OGIRC/CICMR Second Joint Conference
“Production, Pharmacology and Health Benefits of Traditional Medicines”
October 23-25, 2010 - Lamoureux Hall, University of Ottawa

Program Committee

Scientific program: Ed Lui and John Arnason

Program book: Chieu Anh Ta

Organizing committee: Jen Heidenheim, Wendy Ambrose-Hope, Martha Mullally, Jose Antonio Guerrero, Carolina Cieniak, Brendan Walshe-Roussel, Gabriel Picard, Baillie Redfern, Ed Lui, John Arnason

Thank you to our sponsors!
OGIRC/CICMR Second Joint Conference
“Production, Pharmacology and Health Benefits of Traditional Medicines”
October 23-25, 2010 - Lamoureux Hall, University of Ottawa

Program

Saturday, October 23, 2010

<table>
<thead>
<tr>
<th>Time (pm)</th>
<th>Description</th>
<th>Location</th>
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<tbody>
<tr>
<td>4:30–5:30</td>
<td>Registration</td>
<td>University Centre</td>
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<tr>
<td>5:30–7:15</td>
<td><strong>Welcome Reception &amp; Dinner</strong></td>
<td>Jazzy Café, University Centre</td>
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<td><em>Honouring Dr. Patrick Choy</em>, retiring Associate</td>
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<td>Dean at the University of Manitoba, CICMR</td>
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<td>founding member and Honorary Chairman</td>
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<tr>
<td>7:30–7:45</td>
<td>Welcoming &amp; Introductory Remarks</td>
<td>Lamoureux Hall, Rm. 122</td>
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<tr>
<td>7:45–8:45</td>
<td><strong>Plenary Lecture</strong></td>
<td>Lamoureux Hall, Rm. 122</td>
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<td><strong>O-1: Dr. Tommy Cheng</strong>, Professor, Yale University and Chairman, Consortium for Globalization of Chinese Medicine (CGCM)</td>
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<td>&quot;Potential, bottlenecks and approaches to develop future medicine based on traditional Chinese medicine”</td>
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<tr>
<td>9:00–10:00</td>
<td>CICMR Executive Meeting (closed)</td>
<td>TBA</td>
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### Sunday, October 24, 2010

<table>
<thead>
<tr>
<th>Time (am)</th>
<th>Speaker &amp; Title</th>
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<tr>
<td>7:30 – 8:30</td>
<td>Registration</td>
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| 8:30 – 9:00| **Opening remarks**  
*Dr. Roman Szumski* - National Research Council, VP Life Sciences Division,  
*Tommy Cheng*, Professor, Yale University and Chairman, Consortium for Globalization of Chinese Medicine (CGCM) |

#### Symposium I: Agriculture, Botanical and Development Perspectives
Lamoureux Hall, Rm. 122. Chairs: C. Siow and J. Tai

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<th>Time (am)</th>
<th>Speaker &amp; Title</th>
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| 9:00 – 9:25| **O-2:** *Priti Krishna* - University of Western Ontario  
“Transcriptome analysis of polyunsaturated fatty acid-enriched seed of sea buckthorn (*Hippophae rhamnoides* L.) using 454 high-throughput sequencing” |
| 9:25 – 9:50| **O-3:** *Dan Brown* - Agriculture & Agri-Food Canada; Associate Director, OGIRC  
“North American ginseng germplasm characterization and development” |
| 9:50 – 10:15| **O-4:** *Alain Cuerrier* - Montreal Botanical Garden  
“*Rhodiola rosea*: from Ethnobotany to Taxonomy Using Molecular Tools” |
| 10:15 – 10:30| Break                                                                 |

#### Symposium II: Industry and Growers Perspective
Lamoureux Hall, Rm. 122: Chairs: P. Charpentier and J. Zhu

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<tr>
<th>Time (am)</th>
<th>Speaker &amp; Title</th>
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| 10:30 – 10:55| **O-5:** *Anthony Windust* - NRC Institute for National Measurement Standards  
“Analytical Standards for ginseng and other natural health products” |
| 10:55 – 11:20| **O-6:** *Sharla Sutherland* - Afexa Life Sciences Inc.  
“Polymolecular botanical drug discovery and development” |
| 11:20 – 11:45| **O-7:** *Kan He* - Naturex  
“Design and development of herbal products – the example of FraxPure” |
| 11:45 – 12:00| **O-8:** *Jim Todd & Sean Westerveld* - Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)  
“Production of non-traditional crops for the health market” |
| 12:00 – 1:00| Lunch, OGIRC Research Committee Meeting (closed)                                    |
# Symposium III: Pharmacology and Health Benefits of Traditional Medicines

Lamoureux Hall, Rm. 122  Co-Chairs:  J. Henry and D. Bailey

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<tr>
<th>Time (pm)</th>
<th>Speaker &amp; Title</th>
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| 1:00 – 1:30 | **O-9:** Ping Li – China-Japan Friendship Hospital Beijing  
Effect of Tangshen Formula on Type 2 Diabetic Nephropathy patients and study of its action mechanisms by system biology |
| 1:30 – 1:55 | **O-10:** Lina Mussallam – Université de Montréal  
Anti-diabetic action of Cree traditional medicines |
| 1:55 – 2:20 | **O-11:** Brian Foster – Therapeutic Products, Health Canada  
Herb-drug interaction |
| 2:20 – 2:40 | **O-12:** Siyaram Pandey – University of Windsor  
Non-toxic natural compounds as selective inducers of apoptosis in cancer cells |
| 2:40 – 3:00 | Break |

## Contributed Sessions and Workshop

Lamoureux Hall, Room 122

<table>
<thead>
<tr>
<th>Time (pm)</th>
<th>Speaker &amp; Title</th>
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| 3:00 – 3:15 | **O-13:** Melissa Moey - University of Western Ontario  
Ginsenosides from *Panax quinquefolius* (North American ginseng) attenuate leptin-induced neonatal ventricular cardiac hypertrophy though inhibition of the RhoA/ROCK |
| 3:15 – 3:30 | **O-14:** Yan Wu - University of Western Ontario  
Ginseng protects the heart from ischemia and reperfusion injury by activating PI3K/Akt-dependent eNOS pathway |
| 3:30 – 3:45 | **O-15:** Chike Azike - University of Western Ontario  
The Yin and Yang actions of North American Ginseng root in modulating the immune function of macrophages |
| 3:45 – 4:00 | **O-16:** Pamela Ovadje - University of Windsor  
Selective Induction of Apoptosis through Activation of Caspase-8 in Human Leukemia cells by Dandelion Extract |
| 4:00 – 4:15 | **O-17:** Martha Mullally - University of Ottawa  
Pharmacology of *Souroubea sympetala*: Evidence for interaction with the GABAa Benzodiazepine Receptor |
| 4:15 – 4:30 | **O-18:** Ammar Saleem - University of Ottawa  
The role of analytical techniques in Natural Product metabolite profiling |
| 4:30 – 4:45 | **O-19:** Cory Harris - McGill University  
Non-specific effects in Traditional Medicine: the power of 'placebo' |
| 5:00 – 6:00 | Nana Bafi Yeboa - Natural Health Product Directorate  
Graduate Student Workshop on NHP regulations (Everyone welcome!) |
5:00 – 6:00  CICMR Annual Meeting (open) Lamoureux Hall, Room 121

Evening Sessions

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<thead>
<tr>
<th>Time (pm)</th>
<th>Description</th>
<th>Location</th>
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<tr>
<td>6:00 – 7:00</td>
<td>Dinner</td>
<td>Jazzy Café, University Centre</td>
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<tr>
<td>7:00 – 9:00</td>
<td>Poster Presentations (coffee and dessert)</td>
<td>Lamoureux Hall (hallway)</td>
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</table>

List of Posters (alphabetic by presenter- and poster # grouped by subject)

P1-P14 Pharmacology and Health, P15-P25 Ethnobotany, Production and Phytochemistry

*Barnes et al.* Effects of North American Ginseng on Sexual Behavior and Erectile Function in Healthy Rats  **P1**

*Carruthers et al.* North American Ginseng Reduces Expression of Vascular Cell Adhesion Molecule-1 and Intercellular Adhesion Molecule-1 *in vitro* **P2**

*Cayer et al.* Anxiolytic Properties of *Souroubea Sympetala* **P3**

*Chung et al.* The Status if Cultivated Mountain Ginseng Management in South Korea  **P15**

*Guerrero-Analco et al.* Development and Validation of a Novel Analytical Method by LPLC-ELSD for Identification and Quantitation of Monosaccharides from the Polysaccharides Fraction in Roots of American Ginseng  **P16**

*Guo et al.* Ginseng Inhibits Cardiomyocyte Hypertrophy and Heart Failure *via* NHE-1 Inhibition and Attenuation of Calcineurin Activation  **P4**

*Guo et al.* High Mass Resolution Profiling of Ginsenosides in Plant Extracts  **P17**

*Hou et al.* NA Ginseng extracts protect against vascular injury induced by chronic Homocysteine treatment in rats.  **P5**

*Harbilas et al.* Treatment of Obesity and Diabetes by a Canadian Aboriginal Medicinal Plant in a Mouse Model of Diet-Induced Obesity  **P6**

*Kong et al.* Long Term Treatment of Astragalin Extract Enhances Sensitivity of Mouse Hepatoma Cells to Chemo and Radiation Therapies  **P7**
Li et al. Homocysteine Induces Bone Morphogenetic Protein-13 Expression in Rat Hepatic Stellate Cells P8

Liu et al. The Effect of Anti-Diabetic Traditional Chinese Medicine on Repaglinide Metabolism P9

Lui et al. Ginseng and In Vitro Inhibition of CYP3A4-Mediated Drug Metabolism P10

McIntyre et al. Analysis of Malonyl Ginsenosides in Ontario Ginseng (Panax quinquefolius) using LC/MS/MS P18

Migchels et al. Chronic North American Ginseng Administration Alters Arterial Pressure and Metabolic Variables in the Rat P11

Mollick et al An ethnopharmacological survey of Daulatdia Ghat area, Kushtia district, Bangladesh used for treatment of “hard to cure” diseases P19

Mollick et al Studies of development of technologies for processing and physicochemical screening of plants collected from the Lawacherra Rain Forest of Bangladesh used as remedy for diabetes mellitus P20

Moy et al. LC-MS/MS Study Of Ginseng Metabolism P21

Ning et al. Health Care Providers’ Perspectives on Standards of Evidence in TCM Practice and Ginseng Use P12

Rapinski et al. Latitudinal Variation of Phytochemical Compounds in Labrador Tea, Rhododendron Groenlandicum, and Pitcher Plant, Sarracenia Purpurea P22

Salarian et al. An Injectable Angiogenic Poly(Propylene Fumarate)/TiO₂ Based Bone Cement P13


Shang et al. Bioassay-Guided Identification of Anti-Diabetic Principles of AD03, a plant from the Eeyou Istchee Cree First Nations of Northern Quebec P24

Singh et al. Effects of American Ginseng on Intestinal Lipid Secretion and Plasma Clearance in PCYT2 Deficient Mice P14

Ta et al. Inhibition of Bacterial Quorum Sensing (QS) by Tropical and Anti-Infective Plants P25

Walshe-Roussel et al. Ethnobotany and Pharmacology of Anti-Inflammatory Botanicals used by the Q’eqchi’ Maya of Belize P27

Zhou et al. Improvement of a Micropropagation Protocol for North American Ginseng (Panax Quinquefolius L.) and Field Performance of Clonal Plants

Zou et al. Screening for Pesticide Residues and Mycotoxins in Ginseng Roots using High Performance Liquid Chromatography-Tandem Mass Spectrometry
**Monday, October 25, 2010**

**Symposium III (continued): Pharmacology and Health Benefits of Traditional Medicines**
Lamoureux Hall, Rm. 122  Co-Chairs: K. Rogers and Q. Feng

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<thead>
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<th>Time (am)</th>
<th>Speaker &amp; Title</th>
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<tr>
<td>8:00 – 8:30</td>
<td>Registration</td>
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<tr>
<td>8:30 – 9:00</td>
<td><strong>O-20:</strong> Ming Zhu – University of Chinese Medicine and Pharmacology, Beijing</td>
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<td>&quot;Historical perspective of integration of NA ginseng in TCM&quot;</td>
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<tr>
<td>9:00 – 9:30</td>
<td><strong>O-21:</strong> Robin Marles – Bureau of Clinical Trials and Health Sciences, Natural Health Product Directorate</td>
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<td>&quot;Clinical trials for Traditional Chinese Medicines&quot;</td>
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<td>9:30 – 9:55</td>
<td><strong>O-22:</strong> Lique Coolen – University of Western Ontario</td>
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<td>&quot;Ginseng effects on erectile dysfunction&quot;</td>
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<td>9:55 – 10:20</td>
<td><strong>O-23:</strong> Morris Karmazyn – University of Western Ontario</td>
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<td>&quot;The Sodium-Hydrogen Exchange Calcineurin Pathway: A Target for the Antihypertrophic Effects of Ginseng</td>
</tr>
<tr>
<td>10:20 – 10:35</td>
<td>Break</td>
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<tr>
<td>10:35 – 11:00</td>
<td><strong>O-24:</strong> Kari Kramp – Loyalist College</td>
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<td>&quot;Supercritical CO₂ Extraction of Select Natural Products&quot;</td>
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<td>11:00 – 11:30</td>
<td><strong>O-25:</strong> Junzeng Zhang – National Research Council Institute for Nutrisciences &amp; Health</td>
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<tr>
<td></td>
<td>&quot;Bioactive components from dietary Chinese herbs&quot;</td>
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<tr>
<td>11:30 – 12:00</td>
<td>Closing Remarks, Photo and Adjournment</td>
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Professor Emeritus

Patrick Choy

B.Sc. (McGill); M.Sc., Ph.D. (North Dakota); M.D. (Hons.) (McGill); F.A.H.A.; F.I.A.C.S.

Department of Biochemistry and Medical Genetics

A distinguished scientist who is an international leader in the study of heart disease, Dr. Patrick Choy joined the University of Manitoba in 1979 as a researcher and professor in the department of biochemistry and medical genetics. He served as head of the department from 1992 to 1999 and as Associate Dean of Research in the Faculty of Medicine from 1999 to 2009. Dr. Choy is currently the new Associate Dean of Development in the Faculty of Medicine. An internationally recognized leader in the study of cardiovascular phospholipids and lipoproteins, Dr. Choy's research established novel connections between lipid metabolism, cardiac arrhythmias and atherosclerosis. His work has been supported by the Canadian Institute for Health Research for 30 years. Dr. Choy established the Centre for Research and Treatment of Atherosclerosis and has served as President of the Canadian Biochemical Society and Vice-President of the Heart and Stroke Foundation of Manitoba.

Thursday, May 13, Faculty of Medicine session
Dr. Roman Szumski

In 2005, Dr. Roman Szumski was appointed the National Research Council's Vice-President, Life Sciences.

