BioImmersion Inc. *Forward Thinking™*

Wild Blueberry

Freeze Dried Extract
Vaccinium angustifolium
Monograph

Wild Blueberry as Observed and Published in Peer Review Journals

Wild Blueberry: Function

- Acts as a potent anti-inflammatory agent as measured by reduction of Nuclear Factor-kappa B in the brain, COX-2 in vitro and Isoprostane in vivo.
- Acts as a potent antioxidant by increasing antioxidant status in vivo as measured by increased ORAC in bloodstream and reduced level of 8-isoprostanes in blood and urine and reduced lipid-oxidation in vivo.

Wild Blueberry *freeze dried extract*: Beneficial Health Effects

- Improvement of short-term memory loss
- Protection against macular degeneration of the retina
- Amelioration of age-related declines in neural and cognitive function
- Reduction of age associated lipid peroxidation, one of the key factors responsible for development of cardiovascular health problems
- Suppression of several types of cancer cell growth as reported in peer-review scientific journals. Based on this observation it is expected that blueberry could be helpful in reducing the risk of some cancers and managing cancerous conditions.

Blueberry Plays an Important Role in the Process of Aging

James Joseph PhD

Chief of the Laboratory of Neuroscience at Tufts University

"The brain is a hotbed of free-radical activity. As we age, we seem to become more sensitive to their damaging effects." Dr. Joseph wanted to see whether blueberries with all their antioxidant activity, could help protect aging brains. His research made headline news by showing that blueberries confer true "anti-aging" benefits. Wild Blueberry, *freeze dried extract* not only helps prevent declines of old age, but actually reverse brain aging.

Reversal of age related changes in neuronal signal transduction, cognitive, motor and behavioral deficits by dietary supplementation with blueberries was reported in the Journal of Neuroscience. Supplementation with antioxidant rich blueberries was found to improve CNS function in aged animals and humans.

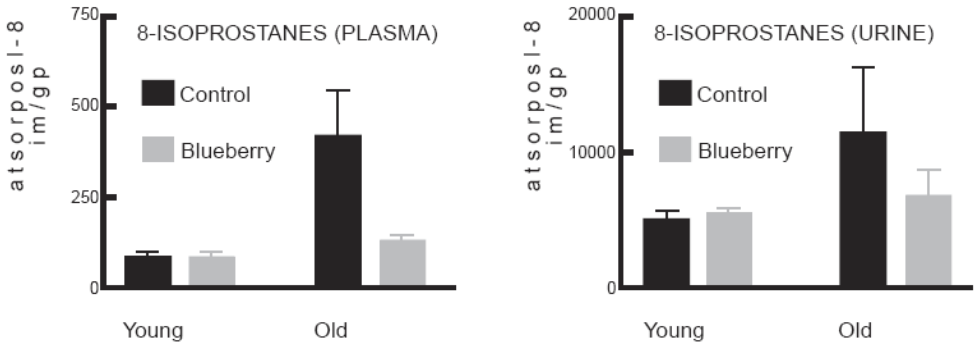

Figure 1. Levels of Isoprostanes in the plasma and urine of young and old rats receiving a normal diet or a diet containing blueberry. Old animals had greater levels of markers of oxidative damage compared to young animals. The blueberry diet reduced this marker for oxidative stress remarkably in the old animals receiving the dietary intervention. Published in: Journal of Undergraduate Research, Volume 2, Issue 5 - February 2001: Nutritional Interventions to Slow Cellular Aging, by Jill Goldstein.

Accumulative Oxidative Damage Causes a "Net Stress" on Normal Body Functions

The result of the Net Stress on the body generates many specific diseases. Net Stress contributes to the general decline of optimum body function and commonly believed to hasten the "aging process". Among the many oxidative stress mediated diseases are: Alzheimer's Disease, Autoimmune Diseases, Cancer, Heart Disease, Cataractogenesis, Diabetes, Macular Degeneration, MS, Muscular Dystrophy, Pancreatitis, Parkinson's Disease and Rheumatoid Arthritis.

