



# Recent Studies Further Demonstrate Astaxanthin's Potential as a Safe and Effective Anti-Inflammatory

## Results Support Cardax Clinical Program

HONOLULU--Recent studies continue to support astaxanthin's broad potential in chronic inflammatory disease, adding to more than 1,000 peer-reviewed papers published on astaxanthin. Selected publications from January 2013 to April 2014 include (i) a thorough review of astaxanthin and its applications, (ii) human studies in cognitive decline, cardiovascular oxidation, and ocular dysfunction, and (iii) animal and mechanistic studies addressing brain health and cognitive decline related pathology, osteoarthritis and joint health, metabolism, cardiovascular health, and related diseases, eye and retinal health, skin protection and health, lung function and health, and kidney function and health (see highlights below).

These studies support the rationale of the Cardax, Inc. ("Cardax" or the "Company") (OTCQB: CDXI) clinical program, which will seek to prove astaxanthin's safety and efficacy in major disease areas with common underlying mechanisms related to inflammation and oxidative stress. Assuming adequate additional funding and/or strategic partnership, the Company plans to conduct a suite of approximately three to five low-risk, high-value human proof-of-concept ("POC") clinical trials in such disease areas.

These human clinical trials would not only advance the Cardax pharmaceutical development program, but could also demonstrate human POC for potential partners and help catalyze nutraceutical sales through the Company's strategic alliance with BASF, under which Cardax will receive tiered royalties on future BASF astaxanthin nutraceutical sales.

### ***Astaxanthin review:***

**Ambati, R.R. et al.** reviews the sources, extraction, stability, biological activities, and commercial applications of astaxanthin.

### ***Human trials utilizing astaxanthin:***

**Hashimoto, H. et al.** evaluated the influence of astaxanthin on oxidative stress in the human eye. Patients (N=35 total) requiring bilateral cataract surgery underwent surgery on one side before and the other side after intake of astaxanthin (6 mg/day for 2 weeks). Aqueous humor was evaluated after each surgery. Astaxanthin significantly reduced total hydroperoxides and significantly increased superoxide scavenging capacity. This effect was more dramatic in diabetic patients and in total supports the capacity for astaxanthin to reduce oxidative stress in the human eye.

In a double-blind, placebo-controlled human study, **Baralic, I. et al.** evaluated the capacity of astaxanthin (4 mg/day for 90 days) to increase paraoxonase (PON1) activity and oxidative stress in young, healthy soccer athletes (N=40 total). PON1 activity has an inhibitory effect on atherosclerosis formation and protects LDL and HDL lipoprotein particles from harmful oxidative damage. Decreased PON1 has been correlated with oxidative stress and cardiovascular risk in humans. Here, astaxanthin significantly increased PON1 activity over the placebo group most likely resulting from elevated protection of PON1 oxidation via astaxanthin-induced increase of sulfhydryl groups.

To evaluate the effect of astaxanthin (in combination with Bacopa monnieri extract, phosphatidylserine and vitamin E) in patients with mild cognitive impairment (MCI), **Zanotta, D. et al.** treated 104 patients for 60 days in a prospective cohort, noncomparative, multicenter trial. Here, the astaxanthin-containing phytotherapeutic compound significantly improved Alzheimer's Disease Assessment Scale-cognitive subscale scores (ADAS-cog) and clock drawing test scores with memory tasks demonstrating the greatest improvement. This study supports the potential for astaxanthin as well as other antioxidants in the treatment of MCI.

#### ***Astaxanthin addressing brain health and cognitive decline related pathology:***

**Wu, W. et al.** utilized a rat model of brain aging to evaluate astaxanthin potential to alleviate aging associated pathology. Here, astaxanthin significantly restored antioxidant marker levels (GSH, SOD, TAC) and decreased oxidative stress markers (MDA, 8-OHdG, protein carbonylation). Astaxanthin also decreased inflammatory effectors including COX-2, ameliorated histological pathology and restored neurotrophic factor BDNF levels supporting the potential for astaxanthin to ameliorate aspects of aging pathology.