A medical doctor and pathologist by training, Dr. Szumski is recognized as a visionary leader and an innovative manager with unique experience in building strategic public-private sector partnerships in the life sciences sector. He was the founding CEO of Calgary Laboratory Services, and more recently Vice-President (Science & Technology) of MDS Inc.

During his years at MDS headquarters, Dr. Szumski held executive-level responsibility for scientific assets of the firm, with more than 10,000 employees, $1.8 billion in annual sales and diverse international business interests. While at MDS, he also led the development of new business initiatives in cancer therapeutics and personalized medicine.

As founding CEO of Calgary Laboratory Services and as President of a private firm, Dr. Szumski championed and built a new collaboration between the Calgary Regional Health Authority and the private sector that facilitated major improvements in service and efficiency in the services offered through seven public and private sector labs. His career at Calgary Medical Laboratories included the roles of pathologist, director of microbiology, and medical director. He also worked in the Department of Pathology at the University of Calgary.

Dr. Szumski is a Fellow of the Royal College of Physicians and Surgeons of Canada, and he holds degrees from Queen's and McGill universities.
Tommy Cheng

My interests are in the development of new drugs and the improvement of the use of clinically proven drugs for the treatment of cancer, and herpes virus, human immunodeficiency virus or hepatitis B virus associated diseases. The types of agents are deoxyribonucleoside analogs, natural products that interfere with DNA and RNA metabolism. Currently we are also interested in the potential uses of Chinese medicines.

There are three (3) drugs currently used in clinic being discovered in this laboratory. This includes: 1) Gancyclovir – For Cytomegaloviral infection; 2) Lamivudine – For Hepatitis B Virus infection; and 3) Clevudine – For Hepatitis B Virus infection. Three additional chemicals and one Chinese medicine formula discovered are currently at different stages of Clinical Trial for the treatment of cancer as well as HIV and HBV infection.

O-1: Potential Bottleneck and Approach to Develop Future Medicine Based on Traditional Chinese Medicine

Tommy Cheng, Yale School of Medicine

The medicines needed today are no longer just for therapeutic purposes but also for the prevention of diseases and improvement for quality of life. The reductive approach with attempts to develop single chemical medicines is not sufficient, particularly for complicated and age-associated diseases such as neuro-degenerative diseases, metabolic diseases, autoimmune diseases and cancer since the etiological factors for the same diseases among different individuals could be different. The holistic/integrative approach using system biology concepts having polychemicals should be considered. Traditional Chinese medicine, has those characteristics and claims for treatment and prevention of symptoms as well as improving the quality of life. It could provide for today’s unmet medical needs and as the basis for development of future medicine.

In order to develop this new paradigm, it is critical to ensure the quality and consistency of TCM products, provide clinical evidence to substantiate the claims, understand of the mechanisms of action as well as the interaction with currently used drugs. Strategies to address all the issues will be discussed based on my laboratory experience in the exploration of PHY906, a TCM formula, as adjuvant therapy for the treatment of patients undergoing chemotherapy. Collaborations among academia, government and industry around the world are critically important to facilitate the development of this new paradigm.
Priti Krishna

Priti Krishna is a professor in the Department of Biology at the University of Western Ontario. Although she did her PhD in medical biochemistry from the University of Calgary, she switched to using plants as model systems for molecular biology during her postdoctoral training at the University of Minnesota, USA. It was then that she was awarded an NSERC faculty award and with this award she joined UWO in 1992 as an Assistant professor. In the last 18 years, her lab has generated results that lead the way in understanding the roles of hsp90 in development and stress responses in plants, and the role of plant steroids, termed as brassinosteroids in stress tolerance. She is recognized as the leader in the brassinosteroids-mediated stress tolerance area. Being intrigued by the nutritional benefits of sea buckthorn and the novelty of applying genomic technologies to this plant to harness new information, she initiated a project on sea buckthorn in 2009.

O-2: Transcriptome analysis of polyunsaturated fatty acid-enriched seed of sea buckthorn (Hippophae rhamnoides L.) using 454 high-throughput sequencing

T. Fatima1, WR. Schroeder2, R. Weselake3, and P. Krishna1*

1Department of Biology, University of Western Ontario, London, ON, N6A 5B7; 2Agriculture and Agri-Food Canada, SK, S0G 2K0, 3University of Alberta, Edmonton, AB T6G 2P5, Canada

Sea buckthorn (Hippophae rhamnoides L.) is a hardy, fruit producing plant known historically for its medicinal and nutraceutical properties. The most recognized product of sea buckthorn is its fruit oil, comprised of seed oil that is enriched in essential fatty acids linoleic (ω-6) and α-linolenic (ω-3) acids, and pulp oil that contains high levels of palmitoleic acid. Since sea buckthorn is fast gaining popularity as a source of functional food and nutraceuticals, but currently has very limited information at the gene level, we determined its seed transcriptome using the 454 GS FLX Titanium sequencing technology, as well as the fatty acid composition of four Canadian grown cultivars (ssp Mongolica).

GC-MS and NMR profiling of fatty acids in seeds and pulp of berries collected from the Canadian grown cultivars indicated that the seed oil was comprised of linoleic and α-linolenic acids at 33-34% and 46-49%, respectively, while the pulp oil was comprised of palmitoleic acid at 30-40%. 454 sequencing of sea buckthorn cDNA collections from mature seeds yielded 500,392 sequence reads, which assembled into 37,482 contigs, 51,659 singletons and 89,141 putative unigenes. Functional annotation by Gene Ontology and computational prediction of metabolic pathways indicated primary metabolism and fatty acid and lipid biosynthesis pathways as highly represented categories. Sea buckthorn sequences related to fatty acid biosynthesis genes in Arabidopsis were identified and a subset of these was examined for transcript expression at four developing stages of the berry. This study provides the first comprehensive genome sequences for sea buckthorn, while also confirming the unique composition of sea buckthorn oil in the Canadian grown cultivars. These data will catalyze further studies on sea buckthorn oil and enzymes involved in its biosynthesis, as well as on the genes involved in the general hardiness of sea buckthorn against environmental conditions.
Dan Brown

Dan Brown is a research scientist with Agriculture and Agri-Food Canada, based in London Ontario as well as an Adjunct Professor with the Biology Department at the University of Western Ontario, London, Ontario, Canada and serves as the Associate Director of the Ontario Ginseng Innovation and Research Consortium. He has extensive experience in developmental plant physiology and the application of plant biotechnology in a range of crop plants including alfalfa, soybean, wheat, tobacco, plum, peach, feverfew and North American ginseng.

O-3: North American Ginseng Germplasm Characterization and Development

Daniel C.W. Brown¹*, Sijun Zhou², Chris Siow⁴, Ed Lui², John T Arnason³ and Kristina L. McIntyre³

¹Agriculture and Agri-food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford Street, London, Ontario, Canada N5V 4T3; ²Ontario Ginseng Innovation and Research Centre, University of Western Ontario, Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, London, Ontario, Canada N6A 5C1; ³Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5; ⁴Agriculture and Agri-food Canada, Canadian Centre for Agri-Food research in health and Medicine, St Boniface Hospital, Winnipeg, Manitoba R2H 2A6

North American ginseng (Panax quinguefolius L.) is genetically heterogeneous and despite its long history of cultural use and declared wide range of pharmacological activities associated with specific extracts or ginsenosides, the active constituents of ginseng and evidence supporting its traditional health claims have been inconsistent. An initiative now under way to exploit biotechnology to establish cultivars for the production of Ontario ginseng with predictable quality, safety, and medicinal properties consists of a three-pronged approach: i) selection of genetic stocks with documented agronomic and phytochemical properties, ii) constituent and genomic analyses of the plant materials and, iii) screening and correlations for pharmacological activities in vitro and in vivo. This information and the use of newly developed micropropagation and genetic characterization techniques is the basis of the accelerated development of “cultivars” for the production of Ontario-grown ginseng and its branding based on unique genomic, phytochemical and pharmacological characteristics. An efficient six-step in vitro tissue culture system has been developed which can produce large numbers of genetically identical (clonal) plants in about 24 weeks. Field grown and clonal lines have been analyzed for their respective major ginsenoside contents and individual lines show considerable variability in ginsenoside content and ratios as well as other measured constituents. Both seed-derived and micropropagated lines show similar field performance and constituent variability and appear to have the basis for developing superior commercial lines.
Alain Cuerrier

Alain Cuerrier is an ethnobotanist and plant taxonomist involved with the First Nations of Eastern Canada. He holds a research position at the Montreal Botanical Garden and Plant Biology Research Institute (University of Montreal). He is a member of the CIHR Traditional Aboriginal Antidiabetic Medicines Team. His works encompass antidiabetic traditional medicines, impact assessment of harvesting medicinal plants. Earlier works have pertained to Inuit traditional medicine (TM). Alain is vice-president of the Natural Health Product Research Society of Canada and Regional Representative for North America for the International Society of Ethnobiology. He is helping First Nations and their TM to be recognised.

O-4: *Rhodiola rosea*: From Ethnobotany to Taxonomy Using Molecular Tools

Alain Cuerrier*, Mariannick Archambault, and Anne Bruneau

Jardin botanique de Montréal, Institut de recherche en biologie végétale

Member of the family Crassulaceae, *Rhodiola rosea* L. has an Amphi-Atlantic distribution. In Canada, it is located on the East coast of Ungava Bay as well as coastal Labrador, Newfoundland, Nova Scotia and New Brunswick. Used by the Inuit and other First Nations for its diverse properties, this adaptogenic plants is now becoming well-known and used in the CAM sector. After a brief account of its uses by First Nations, especially by the Nunavimmiut, we expand the ethnobotany to other species, including *R. integrifolia* distributed in the western part of Canada, with which there is still some taxonomical confusion. Russian researchers as well as NHP industries have been advocating the use of Siberian *Rhodiola* as the only good *R. rosea* on the market. In order to understand whether Canadian populations differ phytochemically and genetically to Eurasian populations, we have collected and analysed samples from a number of sites in Canada and elsewhere. Based upon DNA sequences (*trn*L-F and ITS), we were able to pinpoint that Canadian populations are taxonomically very close to Eurasian ones, although the presence of two duplications found in *trn*L-F suggest a closer relationship between North American and North Scandinavian populations than with Alpine Eurasian populations, which lack duplications. AFLPs were also used for identification and to establish relationships among populations at different geographical scales. We found a positive and significant correlation between geographic and genetic distances. Phytochemical studies support the relationship between *R. rosea* of Siberian and Canadian origins, although there are discrepancies.
Anthony Windust

Anthony Windust graduated with a Ph.D. from Dalhousie University in 1998. Until 2001 he was a NSERC postdoctoral fellow with Ocean Nutrition Canada where he conducted research and development on novel lipid antioxidants and marine Natural Health Products (NHPs). In 2001 he joined the National Research Council Canada to work on the Certified Reference Materials Program for microalgal toxins at the Institute for Marine Biosciences in Halifax. In 2003 he transferred to the Institute for National Measurement Standards in Ottawa to work on reference materials directed at NHPs. His current focus is on American ginseng and the biosynthetic production of $^{13}$C labelled polyunsaturated fatty acids as research tools.

O-5: Analytical standards for ginseng and other natural health products

Anthony Windust


Natural Health Products (NHPs) typically yield numerous, potentially active, compounds with complex and diverse chemical structures. This presents a considerable challenge to the development of reference materials for instrument calibration and research purposes. The Institute for National Measurement Standards is currently producing calibration standards directed at several NHPs important to the Canadian economy: North American ginseng (*Panax quinquefolius* L.), goldenseal (*Hydrastis canadensis* L.) and isotopically labelled long chain polyunsaturated fatty acids including EPA and DHA. When the compounds of interest can not be readily obtained from commercial sources, semi-synthetic, biosynthetic, and natural product isolation routes may be employed. Isolation and purification is primarily done using several approaches including, open column chromatography, preparative HPLC and crystallization. Confirmation of identity is done by spectroscopic methods including 1D and 2D NMR and precision mass measurements.

The determination of the mass fraction content of the candidate reference compounds is a crucial step prior to the preparation of certified reference material solutions. This is done using quantitative $^1$H-NMR (qNMR) using either internal or external standards representing a "fit for purpose", primary ratio method, traceable to the SI and further supported by other analytical techniques including GC-FID/MS, LC-UV/MS, Karl Fisher titration and TGA. Typically, mass fractions can be assigned with relative standard uncertainties of 1% or less.
Sharla Sutherland

Sharla Sutherland is the Vice President of Scientific and Regulatory Affairs at Afexa Life Sciences (formerly CV Technologies, Inc.), a Canadian company, headquartered in Edmonton, which develops evidence-based naturally-derived therapeutics for disease prevention and recovery. She has been an instrumental part of both the research and commercialization efforts behind the success of Canada's #1 selling cold and flu product, COLD-FX®. Sharla completed her Ph.D. in Physiology at the University of Alberta. She has received numerous academic awards including from the National Research Council (NRC), Natural Sciences and Engineering Research Council of Canada (NSERC), and the Alberta Heritage Foundation for Medical Research (AHFMR). Following her Ph.D. Dr. Sutherland worked in Montreal as a medical writer in the field of preventative medicine, drug metabolism, nutrition, and pharmacogenomics. She currently heads Afexa’s clinical, regulatory, quality, and general scientific affairs and awareness programs. She has managed numerous research projects, including one of the largest Canadian natural health product clinical trials - a multi-centre study of 780 Canadians which demonstrated added benefits of COLD-FX in an influenza vaccinated population. She has lead a department to achieve rare and novel regulatory approvals for key products and serves on various academic and government committees including the Expert Advisory Panel for the Ontario Ginseng Growers Association and the BioFutures initiative. Sharla is a Fellow of the International College of Nutrition and a member of the Regulatory Affairs Professionals Society, the American Society of Quality, and the Society of Clinical Research Associates.

O-6: Botanical Drug Discovery From Traditional Ingredients – Polymolecular Therapeutic Opportunities

Sharla K. Sutherland*, Christine Lutsiak, Lei Ling, and Jacqueline Shan; Afexa Life Sciences Inc.

