THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 19, Number 6, 2013, pp. A1–A10
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DOI: 10.1089/acm.2013.1501

Winning Abstracts from the 10th Annual Natural Supplements Research Competition

January 30-February 2, 2013

Scripps Center for Integrative Medicine
La Jolla, CA

Contents

THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 19, Number 6, 2013, p. A3
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Meeting Report

Research Awards from the 10th Annual Natural Supplements Research Competition

Robert Alan Bonakdar, MD

The 10th Annual Natural Supplements Research Competition was hosted by the Scripps Center for Integrative Medicine, located in La Jolla, CA, on January 30-February 2, 2013, and took place as part of the conference *Natural Supplements: An Evidence-Based Update*. This Scripps-sponsored Continuing Medical Education (CME) conference brought together a group of more than 400 health care providers to discuss emerging trends in the field of dietary supplements.

The research presentations took place throughout the weekend of the conference, with accepted posters eligible for awards in faculty and student categories. Research in all categories, including original clinical research as well as basic science and review entries, were considered. The posters were judged by a multidisciplinary team in areas including originality, study design, interpretation, and relevance to the conference theme, with the top posters in each category receiving cash awards.

Posters accepted included entries from the National Institutes of Health, The Salk Institute, and university centers involved in natural supplements research, including the University of California, Los Angeles and San Diego; Bastyr University; and the New Jersey Neuroscience Institute at JFK Medical Center. Several of the entries involved international collaboration as well as collaborative efforts between academic and private entities. Brief overviews of the research winners as well as their abstracts are listed below. The next Natural Supplements Research Competition will take place in conjunction with the 11th Annual Natural Supplements: An Evidence-Based Update conference, January 29–February 1, 2014, in San Diego, CA. Further information, including research entry forms, can be obtained prior to the conference at www.Scripps.org/conferenceservices

For this year's conference, winners in the faculty division highlighted natural supplements in the area of joint health and skin aging as well as a capsaicin compound in the area of weight management. In the basic sciences and review category, research examined the immune-modulating effects of mushroom-derived compounds, cancer cell apoptosis induced by kinase inhibitors derived from milk, and up-regulation of transcription factors related to longevity. The student category highlighted basic sciences advances, including the potential of the naturally occurring flavonoid, fisetin, in the area of diabetic complications and artemisinin in modulating PSA.

The honorable mention category acknowledges additional accepted entries including those that were not eligible for awards, including those from the *National Institutes of Health,* Office of Dietary Supplements (ODS) describing its Analytical Methods and Reference Materials Program, and the ODS/*Centers for Disease Control and Prevention* Vitamin D Standardization Program.

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Category I, First Place

UC-II(R) FOR JOINT HEALTH: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, ADAPTIVE DESIGN, PILOT STUDY

Jay Udani, MD
Medicus Research

Objective: To evaluate the efficacy of UC-II® in alleviating joint discomfort in healthy subjects who experience joint discomfort upon strenuous exercise.

Design: Randomized, double-blind, placebo-controlled, adaptive design, pilot study.

Interventions: The investigational product for this study was UC-II® (InterHealth Nutraceuticals Inc.), an ingredient supplement containing 40 mg UC-II® which provides 10 mg undenatured type-II collagen per day.

Results: One hundred six subjects reporting joint discomfort were screened and 55 were randomized to placebo (n=28) and product (n=27). The Minimum Time to Onset of Joint Discomfort, compared to baseline, was significantly greater in the UC-II® group at V6 (Day 90) (p=0.041) and V7 (Day 120) (p=0.019), while no significant changes were observed in the placebo group. Further, the UC-II® cohort recovered from Joint Discomfort significantly faster at Day 60 (p=0.003), at Day 90 (p=0.000), and at Day 120 (p=0.000), compared to baseline.

The Average Knee Extension at Day 120 (p=0.011) was significantly greater for the UC-II® cohort as compared to placebo. Furthermore, the UC-II® group demonstrated a greater increase in Average Knee Extension at Day 90 (p=0.045) and Day 120 (p=0.002), which was statistically significant compared to baseline.

Conclusions: In a population of healthy subjects who only experienced joint discomfort upon physical activity, the active product, UC-II® appears to be effective in alleviating joint discomfort. UC-II® was able to significantly improve the Average Knee Extension. Supplementation with UC-II®, an ingredient consisting of undenatured type-II collagen, for seventeen weeks was well-tolerated and more effective than placebo in supporting joint comfort, flexibility and mobility.

