Pre-Clinical Workshop

“Moving from Pre-Clinical Research to Clinical Trials”

Wednesday, December 1, 2010
11:00 am – 4:00 pm

Physiology and Pharmacology Boardroom
Medical Sciences Building, Room 212B
UWO Campus
# Workshop Agenda

<table>
<thead>
<tr>
<th>Time</th>
<th>Topics</th>
<th>Speaker</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.00 am</td>
<td>Welcome &amp; introduction</td>
<td>E. Lui</td>
<td>UWO (Phys/Pharm)</td>
</tr>
<tr>
<td></td>
<td><strong>Metabolic syndrome, diabetes complication, erectile dysfunction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.10</td>
<td></td>
<td>M Bakovic</td>
<td>U Guelph (Health &amp; Nutrition)</td>
</tr>
<tr>
<td>11.20</td>
<td></td>
<td>C Chakrabarti</td>
<td>LHSC/UWO (Pathology)</td>
</tr>
<tr>
<td>11.30</td>
<td></td>
<td>L Coolen</td>
<td>UWO (Mic/Imm; Phys/Pharm)</td>
</tr>
<tr>
<td>11.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.30 – 1.00</td>
<td>Discussion: D Spence¹, group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.00</td>
<td></td>
<td>K Rogers</td>
<td>UWO (Anatomy &amp; Cell Bio)</td>
</tr>
<tr>
<td>12.10</td>
<td></td>
<td>E Lui</td>
<td>UWO (Phys/Pharm)</td>
</tr>
<tr>
<td>12.20</td>
<td></td>
<td>QP Feng</td>
<td>UWO (Phys/Pharm)</td>
</tr>
<tr>
<td>12.30</td>
<td></td>
<td>M Karmazyn</td>
<td>UWO (Phys/Pharm)</td>
</tr>
<tr>
<td>12.30 – 1.00</td>
<td>Discussion: J. Younus², group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td></td>
<td>Madrenas</td>
<td>Robarts/UWO (Micro/Imm)</td>
</tr>
<tr>
<td>1.10</td>
<td></td>
<td>Lui</td>
<td>UWO (Phys/Pharm)</td>
</tr>
<tr>
<td>1.20</td>
<td></td>
<td>E. Noble</td>
<td>UWO (Kinesiology)</td>
</tr>
<tr>
<td>1.30 – 1.50</td>
<td>Discussion: J. Younus², group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Cardiovascular health

<table>
<thead>
<tr>
<th>Time</th>
<th>Topics</th>
<th>Speaker</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Immunity & stress

<table>
<thead>
<tr>
<th>Time</th>
<th>Topics</th>
<th>Speaker</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00 – 4.00 pm</td>
<td><strong>Moving forwards</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NHPs sector perspective</td>
<td>G. Leong</td>
<td>Jamieson Labs (VP Sci &amp; Reg)</td>
</tr>
<tr>
<td></td>
<td>Areas of focus, commercialization</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Funding opportunities and challenges</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical-basic integration</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trial design: multiple targets, N=1 study</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metabolomics</td>
<td>S Ramagiri</td>
<td>AB-Sciex</td>
</tr>
<tr>
<td></td>
<td>Test materials: criteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.00 pm</td>
<td>Adjournment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Dr. J. David Spence, dspence@robarts.ca  
Div of Neurology, Dept Clinical Neurological Sciences, SSMD  
Div of Clinical Pharmacology, Dept of Medicine, SSMD  
Robarts Research Institute - Director, SPARC (Stroke Prevention & Atherosclerosis Research Centre)

²Dr. J. Malcolm O. Arnold, malcolm.arnold@lhsc.on.ca  
Division Cardiology, Dept of Medicine - SSMD  
Dept Physiology & Pharmacology - SSMD  
LHSC-UH - Cardiologist  
LHRI Scientist -Circulation program leader  
*Heart failure, Cardiology, Drugs and heart disease, Blood vessel function, Clinical trials*
**Research Summaries**

**Investigator:** Marica Bakovic, University of Guelph  
**Title:** Effects of American Ginseng on Intestinal Lipid Secretion and Plasma Clearance in Pcyt2 Deficient Mice

Initially we 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+/−). We further investigated if ginseng treatments could reduce the elevated TG lipid content in the plasma of the $\text{Pcyt2}^{+/−}$ mice. The $\text{Pcyt2}^{+/−}$ 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 $[^{3}H]$-glycerol trioleate (TO). The obtained data demonstrate that the intestinal, postprandial release of $[^{3}H]$-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 $[^{3}H]$-TO labelled VLDL-like particles. Based on the rate of $[^{3}H]$-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 $\text{Pcyt2}^{+/−}$ mice.

