Abstracts

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Functional food and nutraceuticals course at masters level: experience sharing from teaching and course development perspectives

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The advent of functional food and nutraceuticals has given rise to many courses on this subject being included in food and nutrition, agriculture, health and life sciences postgraduate programmes. A survey of various programmes suggests that each course has its unique emphasis determined by the focus of the parent programme, such as identification of bioactive compounds, product development and health promoting applications. The author has been involved in the design and delivery of a 30-credit course on Natural Health Products (NHP) taught as part of the University of Sunderland MSc in International Pharmaceutical Science programme offered in Hong Kong. The programme targets in-service pharmaceutical business personnel and thus requires a practical slant in the design of course contents and delivery methods. In line with this consideration, the NHP course combines four elements into the curriculum planning process – products, technology, markets and regulation, and the contents are divided into two parts: Western herbal medicines and functional food/nutraceuticals, to facilitate the identification of suitable teaching expertise. This presentation recounts the experience of teaching and developing the contents for the second part of the course on functional food/nutraceuticals which is taught through lectures and student workshops. The teacher-centered lectures focus on developing students' understanding of conceptual issues about the science and regulatory aspects, while student workshops are used to increase understanding of the quality, safety and efficacy issues of named functional food/nutraceutical bioactive compounds and products. This dual method helps to ensure a balance of didactic and interactive approaches in teaching delivery. Students are assessed through a portfolio emulating submission of a new product for health claim application to FDA. They are paired in advance to comment on each other's submissions and act as proponents and opponents of the filed applications during moderated debates taking place in class. In this manner, not only the writing and oral skills are being developed, but also students are exposed to the intricacies of establishing the validity of scientific information in the face of administrative procedures set by regulatory bodies. Comments received from student evaluation have been positive but due to the small class size (<20), further teaching experience accumulation and learning from other successful courses are required in order to continuously modify both content and teaching methods as the subject matter of functional food/nutraceuticals is fast-changing and instruction needs to keep pace with it.

Proposing a flexible curriculum for teaching natural health products at different academic levels

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This presentation aims to share our experience in teaching natural health products (NHP) which has been delivered to a range of students ranging from bespoke training courses to a module at Masters programme. We propose that the subject matter of NHP can be divided into three parts: a background knowledge base equating to the minimum underpinning in concepts and related theories necessary for understanding how these products work; a products usage knowledge base; and an advanced knowledge base relating to the various regulatory, technical and safety aspects of product commercialization. The scope of the background knowledge base include the following topics: definition of NHP terminologies and description of market characteristics; concepts of health and wellbeing, basics of nutrition, rationale for using NHP; antioxidants, antiaging, detoxification; basic immunological responses and pathophysiological processes. The products usage knowledge base can be categorized either by product functions or target group applications. The advanced knowledge base includes analysis and comparison of regulatory requirements; product standards, quality assurance and methods of analysis; safety issues; new ingredients and markets. Typically, we tailored the above to produce company-specific training

courses of 10-12 hours, a generic Certificate in NHP of 70 hours for in-service marketing and sales personnel working in this sector, a 36-hour course at Higher Diploma level, and a 20-hour course at Masters level. We used variations in learning activities and assessment methods to reduce the number of didactic teacher-led sessions at higher academic levels. Our experience suggests that the subject matter of NHP can be taught to a wide range of students at different academic levels by adjusting the curriculum to deliver essential contents which appear recurrently throughout each academic levels but in different degrees of depth and difficulty. Teaching in this manner thus follows the educational concept of a "spiral curriculum" which advocates revisiting the basic subject matter at different academic levels so that those who cannot grasp it this time will be able to do so later on.

Promoting good health with healthy food and physical activity

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Introduction: The combination of healthy food and physical activity are suggested to be a primary strategy for reducing many of the chronic diseases. People who follow a healthy diet would also have higher level of physical activity. With the implementation of healthy food and physical activity in daily lifestyle, the promotion of good health could be achieved. The aim of this study is to review the strategies used to improve the healthy food and physical activity behavior for promoting good health. **Methods:** A systemic search of published literature was conducted using the following databases: Medline, Cinahl and Embase. **Results:** Research suggested that intervention programs showed positive results in improving peoples' behaviors toward healthy food and physical activity. The intervention programs including (1) live lectures with topics like modern medicine and health myth, obesity, dietary fiber, dietary fat, exercise, lifestyle and health; (2) face-to-face visits of self-management support and community linkage sessions; (3) follow-up phone calls to reinforce goal attainment and problem-solve barriers; (4) newsletter with behavior goals; (5) workbooks and assignments with topics on healthy food and physical activity issues. Moreover, school intervention with curriculum component focusing on knowledge and skills development related to healthy eating, physical activity and diabetes education; family and peer component with the support from parents and peers as role models; environment component and healthy school program focusing on healthful eating were suggested to improve peoples' overall health knowledge. To conclude, intervention programs showed beneficial results in health knowledge, physical activity level, dietary behavior, and multilevel support for healthy lifestyles. Thus, it is recommended that information on healthy food and physical activity were important components for intervention programs at promoting good health.

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Green heterogeneous catalyst for biodiesel synthesis

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Green biodiesel synthesis from biomass like algae oil has drawn an increasing attention recently due to its sustainability, renewability and biodegradability (1, 2). Current biodiesel production involves the use of homogeneous strong base and acid that is non-renewable and causes serious pollution which triggered us to search for a greener alternative. Heterogeneous metal oxides have been proved to be an effective replacement because of their high catalytic activity, non-polluting nature, reusability and low processing cost. Among all metal oxides, calcium oxide was found to be one of the most active candidates due to its high basicity for effective deprotonation of MeOH. However, it loses its catalytic activity upon prolonged use as Ca ions leach into the reaction medium (3). We are interested in the development of long-lived and effective alkaline earth based metal oxide nanostructures catalyst for continuous green biodiesel synthesis using microwave irradiation as heating source. Microwave irradiation heating was found to be more energy efficient than traditional heating with significant reduction of reaction time, which opens up its application for fixed-bed continuous biodiesel synthesis.

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Microalgae, the multi-purpose solution for sustainable development of our water resources and biofuel

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Harmful algal bloom (HAB) describes massive growing of microalgal cells over a localized coastal area that kill marine creatures and affect fish-farming industries. In Hong Kong and elsewhere, frequent occurrence of HAB presents a continuous threat to sustainable development of our water resources as well as other commercial activities. However, microalgae, a HAB-causative agent, may help in removing heavy metals from the sea and thus improve seawater quality. We have studied if *Prorocentrum triestimum* (strain AD1), a HAB-causing dinoflagellate can be used for the removal of cadmium metal (Cd) from polluted seawater. The Q_{max} value of cadmium removal was 0.018 mmol/g biomass after calculation using the Langmuir adsorption model. The efficient biomass concentration for bioadsorption was found to be 5 g/L and the efficient time for bioadsorption was estimated to be around 3 hours. Moreover, the removal of algal biomass could be enhanced after the addition of polyethyleneimine (PEI). Lastly, due to the large oil content in HAB-causing microalgae, the large amount of biomass collected could also be used for the extraction of lipids for biodiesel production. Besides the removal of heavy metal ions from polluted seawater, microalgae can also be used to treat domestic sewage water. We have found that the growth of AD1 was promoted in culture medium containing 20% non-autoclaved sewage water collected from The Shatin Sewage Treatment Works site. Therefore, microalgae can be a multi-purpose solution for heavy metal removal, sewage treatment, as well as a source of biodiesel production.

