Pycnogenol®, French Maritime Pine Bark Extract

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INTRODUCTION

Pycnogenol® is the registered trade name for a special standardized extract from the bark of the French maritime pine (*Pinus pinaster* ssp. *atlantica*), distributed by Horphag Research, U.K. The spray-dried, powdered extract is marketed worldwide as a food supplement, a herbal medicine, an ingredient in cosmetics, and as a food additive.

The traditional use of pine bark was in the treatment of scurvy and wound healing. Currently, Pycnogenol is used to restore capillary integrity in cases of gingivitis, retinopathy, or edema formation. Other fields of application are directed to protection of the circulation by inhibiting platelet aggregation, lowering of cholesterol levels, and high blood pressure. The procyanidins, in particular, have shown anti-inflammatory and radical-scavenging activity, leading to treatment of melasma and prevention of ultraviolet (UV)-induced damage of the skin. The application of Pycnogenol in reducing menstrual cramps and pain is probably caused by the spasmolytic activity of the phenolic acids. Very recently, a glucose lowering effect of Pycnogenol has been shown. Safety of Pycnogenol is documented by its generally recognized as safe (GRAS) status in the United States. Unwanted effects are mild and transient, and no interactions with drugs have been reported.

BACKGROUND

Raw material:

- Family: Pinaceae.
- Genus: *Pinus*.
- Species: *Pinus pinaster* Aiton, ssp. *atlantica* D. del Villar.
- Part used: Outer bark.

P. pinaster ssp. *atlantica*, the Atlantic maritime pine, is cultivated in large monocultures in South Western France in the Biscay area (Fig. 1). It is distinguished from other species by the thick, deeply fissured, reddish bark, representing a geographic race adapted to harsh climate and sandy soil. Trees are cut for timber production after cultivation for 30–50 yr, and the fresh outer bark is used for extraction throughout the year. The bark pieces are 1–3 cm thick, and they are formed from up to 50 mussel-shaped, deep red or light brown layers. The inner side of the bark is slightly concave and plane, while the outer part is irregular with deep cut V-shaped fissures.

Traditionally, preparations from pine bark had been used in the Middle Ages for wound healing, as

Fig. 1 Forest of French maritime pine trees.

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Encyclopedia of Dietary Supplements DOI: 10.1081/E-EDS-120022123

referred to in the *Thesaurus Medicaminum* of the Zurich pharmacist H. Minner (1479). Also, in North America, Native Americans used bark from conifers for wound healing, and to treat scurvy.[1]

CHEMISTRY AND PRODUCTION

Composition

Pycnogenol represents a concentrate of phenolic compounds, consisting of phenolic acids, catechin, taxifolin, and procyanidins.[2]

The phenolic acids are derivatives of benzoic acid—*p*-hydroxybenzoic acid, protocatechic acid, vanillic acid and gallic acid—or of cinnamic acid, *p*-cumaric acid, caffeic acid, and ferulic acid. Glycosides and glucose esters of these phenolic acids have been identified.

Catechin is found as the main monomeric procyanidin in Pycnogenol, while epicatechin is present in traces. Another flavonoid, taxifolin, is available in free form and as taxifolin glucoside.

The main constituents of Pycnogenol are procyanidins, biopolymers consisting of catechin and epicatechin subunits (Fig. 2). Chain lengths from dimers up to 12 monomeric units are present. The catechin–epicatechin units can be linked by C4–C8 bonds or by C4–C6 bonds, with the C4–C8 linked isomers predominating.