Source of Funding: InterHealth Nutraceuticals, Inc., Benicia, California.

Category I, Second Place

TOLERABILITY AND EFFICACY OF CAPSIMAX™; A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, DOSE-FINDING, ADAPTIVE DESIGN STUDY

Jay Udani, MD
Medicus Research

Objective: To evaluate the efficacy of two-dose levels of a highly concentrated natural capsicum fruit extract containing capsaicinoids product compared to placebo in overweight female subjects.

Design: Randomized, double-blind, placebo-controlled, dose-finding, adaptive design study.

Interventions: The investigational product for this study was Capsimax™ (OmniActiveHealth Technologies Ltd.), a proprietary encapsulated form of Capsicum extract in beadlet form supplemented at 2 mg and at 10 mg of capsaicinoids, taken daily for thirteen weeks.

Results: One hundred nineteen overweight female subjects with body mass index between 25 and 34.9 kg/m^2 were screened and 51 were randomized to placebo (n=18), 2 mg treatment group (n=18) and 10 mg treatment group (n=15). The 2mg treatment group demonstrated a nearly significant decrease of Body Fat Percentage at Week 6 (p=0.099) compared to placebo; significant decrease of Body Fat Percentage from baseline to Week 12 (p=0.020) and, significant decrease of Body Mass Index at Week 3 (p=0.036), Week 6 (p=0.039), Week 9 (p=0.032), and Week 12 (p=0.035) compared to the placebo group.

Subjects who received the 2 mg treatment group showed significant decrease of Waist Circumference from baseline to Week 12 (p=0.013). The 2 mg treatment group demonstrated a significant decrease of Hip Circumference at Week 12 (p=0.028) compared to the placebo group. The 10 mg treatment group showed significant decrease of Thigh Circumference from baseline to Week 6 (p=0.045) and from baseline to Week 12 (p=0.045). No significant results were found between 10 mg treatment group and placebo group as well as 10 mg and 2 mg treatment group.

Conclusions: Treatment with a highly concentrated natural capsicum fruit extract containing capsaicinoids with OmniActive's proprietary and patent-pending encapsulation system, for thirteen weeks was well tolerated and more effective than placebo in helping manage appetite and supporting healthy metabolism in overweight subjects.

Source of Funding: OmniActive Health Technologies Ltd., Thane (West), India

ISRCTN49036618 http://www.controlled-trials.com/ISRCTN49036618/

Keywords: Overweight, Capsimax™ 2 mg, Capsimax™ 10 mg, Capsaicinoids

Category I, Third Place

A SINGLE CENTER, PILOT, DOUBLE-BLINDED, RANDOMIZED, COMPARATIVE, PROSPECTIVE CLINICAL STUDY TO EVALUATE IMPROVEMENTS IN THE STRUCTURE AND FUNCTION OF FACIAL SKIN WITH TAZAROTENE 0.1% CREAM ALONE AND IN COMBINATION WITH GLISODIN ADVANCED ANTI-AGING FORMULA

Lawrence D. Goldberg, MD, MBA
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Background: Superoxide dismutase (SOD) reduces reactive oxygen species formation associated with oxidative stress. An

imbalance between free radicals and antioxidants can lead to accelerated aging. Additionally, aging decreases SOD activity and increases sensitivity to oxidative stress. GliSODin® Advanced Anti-Aging Formula [ISOCELL Nutra, Paris, France] (GAAF) is a SOD-containing dietary supplement formulated with ingredients that promote improvements in the structure and function of the skin including hydration, elasticity, structural integrity and photoaging caused by oxidative stress. Tazarotene cream, 0.1% [AVAGE®, ALLERGAN, Irvine, CA USA] (TAZ) is an FDA-approved drug indicated for use in the mitigation of facial fine wrinkling, facial mottled hyper- and hypopigmentation, and benign facial lentigines in conjunction with a comprehensive skin care and sun avoidance program.

Objective: The purpose of this study is to determine if the antioxidant properties of GAAF will complement the retinoic actions of TAZ to improve the structure and function of facial skin.

Design: IRB-approved, prospective, single center, double-blinded, randomized, 90-day comparative study with monthly assessments of ten subjects with facial photodamage, set in a private aesthetic medicine practice and research center.

Intervention: Daily topical application of TAZ in combination with 3 capsules of GAAF (780 mg each) or placebo orally per day with food per the randomization allocation.