Fast identification and detection of harmful algal blooming species and marine biotoxins by using MALDI-TOF MS

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Algal bloom is a term used to describe the rapid growth of certain phytoplankton groups in both marine and fresh waters worldwide. Given the ever increasing population of Hong Kong and South-Eastern China, problems with algal blooms have been worsening with time. The recent fresh water blue-green algal bloom that occurred in Jiangsu, China (starting end of May 2007) is a classic example on how serious the effects could be on humans. More than one million people who live around Taihou do not have enough fresh water for their daily consumption. Locally, more than 70 red tide causative species have been found. About 20 cases occurred annually since 1994. One of the most massive blooming occurred in 1998. This extraordinary algal bloom had invaded nearly all corners of the coastal waters of Hong Kong, including 1260 fishery households that were affected, 2500 tons of fishes were killed, and a direct economic loss of HK$ 250 million (about US$ 32 million) was estimated by the fish farmers. Other indirect costs such as cleaning up and compensation programs have not been counted. Furthermore, it should be stressed that some of the harmful algal species produce biotoxins that can cause human illness or even death after breathing through aerosols or consumption. Contaminations of shellfish and fish by such biotoxins have been negatively affecting the shellfish and aquaculture industries. These reiterates the importance of having a fast detection methodology for the identification and detection of harmful algal blooming species as well as the biotoxins. It would allow fish-farmers to have more time to react to an algal bloom. The identification and detection of harmful algal bloom (HAB) causative agents and their corresponding biotoxins is usually achieved with morphology-based techniques and HPLC respectively. These techniques are either time-consuming, labor intensive, and/or technically demanding. In the present study, we demonstrated a rapid and simple methodology for the identification and detection of harmful algal blooming species and their corresponding biotoxins by using MALDI-TOF MS. Our results show that various freshwater and marine harmful algal species could be easily distinguished from each other based on their specific protein mass profiles (PMP). Biotoxins from toxic species such as some primary paralytic shellfish toxins (STX, GTXs) could also successfully be detected based on their specific molecular mass. This rapid protocol is easily operated and both species identification and biotoxins detection could be done within hours. The MALDI-based identification/detection method is praised as objective, fast, simple and reliable. It usually requires minimal amounts of biological material and is suitable for high-throughput routine analysis. Therefore, it has great potential for applications in clinical microbiology, food industry and environmental monitoring.

Toxin production and endogenous arginine level of *Gymnodinium catenatum*, a marine dinoflagellate

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Gymnodinium catenatum is a widespread marine dinoflagellate commonly found in temperate coastal waters. It is a bloom forming and toxin-producing species. These are known to produce paralytic shellfish toxins (PSTs) and their effects could be lethal. Because of these specificities, this species has great environmental and economical impacts. Correspondingly, it displayed much hindrance to the sustainable development of our water resources. From the literature, it is known that arginine is the main precursor for the synthesis of PST. Much research was performed aiming at understanding the mechanism of PST production as well as the pathway(s) involved. However, much of these are still unclear. In our study, under 25°C culturing condition, *G. catenatum* grew slower than a toxic *Alexandrium spp.* with a lower maximum cell density. Intracellular toxin content was about 22-35 *f* mol cell^{-1}. C1 and GTX5 toxins are the major groups of toxins produced. Endogenous arginine was found to remain constant at around 0.4-1.7 mM during exponential growth. However, its levels rose to 3.8 mM in the lag phase and remained at 3.5 mM in the stationary phase. The trends of changes in the arginine and toxin levels, with respect to time exhibited both similarities and differences between these two curves. These indicated that toxin production is not solely dependent on the availability of endogenous arginine.

Toxin production in dinoflagellates may not be controlled by genes in the ITS and 28S regions

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Due to rapid urbanization and industrialization, availability of water resources in our society is of very serious concern. Correspondingly, the sustainable development of water resources becomes increasingly important. However, climate changes and eutrophication further worsen the situation. Harmful algal blooms (HABs), commonly called red tides, are a naturally occurring phenomenon. Mechanism of blooming is currently unclear and we are uncertain how nutrients interact to produce blooming. Nevertheless, these algal blooms represented by rapid increase in the populations of microalgae can be found in both freshwater and marine environments. Diverse species of organisms are involved in HABs and they include *Alexandrium* spp. and *Gymnodinium* spp. Some of these microalgae produce toxic substances which could bio-accumulate in the food chain, causing various acute poisonings. These included amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and paralytic shellfish poisoning (PSP). *Alexandrium catenella* is a type of dinoflagellate that produces saxitoxin (STX) and gonyautoxins (GTXs), causing symptoms collectively known as PSP. These dinoflagellates can be found in different regions of the world such as South China and Australia. On the other hand, *Alexandrium tamarenase* is another dinoflagellate known to produce STX. However, in our hands, all the *Alexandrium tamarenase* cultures were non-toxic. Nonetheless, in order to know more about the controlling mechanism of toxin production, different strains of these two species isolated from various regions were compared with respect to their toxicity profiles, toxins compositions and respective DNA sequences in the ITS and 28S regions. We found that in both toxin-secreting *Alexandrium catenella* as well as those that do not secrete any toxin, DNA sequences in their ITS and 28S regions showed a very high degree of similarity (>97%). Hence, if one is going to find genes that control the secretion of these toxins, they should not be found in the ITS as well as 28S regions!

Homogeneity and stability of microbiological proficiency testing in cosmetic products

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The Division of Cosmetics and Hazardous substances is appointed to be the ASEAN Cosmetic Reference Laboratory (ACRL) by ASEAN Cosmetic Committee. Its responsibility is to run the Microbial Limit Test of cosmetic products. The ACRL acts within a national proficiency testing (PT) scheme as PT provider which is to prepare and distribute PT samples to participant laboratories. PT samples should be in good homogeneity and stability. The aim of this research is to prepare artificially contaminated cosmetic samples with target microorganisms for quantitative and qualitative microbiological proficiency testing in cosmetic products. To prepare PT samples, cosmetic matrices with preservatives were mixed with the mixture of target bacteria, yeasts, and moulds. Target microorganisms, *Salmonella choleraesuis* ATCC 10708, *Escherichia coli* ATCC 8739, and *Aspergillus niger* ATCC 16404 were selected for quantitative method (enumeration) and *Pseudomonas aeruginosa* ATCC 9027 was selected for qualitative method (detection). The cosmetic