Development of proprietary ingredients from traditionally used herbal sources into polymolecular botanical drug products with therapeutic claims, supported with the highest levels of evidence, is the major focus of Afexa product development. The therapeutic claim ‘Helps reduce the frequency, severity, and duration of cold and flu symptoms by boosting the immune system’ was issued to Afexa’s lead product – COLD-FX – based on randomized, double-blind, placebo-controlled trials. Additional therapeutic indications are being explored for the active ingredient in COLD-FX (CVT-E002). Pre-clinical studies demonstrated that CVT-E002 acts through toll-like receptors 2, 4, 1/2, and 2/6 in a MyD88-dependent manner. Activation of these TLRs initiates production of Th1-type cytokines. It is hypothesized that CVT-E002, by shifting the immune response to Th1-type, could treat/prevent development of allergies and asthma. In an OVA-sensitized animal model, CVT-E002 was shown to decrease airway responsiveness and inflammation. A double-blind placebo-controlled clinical trial is in progress to evaluate the therapeutic potential of CVT-E002 in seasonal allergic rhinitis. Similar product development activities with multiple polymolecular drug candidates are on-going in other therapeutic areas, including cancer (chronic lymphocytic leukemia) and lipid management. Products of this nature represent an uncommon subset of NHPs and a regulatory “grey zone”. The Natural Health Products Directorate (NHPD) reviews all Natural Health Products (NHPs) for sale in Canada and issues a license (Natural Product Number- NPN) to products that meet regulatory standards for quality, efficacy, and safety. The issuance of an non-traditional NPN is linked to one of several types of allowed health claims: structure-function claims, risk-reduction claims, and therapeutic claims with the type of claim allowed depending on the evidence provided. Evidence-based patentable polymolecular botanical drugs for therapeutic indications represent a major opportunity for Canadian medical innovation. As such, regulatory policy and practices may play a key role in promoting development of products of this nature. Support of clinical development in therapeutic reas and product licencing approaches which encourage novel proprietary products with unique research is needed to enable growth of this field.
Kan He

Kan He is currently a Director of Research and Development for Naturex/PureWorld. He is responsible for new product and new process development, including plant extraction, purification, and chemical characterization of standardized herbal extracts. He is the author and coauthor of over 60 peer-reviewed papers and holds nine US patents on the herbal product and process development. He earned his Ph.D. from the University of Arizona in pharmaceutical sciences, master and bachelor degrees from the Shanghai University of Traditional Chinese Medicine. He completed his postdoctoral research program at Purdue University before he joined Naturex/PureWorld.

O-7: Developing Herbal Products for the Dietary Supplement Market; Example of FraxiPure™

Kan He*, Naisheng Bai, Alvin Ibarra, Antoine Bily, Marc Roller

Naturex, Inc.

In the United States, annual retail sales of botanical products rose from a meager $200 million in 1988 to $5 billion in 2009. Herbal products are categorized as dietary supplements in the Dietary Supplement Health and Education Act (DSHEA). Under DSHEA law, the products are produced now with the assurance of compliance of cGMP for all companies involved in manufacturing and distributing nutrition supplements. However, there is no premarket review required for these products. The burden of proof for product safety lies on the FDA, rather than on the manufacturer, thus, it is relative easy to get herbal products into the US marketplace. Whether a product is sustainable in the market depends on its safety, quality, and efficacy, which are always the important criteria during new product development. The interests of using natural products for medical purpose are continuously growing. Meanwhile, the demands for more safety and high quality products, as well as more rigorous regulatory requirements place both challenges and opportunities for developing new herbal products. Steps and results of developing herbal products, FraxiPure™, an extract of *Fraxinus excelsior* (FE) seed are illustrated and discussed. FE have been used as hypoglycemic agents in Morocco. In vitro screening found that FE inhibited adipocyte differentiation in 3T3-L1 and activated PPARα reporter cell systems. FE exhibited the activities of reducing bodyweight and decreasing blood glucose in male C57BL/6J mice. In a preliminary clinical assessment, FE lowered the incremental postprandial plasma glucose concentration as compared to placebo.
Jim Todd & Sean Westerveld

Sean Westerveld holds a B.Sc. (Agr.), M.Sc. and Ph.D. in Horticultural Science from the University of Guelph. After leading the Vegetable and Non-Traditional Crops Research Program at the University, Sean joined OMAFRA in 2008 to support the ginseng and herb industries. He is also an adjunct professor in the Dept. of Plant Agriculture at the University of Guelph.

Jim Todd holds a BSc from the University of Waterloo and a Ph.D. from the old horticulture department at the University of Guelph. A series of post-doctoral positions ultimately led to a position at Monsanto in St. Louis. While there, he worked on projects ranging from large scale gene expression analysis to methods to increase the protein content of corn seed. In 2005, Jim started as OMAFRA’s transition crop specialist where he works on identifying crops with potential to serve as agricultural feedstocks for Ontario’s developing bioeconomy.

O-8: Production of Non-Traditional Crops for the Health Market

Sean Westerveld and Jim Todd

Ginseng and Medicinal Herbs Specialist, OMAFRA; JT: Transition Crops Specialist, OMAFRA

Ontario has a suitable climate to grow a wide range of medicinal plants such as ginseng and Echinacea, nutraceuticals such as seabuckthorn and goji, and industrial plants for pharmaceutical uses such as daffodils and tobacco. While there are well established industries for a few of the medicinal plants including ginseng, goldenseal and Echinacea, there is potential for expansion of the industry for most crops in this sector. Growers of non-traditional crops deal with many production challenges including lack of local knowledge of agronomic practices and few resources to deal with pest issues. However, the largest barrier to growth of these crops is the lack of a secure market. Due to limited market size, small changes in acreage of many of these crops can result in large fluctuations in price. Improving the linkages between the healthcare industry and growers could provide more stability in the marketplace, more traceability through the value chain, and more consistent product quality and supply. Production and marketing research is required for many of these crops, and industry support is often necessary to secure research funding. There is also a need to inform consumers of the agricultural origin of medicinal herbs and supplements so the industry can benefit from the buy-local and organic movements.
Ping Li

O-9: Effect of Tangshen Formula on Type 2 Diabetic Nephropathy patients and study of its action mechanisms by system biology

Ping Li

Institute of Clinical Medical Science, China-Japan Friendship Hospital, Beijing 100029, China

Objective: To evaluate the efficacy and safety of Chinese herbal medicine Tangshen formula (TSF) in treatment of type 2 diabetic kidney disease (DKD). Methods: Stratified blocked randomization method was adopted to allocate 192 seeds into the treatment arm and placebo arm, patients number ratio 2:1, with allocation concealment conducted in the drug dispensing procedure. 181 patients with type 2 DKD were enrolled in the trial. After a 2-week of run-in period, during which patients’ blood pressure was controlled under 140/90mmHg and their fasting blood glucose under 7.8mmol/L, then patients were randomized to receive 6 months of treatment with TSF(n=122) (16g daily ) or placebo (n=59), in addition to basic western medicine treatment. The primary outcomes were urinary albumin excretion rate (UAER) for microalbuminuric patients and 24-hour urinary protein (24h-Upro) for macroalbuminuric patients, measured every 3 months. Results: The baseline characteristics of the two groups were comparable. In the microalbuminuric cohort, repeated measures data analysis revealed that there was significant reduction of UAER in TSF group (compared with placebo group, P=0.0323 ). In the macroalbuminuric cohort, 24h-Upro was significantly decreased in treatment group (compared with placebo group, P=0.0153). There was a reduction of serum creatinine level (P=0.0192), and elevation of eGFR (P=0.0216) in the treatment group compared with placebo. There was significant elevation of HDL in patients with macroalbuminuria (P=0.0107) in the treatment group compared with placebo. No adverse effect was found in the trial. Conclusion: TSF may have beneficial effects in patients with DKD who are receiving recommended therapy, and there is synergistic effect of TSF with western medicaiton. However, longer periods of follow-up with endpoint outcomes evaluation are still needed.
Lina Musallam

Lina Musallam is the research coordinator for the **CIHR Team in Aboriginal Antidiabetic Medicines (TAAM)** as well as for the laboratory Dr Pierre Haddad, the Team’s leader. She obtained a BSc in Biology from the Université de Montréal in 1997 and a PhD in Pharmacology from the same university in 2003. She then carried out a 4-year postdoctoral fellowship at the Molecular Oncology Group at McGill University, Montreal. From 2008-2009, she worked as a research consultant for the Quebec Ministry of Health to develop practice guidelines for oncologists about established and emerging therapies. She joined the **CIHR TAAM** in early 2010 and coordinates a cohesive research effort involving seven laboratories in three major Canadian universities to rigorously assess the antidiabetic potential of plants stemming from the Canadian aboriginal traditional pharmacopeia.

O-10: Anti-diabetic action of Cree traditional medicines

Lina Musallam and Pierre Haddad

Department of Pharmacology, Université de Montréal, CIHR Team in Aboriginal Antidiabetic Medicines

Obesity and Type 2 diabetes are considered global epidemics by WHO. Aboriginals such as the Cree of Eeyou Istchee (James Bay area of northern Quebec) are particularly affected. A multidisciplinary team was therefore put together to explore the antidiabetic potential of Cree Traditional Medicine (TM) involving Boreal forest plants. The team is composed equally of scientists as well as Cree Elders and members of various Cree health institutions. A novel ethnobotanical approach based on diabetes symptoms was used to identify potential antidiabetic plants, and a total of 17 species were characterized phytochemically. Each species was screened for primary antidiabetic activity using *in vitro* bioassays such as glucose transport, adipogenesis and hepatic glucose production Secondary antidiabetic activity screening included pro- or anti-inflammatory, anti-oxidant, anti-glycation and neuro-protective activities. Toxicological potential was assessed on recombinant cytochrome P450 isoforms. Ten plant extracts increased glucose transport in muscle cells and adipocytes while exhibiting weak to moderate inhibition of CYPs. Detailed studies revealed that these promising species exert their effect through Metformin-like mechanism. For several of these species, active principles have been identified using bioassay-guided fractionation. Bioavailability, antihyperglycemic and/or anti-obesity efficacy has been confirmed for 6 plants using in vivo animal models of obesity, insulin resistance or diabetes. Clinical studies are also underway to document the safety and efficacy of Cree TM using a culturally-adapted, all-inclusive, observational protocol. Finally, our project represents a pilot study for the integration of Cree TM into diabetes care for the Cree Health Board. Funded by the Canadian Institutes of Health Research.
Brian Foster

Brian Foster (Ph.D. Medicinal Chemistry, University of Alberta) is a Senior Science Advisor in the Office of Science, Therapeutic Products Directorate, Health Canada and an Adjunct Professor, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa. His research interests include alternative models for drug interactions and disposition. Since joining Health Canada, his research has been in the area of drug metabolism, pharmacogenetics, and how natural health products or other xenobiotics affect the safety and efficacy of conventional therapeutic products. He currently has 4 graduate students in a joint Health Canada - University of Ottawa laboratory.

O-11: Herb-Drug Interactions

Brian C. Foster
Health Canada and the University of Ottawa

Traditional use of most natural health products (NHPs) has proven safety, but their modern/current pattern of consumption in the global context has changed. Anecdotal and published reports suggest that NHPs can affect drug disposition with interactions occurring with concomitant use of drugs and other health products which compete for the same active sites, or shunt the products and their metabolites through alternative pathways. Roughly 95% of the 450+ NHPs such as goldenseal, St Johns wort, valerian root, garlic, ginger, red wines, many beers, some leisure and medicinal teas, and Echinacea markedly inhibited one or more P450-mediated reactions. Many NHPs also strongly affected P-glycoprotein-mediated transport. Although SJW and fresh garlic extracts stimulated the sensitivity of some antibiotics in the cultures tested, other antibiotics were less effective. Some products, on prolonged administration, were found to have a time and concentration dependant effect similar to grapefruit. This presentation will examine the potential of NHPs to affect the metabolism of human cytochrome P450 isoforms in vitro and how to relate these findings to ascertain the potential risk of generating clinically important adverse interactions. Findings here and elsewhere indicate that NHPs can affect more than one P450 isozyme and that clinically relevant interactions either adversely or positively affecting the safety and efficacy of health products are possible, particularly in populations on polypharmacy or with polymorphisms affecting drug disposition.
Siyaram Pandey

Siyaram Pandey is a professor in the Department of Chemistry & Biochemistry at the University of Windsor. He received his MSc from Banaras Hindu University (1984), Varansi, India and his PhD from Jawaharlal Nehru University/CCMB (1992), New Delh. He did is postdoctoral training at McGill and joined NRC, Ottawa as a research officer (1993-2000). He joined the University of Windsor in 2000. Dr. Pandey’s research is focused on apoptosis (cell suicide), which is central to various aspects of human health including neurodegeneration, stroke and cancer. He has been using various natural extracts and compounds in cellular and animal models of cancers.

O-12: Non-toxic natural compounds as selective inducers of apoptosis in cancer cells

Siyaram Pandey, Carly Griffin-Moyusik, Pamela Ovadje, Dennis Ma, Philip Tromblay, and Pardis Akabary

Department of Chemistry and Biochemistry, University of Windsor, Windsor ON

Exploration of natural compounds that could selectively target cancer cells may provide an alternative to genotoxic chemotherapies. We have shown that Pancratistatin (PST), a natural compound isolated from the Hawaiian spider lily by Petit et al. in 1992, induces apoptosis in numerous human cancer cell lines, including colon, breast, leukemia and melanoma, with no toxicity to non-cancerous cells. We have demonstrated the non-genotoxic nature of PST; it kills cancer cells without targeting their DNA. Although the target of PST remains unknown, our results show that it causes increased production of reactive oxygen species (ROS) and decreased generation of ATP, suggesting that PST targets cancer cell mitochondria. Moreover, we have reported that PST acts synergistically with estrogen-receptor (ER) antagonist Tamoxifen to destabilize the mitochondria in ER negative and ER positive cancer cells, which leads to increased apoptosis. In parallel to PST, we have also demonstrated that aqueous dandelion root extract (DRE) induced receptor mediated cell death in human leukemia (Jurkat), chronic monocytic myeloblast leukemia (CMML) cells. Furthermore, DRE and curcumin both were shown to effectively induce apoptosis in aggressive cancer cell lines that we tested, such as melanoma, pancreatic cancer and osteosarcoma. Interestingly, the growth and survival rate of non-cancerous cells exposed to either DRE or curcumin was unaffected. Our results indicate the very high potential of natural extracts and compounds for targeted treatment of a multitude of carcinomas in human.
O-13: Ginsenosides from *Panax quinquefolius* (North American ginseng) attenuate leptin-induced neonatal ventricular cardiac hypertrophy though inhibition of the RhoA/ROCK and associated MAPK pathways

Melissa Moey*, Venkatesh Rajapurohitam, Asad Zeidan, Juan Guo and Morris Karmazyn

**Introduction:** Leptin, a product of the obesity gene, has been shown to be a contributing factor in cardiac hypertrophy through the activation of the small G-protein RhoA/ROCK and MAPK pathways. Although the mechanism of RhoA activation by leptin is still not clearly defined, evidence from our preliminary studies suggest the involvement of guanine nucleotide exchange factors (GEFs), small G-proteins regulators, in leptin-induced RhoA activation. Previous research have demonstrated the anti-hypertrophic effects of ginsenosides, the active constituents of ginseng, however their effects in leptin-induced cardiac hypertrophy have yet to be determined. In this study, the effects of total ginsenosides from North American ginseng in leptin-induced cardiac hypertrophy and the molecular mechanisms involved were investigated. **Results:** Treatment of neonatal ventricular cardiomyocytes with leptin (3.1nM) increased cell size and expression of gene markers of hypertrophy by 50% (p<0.05), which was significantly attenuated with pre-treatment of ginsenosides. Leptin-induced cells exhibited an increase in p38 phosphorylation (50%; p<0.05) and nuclear translocation (25%; p<0.05), which were abolished by ginsenosides. Real-time polymerase chain reaction analysis also showed that ginsenosides significantly abolished the 4-fold increase (p<0.05) of p115RhoGEF gene expression in leptin-induced cells. RhoGEF co-localization with RhoA and translocation to the membrane leptin-induced cells was similarly attenuated by ginsenosides. Leptin-induced RhoA/ROCK pathway activation as seen by increased RhoA-GTP (20%; p<0.05), coflin-2 phosphorylation (25%; p<0.05) and decreased G/F actin, which were all inhibited by ginsenosides. **Conclusion:** Ginsenosides are an effective treatment of leptin-induced cardiac hypertrophy by inhibition of the MAPK pathways and p115RhoGEF activation of the RhoA/ROCK signaling cascade.