Blueberry Enhances Antioxidant Potency in the Human Body

ORAC - The Scientific Standard for Measuring the Anti-Free Radical Potency

The scientific standard for measuring the antioxidant potency of foods is currently expressed in ORAC units (Oxygen Radical Absorbent Capacity). The ORAC assay has been extensively utilized in the field of antioxidant and oxidative stress medical research. It gives science the ability to quantify total antioxidant strength of any food, tissue or fluid. Simply explained, a high ORAC score indicates a high total antioxidant capacity.

Blueberries Tested #1 for Antioxidant Activity

The US Department of Agriculture's Center for Aging at Tufts University measured the ORAC activity of more than 40 commercially available fruits and vegetables. The blueberry ranked highest of all.

North American Wild Blueberry has 25-30 different kinds of Anthocyanins

An especially potent blueberry concentrated for its antioxidant benefits, wild blueberry is found in the coastal field and barrens of Maine and Eastern Canada. Only the finest, ripest North American wild blueberries qualify to be used in our product. Our 'Wild Blueberry' extract has been put through a unique propriety process that has concentrated the phytochemicals responsible for free radical absorption. Most fruits have 3-5 types of anthocyanins; our wild blueberries have 25-30 different kinds. **Wild Blueberry**, *freeze dried extract*, powerful health benefit is attributed to exceptionally high levels of unique anthocyanins and chlorogenic acid.

Anthocyanins from Wild Blueberry, *freeze dried extract* are Bio-Available

It is well known that Vitamin C and Vitamin E are readily bio-available compared with anthocyanins which are more difficult to absorb. The anthocyanin molecule is a vastly more powerful free radical scavenger than vitamins C and E. However, in food there is .1 to .3 % of anthocyanin and the final amount that reaches the blood is negligible. Therefore, high concentration of anthocyanins must be provided to obtain an appreciable effect. Through the unique proprietary process, **Wild Blueberry**, *freeze dried extract* is highly concentrated with anthocyanins and achieves significant levels of absorption and bioavailability.A study published in 2002 in the Journal of Agricultural and Food Chemistry demonstrated that the serum levels of 19 anthocyanins contained in wild blueberries were elevated significantly after ingestion of **Wild Blueberry**, *freeze dried extract* powder. Additionally, the increase of anthocyanin concentration was directly correlated with an increase in serum antioxidant capacity as measured by the ORAC standard.

Dusan Miljkovic PhD, Internationally Renowned Scientist, Comments on Wild Blueberry, *freeze dried extract*

"A variety in phenolic rings and extra attachment of alkyl groups is key to the added free radical scavenging ability. Another bio-molecule, chlorogenic acid, is concentrated in this product. It is an exceedingly potent antioxidant. In fact when absorbed it converts through hydrolysis into caffeic acid, and caffeic acid has the highest ORAC value ever recorded- 32,000. It is especially effective in crossing the blood-brain barrier. Chlorogenic acid is also anti-diabetic since it prevents the absorption of glucose from the gut."

Blueberry is Important for Management of Inflammatory Conditions

Mild COX-2 Activity of Wild Blueberry, *freeze dried extract*

In studies Wild Blueberry has been found to be a potent inhibitor of COX-2 enzyme in tube test (EC50=53ug/mL) but not COX-1. This observation indicates that Blueberry could be effective in inhibition of COX-2 within human body if very significant amount

of the berry could be consumed. Exploring further anti-inflammatory activity of Blueberry it was found that this material reduces:

1. Nuclear factor kappa B in vivo. This transcription factor is a key regulator of pro-inflammatory conditions
2. 8-isoprostanes in vivo. This molecule reflects oxidative (peroxidation of lipids) and inflammatory status of the body
3. MCP-1 in vivo (Monocyte Chemoattractant Protein -1, one of several chemokines involved in inflammation process). This chemokine is involved in the process of atherogenesis, stroke, arteriosclerosis, and inflammation induced by oxidized LDL, arthritis.

