GranaGuard

Nanomolecular Punicic Acid

OXIDATION

A Natural Chemical Process

VITAMINS

Antioxidants

OXIDATIVE STRESS

A Cause of Illness

SO YOU DON’T FORGET

Introduction

The role of oxidative stress phenomena as etiological agents of illnesses with high rates of incidence and prevalence has led to research aimed at identifying powerful antioxidants that are safe and effective. Meanwhile, because no system, organ, tissue or cell is exempt from oxidative damage, often leading to a pathological process, interest among researchers has also focused on ensuring that the sought-after antioxidant molecule also has high bioavailability, and that its distribution in the body is not limited by barriers and membranes or their physical chemical characteristics. Thus, the development of new pharmaceutical forms is essential.

Significant research has been conducted in the last decade regarding a powerful phytochemical antioxidant extracted from pomegranate seed oil (PSO) called punicic acid, which, according to research, is considered one of the most powerful antioxidants. Some studies report that it is seven times more powerful than green tea, and at least six times more powerful than grape seed extract. Thanks to recent advances in nanotechnology, nanoemulsion of PSO has been made possible, giving us the best of both worlds in one form: the best natural antioxidant in nanoemulsion galenic formulation, the most advanced in galenics.

This document presents a review of state-of-the-art approaches to oxidative stress, the link between oxidative stress and illnesses, and information about the promising Nano-PSO GranaGard® as part of treatments.

Contents

Introduction 2

Oxidation: A Natural Chemical Process 4

Oxidation in the Body 4

ROS and Free Radicals 5

Antioxidant Defense System 5

Antioxidant Enzymes 6

Antioxidant Vitamins 6

Antioxidant Phytochemicals 7

Runaway Oxidation: Oxidative Stress 7

Oxidative Stress as a Cause of Illness 8

Alzheimer’s Disease 8

Arteriosclerosis 10

Arterial Hypertension 10

Diabetes Mellitus 11

Cancer 11

Fatty Liver 12

Cirrhosis and Alcoholic Hepatopathy 13

Aging 14

Other Pathologies Linked to Oxidative Stress 14

Pomegranate Seed Oil (PSO) 16

Pro- and Antioxidant Activities 17

Metabolism of Punicic Acid 18

Toxicity 18

GranaGard®: The Powerful Nanoemulsified Antioxidant 19

Bibliography 21

LIVE EACH MOMENT

Oxidation: A Natural Chemical Process

The term oxidation was originally used to refer to the combination of oxygen with other elements. There were many known examples of this, such as rusting iron or burning coal. When iron oxidizes, oxygen slowly combines with the metal, forming ferrous oxide (Fe2O3). In combustion, oxygen combines rapidly with coal to form CO2. The observation of these reactions led to the terms “slow” and “fast” oxidation.

However, chemists observed that other, non-metallic elements combined with substances in the same way as oxygen. Reactions such as that of antimony and sodium (which burn in gaseous chlorine) or iron in the presence of fluoride present certain similarities with what was observed in oxygen, and hence chemists created a more general definition of oxidation. Reactants such as O2 eliminate electrons from each element. Hence, oxidation was defined as the process of an atom or an ion losing electrons.

In biochemistry, the science dedicated to the study of chemical reactions in living beings and organic products, oxidation refers to a process involving the loss of electrons, capture of oxygen or transfer of hydrogen (dehydrogenation) and reduction through the capture of electrons or loss of oxygen. All oxygenation processes are accompanied by a process of reduction; these are referred to as oxidation-reduction or redox reactions between conjugate pairs.

In nature, nearly everything becomes oxidized by oxygen, as when fats become rancid, rubber loses its elasticity, paper yellows, apples become browned, etc. Meanwhile, these oxidation-reduction processes are very important in biochemistry, given that living beings obtain the majority of their energy through them: in photosynthesis, solar energy promotes the reduction of CO2 and the oxidation of H2O, forming carbohydrates and O2; in aerobic metabolism by eukaryotes and many prokaryotes, an inverse process occurs, allowing for the storing of free energy produced through the oxidation of carbohydrates and other organic compounds, in the form of ATP. In our body, metabolism and respiration are continual processes through which we generate energy, and there is a constant demand for them in order to keep us alive.