**Ye, Q. et al.** evaluated astaxanthin potential as a neuroprotective in a cell model of Parkinson's Disease. Astaxanthin increased cell viability following induction of cell damage (MPP) and decreased proteins involved in mediating cell dysfunction (Sp1, NR1). This study supports the capacity for astaxanthin to increase neural cell survival and regulate pathways that respond to oxidative stress by inducing neural cell death.

Early brain injury (EBI) refers to the acute injuries to the whole brain within 72 hours following aneurysmal subarachnoid hemorrhage (SAH) and is the primary cause of death in patients with SAH. **Zhang, X.S. et al.** used two animal models of SAH (rats and rabbits) to evaluate the capacity of injected or orally delivered astaxanthin to ameliorate oxidative stress and associated pathology in early brain injury following SAH. Astaxanthin intracerebroventricular injection post-SAH in rats significantly attenuated EBI (measured as brain edema, blood-brain barrier disruption, neural cell apoptosis and neurological dysfunction). Likewise, oral astaxanthin administration 3 hours post SAH was also neuroprotective in both rat and rabbit models.

#### ***Astaxanthin addressing osteoarthritis and joint health:***

**Chen, W. P. et al.** recently investigated the potential for astaxanthin to alter expression of matrix metalloproteinases (MMPs) in human chondrocytes. MMPs are pathologically upregulated in inflammatory diseases such as osteoarthritis and are critically responsible for degradation of joint matrix structure leading to more severe joint dysfunction. Astaxanthin treatment of human joint cells significantly reduced MMP expression levels (MMP-1, 3, 13)

following inflammatory induction of MMP upregulation (IL-1 $\beta$ ). This study supports the influence of astaxanthin on decreasing inflammatory signaling, particularly pathways leading to OA-related pathology.

To further assess astaxanthin influence on joint health, **Kimble, L.L. et al.** treated a human chondrosarcoma cell line with astaxanthin and measured various markers of inflammation induced by interleukin 1- $\beta$  treatment. In addition to significantly decreasing induced oxidative stress and maintaining activity of the antioxidant GPx, astaxanthin treatment significantly prevented IL-1 $\beta$ -induced upregulation of several inflammatory mediators critical to arthritis pathology including; MMP-13, IL-6, TNF- $\alpha$ , and PGE2. Additionally, astaxanthin treatment significantly inhibited NF- $\kappa$ B pathway activation and attenuated AP-1 activation. Similarly to Chen et al. (above), here, treatment of an arthritis-relevant cell type underscores the significant capacity of astaxanthin to diminish pathways of inflammation and attenuate levels of many inflammatory mediators intrinsic to arthritis pathogenesis.

#### ***Astaxanthin addressing metabolism, cardiovascular health and related diseases:***

**Liu, P.H. et al.** investigated the effect of astaxanthin on lipid metabolism in both sedentary and exercising mice (N=8/group). Here, astaxanthin (0.02% w/w for 2 weeks) reduced plasma fatty acids by 17% in sedentary mice and significantly by 21% in exercising mice. Astaxanthin increased intracellular pH levels indicating increased fat utilization in contrast to carbohydrate metabolism during exercise. Importantly, astaxanthin administration also significantly increased PGC-1 $\alpha$  (peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$ ) levels. PGC-1 $\alpha$  is a master controller of mitochondrial biogenesis and aerobic metabolism and directly upregulates critical mitochondrial genes also confirmed induced by astaxanthin in this study (FNDC5, Cytochrome C).

**Aoi, W. et al.** published an important review of oxidative stress during exercise and described the resulting effects on mitochondrial function. Here, studies are reviewed that support the significance of astaxanthin in mitochondria function (see Liu et al. above) as astaxanthin decreases oxidative stress-driven modification of the metabolic regulatory protein carnitine palmitoyltransferase-1 (CPT-1) and its interaction with FAT/CD36 as a limiting step to fat metabolism during exercise.