Zbigniew Pietrzkowski PhD, Comments on Arthritis and Inflammation

Dr. Zbigniew Pietrzkowski, a world class molecular and cell biologist, comments on blueberry's ability to reduce isoprostanes, a very important marker for pro-inflammatory prostaglandins: "Inflammatory prostaglandins are the result of the enzymatic breakdown of arachidonic acid, and a major causative factor in arthritic disease. Isoprostanes are the downstream marker of this oxidative process. Blueberry's ability to reduce levels of Isoprostanes indicates its usefulness in mediating this chronic inflammatory condition."

Cancer and Inflammation

Development of cancer is associated with local pro-inflammatory conditions and often pro-inflammatory proteins are over-expressed and secreted by a wide variety of neoplasias (formation of tumors) such as colorectal, gastric, liver, pancreas, esophagus, lung, skin, breast, bladder and prostate, as well as adenomas in vivo. However, Blueberry may inhibit growth of several types of cancer cells by some other mechanism not necessarily connected to inhibition of pro-inflammatory proteins as published in several peer-review journals. This observation is currently investigated in order to understand the principle of such inhibition.

Aging and Inflammation

Increased inflammation level within the body is observed with progression of age. Preventing and/or reducing inflammation conditions are commonly recognized as important strategy in slowing the process of aging. A new study recently presented from the John Hopkins School of Medicine confirmed the observation published by Joseph et, al showing that blueberries provide safe COX-2 inhibition, improve cognition, and can even reverse-age related declines in brain function. However, isoprostanes represent now the most significant role in the process of aging confirming that anti-oxidant status and inflammation caused mainly by oxidized LDL are a key factor contributing to accelerated aging and progression of Alzheimer disease (see last two references).

Prevent and Even Reverse the Accelerating Rise of Today's Complex Diseases

The USDA and Tufts University are continually publishing new research on the possible health benefits of Wild Blueberry for anti-aging, cancer, ORAC, vision and UTI. The *5 A Day For Better Health* government/industry campaign intends to help people eat 5 servings of fruits and vegetables a day. The National Cancer Institute found that 42% eat less than 2 servings a day. The average ORAC value of five servings of fruits and vegetables is 2500. The average American serving per day can be as low as 300 ORAC units. One gram (2 capsules) of **Wild Blueberry,** *freeze dried extract* provides 6500 ORAC units of antioxidant capacity. One capsule of **Wild Blueberry,** *freeze dried extract* provides 3250 ORAC which is 150% more than the average American diet, and 30% more then the *Five A Day* recommended serving.

Wild Blueberry, *freeze dried extract* is 100% Pure

BioImmersion, Inc. Manufactures and encapsulates Wild Blueberry without any added excipients: fillers, binders and flowing agents (such as magnesium stearate). Each vegetarian capsule is only filled with 100% pure **Wild Blueberry**, *freeze dried extract*.

BioImmersion Inc. technologically advanced Therapeutic Foods range of products responds to the reality of today's negligible dietary patterns and answers the

required nutritional needs to achieve and preserve good health. Our products are extensively and properly analyzed and documented to ensure consistent delivery of the highest levels of active ingredients. **Wild Blueberry**, *freeze dried extract*, provides the highest potency ORAC value in the market today.

Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model.

J Cereb Blood Flow Metab. 2002 Mar;22(3):308-17.

Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C.

Nervous System Research, Core Technology Area, Novartis Pharma AG, Basel, Switzerland.

Inflammatory processes have been implicated in the pathogenesis of brain damage after stroke. In rodent stroke models, focal ischemia induces several proinflammatory chemokines, including monocyte chemoattractant protein-1 (MCP-1). The individual contribution to ischemic tissue damage, however, is largely unknown. To address this question, the authors subjected MCP-1-deficient mice (MCP-1-/-) to permanent middle cerebral artery occlusion (MCAO). Measurement of basal blood pressure, cerebral blood flow, and blood volume revealed no differences between wild-type (wt) and MCP-1-/- mice. MCAO led to similar cerebral perfusion deficits in wt and MCP-1-/- mice, excluding differences in the MCA supply territory and collaterals. However, compared with wt mice, the mean infarct volume was 29% smaller in MCP-1-/- mice 24 hours after MCAO ($P = 0.022$). Immunostaining showed a reduction of phagocytic macrophage accumulation within infarcts and the infarct border in MCP-1-/- mice 2 weeks after MCAO. At the same time point, the authors found an attenuation of astrocytic hypertrophy in the infarct border and thalamus in MCP-1-/- mice. However, these effects on macrophages and astrocytes in MCP-1-/- mice occurred too late to suggest a protective role in acute infarct growth. Of note: at 6 hours after MCAO, MCP-1-/- mice produced significantly less interleukin-1beta in ischemic tissue; this might be related to tissue protection. The results of this study indicate that inhibition of MCP-1 signaling could be a new acute treatment approach to limit infarct size after stroke.