Oxidation: A Natural Chemical Process

Our physiological metabolic processes, such as processing the carbohydrates we ingest, generate the production of ROS and free radicals.

Reactive oxygen species (ROS) are a set of reactive molecules produced in some metabolic processes involving oxygen. ROS are highly reactive molecules, including oxygen ions, free radicals and peroxides. Their reactivity is the consequence of their missing electrons that cause them to react with other organic molecules in oxide reduction processes. The different reactive oxygen species can participate in different types of reactions that can involve oxidation or reduction. The ROS, from highest to lowest level of reduction, are:

Superoxide anion O2, which is a powerful oxidant agent that is highly reactive with water.

Hydrogen peroxide H2O2.

Hydroxyl radical OH, which is the most reactive. Upon accepting one more ion, the hydroxyl radical becomes a water molecule.

ROS is a collective term that is widely used and encompasses all those reactive species—whether free radicals or not—whose reactivity centers on an oxygen atom. However, often included under the name ROS are other chemical species whose reactivity centers on or is derived from atoms other than oxygen, as is the case with species whose reactivity derives from or centers on atoms such as nitrogen or chlorine and are referred to as Reactive Nitrogen Species (RNS) and Reactive Chlorine Species (RCS), respectively.

Antioxidant Defense System

Oxidation reactions are essential in processes of cellular metabolism. Said reactions involve the transfer of electrons that produce free radicals (1).

Life would not be possible were it not for defense mechanisms that neutralize free radicals. These defenses are called antioxidants, and include any substance that, in normal concentrations, possesses a greater affinity than any other molecule to interact with a free radical.

Upon coming into contact with the free radical, the antioxidant cedes an ion, becoming oxidized and transformed into a weak, non-toxic free radical (2, 3-5). Not all antioxidants behave in this way; those that we refer to as “enzymatic” catalyze or accelerate chemical reactions that utilize substrates that react with the free radicals. As suggested above, antioxidants can be either enzymatic or otherwise. Antioxidants are defined as endogenous when they are found in the body and synthesized by its cells, and exogenous when they enter through the diet.

Antioxidant Enzymes

Antioxidant enzymes are those antioxidants that are found in the proteins and minerals we consume as part of our diet. These enzymes are synthesized in the human body and include superoxide dismutases (SOD) (which eliminate the superoxide anion), glutathione peroxidase, glutathione reductase, and catalases (which prevent the reduction of hydrogen peroxide to form the hydroxyl radical) (6-8).

In order for antioxidant enzymes to provide optimal antioxidizing energy, they require cofactors such as iron, copper, selenium, magnesium and zinc (7-8). The quality of the source of proteins is directly related to the quality of the antioxidant enzymes.

Antioxidant Vitamins

The human body does not produce antioxidant vitamins naturally, and it is therefore essential to include these vitamins as part of our diet, whether through foods themselves or through vitamin supplements. The most common antioxidant vitamins include vitamin C, vitamin E, folic acid and beta-carotene.

Vitamin E is important for maintaining healthy blood vessels and skin. It neutralizes singlet oxygen and captures hydroxyl radicals and the superoxide anion. It also neutralizes peroxides.

Vitamin C helps to protect against UV rays from sun, promotes better iron absorption, boosts resistance to infection, and helps control blood cholesterol. It also neutralizes singlet oxygen, captures hydroxyl radicals and regenerates the oxidized form of Vitamin E.

Meanwhile, folic acid is important for women of reproductive age, particularly to prevent neural tube birth defects in a fetus. Beta-carotene is a powerful carotenoid (a type of phytochemical) that is known to offer good protection against molecular oxygen and other free radicals. This vitamin is most commonly found in orange-colored vegetables such as carrots, pumpkin and potatoes, and in dark-green vegetables such as spinach and kale.

The coenzyme Q10 (or CoQ10) is a substance similar to vitamins that is produced in the body and has been shown to be a necessary component of basic cell functioning. The production of this substance naturally lowers with age, and its reduction has been associated with the development of several illnesses and conditions related to aging.