matrices with preservative were mixed with the target microorganisms at high, medium and low concentration by stomacher then were stored until the microbial population in the matrices reached equilibrium (1). Homogeneity tests of the PT samples were done by duplicate analysis of 10 portions of test samples. The variance of total aerobic plate count, mold count and *P. aeruginosa* detection were evaluated for homogeneity test. Stability of PT samples at room temperature and 2-8˚C were analyzed by total aerobic plate count, mold count and *P. aeruginosa* detection (2, 3). The colony count should not be more than ±0.5 $\log_{10}$cfu. for stability test. The results found that PT samples were homogenous with target microorganisms, *Salmonella choleraesuis* ATCC 10708, *Escherichia coli* ATCC 8739, *Aspergillus niger* ATCC 16404 and *Pseudomonas aeruginosa* ATCC 9027 under all of high, medium and low levels of those microorganisms. Moreover high level of target bacteria in PT samples could be stable at 2-8˚C after 72 hours of incubation and the target mold, *Aspergillus niger* ATCC 16404 could be stable until 16 days. For the detection of *Pseudomonas aeruginosa* ATCC 9027 we found that it could be stable at high level until 16 days of incubation at room temperature. So the cosmetic matrices with preservatives could be used as the PT samples with good homogeneity and stability at 2-8˚C. The future study will attempt to prepare PT samples with target bacteria and molds for quantitative and qualitative microbiological proficiency testing in cosmetic products at room temperature.

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Traditional chinese medicine coherent consensus-certified knowledge engineering

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In the living ontology framework coherent consensus-certified knowledge engineering is semi-automatic. The knowledge engineering process can be better explained by using the master aliases table (MAT). The living ontology is supported by the information management activities in the MAT. The enterprise ontology is abstracted by two forms here: Enterprise vocabulary or {V}: This contains all the names of entity in the enterprise ontology but not their associations within the ontological subsumption hierarchy. Semantic net: This is the middle layer of any ontology-based system (e.g. UMLS [UMLS]) in the context of this book for machine understanding and processing (i.e. parsing). According to the meta-interface concept any ontology-based system can be automatically generated from the given iconic specification. This specification is a collection of icons and each icon is a semantic path embedded in the ontological subsumption hierarchy in the given master ontology. If the given master ontology is updated, the same iconic specification will generated the updated target system. The semantic knowledge in part (A) is static in the sense that it is the last consensus-certified version. Part (B) represents the newly acquired knowledge from open sources and this knowledge is not consensus-certified al all. Advancing the old part (A) to a new part (A) by the process of consensus certification can be represented logically by, where: i) is the new part (A); ii) is the old part (A); and iii) is the pruned information from the temporary part (B). There is nil contents in the temporary part (B) when has just come into existence. Therefore, the MAT facilitates coherent consensus-certified knowledge engineering by accumulating relevant useful information into the temporary part (B) in a real-time manner.

The relationship between part (A) and part (B) lies in the concept of referential context (RC), and in this case an RC is always the illness identified from part (A). Every RC (e.g. flu) in the MAT has three relevant tables: i) illness table that contains relevant information such as semantic symptoms, standard prescriptions and their use frequencies; ii) table of aliases that has two parts: consensus-certified aliases transformed directly from part (A) by the ASA (automatic semantic aliasing) mechanism; and new aliases (not consensus-certified) data-mined from open sources in a real-time fashion; and iii) table of relevance indices (RI) that indicate the degrees of similarity (i.e.) between the aliases and the RC. The MAT implementation is an open issue; it can be centralized or distributed/pervasive depending on the operation environment.

Evolution of dietary antioxidants

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The evolution of oxygen-producing cells was probably one of the most significant events in the history of life. Oxygen is a potent oxidant whose accumulation in terrestrial atmosphere resulted from the development of photosynthesis over three billion years ago, in blue-green algae (*Cyanobacteria*), which were the most primitive oxygenic photosynthetic organisms. Brown algae (seaweeds) accumulate inorganic iodine to more than 30,000 times the concentration of this element in seawater, up to levels as high as 1-4% of dry weight. Protective endogenous antioxidant enzymes and exogenous dietary antioxidants helped to prevent oxidative damage (1, 2). In

particular, mineral inorganic antioxidants present in the primitive sea, as some reduced compounds of metalloproteins of Rubidium, Vanadium, Zinc, Iron, Copper, Molybdenum, Selenium and Iodine, which play an important role in electron transfer and in redox chemical reactions. Most of these substances act in the cells as essential trace-elements in redox and antioxidant metalloenzymes. When about 500 million years ago plants and animals began to transfer from the sea to rivers and land, environmental deficiency of marine inorganic antioxidants and iodine, was a challenge to the evolution of terrestrial life (1). Terrestrial plants slowly optimized the production of "novel" endogenous organic antioxidants such as ascorbic acid, polyphenols, flavonoids, tocopherols, etc. A few of these appeared more recently, in the last 200-50 million years ago, in fruits and flowers of angiosperm plants. In fact Angiosperms (the dominant type of plant today) and most of their antioxidant pigments evolved during the late Jurassic period. Plants employ antioxidants to defend their structures against reactive oxygen species (ROS) produced during photosynthesis (3), and formed a part of the human healthy diet. Chordates, the primitive vertebrates, began to use also the "novel" thyroidal follicles, as reservoir for iodine, and to use the thyroxine in order to transport antioxidant iodide. Iodide is one of the most abundant electron-rich essential elements in the diet of marine and terrestrial organisms. Iodide, which acts as a primitive electron-donor through peroxidase enzymes, has an ancestral antioxidant function in all iodide-concentrating cells from primitive marine algae to more recent terrestrial vertebrates (2, 3). Recently, we hypothesized that in the wide range of antioxidants there might be an "evolutionary hierarchy", where the most ancient might be more essential than the recent antioxidants in the developing stages of animal and human organisms (3).

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Dose-dependent effects of green tea on human DNA *in vitro*

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Regular intake of green tea is associated with lower basal oxidation-induced damage to DNA and increased resistance of lymphocytic DNA to oxidant challenge. However, *in vitro* and *in vivo* pro-oxidant, genotoxic and cytotoxic effects of green tea polyphenols at high concentrations have been reported. We investigated at what concentration the damaging pro-oxidant effects overwhelm potential protective effects of green tea. Pooled lymphocytes from healthy volunteers were incubated for 30 min at 37°C in freshly prepared green tea infusions (0.005%, 0.01%, and 0.05% w/v in PBS, with PBS as control), washed, and the comet assay for DNA damage then run after cells were challenged with 30 μM H_2O_2 for 5 min on ice, or treated with an enzyme (Fpg) which reveals oxidation-induced lesions. H_2O_2 generation in tea infusions was measured. Results showed that oxidation-induced lesions were 5-fold higher ($P<0.05$) with 0.05% green tea, but ~20% lower in cells pre-treated with 0.005% and 0.01% tea. After oxidant challenge, cells pre-treated with 0.005% and 0.01% showed significantly ($P<0.05$) less DNA damage than challenged control cells, indicating increased resistance to challenge. H_2O_2 concentrations of 0.005%, 0.01% and 0.05% green tea infusions after 30 min at 37°C were, respectively, 6, 9 and 55 μM. Results indicate that the genoprotective effects of green tea are seen only at very low (≤0.01%) concentrations and that damaging pro-oxidant effects are marked at a concentration of 0.05%. Generation of H_2O_2 by green tea polyphenols may be responsible for both protection and damage: very low concentrations may provide direct and/or indirect antioxidant protection against oxidant challenge, such as faster DNA repair as a consequence of induction of the redox sensitive antioxidant response element (ARE) by low amounts of H_2O_2 generated from low dose tea.

