# INTRODUCTION

## "Mechanism-based Development of Natural Products for Human Health"

This Special Issue *of the Journal of Pharmacy and Pharmaceutical Sciences* arises from an inaugural conference on "Mechanism-Based Natural Product Development" held at Whistler Mountain, Canada on September 21-22, 2012.

The aim of the conference was to bring together scientists from various disciplines to discuss the development of new therapeutic products from natural medicines based on mechanistic and related scientific studies. It provided an opportunity to explore new directions in natural medicine research and development, with the ultimate objective of leading to greater integration of natural and conventional synthetic pharmaceutical medicines for the health of the community worldwide.

The concept for this conference and the Special Issue has come from the growing interest internationally in traditional and natural medicinal health products in recent years. It may, at first, appear surprising that developed countries are re-embracing natural product research and development. There are of course many reasons for this, including growing community interest in natural products providing improved health and wellbeing, the growing difficulty and cost of maintaining a pipeline of effective and, above all, safe new products for chronic diseases in the mainstream pharmaceutical industry, and the knowledge that many of our small drug pharmaceutical medicines have come from plants and other organisms. But as traditional natural product medicines increasingly enter the mainstream, the call for evidence to support their use also grows louder. Essential to the acceptance of natural medicines are the validation of their traditional uses and identification, isolation and structural characterization of their active components, together with the elucidation of their mechanisms of biological action, adverse effects, and identification of their molecular targets. These requirements provide the focus of this Special Issue.

Scientific investigation and development of new health products requires the joining together of many disciplines, including chemistry, pharmacology, pharmacognosy and cell and molecular biology, as well as integration with clinical medicine. Natural product medicines are expected to be multi-component and multi-targeted. Are they effective, safe and properly standardized in their existing formulations? Are there opportunities to isolate single active components for standardization and conventional drug discovery and development? Answering these questions requires collaboration between scientific disciplines focused on a common goal.

In line with the aims of the conference, the Special Issue has incorporated review and original research articles related to mechanisms of action in a number of therapeutic areas, mostly from invited speakers at the Whistler conference. Other articles were unsolicited submissions to the Journal that satisfy the scope of the issue. Articles range from reports on efforts to work with traditional owners in the appropriate cultural context, to develop new therapeutics based on traditional literature, to discover new medicinal products, to develop new pharmaceuticals based on the isolation of active chemical components, to develop new methods of delivery, and to identify mechanisms of action. The medical 'territory' includes cancer, heart disease, diabetes and related chronic inflammatory diseases, pain pathways, deafness and infertility. Other articles investigate the quality and safety of

products by the application of current analytical methods, the potential for interactions of natural products (e.g., cranberry) with pharmaceutical medicines and the variability of a selection of similar natural product medicines with regard to their contents of therapeutically beneficial and marker compounds, as claimed on the product labels. In addition, the issue includes the abstracts of posters that formed an important part of the conference, especially from postgraduate students and postdoctoral fellows. These are included as Proceedings.

We are indebted to the scientists who willingly gave their time and resources to attend the Whistler conference and, in many cases, submitted manuscripts for inclusion in this Special Issue. The delegates and contributors came from many places, near and far, to make this inaugural conference on mechanism-based natural product development a success.

Such a conference and the subsequent proceedings in the Special issue are not possible without the work of many. We thank the organizing and scientific committee for their support and valuable suggestions. A conference of this scope would not be possible without sponsorship; this includes especially the National Health Products Research Society of Canada, the Canadian Society for Pharmaceutical Sciences and several Universities. Major support, without program or editorial input, was provided by SOHO Flordis International (SFI). We commend them for believing in the quest for clinically proven and research-based products based on an understanding of underlying mechanisms. The support of Purapharm International is also gratefully acknowledged.

We would like to dedicate this Special Issue to Professor Allan SY Lau of the University of Hong Kong, who contributed enthusiastically to the organization of the conference and its program, but was ultimately unable to attend due to his untimely passing. Professor Lau was a pioneer in the integration of natural and orthodox medical studies and his inspiration will be greatly missed.

Basil D Roufogalis, Arthur D Conigrave and Emanuel E Strehler
Co-Guest Editors

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# Treatment of Cancer Drug Resistance: microparticle-mediated resistance

**Mary Bebawy,** Ritu Jaiswal[2] Joyce Gong[2],and Georges Grau[2]

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**Introduction:** Cancer drug resistance is a major obstacle to treatment success. Multidrug resistance (MDR) is a unique form of resistance attributed to the overexpression of multidrug efflux transporters in affected cells. We recently discovered a novel intercellular pathway for the acquisition and dissemination of MDR mediated by microparticles (MPs). We now report that this pathway plays an even more significant role in ensuring the dominance of the resistant trait in cancer cell populations.

**Methods**: MPs were isolated from resistant leukaemic ($VLB_{100}$) and breast epithelial (MCF-7/DX) cells by differential centrifugation. Western blot and flow cytometry were used to detect the resistance proteins on MPs and on drug-sensitive recipient cells (CCRF-CEM and MCF-7) following co-culture. The nucleic acid contents of MPs and cells were determined using qPCR. Functionality of transferred proteins and MP drug sequestration was established using dye exclusion techniques.

**Results and Discussion**: MPs shed from MDR cells transfer functional resistance proteins to recipient cells, and confer MDR within 4 h. The transfer of the MP nucleic acid cargo re-templates the transcriptional landscape of recipient cells to reflect that of the donor cells. MPs also sequester the anthracycline class of chemotherapeutics through nucleic acid trapping, thus constituting a parallel pathway limiting drug exposure to cancer cell populations. This work defines a novel pathway whereby MPs serve as vectors in the intercellular transfer and dominance of resistance between cells. This pathway introduces new therapeutic strategies for the circumvention of cancer resistance clinically.

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# $GABA_A$ receptors and flavonoids: the 'natural' benzodiazepines?

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Recent genetic and pharmacological studies have demonstrated that targeting $\alpha_2$- and $\alpha_4$-containing $GABA_A$ receptors may mediate the anxiolytic effects of benzodiazepines and other agents without inducing sedation. Flavonoids are a class of polyphenols found in plants and have a range of pharmacological actions including anti-anxiety effects. In our laboratory, we have developed a number of synthetic flavonoids including 2'methoxy-6-methylflavone (2'MeO6MF) and 3-hydroxy-2'methoxy-6-methylflavone (3-OH-2'MeO6MF) that mediate their action specifically via $\alpha_2$- and $\alpha_4$-containing $GABA_A$ receptors when evaluated on over 40 human recombinant GABAA receptors expressed in *Xenopus* oocytes using 2-electrode voltage clamp methods. Intraperitoneal injection of 1-100 mg/kg of either compound to Balb-C mice showed a significant ($p^*<0.05$, $p^{**}< 0.01$ One way ANOVA, n=8) dose dependent increase in the both the number of open arm entries and the time spent in open arms in the elevated plus maze (EPM) and increase time spent in the light compartment and number of transitions in the light dark tests. Sedative effects were only observed with 2'MeO6MF at the higher doses. No myorelaxant effects were observed with any agent in the horizontal wire test. The anxiolytic effects of both agents were not reversed by the benzodiazepine antagonist flumazenil in the EPM showing that the effects are mediated via nonbenzodiazepine allosteric sites of $GABA_A$ receptors. This study highlights the fact that targeting non-benzodiazepine allosteric sites on $GABA_A$ receptors can lead to new anxioselective agents with fewer side-effects.

# Profiles of surface mosaics on chronic lymphocytic leukemias distinguish stable and progressive subtypes

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Chronic lymphocytic leukemia (CLL) is a heterogeneous disease. Whereas some patients survive for many years, 20-30% of patients progress and may die within several years. Currently, there is not a single procedure that enables accurate prognosis and triaging of those patients who need immediate and aggressive treatment. All CLL cells are characterised by the expression of the B-cell antigens CD19, CD20, CD21, CD22 and CD23, with aberrant expression of the T-cell antigen CD5. We have developed a CD antibody microarray (DotScan) containing 182 immobilised CD antibodies that has been used to obtain extensive surface profiles of CLL cells obtained from 100 patients. Of these 182 antigens, 27 were differentially expressed between 3 clinical disease categories, stable, stable-progressive and progressive. Some of these antigens are not expressed on normal B-cells and may be novel targets for therapeutic antibodies against CLL. Unsupervised hierarchical clustering of the surface profiles from 100 patients showed that those with progressive CLL could be distinguished based solely upon this 'disease signature'. The sensitivity (proportion of actual positives correctly identified) was 67.9%, and the specificity (proportion of negatives correctly identified) was 77.5%.

Many of the surface proteins on human cells are N-linked glycoproteins, enabling their purification by hydrazide coupling. LC-iTRAQ-MS has been used to identify novel surface proteins that are differentially abundant between stable and progressive CLL. CLL is often treated with fludarabine in combination with cyclophosphamide and rituximab (FCR). Fludarabine increases the levels of several CD antigens, providing the opportunity for synergistic treatment with therapeutic antibodies.

# Calcium-sensing receptor regulation and modulation of micronutrient and macronutrient metabolism


## Arthur D Conigrave

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The calcium-sensing receptor mediates feedback control of extracellular calcium homeostasis. It mediates the homeostatic response to hypocalcemia by disinhibiting PTH secretion from cells of the parathyroid gland. The resulting elevations in serum PTH levels stimulate: (i) the release of $Ca^{2+}$ ions from bone; (ii) $Ca^{2+}$ reabsorption from the distal nephron; and (iii) the synthesis of 1,25-dihydroxyvitamin $D_3$, which promotes small intestinal $Ca^{2+}$ absorption. It also mediates the homeostatic response to hypercalcemia by: (i) suppressing $Ca^{2+}$ reabsorption in the renal thick ascending limb; (ii) stimulating calcitonin release from thyroid C-cells, thereby suppressing osteoclastic bone resorption; and (iii) suppressing serum PTH levels. Widespread expression in cells and tissues with no known roles in calcium homeostasis, however, points to additional physiological roles.

While many receptors have just one known ligand and thus a defined spectrum of biological actions, class C G-protein coupled receptors (GPCRs), to which the CaSR belongs, respond to multiple ligands via several distinct binding sites. Consistent with these observations, recent work demonstrates that the CaSR also recognizes several distinct ligands including aromatic L-amino acids and neuromodulator glutathione peptides, which markedly stimulate $Ca^{2+}{}_i$ mobilization and associated signaling pathways. In the small intestine, aromatic L-amino acid-dependent activation of the CaSR promotes the secretion of the gut hormone CCK, thereby coupling protein nutrition to nutrient-dependent digestion, absorption and satiety. The binding sites for amino acids and peptides are located in the receptor's VFT domain and both ligands act as positive allosteric modulators of the receptor. Receptor activation by amino acids or glutathione peptides appears to require a discrete post-translational modification of the receptor, which directs signaling outcomes and suggests new paradigms of receptor action in various gastro-intestinal, endocrine and nervous tissues, as well as those primarily involved in calcium homeostasis.

# Using Technology to Explore the "Treasure House of Traditional Chinese Medicine": Lessons learned from a Sino-American Research Collaboration*

**David M. Eisenberg**

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**Introduction:** While the popularity of and expenditures for herbal therapies in general, and especially herbal therapies from East Asia (e.g., China), have increased dramatically in recent years, their efficacy, safety, mechanisms of action, potential as novel or adjunctive therapeutic agents, cost effectiveness or lack thereof, still remain poorly defined and controversial.

In addition, clinical botanical research in recent years has suffered from the lack of a cohesive research strategy, which draws on the necessary expertise of all relevant specialties.