**Park, J.S. et al.** investigated the influence of astaxanthin (0, 20 mg/day for 16 weeks) on mitochondrial function in dogs (N=14/group). Dogs treated with astaxanthin exhibited greater mitochondrial function observed as increased mitochondrial mass, ATP production and cytochrome c oxidoreductase activity. Ratios of reduced to oxidized glutathione levels increased indicating restoration of antioxidant function. This study supports the capacity for astaxanthin to increase mitochondrial function in vivo in support of Aoi et al. and Liu et al. as described above.

**Bhuvaneswari S. et al.** evaluated the effects of astaxanthin (2 mg/kg/day for 45 days) on cellular stress and inflammation in mice on a high fat diet (N=6/group). This diet induces oxidative stress, fatty liver, inflammation and endoplasmic reticulum stress (ERS plays a role in many diseases including insulin resistance). Here, astaxanthin was shown to significantly reduce oxidative stress, fatty liver content, proteins induced by ERS and inflammatory pathway activation (JNK and NF $\kappa$ B) supporting the important anti-inflammatory role of astaxanthin in modulation of diseases related to ERS and inflammation including cardiovascular disease, liver disease, diabetes, etc.

**Xu, J. et al.** treated rats (N=10/group) on a high fat diet with astaxanthin in flaxseed oil (0, 50, 100, 200 mg astaxanthin/kg/day) for 10 weeks and evaluated oxidative stress, lipids and inflammatory markers. Astaxanthin treatments resulted in significant reductions in lipid measures including; triglycerides, total cholesterol and LDL-C. Inflammatory markers IL-6 and C reactive protein (CRP) were also significantly reduced. Measures of antioxidant capacity significantly increased with astaxanthin treatment (SOD, catalase, glutathione, GPx, TAC) and oxidative stress significantly decreased (TBARS). This study supports the role of astaxanthin in restoring lipid profiles and reducing inflammation in metabolic disease.

**Ishiki, M. et al.** evaluated the capacity of astaxanthin to alter insulin-related signaling in rat muscle cells. Astaxanthin treatment significantly increased insulin-induced responsive pathways leading to enhanced activation of IRS-1 and AKT, increased translocation of GLUT4 glucose transporter, increased glucose uptake as well as decreased activation of JNK pathways and inhibitory IRS-1 phosphorylation. This study mechanistically supports previously published studies utilizing animal models to demonstrate the potential for astaxanthin to ameliorate insulin resistance and related diabetic pathophysiology.

#### ***Astaxanthin addressing eye and retinal health:***

**Otsuka, T. et al.** investigated the influence of astaxanthin (100 mg/kg administered 8 times over 3 days) in a mouse model of retinal cell death resulting from intense light exposure (N=10-18/group). Astaxanthin significantly reduced rod/cone dysfunction (ERG measures) and protected against rod/cone cell death (histological and apoptosis measures). In support, astaxanthin also protected cone cells from light-induced death and lowered the resulting oxidative stress in cell culture.

**Dong, L.-Y. et al.** utilized a mouse model of diabetic disease and retinopathy to evaluate astaxanthin capacity to diminish retinal ganglion cell death (RGC)(N=8/group). Astaxanthin treatment (25, 50 mg/kg/day for 8 weeks) significantly reduced RGC cell death and decreased markers of oxidative stress including MDA, SOA and 8-OHdg and increased MnSOD antioxidant levels supporting the potential for astaxanthin to influence oxidative stress associated with diabetic retinal disease.

**Li, Z. et al.** treated a human cell type (RPE) lost in age-related macular degeneration with astaxanthin and noted that astaxanthin protected the cells against death and decreased oxidative stress following induction with hydrogen peroxide (ROS). Additionally, they deduced the astaxanthin mechanism of action for protection was activation of the insulin-responsive signaling pathway PI3K/AKT which in turn activates an antioxidant protection system (Nrf2).

In support of Li et al. observations (above), **Saw, C. L. L. et al.** observed activation of Nrf2-pathway antioxidant response elements (ARE) in cells treated with astaxanthin. Downstream genes activated by the Nrf2-ARE axis included heme oxygenase 1 (HO-1) and several other important protective antioxidant functions.