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A novel ex vivo focal ischemic stroke model for discovery

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During an occlusive stroke, blood flow to an area of the brain is blocked (focal ischemic insult) and consequently brain tissue located downstream of the blockage rapidly dies. The initial compromised area of neuronal death is called the core region. As neurons within the core succumb to death, their cell membranes become compromised and as such, release their contents into the extracellular space. This leads to further neuronal death to occur well outside of the core region. The region of secondary damage surrounding the core is called the penumbra and the extent of neuronal death in this region in addition to the core defines the total stroke volume and hence the extent and severity of the stroke. There is great interest in using stroke models to screen compounds for their ability to reduce the rate of spread subsequent to a focal ischemic insult. As most survivable strokes are focal in nature, alternative experimental models that best mimic this scenario are highly desirable. In vivo

focal stroke models are extensively used to screen and study neuroprotectants to reduce total stroke volume (core + penumbra). They are high cost, invasive, technically challenging, and low in throughput. *In vitro* stroke models overcome some of these problems but models are global in nature as they involve applying oxygen-glucose deprived (OG-) media to an entire brain slice or to a well of cultured neurons. These fail to accurately mimic the more localized, focal nature of most survivable stroke. We have developed a novel *in vitro* brain slice model of focal stroke that facilitates the investigation of events occurring in the penumbra, core, and normal tissue in an ex vivo slice preparation. We focally applied OG- medium to a small region of the cortex and perfuse the rest of the brain slice with OG+ACSF. This technique produces a focal infarct in the brain slice that increases in severity as a function of time in OG-. In addition, whole cell patch recordings were made from cortical neurons in the slice as a correlative measure physiologically measure and detect changes in neuronal as measured in real time via electrophysiological methods. High fidelity intracellular recordings were made from neurons before and in OG- in the core region. Within 2 minutes of OG- caused a rapid and irreversible anoxic depolarization (AD) occurred whereas recordings of cortical neurons located in the penumbra showed a delay in observing the onset of AD. Neuroprotection was also demonstrated using this model via where we pretreated slices with Edaravone (EDV @30 &100 M) and observed dose-dependent delay in AD in both the core and penumbra. Effects of OG- on synaptic functionality via OG- induced effects was also examined and we found that synaptic currents (EPSCs) were reduced by 70% after 6 minutes in the core and after 50 min in the penumbra. This novel *in vitro* focal stroke model mimics *in vivo* focal ischemia and can be used to rapidly evaluate and screen for potential neuroprotectants and therapeutics on the core and penumbra in a semi-intact nervous system and at a low relative cost.

Antiaging effects of vegetable extracts on oxidative stress and antioxidant enzymes of *Drosophila melanogaster*

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Antioxidants can prevent the oxidation of sensitive biological molecules by free radicals and decelerate the process of aging. Previous studies have demonstrated that cruciferous vegetables show preventive effects on degenerative diseases and their daily consumption can significantly reduce mortality due to cancer, cardio- and cerebro-vascular diseases. These effects are attributed to the different biologically active components contained in cruciferous vegetables, which include phenolic and sulfur containing phytochemicals like glucosinolates, isothiocyanates, dithiothiones, inodoles, sulfonates and flavonoids. Cruciferous vegetables such as Brussels sprouts, broccoli and cauliflower can prevent against the harmful effects of chemical carcinogens and oxidative damage to DNA as reported in many animal studies (1, 2). Among those, broccoli was shown to have the highest antioxidant capacity by FRAP assay (3). The present study was conducted to assess the antioxidant capacity of cruciferous vegetables such as cabbage, Chinese cabbage, carrot and broccoli on the *Drosophila* model in response to lipid hydroperoxide attack induced by paraquat and hydrogen peroxide. In addition, the effects on antioxidant enzymes SOD and catalase were also measured. We found that the antioxidative effects of broccoli extract were dose-dependent. Broccoli extract also enhanced the oxidative resistance of *Drosophila* against superoxide and hydrogen peroxide radicals. However, for the other cruciferous vegetables, only carrot extract, which has a high vitamin A content showed a slight increase in resistance against paraquat-induced oxidative stress.

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Suppression of luteolin on diet-induced atherosclerosis in rats

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Hypercholesterolemia has been associated with many diseases, notably cardiovascular including atherosclerosis. Luteolin is a multibiological activity flavonoid, widely distributed in vegetables, fruits, red wine, olive oil and Traditional Chinese Medicine herbs. To evaluate its anti-atherosclerosis effect and mechanism, male Sprague-Dawley (SD) rats were fed with normal diet, high cholesterol diet (HCD), HCD and luteolin (300, 100, 30 mg/kg/day, p.o.) isolated from peanut hull), or HCD and simvastatin (10 mg/kg/day, p.o., positive control) for 38 days. Results indicated that luteolin, at 300 mg/kg/day, markedly reduced the total cholesterol (TC) in serum, decreased the atherogenic index, improved acetylcholine-induced relaxation on isolated aorta rings, prevented the deterioration of nitric oxide synthase (NOS), and up-regulated the endothelial nitric oxide synthase (eNOS) mRNA expression in rats fed with HCD. Luteolin and positive control drug simvastatin showed a similar cardiovascular protection against atherogenesis in-

duced by hypercholesterolemia. It shows that significant up-regulation of the gene expression of eNOS leading to improvement of availability of NO, and reducing the TC is, at least, a partial mechanism attributable to luteolin to decrease the atherogenic risk induced in rats by HCD. Our findings suggest that the daily intake of some vegetables known to be rich in luteolin is a safe and easy way to reduce the atherogenic risk caused by hypercholesterolemia.

Antiviral activity of a composition of *Gentiana lutea* L., *Primula veris* L., *Sambucus nigra* L., *Rumex spec.* and *Verbena officinalis* L. (BNO 1010) against viruses causing respiratory infections

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BNO 1010 (Sinupret®) is a herbal medicinal product of five different plants used for the treatment of acute and chronic rhinosinusitis and respiratory viral infections such as common cold. Antiviral activities of BNO 1010 against a broad panel of human pathogenic enveloped and non-enveloped RNA and DNA viruses causing infections of the upper respiratory tract were performed in this study. The results showed that antiviral activity of BNO 1010 could be detected independent of the type of the virus in RNA as well as in DNA virus infected cell cultures and also against coated and uncoated viruses. A very strong inhibitory activity was determined against adenovirus and RSV infections. These results demonstrate that BNO 1010 showed inhibitory activity against an exceptionally broad spectrum of viruses with relevance for respiratory tract infections. The basic mechanisms have to be elucidated in further studies.