Aging, gender and APOE isotype modulate metabolism of Alzheimer's Abeta peptides and F-isoprostanes in the absence of detectable amyloid deposits.

J Neurochem 2004 Aug;90(4):1011-8.

Yao J, Petanceska SS, Montine TJ, Holtzman DM, Schmidt SD, Parker CA, Callahan MJ, Lipinski WJ, Bisgaier CL, Turner BA, Nixon RA, Martins RN,

Ouimet C, Smith JD, Davies P, Laska E, Ehrlich ME, Walker LC, Mathews PM, Gandy S

Aging and apolipoprotein E (APOE) isoform are among the most consistent risks for the development of Alzheimer's disease (AD). Metabolic factors that modulate risk have been elusive, though oxidative reactions and their by-products have been implicated in human AD and in transgenic mice with overt histological amyloidosis. We investigated the relationship between the levels of endogenous murine amyloid beta (Abeta) peptides and the levels of a marker of oxidation in mice that never develop histological amyloidosis [i.e. APOE knockout (KO) mice with or without transgenic human APOEepsilon3 or human APOEepsilon4 alleles]. Aging-, gender-, and APOE-genotype-dependent changes were observed for endogenous mouse brain Abeta40 and Abeta42 peptides. Levels of the oxidized lipid F_2-isoprostane (F_2-isoPs) in the brains of the same animals as those used for the Abeta analyses revealed aging- and gender-dependent changes in APOE KO and in human APOEepsilon4 transgenic KO mice. Human APOEepsilon3 transgenic KO mice did not exhibit aging- or gender-dependent increases in F_2-isoPs. In general, the changes in the levels of brain F_2-isoPs in mice according to age, gender, and APOE genotype mirrored the changes in brain Abeta levels, which, in turn, paralleled known trends in the risk for human AD. These data indicate that there exists an aging-dependent, APOE-genotype-sensitive rise in murine brain Abeta levels despite the apparent inability of the petide to form histologically detectable amyloid. Human APOEepsilon3, but not human APOEepsilon4, can apparently prevent the aging-dependent rise in murine brain Abeta levels, consistent with the relative risk for AD associated with these genotypes. The fidelity of the brain Abeta/F_2-isoP relationship across multiple relevant variables supports the hypothesis that oxidized lipids play a role in AD pathogenesis, as has been suggested by recent evidence that F_2-isoPs can stimulate Abeta generation and aggregation.

Measurement of F_2-Isoprostanes Unveils Profound Oxidative Stress in Aged Rats. BBRC 2001, 287(1), 254-256

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Free radicals have been theorized to play a causative role in the normal aging process. To date, methods used to detect oxidative stress in aged experimental animals have only detected 2-to 3-fold differences or less between young and aged animals. Measurement of F_2-isoprostanes has emerged as probably the most reliable approach to assess oxidative stress status *in vivo.* Therefore, we measured levels of F_2-isoprostanes free in plasma and levels esterified in plasma lipids in young rats (3–4 months of age) and aged rats (22–24 months of age). Plasma concentrations of free F_2-isoprostanes were increased dramatically by a mean of 20.3-fold (range 4.3 to 42.9-fold) and levels esterified in plasma lipids were also strikingly increased by a mean of 29.9-fold (range 15.8 to 50.0-fold). These findings unveil profound oxidative stress in aged rats which adds considerable support for the free radical theory of aging.

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