O-14: Ginseng protects the heart from ischemia and reperfusion injury by activating PI3K/Akt-dependent eNOS pathway

Yan Wu*, Xiangru Lu, Fuli Xiang and Qingping Feng

Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

Ginseng has been shown to have cardioprotective effects. However, molecular mechanism responsible for its cardioprotection is not fully understood. We have demonstrated that endothelial nitric oxide synthase (eNOS) mediates cardioprotective effects during myocardial ischemia and reperfusion (I/R). The present study was to test the hypothesis that ginseng protects the heart from I/R injury via activation of PI3K/Akt/eNOS signaling pathway. Wild-type (WT) and eNOS−/− mice were pretreated with Ginseng root aqueous extract (50 mg/kg/day) by oral gavage or drinking water for one week. Mice were subjected to 45 min of myocardial ischemia followed by 3 hours of reperfusion. Infarct size was assessed by triphenyltetrazolium chloride (TTC) staining. Our results showed that pretreatment with ginseng significantly decreased infarct size after I/R compared with non-treated
mice (31.6±2.1% vs. 49.4±2.4%, P<0.01). However, this effect was abrogated in eNOS−/− mice (P<0.01). To study the role of PI3K/Akt signaling, WT mice were pretreated with ginseng in the presence of a PI3K inhibitor, LY294002. Treatment with LY294002 abolished the effect of ginseng on infarct size reduction in WT mice (P<0.01). To further investigate PI3K/Akt/eNOS signaling, Akt and eNOS phosphorylation was determined by western blot analysis. Pretreatment with ginseng significantly increased Akt and eNOS phosphorylation in the WT mouse myocardium (P<0.01). We conclude that ginseng protects the heart from I/R injury in mice. The cardioprotective effects are mediated by activation of PI3K/Akt/eNOS pathway.

**O-15: The Yin and Yang actions of North American Ginseng root in modulating the immune function of macrophages**

Chike Godwin Azike*, Paul Charpentier, Hou Jirui, Pei Hua and Edmund MK Lui

University of Western Ontario

Previous studies by different investigators have demonstrated immuno-stimulatory and anti-inflammatory (immunosuppressive) effects of ginseng. The objective of this study was to investigate the mechanism underlying these apparent paradoxical effects by examining the immunomodulatory effect of aqueous (AQ) and alcoholic (ALC) extracts prepared from 4 year old Ontario grown North American ginseng roots in RAW 264.7 murine macrophages. Our results showed that AQ extract alone upregulated production of NO and TNF-α while ALC extract has no such effect. On the other hand, ALC extract suppressed LPS-stimulated NO and TNF-α production when given 2hr before LPS challenge, suggesting an acute direct anti-inflammatory action. This effect was not however observed with AQ extract. Indirect anti-inflammatory property was evaluated by incubating macrophage for 24 hr with the extract before LPS challenge; and both types of extracts exerted an indirect anti-inflammatory effect. These data suggest that AQ extract is proinflammatory in nature and it also possess indirect anti-inflammatory effect by reducing the responsiveness of macrophages to inflammatory stimulus. In contrast, ALC extract have both direct and indirect anti-inflammatory effects. The observed immune-stimulatory and anti-inflammatory effects of ginseng can be considered as the yin and yang action of ginseng. Analytical size exclusion chromatography of immunostimulatory crude polysaccharides isolated from AQ extract gave molecular weight of >75,000 with random coil solution conformation. The extract-specific immunomodulatory effects will be discussed in light of the observed differences in phytochemical characteristics and bioactivity of the subfractions following Sephadex G-75 chromatographic fractionation of the ginseng ALC and AQ extracts.
O-16: Selective Induction of Apoptosis through Activation of Caspase-8 in Human Leukemia cells by Dandelion Extract

Ovadje, P.*, Chatterjee S., Griffin C., Tran C., Hamm, C., and Pandey, S.

University of Windsor

Natural compounds have been used as anti-oxidant, anti-inflammatory and anti-viral agents in traditional medicine for centuries to treat various ailments. Substantial research has been done to delineate the medicinal components of natural extracts for clinical use, especially for the field of cancer.

Cancer, responsible for 1 in 4 deaths in Canada, is estimated to have caused over 75,300 deaths in 2009 alone. Most chemotherapeutics used to treat cancer generate severe side effects due to their toxicity to non-cancerous cells. Development of an effective chemotherapy that selectively targets cancer cells is of great importance. Our group is studying the anti-cancer properties of dandelion root extract (DRE), which is currently marketed for management of gastrointestinal and liver disorders. We previously reported that aqueous DRE effectively induces apoptosis in human leukemia (Jurkat) cells in a dose and time dependent manner by rapidly activating the extrinsic pathway of apoptosis. Non-cancerous peripheral blood mononuclear cells (PBMCs) remained unaffected. Chronic MyeloMonocytic Leukemia (CMML) one of the most resistant, non-responsive forms of leukemia also responded to DRE in a dose and time dependent manner and apoptosis was measured using standard apoptotic markers. Very early activation of caspase-8 in these cells confirmed activation of the extrinsic pathway of apoptosis. Solvent extracts of our dandelion root have been tested on our cell lines to determine the biologically active component(s). Our results suggest that DRE (both aqueous and solvent extracts) contains components that induce apoptosis selectively in human leukemia cells presenting a novel non-toxic alternative to conventional leukemia therapy.

O-17: Pharmacology of Souroubea sympetala: Evidence for interaction with the GABAa

Martha Mullally1*, Chris Cayer2, Kari Kramp3, Ammar Saleem1, Calum McRae4, John Baker4, Marco Otorola5, Pablo Sanchez5, Mario Garcia5, Luis Poveda5, Zul Merali2, Tony Durst6, Vance L. Trudeau1, and John Thor Arnason1

1Department of Biology, University of Ottawa; 2Department of Psychology, University of Ottawa; 3Loyalist College; 4Bioniche Life Sciences Inc.; 5Herbario Juvenal-Rodriguez, Universidad Nacional de Costa Rica; 6Department of Chemistry, University of Ottawa

Anxiety is a serious form of mental illness that affects 12% of Canadians (1). The major pharmacological therapeutics available to treatment anxiety are the benzodiazepines, drugs associated with deleterious side-effects and not recommended for long-term use. Patients who suffer from anxiety disorders are major consumers of natural health products (NHPs), with an estimated 40% using NHPs to treat their anxiety (2). These factors highlight a need for additional anxiolytic plants to be identified and investigated as phytomedicines to treat anxiety. As part of a natural product investigation to find anxiolytic plants, we identified the genus Souroubea. Souroubea is a group of woody vines belonging to the neotropical family Marcgraviaeae with a tradition of use...
in both Belize and the Amazon to treat susto (fear), a folk-illness associated with anxiety (3). Initial investigations of *Souroubea* identified a triterpene-enriched fraction that reduced anxiety-like behaviour in rodents in a dose-responsive manner (4). The bioactive has since been identified as betulinic acid (BA). The purpose the present study is to further characterize the anxiolysis of *S. sympetala* plant parts (leaves and bark), extracts and the pure compound, BA. *S. sympetala* plant parts, extracts and BA were compared for their effect in anxiety-like behavioural assays. The *S. sympetala* extracts and BA were also examined for their capacity to interact with the GABAa benzodiazepine receptor (GABAa-BZDR) in vitro, with a competitive receptor binding assay; and in vivo, by pre-treating animals with a GABAa-BZDR antagonist, flumanzenil, and the capacity of the antagonist to extinguish anxiolysis determined. In this presentation we provide evidence that the anxiolysis observed after treatment with *S. sympetala* extracts and BA are due to interaction with the GABAa-BZDR.


**O-18: The role of Analytical Techniques in Natural Products Metabolite Profiling**

Ammar Saleem, Kristina McIntyre, John Thor Arnason.

Department of Biology, University of Ottawa

Presently, the hyphenation (combination) of analytical techniques is one of the most focused areas in the filed of analytical chemistry. With the advent of the era of faster data processing systems combined with high throughput autosamplers, scientists are getting closer to hyphenate analytical systems to generate 'directional' data at proteomic and metabolomic levels. In the field of natural products chemistry these analytical platforms are expected to generate authentic data to address several pressing questions related to species identification and quality control. The application of these techniques at systems biology level is expected to open up several horizons to bring back natural products at the forefront of therapeutic screening programs.

This presentation will highlight some of the latest advancements in the field of instrument hyphenation. In addition, major challenges and limitations of this approach will be discussed.
O-19: Non-specific effects in Traditional Medicine: the power of 'placebo'

Cory S. Harris*, Amir Raz & Timothy Johns

McGill University

Appearing increasingly often in both scientific literature and popular media, the subject of placebos constitute an emerging field of study garnering the attention of physicians, scientists, policy makers, and the general public. With many recent publications equating the majority of alternative medicines with placebos, the topic is particularly pertinent to Traditional medicine practitioners and their patients. Though they exist in many forms, placebos are often conceived as sugar pills or saline injections and carry negative connotations of deception and ineffectiveness. This presentation, however, moves beyond reductionist conceptualizations to explore the varied aspects and impacts of placebo research in Traditional medicine. After reviewing recent advances in the field, the presentation will highlight elements of particular relevance to the practice of traditional medicine, such as the patient-care provider relationship, treatment expectancies, and ‘non-specific’ effects, before comparing the cross-cultural strengths and weaknesses of different medical systems (eg. TCM and biomedicine) within the context-dependent framework of placebos. Finally, drawing on clinical and experimental evidence, the presentation will outline future research avenues directed toward harnessing placebo effects for the benefit of patients and health practitioners of Traditional medicine and biomedicine alike.
Monday, October 25

Ming Zhu

Ming Zhu is a professor and doctor-in-chief at the Beijing University of Chinese Medicine and Pharmacology (BUCMP) since 2008. Currently, Ming teaches at various Academic Schools of TCM and TCM Clinical Case Study. The focus is on comparative studies of Western medicine and Traditional Chinese Medicine. Ming was a visiting professor at the Samra University of Oriental Medicine (USA), the Pharmacy Centre at Vienna University, and the Life Science Institute of Nanyang Technical University in Singapore. Currently, Ming uses Chinese herbs to treat 3500 to 4000 patients with various sorts of illnesses annually. Ming has published 10 books and around 50 periodical papers. Ming has supervised 8 graduate students who have earned their Masters degree.

O-20: Historical perspective of integration of NA ginseng in TCM

Ming Zhu

Beijing University of Chinese Medicine & Pharmacology (BUCMP)

The clinical use of North American Ginseng in TCM comprises an extensive category in life sciences. With over 100 years of traditional knowledge, what effect does NA Ginseng have on the Chinese market? Specifically, how has it been integrated into the Chinese medical system and prescribed as herbal formulations to treat various illnesses such as apoplexy, types 2 diabetes mellitus, insomnia, carbon monoxide poisoning, hypertension (certain differentiation phase), nasal bleeding, fatigue or lassitude, stomach ache, cancer with indolence (breast cancer with rankle), etc? Currently, it is essential to elucidate its bioactivities with modern technology to provide the pharmacological plausibility for its medicinal use. Comparative studies have been made to discern unique features among Chinese ginseng (Radix et Rhizoma Ginseng), San Qi (Radix et Rhizoma Notoginseng) and NA ginseng (RadixPanax Qinquefolii), which come from the same botanical genus. Many famous Chinese physicians began to use NA Ginseng to compose their herbal formulations in the last two centuries, which accelerated the wide-spreading of NA Ginseng in China. Herb-herb interactions are the highlight of the study for the herbal combinations. Its contraindications and preparation techniques are also taken into great considerations. Some information concerning its cultivation, marketing, and health effects in mainland China will be also introduced.
Robin Marles

Robin Marles is the Director of the Bureau of Clinical Trials and Health Sciences, Natural Health Products Directorate, Health Canada. His responsibilities have included the safety, efficacy and quality assessment of natural health products (e.g. herbs, vitamins, minerals, amino acid and fatty acids, probiotics) for product licensing, clinical trial authorizations, health risk assessments, monographs and ingredient database development. From 1992-2003 he was a professor of botany and biology at Brandon University, Manitoba. He holds a B.Sc. and M.Sc. in Biology and a Ph.D. in Pharmacognosy. Research interests include the chemistry, pharmacology, quality and development potential of traditional medicines.

O-21: Clinical Trials for Traditional Chinese Medicines

Robin J. Marles*, Thérèse Desjarlais-Renaud, Elise Cogo and Helmi Hussien

Bureau of Clinical Trials and Health Sciences, Natural Health Products Directorate, Health Canada, 2936 Baseline Road (A.L. 3302C), Ottawa, ON K1A 0K9

In Canada, Phase I, II, and III clinical trials of natural health products (NHPs), including Traditional Chinese Medicines (TCM), require authorization from Health Canada's Natural Health Products Directorate. In accordance with the NHP Regulations (NHPR), applications for authorization of clinical trials (CTAs) must be accompanied by an Investigator's Brochure providing the manufacturing, identity, purity, quantity/dose, stability, known pharmacology and toxicology of the trial products, a Protocol with the trial design and methods, Informed Consent Form for the subjects, and an attestation to compliance with international standards of Good Clinical Practices. Approval by an independent Research Ethics Board is required before commencement. CTAs are reviewed by staff with expertise in NHPs, e.g. for TCM a reviewer with formal training in the traditional paradigm, complex formulations and individualized treatment approach of TCM will be involved. TCM practitioners as well as physicians and scientists are anticipated to be on the clinical team and the investigation can be based in the TCM paradigm rather than just a pharmacological investigation of TCM herbs. Authorization is based on evidence that the clinical trial will not endanger the health of the subjects, that it will not be contrary to their best interests, and that there are reasonable grounds to believe that it will achieve its objectives. Any serious adverse reactions occurring during the trial must be reported. The NHPR thus provide a supportive framework for clinical research on safe, effective, high quality NHPs while allowing freedom of choice and respecting cultural and philosophical diversity, such as TCM.
Lique Coolen

Lique Coolen received her Ph.D. at the University of Nijmegen, the Netherlands, and her postdoctoral training at Yale University and the University of Baltimore in Maryland. She is currently a Professor in Anatomy & Cell Biology and Physiology & Pharmacology at The University of Western Ontario and Canada Research Chair in the Neurobiology of Motivation and Reward. She has authored over 70 articles and is an expert in the neurobiology of male sexual behaviour.