As inorganic ions, calcium, potassium, and iron are present, together with traces of manganese, zinc, and copper.[3]

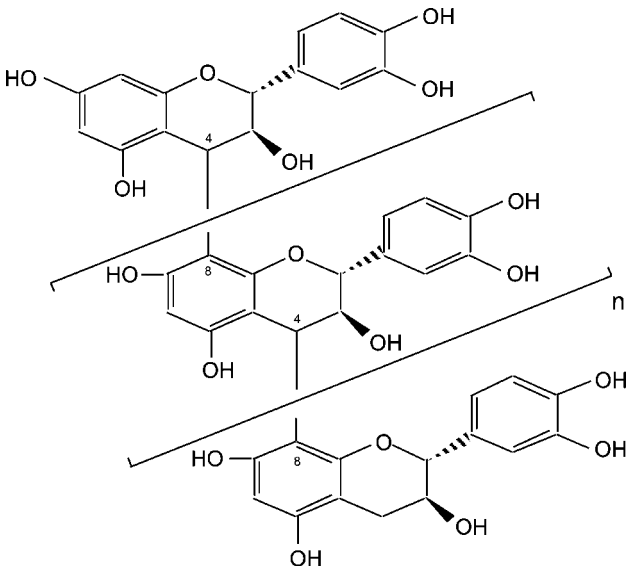

Fig. 2 Procyanidin with type 4–8 bonds. Monomers could be catechin or epicatechin units.

Production

The extract is prepared from fresh sorted, cleaned, and crushed bark. The patented extraction process chain uses ethanol and water as solvents in a multistep process. The purified aqueous extract is spray dried and represents a very fine, brownish-colored powder with an aromatic smell and astringent taste. It is soluble in water, methanol, and ethanol, and insoluble or sparingly soluble in oils. One thousand kilogram bark is needed to produce 1 kg Pycnogenol.

Formulations

Pycnogenol is available as tablets or capsules of 20–100 mg. For oral health care, a mouth spray delivering 2 mg per actuation and a chewing gum containing 5 mg Pycnogenol are available. In concentrations between 100 and 170 mg/L, it is formulated as an aromatized water. A wide range of cosmetic products containing the extract is sold worldwide.

Analysis

The quality of the bark of *P. pinaster* is controlled according to the monograph "Maritime Pine" of the *National Formulary of the US Pharmacopoeia*.[4]

The standardized extract, Pycnogenol, corresponds to the monograph: "Maritime Pine Extract" of the *National Formulary of the US Pharmacopoeia*[4] in terms of identity and purity using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The content of procyanidins (between 65% and 75%) is quantified colorimetrically after oxidative hydrolysis.

Stability

Pycnogenol, stored and protected from light and humidity in well-closed containers at room temperature, is stable over a period of 3 yr.

PRECLINICAL STUDIES

Circulatory Function

Stimulation of e-NOS

Pycnogenol stimulates the activity of endothelial nitric oxide synthase (e-NOS) in vitro and in vivo (Fig. 3).

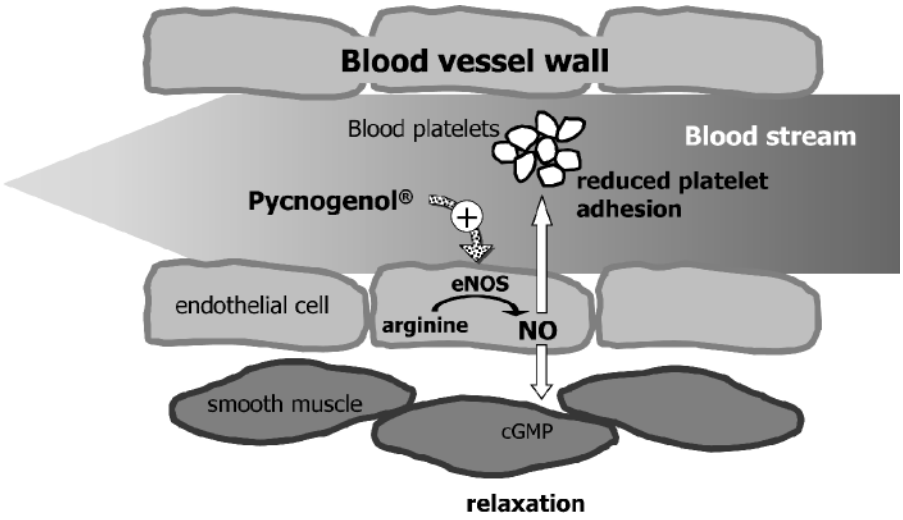

Fig. 3 Scheme for enhanced NO production by increasing synthesis of endothelial NOS.

