



*Please note: The following presentation summaries from the **First International Symposium on ELDERBERRY** held June 9-14, 2013 and hosted at Stoney Creek Inn, Columbia, MO by the University of Missouri. The first half of each entry quotes directly from the printed presentation abstracts provided by the authors in bold font. My personal evaluation of potential present and future relevance to elderberry growers and consumers of elder fruit and flower products follow each quotation in regular font.*

*Of course, in both cases a large amount of information has been left out due to my editorial objectives of reporting concise statements of key learnings. My selection of what was important represents my own experiential bias without intentional critique of anyone's research. Also, I could not physically attend all presentations; therefore, some presentations will have more commentary due my including information derived from the questions and answers that occurred after each presentation. The full presentations will be publish in a special edition of **Acta Horticulturae**, hopefully by the middle of 2014.*

## **Effect of Berries on Cognitive and Neurochemical Functions**

Shibu M. Poulouse and Barbara Shukitt-Hale, Human Nutrition Research Center on Aging at Tufts University, USDA-ARS, Boston, MA, USA

**Berries are an excellent source of numerous, unique, bioactive compounds, which play a pivotal role in attenuating stress-associated biological dysfunctions. The protective effects of berries play even more significance in brain health as brain alone utilizes from 10-50% of the body's total oxygen supply...**

**...Elderberry (*Sambucus* spp.) like blueberries, blackberries and other berry species, contains large amount of flavonoids, particularly catechins and anthocyanins. Our studies have shown that improvement of brain health by berry diets is a result of reduced oxidative stress and inflammation, and, at the same time, an upregulation of the endogenous defense system to ward off toxic protein accumulations, an improvement in neuronal signaling, and a promotion of adult neurogenesis in critical brain regions. We have show feeding aged rats (19-21 months old) with berries high in antioxidants is able to prevent and reverse the neurochemical and behavioral changes that occur in aging, such as loss of memory, cognition and motor functions. Therefore, it is critical to highlight the nutritional interventions of diets rich in nuts, berries, fruits and vegetables which may reverse or allay age-related motor or cognitive deficits, delay the onset of age-related neurodegenerative diseases and convey long-term health dividends in an aging population.**

Read this abstract again and put its key points to work – especially that last sentence! Variety of high quality nutrient dense foods like berries, other fruits and nuts can even REVERSE brain damage due to aging. Of course, prevention is much better, but this study informs me that one can never start too late to improve his or her diet.

The advantage of elderberry, is its extremely high concentration of these indicated antioxidant and anti-inflammatory flavonoids, so that consuming small quantities of elderberry juice/flower products goes a long way to providing potentially effective nutritional boosts in the identified bioactive compounds. Since demographic trends indicate that older people will make up an increasing proportion of the population, the

marketing future for dark berries is bright, if people can be educated about the huge potential for a superior quality of life due to a better diet rich in nuts, fruits, vegetables and (especially dark) berries.

## **Neuroprotection by Berry-enriched Diets: Panacea or Methodological Conundrum?**

Susan O. McGuire, Samantha Sumait, Juan Ortega, and Ian Vaagenes. Rehabilitation Research, Edward Hines, Jr. Veterans Hospital, Hines, IL, USA and School of Medicine, The University of Illinois at Chicago, Chicago, IL, USA.

**Multiple Sclerosis (MS), a neurodegenerative disease resulting from autoimmune destruction of myelin, is associated with progressive physical disability as well as fatigue, cognitive dysfunction and depression. Although MS has varied presentations and progressions, inflammation is a common element found in all disease states. Treatment strategies that decrease inflammation would be expected to improve symptoms and/or slow disease progression. Blueberries contain high levels of the antioxidant anthocyanin flavonoids and have been reported to possess immunomodulatory and anti-inflammatory properties in multiple models of aging and neurodegeneration.**