O-22: Effects of Ginseng on sexual function

Lique Coolen

University of Western Ontario

Erectile dysfunction greatly decreases the quality of life in men. Ginseng has been demonstrated to improve sexual function in male rats and may be effective in decreasing the severity of erectile dysfunction. We hypothesized that Panax quinquefolius, North American (NA) ginseng, will increase the frequency of erections and improve sexual behavior in healthy and in diabetic male rats. Data will be presented showing that oral injections for 28 days of different doses of NA Ginseng (125, or 250 mg/kg) improved erectile function, evidenced by increased copulatory efficiency and reduced numbers of mounts, in young healthy male Long Evans rats. In addition, NA Ginseng treatment increased gene expression for endothelial and neuronal Nitric Oxide Synthase in erectile tissue, suggesting that Ginseng may affect sexual function by increasing nitric oxide signaling in smooth muscle cells of erectile tissues. Finally, preliminary data will be presented showing effects of NA Ginseng on improved sexual function in the type 2 diabetic Zucker rat. Together, these data support the potential use of NA Ginseng in treatment of erectile dysfunction.
Morris Karmazyn

Morris Karmazyn obtained his MSc and PhD degrees from McGill University, carried out postdoctoral research at the University of Manitoba and was a Visiting Scientist at the Weis Center for Research in Danville, PA. He has published over 190 papers and has won a number of awards including a Career Investigator Award from the Heart and Stroke Foundation of Ontario and currently holds a Tier1 Canada Research Chair in Experimental Cardiology. He has presented nearly 200 invited lectures and is listed in both American Men and Women of Science and The Canadian Who’s Who. Morris’ primary research interests involve mechanisms of cardiac hypertrophy and he holds funding from both the HSFO and CIHR.

O-23: The Sodium-Hydrogen Exchange Calcineurin Pathway: A Target for the Antihypertrophic Effects of Ginseng

Morris Karmazyn

Department of Physiology and Pharmacology, the University of Western Ontario, London, Ontario

Cardiac hypertrophy represents an important contributor to heart failure and is an important target for therapeutic intervention in the treatment of heart failure. The mechanisms underlying cardiac hypertrophy are multifaceted and complex. A major mediator of hypertrophy is the sodium hydrogen exchanger isoform 1 (NHE-1), a 91 kDa protein which is the primary proton extruder in the cardiac cell. NHE-1 specific inhibitors are effective antihypertrophic agents and are also effective in reducing heart failure. How NHE-1 induces hypertrophy is not known with certainty but recent evidence suggests a calcium-dependent activation of the protein phosphatase calcineurin which in turn activates the transcriptional factor NFAT. Using both in vivo and in vitro approaches we have shown that ginseng can markedly inhibit this pathway and reduce hypertrophy and heart failure (Guo et al., in press and this congress). However, the salutary effects of ginseng may not be restricted to this pathway as we have also found that it prevents leptin-induced hypertrophy through an alternate pathways involving RhoA activation (Moey et al., this congress). Taken together, our results demonstrate a potent antihypertrophic effect of ginseng likely acting at multiple sites. (Supported by OGIRC and the CIHR)
Kari Kramp is a professor and the coordinator of Biosciences and Principal Researcher in the Supercritical CO\textsubscript{2} Extraction (SFE) Laboratory at Loyalist College. Her Ph.D thesis (2010) provided an in depth assessment of the diversity of biosynthetic classes of compounds that SFE can be effectively used with, as well as provided methods to prepare and evaluate biologically active extracts for emerging products of interest. The laboratory has recently completed an investigation optimizing SFE for the removal of organochlorine pesticides from North American ginseng and is currently collaborating with the University of Ottawa and a regional industry partner to investigate the utilization of SFE for the development of several high-quality natural health products.

**O-24: Supercritical CO\textsubscript{2} Extraction of Select Natural Products**

Kari Kramp

Loyalist College, Belleville, Ontario

Results of the supercritical fluid extraction (SFE) of targeted secondary metabolites from select natural products will be discussed. In addition, a recent study will be reviewed which investigated SFE method optimization for the removal of organochlorine pesticides from North American ginseng.
Junzheng Zhang

Junzheng Zhang is a research officer at NRC Institute for Nutrisciences and Health and an adjunct professor at University of Prince Edward Island. He has a background in traditional Chinese medicine and a Ph.D. in natural products chemistry. Prior to NRC, Dr. Zhang had worked in industry for 6 years on natural health products research and development. He is a board member of Canadian Institute of Chinese Medicinal Research and Natural Health Products Research Society of Canada. His research interest is on discovery and development of bioactive compounds from Canadian bioresources and Chinese medicine for anti-diabetic/obesity, anti-allergy, and neuroprotective applications.

O-25: Bioactive components from dietary Chinese herbs

Xiaoli Sun1,2,3, Kaibin Zhang1,2, Xiuhong Ji1, Yanwen Wang1, Jeffrey Zidichouski1, Zhimin Wang2, and Junzeng Zhang1*

1Institute for Nutrisciences and Health, National Research Council Canada, Charlottetown, PE, Canada; 2Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China; 3School of Traditional Chinese Medicine, Capital Medical University, Beijing, China

Some herbs commonly used in traditional Chinese medicine (TCM) for prevention, early intervention, and treatment of diseases have been integrated into the present day Chinese diet. This convergence of food and medicine is also reflected in the current regulatory framework in China for selected herbs used as food or source materials for manufacturing natural health products, including functional foods and nutraceuticals. The presentation will provide an overview on the dietary Chinese herbs and the recent progress on bioactive components research with selected herbs. Specific research activities and some preliminary results from our lab in this area will also be briefly introduced.
Poster abstracts

EFFECTS OF NORTH AMERICAN GINSENG ON SEXUAL BEHAVIOR AND ERECTILE FUNCTION IN HEALTHY RATS

Matthew Barnes* and Lique M. Coolen

University of Western Ontario

Erectile dysfunction (ED) greatly decreases the quality of life in men. Currently, men experiencing ED are treated with phosphodiesterase type 5 (PDE5) inhibitors, however, approximately 10% respond poorly to PDE5 inhibitors and this percentage increases with age and concurrent cardiovascular pathologies. Ginseng has shown promise in decreasing the severity of ED. We hypothesized that Panax quinquefolius, North American (NA) ginseng, will increase the frequency of erections and improve sexual behavior in healthy male rats. After oral injections for 28 days of ethanol extract (0, 125, or 250 mg/kg), male Long Evans rats were tested for improvements in sexual behavior and motivation, as well as erectile function. Results showed that 125 and 250 mg/kg of ginseng improved copulatory efficiency by reducing numbers of mounts, which is indicative of improved erectile function. In addition, sexual arousal was enhanced in sexually naive males by the higher dose of ginseng. However, ginseng did not affect sexual motivation assessed by the runway test, or psychogenic erections in non-contact erection tests. Hence, ginseng improved sexual function only when males were allowed direct encounter with a receptive female. Overall, these experiments show that the ethanol extract of NA ginseng is effective at improving erectile function and sexual arousal in young adult male rats.

NORTH AMERICAN GINSENG REDUCES EXPRESSION OF VASCULAR CELL ADHESION MOLECULE-1 AND INTERCELLULAR ADHESION MOLECULE-1 IN VITRO

Colin Carruthers*, Jessica Y. Davie, and Kem A. Rogers

University of Western Ontario

Atherosclerosis is a major cause of cardiovascular morbidity and can lead to complications such as stroke and myocardial infarction. During initiation of atherosclerosis, there is an upregulation of pro-inflammatory adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), followed by macrophage infiltration and lesion formation. Previous research suggests ginseng extract may reduce the inflammatory response in atherosclerosis. After pre-treatment with 0, 50, 125 or 250 μg/mL North American ginseng extract, porcine aortic endothelial cells were stimulated with interleukin-1β (IL-1β) to induce an inflammatory response. Immunofluorescent staining and confocal microscopy revealed that high dose (125 and 250 μg/ml) ginseng pre-treatment effectively reduces IL-1β-dependent upregulation of VCAM-1 and ICAM-1. To determine if ginseng was equally effective in vivo, Long Evans rats were fed an atherogenic diet (4% cholesterol, 1% cholic acid and 0.5% propylthiouracil) for 17 days following a 38
day pre-treatment with 0, 125, or 250mg/kg/day ethanol extracted North American ginseng (n=10, 9, and 11, respectively). Total serum cholesterol levels in ginseng treated rats were not significantly different from the control group. Furthermore, there were no significant differences between treatment groups with respect to monocyte recruitment into the aortic intima. While it has been shown by others that ginseng has anti-atherogenic effects, it would appear that despite seeing a downregulation of VCAM-1 and ICAM-1 in vitro, ginseng is not effective in downregulating monocyte recruitment into the vessel wall of hypercholesterolemic rats. Further studies are therefore required to determine the mechanism by which ginseng influences atherogenesis.

ANXIOLYTIC PROPERTIES OF SOURTOEBEA SYMPETALAE

C. Cayer1,4,5*, P. Kent1,6, M. Mullally2, L. Poveda-Alverez7, P. Sanchez Vindas7, T. Durst3, Z. Merali1,4,5, and J. T. Arnason2

1Department of Psychology, University of Ottawa, 2Department of Biology, University of Ottawa, 3Department of Chemistry, University of Ottawa, 4Department of Psychiatry, University of Ottawa, Ottawa, 5Department of Cellular and Molecular Medicine, University of Ottawa, 6University of Ottawa Institute of Mental Health Research, 7Herbario Juvenal-Rodríguez, Universidad Nacional de Costa Rica, Heredia, Costa Rica

Anxiolytic properties of Souroubea gilgii on the ratAbstract: As part of our ongoing research into phytomedicine therapies for mild to moderate anxiety, the neotropical vine Souroubea spp. was chosen for study as part of a phytochemical discovery strategy focusing on rare plant families in Central America. Ethnobotanical reports suggested psychopharmacological activities related to fear, anxiety and sleeplessness. The first phase of our study involved a characterization of the behavioral effects of crude ethanol extracts and several isolated fractions from leaves of Souroubea gilgii in rats. Male Sprague Dawley rats were administered either the crude ethanol extract (25, 50 or 100 mg/kg), one of four isolated fractions or vehicle (50% sweetened condensed milk solution) using a non aversive oral administration technique and were then tested (60 min post-drug administration) in several behavioral paradigm that assess levels of anxiety and/or fear including the elevated plus maze, social interaction test, vogel conflict test and fear potentiated startle. Results showed that in all four paradigms, treatment with the crude ethanol extracts and the ethyl acetate fraction reduced anxiety and/or fear-related behavior. The next phase of the study involved bioassay guided isolation (using HPLC-MS) of the active principle, the pentacyclic triterpene, betulinic acid and its derivatives. Similar to the findings with the crude ethanol extracts, treatment with betulinic acid (0.5 mg/kg) reduced anxiety/fear in the behavioral paradigms. In addition, chronic oral administration of the extract and its bioactive compound did not show any deleterious effects in locomotor activity, weight gain, and organ weights and showed no withdrawal symptoms behaviorally. Together, these findings suggest that further research is warranted to investigate the safety and efficacy of Souroubea spp. and its active ingredient, betulinic acid, as an effective treatment for anxiety disorders. and the identification of its main active ingredient: Betulinic acid.
THE STATUS OF CULTIVATED MOUNTAIN GINSENG MANAGEMENT IN SOUTH KOREA

Joosang Chung*, Hee Han, and Hyejean Chung

Department of Forest Sciences, Seoul National University, Seoul, Korea

Recently the cultivated mountain ginseng (Panax ginseng) is rising as one of the most profitable forest products in South Korea. It is a popular restorative well-being food without any detrimental chemical elements. According to the National Statistics, 42,721kg of commercial mountain ginseng was produced from about 5,500ha of mountain forests all over the country. This amount means about 200 times of growth within only last 9 years and still the cultivation area expands so fast. As the market increases, raised are various issues, among customers, about the origins of seeds and products (domestic or imported), the amount of residuals of agricultural chemicals and detrimental heavy metals, and the age of products. In order to solve the problems and to promote the competitiveness of Korea mountain ginseng on domestic and international market, Korea government is developing an institutional production certification system by adopting a reliable traceability system. In this presentation, the current status and environment of mountain ginseng production management in South Korea will be introduced.

DEVELOPMENT AND VALIDATION OF A NOVEL ANALYTICAL METHOD BY LPLC-ELSD FOR IDENTIFICATION AND QUANTITATION OF MONOSACCHARIDES FROM THE POLYSACCHARIDES FRACTION IN ROOTS OF AMERICAN GINSENG

José A. Guerrero-Analco*, Kristina McIntyre, Ammar Saleem, and John Thor Arnason

Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, ON, K1N6N5, Canada

Extracts of North American ginseng (Panax quinquefolius L., Araliaceae) containing polysaccharides and oligosaccharides have been shown to have different immunomodulatory effects. However, chemical characterization of the sugar components has been undertaken due the structural complexity of the saccharides. Our team at University of Ottawa has developed a new analytical method to determine the chemical composition of water soluble components. We developed and validated a new two dimensional liquid chromatographic analytical method by using Low Pressure Liquid Cheormatography-Evaporative Light Scattering Detection (LPLC-ELSD). This method enabled us to identify and quantify the major monosaccharides present in ginseng samples collected in various locations of Ontario. Glucose was found to be the major neutral monosaccharide (14.98- 23.59 mg/ g dry weight), galactose (0.48- 1.46 mg/g dw) and arabinose (0- 0.79 mg/g dw) were present in similar amounts and galacturonic acid (5.54- 14.63 mg/g dw) was also identified. Rhamnose was detected only in two of the five Ontario ginseng samples. Mannose and xylose were also monitored but were not detected. The developed method has enabled us to determine the monosaccharide composition in P. quinquefolius and could act as a tool for quality control and quality assurance of ginseng products commercially available in North America.
GINSENG INHIBITS CARDIOMYOCYTE HYPERTROPHY AND HEART FAILURE VIA NHE-1 INHIBITION AND ATTENUATION OF CALCINEURIN ACTIVATION

Juan Guo*, Xiaohong Tracey Gan, James V Haist, Venkatesh Rajapurohitam, Asad Zeidan, Nazo Said Faruq, and Morris Karmazyn

Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada

Ginseng is a highly valued medicinal plant gaining popularity as a therapeutic agent. The pharmacological properties of ginseng are attributed to its bioactive ginsenosides, the principal bioactive constituents in ginseng. The present study was carried out to determine whether ginseng exerts a direct antihypertrophic effect in cultured cardiomyocytes and whether it modifies the heart failure process in vivo. Moreover, we determined the potential underlying mechanisms for these actions. Experiments were performed on cultured neonatal rat ventricular myocytes as well as adult rats subjected to coronary artery ligation (CAL). Treatment of cardiomyocytes with the \( \alpha_1 \) adrenoceptor agonist phenylephrine for 24 h produced a marked hypertrophic effect as evidenced by significantly increased cell surface area and ANP gene expression. These effects were attenuated by ginseng in a concentration-dependent manner with a complete inhibition of hypertrophy at a concentration of 10 µg/ml. Phenylephrine-induced hypertrophy was associated with increased gene and protein expression of the Na\(^+\)-H\(^+\) exchanger 1 (NHE-1), increased NHE-1 activity, increased intracellular concentrations of Na\(^+\) and Ca\(^{2+}\), enhanced calcineurin activity, increased translocation of NFAT3 into nuclei and GATA-4 activation, all of which were significantly inhibited by ginseng. Upregulation of these systems was also evident in rats subjected to 4 weeks of CAL. However, animals treated with ginseng demonstrated markedly reduced hemodynamic and hypertrophic responses which were accompanied by attenuation of upregulation of NHE-1 and calcineurin activity. Taken together, our results demonstrate a robust antihypertrophic and antiremodeling effect of ginseng which is mediated by inhibition of NHE-1 dependent calcineurin activation.