Investigation of the immunomodulatory effects of *Radix Astragali* based on the full chemical fingerprinting using the QPAR

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Introduction: Huangqi, or *Radix Astragali* (RA) has been traditionally used in Chinese Medicine to enhance the body's general well-being. Some chemotherapy-treated cancer patients also use RA as complementary therapy and claimed improved clinical outcomes. Dendritic cells (DC), as professional antigen presenting cells, play a pivotal role in the regulation of tumour-specific immune responses. However, it has been shown that cancer-associated microenvironment adversely affect DC-related immune-surveillance system, and thus ensue tumor escape. It has been reported that these defective DCs in cancer failed to up-regulate two important co-stimulatory surface markers of CD40 and CD80. In this work, we measured the change of CD40 and CD80 expression in a DC experimental model treated with three groups of RA (RA-A, RA-B and RA-C; 72 RA samples in total). At the same time, chemical fingerprints of these RA samples were also acquired by a HPLC-DAD-MS instrument. In this study, we have also applied a newly developed chemometrics method that based on the Quantitative Pattern-Activity Relationship (QPAR) approach, to correlate the chemical fingerprinting and the CD80 bioactivity data. This CD80-QPAR model can accurately predict the overall immunomodulatory effects on CD80 expression with their respective chemical profiles in the RA samples. **Aims:** This study aimed at establishing a bioactivity profiling model by correlating the biological fingerprinting data of immunomodulatory effects of RA on DC, and the corresponding RA chemical fingerprinting data. Such newly developed chemometrics CD80-QPAR model may then subsequently be used to predict the CD80 immunomodulatory effects with input of chemical fingerprinting profiles. **Methodology:** THP-1 was used as a convenient robust source of DC in this *in vitro* DC functionality flow cytometric study. DCs were treated with aqueous preparations of RA samples of different sources of origin in combination with different refluxing durations. Flow cytometric analysis was performed after cells stained with fluorescence-conjugated monoclonal antibodies of specificity against CD40 and CD80. LC-DAD-MS chromatographic profiles were also obtained. As for the QPAR data processing, three different PLS (Partial Least Squares) methods of PLS, UVEPLS and EN-PLS were applied to the data sets obtained from the 72 RA samples. **Results**: The three QPAR methods PLS, UVEPLS and EN-PLS were proposed and applied to process the CD80 data with corresponding chemical fingerprints of the RA samples. The chemical and CD80 data pairs obtained from experiments were divided into two groups: a training set (48 samples) and a test set (24 samples). The training set was used to build models by chemometrics methods, while the root mean squared errors of prediction (RMSEPs) obtained from the test set using these QPAR models was utilized to evaluate the performance of these methods. In this study, the EN-PLS method was found to out-perform the other two with the agreement between the predicted values and experimental CD80 data of the test set better than 92%. The DC flow cytometric analysis of five RA samples was selected to illustrate the range of CD40 and CD80 up-regulation as well as down-regulation by different RA preparations. Negative control was the untreated cell preparation, while the positive control was treated with LPS where both CD40 and CD80 were up-regulated by 87.3% and 43.1%, respectively, compared with untreated counterpart. THP-1 treated with fraction RA-A23 showed the highest up-regulation of CD40

expression (91.0%); whereas that of fraction RA-C8 had the highest CD80 expression (57.3%). For treatments with fractions RA-14 and RA-C8, THP-1 showed elevated CD80 expressions of 45.0% and 57.3% respectively which were higher than that of LPS. Most interestingly, when THP-1 was treated with fraction RA-1, CD40 and CD80 expression were down-regulated by 44.5% and 49.8%, respectively, in comparison to their untreated controls. This result was consistent with a tolerogenic immunological effect. **Discussion and Conclusion:** In this study, we demonstrated that different preparations of the same herb may have opposing immunological effects as shown by above-mentioned CD40 and CD80 expression changes, in relation to the different chromatographic profiles. This understanding of the interrelationship between the chemical constituents profile with its corresponding effects on DCs may provide useful insights into herbal vaccination-adjuvants, and may lead to a more effective DC immunotherapy. This chemometrics methodology can also be further applied as a quality control platform to provide a quantitative assessment of the immunomodulatory bioactivity profiling of different RA products. This CD80-QPAR model based on the EN-PLS method predicted well the DC immunomodulatory bioactivity based on chemical fingerprinting profile. Such model can be further extended for use as an effective and accurate bioactivity indicator for any herbal product by merely examining their chemical fingerprinting.

Effect of immunomodulating and antiviral agent Immune Assist 24/7™ on CD4+ T- lymphocyte counts of HIV infected patients

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Immune enhancement through the use of natural products is a potentially valuable therapeutic modality in HIV infected people, especially those who are not good candidates for aggressive ARV therapy. One such immune enhancement medicinal mushroom product from the United States is Immune Assist 24/7™. In this study the effect of Immune Assist 24/7™, which is a naturally derived immune-modulating and antiviral agent on CD4+ T-lymphocyte counts, was evaluated in eight HIV infected patients at the Sunyani Regional Hospital (Ghana). The subjects were administered three capsules of 800 mg Immune Assist 24/7™ once a day (2400 mg/day) and peripheral blood samples were withdrawn at baseline, day 30, and day 60, and the CD4 count measured. The study revealed that Immune Assist 24/7™ used as a sole therapeutic agent without additional ARV drugs, significantly increased CD4 T-lymphocyte populations in all of the patients. In one patient, the CD4 T-lymphocyte count went from four (4) at the baseline, to 170 cells in 60 days, representing an increase of more than 4000%. In another patient, the CD4 count went from 88 to 470 cells within the same period. Even in the patients with the highest CD4 counts of around 800, there was a significant elevation in the CD4 count noted. This study did not deal with the effect of Immune Assist 24/7™ on other immune parameters such as CD3+ T-lymphocyte count, natural killer cells count or viral load among HIV infected patients. These initial results are promising, and indicate the potential value of further evaluating the effects of Immune Assist 24/7™ on other immune parameters and viral load among HIV patients treated either as a sole therapeutic agent, used adjunctively with standard ARV therapy or in comparison with standard ARV therapy alone.

Evaluation of *in vitro* immunomodulatory activities of *Astragalus* extracts

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Astragalus membranaceus (Fisch.) Bunge (Huang Qi) is a widely used herb in traditional Chinese medicine. It is also a common ingredient in folk remedies for making soup and tincture. The extracts of *Astragalus membranaceus* and their active components have been shown to exert immunostimulating and immunosuppressive activities. Being a component of our wheeze-relief formula, the immunomodulatory activities of *Astragalus membranaceus* extracts have been examined using human peripheral blood mononuclear cells (PBMC) in the present study. Our results showed that the ethanolic crude extract of *Astragalus membranaceus* (50-200 μg/ml) exhibited significant inhibitory effects on phytoagglutinin(PHA)-stimulated PBMC proliferation, as well as TNF-α and IFN-γ production ($p<0.05$) as assessed by [methyl-^{3}H]-thymidine incorporation assay and ELISA, respectively. In an attempt to isolate the active fractions responsible for the activities, three sub-fractions were obtained by further column chromatography using D101 resin. One of the fraction which contained mainly phenolic compounds and flavonoids showed significant inhibitory effects on PBMC proliferation, TNF-α and IFN-γ production ($p<0.05$) in a concentration-dependent manner without cytotoxicity (25-200 μg/ml inhibited 41.5-66.8% of PHA-stimulated PBMC proliferation when compared with untreated control cells). On the other hand, the saponins-enriched fraction did not show significant changes in PBMC proliferation ($p>0.05$). In conclusion, our study reported for the first time the immunosuppressive effects of *Astragalus* ethanolic extract sub-fraction in human peripheral blood mononuclear cells.