**...Disease incidence was decreased 40% in blueberry-supplemented animals. Whereas no mortality was noted in the blueberry-supplemented mice, 16% of non-supplemented mice succumbed to EAE. Blueberry supplementation also was associated with decreased cumulative and final disease scores...These data strongly suggest that dietary supplementation with WBBP can reduce disease incidence and severity but that the mechanism behind such protection is complex and must be determined before WBBP can be used in human MS trials.**

EAE = Experimental Autoimmune Encephalomyelitis

WBBP = freeze dried blueberry powder

The mice were fed a rodent chow supplemented with about 1% WBBP. Higher quantities of WBBP did not result in superior results, which indicate an indirect effect of anthocyanins of the immune system. In other words, eating dark berries triggers some complex defense mechanism in the body or helps the immune system to better balance and regulate itself.

The consumer cannot wait on medical research to provide all the answers before acting to improve his/her life today through better diet. Dark berries decrease inflammation and positive help the body to repair itself in ways not fully understood yet. Elderberry has demonstrated substantially higher concentrations of these valuable nutrient compounds than blueberries.

The consumer should consume at least some dark berries or pure berry juice almost every day. Since no one knows precisely how much is best to deal with the various auto-immune diseases and aging, we are left to try different whole natural foods/juices in varying amounts for some reasonably extended time (weeks to months) and discover our personal results. Since the “mechanisms behind such protection is complex” whole foods would seem to be a superior alternative to extracted supplements.

## **Probing Action of Botanical Polyphenols on Oxidative and Inflammatory Signaling Pathways in Microglial Cells**

Grace Y. Sun, Jinghua Jiang, Yijia Zong, Dennis Y. Chuang, Agnes Simonyi, Zezong Gu, Kevin L. Fritsche, William R. Folk, and Dennis B. Luhan. Biochemistry Department and Center for Botanical Interaction Studies, University of Missouri, Columbia, MO, USA

**Many berry species and fruits are enriched in polyphenols and their consumption has been shown to offer health effects including fighting against infectious and inflammatory diseases. In the central nervous system, microglial cells constitute a unique class of immune cells and they exhibit characteristic properties to carry out multifunctional duties in the brain. Our recent studies have focused on signaling pathways underlying microglial response to proinflammatory cytokines and bacterial endotoxin (lipopolysaccharides, LPS).**

**...Using the microglial cell model, botanical phenolic compounds, such as epigallocatechin-gallate (EGCG) from green tea, honokiol from Magnolia bark, and even ethanol extract of *Sutherlandia frutescens* (a South African “cancer bush”), showed ability to inhibit oxidative and inflammatory responses in microglial cells through modulating pathways involving ERK1/2. Many species of berries, including elderberries (*S. nigra* subsp. *canadensis*) are enriched in anthocyanins, compounds known to contribute to the purple color of the fruit. Cyanidin-3-glucoside is the most common anthocyanin in the elderberry fruit and has high antioxidant activities. Our study indicated ability for cyaniding-3-glucoside to inhibit IFN $\lambda$ -induced NO and ROS production in microglial cells.**

Using North American elderberries, researchers probed the cell level activity of cyaniding-3-glucoside, one of seven antioxidants present in *S. canadensis*, on microglial cells, which form important signaling pathways in the central nervous system. They want to understand the cell level mechanisms behind the beneficial effects of elderberry anthocyanins in protecting and restoring brain/central nervous system performance. This study explores a specific indirect immune system response mechanism activated by elderberry.

## **Quantitative Proteomic Analysis for the Action of Botanicals on Nitrosative-oxidative Stress Signaling**

Zezong Gu. Department of Pathology and Anatomical Sciences, Center for Translational Neuroscience, Center for Botanical Interaction Studies, University of Missouri School of Medicine, Columbia, MO, USA

**Consumption of anti-oxidants...may reduce the risk of stroke and other age related neurodegenerative disorders. However, our understanding of the action of botanical anti-oxidants upon brain health is lacking...**