Results: TAZ alone and in combination with GAAF improved fine wrinkles [FW] (↓1.2 vs. 2.0), mottled hyperpigmentation [MH] (↓2.2 vs. 2.8) and overall photodamage [OP] (↓1.0 vs. 1.8) as well as patient-reported response to treatment [RTT] (↓2.0 vs. 1.6). At week 12, success of TAZ/GAAF combination treatment (Group A) versus TAZ treatment alone (Group C) was of significant clinical benefit, as measured by both absolute and relative comparative improvement, with respect to the parameters of fine wrinkling [FW] (14.7%/41.7%), overall photodamage [OP] (15.6%/53.0%), skin moisture [M] (19.1%/103.2%), skin elasticity [E] (12.8%/87.7%) and response to treatment [RTT] (8.8%/21.4%). Local adverse events were mild and there were no recorded events of medication discontinuation or study withdrawals.

Conclusions: The results of this study suggest that the addition of the antioxidant GAAF to the treatment of facial photodamage with TAZ is safe and provides significant clinical benefit with respect to key observed physician reported scales and patient reported response to treatment, with relative improvement in facial fine wrinkling, overall photodamage, skin moisture and elasticity.

Category II, First Place

DIETARY SUPPLEMENTS MAY INCREASE LONGEVITY BY UP-REGULATION OF DAF-16/FOXO GENE

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Purpose: Research data from nested, case-control *Hawaii life span study* provided support for a genetic basis in the aging process, mainly concerning a role of FoxO3A in longevity, a member of the FoxO family of transcription factors. FoxO protein regulates transcription of numerous cellular processes, including glucose metabolism, angiogenesis, detoxification of reactive oxygen species (ROS), DNA repair, apoptosis, stem cell maintenance, and immune, muscular and neuronal functions. Gene modification of *C. elegans daf-16* (the equivalent of mammalian FoxO) has been shown to extend lifespan. Recent advances in genetic and biochemical studies have made elucidation of these complex biological pathways and processes possible. In this study we aim to review potential of dietary factors with significant effects on modulating cellular transcription of *daf-16* with implication to modify aging in this model system.

Methods: Systemic search of the literature was conducted in electronic databases of MEDLINE. The reference lists of all papers and our files were searched for relevant publications. Experts in the field and manufacturers of dietary supplements were contacted for published and unpublished data. No language restrictions were imposed.

Results: In some trials, resveratrol (red wine, berries, grapes, peanuts) in diet was associated with slight lifespan increases (10%) in *C. elegans*. Research data showed acetic acid (vinegar) extended *C. elegans* lifespan by 25%, while Vitamins C and E, and vitamin B complex showed less significant effect (<3%). Mushroom extracts, including Reishi F-3 polysaccharide (*Ganoderma lucidum*), mycelium fractions of Chang-Chih (*A. camphorata*), and lion's mane *(H. erinaceus*) were associated with significant increase of lifespan, ranging from 25% to 35%. A synergistic, life-extending effect was found when acetic acid and RF-3 were combined (40%).

Discussion: From yeast to mammals, calorie restriction (CR) delays age-associated pathologies and promotes lifespan up to 50%. CR is thought to increase daf-16/FoxO expression through energy sensing AMP-activated protein kinase, which is also necessary for lifespan extension elicited by resveratrol. On the other hand, glucose shortens the lifespan of *C. elegans* (20%) by down-regulating daf-16/FoxO activity and aquaporin gene expression. Both Chang-Chih mushroom extract and RF3 polysaccharide were shown to up-regulate *daf-16* expression via TIR-1 and other MAPK pathway, distinct from the well-known conserved insulin/IFG-1 *daf-2* signaling pathway inhibited by acetic acid and lion's mane extract, resulting in increased expression of the longevity factor *daf-16*.

Category II, Second Place

MULTI-KINASE INHIBITOR AX-3 FROM MILK EXERTS CANCER CELL APOPTOSIS EFFECT *IN VIVO* AND REDUCES SERUM LEVELS OF PRO-INFLAMMATORY FACTORS TNF-ALPHA, MCP-1 AND RANTES

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Background: Research in the past decade has shown that many bioactive peptides encrypted in major milk proteins only become active when released proteolytically. We have isolated a novel milk peptide mixture (designated AX-3) that inhibits the tyrosine kinase activity of epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor 2 (VEGFR2), and insulin receptor (IR). *In vitro*, this multi-kinase inhibitor causes apoptosis in various cancer cell lines, and a phase I/II open label veterinary oncology clinical study shows promising anti-cancer effect against several canine tumor types. A six week double-blind, placebo-controlled human study conducted at Baylor University involving 73 healthy volunteers demonstrated that AX-3 is safe to consume orally. There was evidence of improved insulin sensitivity, neutrophil-to-lymphocyte ratio (NLR), and quality of life assessment of role of physical function.