Our data suggest that ginseng may play multiple roles in reducing plasma lipolytic activity and TG content, and as such it could represent a valuable application for the treatment of human hyperlipidemia.

We will compare those data with our previous trials when we observed that ginseng also reduce liver steatosis and insulin resistance.
Investigator: Subrata Chakrabarti, Department of Pathology, UWO
Title: Prevention of Chronic diabetic complications by ginseng.

Diabetic complications affect various organs including retina and kidney. We have previously demonstrated that glucose-induced endothelin-dependent increased synthesis of fibronectin (FN) and its splice variant EDB+ FN are mediated via the activation of NFκB and AP-1. Hyperglycemia causes several metabolic defects and produce oxidant injury. In diabetes, due to oxidative stress, increased DNA strand breaks and the activation poly(ADP-ribose) polymerase (PARP) may activate NFκB. Ginseng is a known antioxidant has been shown to prevent oxidative lipid peroxidation and kidney damage in rats. Therefore we propose to study ameliorative effects of ginseng on diabetes induced biochemical, functional and structural changes in the retina and kidney. Initially we will establish these mechanisms in an endothelial cell culture. For in vivo studies, will use both type 1 (STZ-induced) and type 2 (db/db) mice models. Ginseng will be administered daily (200mg/kg by gavage). Blood, urine, retina, heart and kidney will be collected. Blood glucose, glycated hemoglobin, albumin, creatine and ketone bodies will be measured. Cardiac hemodynamic analysis will be carried out. mRNA and protein levels of FN, EDB+FN, endothelin, nitric oxide synthase and vascular endothelial growth factor will be quantitatively analyzed by real time RT-PCR and western blotting. Oxidative stress markers, namely 8-OHdG and nityrotyrosine will be analyzed. PARP activation will be measured by [3H]–NAD incorporation and ss-strand breakage. Morphological and morphometric analysis of the tissues will be performed for glomerular basement membrane thickening and mesangial matrix expansion. Initial studies demonstrate that ginseng has significant preventive effects on the development of chronic diabetic complications both in vivo and in vitro. We will continue to work and complete these projects.

Investigator: Lique Coolen, Depts. Anatomy & Cell Biology; Physiology and Pharmacology, UWO
Title: Effects of ginseng preparations on sexual function and libido
Research summary not available

Investigator: David Mutch, University of Guelph
Title: Investigating the effects of ginseng extracts on adipocyte function using nutrigenomics

Current HQP involved in project: Sarah Wilson (undergraduate student)
Past HQP involved in project: Anna Deboer (former undergraduate student)
Summary of activities: Ginseng (Panax quinquefolium) is commonly used throughout traditional Chinese medicine for the purposes of modulating the immune system, regulating blood glucose homeostasis, etc. Existing evidence suggests that specific ginseng extracts have immunomodulatory activity in macrophage; however, the role of ginseng in adipocytes remains largely unexplored. Our current activities are focused on the study of ginseng on adipokine expression and secretion. We are using
differentiated 3T3-L1 adipocytes to study the effect of both the ethanol (EtOH) and aqueous (Aq) ginseng extracts. Cells were treated with extracts for 48hrs in serum-free media and tested in a range of doses: 12.5 – 50 µg/mL. These doses were found to be non-toxic using an enzymatic kit. Messenger RNA is extracted and real time RT-PCR was used to analyze the expression of adipokines, including leptin, adiponectin, IL-6, NFkB, TNFα and iNOS. We are also confirming changes in gene expression by assaying adipocyte secretion in the media of adipocytes (ongoing). The Aq extract significantly increased adipocytokine expression in a dose-dependent manner (α = 0.01; n = 8). At the maximum dose of 50µg/mL, the Aq extract stimulated the expression of IL-6 by approximately 270-fold, TNFα by 4.6-fold, NFkB by 7.3-fold and iNOS by more than 40-fold. There was no effect on leptin or adiponectin expression. The EtOH ginseng extract had no affect on these markers. We plan to examine whether the Aq extract signals via the TLR4 pathway. Currently, we are validating a commercially available TLR-4 chemical inhibitor that we plan to use in our experiments (ongoing). We are also performing studies to determine whether the Aq and EtOH extracts affect lipid accumulation in adipocytes by studying their effect on differentiation. This will be determined by assaying gene expression for markers of differentiation, as well as measuring lipid profiles by gas chromatography (planned for the first quarter of 2011).

