ALOE VERA IN THE MANAGEMENT OF OXIDATIVE STRESS

Author: Ken Jones, Chief Science Officer, ALOECORP INC.

Oxidative stress

Oxidative stress can be defined as an imbalance between the production of reactive oxygen species (ROS) in the body that disrupts its ability to detoxify reactive intermediates, or to repair the damage to organ and cellular systems that can be caused by ROS. Intracellular redox* balance (the dynamic balance between oxidizing and reducing species within cells) is closely coupled to the antioxidant peptide **glutathione**; intracellular levels of this compound are highly regulated by enzymes to maintain a reducing environment. Some of these reactive oxygen species are constantly produced at low levels as by-products of normal metabolic reactions, and are kept in check by the enzymes that maintain the intracellular redox balance. When redox balance is upset, these moderately reactive species can interact with transition metals or other components of the redox cycle to produce highly reactive oxygen species that can cause extensive damage to cell membranes lipids, proteins, DNA, and cellular organelles (Table 1).

Table 1: Reactive oxygen species (ROS) originating from intracellular redox metabolism (1,2,3)

●O_2^- Superoxide anion	One-electron reduction state of O_2 formed in many autooxidation reactions and in the electron transport chain.
H_2O_2 Hydrogen Peroxide	Two-electron reduction state, formed by dismutation of **●O_2^-** or by direct reduction of O_2.
●OH Hydroxyl Radical	Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components.
ROOH Organic Hydroperoxides	Formed by radical reactions with cellular components such as lipids and nucleosomes.
ROO●, **RO●,** **Alkoxy**- Peroxy Radicals	Oxygen-centered organic radicals. Lipid forms are involved in lipid peroxidation. Produced in presence of oxygen by radical addition to double bonds or hydrogen abstraction.
HOCl Hypochlorite	Formed from H_2O_2 by myeloperoxidase†. Lipid soluble and highly reactive. Will readily oxidize protein constituents.
OONO- Peroxynitrate	Formed in a rapid reaction between •O_2- and NO•. Lipid soluble and similar in reactivity to hypochlorite.

Not all ROS or oxidation reactions in living systems are harmful. ROS (and RNS, Reactive Nitrogen Species) play important roles in the defense against infectious agents, as secondary messengers in intracellular signaling cascades (redox signaling) and in induction of the mitogenic response. ROS-mediated reactions can even act to protect cells against oxidative stress and re-establish or maintain "redox homeostasis."

* "redox" short for oxidation/reduction reactions, refers to chemical reactions in which compounds have their oxidation state changed. In the simplest case, oxidation describes the loss of electrons by a molecule, while reduction involves the gain of electrons.

† A peroxidase enzyme most abundant in neutrophil granulocytes

Oxidative stress and disease

Damage to cellular, tissue, and organ systems as a consequence of oxidative stress has been linked to a number of serious diseases, including cancer, cardiovascular diseases such as hypertension and atherosclerosis, neurodegenerative diseases such as Parkinson's disease and Alzheimer's dementias, diabetes, ischemia/reperfusion injuries, rheumatoid arthritis, and even the process of aging. (4)

Inflammation

Inflammation is a disease state that exemplifies the two-edged nature of ROS in living systems. Chronic inflammatory states have been linked to diseases as diverse as cancer, atherosclerosis, Alzheimer's, and diabetes. Inflammation is an immune-mediated response to infection or irritation, characterized by redness, swelling, pain, and dysfunction of the affected organs. The cellular inflammatory response involves the movement of several types of white blood cells (leukocytes) into the injured or infected tissues. Free radicals and other ROS are thought to play a role in the inflammatory response. Specific antioxidant enzymes, such as Superoxide Dismutase (SOD) or catalase (CAT) attenuate the injury and inflammation in animal models of ischemia/reperfusion, arthritis, chronic gut inflammation, and immune-complex induced pulmonary injury. This suggests that ROS may directly or indirectly influence the inflammation and tissue dysfunction associated with these conditions. The likely sources of these ROS are the phagocytic leukocytes. Activation of phagocytes give rise to a cascade of reactive and radical species such as the superoxide anion radical, hydrogen peroxide, hydroxyl radical, directly injure cells and tissues at the site of injury or infection. They may also indirectly initiate or amplify the inflammation via the activation of transcription factors such as NFkB, which amplifies the inflammatory response by up regulating various proinflammatory cytokines. A number of antioxidants are able to inhibit NFkB activation, and this, coupled with the fact that oxidants can activate it, suggests a common signaling pathway for reactive oxygen species. Chronic, systemic inflammatory states in combination with oxidative stress may be the common thread that has implicated these processes in the pathogenesis of many diseases including atherosclerosis, obesity, insulin resistance, and diabetes. (5)