In isolated aortic rings from rats[2] as well as in human sperms,[5] it stimulated endothelial NO production from natural substrate L-arginine by e-NOS. Nitric oxide (NO) initiates release of cGMP in smooth muscle cells and leads to vasorelaxation. Furthermore, NO reacts with blood platelets and prevents their aggregation.

Inhibition of adrenaline-induced vasoconstriction

Adrenaline as well as noradrenaline 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 the endothelium.[2]

Antihypertensive effect

In vitro, Pycnogenol inhibits the angiotensin-converting enzyme (ACE), while in rats, blood pressure could be reduced significantly after i.v. injection.[2]

Capillary Integrity

Protein binding

Procyanidins, the main constituents of Pycnogenol, belong to the class of nonhydrolyzable tannins and have a high affinity to proteins. Pycnogenol binds selectively to collagen, elastin, and skin powder, whereas binding to egg albumin is low.[6] Also, the interaction with enzymes is specific, as a result of the differing IC_{50} values[3] for inhibition of various enzymes. Stabilization of membranes of erythrocytes via protein binding may be the cause of prevention of hemolytic injury in glucose-6-phosphate dehydrogenase deficient human erythrocytes.[7]

Capillary sealing

Spontaneous hypertensive rats show a pathologically high leakage of capillaries. Feeding with Pycnogenol produced a long-lasting, dose-dependent increase of capillary resistance against a topically applied vacuum.[2]

Anti-inflammatory Activity

Radical-scavenging activity

In several in vitro models, Pycnogenol inactivated superoxide- and hydroxy-radicals as well as inhibited the formation of singlet oxygen and nitric oxide-radicals.[2,3] The superior capacity of procyanidins in radical scavenging is based on their ability to retain scavenging activity by intramolecular rearrangements.[2] The lifetime of the ascorbyl-radical is prolonged by Pycnogenol to a greater extent than by other bioflavonoids,[3] and the oxidation of low density lipoprotein (LDL) was inhibited in vitro.[3] DNA was protected against iron/ascorbate-induced strand breaks by Pycnogenol.[3] Toxicity of free radical producing antitumor drugs was reduced by pretreatment of mice with Pycnogenol without reducing the anticancer activity of doxorubicin and cyclophosphamide.[8]

Antioxidative effects in biological systems

Incubation with Pycnogenol protected α-tocopherol in endothelial cells against oxidation by peroxynitrite,[3] protected nerve cells against β-amyloid- or glutamate-induced toxicity,[2,3] and inhibited peroxidation of retinal lipids more efficiently than vitamins E and C.[2] Neurons were protected from amyloid-β-peptide-induced apoptosis.[9]

Stimulation of synthesis of antioxidative substances

Pycnogenol incubation doubles the concentrations of antioxidative enzymes in vascular endothelial cells.[2] Synthesis of proteins in macrophages is increased, and activity of antioxidative enzymes, like catalase or superoxide dismutase, is dose dependently enhanced.[2]

Interaction with NF-κB

In a murine macrophage cell line, preincubation with Pycnogenol blocked the activation of nuclear factor kappa B (NF-κB) and the activator protein (AP-1), major transcription factors centrally involved in inflammatory processes.[10] In a human lymphocyte cell line, the extract inhibited the transcription factors NF-AT and AP-1.[10] It also prevented the UV-induced activation of transcriptional factors NF-κB and AP-1 in human cell lines from fibroblasts and keratinocytes.[2] In human endothelial cells, pretreatment with Pycnogenol suppressed the activation of NF-κB by tumor necrosis factor-α (TNF-α).[2] These results indicate that proinflammatory responses can be inhibited by Pycnogenol early in the biochemical reaction chain at the transcriptional level.