HIGH MASS RESOLUTION PROFILING OF GINSENOSIDES IN PLANT EXTRACTS

Xu Guo*, Eva Duchoslav, Yunyun Zou, Robert Ellis, Tom K. Moy and Takeo Sakuma

AB SCIEX

Ginseng grows in the NE part of Asia in cooler climate, and is also cultivated in North America. Its root has been used for thousands of years to improve the overall health. Ginsenosides (Rb\(_1\), Rb\(_2\), Re, Rf, et al) are major active components. Types and distributions of gensenosides vary by Panax species, geographic location, soil, climate, or even extraction method. Therefore, it is important to develop an accurate and sensitive quantitative and qualitative analytic method for the characterization of ginsenosides from various ginseng samples. In this study, we present a profiling method using an ultra high performance liquid chromatograph combined with a triple-quadrupole-time-of-flight mass spectrometry system. This combination will produce fast and high mass accuracy data. Two batches
of American ginseng extracts, using either alcohol or water, from Ontario farms were examined. A multivariate statistical software program and a “formula finder” utility program were used for data analysis. The preliminary results indicate that (1) the protonated, sodiated precursor ions and doubly charged sodiated ions species were observed from ginsenoside standards (2) the triple-quadrupole-time-of-flight mass spectrometry exhibited high resolution (~ 35,000 full width at half mass) across the whole mass range we examined (m/z =100 – 1,200). Typical mass accuracy is less than 2 RMS with external calibration. We obtained formula of ginsenosides based on accurate mass. (3) Alcohol extracts produced higher amounts of Ginsenosides: Rb1, Rb2, Rc, Rd, Re, Rg1 and Rf than water extracts. However, doubly charged ions of ginsenosides were observed prominently from water extracts.

NA GINSENG EXTRACTS PROTECT AGAINST VASCULAR INJURY INDUCED BY CHRONIC HOMOCYSTEINE TREATMENT IN RATS

Jirui Hou*, Hua Pei, Chike Azike, and Edmund MK Lui
University of Western Ontario

Homocysteine (Hcy) is an independent risk factor for atherosclerosis and other vascular lesions. It causes endothelial dysfunction and vascular injury. Ginseng compounds have the properties of protection against vascular injury. The purpose of this study was to determine the effects of water (Aq) and hydro-alcoholic (HA) ginseng extracts on vascular injury induced by chronic Hcy treatment in young adult male rats.

Daily treatment with Hcy (50 mg/kg) by gastric gavage for 42 days significantly elevated plasma Hcy levels by more than 2 fold over control levels, but it was significantly reduced to the control level by daily treatment with 150 and 500 mg/kg of both types of ginseng extracts. The body and major organ weights were not affected by Hcy or ginseng treatment.

Hemodynamic study was conducted in intact animals under anesthesia Hcy induced elevation of mean arterial pressure and heart rate without altering the rate of left ventricular pressure; and these Hcy effects were reversed by both Aq extract and HA extract.

Modulation of vascular reactivity study showed that Hcy group significantly reduced the contractile response to phenylephrine (PE) and impaired Acetylcholine- and Isoproternol-induced vaso-relaxation of aortic ring in an organ bath-based bioassay. The reduced contractile-relaxation response was, however, not observed in rats treated with Aq extracts.

Histology study of aortic ring tissues stained with hemotoxylin & eosion showed that Hcy treatment produced an irregular abluminal surface and the presence of damaged endothelium cells as well as fat accumulation in the smooth muscle compartment. These Hcy-induced morphological changes were apparently absent in rats treated with Aq extract.

Our study showed that Aq ginseng extract was effective in protecting rat aortic tissues from injury induced by chronic Hcy treatment. The effect was extract-specific in that AH extract was not effective.
TREATMENT OF OBESITY AND DIABETES BY A CANADIAN ABORIGINAL MEDICINAL PLANT IN A MOUSE MODEL OF DIET-INDUCED OBESITY

Despina Harbilas*, Antoine Brault, Diane Vallerand, , Ammar Saleem, John T. Arnason, Lina Musallam, Pierre S. Haddad
Université de Montréal, INAF, MDRC, University of Ottawa

The prevalence of the metabolic syndrome is increasing among the Cree of Eeyou Istchee (CEI - Northern Quebec) as a result of increases in obesity and insulin resistance. Non-traditional diet and sedentary lifestyle along with cultural disconnect of modern type 2 diabetes (T2D) therapies are involved. Exploring treatments from within CEI traditional pharmacopeia represents a valuable alternative. W7, a CEI plant from the Canadian Boreal Forest, demonstrated anti-obesity properties in vitro and its active principle was identified through a bioassay-guided fractionation approach. Therefore, the aim of this study is to study the potential effects of W7 and its active in a mouse model of diet-induced obesity and T2D. In the first study C57/BL6 mice were subjected to high fat diet (HFD) for eight weeks to which W7 was incorporated. In the second study, the mice were subjected to HFD for sixteen weeks resulting in obesity, hyperinsulinemia and mild steady-state hyperglycemia. W7 or its active compound was introduced in the HFD for the last eight weeks. In both studies, treatment with W7 or the active effectively reduced body weight, retroperitoneal fat pad weight, and lipid content of the liver, as compared to HFD controls. No statistical difference was observed in water or food intake. Glycemia, insulinemia, G/I index (indicator of insulin resistance), leptin and adiponectin levels were also improved as compared to HF controls. W7 and its active thus exhibit promising anti-obesity and consequently anti-diabetic effects. Mechanisms remain to be elucidated but current results point towards a stimulation of metabolic rate. Funded by the CIHR.

LONG TERM TREATMENT OF ASTRAGALIN EXTRACT ENHANCES SENSITIVITY OF MOUSE HEPATOMA CELLS TO CHEMO AND RADIATION THERAPIES

Weisi Kong1*,  Dashang Zhang2, Shu Zhou2, Frank Burczynski1, Patrick Choy1, and  Yuewen Gong1
1University of Manitoba, Winnipeg, Canada and 2China-Japan Friendship Hospital, Beijing, China

Background: Astragalin is a common herb used in traditional Chinese medicine (TCM) to tonify qi. It has been used in TCM clinical practice to treat certain kinds of cancer in China. Although the precise mechanism of astragalin in cancer treatment remains unclear, it has been suggested that astragalin can regulate immune system and enhance activity of cytotoxic T lymphocyte. In the current study, we employed astragalin extract and mouse H22 hepatoma cells to investigate the mechanism of astragalin anti-cancer activity. Methods: BALB/c mice were inoculated with mouse H22 hepatoma cells in the peritoneal cavity. The cells were inoculated into 20 rats in two groups with and without treatment of astragalin extract. The cells were then inoculated into mice with and without treatment of cyclophosphamide or X-ray. Results: Compared to cyclophosphamide treatment groups, H22 cells treated with astragalin extract had significant lower tumor formation in the mice than H22 cells treated without astragalin extract (3.6% vs 92.6%). Moreover, after radiation with 8000CGV, the
incidence of hepatoma in mice was 26.5% for astragalin treated cells and 95% for astragalin untreated cells. Furthermore, astragalin treatment rendered H22 cells staying in the G1 phase instead of the S phase. Conclusion: Long term treatment of astragalin increases sensitivity of mouse H22 hepatoma cells to chemo and radiation therapies and it may be related to its regulation of cell cycle.

HOMOCYSTEINE INDUCES BONE MORPHOGENETIC PROTEIN-13 EXPRESSION IN RAT HEPATIC STELLATE CELLS

Yining Li*, Karmin O, Patric Choy, and Yuewen Gong

University of Manitoba

Background: Homocysteine (Hcy) is a metabolic product of essential amino acid - methionine. It has been documented that elevated Hcy may be involved in the development of liver diseases. However, it is still unclear what the role of Hcy in hepatic fibrogenesis is. Since hepatic stellate cells are the type of cells that responsible for liver fibrosis, in the current study rat hepatic stellate cells are incubated with Hcy and the expression of bone morphogenetic proteins are investigated. Method: Two rat hepatic stellate cell lines (CFSC-5H cells and CFSC-8B cells) were cultured in DMEM plus 5% fetal bovine serum and treated with Hcy for different time intervals and concentrations. Total RNA and protein of CFSC-5H and CFSC-8B cells were extracted respectively. RT-PCR and Western blot were employed to analyze the expression of bone morphogenetic proteins. Results: After incubation of Hcy with CFSC-5H cells and CFSC-8B cells for 24 hours and 48 hours, BMP-13 expression was significantly increased. Furthermore, the expression of other BMPs remained the same after incubation with Hcy. Conclusion: Hcy may mediate the development of liver fibrosis through its regulation of BMP-13 gene expression in hepatic stellate cells.

THE EFFECT OF ANTI-DIABETIC TRADITIONAL CHINESE MEDICINE ON REPAGLINIDE METABOLISM

Rui Liu1,2, Ammar Saleem2, Anthony Krantis1,2, John T. Arnason2, Brian C. Foster1,2,3*
1Department of Cellular and Molecular Medicine, University of Ottawa, 2Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, 3Therapeutic Products Directorate, Health Canada

Background: Many traditional Chinese medicines (TCM) are reputed to have anti-diabetic activity. They have been widely used as alternative or complementary medicine for diabetic patients. However these TCM products may affect metabolism of other anti-diabetic drugs and health products, potentially affecting the safety and efficacy of these drugs.

Purpose: This study was undertaken to characterize the effect of selected TCM products on the metabolism of repaglinide, one of the widely used meglitinide class of blood glucose-lowering drugs.

Methods: Ginseng (both Asian and American species) and Goji sample were purchased from local manufacturers (Ontario, Canada). Ge gen and Dang shen were purchased from Sanjii Medical & Pharmaceutical Co (P. R. China). Water extracts and 95% ethanol extracts (20 mg/ml) were prepared fresh. Extracts were examined for their effects on the formation of major repaglinide metabolites by using established in vitro bioassays with human liver microsomes.
**Results:** Our data indicates that GoJi, and both American and Asian ginsengs did not alter the formation of major repaglinide metabolites. However, ethanolic extracts of Ge gen and aqueous extract of Dang shen significantly decreased the formation of repaglinide metabolites.

**Conclusions:** According to our study, Ge gen and Dang shen exhibit the potential to interact with repaglinide. They may inhibit the metabolism of repaglinide and cause a overdose. Further studies are warranted to determine if these effects are clinically significant.

**GINSENG AND IN VITRO INHIBITION OF CYP3A4-MEDIATED DRUG METABOLISM**

Cheryl Lui*, J. Malcolm, O. Arnold, and David G. Bailey

McMaster University

Ginseng is a popular medicinal herb extensively grown in Ontario for export to eastern Asia. Since individuals taking ginseng might also receive a prescription or over-the-counter medication, this would create the risk for an herbal – drug interaction and unwanted clinical effect. Drug metabolism is a fundamental biological mechanism for the inactivation of many drugs. The most important drug metabolizing enzymes constitute the family known as Cytochrome P450s (CYPs) and the enzyme, CYP3A4, contributes to the inactivation and elimination of an estimated 50% of all drugs, which makes inhibition of it an important source of drug interactions. Alcoholic and aqueous extracts of ginseng root randomly selected from 5 farms before institution of the OGIRC program (Pre-Study Samples) and 9 farms participating in the program (First Harvest Samples) were assessed for competitive (reversible) and mechanism-based (irreversible) inhibition of in vitro metabolism of felodipine, a drug probe for CYP3A4 activity. The alcoholic extract of Pre-Study Samples produced modest competitive inhibition (10% - 35%) and marked mechanism-based inhibition (62% - 100%) at the highest tested concentration (100 ug/ml). A relationship between concentration of extract and extent of mechanism-based inhibition was apparent. The aqueous extract produced much less inhibitory activity. The alcoholic and aqueous extracts of First Harvest Samples caused negligible competitive or mechanism-based inhibition (0% - 14%) at the highest tested concentration (100 ug/ml). Ontario grown ginseng has the potential to cause an important pharmacokinetic interaction and excessive drug effect. The ginseng produced under the OGIRC program might not have this concern.

**ANALYSIS OF MALONYL GINSENSOSIDES IN ONTARIO GINSENG (PANAX QUINQUEFOLIUS) USING LC/MS/MS**

Kristina McIntyre*, Ammar Saleem, and John Thor Arnason

Department of Biology, University of Ottawa

Ginsenosides are the most commonly studied compounds in ginseng as they are commonly believed to be responsible for most of ginseng's biological activity. Although greater than 150 ginsenosides have been identified, most studies examine only 5 or 6 of the major ginsenosides by chromatographic
methods. Many important ginsenosides, including malonyl ginsenosides, are rarely assessed. Malonyl ginsenosides can contribute to a significant proportion of total ginsenoside levels in American ginseng and ignoring them can lead to underestimating ginsenoside levels. Furthermore, as malonyl ginsenosides are converted to neutral ginsenoside under gastric conditions, failure to include malonyl ginsenosides in analysis would lead to underestimating the amount and composition of individual ginsenosides that would effectively be taken up by the body. In this study LC/MS/MS was used to examine a wider range of ginsenosides in Ontario ginseng including Rg1, Re, Rb1, Rb2, Rc, Rd, Ro, and malonyl ginsenosides mRb1, mRb2, mRc, and mRd. Neutral ginsenoside composition generally followed the trend: Rb1 > Rd~ Re > Rc > Ro > Rg1 > Rb2~ Rg1. Malonyl ginsenosides were shown to occur in amounts from 1.14–7.32 mg/g dry weight. Of the malonyl ginsenosides, mRb1 was present in the highest quantities and together malonyl ginsenosides accounted for up to 16.45% of total ginsenoside content. This showed that malonyl ginsenosides can make up a significant percentage of total ginsenoside content and it is important to include these compounds in ginsenoside quantification.