Antioxidant Phytochemicals

Phytochemicals are the naturally occurring antioxidants utilized by plants to protect themselves against free radicals. Research demonstrates that humans who consume vegetables rich in phytochemicals also benefit from their antioxidant properties. Phytochemicals are divided into the following categories:

* Carotenoids
* Flavonoids
* Allyl sulfide
* Polyphenols

Most natural whole foods such as whole grains, fruits and vegetables contain phytochemicals, while processed or refined foods contain little to none.

Our own physiological antioxidant system would be sufficient if there were only a need to neutralize the free radicals generated by our body’s metabolic processes; however, the antioxidant system must also neutralize the free radicals generated by exogenous factors such as pollution, UV rays, bad habits such as smoking or alcohol consumption, or the chemicals we ingest through our diet. Free radicals multiply rapidly, and once an antioxidant molecule neutralizes a free radical, it loses its neutralizing capacity. When the free radicals are not being neutralized by endogenous antioxidants and when the quantity of exogenous antioxidants in our diet is insufficient, this produces what is known as oxidative stress.

Oxidative Stress as a Cause of Illness

In 1954, Dr. Rebeca Gerschman was the first to suggest that ROS are toxic agents that generate pathologies. She postulated that:

1. Free radicals are a common molecular mechanism of damage in bodies subject to high oxygen pressure and ionizing radicals.
2. An imbalance between oxidants and antioxidants produces toxic effects.
3. The production of free radicals is a constant phenomenon with implications for the production of pathological states.

In 1956, two years after Dr. Gerschman’s findings, Dr. Denham Harman proposed his theory of free radicals as a cause of aging (10). The work of these two pioneers formed the “tip of the iceberg” of what today, 70 years later, constitutes “the catalogue” of all the conditions and illnesses that are produced and/or exacerbated by oxidative stress. Oxidation is a systemic process that occurs in our body, affecting it in a chronic way; it is therefore possible to identify pathological manifestations caused by an excess of oxidation in all organs and systems throughout the body.

There are a series of illnesses related to attacks by free radicals (6). Ongoing research has provided evidence of this for many of the most common pathologies or conditions, several of which are described below.

**Alzheimer’s Disease**: Alzheimer’s disease is the leading cause of disability among adults over the age of 65 around the world. This illness is characterized by abnormal deposits of amyloid-β peptides (βA) and the intercellular accumulation of neurofibrillary tangles comprised of the hyperphosphorylated Tau protein. Both are diagnostic criteria for the illness and are mediators of the neurodegenerative process. These processes are initiated and exaggerated by oxidative stress.

The brains of patients suffering from Alzheimer’s show a significant level of oxidative damage associated with the abnormal and marked accumulation of βA and deposits of neurofibrillary tangles (11). Growing evidence suggests that biometals including iron, zinc and copper play an important role in βA production and neurodegeneration (12). In accordance with these findings, there are high-affinity binding sites for copper and zinc in the N-terminal area of the amyloid-β peptide and its precursor, APP (13, 14). Meanwhile, copper is a powerful mediator of the highly reactive hydroxyl radical (OH), and hence contributes to the increased oxidative stress characteristic of a brain with Alzheimer’s disease (15). This is in accordance with the high concentration of copper that is found in amyloid plaque (16).

This appears to be associated with the length of amyloid-β peptides, specifically the βA of 42-amino acid, which is the most toxic as it is less soluble than βA of the 40-amino acid and is also the most probable candidate for the regeneration of hydrogen peroxide and other ROS (17). Additionally, it has been associated with high concentrations of zinc in areas of the brain related to cognitive functions and memory, including the cerebral cortex, amygdala and hippocampus, which are primarily affected by Alzheimer’s disease (18, 19). This union of zinc has a highly ordered conformation for the βA of the 40-amino acid, which leads to the production of toxic amyloid-β fibrillary aggregates, and, as a result, the immunological/inflammatory response against the insoluble βA plaque implies the alternation of zinc homeostasis, followed by the uncontrolled accumulation of zinc or βA, leading to oxidative stress and cytotoxicity induced by the zinc and mediated by βA (20).