**Discussion:** With this as background, US (Harvard) and Chinese (Beijing University of TCM and Hong Kong Baptist University) co-investigators with expertise in Traditional Chinese Medicine (TCM), botany, chemistry, and drug discovery, jointly established a prototype library of authenticated TCM medicinal plants that collectively represent the majority of all commonly prescribed TCM herbal prescriptions. This presentation will summarize the rationale for, methods, and preliminary "proof of principle" for the establishment of this prototype authenticated medicinal plant library; and, the establishment of a research strategy whereby medicinal plants can be systematically evaluated using state-of-the-science technologies as well as established (NIH) policies and procedures for the design of acceptable randomized controlled clinical trials.

It is hoped that these proposed methods will foster discussion among fellow botanical researchers; and, will lead to scientific discoveries while enhancing efforts to evaluate commonly used herbal therapies worldwide.

*This abstract and presentation are based on the peer reviewed publication: Eisenberg, DM et al, Fitoterapia 82 (2011) 63-79, "Developing a library of authenticated TCM plants for systematic evaluation-Rationale, methods and preliminary results from a Sino-American Collaboration".

# Regulation of cell surface microdomains important for cholesterol and glucose homeostasis


## Thomas Grewal

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**Introduction:** Cholesterol-rich membrane domains at the cell surface regulate the activity of key molecules in lipid and glucose homeostasis, including cholesterol- and GLUT4 transporters. It is well known that cholesterol regulates caveolin transport and SNARE function, which determine the formation of cholesterol-rich caveolae at the plasma membrane and the docking of secretory vesicles, respectively. However, the molecular mechanism and cellular cholesterol pools that determine the localization of caveolin and assembly of the SNAREs machinery are largely unknown.

**Methods:** Chinese hamster ovary cells (CHO) were treated with amphipathic tertiary amine (U18666A), methylarachidonyl fluorophosphate (MAFP*)* or were stably transfected with annexin A6 expression vector. Cholesterol distribution, caveolin and SNARE localization in these cells upon addition of exogenous cholesterol and/or NPC1 overexpression was analysed biochemically, by western blot analysis of cell lysates and subcellular fractions, and by fluorescence microscopy. Caveolae formation was determined by EM analysis. Localization of Glut 4 and eNOS was analysed by fluorescence microscopy. Secretion of fibronectin and cytokines was determined using ELISA assays. Cell surface integrin expression was compared by western blotting of plasma membrane fractions.

**Results:** We showed that pharmacological accumulation of late endosomal cholesterol by U18666A reduces cholesterol in the Golgi and plasma membrane. This leads to an impaired supply of cholesterol needed for cytosolic phospholipase A(2) (cPLA(2)) to drive Golgi vesiculation and caveolin/SNARE protein transport (t-SNAREs: SNAP23, syntaxin-4) to the cell surface. Pharmacological inhibition of cPLA(2) using MAFP and overexpression of annexin A6 induce a similar phenotype. Ectopic expression of Niemann-Pick C1 (NPC1) or exogenous cholesterol restores the location of caveolin and SNARE proteins within the PM. Importantly, mislocalization of caveolin and t-SNAREs correlates with reduced caveolae formation and secretion of cargo via exocytic pathways. These findings correlate with altered cell migration, and mislocalization of key molecules involved in cholesterol and glucose homeostasis.

**Discussion:** We conclude that changes in cholesterol levels in the Golgi provides opportunities to modulate the activity of various biological processes in cardiovascular disease, fatty liver disease and the development of insulin resistance, as the activity of cholesterol transporters, growth factor receptors, NO production, cytokine secretion and GLUT4 translocation is affected.

# Ginsenosides: pharmacokinetics and metabolism in prostate cancer

**Emma S. Guns**

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**Introduction:** Within the context of integrative cancer care, combinations of non-toxic phytochemicals with established chemotherapeutic agents could offer the potential to enhance drug efficacy while alleviating toxic side effects. Rh2, a 20(S)-protopanaxadiol type and its aglycone aPPD are major *in vivo* metabolic products of orally ingested ginseng. We have assessed their *in vitro* and *in vivo* therapeutic potential, alone and in combination with docetaxel (Taxotere) for the treatment of prostate cancer. We have also evaluated the pharmacokinetic profiles of Rh2 and aPPD in mice and delineated the primary route of metabolism of aPPD *in vitro*.

**Methods:** A novel oral dosage formulation was developed for Rh2 and aPPD and pharmacokinetics determined for the two compounds in serum and tissues following administration to nu/nu nude mice. Efficacy of treatment with the two compounds alone and in combination with docetaxel was also determined *in vitro,* in PC-3, DU145 and C4-2 prostate cancer cell lines, and *in vivo*, in an established PC-3 human prostate cancer model. Primary routes of ginsenoside metabolism were also evaluated *in vitro*.

**Results and Discussion:** Rh2 and aPPD can be combined with docetaxel to yield additive or synergistic activity *in vitro*. Pharmacokinetic studies demonstrate the ability of both Rh2 and aPPD to be absorbed and widely distributed to major organs following oral administration. At the oral doses used, both Rh2 and aPPD procured significant growth inhibition of PC-3 tumors. *In vitro* studies using human liver and intestinal microsomes (HLM/HIM) indicated that CYP3A4 and CYP3A5 isoforms are the predominant enzymes responsible for hydroxylation of aPPD.

# Glucosamine: from Nutraceutical to Pharmaceutical

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**Introduction:** Glucosamine (GlcN) is a naturally occurring amino sugar that is widely used to treat osteoarthritis despite controversial clinical trial results. Animal studies, on the other hand, unequivocally suggest anti-inflammatory and disease modifying effects for GlcN. Many explanations have been offered as to the root of the controversy. They include superiority of a crystalline sulphate salt over HCl, industry bias, insensitive assessment metrics and poor clinical methodology.

**Methods:** We have ruled out the difference in bioequivalence between GlcN salts and have suggested that the main problem lies in patient under-dosing (1). GlcN has a low bioavailability due to its loss in the gut before its absorption that is facilitated by glucose transporters.

**Results:** Consequently, using a rat animal model, we have developed a GlcN-peptide pro-drug with 3-fold greater bioavailability and potency than GlcN in treating adjuvant arthritis, a model of human rheumatoid arthritis. Preliminary results from a study in humans also suggest three-fold increased bioavailability of glucosamine after administration of the pro-drug.

**Conclusion:** Our data suggest a safe alternative for the treatment of various forms of arthritis.

**Reference**

1) Aghazadeh-Habashi A, Jamali F. J Pharm Pharm Sci. 2011;14:264-73.

# TRP channels and natural products: research tools in studies for pain relief

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Transient Receptor Potential (TRP) ion channels expressed in the somatosensory system are attractive targets for pain relief. A number of natural products traditionally known to relieve pain target these TRP channels and modulate the somatosensation of pain. These studies clearly provide templates for developing novel analogs of the analgesic natural compounds by targeting specific TRP channels that serve as versatile somatosensors. An important step during novel drug development is to test them mechanistically *in vitro* (cells, systems) and *in vivo* (whole animal). We have used spinal cord preparations from neonatal mice as an *in vitro* bioassay to study the role of TRP channels in the control of sensorimotor function relevant to central mechanisms under physiological and pathological conditions. We are currently extending these observations to whole animal central injury models of neuropathic pain and motor dysfunction. This presentation will discuss a brief overview of our research models and outcomes.

# Herb-Drug interactions: mechanisms and significance

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**Introduction:** Herbal medicines and nutraceuticals are widely used in the community and used concomitantly with conventional medicines. We have critically analysed studies investigating interactions between clinically used substrates of the organic anion transporting polypeptide (OATP) and selected phytochemicals (found in herbal medicines and fruit juices). The mechanism of these interactions and clinical significance will be discussed.

**Methods:** Systematic literature search (with defined terms) to identify and critically appraise relevant clinical studies.

**Results:** The constituents of orange and apple juice (also found in some herbal medicines) have been shown to inhibit intestinal influx transporters (OATPs) leading to reduced bioavailability. Significant reductions in systemic exposure with fruit juice co-administration have been demonstrated for atenolol, talinolol, ciprofloxacin, ivermectin and etoposide, with major reductions (more than half) in bioavailability for fexofenadine, celiprolol and aliskiren.

**Discussion:** Differences in juice and herbal medicine composition are a significant confounding factor in the assessment of herb-drug interactions. Concentrations of flavonoids in fruit juices and products may vary based on fruit/plant species, geographic origin, maturity, manufacturing processes, storage conditions and seasonal variability. It is likely that these factors contribute to the highly variable concentrations of flavonoids observed in fruit juices and herbal medicines.

**Conclusion:** OATP-mediated fruit juice/herb-drug interactions represent a clinically important, rapidly developing area of research, helping to inform the optimal use of medicines. Effective communication is essential for successful translation of these new understandings of herb-drug interactions into clinical practice.

# Molecular processes regulating glucose transport by skeletal muscle in response to conjugated linoleic acid isomers

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**Introduction:** Conjugated linoleic acids (CLAs), *n*-7 fatty acids in milk and ruminant meat, are putative anti-diabetic/obesity agents that promote skeletal muscle glucose transport. However, their mechanisms of action are unknown. Our study investigated the cellular dynamics of the effects of cis-9, trans-11 (c9,t11) and trans-10, cis-12 (t10,c12) CLA isomers in L6 myotubes.

**Methods:** Rat L6 myoblasts ($1\times10^3$ cells/ml) were grown in cell culture dishes containing DMEM supplemented with 10% fetal bovine serum (FBS). At 60–70% confluency, cells were differentiated into myotubes using FBS-free DMEM containing 2.5% horse serum. Cell fusion and formation of multi-nucleated myotubes were monitored by phase contrast microscopy. L6 myotubes were placed in serum-free medium for 24-36 h then incubated in the absence or presence of CLA isomers (60 μM) for 15 min. They were monitored for glucose uptake using isotope/fluorescently-labelled 2-deoxyglucose, intracellular $Ca^{2+}$ ($Ca^{2+}{}_i$) release using Fluo-4 AM, protein phosphorylation by Western blotting, and GLUT4 translocation by fluorescence microscopy.

**Results and Discussion:** Acute exposure of L6 myotubes to either c9,t11-CLA (60 μM) or t10,c12-CLA (60 μM) for 15 min stimulated GLUT4 trafficking and glucose uptake by activating insulin-dependent signals including phosphatidylinositol 3-kinase (PI3-kinase) and Akt substrate-160 kDa (AS160). Intriguingly, t10,c12-CLA also stimulated $Ca^{2+}{}_i$ release and phosphorylation of $Ca^{2+}$/-calmodulin-dependent protein kinase II (CaMKII) and AMP-activated protein kinase (AMPK) in a concentration-dependent manner (0-240 μM), whereas c9,t11-CLA had little or no effect. Inhibition of PI3-kinase by LY294002 (10 μM), $Ca^{2+}{}_i$ release by BAPTA/AM (1 μM; 15 min), CaMKII by either KN-93 (10 μM) or autocamtide-2-inhibitory peptide (1 μg; 30 min) and AMPK by either dorsomorphin (1 μM) or dominant-negative AMPKα adenovirus (multiplicity of infection 100; 36 h) abrogated t10,c12-CLA-induced AS160 phosphorylation and glucose uptake by L6 myotubes. Genetic knock-down of CaMKII using siRNA completely abrogated both basal glucose uptake as well as stimulated glucose uptake in response to both CLA isomers, suggesting that CaMKII expression has an important permissive role. Evidence that CaMKII protein kinase-selective inhibitors suppressed t10,c12-CLA-stimulated AMPK indicates that CaMKII acts upstream of AMPK in the t10,c12-CLA-mediated pathway. The findings demonstrate that t10,c12-CLA stimulates skeletal muscle glucose transport via $Ca^{2+}{}_i$-CaMKII-AMPK-AS160. However, the mechanism of action of c9,t11-CLA remains undefined.