Cancer

ROS are potential carcinogens because they facilitate mutagenesis and promote tumor formation and growth (6). These growth-promoting actions are mediated via redox-sensitive signaling cascades. Hydrogen peroxide and superoxide can trigger cell proliferation and expression of growth-related genes. Malignant cells themselves produce ROS following expression of genes associated with a transformed phenotype including the proto-oncogenes H-Ras or mox1. Oncogenic mutations in Ras proteins are present in about 30% of malignancies, and mutated gene products can stimulate cell proliferation and inhibit apoptosis. Another mechanism also implicated in cancer, as well as other states of oxidative stress, is a shift in the thiol/disulfide redox state. Glutathione is present in cells mainly in reduced form (GSH) because glutathione reductase is induced by oxidative stress. Cancer cells characteristically display decreased clearance of glucose, and high levels of anaerobic glycolysis can stimulate lactate production, decreasing intracellular levels of reduced glutathione.

Cardiovascular disease

Atherosclerosis is a chronic disease characterized by hardening and thickening of the arterial wall. It is currently understood as a chronic inflammatory disease and is associated with risk factors such as hyperlipidemia, hypertension, and diabetes. Excessive ROS production is implicated in the pathogenesis of all of these conditions. Oxidative stress induces the production of intracellular adhesion molecules that facilitate the invasion of the arterial wall by monocytes and lymphocytes that have 'scavenger' receptors for oxidized low-density lipoprotein (LDL), as do smooth muscle cells. Binding of oxidized LDL activates macrophages and monocytes that stimulate the expression of SOD, which catalyzes the breakdown of the highly reactive superoxide anion into hydrogen peroxide and oxygen. Increased hydrogen peroxide production can disturb steady state levels of ROS, leading to massive macrophage apoptosis (programmed cell death), a process that is associated with the production of atherosclerotic lesions. (7)

Neurodegenerative disease

Disruption of oxidative homeostasis has also been implicated in several neurodegenerative diseases, including Parkinsonism, Alzheimer's dementia, ALS, and other neuropathologies including Bovine Spongiform Encephalopathy (BSE). ROS generated by the oxidation of dopamine in the central nervous system has been linked to the age-related destruction of dopamine neurons (8). Post-mortem Alzheimer's patients typically have elevated levels of lipid peroxidation in the brain and increased levels of 4-hydroxynonenal: a by-product of lipid peroxidation in cerebral spinal fluid. ROS have also been found to mediate neuronal damage caused by ß-amyloid protein. (9).

Rheumatoid arthritis

This autoimmune disease is characterized by chronic joint inflammation with infiltration of macrophages and activated T cells. Abnormal induction of redox-sensitive pathways can lead to abnormal expression of adhesion molecules, stimulating the migration of monocytes and lymphocytes into the rheumatoid arthritis synovium. (6)

Ischemia/reperfusion injury

Ischemia is a restriction in blood supply to tissues or organs that can cause injury resulting from cell death due to hypoxia or lack of oxygen and nutrients. Reperfusion injury results when the blood supply returns to the tissue following ischemia. The restoration of circulation triggers a massive inflammatory response and markedly stimulates production of ROS. Antioxidant treatment has been shown to attenuate both reperfusion injury and leukocyte adhesion to the endothelium. (6)

Diabetes

Diabetes is a state of chronic hyperglycemia, which can trigger a pro-oxidative shift in the glutathione redox state in the blood via several mechanisms. Glucose auto-oxidation is associated with the formation of glycated proteins and the production of superoxide; interactions of the glycated proteins with cell surface receptors stimulate ROS production and decreases in intracellular glutathione. Hyperglycemia also enhances cell-mediated