Thus, given that the phospholipids in the brain membranes are composed of polyunsaturated fatty acids, this organ is particularly vulnerable to attacks by free radicals.

Their double bonds allow for the elimination of hydrogen ions (21) and an increase in lipid peroxidation, which is the most prominent characteristic of degenerative change and the most pronounced one occurring in a brain with Alzheimer’s disease (22). Additionally, the oxidation of proteins by free radicals can be an important factor in Alzheimer’s disease; protein oxidation in the brain could affect enzymes that are critical for neural and glial functions.

This is true of two enzymes that are particularly sensitive to oxidative modification: glutamine synthetase and creatine kinase, both of which are notably reduced in brains affected by Alzheimer’s disease (23).

**Arterial Hypertension**: Currently, oxidative stress appears as one of several metabolic alterations described in essential arterial hypertension. It has been shown to be implicated both in endothelial dysfunction and in the hypertrophy of vascular smooth muscle cells, with strong evidence that a disorder in the endothelium-dependent vasodilation could be the primary phenomenon. The endothelial cells produce and release substances that are vasodilators and vasoconstrictors. Vasodilators include nitric oxide (NO), the endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin. Vasoconstrictors include endothelium and thromboxane A2. Lowered production of NO or its low bioavailability lead to vasoconstriction, and hence an increase in peripheral vascular resistance, causing hypertension. Low bioavailability of NO has been linked to an increase in ROS production. Other studies suggest that increased ROS can stimulate hypertrophy and hyperplasia of vascular smooth muscle cells. Increased peroxidation of lipids has been found in arterial hypertension, both in plasma as well as in cellular membranes, as well as an increase in the total quantity of lipids and a reduced antioxidant capacity (25, 31). Arterial hypertension predisposes to and accelerates arteriosclerosis, at least in part due to the synergy between pressure in blood vessels and other arteriogenic stimuli that induce oxidative stress in arterial blood vessels (32).

The formation of arterial plaque begins with the capture of LDL cholesterol by macrophages, which are transformed into spongy cells. These cells are captured by the endothelium through adhesion molecules and accumulate in the subendothelial space, where they induce the migration, proliferation and hypertrophy of muscular cells (24, 25). In certain oxidative conditions, the lipoproteins that accompany cholesterol are fragmented and form amino acid residue (26). These oxidized LDL, or the products released by them, will have greater ateriogenic power, as they are more rapidly captured by the macrophages, are cytotoxic for the endothelium and stimulate the production of vasoactive factors, adhesion, thrombolytics and the proliferation of vascular smooth muscle cells, initiating or extending arteriosclerotic lesions (25, 27, 28). A close link has been demonstrated between ROS and low-density lipoproteins (LDL), and it is known that its increase has a known direct predictive value in the appearance of arteriosclerosis (29, 30). Several risk factors for arteriosclerosis have been identified, such as smoking, hypertension, hypercholesterolemia—and these can initiate a pro-oxidative and anti-oxidative imbalance (30).

**Diabetes Mellitus**: The high glucose levels characteristic of diabetes leads to non-enzymatic glycosylation of proteins. This in turn alters the structure and function of the proteins (33). The oxidation of sugars generates types of ROS. High glucose concentrations, in the presence of transition metals, increase ROS production (34). However, the rise in oxidative stress seen in diabetics is not only related to accelerated ROS production, but also reduced antioxidants (35). The polyol pathway is a possible mechanism through which hyperglycemia can alter the structure and function of cells affected by diabetic complications. The activation of the polyol pathway would lower NADPH and glutathione levels, and hence diabetic patients would have an imbalance between antioxidants and oxidants, increasing their oxidative stress (36).