# Mouse ES cells as a model for embryo development

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**ES cells:** The phenotypic status of ES cells is controlled in part by signalling pathways, which translate inputs mediated by extracellular molecules. Here we show that the cytokine LIF, together with L-proline, promotes neurogenesis of mouse ES cells via a series of embryologically relevant cell types. L-proline is first taken up into ES cells via a specific transporter, SNAT2. Inhibitor studies and kinome array analysis show that it then (i) changes the activity of signalling pathways already stimulated by LIF and (ii) activates additional signalling pathways. Thus, L-proline acts in a growth-factor-like manner in which its signalling cascades are 'co-ordinated' with those of LIF to alter the emergent properties of the system, including the stimulation of differentiation and cell cycle, and changes in cell shape.

**Preimplantation embryos:** Selected amino acids, including L-proline and L-glutamine, also stimulate development of cultured pre-implantation mouse embryos. The mechanism(s) appears to be autocrine-like, promoting the development of embryos when they are cultured at low density but not when they are cultured at high density. As with the differentiation-inducing effects of L-proline in ES cells, L-proline and L-glutamine must first be taken up into the blastomeres of the embryo via specific amino-acid transporters.

Together these results indicate that selective addition of amino acids promotes development in early embryos and that the mechanisms may be similar to those driving ES-cell differentiation. They also point to a cheap and reliable means of improving the culture conditions of embryos to be used for assisted reproduction in humans and livestock.

# *Zingiber officinale* (Ginger) improves lipid and glucose metabolism in *in vivo* and *in vitro* models of metabolic syndrome

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**Introduction:** Metabolic syndrome, including type 2 diabetes, insulin resistance, obesity and dyslipidemia, is a major disease problem around the world and a plethora of herbal medicines claim effectiveness in controlling these disorders. The rhizome of *Zingiber officinale*, is widely used as a herbal medicine for the control of diabetes and dyslipidemia.

**Methods:** The protective effects of a characterized and standardized extract of *Zingiber officinale* on the development of metabolic syndrome was investigated using in vivo (high fat diet-fed rats) and in vitro (cultured HuH-7 cells; L6 skeletal muscle cells) models of deranged glucose and lipid metabolism.

**Results and Discussion:** *Zingiber officinale* decreased weight gain, and improved the circulating and hepatic lipid profiles (LDL cholesterol, triglycerides, total cholesterol) in high fat diet-fed rats. In parallel, ginger up-regulated the protein and mRNA expression of LDL receptor while down-regulating HMG-CoA reductase, thus the lipid lowering effects of ginger are attributable to decreased cholesterol biosynthesis and increased hepatic uptake of circulating LDL cholesterol. *Zingiber officinale* also decreased serum glucose levels and alleviated insulin resistance in vivo with concomitant decreases in hepatic expression of TNFα and IL-6; suppression of NF-κB activity; and increase in glucose uptake, and GLUT4 expression in skeletal muscle cells. The study demonstrates that *Zingiber officinale* improves glucose metabolism and insulin sensitivity at least in part through increased expression of GLUT4 transporters, decreased expression of hepatic cytokine genes and decreased activation of NF-κB. Thus the present investigation provides a rational explanation and scientific evidence for the use of ginger in alleviating and possibly preventing metabolic disorders.

# Drug Discovery from Ayurvedic Experience

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Traditional medicine like Ayurveda is fertile ground for modern drug development through a reverse pharmacology approach in which the clinical evidence comes as presumption. Unlike conventional drug discovery, in Ayurveda-directed drug discovery, clinical experience, observations and data provide the starting point. This is economic and time-sparing with reduced bottle-necks. Reverse pharmacology is more exhaustive and requires the investigation of clinical/experiential leads and involves trans-disciplinary exploratory and experimental research. It also requires evaluation at multiple levels of biological organization to understand mechanisms of action and optimize safety, efficacy and acceptability of the lead modality. Several molecules have been isolated based on Ayurvedic experience including Rauwolfia alkaloids for hypertension, Psoralens for vitiligo, Holarrhena alkaloids for amoebiasis, Guggulosterones for hyperlipidemia, Mucuna pruriens for Parkinson disease, Piperidines for bioavailability enhancement, Baccosides for memory enhancement, Picrosides for hepato-protection, Phyllanthins for viral infections, Curcumins for inflammation, Withanolides for adaptogenic effects among others.

Darakchasava, an Ayurvedic medicine wherein the main constituent is *Vitis vinifera* L is prescribed as a cardiotonic in India. A major component of this medicine is Resveratrol. Today, Resveratrol is widely used as a cardio-protective agent. A preliminary clinical study carried out by the author reveals that oral supplementation of resveratrol (250 mg) along with anti-diabetic agents is effective in patients with type-2 diabetes and improves vascular risk factors namely, body weight, oxidative stress and lipid profile in addition to improving blood glucose level. Drugs that are isolated from Ayurvedic experience have been elaborated within living systems and are frequently more “biologically friendly” when compared to synthetic sources.

# Plasma Membrane Calcium-ATPase 4 (PMCA4) Inhibitors to Prevent and treat Cardiac Hypertrophy

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The plasma membrane calcium/calmodulin dependent calcium pumps have traditionally been viewed as 'housekeeping' enzymes that keep intracellular calcium within physiological limits.

Because of the presence of a sodium/calcium exchanger, the role of the PMCAs in excitable tissues has been even less clear. In contrast to the assumption that PMCAs had little role in the myocardium, over the last years our group has shown that PMCAs do have unexpected functions in signalling as well as in cardiac and vascular contraction. In addition, genome-wide association studies have demonstrated the relevance of single nucleotide polymorphisms in the PMCA1 and 4 genes in cardiovascular disease.

Specifically, from our work a picture emerges in which PMCA4 in the myocardium is a regulator of NO production. It is part of the dystrophin/syntrophin complex in caveolae, binds to nNOS via a PDZ domain and regulates its activity through the adjustment of the local calcium level (nNOS is calcium/calmodulin dependent, as is PMCA4). In addition, PMCA4 binds to RAASF1 (Ras-Associated Factor 1), a suppressor of the Ras pathway, which is relevant for growth in the heart (and tumorigenic in other tissues, when mutated).

Based on these findings, we have screened medically optimised chemical libraries and identified several high-affinity PMCA4 inhibitors that are able either to prevent or even to treat pre-existent cardiac hypertrophy, a key step to heart failure, one of the most prominent causes of mortality worldwide. We will also present initial data on the role of PMCA1 in cardiac contraction, a hitherto unrecognised function of the enzyme.

# The neurocognitive effects of Ginkgo and Ginseng alone and in combination

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Both ginkgo biloba and Panax ginseng have long histories of use in traditional medicine systems, including uses as cognitive enhancers. The recent availability of standardised extracts has allowed reproducible research, including in the context of their traditional use. In consequence a number of randomised, placebo-controlled studies into the acute and chronic neurocognitive effects of Ginkgo and Ginseng, including their effects in combination have been successfully completed.

Brain imaging studies demonstrate that Ginkgo (extract G501) and Ginseng (G115) are both absorbed and have central activity with distinct but overlapping effects. GK501 improves memory function in the hours following a single dose, with 120 mg the optimal dose. G115 improves cognitive function in the hours following a single dose with dose-dependent effects on memory and working memory processes. Ginkgo and Ginseng in combination (Gincosan™) appear to improve working memory function, especially under conditions of high mental effort. There is also evidence for cognitive benefits following chronic administration of Gincosan™ in middle-aged populations and in those with fatigue.

These data provide an evidence base demonstrating the efficacy of specific extracts for improving aspects of cognitive function. Interestingly these effects are largely consistent with their uses in traditional medicine systems. They also illustrate synergistic effects of herbal extracts. Future studies should focus on exploring underlying mechanisms of these effects by co-monitoring appropriate biomarkers and neuroimaging.

# Learning from both sides: experiences and opportunities in the investigation of Australian Aboriginal medicinal plants

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**Introduction:** With one of the oldest surviving cultures in the world, Australian Aboriginal people have developed immense knowledge about the diverse Australian flora. Western scientific investigation of some Australian Aboriginal medicinal plants has demonstrated interesting pharmacological activities and chemistry, however the majority of these species have not yet been extensively examined. Research that is locally initiated and driven by Indigenous traditional owners in collaboration with Western scientists has significant potential to develop new plant-based products.

**Methods:** Research that is locally driven by Indigenous traditional owners and in which traditional owners work as researchers in collaboration with University-based colleagues in the investigation of medicines rather than "stakeholders", or "informants" is one model that may be used in characterising plants with the potential to be developed into sustainable plant-based medicinal products with commercial value. Our team has taken this approach in research located both on traditional homelands and in the laboratory.

**Results:** Research being conducted by the University of South Australia and Chuulangun Aboriginal Corporation has led to patent filing for protection of intellectual property associated with novel compounds and extracts with the potential for development through cosmetic, complementary medicine and pharmaceutical routes. Ongoing research examining safety issues, mechanism of action, compound supply and sustainable harvesting of plant materials on homelands is being undertaken.

**Discussion:** By way of example, a collaborative initiative between the Chuulangun Aboriginal Corporation and scientists from the University of South Australia aimed at the development of enterprises based on traditional medicines will be discussed. This work serves as a model for collaborative engagement opportunities through the investigation of Australian Aboriginal medicinal plants using an Indigenous-guided approach. This presentation will address the lessons learnt and building of cultural reciprocity during this time.

# Intake of Boswelan® with food improves the bioavailability of 11-Keto-β-Boswellic Acid after a single dose in healthy males, but less so for simulated steady-state exposure.

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**Introduction:** Boswelan® is a novel solubilized frankincense extract from the *Boswellia serrata* tree. Boswellic acid formulations are proposed to alleviate inflammatory conditions although the exact mechanism is unclear. The objectives were to evaluate the dose-exposure relationship for fed versus fasting conditions of 11-keto-beta-boswellic acid (KBA) and 3-acetyl-11-keto-beta-boswellic acid (AKBA) after oral administration of Boswelan®, to develop a population-based pharmacokinetic model, and to explore drug effects.

**Methods:** After regulatory approval, 12 healthy male volunteers were administered 2 soft gelatine capsules Boswelan® (22.16 mg KBA/21.18 mg AKBA in 800 mg native *Boswellia serrata* gum resin extract) after a standardized breakfast or during fasting in a randomized, open cross-over study. LC-MS/MS-determined KBA/AKBA plasma concentrations were fitted using nonlinear, mixed-effect pharmacokinetic models. Pharmacodynamic markers were leukotriene $B_4$, thromboxane $B_2$, ex vivo LPS-induced prostaglandin $E_2$, platelet aggregation, thrombin, and cathepsin-G proteolytic activity.

**Results and Discussion:** Food increased the systemic exposure of KBA by 27% (mean ± SD area-under-the-curve 786.2±363.4 versus 1058.3±582.8 ng/ml*h, $p < 0.01$), but this interaction seems to lose its relevance for the simulated repeated-dose scenario. AKBA appeared to increase after food but the effect was not statistically significant (48.37±27.58 versus 98.92±63.41 ng/ml*h, $p = 0.23$). The KBA plasma concentration-time-course followed a two-compartment model with single first-order absorption phase. These observations suggest that KBA/AKBA's bioavailability from Boswelan® is less variable than from dry extract preparations. Directional drug effects for inflammatory and platelet aggregation markers were not detected except a trend for decreased cathepsin-G activity, a potential anti-inflammatory target. Projections of KBA/AKBA *in vivo* plasma concentrations in reference to recognised *in vitro* molecular targets do not identify particular drug exposure-mechanism relationships.

# Ten years of research on the effects of Bacopa *monnieri* (Keenmind, CDRI08) on neurocognition

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Bacopa *Monnieri* has been used in traditional Ayurvedic medicine for various indications including memory decline, inflammation, pain, pyrexia, epilepsy and as a sedative. While Bacopa has been reported to have many actions, its memory enhancing effects have attracted most attention. Although the exact mechanisms of action remain uncertain, evidence suggests that Bacopa may modulate the cholinergic system and/or have antioxidant or metal chelating effects. Bacopa may also have anti-inflammatory, anxiolytic and antidepressant actions, relaxant properties in blood vessels and adaptogenic activity. In this talk I discuss the consistent results from human clinical trials in which cognition has been assessed together with putative mechanisms of action for enhancement of cognition. These mechanisms may be different for different age cohorts.