LDL peroxidation in endothelial cells, a process that can eventually lead to the formation of atherosclerotic lesions in arterial walls. (6)

Aging

The free radical theory of aging postulates that damage to cells and tissues induced by free radicals can cause age-related degenerative processes. Aging involves progressive dysfunctional changes in free-radical mediated signaling cascades that are linked to altered gene expression. Ultimately, increased production of ROS in aging can lead to a pro-oxidative shift in redox states, indirectly manifesting oxidative stress in the form of lipid peroxidation, DNA and protein oxidation. This oxidative shift may alter the set points of redox sensitive signaling pathways, accounting for age-related immunological dysfunctions and inflammatory processes. (6)

Protection from Oxidative Stress

Oxidative stress results from the disruption of the regulatory signaling processes that maintain the reducing environment of the intracellular redox state. Loss of this homeostasis and the resulting pro-oxidative shift in redox balance has been implicated in the etiology of many diseases, as discussed above. Ultimately these disease states are the outcome of chronic states of oxidative stress. Nature has evolved elegant regulatory mechanisms for preserving antioxidative redox states. These primarily involve **antioxidants**, some of which are part of the body's redox regulating machinery, while others are derived from dietary sources. *Aloe vera* is one example of a functional food that can play a significant role in protection from oxidative stress. Not only does Aloe gel contain a number of antioxidant constituents, but it is able to activate the body's endogenous ROS protective systems. These results are discussed further below.

Antioxidants

Antioxidants are reducing agents that can slow or stop oxidation reactions directly by interacting with intermediates of the reaction, or by reacting with the oxidizing agent and preventing the reaction.

Endogenous antioxidant enzymes

The body's protective antioxidant systems are mainly enzymes that also participate in redox-mediated signaling processes and help maintain redox balance (10). There are five major families of cellular antioxidant enzymes:

- The **thioredoxin system** consists of thioredoxin and thioredoxin reductase. Thioredoxin acts as an efficient reducing agent, scavenging reactive oxygen species and maintaining other proteins in a reduced state.
- The **glutathione system** consists of glutathione, glutathione reductase, and glutathione peroxidase. Glutathione peroxidase catalyzes the breakdown of hydrogen peroxide to water and reduces lipid peroxides. Oxidized glutathione (glutathione disulfide) is reduced by glutathione reductase.
- **Superoxide Dismutases** (SODs) are a family of closely related enzymes that catalyze the conversion of superoxide anion into oxygen and hydrogen peroxide.

- **Catalase** catalyzes the conversion of hydrogen peroxide to water and oxygen, but also oxidizes toxins such as formaldehyde, formic acid, and alcohols. Catalase becomes activated when the glutathione peroxide pathway approaches saturation.
- **Peroxyredoxins** catalyze the reduction of peroxides.

Antioxidants in food

Protection of cellular redox balance can be enhanced through the consumption of dietary antioxidants. Epidemiologic evidence indicates that consumption of a wide variety of fruits and vegetables is associated with reduced risk of chronic diseases, most of which are etiologically related to oxidative stress. Fruits, vegetables, and whole grains contain an enormous variety of antioxidant chemicals such as flavonoids, carotenoids, nitrogen-containing compounds, and organosulfer compounds (11). Dietary antioxidants work primarily by scavenging free radicals (12). Studies have demonstrated an inverse relationship between dietary antioxidant intake and the development of cardiovascular diseases (13,14,15). Dietary antioxidants also play an important role in the reduction of the risk of cancer. An epidemiologic review of 200 cancer studies (16) found that fruit and vegetable consumption was correlated with reduced risk of cancers of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary. Still other studies have shown preventive or attenuating effects of dietary antioxidant consumption in virtually every other chronic disease related to oxidative stress.