**These approaches enable us to investigate multi-target actions of botanicals on nitrosative-oxidative stress signaling in disease. In our studies we applied these methods and examined the effects of anti-oxidants from botanicals, including *Sambucus nigra* subsp. *canadensis* (elderberry), *Carnelia sinensis* (green tea), *Allium sativum* (garlic), and *Sutherlandia frutescens* (Sutherlandia) on either endotoxin lipopolysaccharides-induced activation of microglia and/or neurovasculature against ischemia in mice. We observed more proteins with significant alteration in the ischemic brains of mice fed the elderberry diet (2% of freeze-dried whole fruit) as compared to the AIN-93G control diet. We identified unique SNO-proteins and NO adduct on the specific cysteine of SNO-proteins. Characteristic of these SNO-proteins and elucidation of the network of nitrosative-oxidative stress signaling provide valuable insights into molecular mechanism of the botanical anti-oxidants on neuroinflammatory responses and against neurovascular impairment in the prevention of stroke.**

Anti-oxidants reduce the number of free radicals that seem to contribute to inflammation and the build-up of the fatty acids that narrow and block blood vessels. Microglia are dormant in the brain unless turned on by the central nervous system's need for protection. If botanical anti-oxidants reduce inflammation and the fatty build-up, then less/fewer signals will be sent to call the microglia into action.

The researchers want to learn about the chemical communication going here through the proteins in order to understand the precise way anti-oxidants found in plants and fruits reduce and reverse nerve and blood vessel inflammation to reduce the risk of ischemic stroke.

## **Beneficial Effects of Botanical Polyphenol Diets and Exercise on Behavior in Alzheimer's Disease Mouse Models**

Jennifer M. Walker, Agnes Simonyl, Grace Y. Sun, Todd R. Schachtman. University of Missouri Department of Psychological Sciences and Department of Biochemistry, Columbia, MO, USA

**Alzheimer's disease (AD) is one of the most debilitating age-related disorders and is the sixth-leading cause of death with an estimated global prevalence of 30 million, and more than 5 million currently in the United States. Preventing or postponing the onset of AD, as well as**

**delaying or slowing its progression would lead to an improvement of health status and quality of life for the elderly...Amyloid plaques and neurofibrillary tangles are well-established diagnostic markers of AD, and other features commonly seen include oxidative damage and inflammation...Rigorous nutritional modifications which are pleiotropic by nature, may counteract chronic subclinical processes aiding the aging brain's endogenous defenses...**

**Research from our laboratory, using animal models, has suggested that botanical polyphenols and/or exercise can be effectively used as a preventative measure for disease onset, as well as for slowing disease progression. We have shown that both dietary polyphenols and exercise are able to improve learning and memory performance in an AD animal model...Our studies have found age-dependent changes in cognitive and non-cognitive behaviors in these mice, and demonstrated that diet/exercise prevented or reversed some of these changes. These results, together with epidemiological and clinical studies in humans, suggest that dietary polyphenols and exercise may have beneficial effects on brain health and slow the progression of AD.**

AD model mice fed Missouri grown elderberry soaked foods exhibited greater resistance to onset and stronger recovery – reversal of symptoms – than the control group fed the non-elderberry diet. The research team observed significant improvement in behavior as well as evidence of reversal in study of brain sections. Of course, much more work needs to be done, but the evidence of damage reversal in AD seems parallel to the reversal of stroke damage found in a related but separate study. [The farm providing the elderberries is also a primary source of berries used in making River Hills Harvest elderberry juice products.]