#### ***Astaxanthin addressing skin protection and health:***

**Rao, A.R. et al.** evaluated the potential for astaxanthin to inhibit or attenuate skin cancer development in a rat model of chemical-induced skin carcinogenesis. Rats (N=6/group) treated with astaxanthin (0.1, 0.2 mg/kg/day for 60 days) showed significant reductions in

skin tumor frequency as high as 96%. Normalization of tyrosinase and antioxidant levels was also observed. This study supports astaxanthin in skin protection and chemoprevention.

#### ***Astaxanthin addressing lung function and health:***

**Wang, M. et al.** utilized a chemically-induced rat model of lung fibrosis to evaluate the potential of astaxanthin to ameliorate lung disease pathology. Astaxanthin was administered (24 days at 0, 0.5, 1, 2 mg astaxanthin/kg/day or dexamethasone 1mg/kg/day)(N=10/group) and was found superior to dexamethasone in significantly ameliorating the induced lung fibrosis, decreasing edema and thickness in the pulmonary alveoli and increasing gas exchange. This study supports the potential for astaxanthin to ameliorate lung pathophysiology in vivo.

#### ***Astaxanthin addressing kidney function and health:***

Colistin methanesulfonate (CMS) is one of the few remaining therapeutic options for treatment of life-threatening infections caused by multi-drug resistant pathogens but often induces serious nephrotoxicity. **Ghlissi, Z. et al.** investigated the ability of astaxanthin to ameliorate renal toxicity from CMS in a rat model (N=6/group). Astaxanthin treatment (20 mg/kg/day for 7 days) significantly reduced histopathological damage and restored pathological biochemical parameters including MDA, SOD, catalase, GPx, GSH to more physiological levels. This study supports the potential for astaxanthin to reduce renal toxicity.

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### About Cardax

Cardax is a development stage life sciences company that devotes substantially all of its efforts to developing nutraceutical and pharmaceutical products that provide the anti-inflammatory benefits of steroids or NSAIDS, but with exceptional safety profiles, as conferred by U.S. Food and Drug Administration ("FDA") Generally Recognized as Safe ("GRAS") designation at certain doses. Cardax is preparing proprietary nature-identical products and related derivatives by total synthesis to provide scalable, pure, and economical therapies for diseases where inflammation and oxidative stress are strongly implicated, including, but not limited to, osteoarthritis, rheumatoid arthritis, dyslipidemia, metabolic disease, diabetes, cardiovascular disease, hepatitis, cognitive decline, macular degeneration, and prostate disease. The initial primary focus of Cardax is its astaxanthin technologies. Astaxanthin is a powerful and safe naturally occurring anti-inflammatory and anti-oxidant without the adverse side effects typical of anti-inflammatory treatments using steroids or NSAIDS, including immune system suppression, liver damage, cardiovascular disease risk, and gastrointestinal bleeding.

### Safe Harbor

This release may contain certain forward-looking statements regarding our prospective performance and strategies within the meaning of Section 27A of the Securities Act of 1933, as amended, and Section 21E of the Securities Exchange Act of 1934, as amended. We intend such forward-looking statements to be covered by the safe harbor provisions for forward-looking statements contained in the Private Securities Litigation Reform Act of 1995, and are including this statement for purposes of said safe harbor provisions.

Forward-looking statements, which are based on certain assumptions and describe future plans, strategies, and expectations of our company, are generally identified by use of words "anticipate," "believe," "estimate," "expect," "intend," "plan," "project," "seek," "strive," "try," or future or conditional verbs such as "could," "may," "should," "will," "would," or similar expressions. Our ability to predict results or the actual effects of our plans or strategies is inherently uncertain. Accordingly, actual results may differ materially from anticipated

results. Some of the factors that could cause our actual results to differ from our expectations or beliefs include, without limitation, the risks discussed from time to time in our filings with the Securities and Exchange Commission.

Readers are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date of this release. Except as required by applicable law or regulation, we undertake no obligation to update these forward-looking statements to reflect events or circumstances that occur after the date on which such statements were made.

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