Synergy in antioxidant action

The benefits of dietary antioxidants obtained from whole foods are clear but there remains controversy around the consumption of single, highly purified antioxidants in the form of dietary supplements. Supplement makers often promote their products by citing the ORAC (Oxygen Radical Absorbance Capacity) assay (17), an *in vitro* measurement of antioxidant capacity. A high *in vitro* ORAC value, however, may not accurately reflect the capacity of an antioxidant supplement to alleviate oxidative stress *in vivo*. Caution must be used in extrapolating the results of *in vitro* ORAC assays. For example, increased ORAC values in plasma or serum may not be desirable if they reflect increased oxidative stress; similarly, decreased plasma ORAC values could reflect a decreased production of reactive species, a desirable outcome (17). The ORAC value of a given supplement is meaningless unless taken in the context of its bioavailability, interaction with other nutrients and its effect on cellular redox systems. Furthermore, mounting evidence indicates that individual antioxidants, taken alone, do not have the same benefits as a diversity of antioxidants derived from whole foods (12,11). In some cases, consumption of single antioxidants may even have deleterious effects. For example, numerous studies have shown cancer protective effects from the consumption of green and yellow vegetables and fruits, known to be high in the carotenoid antioxidant, ß-carotene. However, one clinical trial with a combination of retinol (Vitamin A) and ß-carotene indicated that not only was there no benefit, but supplementation appeared to increase the risk of death from lung cancer and heart disease (18). In the Heart Outcomes Prevention Evaluation (19) patients given 400 IU of vitamin E daily for 4.5 years had no difference from placebo in deaths from cardiovascular disease, myocardial infarctions, or stroke. Vitamin C supplements also did not lower the incidence of cancer or CVD (20,21). In light of these studies, many health care providers now argue that including a

wide variety of whole foods, especially fruits and vegetables, in the diet is a superior strategy for protecting the body from chronic oxidative stress and its associated disease states. In this context, *Aloe vera* gel emerges as a unique multi-functional food for protection from oxidative stress; Aloe contains a variety of chemically and functionally distinct antioxidants in a complex matrix; additionally, it activates the body's endogenous antioxidant systems to maintain or restore redox balance, and it activates Phase II metabolism to facilitate detoxification and elimination of reactive oxygen species.

Xenobiotic metabolism: Phase I and Phase II metabolism

Humans, like all organisms on the planet, live in a chemical ecology where they are at risk of exposure to thousands of potentially harmful chemicals, ranging from toxins in the diet, water, and air, to a plethora of toxins and pollutants of human origin. These chemicals are called 'xenobiotics' because they are "foreign" to human metabolism, that is, they are not produced endogenously as products of metabolism. Although many xenobiotics, such as carcinogenic Polycyclic Aromatic Hydrocarbons (PAHs) can be harmful, others, such as synthetic medicines, may be beneficial. The outcome is largely determined by how the body transforms and detoxifies these compounds, and this process is largely determined by Phase I and Phase II metabolism. Along with endogenous antioxidant enzyme systems and dietary antioxidants, the xenobiotic metabolism and detoxification processes governed by Phase I and II systems constitute yet another important means by which the body maintains homeostasis and protects itself from the damaging effects of "foreign" chemicals (22).

Cytochrome P450 Drug Metabolizing Enzymes

Collectively, drug metabolizing enzymes are called mixed-function oxidases (MFO) or monooxygenases, and include various cytochromes such as cytochrome P450, cytochrome B_5, NADPH cytochrome reductase, and others. The cytochromes (CYP) are a multi-gene 'family' of enzymes that catalyze the oxidation and reduction of numerous structurally diverse substances of both exogenous and endogenous origin. Although the outcome of mixed-function oxidase metabolism is ultimately directed toward polar end products that are readily excreted, other reactions in the pathway can produce highly reactive intermediates that can damage cells if not shunted toward detoxifying reactions. Which process wins out depends on cellular levels of the xenobiotic metabolites, but also the activity, levels, gene expression, and compartmentalization of the various cytochrome isozymes (22).