## **Protective Effects of Elderberry against Cerebral Ischemic Injury**

Dennis Y. Chuang, Jiankun Cui, Agnes Simonyi, Victoria A. Engel, Shanyan Chen, Orr Hadass, Kevin L. Fritsche, William R. Folk, Andres L. Thomas, Wendy L. Applequist, George E. Rottinghaus, Dennis B. Lubahn, Zezong Gu, and Grace y. Sun. University of Missouri, Center for Botanical Interaction Studies, Columbia, MO, USA

**...In this study, we focus on the effects of *S. nigra* subsp. *canadensis* (elderberry), known to contain bioflavonoids and cyanindin-3-glucosides which are considered potent anti-oxidants. Previous studies have demonstrated protective effects of botanical polyphenols including resveratrol and curcumin on cerebral ischemia-reperfusion damage. In this study, we aimed to investigate whether supplementation with elderberry diet can similarly ameliorate brain damage using a transient global cerebral ischemia model in mice induced by a 30 min bilateral common carotid artery (BCCA) occlusion...mice were fed with either the control AIN93 diet or an AIN93 diet with 2% elderberry in dried mass weight for 2 months.**

**...Administration of elderberry diet partly ameliorated this deficit by prolonging the rotarod latencies. Quantitative digital pathology assessment of cresyl violet-stained stereological sections showed significant reduction in neuronal cell damage in the elderberry-treated group as compared with controls fed with the diet without elderberry...Taken together, our results demonstrated protective effects of elderberry diet in a mouse model of cerebral ischemic stroke and its potential translational value as dietary supplement against ischemic stroke.**

This study used multiple technical evidence sets to demonstrate that a diet providing regular supplementation of elderberry protected the brains of these specially bred mice against the damage caused by induced stroke. Mice fed elderberry also suffered observably and notably less brain damage. Of course, much more work needs to be done, but the evidence is encouraging. [The farm providing the elderberries is also a primary source of berries used in making River Hills Harvest elderberry juice products.]

## **Quantitative Proteomic Analysis for the Effects of Elderberry on Global Cerebral Ischemia in Mice**

Hui Zhou, Zhe Qu, Jiankun Cui, Agnes Simonyi, Victoria A. Engel, Shanyan Chen, Shuwei Li, Jilong Li, Jianlin Cheng, Michael Greenlief, Andrew L. Thomas, Kevin L. Fritsche, William R. Folk, Dennis B. Lubahn, Grace Y. Sun, Zezong Gu. Center for Translational Neuroscience, Center for Botanical Interaction Studies, Departments of Pathology and Anatomical Sciences, Biochemistry, School of Medicine, Departments of Computer Science, Informatics Institute, Chemistry, Southwest Research Center, Division of Animal Sciences, College of Agriculture, Food and Natural Resources, University of Missouri, Columbia, MO, USA. Department of Chemistry, University of Maryland, College Park, MD, USA.

**Stroke is the third leading cause of death and disability in aging adults. It is commonly caused by interference in blood flow to the brain that results in considerable brain damage. Elderberry has been found to exhibit benefits in human health through its antioxidant properties, which are assumed to offer beneficial effects on antagonizing inflammatory responses and preventing neurodegenerative diseases. Although interest in neuronal-related actions of elderberry is increasing, the molecular and cellular mechanisms underlying their mode of action have not been elucidated. In the present study, we selected a global cerebral ischemia mouse model. Mice were fed a semi-purified control diet (AIN-93G) for one of two experimental diets that contained elderberry (2% freeze-dried whole fruit) for 2 months prior to 30-min bilateral common carotid artery (BCCA) occlusion followed by 3-day reperfusion. We then conducted a comprehensive proteomic analysis to investigate the overall changes in protein levels in various regions of ischemic injury mice brain...**

**...In addition, with the proteins exhibiting significant protein level changes, Ingenuity Pathway Analysis (IPA) was used to identify multiple molecular targets and predict signal transduction pathways affected by elderberry. Our study may provide important insights into the molecular events underlying the treatment of elderberry and allow the identification of novel therapeutic targets.**

This research took a deeper, more granular look at the positive effects of elderberry against stroke damage at the molecular level with emphasis on the protein changes and the molecular pathways used in effecting those changes. Key learnings here at this point are further support of the principle of using elderberry as a regular part of one's diet to reduce or prevent various disease symptoms or injury, elderberry impacts brain and central nervous system function at the protein level, and the evidenced potential to develop elderberry-based agents or drugs for specific, targeted medical treatment is enough to justify further research.