Aim: To understand the pharmacodynamics of AX-3 in human.

Methods: Four healthy adult volunteers ingested 1g of AX-3 daily for six days. Prior to the experimental phase, blood and saliva was collected as controls. Fasting blood and saliva was collected at 2hr, 4hr, 6hr, 12 hr, 24 hrs, 4 days, and 6 days. AX-3 anti-cancer effects in serum and saliva were measured by incubating samples with PaCa-2 pancreatic cancer cell line, LNCaP prostate cancer cell line, and T-47D breast cancer cell line; percent cell death was calculated using TUNEL assay. Serum levels of pro-inflammatory factors TNF-alpha, MCP-1 and RANTES were measured using ELISA assays.

Results: Significant cancer cell apoptosis activity was detectable in both blood serum and saliva after 4 hrs of AX-3 ingestion and peaked by 12th hr. After six day ingestion, average serum TNF-alpha level decreased by 78% ($p = 0.022$), chemokines MCP-1 and RANTES levels decreased by 19% ($p = 0.017$) and 47.9% ($p = 0.039$) respectively.

Conclusion: Cancer cell growth and inflammation are hallmarks of cancer disease progression. AX-3 has detectable cancer cell apoptosis activity in blood and saliva, and lowers pro-inflammatory factors TNF-alpha, MCP-1, and RANTES. These may explain the tumor regression observed in the veterinary oncology study. Since TNF-alpha, MCP-1 and RANTES are also implicated in the progression of several inflammatory diseases, AX-3 may also be useful as a therapeutic agent for treating inflammatory diseases.

Category II, Third Place

THE USE OF MUSHROOM-DERIVED DIETARY SUPPLEMENTS AS IMMUNO-MODULATING AGENTS: AN OVERVIEW OF EVIDENCE-BASED CLINICAL TRIALS ALONG WITH THE MECHANISMS AND ACTIONS OF MUSHROOM CONSTITUENTS

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Historical and archeological records reveal that humans have been consuming mushrooms for thousands of years; as food, medicine and sacred items. The more recent history of mushrooms as medicine, however, has come from just the past few hundred years, primarily within the medicinal traditions of Japan, Korea, China and Eastern Russia. Today, mushrooms and mushroom constituents are commonly used as "dietary supplements" in the U.S. and around the globe; in the form of whole mushroom powders, powdered mycelia, extracts and isolated compounds from shitake, maitake, reishi, oyster, Brazilian and other edible mushroom species.

Here we review a number of the most common mushroom species used in the United States dietary supplement market, focusing on the mechanisms of action and the published clinical trials. Mushroom bio-actives mainly include alpha and beta glucans and related proteoglycans, such as polysachharide peptide complexes (PSP, PSK). Other non-polysaccharide constituents are also described (triterpenes, cordycepin etc.). These constituents have been shown to have direct properties; such as anti-bacterial, anti-viral and anti-cancer effects; but most of their immuno-modulating activity is derived through triggering the cells of the innate (non-specific) and adaptive (specific) immune system. Pre-clinical studies have shown that upon oral consumption of mushroom products, the bio-active beta-glucan, for example, gets transported from the intestinal lumen to the gut associated lymphoid tissue (GALT) via microfold cells of the intestinal epithelium. Beta-glucans and other bio-actives then trigger GI macrophages and dendritic cell activities by binding cellular pattern recognition receptors (PRR), such as toll like receptors (TLRs), dectin-1, complement-3 (CR3) and scavenger receptors. Binding of beta-glucans to these cell surface receptors, stimulates signal transduction cascades, inflammatory pathways and cytokine production.

Immuno-modulating effects of mushroom bio-actives in humans and animals vary, depending on their method of preparation, dosage and route of administration. These effects can also be measured by using immune functional bioassays such as NK (natural killer cell) cell cytotoxicity, Th1 and Th2 (T helper cells) assays, and by flow-cytometry. Available published clinical trials are outlined, where various mushroom-based preparations have been used to improve outcomes in various patients, with an emphasis on a wide range of cancer patients. The potential for these agents to be a useful adjunct for immunomodulation within a thriving integrative medicine practice, is summarized.