The microsomal cytochromes are inducible; exposure to substrates for the enzymes can activate genes coding for cytochromes that have selective substrate specificities for the inducing chemicals. In cases where the cytochrome activates the substrate to reactive intermediates, as exemplified by PAH activation by Cytochrome P450 1A, the effect can be harmful; exposure to PAHs in the environment induces the enzymes that ultimately convert them to carcinogens. On the other side, the inducible MFO system is probably one of the primary adaptations that have enabled humans to adopt an omnivorous diet. Food, especially plants, is full of xenobiotic compounds that can be harmful or beneficial depending on how they are metabolized. Chemicals in the diet can effect the expression

of inducible cytochromes, activating pathways that enable detoxification of toxins in food (22).

Phase II metabolism

The enzymes of Phase II metabolism constitute the other side of the "coin" of xenobiotic metabolism. In general, enzymes of Phase II metabolism act on reactive metabolites in a manner that leads to inactivation, conjugation, and elimination. Phase II enzymes work together with endogenous antioxidants to maintain cellular redox balance.

Table 2: Enzymes of Phase II metabolism (22)

Name	Function
glutathione-S-transferase (GST)	Conjugates activated P450 metabolites with reduced glutathione (GSH), leading to inactivation and elimination.
DT-diaphorase	Catalyzes conversion of reactive quinones to hydroquinones; the stable hydroquinone can be conjugated by glucuronide or sulfate and excreted.
UDP-Glucuronyl Transferase	Conjugates endogenous compounds and xenobiotics having a carboxylic acid with D-glucuronic acid.
Sulfotransferases	Catalyzes sulfation of drugs with inorganic sulfate.
N-acetyltransferase	Acetylates amines, preventing their bioactivation by N-oxidation.
Epoxide hydrolase	Converts epoxides formed in Phase I metabolism of aromatic compounds to trans-dihydro diols that can be conjugated and excreted.

As a consequence of genetic polymorphisms, the enzymes that mediate the activation, biotransformation, and detoxification of xenobiotics, dietary constituents, and endogenous substances, as well as enzymes regulating redox reactions, are differentially expressed in each individual. These individual differences in the metabolism of toxins and drugs are important determinants of individual susceptibility to diseases in which xenobiotic metabolism and oxidative stress are implicated. The new field of pharmacogenetics (23) studies genetically-determined individual biochemical variability in metabolism of drugs and toxins

Chemoprotective and antioxidant properties of *Aloe vera* gel

Aloe vera is an ancient medicine whose anti-inflammatory, antiseptic, wound-healing, antidiabetic, antioxidant, and cancer chemopreventive qualities have attracted recent scientific interest (24).

Topically applied *Aloe vera* gel has remarkable wound healing and anti-inflammatory properties, but other evidence indicates its medicinal properties are even more remarkable when the "gel"[‡] is orally ingested, as a juice or in dried form.

[‡] *Aloe vera* "gel" refers to the mucilaginous, translucent material that constitutes the inner pulp of the succulent leaves.

Both clinical studies of diabetic patients and animal studies have demonstrated that ingested *Aloe vera* gel is able to reduce blood glucose levels and plasma triglycerides, as well as many of the secondary symptoms of diabetes that are associated with oxidative stress (25,26,27,28). Other animal studies have included irradiated rats (29) and aged rats (30). All of these studies have consistently shown that *Aloe vera* gel significantly ameliorates oxidative stress, by activating endogenous antioxidant systems, and via the antioxidant constituents present in *Aloe vera* gel (Table 3).

Table 3. Bioactive constituents isolated from *Aloe vera* gel

Constituent	Class	Function	Reference
Mannose-6-phosphate	Polysaccharide	Anti-inflammatory; hypoglycemic	31,32
Acemannan	Polysaccharide	Accelerates wound healing; immune stimulant; anticancer; antiviral	33,34
Uncharacterized polysaccharide	Polysaccharide	Reduced oxidative DNA damage; induced GST activity	35
8-C-ß-D-[2-O-(*E*)-coumaroyl] glucopyranosyl-2-[2-hydroxy]-propyl-7-methoxy-5-methylchromoone	Phenolic glycoside	Inhibits lipid peroxidation	36
Dihydrocoumarins	Phenolic glycoside	Stimulates phagocytosis and O_2 respiratory burst	37
5 phytosterol derivatives	Triterpenes	Hypoglycemic	38