The immunosuppressive and anti-inflammatory activities of this sub-fraction may help to ameliorate the symptoms in allergic disease, such as asthma.

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Screening putative cytoprotectants based on inhibition of β-amyloid and human islet amyloid polypeptide aggregation

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Alzheimer's disease (AD) is a progressive and debilitating neurodegenerative disease clinically diagnosed in humans by the presence of significant amounts of neurofibrillary plaques in the brain (post mortem). These plaques are known to cause cellular damage and neuronal dysfunction due to the ongoing process of the aggregation of β-amyloid (Aβ) peptides. Therefore, an intervention that effectively inhibits Aβ aggregation and thus plaque formation is viewed as viable interventional approach that could potentially slow progression of AD. Similarly, the aggregation of human islet amyloid polypeptide (IAPP) and the deposition of extracellular amyloid plaques in pancreatic islets have been associated with beta-islet cell apoptosis. Hence, the inhibition of HIAPP aggregation is also considered to be novel and potential therapeutic approach towards the treatment of diabetes. Accordingly, we have screened over 200 extracts derived from Atlantic Canada plants and other selected TCM-based natural compounds for their respective abilities to inhibit Aβ using Thioflavin T (ThT) fluorescence-based assay. Our testing showed that a number of extracts demonstrated strong inhibitory effects on the aggregation of Aβ and human IAPP. Any positive result derived from the primary ThT assay was further tested via a counter assay that utilized electron microscopic examination to further validate our results. A neuronal based cell culture assay (using human neuroblastoma SH-SY5Y cell line) was also performed to test several lead extracts for potential neuroprotective effects against Aβ-induced cytotoxicity. In this series of experiment preliminary results showed that pre-incubation of SH-SY5Y cells with selected extracts significantly reduced Aβ cytotoxicity. Taken together, these 3 assays can be used to test and rapidly screen and identify natural extracts or select bioactive compounds that may slow or mitigate aberrant protein aggregations and the downstream cytotoxic effects that are known to occur as a result the formation and deposition of amyloid plaques. Further studies to characterize and purify the putative bioactive components are therefore warranted.

AgeLOC vitality improves exercise endurance, glucose metabolism and antioxidant capacity in an aging model

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A number of compounds were screened for their anti-aging effects through a high-throughput gene expressions platform. On the basis of the differentiated gene expression patterns in skeletal muscle, heart muscle and brain between the young (3 months of age) and old (24 m), an ageLOC Vitality product was formulated, including *Cordyceps sinensis* Cs-4 (a mycelial, fermentation product of *C. sinensis*), GinsengMax (a standardized extract of ginseng), and a pomegranate extract (>85% polyphenols). We examined in this study the effect of ageLOC Vitality (400 or 800 mg/kg BW) in improving endurance exercise in ICR mice (10 months of age). Daily exercise training on treadmill reduced body weight, body fat, liver and gastrocnemius glycogen and increased blood lactate ($p<0.05$). Consecutive 47 days of Vitality consumption (800 mg/kg) increased weight-loaded swim time to exhaustion in mice (+22% compared to exercise controls; $p=0.024$). Vitality consumption (800 mg/kg) for 14 days increased gastrocnemius glycogen (+28% compared to exercise controls; $p=0.049$) and liver glycogen (+121% compared to exercise controls; $p=0.032$), and reduced cellular ROS species concentration in post-exercise muscle tissues in a dose-dependent manner (-32% compared to exercise controls; $p=0.005$). In conclusion, ageLOC Vitality extends endurance swim time to exhaustion with increased cellular glycogen preservation and antioxidant capacity in old mice, suggesting the anti-aging benefit of ageLOC Vitality in enhancing exercise endurance in advanced ages and preventing muscle aging.

***Cordyceps sinensis* CS-4 restores aging-associated changes in gene expression and extends lifespan in normal aged mice**

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Cordyceps sinensis is believed traditionally to be an anti-aging traditional Chinese herb. We previously reported that *C. sinensis* Cs-4, a mycelial fermentation product of *C. sinensis*, improves glucose, lipid and energy metabolisms and has antioxidant, anti-fatigue and endurance enhancement effects. In this study we examined gene expression (GE) profiles of neocortex and gastrocnemius from young

(5 months of age), old (25 mo) and old Cs-4 treated (0.3 g/kg) C57Bl/6 mice. Of the 20,687 gene transcripts examined, 1241 were classified with the mitochondria in some fashion (structural, enzymatic, signal transduction, etc). Of them, 172 changed in GE with aging in brain and 220 in muscle tissues when compared GE in old controls vs. young controls. Age-related changes in mitochondria related GE were clustered (mtYGCs). Cs-4 strongly opposed mtYGCs of many changes that occurred with age ($p<0.05$). This anti-aging effect at the gene transcription level was examined in a lifespan study in male & female ICR mice fed either control or Cs-4 (0.5, 1.0 or 1.5 g/kg; n=48 each group) beginning at 1 year of age. Calorie intake was adjusted twice a week to match the controls. No differences in body weight were noted among the groups. All control mice died before 3 years of age, with a median lifespan of 755 days and the longest lifespan of 1061 days. The average lifespan of the longest 10% mice was 1028 days, or of the longest 20% mice 969 days. The lifespan for Cs-4 group was extended 10-66 days at 50% survival and 45-153 days at 10% survival. The age of the oldest surviving mice was extended 258 and 152 days at the dose of 0.5 and 1.5 g/kg, respectively, and >278 days (1 mouse still alive) for 1.0 g/kg. Kaplan-Meier analysis revealed the extended lifespan and reduced the risk of death in mice receiving Cs-4 0.5 g/kg ($p=0.03$). In conclusion, *C. sinensis* Cs-4 reverses age-related changes in GE and extends the lifespan of mice, supporting the traditional belief that *C. sinensis* Cs-4 conveys anti-aging benefits to humans.