Category III, First Place

FISETIN: FLAVONOID AND FIGHTER AGAINST DIABETIC COMPLICATIONS

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Background: An estimated 23.6 million Americans (8% of the population) suffer from diabetes and an estimated 57 million Americans have prediabetes, a condition which increases the risk of developing diabetes. Diabetic nephropathy (DN), a complication of diabetes, is a condition that often

ends in kidney failure and has no known therapy. During the early stages of DN, clusters of kidney blood vessels (glomeruli) undergo hypertrophy and a thickening of the basement membrane, resulting in increased levels of albumin in the urine. Another diabetic complication is diabetic neuropathy: nerve damage, often presenting itself early as tingling and burning in the toes and feet, that results in diabetic patients due to high blood sugar levels. An estimated half of diabetic patients develop diabetic neuropathy.

Aim: To study the ability of orally active natural product and flavonoid, fisetin, to protect diabetic animals against diabetic complications.

Methods: Compared to wild-type mice, Akita mice develop renal hypertrophy, increased glomerular volume, and increased urine protein secretion; thus, this animal model was used in our studies as a diabetic animal model. Mice were fed 0.05% fisetin in their food for 18 weeks at which point we assayed urinary albumin levels and weighed kidneys to determine fisetin's effect on diabetic nephropathic conditions. In addition, we measured osteopontin, a key regulator of the pathophysiological changes associated with diabetic nephropathy (*Nicolas et al., Kidney Int., 2010*) in mouse kidneys and analyzed via Western blotting. Likewise, C-reactive protein, a serum inflammation marker elevated in both human disease and in diabetic mice, was measured and analyzed via Western blotting. Finally, fisetin's effect on diabetic neuropathy was probed by measuring motor nerve conduction and thermal latency response.

Results: Fisetin feeding of Akita mice prevents both the increase in kidney weight and decreased urinary protein secretion compared to untreated Akita mice. Fisetin treatment significantly reverses upregulation of osteopontin in Akita mice and decreases serum inflammation marker C-reactive protein, but had no effect on the large increase in blood glucose levels in the Akita diabetic mice. Finally, fisetin treatment ameliorates the diabetes-induced slowing of the motor nerve conduction velocity and decreased sensitivity to thermal stimuli. Also, fisetin improves peripheral nerve function in diabetic mice.

Conclusion: Fisetin restores levels of the diabetic complication markers to those similar to wild-type mice. Therefore, fisetin shows significant promise as a treatment to ameliorate the consequences of diabetes.

Category III, Second Place

A COMPREHENSIVE APPROACH FOR KNEE OSTEOARTHRITIS PAIN

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Background: Treating osteoarthritis in a comprehensive manner includes addressing the structural and chemical elements contributing to pain and pathology. Effective therapy must treat both the primary and secondary etiologies. On a large scale the primary cause of pain is structural in nature, while chemical contributions are secondary. The pathophysiology of osteoarthritis involves a complex balance of structural integrity, inflammation, and fluid movement. The purpose of this work is to provide a systematic conservative approach to treating osteoarthritic knee pain using knowledge of functional anatomy and physiology.

Methods: A focused literature review of Ovid Medline, Cochrane, and Pubmed was conducted investigating the anatomical, nutritional, and supplemental therapies use to treat knee osteoarthritis.

Summary: Assessing the structural elements of pain is a complex art. The knee joint is largest joint of the body. It has numerous structures that can contribute to pain. The knee is primarily a synovial hinge joint that articulates in flexion and extension, but there are also minor movements involved within this joint. Full range of motion in all of the major and minor joints is necessary for a smooth motion of the knee, but this is not the only joint that contributes to the gait pattern. The hip, spine, ankle, and multiple other joints could be compromised resulting in a compensated gait with the end result of knee pain. Additionally, Pressure = Force/Area clearly illustrates that excess body weight will increase the load on an unchanging surface area resulting in accelerated degeneration. Furthermore, the omental fat of obesity enhances a pro-inflammatory endocrine environment that accelerates the course of osteoarthritis. Conservative therapies to treat the structural elements of knee pain include osteopathic manual medicine, physical therapy, exercise, weight loss and acupuncture.

Once the structural elements have been addressed the physiology of pain can be investigated. This work focuses on the safety and efficacy of nutritional supplements in the treatment knee osteoarthritis. The nutritional supplements frequently used alter the course of osteoarthritis work by altering the inflammatory profile, supporting the cell and tissue integrity, and by replacing nutrients found to be deficient in the disease process. Omega 3 (EPA/DHA), Chondroitin, Glucosamine, Hyaluronic Acid, Avocado-Soybean unsaponifiable fraction, Methylsulfonylmethane, S-Adenosyl-Methionine, Ginger, and Turmeric have all demonstrated efficacy and safety in treating knee osteoarthritis. This work summarizes the mechanism of action, dosage, safety, adverse effects, and efficacy of these commonly used supplements.