Inhibition of inflammatory mediators

At the level of cytokines, Pycnogenol blocks production of interleukins 1, 2,[10] 6, and 10.[2] Synthesis of inducible nitric oxide synthase is blocked by preincubation of macrophages with Pycnogenol,[3] and the release of histamine from mast cells is inhibited in vitro.[2]

Inhibition of adhesion molecules

Intercellular adhesion molecules are necessary for tissue invasion of leukocytes in inflammatory processes. Pycnogenol pretreatment downregulates expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[2]

Inhibition of matrix metalloproteases

Matrix metallo-proteases (MMPs) destroy collagen and elastin. Pycnogenol and its metabolites inhibit MMP1, MMP2, and MMP9, and furthermore prevent the release of MMP9 from human monocytes.[6]

Inhibition of UV-induced damage

Topical application of a Pycnogenol-containing gel significantly prevented erythema formation after UV radiation[2] and inhibited photocarcinogenesis.[11] Wound healing was accelerated and scar formation reduced following application of 1–2% Pycnogenol in gels.[12]

Age-Related Degenerative Processes

Stimulation of immune system

Incubation with Pycnogenol augmented phagocytosis in macrophages.[13] Feeding of the extract to immunosuppressed mice provided protection against protozoal infection,[14] and to senescence-accelerated mice restored levels of progenitor cells and β- and T-lymphocytes in a dose-dependent manner.[2] Oral administration of Pycnogenol to retrovirus infected mice[2] or mice inoculated with cancer cells[15] showed enhanced natural killer cell activity.

Enhancement of cognitive function

Administration of Pycnogenol to senescence-accelerated mice significantly enhanced the memory retention rate in a dose-dependent manner compared to non-supplemented mice in step-through and step-down tests. The treatment also significantly improved the cognitive behavior in the shuttle box test.[2]

Antiaging effects

The life span of mice was prolonged after oral administration of a Pycnogenol-containing combination of antioxidants.[16] That of drosophila was increased after feeding the extract.[17]

Spasmolytic Activity

Phenolic constituents of Pycnogenol, ferulic acid and caffeic acid, possess spasmolytic activity as demonstrated in vivo on the isolated rat uterus[2] as well as in vivo in experiments with rats.[2]

Blood Glucose Lowering

In streptozotocin-induced diabetic rats, Pycnogenol feeding lowered blood glucose concentrations and enhanced concentrations of antioxidative substances in blood.[18]

Proposed Mechanisms of Action

Based on the support of nitric oxide production, Pycnogenol offers a range of pharmacological effects on the vascular system, such as increased microcirculation, improvement of erectile function, antihypertensive effects, and inhibition of platelet aggregation. The enhancement of capillary integrity results in an antiedema effect and prevents microbleedings. Its radical-scavenging potency may contribute to the anti-inflammatory actions and beneficial effects in degenerative diseases. The spasmolytic activity of phenolic acids contained in Pycnogenol is probably related to its activity in reducing premenstrual cramps and pain. The lowering of blood glucose in streptozotocin-induced diabetic rats points to an antidiabetic effect.

Safety Studies (On File at Horphag Research Ltd., U.K.)

Animal toxicology

The *absence of mutagenic* effects has been shown using the Ames test, the chromosome aberration assay in human lymphocytes and the micronucleus test in mice.

Acute toxicity is very low. Fourteen acute toxicity tests had been performed using three different species and oral, subcutaneous, intraperitoneal, and intravenous routes of administration. LD50 data varied after oral administration from 1000 to 4000 mg/kg.

Chronic toxicity had been tested in three species after oral administration. The no-adverse-effects' level (NOAEL) was established as 100 mg/kg/day.

In six *reproduction toxicity* studies with three species no teratogenic effects were detected, no signs of perinatal toxicity or negative effects on fertility were noted.

Tolerance after topical application was tested in several models. Skin and eye irritation tests in rabbits and contact hypersensitivity test in guinea pigs showed that Pycnogenol is nonirritating. In human volunteers, no skin irritation was found with the patch-occlusion test.