Although most research on Bacopa has focussed on its chronic effects in humans, recently our centre has completed a number of acute dose-ranging studies on cognition in both the young and the elderly. The results of these and other studies from our centre using the specific Bacopa extract CDRI08 (Keenmind), as well as current and future studies, which focus on the treatment of dementia as well as age-related cognitive decline are also discussed. The CDRI08 extract is presently the best validated Bacopa extract available.

# Plasma Membrane Calcium ATPases: Important Players in Cellular Calcium Signaling and Candidates for Therapeutic Agent Development

**Emanuel E. Strehler**

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Plasma membrane calcium ATPases (PMCAs) play a dynamic role in local $Ca^{2+}$ transport and $Ca^{2+}$ signaling at the membrane. PMCAs are encoded by 4 genes in humans, and additional diversity is generated by alternative RNA splicing. Different PMCA isoforms are implicated in various tasks of $Ca^{2+}$ regulation; their loss or inappropriate function results in specific pathologies such as deafness, ataxia, male infertility, and cardiovascular disease. PMCAs are highly regulated transporters responsible for $Ca^{2+}$ extrusion from the cell and are integral components of multi-protein signaling complexes in distinct membrane compartments. Compared to $Ca^{2+}$ influx channels, the PMCAs have lagged far behind as targets for drug development, mainly because of a lack of detailed understanding of their structure and and tissue- and cell-specific function. This is rapidly changing due to integrated efforts combining biochemical, structural, cellular and physiological studies suggesting that selective modulation of PMCA isoforms may be of therapeutic value in the management of different diseases. Both structurally informed rational drug design and high-throughput small molecule screenings are promising strategies that should lead to specific modulators of PMCA activity. Targeting the PMCAs as the major $Ca^{2+}$ efflux system of cells and as integrated components of nanodomain signaling complexes will add important new tools to deal with altered $Ca^{2+}$ handling in disease. Agents that stimulate or inhibit PMCA isoforms selectively will have broad applications and should provide a useful complementary approach to the current use of $Ca^{2+}$ channel modulators.

# Drug Discovery: Searching for Life in the Chemical Universe

**David J. Triggle**

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A question posed with increasing frequency by the pharmaceutical industry – "Where have all the molecules gone"? From the perspective of new drug discovery - decreased productivity, increased costs, patents running out on many highly profitable "blockbuster" drugs, forthcoming major medical and public health problems of neurodegenerative disorders of aging populations and the rising prevalence of such life style disorders as obesity and diabetes the problems escalate. This situation cannot continue: both new approaches and new organizational models for drug discovery are needed.

Only an extremely small fraction of theoretically available chemical space has thus far been explored and it is likely that synthetic constraints and bias to existing frameworks and scaffolds are contributing factors. Chemical space, whether defined by small molecules or large macromolecules, is far larger than can ever be completely explored and it is clear that we must reconsider the rationale of chemical space exploration. Several approaches are now being employed that may yield more fruitful paths to drug discovery. These include acceptance that existing therapeutic molecules occupy already validated pharmacological space and thus are useful leads for new areas, the use of molecular fragments that permit a broader exploration of chemical space, the role of biological templates that permit fragment self-organization to yield active molecules and a new focus on natural product-like scaffolds. The latter approach deserves far greater attention since Nature's molecules are those "forged in the crucible of evolution". And new vistas of chemical space may be explored with the creation or discovery of non-DNA based genetic codes.

However, the exploration of chemical space alone will not solve the current deficit in new drug discovery productivity. As others have noted the reductionist approach to drug discovery has also contributed to this deficit by ignoring the essential integrative nature of biological systems. This too must be addressed.

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# Regulation of the epidermal growth factor receptor by the $Ca^{2+}$/calmodulin complex

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**Introduction:** This study shows the role of calmodulin (CaM) on the regulation of the epidermal growth factor receptor (EGFR/ErbB1/HER1) and other ErbB receptors using *in vitro* assay systems and living cells. RESULTS: It demonstrates that both the EGFR and ErbB2/HER2 are $Ca^{2+}$-dependent CaM-binding proteins, identifying a basic amphiphilic CaM-binding domain (CaM-BD) located at their respective cytosolic juxtamembrane regions. It shows also in living cells that: i) distinct plasma membrane-permeable CaM antagonists; ii) down-regulation of CaM expression in conditional CaM-knockout cells; and iii) loading cells with a $Ca^{2+}$ chelator, all decrease ligand-dependent EGFR activation. Site-directed mutagenesis of the CaM-BD of the EGFR, substituting six basic residues to alanine, again results in the inhibition of the ligand-dependent activation of the receptor and its downstream signaling pathways.

**Discussion:** Overall, these results suggest that CaM is a physiological positive modulator of the EGFR in living cells. A mechanism on how CaM might play its activating role on the EGFR will be presented. This study opens the possibility for the development of drugs targeting this novel regulatory mechanism mediated by the $Ca^{2+}$/CaM complex, what could be useful against solid tumors over-expressing and/or with hyperactive mutated forms of the EGFR and/or the ErbB2 receptor.

# Drug Discovery Inspired by Mother Nature: Seeking Natural Biochemotypes and the Natural Assembly Rules of the Biochemome

**Jun Xu**

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Hitherto the primary paradigm of drug discovery has relied on laboratory-based syntheses of artificial compounds and analyses of their physical, chemical, and biological properties. Our work focuses on new opportunities for drug discovery based on a systematic consideration of the bio-molecular building blocks of natural compounds together with knowledge derived from Traditional Chinese Medicine (TCM), which stretches back more than one-thousand years.

The Human Genome Project is producing a new biological 'periodic table'. We now need to consider whether to initiate a *biochemome project* aimed at discovering biochemistry's 'periodic table', which defines islands of chemical diversity for the design and development of new drug molecules.

The Biochemome should be the complete set of chemical building blocks in an organism and Biochemomics should study the rules governing their assembly and their evolution, together with the relations between the biochemome and drug targets. This approach has the potential to provide a new drug discovery paradigm based on a comprehensive knowledge of the synthetic origins of biochemical diversity and help to direct biomimetic syntheses aimed at assembling quasi-natural product libraries for drug screening.

We are presently engaged in identifying natural biochemotypes and the natural assembly rules of the biochemome by data-mining the TCM and other natural product databases.

# Calcium channels as targets for pain therapeutics


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Chronic pain is a disabling condition defined as pain lasting for at least six months. This often invisible disorder frequently leads to intractable daily pain, reducing quality of life and the ability to contribute productively to society. While we have witnessed significant advances in basic pain research, including the development of important theories regarding pain mechanisms, these developments have been translated into clinical practice with limited success, and few new analgesics have been approved for use in humans.

Painful stimuli are detected by peripheral nociceptors, leading to the firing of action potentials in primary afferent dorsal root ganglion neurons. These form synaptic connections in the dorsal horn of the spinal cord with neurons that ultimately project to higher brain centres where pain is perceived as an unpleasant experience. Numerous types of ion channels contribute to the transmission and processing of pain signals at the peripheral level. These include voltage-activated T-type calcium channels, which regulate neuronal excitability and synaptic transmission in dorsal horn synapses, and N-type calcium channels, which are responsible for the release of glutamate and substance P at these synaptic terminals. Both N-type and T-type channels are possible targets for the development/identification of analgesics. This includes both natural and non-natural products. In this presentation, I will provide an overview of the roles and molecular pharmacologies of T-type and N-type calcium channels, and highlight some of the natural compounds that block their activities. This will set the stage for a later plenary presentation by Prof. David Adams.

# Hops, A Potential Treatment for Tuberculosis?

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**Introduction:** Hops used in the brewing process as a bittering and flavouring agent also have a number of antibacterial phytochemicals, which are utilised as a preservative to increase the longevity of beer. In preliminary studies at least one of these compounds has been shown to have activity against *Mycobacterium tuberculosis* (MTB), a bacterium estimated to infect 1/3 of the world's population. While antibiotics are available, the emergence and spread of resistant strains has prompted researchers to develop novel therapeutics.

**Methods:** Using an agar-based method we have screened forty commercially available hop variants for their ability to inhibit the growth of *Mycobacterium smegmatis* (MTS assay). The method was also used to determine synergy between the hops and anti-mycobacterial antibiotics including rifampicin, isoniazid, pyrazinamide and ethambutol. Hops variants with the greatest activity were further characterised using high performance counter current chromatography (HPCCC). Finally, a THP-1 (differentiated) macrophage infection model was used to determine the ability of hop-derived compounds to enhance bacterial clearing.

**Results:** Hops varied in their ability to inhibit mycobacterial growth in a manner independent of their alpha acid (bitter tastant) content. Preliminary studies found no evidence of synergy between crude hop extracts and any of the antibiotics tested. HPCCC methods have been developed and isolated compounds are being assessed for activity.

**Discussion:** Testing is ongoing to relate antimycobacterial activity to known phytochemicals and it was also shown that there was no antagonism between hops and TB antimicrobials. Using the HPCCC fractionation of hops the performance of isolated phytochemicals is being evaluated using the macrophage model.

# α-tocopherol and quercetin modulate RAGE and other markers of oxidative stress induced by advanced glycation endproducts in human endothelial cells.

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**Introduction:** Advanced glycation end-products (AGE) are proteins that are glycated upon exposure to reducing sugars or lipids. AGEs are prevalent in the diabetic vasculature and contribute to the development of atherosclerosis. Engagement of the receptor for advanced glycation end-products (RAGE) by AGEs induces cellular oxidative stress (OS) and causes upregulations of the transcription factor nuclear factor-κB and its target genes. α-Tocopherol is known to act as an anti-glycating factor *in vitro* but information concerning the role of quercetin in AGE-mediated responses is lacking. This study investigated the ability of Trolox (water-soluble α-Tocopherol analog), quercetin, and quercetin metabolites to modulate AGE-induced increases in oxidative stress and markers of inflammation in human endothelial cells.

**Methods:** Human aortic endothelial cells (HAoEC) were purchased from PromoCell GmbH (Heidelberg, Germany) and used from passage 4-8. Confluent cells were pre-treated dose-dependently with (a) Trolox, (b) quercetin, (c) a mixture of Trolox and quercetin, (d) quercetin metabolites (quercetin 3-glucuronide-Na and quercetin 3'-sulfate-Na) or (e) a mixture of quercetin metabolites and Trolox. Pre-treated cells were stimulated with endotoxin-depleted BSA and AGE-BSA (200 μg/mL protein) for 24 h.

**Results and Discussion:** Trolox and quercetin pre-treatment significantly attenuated AGE-induced ROS (hydrogen peroxide and superoxide) production. This resulted in a concentration- and time-dependent decrease in RAGE, a marker of the mitogen activated protein kinase pathway (MAPK-1) and tumor necrosis factor alpha (TNF-α) mRNA expression, as well as p65 NF-κB activation. Mixtures of Trolox and quercetin showed either additive or synergistic down-regulations in the observed endothelial dysfunctions. Quercetin 3-glucuronide and quercetin 3'-sulfate (40 or 200 μM) with or without Trolox also attenuated RAGE, ROS and pro-inflammatory markers. The study demonstrates a potential role for plant polyphenols in the regulation of AGE receptors and subsequent cell signalling pathways associated with pro-inflammatory responses.

# Effect of dietary DHA supplementation on orchidectomy-related changes in endothelium-dependent vascular responses

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**Introduction:** Loss of gonadal function induces vascular dysfunction by altering the actions of endothelial factors controlling vascular tone. Although the cardioprotective effect of *n-3* polyunsaturated fatty acids has been documented in different vascular alterations (review: Das, 2004), the specific effect of docosahexaenoic acid (DHA) in the vasodilator responses of orchidectomized rats remains to be analyzed.