Table 4. Bioactivity of *Aloe vera* Gel in Glucose and Lipid Metabolism, Oxidative Stress, and Phase II Metabolism

Action on Glucose and Lipids	**Effect**	**Reference**
Insulin	↑ stimulates	25
Blood Glucose	↓ reduces	26
Cholesterol	↓ reduces (after 7 months)	30
Action on Oxidative Stress	**Effect**	**Reference**
GSH (reduced glutathione)	↑ elevates	39
Glutathione Peroxidase	↑ elevates	39
Glutathione Reductase	↑ elevates	39
Superoxide dismutase	↑ elevates	39
Catalase	↑ elevates	39
Lipid peroxidation	↓ inhibits	39
Lactate dehydrogenase	↓ inhibits	39
Action on Phase I/II	**Effect**	**Reference**

Metabolism		
DT diaphorase	↑ induces	39
Cytochrome P450	↓ reduces	39
Cytochrome b5	↓ reduces	39
Cytochrome P450 reductase	↑ induces	39
Cytochrome b5 reductase	↑ induces	39
Glutathione S-transferase	↑ induces	39

In rats induced with chemical hepatocarcinogenesis by co-administration of diethylnitrosamine (DEN) and 2-acetylaminofluorene (AFF) *Aloe vera* gel and vitamin C reduced induction of enzymes associated with carcinogenesis and normalized cellular architecture (40). The authors remarked that the actions of Vitamin C and *Aloe vera* were similar, but did not investigate combinations of the two. Vinson et al. (41) measured plasma levels of vitamin E and C in normal fasting human subjects. Supplementation with *Aloe vera* gel increased the plasma levels of Vitamin E and C by 369% and 304%, respectively. The *Aloe vera* extracts also slowed down the absorption of both vitamins by 2 to 4 hours compared to controls, indicating that *Aloe vera* supplementation prolonged the half-lives and elevated the bioavailability of these antioxidants.

Taken together, these *in vitro*, animal, and clinical studies on *Aloe vera* provide evidence that it is a multi-functional food capable of stabilizing and normalizing a number of chronic disease states that are directly or indirectly related to oxidative stress. The key aspects of its functionality include:

- Normalization of blood glucose, plasma triglycerides, and cholesterol.
- Restoration of the cellular redox balance via activation of endogenous antioxidant enzyme systems.
- Antioxidant constituents of *Aloe vera* gel also contribute to the maintenance of a healthy oxidative redox balance.
- *Aloe vera* gel also regulates Phase I/II metabolism by reducing the cellular levels of cytochromes involved in Phase I metabolism, while inducing the enzymes of phase II metabolism that neutralize and detoxify ROS by shunting them in the direction of conjugation and elimination.
- *Aloe vera* gel displays “antioxidant synergy” not found with single, highly purified antioxidants, by prolonging the half-life and increasing the bioavailability of Vitamin C and E, and possibly others.

Oxidative stress is a cellular metabolic imbalance that has been implicated in numerous chronic disease states, from atherosclerosis and cancer to the aging process. Due to our life-style and diet, oxidative stress is extremely common. It is to be hoped that further research on this remarkable plant will lead to simple, effective, natural, and inexpensive solutions to this problem, which has an incalculable impact on human health.