Absorption and metabolism

After oral intake, four metabolites could be found in the urine of a human volunteer. Taxifolin and ferulic acid were excreted as glucuronides or sulfates after 1–4 hr.[2] Another investigation found a maximum excretion of ferulic acid after intake of Pycnogenol at 17 hr.[3] Procyanidins had been metabolized to valerolactones, which are excreted as glucuronides after 8–15 hr.[2]

CLINICAL STUDIES

Circulatory Function

Improved microcirculation

By microscopic observation of blood capillaries through fingernails it was found that the capillary diameter was increased significantly compared to placebo following intake of Pycnogenol. Supplementation of 60 cardiovascular patients with the extract for 1 mo improved microcirculation significantly as a result of increased vasodilation. The rate of cardiovascular events diminished significantly in the Pycnogenol group compared to placebo.[2]

Antihypertensive effect

In a double-blind, placebo-controlled crossover study with 11 patients, supplementation with 200 mg Pycnogenol normalized blood pressure of patients with mild hypertension and lowered thromboxane levels.[2] In another double-blind, placebo-controlled trial with 58 subjects, intake of 100 mg allowed to reduce significantly the dosage of the calcium channel blocker nifedipine required for treatment of patients with hypertension. Plasma levels of endothelin-1 were reduced, and concentrations of prostacyclin were elevated.[19]

Inhibition of platelet aggregation

Smoking produces an activation and aggregation of blood platelets. This platelet aggregation was inhibited

in smokers dose dependently by Pycnogenol,[2] and the effect persisted over several days. In another group of smokers, thromboxane levels were decreased in blood in addition to the inhibition of platelet aggregation.[2] Also, in cardiovascular patients, platelet aggregation was inhibited following intake of the extract.[2]

Improved erectile function

A double-blind, placebo-controlled study demonstrated a significant improvement of erectile function following supplementation with Pycnogenol in men with mild erectile dysfunction.[20] Supplementation with L-arginine, the substrate for NO production, led to no significant improvement of erectile function in another clinical study with patients of similar condition.[21] However, if the same subjects were given Pycnogenol in addition to L-arginine, the percentage of those with completely restored sexual function increased dramatically. Continuation of treatment with the combination of L-arginine and Pycnogenol in a higher dose yielded a response rate of 80%. Intensity and duration of erectile function were quite significantly improved.[21]

Cholesterol lowering

In an open, controlled study with 25 volunteers, LDL was lowered significantly after 4 weeks supplementation with 150 mg Pycnogenol, while high density lipoprotein (HDL) as well as overall radical absorbance capacity of blood was increased.[22] A double-blind, placebo-controlled study with 21 patients showed significant reduction of total cholesterol and LDL after intake of 120 mg Pycnogenol.[20] In a comparative, controlled study, total cholesterol and LDL were significantly lowered.[23]

Capillary Integrity

Gingival bleeding

In a placebo-controlled study with dental students, a Pycnogenol-containing chewing gum reduced gingival bleeding and plaque formation compared to regular sugar-free chewing gum.[24]

Effects in vascular retinopathy

Diabetic microangiopathy causes leakage of retinal capillaries. Two open-case experiments, two double-blind, placebo-controlled trials, and a multicenter field study with a total number of 1289 patients showed unequivocally that Pycnogenol retained progressions of retinopathy and partly improved visual acuity. It restored capillary integrity and reduced leakage of blood into the retina.[25]

Inhibition of edema formation

Chronic venous insufficiency is associated with edema formation in the lower legs, leading to the feeling of heavy legs, swelling, cramps, and pain. In five placebo-controlled, double-blind studies and three double-blind, controlled experiments, these symptoms had been significantly reduced.[2] Findings could be objectivated by measuring the circumference of lower limbs[23] and demonstrated superior activity compared to a commercial horse chestnut seed extract, a remedy for venous disorders.[23]

Prevention of deep vein thrombosis

In a double-blind, placebo-controlled, randomized trial with 198 passengers, 400 mg Pycnogenol prevented thrombus formation after long-haul flights in the verum group. In the placebo-group, 5 cases of thrombosis were observed, and none in the verum group.[26]