**Dissemination of Results:** Abstract for a poster presentation will be submitted shortly for the upcoming Canadian Obesity Network meeting in Montreal (April/May 2011). A manuscript is anticipated by the end of summer 2011.

---

**Investigators:**

Kem Rogers (Dept. Anatomy & Cell Biology, UWO)

Co-authors: Colin P. Carruthers & Jessica Y. Davie

**Title:** The influence of North American Ginseng on the initiation and progression of atherosclerosis.

**Hypothesis**

Ginseng will reduce serum total cholesterol levels, and decrease lipid deposition and plaque formation in a rabbit model of atherosclerosis.

**Objective**

This long-term rabbit study will allow us to assess the effects of ginseng administration on the initiation and progression of later stages of atherosclerosis, including lipid accumulation and plaque formation. Rabbits provide a relevant model of human disease, forming human-like lesions on a diet of normal chow supplemented with 0.25% cholesterol.

**Research Findings**

To determine the effectiveness of ginseng as a preventative treatment, rabbits will be divided into 3 groups (n=10 each). The rabbits will either be fed a high cholesterol diet alone or supplemented daily with 250mg/kg of ethanol or aqueous ginseng extract for a period of four months.
Every two months throughout the study, we will collect blood samples from the rabbits to assess the
effects of ginseng on serum total cholesterol levels. Following sacrifice, we will remove aortas, cut them
longitudinally, and stain with Oil Red O to detect lipid deposition. The aortas will then be pinned out,
and the area of Oil Red O staining compared to total aorta area will allow quantification of lipid
deposition and plaque formation. We will also collect tissue, from the aortic arch, between the
abdominal and thoracic aorta, and from the liver for RNA analysis by quantitative RT-PCR.

Future Directions
A second cohort of rabbits will be sacrificed after 7 months, and will be divided up into 4 groups (n=10
each). The first group will consist of rabbits fed normal chow for the entire 7 months, and the second
fed a high cholesterol diet for the entire 7 months. The last two groups will be fed a high cholesterol
diet for the entire 7 months, but following atherosclerotic lesion formation (4 month time point) they
will begin receiving either 125 or 250mg/kg aqueous ginseng extract daily for the final 3 months. This
model allows us to determine the effectiveness of ginseng as an interventional therapy in
atherosclerosis, and the same analysis as above will be carried out.

This study provides us with a system to assess the effects of ginseng on the initiation and progression of
atherosclerosis, and also the optimal timing of ginseng administration. Since ginseng can be taken by
healthy people, it is critical to know if there are any preventive effects, so that patients in at-risk groups
for atherosclerosis may begin a treatment regimen before experiencing a cardiac event.

Investigator: Ed Lui (Dept. Physiology and Pharmacology, UWO)

I. Cardiovascular health
Title: Effects of water (Aq) and hydro-alcoholic (HA) ginseng extracts on vascular injury induced by
chronic Homocysteine treatment
Homocysteine (Hcy) is an independent risk factor for atherosclerosis and other vascular lesions. It causes
endothelial dysfunction and 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 (low dose)and 500 (high dose) 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 anaesthesia. 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.
Hcy group significantly reduced the contractile response of aortic ring to phenylephrine (PE) and impaired Acetylcholine (ACh)-, Isoproternol (Isop)- and 3-morpholinosydnonimine (SIN-1)- 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 all ginseng treatment groups except the high dose of HA extract. These functional changes were reflected in similar histological findings. In addition, results from Dr Rogers showed that ginseng treatment reduced monocyte adhesion to aortic tissues induced by Hcy by histochemial analysis.