Category III, Third Place

ARTEMISININ DECREASES PSA INDUCED BY TGFβ1 + DHEA IN PROSTATE CANCER EPITHELIAL AND STROMAL CELL CO-CULTURES

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Dehydroepiandrosterone (DHEA) is used as a dietary supplement and can be metabolized to androgens and/or estrogens in the prostate. In previous studies, addition of TGF-β1 to DHEA-treated prostate stromal-epithelial cellco-cultures reproduced a reactive stromal microenvironment and significantly increased the androgenicity of both cell types, as measured by increased PSA and testosterone production. These data suggest that DHEA may be benign in normal prostate but produce paracrine-mediated androgenic effects in the cancer stromal microenvironment. This study aimed to investigate if artemisinin, an isolated alkaloid compound of Artemisia annua, can reverse this effect. The co-culture model used to assess

endocrine (DHEA), immune (TGF-β1) and paracrine (epithelial-stromal) interactions is a complex system which attempts to reproduce prostatic tissue microenvironment in vitro. LAPC-4 prostate cancer cells were grown in co-culture with prostate stromal cells and treated with DHEA + TGFβ1 + /- varying doses of artemisinin. The effects of artemisinin on PSA expression in the co-cultures were compared with those in monocultured epithelial and stromal cells using real time PCR and ELISA. Artemisinin treatment led to a dose-dependent decrease in PSA protein expression induced by TGFβ1 + DHEA. In this co-culture model of endocrine-immune-paracrine interactions in the prostate, artemisinin appears to be effective in decreasing PSA production induced by DHEA + TGFβ1. Further research to assess the potential clinical benefit of Artemisia annua extracts for prostate cancer is warranted.

Category IV, Honorable Mention

VITAMIN D STANDARDIZATION PROGRAM

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Objectives: (1) standardize measurement results of serum 25-hydroxyvitamin D [25(OH)D] in national health surveys, (2) study differences in 25(OH)D values in national health surveys; (3) standardize 25(OH)D measurement results from clinical, commercial, and research laboratories to enable transfer of survey findings to patient care and pubic health activities. The measurands addressed in the program are total 25(OH)D, 25(OH)D_2, 25(OH)D_3 and 3-epi-25(OH)D_3.

Relevance: One of the key recommendations of the Institute of Medicine's recently released "Dietary Reference Intakes for Calcium and Vitamin D" is the need to standardize the measurement of serum 25(OH)D. The widespread variation in measurement results of 25(OH)D confounds international efforts to develop evidence-based clinical guidelines.

Methodology: To assess measurement variability in currently used assays; an interlaboratory comparison study is being conducted that includes laboratories from national surveys as well as research laboratories and assay manufacturers. A formal laboratory standardization program will be implemented using procedures similar to those used in the CDC Hormone Standardization Program (HoSt Program). These standardization activities will use single-donor fresh-frozen serum collected using the CLSI C37 protocol. Values will be assigned to these sera by the reference laboratories at the National Institute for Standards and Technology (NIST) and the University of Ghent (Prof. Dr. Thienpont). The initial assay performance criteria used in this standardization program are $<=10\%$ imprecision and $<=5\%$ bias to the reference values. These performance criteria are based on biological variability data. To increase the comparability of existing data from different national surveys, studies are performed to create master equations that facilitate the conversion of already existing national survey data.

Results: The NIH Office of Dietary Supplements (ODS) and CDC National Center for Environmental Health (NCEH) established a vitamin D standardization program with a Standardization Coordinating Center at CDC. Survey laboratories from national surveys in Canada, Germany, Ireland, UK and USA are participating in this program. Additional national survey laboratories are enrolling. An initial study to establish a master equation between data obtained in the U.S. National Nutrition and Health Examination Survey (NHANES), National Center for Health Statistics, CDC and data obtained in the German National Health Surveys is being conducted.

Conclusions: A Vitamin D Standardization Program has been established by NIH ODS, CDC and NIST. The program will standardize measurement results from national survey laboratories as well as clinical laboratories and assay manufacturers. This program will resolve current discrepancies that limit the use of research data in patient care and public health.