**Methods:** Aortic segments were obtained from 6 month-old male control and orchidectomized Sprague-Dawley rats. Orchidectomy was induced at 18 weeks of age, and the experiments were performed 6 weeks later. Rats were fed a standard diet (diet 2014, Harlan Laboratories) supplemented with either 7.5% (w/w) sunflower oil (control and V-CX groups) or 6.5% sunflower oil plus 1.5% (w/w) DHA (Lipid Nutrition) (DHA-CX group). Aortic segments were analyzed for: (i) the vasodilator response to acetylcholine (ACh) and sodium nitroprusside (SNP); (ii) the effect of NO synthase suppression by L-NAME and cyclo-oxygenase inhibition by indomethacin on the ACh-induced response; (iii) the effect of KCl-precontraction on the ACh- and SNP-induced responses; and (iv) the vasodilator response to the calcium-dependent-potassium ($K_{Ca}$) channel opener NS-1619. The Animal Ethics Committee of the Universidad Autónoma of Madrid approved this study (Ref. CEI-37-829).

**Results:** The vasodilatory response induced by ACh (0.1 nM-10 μM) was similar in all groups, and abolished by L-NAME (0.1 mM). Indomethacin (10 μM) attenuated the ACh-induced response in arteries from control rats. In orchidectomized rats, however, indomethacin had no effect on the ACh-induced response in the V-CX group, and paradoxically potentiated it in the DHA-CX group. KCl (30 mM)-precontraction decreased the ACh-induced response more effectively in the control group than in the V-CX group, and the decreases were similar in the control and DHA-CX groups. The SNP (0.1 nM-10 μM)-induced response was similar in all groups. KCl-precontraction decreased the SNP response in the control and DHA-CX groups but had no effect on the response in the V-CX group. Orchidectomy decreased the vasodilator response induced by NS-1619 (0.1 nM-10 μM), which was partially recovered in the DHA-CX group.

**Discussion and Conclusions:** Orchidectomy decreased the participation of vasodilator prostanoids and hyperpolarizing factors/mechanisms in the vascular response. Dietary DHA supplementation improved hyperpolarizing mechanisms, at least in part, by activating $K_{Ca}$ channels.

**Reference:** Das U.N. (2004) Eur J Clin Nutr 58:195-203

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# Revealing plausible mechanisms of action of Indian medicinal plants through an integrated *in silico, in vitro & in vivo*-based approach

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**Introduction**: The discovery of new drugs is complementary to understandings of disease pathogenesis as well as mechanisms of drug action. Although medicinal plants are extensively used in the treatment of the diseases and have been an important source of novel molecules in modern medicine, their mechanisms of action remain unknown in many instances. *Ficus religiosa* L. (Moraceae) is an important traditional medicinal plant whose bark has been found to have anticonvulsant properties through unknown mechanism(s).

**Methods**: Based on available information regarding the presence of secondary metabolites in *F. religiosa* bark, *in silico* analyses were performed using PASS (prediction of activity spectra for substances) software and PharmaExpert software regarding the identities of metabolites with known anticonvulsant actions together with their mechanisms of action. The predicted actions were further studied *in vitro* using rat brain homogenates and *in vivo* by maximal electroshock- and pentylenetetrazole-induced convulsions models in three month old male Swiss Albino mice (22-25 g). The procedures were approved by the departmental animal ethics committee (Vide protocol approval No. 107/99/CPCSEA/2009-4.3).

**Results and Discussion**: *In silico* analysis reported a high activity score for GABA aminotransferase inhibition (Vigabatrin type) of several secondary metabolites. This was supported by *in vitro* and *in vivo* pharmacological experiments. The extract showed GABA aminotransferase inhibitory activity in *in vitro* studies and showed anticonvulsant effect in both *in vivo* models of convulsions. It is concluded that the integrated approach utilized in the present study may be more generally used to reveal the mechanisms of other bioactive natural products.

Acknowledgement This work was supported by RFBR/DST grant No. 11-04-92713-IND_a/RUSP-1176.

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# Astragalin enhances cancer treatment sensitivity in mouse hepatoma

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**Introduction:** Astragalin is a herb used in traditional Chinese medicine (TCM) to *'tonify qi'* i.e., to alleviate syndromes arising from disease-related organ deficiencies. It has also been used to treat certain kinds of cancer. However, its efficacy and mechanism(s) of action are unclear. In the current study, we investigated the impact of cancer therapies in mice inoculated with H22 hepatoma cells that had first been passaged multiple times through control or astragalin-treated mice *in vivo*.

**Methods:** Distillates for SC injections in mice were prepared by boiling 100 g Astragalin in 500 ml water for 20 min. Mouse H22 hepatoma cells were initially cultured in RPMI-1640 medium with 10% FBS prior to multiple passaging through successive cohorts of BALB/c mice. In the first passage *in vivo*, a total of 20 male BALB/c mice (18-22 g) were divided into two groups. All 20 mice were inoculated with $10^7$ mouse H22 hepatoma cells (0.2 mL) by intra-peritoneal injection on day-1. One sub-group of ten mice received astragalin distillate by SC injection at a dose of 0.6 ml on alternate days starting on day-2 and continuing until day-6. A second sub-group of ten control mice received normal saline by SC injection on alternate days using the same protocol. On day-7, H22 cells were recovered by the collection of 1 mL samples of ascitic fluid from all mice (around $4 \times 10^8$ cells/mL). In the second and subsequent passages, the cell samples collected from each sub-group of ten mice, control and Astrogalin-treated, were combined respectively and inoculated by intra-peritoneal injection ($10^7$ cells; 0.2 mL) into 20 more mice divided as above into two sub-groups. As above, the newly inoculated mice received either astrogalin distillate or saline injections respectively (0.6 mL alternating days from day-2 to day-6). After successive passages *in vivo* totalling 110 weeks, H22 cells from the control or Astrogalin-treated groups were employed in two distinct experimental protocols: (i) a chemotherapy protocol *in vivo*; and (ii) a radiotherapy protocol *in vitro*. For chemotherapy, H22 cells derived from either control or astrogalin-treated donor mice were inoculated into recipient mice and the recipient mice were then treated five times with cyclophosphamide (40 mg/kg by oral gavage) every second day for 10 days. After 10 days, tumour incidence was evaluated by the formation of ascites and the mice were killed by cardiac puncture under anaesthesia. The study was conducted at the China-Japan Friendship Hospital (Beijing, China) with institutional ethics approval. For radiotherapy, $10^7$/ml of H22 cells were placed in a glass tube with a wall thickness of 2 mm. The tubes were then placed in a water container for X-irradiation (ML-20MDX, Mitsubishi Electric) and exposed to multiple cycles of 200 cGy (1 min 'on' then 1 min 'off') to a total dose of 8,000 cGy. After X-irradiation, cells were stained with Trypan blue. Live and dead cells were counted using a hemocytometer. For cell cycle analysis, cells were stained with propidium iodide and evaluated by flow cytometry (FACS440; BD).

**Results and Discussion:** Compared to mice inoculated with H22 cells recovered from control untreated donor mice, mice inoculated with H22 cells recovered from astragalin-treated donor mice exhibited a substantially enhanced response to cyclophosphamide. Thus, tumour formation was 3.6% in the astragalin arm v 93% in control ($p < 0.01$). Moreover, after X-irradiation in vitro, the death rate of cells 20.5 hour after X-irradiation was 95% in the astragalin arm v 27% in control ($p < 0.01$). In cell cycle analyses, astragalin treatment promoted accumulation of H22 cells in G0/G1 phase. The results demonstrate that astragalin has anti-tumour efficacy *in vivo* and indicate that it acts primarily to induce cell cycle arrest in G1. In addition, the results suggest that the efficacy of astragalin in human cancer chemotherapy requires evaluation.

# The Mechanism of Action of Biflavonoids as Amyloid-β Aggregation Inhibitors

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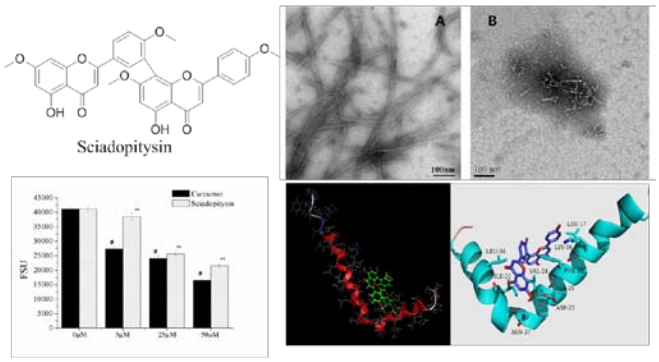
Aggregation and fibril formation of amyloid-β (Aβ) is associated with the neurodegenerative diseases and a cascade of harmful events related to Alzheimer's disease (AD)[1]. Using small molecules to prevent the aggregation of Aβ is an effective therapeutic strategy for AD. Mono-flavonoids and biflavonoids were reported as Aβ aggregation inhibitors and cyto-protectors[2]. But the mechanism of action remains unknown.

In this study, we isolated a biflavonoid from *Taxus chinensis*, and found that it inhibited Aβ aggregation with thioflavin T (ThT) fluorescence assay, circular dichroism spectroscopy (CD) and electron microscopy (EM) experiments. A mechanism of action is proposed based upon molecular docking studies and molecular dynamics (MD) simulations for the interaction of bioflavonoids and Aβ.

ThT experiments were performed to quantify inhibition of Aβ aggregation; CD was used to determine whether Aβ switched conformation from α-helix to β-sheet; and EM was used to detect cessation of Aβ fibril formation. Sciadopitysin, one of the bioflavonoids isolated from *T. chinensis,* exhibited anti-aggregation activity without cytotoxicity. The MD simulations indicate that biflavonoids form hydrogen bonds with Aβ42, stabilize the C-terminal conformation in the Aβ42, and prevent switching from α-helix to β-sheet conformation. This was confirmed by the CD observations. Sciadopitysin formed salt bridges between residues Asp23 and Lys28, reduced the hydrophilicity of Aβ42 C-terminal residues, and stopped Aβ aggregation.

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# Evaluation of anti-nociceptive, anti-inflammatory and anti-pyretic activities of a hydromethanolic extract of *Artemisia scoparia*

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**Introduction:** *Artemisia scoparia (red stem wormwood)* is traditionally used in Asia to alleviate pain, inflammation and febrile conditions. The present study was designed to explore the said pharmacological effects of *A. scoparia* hydromethanolic extract *(ASHME)*, in murine models of inflammation.

**Methods:** *ASHME* was prepared by macerating powdered plant material (700 g) in 80% methanol (1:5 W/V). Sprague Dawley rats (120-220 g) and Balb/c mice (20-35 g) of either sex were used for the in-vivo pharmacological testing (protocol # 2010-08-RIPS) under the NIH (USA) animal care guidelines (ver. 1985). Chemical (1% acetic acid, i.p.) and thermal (hot-plate, 50°C) nociception, carrageenan-induced (1% w/v, s.p.) rat-paw edema and yeast-induced (15% w/v, s.c.) mouse pyrexia models were used to test anti-inflammatory and anti-pyretic activities. The mechanism of anti-nociception in the hot-plate test was further evaluated in the presence of caffeine (10 mg/kg, i.p.), an adenosine receptor antagonist; naloxone (2 mg/kg, i.p.), a mu-opioid receptor antagonist; and mono sodium glutamate (MSG, 1g/kg, i.p.), an m-glutamate and NMDA receptor agonist.