Attenuation of fatty liver and prevention of hypercholesterolemia by extract of *Curcuma longa* through regulating the expression of cholesterol 7 α-hydroxylase, LDL-Receptor, hemeoxygenase 1 and 3-hydroxy-3-methyl-glutaryl-CoA reductase

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Hypercholesterolemia is a major risk factor for the development of the non-alcoholic fatty liver disease (NAFLD). *Curcuma longa,* better known as turmeric and a common food ingredient, was investigated on its potential benefits for regulating plasma lipid, cholesterol levels and improving hepatic conditions in induced hypercholesterolemic rats. Rats fed a high cholesterol diet with turmeric supplements showed a significant decrease in total plasma cholesterol, low density lipoprotein-cholesterol (LDL-cholesterol) but an increase in high density lipoprotein-cholesterol (HDL-cholesterol) when compared with rats fed a high cholesterol diet only. Fatty liver was found to develop in hypercholesterolemic rats treated with high cholesterol diet and this pathological condition was markedly attenuated when rats were provided with turmeric supplements. With turmeric treatment a significant decrease in the total amount of hepatic lipid was found in rats as determined by lipid extraction using diethyl ether. Histological staining of the liver tissues with Sudan III and hematoxylin showed that rats fed a high cholesterol diet only had significant higher number and larger granular fat bodies stained red with Sudan III compared with rats with turmeric treatment. Reverse-transcription polymerase chain reaction (RT-PCR) was used to assess the expression levels of enzymes involved in fat metabolism and cellular homeostasis in liver. The results showed that rats fed a high cholesterol diet supplemented with turmeric extract had a significant increase in the expressions of cholesterol 7 α-hydroxylase (CYP7A1), low density lipoprotein (LDL) receptor and hemeoxygenase 1 (HO1) but we measured a significant drop in 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) level when compared to both the normal diet (control) and high cholesterol diet treated rats. This is the first animal study showing that turmeric can effectively reduce the risk of developing fatty liver and prevents hypercholesterolemia by modulating the expressions of enzymes important in cholesterol metabolism.

PPD-type and PPT-type ginsenosides improve vascular dysfunction induced by diabetes

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Endothelial damage is the primary cause of the vascular complications of diabetes mellitus and the damage is usually not reversible. *Panax ginseng* contains bioactive ginsenosides and is found to be cardiovascular active. To evaluate the cardiovascular protective effect, male Sprague-Dawley (SD) rats were treated to induce diabetes for 2 weeks, and fed with vehicles, PPD-type and PPT-type of ginsenosides for 12 days. Then we isolated the thoracic aorta and investigated the effect on acetylcholine-induced endothelium-dependent vasorelaxation pre-contracted with phenylephrine. Severe impairment of vasorelaxation was found in the diabetic group (62.4% of control in maximum dosage of ACh-induced vasorelaxation) while the groups fed with ginseng extracts (both PPD-type and PPT-type) restored the vasorelaxation (no significant difference with control). Blood profiles showed that the ginseng extracts could not reduce blood glucose and blood cholesterol levels due to diabetes mellitus. Moreover, PPD-type of ginsenosides improved nitric oxide (in terms of nitrite) production stimulated by acetylcholine. We conclude that *Panax ginseng* may have vascular protective effects on diabetic condition, and the protective mechanism may be quite complex.

Study of the ethanol extract of *Fructus Ligustri Lucidi* on Ca^{++} metabolism in different ages of female rats

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Aging might alter the responses of our body to drug treatment. *Fructus Ligustri Lucidi* (FLL) is a commonly prescribed herb in Traditional Chinese Medicine (TCM) to nourish the endocrine and renal systems and to strengthen bones. Our previous studies demonstrated that the ethanol extract of FLL could improve bone properties in the aged female rats, mainly by elevating calcium (Ca^{++}) absorption and balance. The present study was designed to determine the effects of age on the responses of Ca^{++} balance and bone properties to treatment with FLL ethanol extract in four-month mature and eleven-month aged female Sprague Dawley rats. The results showed that aging worsens Ca^{++} balance and bone properties in female rats. FLL ethanol extract had positive effects on Ca^{++} balance and Ca^{++} absorption in both mature and aged female rats, but the actions were more prominent in the aged ones. FLL ethanol extract offered protective effects on bone mineral content (BMC) in diaphysis of the aged rats, but not in mature rats. Our results indicate that the effects of FLL on Ca^{++} and bone metabolism were altered with age and aged rats appear to be more sensitive to the protective effects of FLL treatment.

Recombinant human arginase depletes a single amino acid (arginine) and inhibits the proliferation of human melanoma

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Traditional chemotherapies for melanoma have shown poor response rates and produce deleterious side effects. These tumor cells have been shown to require arginine for growth, thus one innovative anti-cancer strategy is to starve melanoma cells through depletion of arginine – a key nutrient for many cancer cells. Here we show that arginine depletion, using a recombinant form of human arginase I (rhArg), efficiently inhibits the growth of mammalian melanoma cell lines *in vitro*. These cell lines are consistently deficient in ornithine transcarbamylase (OTC) expression, correlating with their sensitivity to rhArg. Cell cycle distribution of A375 human melanoma cells treated with rhArg showed a remarkable dual phase cell cycle arrest in S and G2/M phases. rhArg induced substantial apoptosis in A375 cells, accompanied by global modulation of cell cycle- and apoptosis-related transcription. Moreover, pegylated rhArg dramatically inhibited the growth of A375 tumor xenografts *in vivo*. Our results establish for the first time that pegylated rhArg is a promising candidate for an effective melanoma treatment. Insight into the mechanism behind the anti-proliferative activity of rhArg could inform us in designing combination therapies for future clinical trials.

Arginine depletion: a blessing in disguise?

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The 20 amino acids are the building blocks of proteins. Traditionally, amino acids are classified as essential and non-essential amino acids. Growing evidence shows that some of the amino acids also play an important role in the key metabolic pathways. Arginine is a versatile amino acid which can act directly as a signaling molecule to regulate the mTOR pathway. Besides, it is also the substrate for nitric oxide synthase which will produce nitric oxide, a very important signaling molecule involved in regulating many physiological processes. Recently, studies found that Hepatocellular carcinoma (HCC) has an elevated requirement for arginine. Development of targeted therapeutics can be based on single amino acid depletion by arginase (ARG) and arginine deiminase (ADI), converting arginine to ornithine and citrulline, respectively. A panel of HCC cell lines was exposed to these arginine depleting enzymes and the anti-proliferating effects were determined using MTT cell viability assay. The expression profiles of the enzymes involved in urea cycle were analyzed using RT-PCR which provided information to explain the observed cellular response. The impacts of arginine depletion on cell cycle distribution were analyzed by flow cytometry and through the analysis of transcript levels of cell cycle regulators

would further explain the underlying mechanisms for the observed cell cycle arrest in different cell lines. Except one cell line, ADI is ineffective to most of the liver cancer cell lines possibly due to the expression of arginosuccinate synthetase (ASS) which converts citrulline back to arginine. ARG exhibits a potent growth inhibition in all cell lines tested. Down regulation of ornithine transcarbamylase (OTC) prevents recycling of ornithine, and arrest the cancer cells at G2/M or S phase.