THE NIH/ODS ANALYTICAL METHODS AND REFERENCE MATERIALS PROGRAM: ACCOMPLISHMENTS AND FUTURE DIRECTIONS

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Assuring the quality of natural products is a challenge to manufacturers, researchers, regulators, and consumers. There are numerous ways to describe quality, often starting with botanical identity and sanitation and expanding to include specifications for pesticides, toxic elements, non-target plant material, and content of desirable and undesirable natural chemicals. Because plants contain complex mixtures of secondary metabolites, they pose unique analytical challenges. Evaluation of the accuracy, precision and reproducibility of chemical measurements made in these complex matrices is difficult. In 2002, the NIH Office of Dietary Supplements created a new program, the Analytical Methods and Reference Materials Program (AMRM), to provide tools to help members of the supplement community address these challenges. The program is stakeholder driven, with participation by government agencies, non-governmental organizations, academic researchers, and the private sector. The program provides the user community with several tools for demonstrating the integrity of analytical data: 1) published, publicly available methods that have undergone formal validation studies (useful for analysts just entering the field and for dispute resolution); 2) suites of certified matrix reference materials for use as method development and method evaluation tools; 3) certified calibration standard solutions for use in compound identification and instrument calibration; 4) no-cost laboratory quality assurance programs to assist laboratories in evaluating and improving method, analyst, and laboratory performance. The poster will describe the structure and evolution of the NIH AMRM, provide an overview of accomplishments and resources made available by the program, and review suggestions for future program directions made by an external expert review panel charged with program evaluation.

HEALTH-PROMOTING EFFECTS OF REISHI MUSHROOM POLYSACCHARIDE - A REVIEW

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Objectives: Mushrooms have long been used as food, dietary supplements, and medicine. Polysaccharides have important biological roles as antibiotics, antioxidant, antimutation, anticoagulant, and immunomodulator. Polysaccharides derived from mushrooms appear to enhance innate and cell-mediated immune responses, and exhibit anti-tumor activities through effects on maturation, differentiation, and proliferation of immune cells. We aim to review the potential health benefits of polysaccharides derived from Reishi (G. Lucidum), one of the most potent medical mushrooms.

Search Methods: Literature search was conducted in electronic databases of MEDLINE and our own files. Manufacturers of herbal products were contacted. Only polysaccharides with known chemical structure and mechanisms of action were included in the analysis.

Results: Five polysaccharide protein/peptide conjugates obtained from Reishi (*GLIS, GIPS, GIPP, GLPP, F3*) have known monosaccharide compositions and carbohydrate/protein ratio. *GLIS* inhibited tumor growth and enhanced humoral and cellular immune functions in mouse models. *GIPS* inhibited tumor growth in a murine sarcoma 180 model, the adhesive ability of tumor cells, and induction of lymphocyte proliferation and activation. *GIPP* was suggested to have anti-angiogenic effects on human lung cancer cells. GLPP inhibited the growth of S180, Heps, and EAC tumor cells in mice, and was active in prevention of immunosuppression induced by cyclophosphamide and 60 Co radiation in mice. *F3 was found to* induce macrophage-like differentiation and apoptosis in human leukemia THP-1 cells, and interacted with the Toll-like Receptor 4 in mice, suggesting immunomodulatory effects, and extended the life span of *C. Elegans* by up-regulating the activity of *daf-16*, a nematode homologue of the mammalian FoxO gene, known to control the organism's ability to deal with oxidative stress and energy metabolism associated with aging.

Conclusions: Of the 700 species of mushrooms, 33 may contain anticancer polysaccharides, 13 are edible, and 11 were studied extensively. Similar to ß-glucans such as lentinan, PSK, PSP, which have been used as adjunct agents during chemotherapy, these glucan-protein complexes show great promises as well.

Compared to antioxidant vitamins (B1, B2, B6, C, E) and acetic acid (vinegar), F3 shows greater lifespan-extending effects (35% increase) in *C. Elegans, a* model organism for study of aging, *with* a synergistic effect when combined with vinegar (30–40% increase). These data suggest both vinegar and F3 have potential as diet based methods for altering expression of longevity genes, resulting in better health and longer lifespan.

AGMATINE - UTILITY AS A NUTRACEUTICAL IN NEUROPATHY AND NEUROPATHIC PAIN

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Background: Primary nerve damage, the underlying cause of neuropathies, sets in motion destructive molecular reactions that can cause nerve cell dysfunction which can progress to nerve cell death. Not surprisingly, several molecular mechanisms implicated in nerve degeneration are also incriminated in causing neuropathic pain. This has suggested that treatments aimed at neuroprotection would prove to be a novel strategy for neuropathic pain reduction.