**Results:** In mice, *ASHME* (400 mg/kg and 800 mg/kg, i.p.) inhibited acetic acid-induced writhing by 41.12% and 61.53% respectively ($P < 0.001$), increased the thermal nociception latency time by 18% and 27% respectively ($P < 0.001$), reduced carrageenan-induced paw edema in rats, by 62% and 74% respectively ($P < 0.001$) at 4 hours, and attenuated yeast-induced pyrexia in mice by 80% after 120 minutes ($P < 0.001$). Caffeine and Naloxone, significantly inhibited *ASHME* (400 mg/kg) mediated anti-nociceptive responses while MSG completely abolished its effect.

**Conclusion:** The anti-nociceptive and ant-inflammatory effects of *ASHME* appear to involve the inhibitory opioid and adenosine receptors while the excitatory glutamate and NMDA receptors activation may not be affected by *ASHME components*.

# Wild Honey as a drug discovery tool

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**Introduction:** Honey has been used to treat bacterial infections for thousands of years and has recently found renewed favour due to the emergence of antibiotic-resistant pathogens. Its antibacterial activity arises from various factors that include high sugar content, low pH (3-5), the production of hydrogen peroxide and the presence of bee-derived (cationic peptides) and plant-derived (e.g., methylglyoxal) compounds. Plant-derived antibacterial phytochemicals have potential use as single agents or in combination with other agents.

**Methods:** Processed commercial honey samples (25) including samples of Manuka, and raw, unprocessed samples donated by UK bee-keepers (175) were studied. The antibacterial activity of each honey was assessed against methicillin-resistant *staphylococcus aureus* (MRSA) using an agar-well diffusion assay. The contribution of individual factors to antibacterial activity was determined following successive neutralisation of known antibacterial factors. The main active component hydrogen peroxide was neutralised using catalase.

**Results:** Of the processed honey samples only those derived from Manuka plants demonstrated activity. In contrast, 91% of unprocessed honey samples exhibited inhibitory activity. Following neutralisation, one sample of unprocessed honey retained activity, suggesting the presence of novel phytochemicals.

**Discussion:** The majority of antibacterial activity observed in the raw unprocessed honey samples examined was due to the production of hydrogen peroxide. Only one sample showed evidence of containing an antibacterial phytochemical and studies are in progress to determine the nature of this compound.

# Protective Effects of Polyphenol Mixtures Against Lung Damage Caused by Sulfur Dioxide Derivatives

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**Introduction:** Air pollutants generate intracellular oxidative stress and inflammation that causes lung injury. Due to the complexity of the biochemical mechanisms, individual antioxidant phytochemicals may provide only limited protection for lung structure and function when compared to plant extracts containing synergistic mixtures of phytochemical nutrients. In the current study, we investigated the potential benefits of polyphenol mixtures extracted from Onaway potato cultivars (cv), which were previously found to contain several potentially active components including chlorogenic acid, caffeic acid, ferulic acid and rutin.

**Methods:** The polyphenols of cv Onaway potato extract were re-constituted and tested for protective effects against levels of sulphur dioxide derivatives ($SO_2D$; bisulfite: sulfite = 1:3) that are considered deleterious in humans. The polyphenolic composition of cv. Onaway potato extract consisting of the relative concentrations of chlorogenic acid : caffeic acid : ferulic acid : rutin (21 : 3.7 : 2.7 : 1) was re-constituted with purchased chemicals and tested for protective effects against deleterious exposure levels of $SO_2D$ in differentiated MucilAir™ 3-dimensional lung tissue cultures (purchased from Epithelix Sàrl, Switzerland) that were exposed to 0.1 mM $SO_2D$ for 4 h. Lung tissue cultures were pre-incubated in the absence or presence of the above polyphenolic mixture diluted in culture medium to 20 µM (total polyphenols) for 4 h prior to exposure to $SO_2D$. The release of IL-8 into the medium was quantified using a commercial enzyme immunoassay (BD OptEIA Set for human IL-8, BD Biosciences) and expressed in nanograms of IL-8 release per $10^6$ cells. Intracellular reactive oxygen species (ROS) were quantified using a fluorescent probe, 5-(and 6-) carboxy-2'7'-dichlorodihydrofluorescein diacetate.

**Results and Discussion:** Cell viability was unaffected by 0.1 mM $SO_2D$ or the 20 µM polyphenol mixture. In the absence of pre-treatment, 0.1 mM $SO_2D$ induced a significant 5-fold increase in IL-8 release after 4 h of incubation and a greater than 3-fold increase in ROS. Pre-treatment with the 20 µM polyphenol mixture, however, suppressed $SO_2D$-induced IL-8 release to levels seen in control cultures and markedly blunted the increase in the levels of ROS. The anti-inflammatory potency of the polyphenol mixture appears to arise from synergistic actions of the cv Onaway potato components chlorogenic acid, caffeic acid, ferulic acid and rutin, and provides support for the idea that interactions between key components in plant extracts may be important for their functions *in vivo*.

# Identification of an anti-influenza virus compound from a Chinese medicine decoction

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**Introduction:** Influenza epidemics have become a major public health concern. Recurring emergence of new influenza virus strains that are resistant to currently approved antiviral medications pose a critical need to explore new or alternative medications. A classical Traditional Chinese Medicine (TCM) decoction, named AVD, has a long history for treating respiratory diseases. In the present study, we aim to examine the effectiveness of AVD in treating influenza. The mechanism of action of AVD was also investigated through identifying the active antiviral compound from it.

**Methods:** AVD extract was prepared according to Chinese Pharmacopeia. The effectiveness of AVD was investigated in a mouse model. Briefly, BALB/c mice were infected intranasally with the mouse-adapted influenza virus. They were then administrated with the AVD extracts through oral gavage. Survival rate of the mice and the lung viral loads were determined. The active antiviral compound was identified by using a bioactivity-guided fractionation scheme in which, herbal extracts were fractionated and purified by high-performance liquid chromatography. The resulting fractions were examined for the antiviral effects by carrying out tissue culture infective dose assay. The active fraction showing antiviral effects was subjected to repeated purification until the pure compound was obtained.

**Results:** We showed that AVD increased the survival rate of influenza virus infected-mice with significant reductions in lung viral loads. In addition, we purified an active antiviral compound named as Compound-A1 from AVD. Compound-A1 was identified by determining its chemical structure. Anti-influenza virus effect of Compound-Al has not been described previously. Our present results showed that Compound-A1 suppressed a wide range of influenza virus strains including the oseltamivir-resistant and the pandemic H1N1 viruses. Compound-A1 also increased the survival rates of influenza virus-infected mice. We further demonstrated that Compound-A1 suppressed virus replication by reducing the expression of influenza virus matrix protein.

**Discussion:** TCM has been recognized as an important part in complementary and alternative medicine and it is a plentiful source of antimicrobial drugs. Our study not only supports the efficacy of AVD, but also identified an active antiviral compound from the decoction. This may provide a lead compound for future drug development.

*This project was supported in part by grants from PuraPharm International, Prof Francis S.K. Lau, and Ms Ho Yuk Ching (Ching Ping) Research Funds awarded to Prof A. Lau.*

# Attenuation of Pro-Inflammatory Responses by [6]-S-gingerol via Inhibition of ROS/NF-kappa B/COX2 Activation in HuH7 cells

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**Introduction:** Hepatic inflammation drives the pathogenesis of the chronic diseases such as type-2 diabetes mellitus, insulin resistance, atherosclerosis, and nonalcoholic fatty liver disease (NAFLD). [6]-S-gingerol has been shown to protect against inflammatory insults in various stimulus-induction models. However, it remains unknown whether [6]-S-gingerol protects hepatocytes against cytokine-induced inflammation. Nuclear factor κB (NFκB) is a master regulator of the hepatic inflammatory response. In addition, cyclooxygenase (COX) is among the most important pro-inflammatory mediators and COX-2 is responsible for persistent inflammation. We now explore whether [6]-S-gingerol inhibits IL1β-induced hepatic activation of NFκB and the associated increase in COX2 expression.

**Methods:** HuH7 cells were stimulated with IL1β (8 ng/L) to establish a cytokine-induced cell inflammatory model.

**Results:** Pre-treatment of HuH7 cells with [6]-S-gingerol (100 μM) for 6 h before exposure to IL1β for 3 h significantly attenuated the IL1β-induced inflammatory response and oxidative stress, as evidenced by decreased mRNA levels of inflammatory factor IL6, IL8, serum amyloid A1 (SAA1) and reactive oxygen species (ROS) generation and by increased mRNA level of antioxidant protein, DHCR24. In addition, pretreatment with [6]-S-gingerol (100 μM) markedly reduced IL1β-induced COX2 overexpression as well as NFκB activity. Similar to the protective effects of 6-S-gingerol, both 100 μM NS-398 (a selective COX2 inhibitor) and 50 μM pyrrolidine dithiocarbamate (PDTC; a selective NFκB inhibitor) suppressed mRNA levels of IL6, IL8 and SAA1. Importantly, PDTC attenuated over-expression of COX2 induced by IL1β. Of particular note, butylated hydroxytoluene, a ROS scavenger, conferred a similar protective effect to that observed in response to [6]-S-gingerol against the IL1β-induced inflammatory response.

**Conclusions:** Together, the findings of the present study demonstrate that [6]-S-gingerol protects HuH7 cells against IL1β-induced inflammatory insults through inhibition of the ROS-activated NFκB/COX2 pathway.

# Identification of a novel pathway of [6]-S-Gingerol signalling in HuH7 Cells

**Xiao-Hong Li**[1,2], Kristine McGrath[1,3], Van Tran[2], Colin C Duke[2], Yi-Ming Li[2], Alison Heather[1,3] and Basil D Roufogalis[2]

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**Introduction:** Calcium signals in hepatocytes regulate glucose, fatty acid and amino acid metabolism, and they control cell growth, proliferation, and death. Members of the transient receptor potential (TRP) channel superfamily are candidate $Ca^{2+}$ influx channels. NFκB activation has been demonstrated to be strictly dependent on $Ca^{2+}$ influx and often induces genes favouring cell survival. We previously reported that [6]-S-gingerol is a potent and efficacious agonist of the TRPV1 receptor in capsaicin-sensitive neurones. We now explore whether [6]-S-gingerol activates the TRPV1 receptor and NFκB activation in HuH7 cells.

**Methods:** [6]-S-gingerol induced intracellular $Ca^{2+}$ transients in HuH7 cells were measured using the Fluo-4 $Ca^{2+}$ assay. Changes in TRPV1 receptor mRNA expression were quantified by Real-Time RT-PCR. NFκB activity was identified using HuH7 cells transfected with an NFκB luciferase reporter plasmid and then exposed to [6]-S-gingerol for different time intervals.

**Results:** [6]-S-gingerol (50 μM, 100 μM) rapidly increased intracellular $Ca^{2+}$ in HuH7 cells in a concentration-dependent manner; the increase was transient and blocked by removal of extracellular $Ca^{2+}$ in the presence of EGTA (2 mM). [6]-S-gingerol (100 μM) increased the TRPV1 receptor mRNA level and the TRPV1 receptor agonist capsaicin (10 μM) induced an intracellular $Ca^{2+}$ transient in HuH7 cells. Importantly, the [6]-S-gingerol-induced intracellular $Ca^{2+}$ transient was blocked by the TRPV1 receptor antagonist capsazepine (40 μM) in a manner similar to its inhibition of capsaicin. NFκB was rapidly activated within 7.5 min of exposure to [6]-S-gingerol (100 μM) and reached a peak at 15 min. After a longer incubation of 30 min with [6]-S-gingerol, NFκB activation started to decline, and was deactivated after 120 min.

**Conclusions:** Together, the findings of the present study suggest that the activation of TRPV1 receptor by [6]-S-gingerol acting as an agonist leads to non-genomic and transient activation of NFκB by $Ca^{2+}$, which may be of benefit for cell survival in HuH7 liver cells.

# Bioassay guided isolation and identification of active components in *Zingiber officinale* (ginger) promoting glucose uptake in L6 skeletal muscle cells

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**Introduction:** Ginger (*Zingiber officinale,* Roscoe) has shown promising anti-hyperglycemic properties in clinical and experimental studies. However, the active components and mechanism of action are not fully understood.