[1] Sies H. In *Oxidative Stress;* H. Sies (Ed). Academic Press; London, 1985 pp 1-7
[2] Docampo R. In *Biochemistry and Molecular Biology of Parasites.* Marr J. and Müller M. (Eds). Academic Press; London, 1995 pp 147-160
[3] Rice-Evans C., Gopinathan, V. *Essays Biochem* **1995**, 29, 39
[4] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. *Int J Biochem Cell Biol*. **2007**, 39, 44
[5] Conner E.M., Grisham M.B. *Nutrition* **1996**, 12, 274
[6] Dröge W. *Physiol Rev* **2002,** 82, 47
[7] Molavi B. Mehta J.L. Curr Opin Cardiol. **2004**, 19, 488
[8] Luo Y, Roth GS. *Antioxid Redox Signal.* **2000**, 2, 449
[9] Multhaup G, Ruppert T, Schlicksupp A, Hesse L, Beher D, Masters CL, Beyreuther K. *Biochem Pharmacol*. **1997**, 54, 533
[10] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. *Int J Biochem Cell Biol.* **2007**, 39, 44
[11] Liu RH. *J Nutr*. **2004**, 134, 3479S
[12] Liu RH. *Am J Clin Nutr*. **2003**, 78(suppl), 517S
[13] Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. *Lancet* **1993**, 342, 1007
[14] Knect P, Jarvinen R, Reunanen A, Maatela J. *Br Med J* **1996** 312, 478
[15] Arai Y, Watanabe S, Kimura M, Shimoi K, Mochizuki R, Kinae N. *J Nutr* **2000** 131, 224
[16] Block G, Patterson B, Subar A. *Nutr Cancer* **1992** 18, 1
[17] Prior RL, Cao G. *Free Radic Biol Med.* **1999** 27,1173
[18] Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP,Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. *N Engl J Med.* **1996** 334, 1150
[19] Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. *N Engl J Med.* **2000** 342, 154
[20] Blot WJ, Li JY, Taylor PR *J Nat Cancer Inst.* **1993** 85, 1483
[21] Salonen JT, Nyyssonen K, Salonen R, Lakka HM, Kaikkonen J, Porkkala-Sarataho E, Voutilainen S, Lakka TA, Rissanen T, Leskinen L, Tuomainen TP, Valkonen VP, Ristonmaa U, Poulsen HE. *J Intern Med*. **2000** 248, 377
[22] Sheweita SA. *Curr Drug Metab*. **2000** 1, 107
[23] Koo SH, Lee EJ. *Clin Exp Pharmacol Physiol*. **2006** 33, 525
[24] Reynolds T, Dweck AC. *J Ethnopharmacol*. **1999** 68, 3
[25] Rajasekaran, S, Sivagnanam K, Subramanian S. *Journal of Pharmacy and Pharmacology*. **2005** 31, 241
[26] Rajasekaran, S, Sivagnanam K, Subramanian S. *Pharmacological Reports.* **2005** 57, 90
[27] Yongchaiyudha S, Rungpitarangsi V, Bunyapraphatsara N, Chokechaijaroenporn O. *Phytomedicine.* **1996** 3, 241
[28] Bunyapraphatsara N, Yongchaiyudha S, Rungpitarangsi V, Chokechaijaroenporn O. *Phytomedicine*. **1996** 3, 245
[29] Saada HN, Ussama ZS, Mahdy AM. *Pharmazie* **2003** 58, 929
[30] Lim OB, Choue RW, Kim JD, Yu BP, Jeon TI, Park DK. *J. Nutritional Science & Vitaminology*. **2003** 49, 292

[31] Davis RH, Parker WL, Samson RT, Murdoch DP. *J Am Pediatri Med Assoc*. **1991** 81, 473
[32] Davis RH, Donato, JJ, Hartman GM, Haas RC. *J Am Pediatri Med Assoc*. **1994** 84, 77
[33] Zhang L, Tizard IR. *Immunopharmacology*. **1996** 35, 119
[34] Djeraba A, Quere P. *Int J Immunopharmacol*. **2000** 22, 365
[35] Kim HS, Kacew S, Lee BM. *Carcinogenesis*. **1999** 20, 1637
[36] Lee KY, Weintraub ST, Yu BP. *Free Radic Biol Med.* **2000** 28, 261
[37] Zhang XF, Wang HM, Song YL, Nie LH, Wang LF, Liu B, Shen PP, Liu Y. *Bioorg Med Chem Lett.* **2006** 16, 949
[38] Tanaka T, Misawa E, Ito Y, Habara N, Nomaguchi K, Yamada M, Toida T,Hayasawa H, Takase M, Inagaki M, Higuchi R. *Biol Pharm Bull*. **2006** 29, 1418
[39] Singh RP Dhanalakshmi S, Rao AR. *Phytomedicine* **2000** 7, 209
[40] Shamaan NA, Kadir KA, Rahmat A, Ngah WZ. *Nutrition*. **1998** 14, 84
[41] Vinson JA, Al Kharrat H, Andreoli L. *Phytomedicine*. **2005** 12,760