Our study showed that ginseng extract and in particular the Aq extract was effective in protecting rat aortic tissues from injury induced by chronic Hcy treatment.

Other findings: 1. In vitro studies using cultured aortic rings have been conducted to examine mechanism of action. 2. Direct effect of ginseng on vascular tone: Ginseng extract induced relaxation of PE-precontracted aortic ring: Aq > HA. 3. Contractile –relaxation response has been conducted with tissues of Zucker rats treated with ginseng (via Dr Coolen), and the result is being analysed.

II. Immunity
Title: The Yin and Yang actions of NA ginseng in modulating the immune function of macrophages.

Different and inconsistent immuno-modulatory effects of ginseng have been reported by various investigators, including both immuno-stimulatory and immuno-suppressive effects. The first objective of this study was to characterize the paradoxical effects of ginseng by examining the immuno-modulatory effect of aqueous (AQ) and alcoholic (ALC) extracts of North American ginseng roots in murine macrophages to determine the role of extraction methodology on bioactivity. Results showed that AQ extract possessed immuno-stimulatory effect by up-regulating production of NO, TNF-α and IL-6 in a dose dependent manner, while ALC extract did not. On the other hand, ALC but not AQ was found to suppress the up-regulation of macrophage NO and TNF-α production induced by LPS in a dose-dependent manner. These extract-related anti-inflammatory and pro-inflammatory effects may be considered as the Yin and Yang actions of ginseng. Consistent with this concept, the macrophage-stimulating activity of the AQ extract was found to be inhibited in the presence of ALC extract. The phytochemical basis for this immuno-modulatory phenomenon was studied by examining extract-specific immuno-active chemical constituents of ginseng extracts. Sephadex G-75 chromatographic fractionation of AQ extract revealed the presence of two major peaks (Fraction I and III) at 230 nm with average molecular weights of 76,000 and 33,000 dalton. The first fraction had similar elution volume as the crude polysaccharide (PS) isolated from the AQ extract, and both components were found to stimulate macrophage response, while fraction III was inactive. However, their immuno-stimulatory potency was considerably lower than that of the AQ extract, suggesting that these macromolecular constituents may not be solely responsible for the immuno-stimulatory effect of AQ extract. Parallel fractionation study of ALC extract yielded similar elution profile; however, both sub-fractions were devoid of PS. These two non-polysaccharides macromolecular fractions were found to suppress LPS-induced NO production dose-dependently. It was also observed that small molecular weight ginsenoside-like compounds did not posses immunosuppressive activity. In conclusion, this study
showed that variation in the chemical constituents of ginseng extracts could drastically influence their immuno-bioactivity towards macrophages; and this may have important implications in the production, design, regulation of ginseng extracts regarding their use and misuse by consumers.

Investigator: Qingping Feng (Dept. Physiology and Pharmacology, UWO)
Title: Cardioprotective Effects of North American Ginseng

CURRENT ONGOING STUDIES
1. **Ginseng protects the heart from ischemia and reperfusion injury by activating PI3K/Akt-dependent eNOS pathway**

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.

2. **Effects of Ginseng on Cytokine Expression and Cardiac Function during Sepsis**

Sepsis is a consequence of infectious diseases. Endotoxins or lipopolysaccharides (LPS) of Gram-negative bacteria are pathogens responsible for myocardial depression during sepsis. LPS induces tumor necrosis factor-alpha (TNF-\(\alpha\)) production, a proinflammatory cytokine, which has been proposed as a major mediator responsible for cardiac dysfunction during sepsis. Ginseng has been shown to have anti-inflammatory effects. We are currently studying the effects of ginseng on myocardial TNF-\(\alpha\) expression and cardiac function during sepsis. Preliminary data showed that aqueous ginseng extracts inhibited LPS-induced TNF-\(\alpha\) expression in cultured cardiomyocytes and decreased myocardial TNF-\(\alpha\) expression \textit{in vivo} in mice with endotoxemia. Ongoing work is to study the effects of ginseng on cardiac function during endotoxemia.