**Cancer**: The development of cancer is a complex process involving many phenomena including the presence of cellular necrosis in healthy tissue, uncontrolled growth of cancerous cells, and neovascularization of the affected area to guarantee the supply of oxygen and nutrients to the neoformation. It has been suggested that free radicals are associated with the development of cancer (37). For example, tobacco smoke has long been noted as the most important factor in the development of lung cancer, and its toxic products (in addition to nicotine and tar) include abundant free radicals that attack lung tissue and destroy its protective substances. Hence, we have nitric oxide radicals that form carcinogenic compounds such as nitrosamines with the proteins (38). On the other hand, free radicals stimulate the growth of smooth muscle cells, and hence oxidative stress would play a role in tumor neovascularization or angiogenesis (39). The activation of some early genes that could participate in controlling the transcription of growth factors necessary for tumor development has also been observed. It should also be recalled that oncogenic transformation is conditioned by the presence of mutated genes or oncogenes that control key cellular functions, and this can also influence this state of cellular redox (40). Lowered levels of antioxidant enzymes have been detected in various types of tumorous cells (41), as well as alterations in the state of cellular thiols (42). Antioxidant agents would have an anti-carcinogenic effect (43), and hence their inclusion as part of therapeutic strategies used to treat cancer should be studied.

**Fatty Liver**: The pathogenesis of nonalcoholic fatty liver disease (NAFLD) is not clearly defined. According to the most widely accepted theory, peripheral insulin resistance is considered to be the causal mechanism of simple hepatic steatosis, and probably of steatohepatitis (44-46). It is likely that NAFLD represents the hepatic component of a metabolic syndrome that in its complete phenotypical expression is characterized by the presence of peripheral insulin resistance, obesity, type 2 diabetes mellitus, hypercholesterolemia, hypertriglyceridemia and arterial hypertension.

A theory exists regarding the “second hit” or “additional oxidative damage” needed for the necroinflammatory component of steatohepatitis to be manifested from simple hepatic steatosis (47). The most frequent types of oxidative damage are iron deposits in the liver, leptin (an enzyme produced in the adipose tissue that increases resistance to insulin), antioxidant deficiency, bacterial translocation from the gastrointestinal tract to the liver, and mitochondrial alterations of hepatocytes. The mechanisms that lead to the development of hepatic steatosis, and, later, to the appearance of inflammatory phenomena, are only partially known, although they support the hypothesis of multiple parallel stimuli (48).

**Cirrhosis and Alcoholic Hepatopathy**: It was previously thought that portal pressure in liver diseases was determined solely by the alteration of the liver architecture and splanchnic blood flow. However, studies have demonstrated that portal pressure could turn into alcoholic hepatitis and acute liver failure, which suggests the need for intervention by vasoactive mediators in order to explain the mechanism of this acute portal pressure, and also to explain why patients with cirrhosis develop acute vein hemorrhage in periods of hepatic decompensation. One of these mediators could be the 8-iso-PGF2a, a product of lipid peroxidation by ROS, which has been shown to elevate portal pressure in cirrhotic rats (49). Extrapolating this to patients with cirrhosis, lipid peroxidation due to liver damage though alcohol, sepsis or other hepatic illnesses can produce a sharp increase in portal pressure (through the 8-iso-PGF2a and/or other mediators), as observed in acute liver damage. This elevated portal pressure was blocked with SQ29548, an antagonist of the thromboxane receptor (50). Alcohol-induced liver damage is at least partially related to oxidative stress caused by the ROS production and/or a decrease in antioxidants (51).

**Aging**: While this is not a pathology, it can be difficult to differentiate between the processes involved in aging and pathological processes that develop primarily during aging. Aging and death can be the result of the activation of specific genes at a given moment in the cellular cycle (apoptosis). The ROS theory of aging assumes that this is the result of the accumulation of organic lesions due to ROS (26, 27, 52-55). Lessened proteolytic activity has also been detected compared to in young cells (26), and a reduced concentration of antioxidants and inactivation of detoxifying ROS enzymes (56), as well as the accumulation of non-degraded oxidized proteins (57).

**Other Pathologies Linked to Oxidative Stress**: The list of illnesses that in one way or another involve an imbalance between oxidation and antioxidation grows each day. To complement the details provided in the above paragraphs, just some of those documented are listed here:

Demyelinating diseases (58), muscular dystrophy, arthritis and inflammation (27, 57, 59), pulmonary emphysema (60), amyloidosis, collagenosis, connectopathies (LES, escleroderma, Wegener’s granulomatosis), ulcerous colitis, senile dementia, contact dermatitis, bronchopulmonary dysplasia, adult respiratory distress, mutations, lipofucsinosis, Parkinson’s disease, EPOC, retrolental fibroplasia, Kwashiorkor, cerebral ischemia, glomerulonephritis, myocardiopathy, heart failure (61), porphyria, peptic ulcer, ataxia-teleangiectasia syndromes, Down’s syndrome, Bloom syndrome, Dubin-Johnson-Sprinz syndrome, HIV (62, 63), senile cataract (34, 64), acute liver failure (ALF), chronic liver failure (CLF) (65, 66), etc.