CHRONIC NORTH AMERICAN GINSENG ADMINISTRATION ALTERS ARTERIAL PRESSURE AND METABOLIC VARIABLES IN THE RAT

Megan Migchels*, W. Iqbal, J. M. Moreau and J. Ciriello
University of Western Ontario

Although ginseng has been used for centuries as a medicinal herb, little is known about the effects of North American ginseng (NAG) on cardiovascular disease and obesity. This study was done to investigate the effects of NAG on arterial pressure and metabolic markers in adult male Sprague-Dawley rats on a standard chow diet. Animals were given 250mg/kg of body weight dose of an alcoholic extract of NAG dissolved in 0.9% saline or the vehicle daily for 4 weeks by oral gavage. After the first week of treatment, mean arterial pressure was significantly (p<0.02) reduced in the NAG treated group by -4 ± 3 mmHg compared to controls that increased by 7 ± 4 mmHg. However, by the end of the treatment period arterial pressures were not different (p<0.11) between groups. Cumulative body weight gain over 4 weeks was significantly decreased (120.9 ± 4.4 g) in the NAG treated animals compared to controls (139.2 ± 3.8 g), despite a lack of differences in food or water intake between the groups. Blood glucose (8.4 ± 0.5 mmol/L; 10.7 ± 0.9 mmol/L), plasma insulin (0.76 ± 0.03 ng/ml; 1.9 ± 0.56 ng/ml) and leptin (323.5 ± 81.3 ng/ml; 555.8 ± 79.1 ng/ml) levels were also found to be lower in the NAG treated animals than controls, respectively. Taken together these data suggest that the use of NAG may be an effective therapy for reducing the risk of the metabolic syndrome in adult obesity.

This work was supported by the Heart and Stroke Foundation of Ontario and the Ministry of Research & Innovation Ontario Research Fund-Research Excellence.
AN ETHNOPHARMACOLOGICAL SURVEY OF DAULATDIA GHAT AREA, KUSHTIA DISTRICT, BANGLADESH USED FOR TREATMENT OF “HARD TO CURE” DISEASES

Md. Nur Kabidul Azam¹, Azmal Ibna Hassan¹, Minhajul Islam¹, Md. Nazmul Islam¹, Md. Ziaul Haque¹, Rownak Jahan¹, Mohammed Rahmatullah¹, Majeedul H. Chowdhury²

¹Faculty of Life Sciences, University of Development Alternative, House No. 78, Road No. 11A (New), Dhanmondi R/A, Dhaka-1205, Bangladesh ²Department of Biological Sciences, New York City College of Technology, City University of New York, 300 Jay Street, Brooklyn, NY 11201, USA

Traditional healers, otherwise known as Kavirajes from an important component of the primary health-care system of Bangladesh. The Kavirajes generally possess considerable expertise on use of plants used to treat various ailments. The ailments treated can range from minor ailments like coughs and colds to difficult to treat diseases like diabetes. Since the Kavirajes in various regions of Bangladesh differ in the choice of plants, the objective of this present study was to conduct a survey on the plants used by the Kavirajes of Daulatdia Ghat area, Kushtia district, Bangladesh. Interviews were conducted with the help of a semi-structured questionnaire and the guided field-walk method, where the informant pointed out plants and described their uses. Informed consent of the Kavirajes was obtained prior to the survey. The various plants used in the area (with ailments treated given in parenthesis) included Mucuna pruriens (low sperm count, energy stimulants, vaginal wounds), Pandanus odoratisimus (obesity), Ipomoea mauritiana (to increase breast milk), Cassia occidentalis (boils, skin diseases, coughs, mucus, blood purifier), Geodorum densiflorum (low sperm count), Cereus grandiflorus (acidity), Crinum asiaticum (edema, rheumatism), Withania somnifera (piles, debility), Vernonia patula (to stop menstruation), Azadirachta indica (to regularize menstruation, diabetes), Paederia foetida (coughs, mucus, loss of appetite, rheumatism, dysentery), Diospyros peregrina (leucorrhea, gangrene, mucus, biliary diseases, blood purifier), Cayratia trifolia (to stop bleeding, scorpion-bite, snake-bite, leucorrhea), and Aloe vera (debility, increase semen volume, enlarged spleen, bloating, hepatic diseases, boils). It is expected that scientific studies conducted with the plants can lead to discovery of novel drugs.

STUDIES OF DEVELOPMENT OF TECHNOLOGIES FOR PROCESSING AND PHYSICOCHEMICAL SCREENING OF PLANTS COLLECTED FROM THE LAWACHERRA RAIN FOREST OF BANGLADESH USED AS REMEDY FOR DIABETES MELLITUS

Romeo McField¹, Bivash Chandra Panday¹, Israt Jahan Mukti², Md. Rohimul Faruque³, Md. Badruddaza⁴, A.K.M. Fahmidul Haque⁵, Bulbul Ahmmed⁶

¹Department of Epidemiology, Biostatistics, Community Nutrition and Noncommunicable Diseases, Practical Academy on Wise Education and Research Foundation, Dhaka-1000, Bangladesh ²Department of Health and Nutrition, Biogene Life Care, Dhaka-1000, Bangladesh ³Department of Epidemiology, Biostatistics, Community Nutrition and Noncommunicable Diseases, Peoples Integrated Alliance, Dhaka-1216, Bangladesh ⁴Department of Biotechnology and Genetic Engineering, North South University, Dhaka-1229, Bangladesh ⁵Department of Health and Nutrition, Bangladesh Institute of Medical Science, Dhaka-1207, Bangladesh ⁶Department of Biomedical Engineering, Al-Helal Specialized Hospital Limited, Dhaka-1216, Bangladesh
Diabetes mellitus, a disease characterized by abnormalities in insulin secretion and consequent hyperglycemia affects millions of people world-wide. It has been estimated that 3.8% of the rural population and a larger percentage of the urban population of Bangladesh suffers from this disease, which over the years can lead to hypertension, cardiovascular disorders, obesity, and diabetic nephropathy; to mention only a few.

**LC-MS/MS STUDY OF GINSENG METABOLISM**

Tom Moy*, Takeo Sakuma, Deolinda Fernandes, Suma Ramagiri, Robert Ellis and Carmai Seto

AB SCIEX

Ginsenosides are among a growing class of herbal and vitamin products known as nutraceuticals because they have shown pharmacological benefits to human health. Because ginsenosides appear to affect multiple pathways, their effects are complex and difficult to isolate. With millions of patients at risk of potential adverse drug-herb interactions, there is growing interest in characterizing these compounds. Understanding the ADME properties (adsorption, distribution, metabolism and elimination) of ginenosides is one way of characterizing these compounds. Liquid chromatography-mass spectrometry (LC-MS) is widely used for the identification of metabolites because of the analytical speed, sensitivity and specificity of mass spectrometers, but also the ability to perform structural elucidation. In this presentation, we study the in vitro metabolism of several ginsenosides in preparation for in vivo metabolism in the next phase of our study. Individual ginsenosides were treated with rat liver microsomal incubation in the presence of cofactor NADPH or UDPGA to yield several metabolites. The analysis was performed using a sensitive hybrid linear ion trap – triple quadrupole mass spectrometry system with metabolite identification and method-building software.

**HEALTH CARE PROVIDERS’ PERSPECTIVES ON STANDARDS OF EVIDENCE IN TCM PRACTICE AND GINSENG USE**

Ana Ning¹* and Ed Lui²

¹Department of Sociology, King’s University College at the University of Western Ontario;
²Department of Physiology and Pharmacology, University of Western Ontario

Existing social science studies on TCM in global settings have not explored the complex linkages between TCM, biomedical practitioners and the State in reference to determining appropriate evidence bases for the actual integration of TCM into mainstream health care. Identifying what constitutes “evidence” for evaluating TCM contributes to ongoing debates about the regulation of TCM and other CAM practices in North America. Thus far, in most Canadian provinces, CAM practices remain unregulated and their legitimacy continues to be evaluated according to the biomedical model, which deems randomized controlled trials (RCTs) to be the only appropriate evidence base for assessing the efficacy and safety standards of any therapeutic practice. CAM practitioners however, argue that CAM cannot always meet these standards because it is holistic and individualized in its treatment orientations, and therefore, it requires its own “testing system”. By focusing on more
encompassing constructions of “evidence”, we will document an approach entailing diverse perspectives and methods towards achieving a common goal: to establish the health benefits of traditional medicines by exploring the specific example of Ginseng use. In so doing, this project will have broader practical application by responding to increasing public demand for caring solutions to chronic health problems. For the purposes of this poster presentation, we will present a closed and open-ended approach to surveying diverse health care providers’ knowledge bases, attitudes and standards to evaluate efficacy and safety of Ginseng, among other issues in multi-site research settings. Such a data-gathering process is intended to elicit data on views and experiences integrating diverse health modalities, and the question of appropriate “evidence” for assessing TCM safety and efficacy.

LATITUDINAL VARIATION OF PHYTOCHEMICAL COMPOUNDS IN LABRADOR TEA, RHODODENDRON GROENLANDICUM, AND PITCHER PLANT, SARRACENIA PURPUREA

Michel Rapinski1*, Rui Lilin2, Asim Muhammad3, Lina Musallam4, Ammar Saleem2, Alain Cuerrier1, and John Arnason2
1Jardin botanique de Montréal, Institut de recherche en biologie végétale, 2Department of Biology, University of Ottawa, 3Department of Chemistry, University of Ottawa, 4Department of Pharmacology, Université de Montréal, CIHR Team in Aboriginal Antidiabetic Medicines

Rhododendron groenlandicum and Sarracenia pupurea are used as traditional medicines by the Cree Nation of Eeyou Istchee to treat diabetes related symptoms and have exhibited antidiabetic activity in 3T3 cell adipogenesis and C2C12 cell glucose transport respectively. Through ethnobotanical surveys, it has been mentioned that the plant’s medicinal potential augments in northern community. While there are no studies focused on the effect of a latitudinal gradient on plant biological activity, there is evidence that phenolic compounds are produced in greater quantities to protect the plants from photoinhibition. With longer day times at northern latitudes over the growing season, we hypothesized that northern populations of S. Purpurea and R. Groenlandicum will have a higher concentrations of phytochemicals and a stronger biological activity. Accessions from the surroundings of five Cree communities in the James Bay area were collected and extracted in 80% EtOH. Polyphenols were identified and quantified by applying a novel analytical method using RP-HPLC-DAD-ELSD. Although not linearly related to latitude, phytochemical results indicate a geographical variation in polyphenol concentrations.
AN INJECTABLE ANGIOGENIC POLY(PROPYlene Fumarate)/TiO₂ BASED BONE CEMENT

Mehrnaz Salarian* and Paul A. Charpentier

Department of Chemical and Biochemical Engineering, University of Western Ontario, London, Ontario, Canada

The lack of microcirculation in bone will result in osteonecrosis, which primarily occurs in the weight-bearing region such as anterosuperior aspect of the femoral head. Cementation is a reliable, newly developed approach in treating osteonecrosis of femoral head. Moreover, for treating osteonecrotic lesion neovascularization is required to encourage the healing process and graft incorporation and stimulate osteogenesis. The aim of this research is to prepare a poly(propylene fumarate)/TiO₂ nanotubes composite. Ginsenoside Rg1, a natural herbal compound isolated from ginseng, is loaded into TiO₂ nanotubes based on its activity to promote angiogenesis in vitro and in vivo. The drug release profiles are analyzed and the angiogenecity of released Rg1 is investigated by the assay of tube formation in human umbilical vein endothelial cells (HUVECs). We expect to achieve a significant increase in mechanical properties such as fracture toughness (K IC), flexural strength (FS), and flexural modulus (FM) of PPF based bone cement by providing an enhanced interfacial interaction and strong adhesion between n-TiO₂ and PPF matrix. In addition, this angiogenic bone cement composite may open a new window to treating osteonecrosis of the femoral head.

EXTRACTION OF GINSENOsIDES FROM NORTH AMERICAN GINSENG USING ULTRASOUND-ASSISTED EXTRACTION

Raziye Samimi*, Paul Charpentier, Edmund Lui, and Hua Pei

Department of Chemical and Biochemical Engineering, University of Western Ontario, London, Ontario, Canada

The North American ginsenosides were extracted by means of ultrasound-assisted extraction with methanol, DMSO, THF and acetonitrile as modifiers at the temperature 22-33°C. The HPLC analysis of individual ginsenosides demonstrated that sonication enhanced extraction efficiency especially for thermal unstable ginsenosides. The best extraction efficiency was obtained for most ginsenosides in case of using 80% solvents by HPLC analysis. Moreover, the mono-O-acetylated Rb₁ was identified by means of LC-MS-MS which was extracted with ultrasound-assisted extraction especially in case of DMSO. The direct and indirect anti-inflammatory effects of extracts investigated in RAW 264.7 murine macrophages.
BIOASSAY-GUIDED IDENTIFICATION OF ANTI-DIABETIC PRINCIPLES OF AD03, A PLANT FROM THE EEYOU ISTCHEE CREE FIRST NATIONS OF NORTHERN QUEBEC

Nan Shang1,2*, José-Antonio Guerrero2,3, Lina Musallam1,2, Diane Vallerand 1,2, Ammar Saleem2,3, John T. Arnason 2,3, and Pierre S. Haddad 1,2

1Natural health products and metabolic disease laboratory, Département de pharmacologie, Faculté de médecine, Université de Montréal; 2Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center; 3Medicinal Plant and Ethnopharmacology laboratory, Department of biology, University of Ottawa

Type 2 diabetes is a major health problem all over the world, but certain populations are particularly at risk. Among indigenous populations, the Cree of Eeyou Istchee (CEI) from northern Quebec are more affected by obesity and type 2 diabetes. The average incidence of type 2 diabetes currently nears 20% among adults or 3-5 times higher than the rest of the Canadian population. Through an ethnobotanical study, we identified 17 plants from the Cree pharmacopeia to treat symptoms of diabetes. One of these plants, AD03, demonstrated a strong anti-diabetic potential by stimulating adipogenesis 2.4-3 folds, a surrogate assay of PPAR-gamma agonism. We therefore used the latter assay to isolate the active principles from this plant by using an activity-guided fractionation approach. Adipogenesis was assessed by measuring the accumulation of triglycerides in differentiated adipocytes in culture (3T3-L1 cell line). Five compounds were isolated from the most active fraction of AD03. Two of those had more potent activity than the crude extract (3-4.5 fold increase in adipogenesis), but less than the active fraction (5.5 fold increase). This suggests that the anti-diabetic activity of AD03 could be attributed to multiple compounds exhibiting additive or synergistic actions, rather than to a single active compound. Funded by CIHR and China Scholarship Council.