FUTURE DIRECTIONS
1. Large animal studies (e.g., pigs) to confirm findings from rodents.
2. Potential clinical studies in patients with acute myocardial infarction
3. Potential clinical studies in patients with sepsis
Investigator: Dr. Karmazyn (Dept. Physiology and Pharmacology, UWO)

Title: Antihypertrophic effects of ginseng

The nature of our research involves the potential ability of ginseng to attenuate myocardial hypertrophy, remodelling and heart failure. We have completed or nearly completed two primary studies. In Study 1 (carried out principally by Juan Guo) we investigated the effect of ginseng on hypertrophy and heart failure using both *in vitro* and *in vivo* approaches. In Study 2 (carried out principally by Melissa Moey) the effect of ginseng on the hypertrophic effect of leptin was the principal goal.

**Study 1**

Using both isolated cultured ventricular myocytes as well as an *in vivo* model of heart failure secondary to myocardial infarction we showed that ginsenosides were able to effectively attenuate cardiac hypertrophy and heart failure by maintaining intracellular sodium and calcium homeostasis. Further studies revealed that the anti-hypertrophic effect of ginsenosides was mediated by inhibiting sodium-hydrogen exchange-(NHE-1) activity and blocking calcium mediated signalling which was evidenced by decreased intracellular calcium levels, calcineurin activity and NFAT3 translocation. In support of *in vitro* findings, our *in vivo* studies demonstrated that oral administration of ginsenosides prevented the progression of heart failure by reducing post-infarction myocardial remodelling as evidenced by diminished hypertrophy and improved hemodynamics. Thus, by combining *in vitro* and *in vivo* approaches, we provide the first comprehensive evidence for identifying the molecular mechanisms underlying the beneficial effects of ginsenosides to inhibit cardiac hypertrophy and also improving cardiac function. This work has recently been published on line in *Circulation: Heart Failure*.

**Study 2**

In another study nearing completion we have found that ginseng is also a potent inhibitor of the hypertrophic effects produced by the obesity-related adipokine leptin. The hypertrophic effect of leptin is mediated by complex cell signalling processes although a key factor appears to involve the activation of the RhoA/ROCK pathway which results in the subsequent modulation of actin dynamics which contributes to the hypertrophic program. We have found that ginseng’s ability to abrogate leptin-induced hypertrophy is associated with prevention of RhoA/ROCK activation. Further studies revealed that this was due to prevention of activation of a specific guanine-nucleotide exchange factors (Rho-GEF) which catalyze the exchange of GDP for GTP and which is critical for RhoA activation.

**Preliminary Studies**

We have initiated two preliminary studies. In one, we are determining the role of calcineurin in iNOS upregulation during hypertrophy and the effect of ginseng whereas in the second study we are determining whether ginseng can reverse hypertrophy and heart failure once these are established. Initial pilot data are promising.
Investigator: Joaquín Madrenas (Robarts Research Institute/Dept. Microbiology & Immunology, UWO)  
madrenas@robarts.ca . Telephone: 519-663-5777 ext. 24242, FAX: 519 – 931-5268

Title: Modulatory Effects of Ginseng Extracts on Human Innate and Adaptive Immune Responses