Pomegranate Seed Oil (PSO) and Punicic Acid: Antioxidant and Therapeutic Power

Pomegranate Seed Oil (PSO)

Pomegranate seed oil (PSO) consists of a complex series of fatty acids, of which approximately 80% are 18-carbon molecules with three alternating double bonds, which are also known as trienoic acids. Research has demonstrated that trienoic fatty acids such as those found in the pomegranate seed possess a more powerful physiological activity than the dienoic fatty acids (with two alternating double bonds, also known as conjugated linoleic acid or CLA). The specific trienoic fatty acid found in PSO is known as punicic acid (PA), which is a polyunsaturated fatty acid (18: 3 n-5) also called trichosanic acid, a cis 9, trans 11, cis 13 acid with the name IUPAC 9Z, 11E, 13Z-octadeca-9, 11, 13-trienoic (67). Some authors have referred to it as a “super CLA” whose efficacy is even more potent than that of ordinary CLA (68).

Studies using animal models have reported on the potential therapeutic effects of punicic acid in cancer, obesity, diabetes and cardiovascular diseases. In a study using Otsuka Long Evans Tokushima Fatty (OLTEF) rats (a specific breed used for the study of type 2 diabetes and obesity) found that the rats remained relatively lean when punicic acid was added to their diet and that fatty cells could suffer programmed death when exposed to punicic acid (69, 70). Punicic acid also demonstrated a capacity to reduce glycemia in rats with type 2 diabetes induced by streptozocina (STZ) (71), though not in that induced by alloxan (72). Another study (73) reported on the protection offered by PSO to a group of rats receiving a high-fat diet plus 1% PSO, versus the control group that only received the high-fat diet. After 12 weeks, it was verified not only that the group that received PSO was leaner, but that they had also improved their sensitivity to insulin.

The gamma receptors activated by the peroxisome proliferators (PPAR-gamma) are nuclear receptors found primarily in the adipose tissue, which regulates fatty acid reserves and glucose metabolism, and hence is the target for many anti-diabetic agents. Other researchers (74) describe PPAR molecular objectives for diseases related to obesity and other related disorders considering the side effects of PPAR agonists, suggesting that natural molecules should be sought, and punicic acid is a PPAR antagonist. In a study on oxidative stress and inflammation that compared punicic acid and eleostearic acid, both isomers demonstrated powerful activity against streptozotosin-induced oxidative stress: the peroxidation of lipids was significantly reduced, and levels of the superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX), reduced glutathione (GSH) and nitric oxide (NO) were restored in the pancreas, blood and erythrocyte lysate (75).

It has also been demonstrated that the contents of PSO inhibit the expression of pro-inflammatory cytokines (such as IL-6, IL-8, IL-23, IL-12 and TNF-α) through PPSR and modulation.

Pro- and Antioxidant Activities

Mukherjee et al. (77) have described the antioxidant and hypolipidermic activity of punicic acid. Feeding punicic acid to rats in concentrations of 0.6%, 1.2%, and 2.4% for 14 weeks, they found that total cholesterol levels and LDL cholesterol were significantly lower in the group that received the 2.4% concentration of punicic acid. Pro-oxidant and antioxidant activity under 0.6% and 1.2% concentration was demonstrated through susceptibility to oxidation of lipoproteins and peroxidation of lipids in plasma. The atherogenic index [(CT/HDL-C)-1] and the LDL-C/HDL-C relationship were significantly reduced under 0.6% and 2.4% concentrations. This dual behavior is likely due to a phenomenon of isomerization: the total cis and trans molecular configuration increases from 0.6% to 2.4% through 1.2%. The authors propose that inhibition of the formation of hydroperoxides by punicic acid could be due to the reduction in free radicals production or peroxidation. Additionally, there is a biohydrogenation process (involving the addition of free radicals to one of the conjugated double bonds) forming conjugated dienoics, which would explain their antioxidant function.