**Methods:** Total extract of ginger was prepared from freeze-dried ginger powder (1 kg, provided by Buderim Ginger Pty Ltd) with ethyl acetate (3 L) by continuously stirring at room temperature. The extract was fractionated by a normal phase column chromatographic method. The total ginger extract and fractions were evaluated for their glucose uptake effect in insulin stimulated L6 rat skeletal muscle cells using radioactive labelled 2-[1,2-$^{3}$H]-deoxy-D-glucose. Glucose transporter (GLUT4) expression and translocation were detected by Western blotting and immunostaining.

**Results:** The total ginger extract dose-dependently increased glucose uptake in L6 muscle cells. This activity was found to predominate in two fractions of ginger extract. Analyses of these fractions by nuclear magnetic resonance (NMR) spectroscopy and HPLC revealed that both fractions contained mainly (*S*)-[6]-, (*S*)-[8]- and (*S*)-[10]-gingerols, components which are known to contribute to the pungency of ginger. (*S*)-[8]-gingerol was the most potent compound in enhancing glucose uptake and increasing GLUT4 expression and translocation to the plasma membrane.

**Discussion:** Effective control of blood glucose level is achieved by improving insulin sensitivity and promoting glucose uptake into peripheral tissues in type 2 diabetic patients. The present study has demonstrated that a ginger extract and fractions containing pungent phenolic principles significantly increased glucose uptake in L6 rat skeletal muscle cells. Enhanced glucose uptake by gingerol was associated with translocation of GLUT4 to the surface plasma membrane, as demonstrated by the results with (*S*)-[8]-gingerol. The study provides supportive evidence for the potential of ginger in management of type 2 diabetes. (Supported by an Australian Research Council (ARC) Linkage Grant)

# Anti-inflammatory Mechanisms of Compound Z from Gastrodia & Uncaria Decoction, a Commonly Used Post-stroke Decoction

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**Introduction:** Ischemic stroke is a leading cause of death and long-term disability worldwide. Accumulating evidence implicates tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO), produced by microglial cells, as key mediators in the pathogenesis of the disease. Gastrodia & Uncaria Decoction (GUD), a traditional herbal remedy, has been commonly used in the therapy of post-ischemic stroke in China. In this study, we set out to identify the constituents of GUD that modulate the inflammatory responses.

**Methods:** Murine immortalized microglial BV-2 cells and neuroblastoma cell line Neuro-2a were cultured in Dulbecco's modified Eagle's Minimum Essential Medium and Eagle's Minimum Essential Medium, respectively. The medium was supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin at 37$^{o}$C in a humidified incubator containing 95% air and 5% $CO_2$. Bioactive compounds were purified and identified using chromatographic and spectrometric methods. The inhibitory effects of extracts/compounds on NO production were examined using microglial cells exposed to lipopolysaccharide (LPS) as the inducer. Nitrite levels were determined using Griess reagent. mRNA levels of iNOS and TNF-α were determined by RT-PCR and Q-PCR. iNOS expression and PI3K/Akt phosphorylations were determined by Western blot.

**Results and Discussion:** Compound Z dose-dependently inhibited LPS-stimulated NO over-production. It also suppressed mRNA levels and protein expression of inducible nitric oxide synthase (iNOS) and TNF-α upon LPS-induction. In addition, phosphorylations of phosphoinositide-3 kinase (PI3K) and protein kinase B (Akt) were suppressed. Finally, compound Z protected murine Neuro-2a neuroblasts against neurotoxicity from conditioned media transferred from LPS-challenged BV-2 cells. The results reveal that Compound Z and synthetic analogues have potential for the development of effective new drugs for treating ischemic stroke.

# Pharmacokinetics and Pharmacodynamics of 3-Methoxypterostilbene: A Constituent of Chinese Medicinal Plants

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**Introduction**: 3-Methoxypterostilbene is a stilbene analogue of resveratrol that can be found in *Sphaerophysa salsula*, used in Chinese medicine to treat hypertension, and as an aglycone of a stilbene glucoside found in Chinese rhubarb (*Rheum palmatum*). Presently, very little literature on the bioactivity of 3-methoxypterstilbene is available and no published literature on the pharmacokinetics of 3-methoxypterostilbene currently exists.

**Methods:** A validated, novel and simple isocratic RP-HPLC method to quantify 3-methoxyptoerstilbene in biological fluids (Martinez *et al*., 2012) was used to determine the content of 3-methoxyptoerstilbene in *S. salsula* and *R. palmatum* and the pre-clinical pharmacokinetics of the compound in rats. Male Sprague-Dawley rats were cannulated and dosed either intravenously or orally with 3-methoxypterostilbene in PEG 600 at 10 mg/kg or 100 mg/kg, respectively. Serum and urine samples were collected over a 72h period post-dose. Content analysis was carried out using a simple methanol extraction. Anti-oxidant capacity and COX-1 and -2 inhibition was assed via commercially available assay kits. α-Glucosidase and α-amylase inhibition was assessed using simple colorimetric assays.

**Results and Discussion:** After intravenous and oral administration, 3-methoxypterostilbene was detected in both serum and urine as the algycone and glucuronidated metabolite suggesting that 3-methoxypterostilbene undergoes phase II metabolism. Content analysis showed that 3-methoxyptoerstilbene was present in *S. salsula* and R. *palmatum* in its aglycone and glucoside forms. 3-Methoxypterostilbene demonstrated *in vitro* concentration dependent anti-oxidant, COX inhibitory, α-glucosidase inhibitory and α-amylase inhibitory activities.

**Reference:** Martinez SE, Sayre CL and Davies NM. Analysis of 3-methoxypterostilbene in biological fluids by high-performance liquid chromatography: application to pre-clinical pharmacokinetics. *Biomedical Chromatography* 2012; doi: 10.1002/bmc2749.

# Plasma glucose response to acute oral administration of the α-glucosidase inhibitor, Montbretin A

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Presented at Mechanism-Based Natural Product Development Conference, Whistler, B.C., September 2012

**Introduction:** Postprandial hyperglycemia is a risk factor for cardiovascular disease in diabetic patients. The therapeutic use of α-glucosidase inhibitors, such as Acarbose, are known to reduce elevated plasma glucose levels following a meal but are not well tolerated due to side effects. Research has been on-going to develop products that will minimize or eliminate these side effects and improve patient compliance. Montbretin A (MbA), an extract of the hybrid plant, *Crocosmia crocomiiflora*, has been shown to be a potential α-glucosidase inhibitor *in vitro*. The objective of this study was to determine if MbA is effective orally in lowering plasma glucose levels in diabetes.

**Methods:** Animal models of type-1 (streptozotocin, male, 200-300 g) and type-2 (Zucker Diabetic Fatty rats, male, 400-500 g) diabetes were challenged with a starch load (2 g/kg) immediately followed by MbA (dose range: 0.5 to 10 mg/kg body weight) administered by oral gavage. Plasma glucose levels were determined prior to and at 30, 60, 90 and 120 min following drug administration. Acarbose (10 mg/kg) was used as a positive control for the plasma glucose response to α-glucosidase inhibition. All animal work was approved by the University of British Columbia Ethics Committee; certificate number A08-0221.

**Results:** MbA prevented the starch-induced increase in plasma glucose levels at doses greater than 5 mg/kg in both type-1 and type-2 animal models of diabetes. In type-2 diabetic animals, MbA (7.5 mg/kg) reduced glucose levels by 38 ± 3% as compared to 25 ± 2% for acarbose (10 mg/kg).

**Discussion:** These data show that *in vivo* oral administration of MbA was effective in lowering plasma glucose levels. Further studies are required to determine the chronic effects of oral MbA treatment in type-1 and type-2 diabetes mellitus.

# The anti-inflammatory and neuro-protective effects of herbal compounds in treating neurodegenerative diseases

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**Introduction:** Neuroinflammation and oxidative stress play important roles in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and stroke. The use of traditional Chinese medicine (TCM) for treating neurodegenerative diseases has gained popularity. *Ligusticum chuanxiong* (LCX) is a commonly used TCM with putative analgesic effects while *Cimicifuga racemosa* (CR) (black cohosh) has a long and diverse history of medicinal use in Eastern United States and Canada for treating malaria, rheumatism and menstrual irregularities. Using bioassay-guided fractionation scheme, Z-lig and Cim A with anti-inflammatory effects have been identified from these two herbs, respectively. The aims of the study were to investigate the anti-inflammatory and neuro-protective effects of the bioactive compounds from these two herbs.

**Methods:** The productions of inflammatory mediators including TNF-α and nitric oxide were measured using ELISA and Griess agent, respectively. The gene expressions were examined using RT-PCR. Regarding neuroprotection, the cell viability of neuronal cells was measured using MTT assays while the intracellular reactive oxygen species were studied using DCF-DA fluorescence staining.

**Results and Discussion:** Z-lig and Cim A were found to inhibit the production of TNF-α and nitric oxide in LPS-induced BV-2 microglial cells via the suppression of mRNA of TNF-α and iNOS. Moreover, they prevented hydrogen peroxide-induced cell death of PC12 neuronal cells by suppressing the production of intracellular reactive oxygen species. These findings indicate that Z-lig and Cim A are potential drug candidates for treating neurodegenerative diseases.

# Antipsoriatic potential of *Psoralia corylifolia* seeds

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**Introduction:** Psoriasis is a common skin disease affecting 2% of the world population, characterized by epidermal keratinocyte hyper-proliferation, abnormal keratinocyte differentiation and immune cell infiltration. It is recurrent and debilitating. Existing topical treatments such as emollients, coal tar and dithranol have low efficacy and are cosmetically unacceptable, whereas systemic therapies such as methotrexate, cyclosporine and acitretin have significant side effects. Herbal remedies are promising in the management of dermatological conditions including psoriasis. *Psoralea corylifolia* (Babchi) (Fabaceae) is an important plant in the Indian Ayurveda and Tamil Siddha systems of medicine, and also Chinese medicine. Traditionally, the seed decoction is used for treating psoriasis. However, the antipsoriatic activity of *Psoralia corylifolia* seeds has not been scientifically evaluated. Hence, in the present study, we investigated anti-psoriatic activity of the seed extract using a mouse tail model.

**Methods:** 95% Ethanolic extracts of *Psoralia corylifolia* seeds were prepared by soxhlet extraction and analyzed by high performance thin layer chromatography (HPTLC) for their phytoconstituents. The anti-psoriatic activity of 95% of ethanolic extracts was assessed using a sulphorhodamine B (SRB) assay on HaCaT human keratinocyte cell lines (*in-vitro)* and a mouse tail model (*in-vivo*). In this well-characterised model[1], the mouse tail is considered to have a psoriasis-like condition and is used for evaluating anti-psoriatic activity. Institutional animal ethics approval was obtained for the mouse experiments. About 0.5 ml of the extract or Tazret gel (0.1 % Tazarotene) was applied topically to the proximal part of the tail for about 2.5 cm and allowed to remain in contact for 2 h. The tails were then washed with water. Treatment was given once daily for 14 days. The animals were sacrificed using deep ether anaesthesia and cervical dislocation, 2 h after the last treatment. The tails were then removed by proximal transection and stored in separate containers containing 10% formalin in saline. Longitudinal sections of tail skin were prepared, stained with hematoxylin-eosin and evaluated by histopathological examination.

**Results and Discussion:** The seed extracts gave strongly positive results in phytochemical analysis and were analysed for the presence of gallic acid, a potent phenolic antioxidant. HPTLC fingerprinting confirmed its presence. 95% ethanolic extract-treated groups showed significant epidermal differentiation in the degree of orthokeratosis (85.4 ± 3.6%) when compared to the negative control (17.3 ± 4.1%) and this was comparable to the effect of the positive control, 0.1% Tazret gel (90.0 ± 2.0%). The extract also induced a decrease in relative epidermal thickness when compared to the control group. Thus, the ethanolic extract showed an $IC_{50}$ value of 255 µg/ml, with good anti-proliferant activity when compared to the positive control asiaticoside with an $IC_{50}$ value of 20.1 µg/ml. *Psoralia corylifolia* seed extract showed an overall antipsoriatic activity of 75.9%, when compared with standard Tazret gel (87.9%) and the present study confirms its traditional use in psoriasis treatment.