Abstract

Ginseng (GS) has been used as an herbal remedy for thousands of years based on reported beneficial biological effects including enhanced immunity. The precise cellular and molecular nature of immunomodulatory effects remain unclear because most of the studies to date have used neoplastic cell lines whose results may not be extrapolated to normal human immune cells. Here, we report the effects of standardized North-American ginseng (Panax quinquefolius) extracts (GS) (ethanol, aqueous and crude polysaccharide extracts) on innate and adaptive immune responses of human peripheral blood mononuclear cells (PBMCs) of healthy volunteers. We found that endotoxin (LPS)-free GS by itself induced the production of pro-inflammatory cytokines (IL-1β, IL-6, TNFα) and of IL-10 by PBMCs in a dose-dependent manner. Of the three extracts of GS tested, the aqueous extract was the most potent although variability among the GS sources was observed. GS extracts did not inhibit but rather enhanced the proinflammatory response induced by LPS. However, the T-cell IL-2 response to bacterial superantigens was down-regulated in the presence of GS. The immunomodulatory effects of GS were associated with activation the MAPK (ERK1/2), the PI3K/Akt, and the NF-κB pathways and could be inhibited by pharmacological blockade of these pathways. Preliminary chemical fractionation of GS extracts suggests that its immunomodulatory effects may be mediated by high molecular weight compounds distinct from ginsenosides. Based on these results, we conclude that GS, and in particular its aqueous extract containing high molecular weight compounds, has modulatory properties on innate and adaptive immunity through a complex profile of pro-inflammatory and anti-inflammatory cytokine production. This work should help to focus the search for compounds in these extracts with specific immunomodulatory activities.

Investigators: Earl Noble and John Trevithick

Title: The Effect of Ginseng on Exercise Capacity and Vascular Function in Diabetic Rats/Modeling Experimental Cataract Risk Reduction by Ginseng Extracts

Panax quinquefolius or American Ginseng has been shown in vitro to have anti-inflammatory effects by downregulating the NF-kB pathway at various steps. Since most models studied have used immune cells as a model of inflammation it is of interest to see if similar events occur within muscle cells, and whether or not these effects may enhance physical performance or reduce muscle damage. Our in vivo experiments will look at the effects of a 2 week treatment of 300mg/kg Alcoholic Ginseng Extract (AGE), Aqueous Ginseng Extract (AQE) compared to their complementary placebo or naïve group. Daily body weights, non-invasive blood pressure measurements (twice/week) and fasting blood glucose levels were measured. At the end of the 2 weeks, each treatment group was randomly divided into two groups, a) Exercise and b) Sedentary. 24 hrs after a 1 hour bout of downhill running (-14 degree) designed to cause muscle damage, animals were sacrificed and blood and muscle tissues were harvested for analysis.
Preliminary Findings:
**Body Weights:** No significant differences were observed between groups.
**Blood Glucose:** No significant differences were observed between groups.
**Blood Pressures:** Limited differences were observed between groups.
**Lens Opacification:** No significant differences were observed between groups.
**Lens Focusing Ability (BVD):** Exercise tended to decrease the variability in focusing of rat lenses. This difference was significant for the exercised group receiving the alcohol extract.

Ongoing Measurements:
Creatine Kinase will be analyzed in the blood serum to see if significant structural damage has occurred globally within the muscles. Citrate synthase will also be used as a marker to determine if ginseng had any metabolic effects. H & E staining within the Medial Triceps, Soleus and Red Vastus muscles will be employed to assess local damage. Western Blot Analysis will indicate activation of the NF-kB by examining the phosphorylation of the IKKa/b and IkBa proteins to their activated form; p-IKKa/B and p-IkBa. Translocation of the liberated Nf-κB complex (the p-65-p50 dimer) will be examined by nuclear fractionation. Lastly heat shock proteins which are associated with the stress response and muscle protection will be evaluated.

Biography: Dr. Suma Ramagiri
**Application Scientist, AB SCIEX (Concord, ON)**

Dr. Suma Ramagiri is an application scientist in the AB SCIEX product application lab based in Concord, Canada. Dr. Ramagiri published 11 research articles in internationally peer reviewed journals on the subject of mass spectrometry coupled chromatography techniques to bioanalysis, metabolite identification in drug development, clinical and forensic applications. In this current role, she participates in worldwide efforts for application development and implementation of the AB SCIEX technologies to enhance the quantitative and qualitative bioanalysis.

She obtained B.Sc. M.Sc. and Ph.D. degrees in analytical chemistry from Osmania University, India. She did her post doctoral studies in University of Tennessee Health Sciences, USA. Prior to her current role she worked in analytical R&D, drug discovery and development program towards anti-inflammatory, anti-cancer and radio mitigation drugs in ED Laboratories, USA and Dr. Reddys Laboratories India.