Metabolism of Punicic Acid

Using animal models, Tsuzuki et al. (78) found that punicic acid was converted into rumenic acid (9Z11E-CLA), which indicates that part of the bioactivity of punicic acid could be due to c9t11 CLA. A 28-day study in which rats were given punicic acid orally demonstrated that CLA levels increased from 0.05% to 0.06% of total fatty acids in the control group, while the group receiving punicic acid showed an increase of 0.23%. Meanwhile, punicic acid was also detected in their circulation, which simply suggests its incomplete metabolism in CLA c9t11.

Toxicity

Meerts et al. (79) studied issues of safety and toxicology with regard to PSO through in vivo and in vitro tests. They did not observe mutagenic or clastogenic effects in the absence and presence of metabolic activation of up to 5,000 ug/plate (Ames tests) or 333 ug/mL (chromosome aberration test). There were no significant findings in acute oral toxicity studies with 2g of PSO per kg of weight, hence LD50 can be considered as high as 5g per kg of weight according to test guideline 423 of the OECD (Organization for Economic Cooperation and Development), and no toxicology warning is required on labels. The no observed adverse effect (NOAEL) for punicic acid has been reported at 50,000 ppm, which is equivalent to 4.3g of PSO per kg of weight.

GranaGard®: The Powerful Nanoemulsified Antioxidant

GranaGard is a nanoemulsion of pomegranate seed oil (*punica granatum*). The active ingredient in pomegranate seed oil, or PSO, is a polyunsaturated fatty acid that is also considered to be one of the most powerful natural antioxidants (80). Nanoemulsion of PSO, called Nano-PSO (81), makes it possible to increase the bioavailability and efficiency of PSO. Nanoemulsion permits the supply of PSO as micelles formed by phospholipids or nanodrops (82-84), which allows for the distribution of the oil to other organs besides the liver, increasing blood levels of punicic acid and hence its greater availability in order to achieve the blood-brain barrier. In fact, it has been demonstrated that similar unsaturated fatty acids such as linoleic acid easily surpass the blood-brain barrier (85-87). Punicic acid is present exclusively in PSO (60-80%) and in *trichosanthes kirilowii* (40%) (88). Its efficiency in fatty tissue protection in trials with inflammatory disease has been reported (89). The lack of toxicity and partial bioavailability of PSO has already been established in humans (88). Additionally, another antioxidant, β-sitosterol, which has been shown to accumulate in the plasmatic membrane of the brain (90), is present in PSO in significantly higher concentrations than those found in other plant oils (91). This could indicate that PSO may constitute a natural compound with greater antioxidant activity than its individual components.

Research has compared and demonstrated the superiority and efficacy of Nano-PSO utilizing as a model experimental autoimmune encephalomyelitis induced in rats, demonstrating that those treated with natural PSO showed a certain reduction in the burden of disease, and this beneficial effect increased significantly when the rats were treated with Nano-PSO in concentrations far lower than those of the natural oil. Pathological tests revealed that administering Nano-PSO drastically reduced the demyelination and oxidation of lipids in the brain characteristic of this serious neurological illness (92). In another study designed to prove whether reduced oxidation altered the manifestations of neurodegenerative diseases, TgMHu2ME199K mice were used as models of genetic prion diseases, which were treated with Nano-PSO. The Nano-PSO significantly delayed the onset of illness when administered to asymptomatic mice, and delayed the worsening of the illness in those mice that already displayed symptoms. Analysis of brain samples revealed that treatment with Nano-PSO did not diminish the accumulation of PrP (Sc), but rather, reduced the oxidation of lipids and neuron loss, indicating a strong neuroprotective effect. The authors proposed that Nano-PSO and similar formulations could be beneficial and sufficiently safe to be administered for many years in subjects that are at risk of or already suffering from neurodegenerative illnesses (93).

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ANTI-WRINKLE EFFECTS

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