Concept: It was postulated that a single neuroprotective agent capable of modulating, potentially synergistically, multiple molecular targets - like a 'molecular shotgun' - would be a preferred therapeutic for neuropathies. According to this concept, it was proposed that agmatine, a robust neuroprotective/cytoprotective naturally occurring molecule, might constitute such a candidate. Agmatine [$(NH_2(CH_2)_4NH_2C(NH=)NH$] is a ubiquitous naturally occurring molecule present in small amounts in foodstuff derived from plants, fish, and animal products. It is biosynthesized by decarboxylation of the amino acid arginine, hence known as decarboxylated arginine, and can interact with multiple molecular targets critical for both neuroprotection and neuropathic pain reduction. These include: (A) modulating several neurotransmitter receptors; (B) blockade of key ion transport channels; (C) regulating nitric oxide (NO) production; (D) inhibiting protein ADP-ribosylation; (E) modulating polyamine metabolism; (F) inhibiting matrix metalloproteases (MMPs); (G) blocking advanced glycation end products (AGEs) formation.

Clinical Studies: The cumulative evidence clearly indicated the therapeutic potential of agmatine in neuropathy. This assumption was tested by a set of recently published human clinical trials (Keynan et al., *Pain Med* 11:356–368, 2010). A phase I, open-label study with consecutive cohorts who took escalating regimens demonstrated the safety of oral agmatine sulfate (using the nutraceutical brand, G-agmatine®). This study was followed by a phase II randomized, placebo-controlled trial to assess the effectiveness of oral agmatine as an add-on treatment for lumbar disc-associated radiculopathy, commonly known as sciatica. It was found that patients treated with agmatine experienced significantly more neuropathic pain relief and improved health-related quality of life as compared to placebo. These studies were considered a 'proof-of-concept' landmark for using agmatine in other neuropathies. Accordingly, clinical trials to assess agmatine's effectiveness in various types of neuropathy are underway.

Conclusions: Based on the evidence, oral agmatine is considered safe (lacking adverse effects) and is now available as an effective nutraceutical treatment for neuropathies.

AGMATINE – A PILOT STUDY OF ITS EFFECTIVENESS IN SMALL FIBER NEUROPATHY

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Background: Small fiber neuropathies presenting as pain and burning sensations as well as complications of autonomic dysfunctions are a significant cause of disability. Current medications such as Lyrica, Neurontin, and Amytryptline are often not effective and can cause side effects such as drowsiness and weight gain. Previous clinical studies strongly support the use of oral agmatine, decarboxylated arginine, as a safe (lacking adverse effects) and effective treatment for sciatica. Based on these reports we undertook a prospective study to evaluate the effectiveness of agmatine in painful small fiber neuropathies.

Objective: A case series study to assess the effectiveness of agmatine in painful small fiber neuropathy (Study Registry: ClinicalTrials.gov, System Identifier: NCT01524666). This is an interim report of the first 3 patients entered into the study.

Methods: Patients were recruited from the neuromuscular clinic at the New Jersey Neuroscience Institute if pain in the extremities was diagnosed as secondary to a peripheral neuropathy. Diagnosis of a small fiber neuropathy was confirmed by a skin biopsy and/or Quantitative Sensorimotor Axonal Reflex Testing (QSART). Patients completed the Neuropathic Pain Questionnaire (*Clinic J Pain* 19:306-314, 2003) before and after a 3-month treatment with 2.670 g/day AgmaSet® - the dietary supplement containing the nutraceutical brand of agmatine sulfate, G-agmatine®, and were allowed the use of any concomitant conventional treatment during the study. This questionnaire includes 8 scales with positive correlations and 4 with negative correlations to neuropathic pain (i.e., a high score on the latter scales suggests a non-neuropathic pain).

Results: To date, 3 patients have completed the trial without any significant adverse effects. One patient had marked improvements (over 85%) in all pain scale questions. Another patient reported moderate improvement - indicated by improving in 7 of 8 scales correlated with neuropathic pain and worsening in 3 of 4 scales that negatively correlated with neuropathic pain, thus suggesting significant improvement in the neuropathic pain component. The third patient did not have significant changes in his pain symptoms.

Conclusion: Based on these preliminary results, agmatine sulfate may be considered a safe medication with significant positive effects for patients with painful small fiber neuropathies. This further substantiates agmatine as an effective nutraceutical for neuropathies.