Anti-inflammatory Activity

Reduction of asthma symptoms

The anti-inflammatory effects of Pycnogenol also contribute to its beneficial action on asthma patients. In a placebo-controlled, double-blind, crossover study, asthma symptom scores of 22 patients were significantly lower and lung function parameters higher in the Pycnogenol-treated group, while leukotriene levels decreased.[2] In a double-blind, placebo-controlled study with 60 children, the extract improved pulmonary function and asthma symptoms and reduced use of rescue inhalations. Leukotriene levels in urine were significantly lowered.[27]

Immune modulation

Following supplementation with 60 mg Pycnogenol, apoptosis of lymphocytes and formation of DNA-antibodies were downregulated in patients with lupus erythematosus.[2]

Improvement of quality of sperms

Lack of antioxidants has been connected with malformed sperms. Quality of sperms of 19 subfertile men was significantly improved in terms of morphology and mobility after supplementation with 200 mg Pycnogenol for 90 days.[28] Supplementation with Pycnogenol + L-arginine aspartate and vitamin E or testosterone improved dramatically motility, concentration, and quality of sperms.[5]

Inhibition of UV-induced damages

Oral intake of Pycnogenol strengthens the antioxidative defense system against UV radiation. The minimum erythema doses were significantly increased after intake of 1.11 mg/kg body weight of the extract in 21 volunteers and further enhanced after a larger dose of 1.66 mg/kg.[2] In a study with 30 women, taking 75 mg Pycnogenol for 1 mo,[29] the UV-induced discoloration of sun-exposed skin areas, melasma, could be reduced with respect to size of the affected area and intensity of discoloration.

Antidiabetic Effects

In patients with diabetes type II, Pycnogenol lowers dose dependently fasting and postprandial blood levels and improves endothelial function by lowering endothelin-1 levels and increasing prostacyclin concentrations in blood.[30] Results were confirmed in a placebo-controlled, double-blind study with diabetic patients showing significant decreases of blood glucose and endothelin-1 and increases of prostacyclin after supplementation with 100 mg Pycnogenol.[31]

Reduction of Menstrual Cramps and Pain

Administration of 30–60 mg Pycnogenol to 39 patients with endometriosis and menstrual pain was reported to reduce symptoms in 70% of the subjects in an open clinical study.[2] Supplementation with 60 mg Pycnogenol reduced intake of analgesics, number of days with pain and intensity of low back pain and abdominal pain in 42 patient suffering from menstrual pain.[32]

Efficacy

Pycnogenol has demonstrated antiedema effect in several clinical studies as well as efficacy in inhibiting the progression of retinopathy. Other clinical investigations support the application of the extract to protect the circulation by inhibition of platelet aggregation, lowering of cholesterol, and an antihypertensive effect. An enhanced radical absorbing capacity after intake of Pycnogenol is probably related to protection against UV radiation and inflammatory diseases. The antidiabetic effect and antispasmodic activity has to be confirmed by further clinical and mechanistic studies.

Optimum Intake

Clinical studies suggest an optimum dose range between 40 and 100 mg Pycnogenol/day or 1 mg/kg body weight. Pycnogenol should be taken together with breakfast to minimize gastrointestinal troubles.

Side Effects

The evaluation of clinical studies with more than 2500 patients revealed no serious adverse events related to intake of Pycnogenol. The rate of mild side effects is low, and unwanted effects such as gastrointestinal troubles, dizziness, nausea, headache, or skin sensations were mild and transient in most cases.

Contraindications

To date, no contraindications have been seen.

Observed Drug Interactions

No drug interactions have been reported until now.

Use in Pregnancy and for Children

Despite the fact that teratogenity tests showed no teratogenic effects, the intake of Pycnogenol during the first 3 mo of pregnancy and during breast feeding should be avoided as a general precaution. Children under 12 should not take Pycnogenol because no clinical experience is available with young children.

REGULATORY STATUS

In most countries, for example, in the United States, Australia, the United Kingdom, Belgium, the Netherlands, Finland, Italy, Thailand, Taiwan, P.R.

China, and Japan, Pycnogenol is used as a food supplement. In the United States, Pycnogenol has the status of GRAS. In Greece, Switzerland, Colombia, and Venezuela, it is a nonprescriptional herbal drug.

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