EFFECTS OF AMERICAN GINSENG ON INTESTINAL LIPID SECRETION AND PLASMA CLEARANCE IN Pcyt2 DEFICIENT MICE

Ratnesh Kumar Singh* and Marica Bakovic
University of Guelph

We have initially established an elevated intestinal lipid-triglyceride (TG) secretion and a reduced lipid clearance in a new animal model for the human metabolic syndrome-the CTP: ethanolaminephosphate cytidylyltransferase mice (Pcyt2+/−). In this study, we investigate if ginseng treatments could reduce the elevated TG lipid content in the plasma of the Pcyt2+/− mice. The Pcyt2+/− 32-week old, obese and hyperlipidemic female mice (n=8) were assigned into two groups of which one group served as control (were orally administered only 0.9% saline) and the other group was treated with ginseng ethanol extract at a daily dose of 200 mg/kg for four weeks. The rate of intestinal lipid secretion at different time points was investigated after a single intragastric fat-load of olive oil containing [3H]-glycerol trioleate (TO). The obtained data demonstrated that the intestinal, postprandial release of [3H]-TO into plasma become significantly reduced in the ginseng treated animals compared to the saline treated littermate controls. To investigate the effect of ginseng on
plasma lipid-lipoprotein clearance, at the end of the feeding period the ginseng treated and the saline treated control mice were intravenously injected with $[^3H]$-TO labeled VLDL-like particles. Based on the rate of $[^3H]$-TO disappearance, no difference in plasma lipid clearance between the two groups was found. Ginseng treatments however significantly enhanced the lipoprotein lipase activity in the plasma, liver and heart of Pcyt2$^{-/-}$ mice. Our data suggest that ginseng may play multiple roles in reducing plasma TG content, and as such it could represent a valuable application for the treatment of human hyperlipidemia.

**INHIBITION OF BACTERIAL QUORUM SENSING (QS) BY TROPICAL AND ANTI-INFECTIVE PLANTS**

Chieu Anh Ta*1, Marie Freundorfer1, Ana Gargaun2, Mario Garcia Quesada3, Marco Otarola Rojas3, Pablo Sanchez-Vindas3, Luis Poveda3, Victor Cal4, Tony Durst2, and John T. Arnason1

1Department of Biology, Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Canada; 2Department of Chemistry, D'Iorio Hall, University of Ottawa, Ottawa, Canada; 3Herbario Juvenal Valerio Rodriguez, Escuela De Ciencias Ambientales, Universidad Nacional, Costa Rica; 4Belize Indigenous Training Institute, Punta Gorda, Belize

This is a new approach to the discovery of new phytochemicals from tropical plants that can interfere with the formation of bacterial biofilms. Bacteria use a cell-to-cell communication system known as quorum sensing (QS) to coordinate gene expression for the formations of these biofilms. Ethanolic extracts of 48 Costa Rican plant species and 24 traditional anti-infective Q’eqchi’ plants were screened for QS interference. A modified disc diffusion assay was used to screen ethanolic extracts of different plant parts for QS interference using *Chromobacterium violaceum* ATCC 12472. Disruption of QS is indicated by inhibition of violacein production (under QS control). Six Costa Rican plant extracts from the Piperaceae and Meliaceae three traditional medicines from the Melastomataceae and Combretaceae showed promising activities with zones of inhibition ranging from 10.4.± 0.08 mm to 27.7 ± 0.6 mm. Interestingly, none of these species have been reported to have QS-disrupting activities and very little is known about their phytochemistry.

**IN VITRO PROPAGATION AND CRYOPRESERVATION METHOD FOR THE NORTH AMERICAN GINSENG**

Esther Uchendu* and Praveen Saxena

University of Guelph

Ginseng (*Panax* sp.) is a popular medicinal plant consumed world-wide for its medicinal benefits. We developed an efficient liquid-based propagation system using nodal segments of *Panax quinquefolius* L., quantified phenolic production in vitro by spectrophotometric analysis and determined the effects of phenolic reducing chemicals (activated charcoal, vitamin C, polyvinylpyrrolidone and melatonin) and plant growth regulators on organogenesis. We also tested the effects of sucrose and abscisic acid (ABA) pretreatments on shoot tips and cotyledonary leaf explants both at 4 °C and following storage in liquid nitrogen. Nodes and shoots production was best (81%) with kinetin. The highest number of
shoot production per explant occurred with 2.5 mg/l kinetin. NAA (0.5 mg/l) gave the greatest average root production (78%) followed by IAA (50%). All plants produced had normal appearance and over 90% were established in the greenhouse. Phenolics production significantly increased over a 4-week period. Activated charcoal (50 mg/l) was the most effective treatment and significantly reduced total phenolics from 81ug/ml to 34 ug/ml. Shoot tips and cotyledons had 50-70% regrowth when pretreated with sucrose or ABA for one week at 4°C, warmed at room temperature and moved to recovery medium. Shoot tips pretreated with both ABA and sucrose at 4°C and then cryopreserved by PVS2 vitrification had 60% regrowth. Shoot tips had normal development but cotyledons produced embryogenic callus. Production of shoots in liquid medium from nodal segments is a viable option for the micropropagation of ginseng and shoot tips or cotyledonary leaf explants can be cryopreserved to improve the security of ginseng through long-term ex-situ conservation.

ETHNOBOTANY AND PHARMACOLOGY OF ANTI-INFLAMMATORY BOTANICALS USED BY THE Q’EQCHI’ MAYA OF BELIZE

Brendan Walshe-Roussel1*, Christine Choueiri2, Max London1, Ammar Saleem1, Federico Caal3, Victor Cal3, Todd Pesek4, Tony Durst2, John Thor Arnason1

1Ottawa-Carleton Institute of Biology, University of Ottawa, Ottawa, Ontario, Canada; 2Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada; 3Belize Indigenous Training Institute (BITI), Punta Gorda, Toledo District, Belize; 4Department of Health Sciences, Center for Healing Across Cultures, Cleveland State University, Cleveland, Ohio, USA

Inflammation has been implicated as a causative or contributing factor in the pathology of many disorders and a wide range of symptoms have been associated with inflammatory conditions. As such, immunomodulation represents an important target in attempts to understand and treat many categories of illness. Indigenous pharmacopeias recognize the important role of inflammation in disease, and the Q’eqchi Maya healers of Belize possess a practical understanding of a large number of botanical treatments with immunomodulatory effects. Ethnobotanical interviews were held with 5 Q’eqchi Maya healers using a list of 14 inflammatory symptom categories and one hundred and seven plant species were collected from primary and secondary semi-evergreen rainforest in the Maya mountains of S. Belize. Ethanolic extracts of fifty-five species were assayed for anti-inflammatory activity in a LPS-stimulated THP-1 monocyte assay. 76% of all species assayed demonstrated significant anti-inflammatory activity relative to the vehicle control, and three species displayed anti-inflammatory activity equal to that of the parthenolide positive control. In addition, several sesquiterpene lactones isolated from the traditionally used *Neurolaena lobata* exhibit potent anti-inflammatory activity.
IMPROVEMENT OF A MICROPROPAGATION PROTOCOL FOR NORTH AMERICAN GINSENG (PANAX QUINQUEFOLIUS L.) AND FIELD PERFORMANCE OF CLONAL PLANTS

Sijun Zhou* and Daniel C. W. Brown

University of Western Ontario

A previously established six-step micropropagation protocol was successfully improved by using one to two-week-old seedlings as starting material to induce somatic embryos. Seedlings from ½ MS containing 0.5 mg l⁻¹ GA₃ and 0.1 mg l⁻¹ BA give the best results. Compared to the old protocol, in which embryos were induced from cotyledons excised from stratified seeds on MS medium without regulators, the frequency, number and quality of embryos induced from seedling explants are higher. Embryogenesis frequency was increased from 70% to 95%. Embryos from seedling explants are separate from each other and separate from explants. Quality of somatic embryos was improved by placing cotyledonary-stage embryos on a new maturation medium, ½ MS containing 0.5% activated charcoal and 3% sucrose, and incubating for a prolonged period (2 to 4 months) under 15 °C. Germination rate of the high quality embryos was raised from 54% to 70%. A punched transparent plastic cover on the tray with newly potted plants resulted in an easy and high efficient acclimation of the plants. Field performance results indicated that there were no significant morphological and phytochemical differences between micropropagated plants and seed-derived plants.

SCREENING FOR PESTICIDE RESIDUES AND MYCOTOXINS IN GINSENG ROOTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Yun Yun Zou*, Takeo Sakuma, André Schreiber, Robert Ellis and Tom Moy

AB SCIEX

Mycotoxins are produced by fungi and often found in plants and foods: such as vomitoxin, nivalenol, deoxynivalenol in wheat, aflatoxins in peanuts, rice and ginseng roots. Some of them are highly toxic to human and livestock. Pesticides are widely used in ginseng farms to prevent pests and fungi growth. However, pesticides have potential toxicity to human and animals. Many countries regulate maximum residue limits for both mycotoxins and pesticides. Here we present 2 simple and rapid screening methods for either 171 mycotoxins and their metabolites, or 494 pesticides. American ginseng roots from Ontario farms, white and a red ginseng samples from Korea were extracted and analyzed. Spiked ginseng samples were also analyzed to verify our methods. The analysis was performed using an Agilent 1100 LC with a Phenomenex Gemini C₁₈ column for mycotoxins and Fusion-RP column for pesticides, respectively. Compounds were detected using an AB SCIEX 4000 QTRAP® LC/MS/MS system with electrospray ionization in positive polarity. The hybrid triple quadrupole-linear ion trap system was operated in selective multiple reaction-monitoring (MRM) mode for sensitive quantification and additional enhanced MS/MS spectra were collected for compound identification based on mass spectral library searching. Typical LC-MS/MS analysis takes 20.5 minutes for mycotoxins and 17.5 minutes for pesticides.
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</tr>
<tr>
<td>Ning</td>
<td>King's College University</td>
<td><a href="mailto:aning@uwo.ca">aning@uwo.ca</a></td>
<td></td>
</tr>
<tr>
<td>Pandey</td>
<td>University of Windsor</td>
<td><a href="mailto:spandey@uwindsor.ca">spandey@uwindsor.ca</a></td>
<td></td>
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<tr>
<td>Pei</td>
<td>University of Western Ontario</td>
<td><a href="mailto:hpei@uwo.ca">hpei@uwo.ca</a></td>
<td></td>
</tr>
<tr>
<td>Rapinski</td>
<td>University of Montreal</td>
<td><a href="mailto:mrapi090@uottawa.ca">mrapi090@uottawa.ca</a></td>
<td></td>
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<tr>
<td>Rogers</td>
<td>University of Western Ontario</td>
<td><a href="mailto:krogers@uwo.ca">krogers@uwo.ca</a></td>
<td></td>
</tr>
<tr>
<td>Salarian</td>
<td>University of Western Ontario</td>
<td><a href="mailto:msalaria@uwo.ca">msalaria@uwo.ca</a></td>
<td></td>
</tr>
<tr>
<td>Samimi</td>
<td>University of Western Ontario</td>
<td><a href="mailto:rsamimih@uwo.ca">rsamimih@uwo.ca</a></td>
<td></td>
</tr>
<tr>
<td>Saxena</td>
<td>University of Guelph</td>
<td>psaxena@uoguelph</td>
<td></td>
</tr>
<tr>
<td>Shang</td>
<td>University of Montreal</td>
<td><a href="mailto:nan821208@yahoo.com.cn">nan821208@yahoo.com.cn</a></td>
<td></td>
</tr>
<tr>
<td>Singh</td>
<td>University of Guelph</td>
<td><a href="mailto:ratneshs@uoguelph.ca">ratneshs@uoguelph.ca</a></td>
<td></td>
</tr>
<tr>
<td>Siow</td>
<td>University of Manitoba; AAFC</td>
<td><a href="mailto:YawChris.Siow@agr.gc.ca">YawChris.Siow@agr.gc.ca</a></td>
<td></td>
</tr>
<tr>
<td>Sutherland</td>
<td>Afexa Life Sciences Inc.</td>
<td><a href="mailto:ssutherland@afexa.com">ssutherland@afexa.com</a></td>
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<tr>
<td>Last Name</td>
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<td>Affiliation</td>
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<tr>
<td>Ta</td>
<td>Chieu Anh</td>
<td>University of Ottawa</td>
<td><a href="mailto:chieu.anh@gmail.com">chieu.anh@gmail.com</a></td>
</tr>
<tr>
<td>Todd</td>
<td>Jim</td>
<td>Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)</td>
<td><a href="mailto:jim.todd@ontario.ca">jim.todd@ontario.ca</a></td>
</tr>
<tr>
<td>Uchendu</td>
<td>Esther</td>
<td>University of Guelph</td>
<td><a href="mailto:euchendu@uoguelph.ca">euchendu@uoguelph.ca</a></td>
</tr>
<tr>
<td>Walshe-Roussel</td>
<td>Brendan</td>
<td>Ottawa-Carleton Institute of Biology</td>
<td><a href="mailto:bwals056@uottawa.ca">bwals056@uottawa.ca</a></td>
</tr>
<tr>
<td>Westerveld</td>
<td>Sean</td>
<td>Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)</td>
<td><a href="mailto:sean.westerveld@ontario.ca">sean.westerveld@ontario.ca</a></td>
</tr>
<tr>
<td>Windust</td>
<td>Anthony</td>
<td>NRC Canada</td>
<td><a href="mailto:anthony.windust@nrc.ca">anthony.windust@nrc.ca</a></td>
</tr>
<tr>
<td>Wu</td>
<td>Yan</td>
<td>University of Western Ontario</td>
<td><a href="mailto:ywu287@uwo.ca">ywu287@uwo.ca</a></td>
</tr>
<tr>
<td>Zhang</td>
<td>Junzeng</td>
<td>National Research Council - Institute for Nutrisciences and Health</td>
<td><a href="mailto:junzeng.zhang@nrc.ca">junzeng.zhang@nrc.ca</a></td>
</tr>
<tr>
<td>Zhou</td>
<td>Sijun</td>
<td>University of Western Ontario</td>
<td><a href="mailto:sijun.zhou@agr.gc.ca">sijun.zhou@agr.gc.ca</a></td>
</tr>
<tr>
<td>Zhu</td>
<td>Ming</td>
<td>Beijing University of Chinese Medicine &amp; Pharmacology</td>
<td><a href="mailto:zhutaiping_1930@163.com">zhutaiping_1930@163.com</a></td>
</tr>
<tr>
<td>Zou</td>
<td>YunYun</td>
<td>AB SCIEX</td>
<td><a href="mailto:yunyunzou@absciex.com">yunyunzou@absciex.com</a></td>
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