P, Alberti KGM, Laker MF. Relation of lipid peroxides to macrovascular disease in type 2 diabetes. *Diabet Med* 1991; 8: 752-758.
67. Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P, Halliwell B, *et al.* Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. *Diabetologia* 1997; 40: 647-653.
68. Douillet C, Chanceerelle Y, Cruz C, Marocles , Kergonou

JF, Renaud S, *et al*. High dosage vitamin E. Effect of oxidative stress and serum lipids distribution in Streptoztocin-induced diabetic rats. *Biochem Med Metab Biol* 1993; 50: 265-276.

69. Kahler W, Kuklinsiki B, Ruhlmann C, Pléotz C. Diabetes mellitus - a free radical-associated disease: effects of adjuant supplementation of antioxidants. In: Gris FA, Wessel K, editors. *The Role of Antioxidants in Diabetes Mellitus: Oxygen Radicals and Antioxidants in Diabetes.* Verlag Grouppe 1993; 33-53.
70. Liu X, Ha-Jun Z, Rohdewald P. French maritime pine bark extract Pycnogenol® lowers glucose dose dependently in patients with diabetes type II. *Diabetes Care* 2003; 27: 839.
71. Liu X, Wei J, Tan F, Zhou S, Wèrthwein G, Rohdewald P. Antidiabetic effects of Pycnogenol®, French maritime pine bark extract, in patients with diabetes type II. *Life Sci* 2004; 75: 2505-2513.
72. Park YC, Rimbach G, Saliou C, Valacchi G, Packer L. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF-α secretion, and NF-κB-dependent gene expression in RAW 264.7 macrophages. *FEBS Lett* 2000*,* 465: 93-97
73. Bito T, Roy S, Sen CK, Packer L. Pine bark extract Pycnogenol downregulates IFN-γ-induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression. *Free Radical Biol Med* 2000*,* 28: 219-227.
74. Rihn B, Saliou C, Bottin MC, Keith G, Packer L. From ancient remedies to modern therapeutics, Pine bark uses in skin disorders revisited. *Phytother Res* 2001; 15: 76-78.
75. Virgili F, Ambra R, Canali R, Gulati O. Pycnogenol in cancer chemoprevention. In: Bagchi D, Preuss G, editors. *Phytopharmaceuticals in Cancer Chemoprevention*. 2005; 29; 491-508.
76. Canali R, Ambra R, Gulati O, Virgili F. Antioxidant and gene regulatory properties of procyanidins. Rimbach/ Packer: *Nutrigenomics - The Role of Oxidants and Antioxidants in Differential Gene Expression.* 2005; 18: 379-395.