1. Vogel GH. Drug Discovery and Evaluation: Pharmacological Assays. Springer New York, 2008) pp.1964-65.

# Anti-osteoarthritis activity of a protein fraction (VB-P5) from the fresh water snail Viviparous bengalensis

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**Objective:** To evaluate the anti-osteoarthritic activity of a protein fraction VB-P5 from the fresh water snail Viviparous bengalensis in a rat experimental model.

**Materials & Methods:** Snails were collected and whole body extracts were prepared by homogenization followed by centrifugation at 1500 rpm (bench-centrifuge, Plastocraft). The supernatants were purified by ion exchange chromatography (IEC) and reverse phase high performance liquid chromatography (RP-HPLC). Anti-osteoarthritic activity was assessed in collagen-induced arthritic male Wistar rats. All animal experiments were approved by the departmental animal ethics committee in accordance with the guidelines of the Indian Government's Committee for the Purpose of Control and Supervision of Experiments on Animals (Approval no. PHY/CU/IAEC/20/2008). Results were obtained from the following experimental groups for osteoarthritis (OA) models: Group-1, Sham-injected controls; Group-2, collagen-injected (OA) controls; Group-3, collagen-injected with standard therapy (indomethacin for OA); Group-4, collagen-injected with VB-P5 treatment (1 $mg.kg^{-1}$ i.p. x 15 days); Group-5, collagen-injected with VB-P5 treatment (2 $mg.kg^{-1}$ i.p. x 15 days). Anti-osteoarthritic activity was examined using the following physical (ankle, knee diameter), urinary (hydroxyproline, glucosamine, calcium, creatinine, pyridinoline, deoxypyridinoline), and serum parameters (ACP, ALP, LPO, GSH, SOD, Catalase, and cytokines including TNFα/CINC-1/IL-10/IL-12/Pyridinoline/Deoxy-Pyridinoline/osteocalcin). Active fractions were subjected to UV absorbance scan and fluorescence scan analysis. Data were expressed as means ± SEM (n = 6). Differences between means for different groups were assessed by one-way ANOVA and statistical significance was accepted at $P < 0.05$.

**Results:** Following IEC of snail whole body extracts, peak-5 (VB-P5) was found to possess anti-osteoarthritic activity. Thus, in the collagen-arthritis rat model, physical parameters including body weight/ankle/knee diameters/urinary hydroxyproline glucosamine/pyridinoline/deoxypyridinoline/ serum ACP/ALP/LPO/GSH/SOD/Catalase and cytokine (TNFα/CINC-1/IL-10/IL-12/Pyridinoline/Deoxy-Pyridinoline/osteocalcin) levels were significantly restored after VB-P5 treatment. Both dose regimens were more effective than standard drug treatment with indomethacin and the higher dose was more effective than the lower dose. As VB-P5 is protein in nature, it produced a sharp peak at 280 nm on RP-HPLC and a UV scan of VB-P5 (247 μg/ml) yielded an absorbance peak of 0.167 at 286 nm. In addition, an emission peak was observed at 360 nm when samples of VB-P5 were excited at a wavelength of 280 nm. Finally, CD spectra analysis demonstrated 50.9% strong α-helical structure with random coil.

**Conclusion:** The results show that VB-P5 contains one or more proteins that possess anti-osteoarthritic activity in an experimental rat model. Further studies are in progress to characterize the active compounds and determine their mechanisms of action.

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# Stereospecific Analysis of Hops Flavonoids: 6-Prenylnaringenin, 8-Prenylnaringenin, and Isoxanthohumol: Nutraceutical Content Analysis, Anti-Oxidant, Anti-Inflammatory, and Anti-Diabetic Properties

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**Introduction:** While hops (*Humulus lupulus*) are most commonly associated with beer, they also have a long history as a medicinal herb. Today, hops-based nutraceuticals are commonly taken as natural hormone replacement therapy and menopause symptom remedies as well as sleep aids, relaxants, and anti-inflammatories. Three chiral flavonoids of interest that can be found in hops are 8-prenylnaringenin (the most potent phytoestrogen presently known), 6-prenylnaringenin, and isoxanthohumol. Currently, no validated stereoselective HPLC methods of detection exist for these three compounds in the literature and validated methods of detection are needed to perform content analyses and evaluate the metabolism kinetics of the compounds' enantiomers. Additionally, there is a strong interest to find additional pharmacological uses of these compounds.

**Methods:** Three novel and simple isocratic RP-HPLC methods were developed to separate and quantify the enantiomers of +/- 8-prenylnaringenin, +/- 6-prenylnaringenin, and +/- isoxanthohumol. These methods employed the use of Chiralpac® columns. Content analyses of readily available hops-based nutraceutical products were carried out using the RP-HPLC methods along with a methanol-based extraction. Anti-oxidant capacity and COX-1 and -2 inhibition of the three compounds was assessed via commercially available kits. α-Glucosidase and α-amylase inhibition was assessed using simple colorimetric assays.

**Results and Discussion:** The three RP-HPLC methods of separation and quantification were successfully applied to the content analyses of several hops-based nutraceuticals. All three compounds displayed primarily concentration-dependent anti-oxidant, COX inhibitory, α-glucosidase inhibitory and α-amylase inhibitory activities.

# A validated HPTLC method for quantitative determination of dopamine in nephroprotective plants

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**Introduction**: A simultaneous high-performance thin-layer chromatographic (HPTLC) method has been developed and validated for quantification of the biologically active constituent dopamine, in dried seed powders prepared from widely-used nephroprotective plants.

**Method**: Dopamine stock solutions (1μg/mL) were prepared in double distilled water. Different volumes (0.1, 0.2, 0.4 and 0.5 to 5μl) of the dopamine stock solution were spotted in duplicates on TLC plates to furnish 100, 200, 400 and 500 to 5000 μg/spot. Chromatography was performed on 20 cm × 10 cm HPTLC plates. Standards and samples were applied to the plates as 5 mm bands, 6 mm from each other and 10 mm from the bottom edge of the plate using Camag Linomat V applicator. The plates were developed to a distance of 80 mm, with *n* butanol-acetone-formic acid-water in the ratio of 7.0: 0.5: 1.5: 1 (*v/v/v/v*) mobile phase. Scanning was performed at 280 nm using Camag TLC scanner III. The method was validated for precision, recovery, repeatability, and accuracy in accordance with International Conference on Harmonisation (ICH Q2) guidelines.

**Results**: The results met the acceptance criteria for accuracy, precision, linearity, detection and quantification limit set by ICH guidelines. The method yielded a compact spot for dopamine at an *R*f value of 0.64 ± 0.02. Linear regression analysis demonstrated a good relationship between dopamine content and peak area over the range of 100–5000ng ($r = 0.993$). The apparent limit of detection (LOD) and limit of quantification (LOQ) were, respectively, 9.15 and 27.7 ng. Using this method, the dopamine contents of dried seed powders from *Mucuna pruriens*, *Portulaca oleracea* and in *Delphinium denudatum* were respectively 0.198% (w/w), 1.22%(w/w) and 0.144% (w/w) with low % RSDs in each case.

**Conclusion**: The HPTLC method we have developed provides simultaneous, quantitative, accurate estimation of dopamine with potential application for the quantitation of dopamine in polyherbal formulations.

# A comparative study on the chemical profiles and cytoprotective effects of the roots of *Pueraria lobata* and *Pueraria thomsonii*

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**Introduction:** *Pueraria lobata* (Willd.) Ohwi (PL) and *P. thomsonii* Benth (PT) are traditional Chinese medicines used for treating diabetes and cardiovascular diseases. However, the comparative efficacies of the species are unknown. Here we compared (i) the chemical profiles of PL and PT, and (ii) their cytoprotective effects on human EA.hy926 umbilical vein endothelial cells.

**Methods:** Powdered PL and PT (1 g) were extracted with 46 % ethanol (120 mL) in a sonication bath for 5 min at 40 °C. Extracts were centrifuged and filtered through a 0.22 μm filter then dried. Dried ethanolic extracts of PL (n = 3) and PT (n = 3) were then reconstituted in 46 % ethanol (1 mg/mL) and analysed by Ultra-Performance Liquid Chromatography. In cellular assays, dried ethanolic extracts were dissolved in DMEM-F12 with 10% FBS, and 1% penicillin/streptomycin. After seeding, EA.hy926 cells were incubated with control medium or PL or PT at various concentrations for 24 h. Cytoprotection against hydrogen peroxide (0.4 mM)-induced cell death was measured using the MTT assay.

**Results:** The contents of major chemicals including puerarin, daidzin, daidzein, 3'-hydroxypuerarin and 3'-methoxypuerarin were greater in PL than PT ($p < 0.01$). At a total solute concentration of 100 μg /mL, PL had no effect on cell viability, whereas PT exhibited a cytotoxic effect when compared to the vehicle-only control in the MTT assay. In a dose-dependent manner, PL (100-1000 μg/mL) but not PT protected EA.hy926 cells against hydrogen peroxide-induced cell death.

**Discussion:** This study demonstrates that PL and PT possess distinct profiles with respect to chemical composition, cytotoxicity, and cytoprotective efficacy on hydrogen peroxide-treated endothelial cells. The findings provide new insights into the clinical usage of the two *Pueraria* species.

# The effects of chronic oral administration of Montbretin A, a human pancreatic α-amylase inhibitor, in Zucker Diabetic Rats

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**Introduction:** In 2010 approximately 6.4% of the world population had diabetes mellitus. By 2030 its prevalence is expected to increase to 8% [DOI:10.2337/diacare.27.5.1047]. In addition, it has been estimated that a child born in the USA in 2000 has a greater than 1 in 3 risk of developing diabetes in their lifetime [*www.cdc.gov/**diabetes**/news/docs/lifetime.htm*]. Negative modulation of postprandial glucose release provides a novel approach to the treatment of hyperglycemia in type-2 diabetes. Montbretin A (MbA), a glycosylated acyl flavonol isolated from the plant hybrid *Crocosmia crocomiiflora*, is an inhibitor of human pancreatic α-amylase *in vitro* and has acute oral effects on plasma glucose levels *in vivo*. In this study, we examined the effects of chronic MbA treatment in an animal model of type-2 diabetes, the Zucker Diabetic Fatty (ZDF) rat.

**Methods:** Male ZDF and lean littermate control rats were treated chronically with MbA *ad libitum* in the drinking water at a dose of 7.5 mg $kg^{-1}$ $day^{-1}$ for 9 weeks. Untreated rats had *ad libitum* access to drinking water only. Unfasted plasma glucose levels were measured weekly from blood collected from the saphenous vein. At termination, abdominal fat pads were collected to assess body composition. In addition, plasma was collected for the determination of insulin and lipid levels, and assessment of liver function and oxidative stress. All animal work was approved by the University of British Columbia Ethics Committee; certificate number A08-0221.

**Results:** MbA reduced plasma glucose levels by 46±3% in ZDF rats (n=6). There was no effect of MbA on glucose levels in control-treated animals. In addition, the total plasma cholesterol level was reduced and liver function (alanine transaminase assay) was improved following MbA treatment. There was a significant reduction in oxidative stress is indicated by a reduction in total plasma nitrate/nitrite and plasma 8-isoprostane levels. There were no signs of gastrointestinal side-effects.

**Discussion:** MbA was an effective glucose-lowering agent in ZDF rats. In addition, MbA improved plasma lipid levels and measures of oxidative stress. Further studies are necessary to assess the side effects associated with α-glucosidase inhibition.

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