Starving brest cancer cells through depletion of arginine – a key nutrient for cancer cells

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Arginine is one of the twenty natural amino acids that construct proteins. It plays an important role in various metabolic processes, especially the urea cycle. Although most somatic cells are able to synthesize arginine from citrulline, arginine is still considered as a semi-essential amino acid because the supply of this amino acid would not meet the high demand at certain growth and developmental stage. Thus for specific groups of people such as children and patients, arginine has to be supplied from dietary intake. For many tumor cells, proliferation also depends on the presence of arginine. When arginine is deprived, normal cells simply exit cell cycle and can wait at G_0 phase for weeks until arginine supply resumed. On the other hand, tumor cells will keep cycling to imbalance and die from arginine auxotrophy due to their abnormal cell cycle mechanisms (such as deficient "R" checkpoint). This evidence calls for the attention that arginine-depleting enzymes can be used as targeted cancer therapeutic agents. With the help of modern biological techniques such as pegylation, many shortcomings of arginase (short half-life, immunogenicity etc.) have been overcome so that arginase is now favored over arginine deiminase (ADI) due to its potential efficacy against not only ASS-negative but also OTC-negative cancer cell lines. Arginase originated from *Bacillus caldovelox* (BCA) has been studied in this project. This enzyme has been proved to be very heat-stable, and can be readily produced and purified. Native BCA is found to have inhibitory effect against OTC-negative MCF-7 cell line. Therefore, it is suggested that BCA is very likely to be a potential enzyme drug against cancer and deserves further investigation for its anti-cancer efficacy.

Antimicrobial volatile essential oils in edible films and pouches for produce safety

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Plant-derived essential oils (EOs) and oil compounds, with relatively high vapor pressure, have been evaluated at their liquid and gas phases for their ability to protect food against pathogenic bacteria. The evaluation of antimicrobial effectiveness of EOs in edible films can be done by different methods depending on relevant applications. The overlay method represents the direct contact of the films on food surfaces and the vapor phase method is related to inactivation of pathogens from a distance without direct contact of the films with the contaminated food. In this study, 17 volatile EOs at 3% (w/w) concentration in tomato-based edible films were screened against *E. coli* O157:H7. Oregano, clove bud and allspice EOs showed the highest inhibition area by the overlay and vapor phase methods. Carvacrol, the main active antibacterial component in oregano oil (the most potent antimicrobial EO), was effective in vapor phase against *E. coli* O157:H7 when incorporated in apple or tomato films at concentration as low as 0.5% (w/w). These promising antimicrobial volatile EOs were incorporated into edible films and pouches to evaluate practical applications as non-contact antimicrobial agents on spinach leaves and whole strawberries stored under refrigeration. The antimicrobial data obtained with vapors diffused from edible films and pouches can serve as a guide for selection of appropriate levels of volatile EOs and their active constituents for incorporation into antimicrobial edible films and pouches for not direct contact applications on food. Edible films and pouches containing plant-derived volatile EOs provide new ways to enhance microbial safety and shelf life of produce.

Development and use of a chemically-induced rat osteoarthritis rat model for joint health

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As part of our ongoing program to evaluate joint health supplements for both animal and human species, we developed a chemically–induced rat osteoarthritis model. Use of this model allowed us to evaluate the efficacy of STEADFAST® Equine joint supplement (SFE) and its active components (Natural Eggshell Membrane (NEM®) and TêlaFIRM®, singly and the combination). We

showed these supplements possess anti-inflammatory and/or chondromodulating effects in this rat model of monosodium iodoacetate (MIA)-induced osteoarthritis (OA). Two separate trials were conducted with male Wistar rats fed dietary treatments 28 d prior to MIA injection and rats continued on this diet until the final tissue collection. Osteoarthritis was induced by intra-articular injection of 50 μL MIA through the patellar ligament of the right or left knee (0.6 mg MIA, trial 1; 1 mg MIA, trial 2). The contralateral knee was not injected. In trial 1, 54 rats were fed one of three dietary treatments (n=18/trt): 1) rat AIN-93M diet, 2) As 1 + 1% STEADFAST Equine joint supplement (SFE), or 3) As 1 + 2% SFE. Diets were fed for an additional 28 d following MIA injection. In trial 2, 48 rats were fed one of four dietary treatments (n=12/trt): 1) rat AIN-93M diet, 2) As 1 + 0.6% NEM incorporated into diet, 3) As 1 + 0.75% TêlaFIRM (a proprietary blend containing chelated minerals MINTREX® Mn, Cu & Zn), and 4) As 1 + 0.6% NEM + 0.75% TêlaFIRM. In addition, six rats served as a control (no MIA injections) and were fed treatment 1 for a total of 54 rats. Changes in hind paw weight distribution (HPWD) between the arthritic and contra-lateral control limb were used to assess joint discomfort. In addition, inflammation was measured using calipers (knee swelling). Knee swelling, expressed as the difference between arthritic and control knee, was measured at the same time points as HPWD. Serum biomarkers included CTXII and COMP, markers of cartilage degradation, and PIIANP, a synthetic cartilage marker and were collected on 6 rats per treatment at various time points. Results of trial 1 showed that rats fed 2% SFE were able to bear significantly more weight on their arthritic limb on d 14 post-MIA injection ($P<0.05$) relative to rats fed the other treatments. Inflammation and HPWD were numerically lower for rats fed 2% SFE vs control at all but one time point. CTXII was decreased in rats fed 2% SFE at d 7, 14 and 28 ($P<0.05$) and decreased in rats fed 1% SFE d 28 ($P<0.05$) relative to control. Results of trial 2 indicated a significant improvement in weight bearing ($P < 0.05$) observed d 1 post MIA injection and a trend on d 3 and 7 ($P < 0.10$) for rats fed the combination of NEM®+ TêlaFIRM® relative to rats fed the negative control (AIN-93M). Rats fed NEM only (d 1; $P = 0.10$) tended to have improved weight bearing on arthritic limb relative to rats fed the AIN-93 diet only. Day 3 caliper measurements of knees, likewise, indicated significant reductions in swelling in rats fed the combination of NEM+TêlaFIRM ($P < 0.05$) relative to rats fed NEM or TêlaFIRM only ($P <0.05$). Thus, these two independent measurements, swelling and weight bearing, suggest the combination of NEM + TêlaFIRM is more effective than NEM or TêlaFIRM fed singly. This MIA OA model is a rapid, reproducible animal model that mimics pain & structural changes associated with human OA. Previous studies have demonstrated that this model mimics behavioral, pathologic and pharmacologic features of human OA. The reduction in swelling, pain and degradative cartilage biomarkers (COMP and CTXII) suggests the active ingredients in STEADFAST® (NEM & TêlaFIRM) together may have anti-inflammatory and chondromodulating effects in osteoarthritic animals. Based on the swelling and weight shift measurements, the combination of NEM + TêlaFIRM was more effective than either NEM or TêlaFIRM fed singly.

Synergistic bone anabolic effects, in vitro, of CU409B1 and 1,25α dihydroxyvitamin D3 in rat osteoblast-like cells

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Osteoporosis is a condition affecting the bones with increased risk of bone fracture because of reduced bone minerals and disrupted bone micro-architecture. The condition is mainly due to increased osteoclast activity and/or reduced osteoblast activity. Vitamin D_3 is one of the medications recommended for osteoporosis as it enhances Ca^{2+} absorption from the diet. Previous findings in our laboratory demonstrated that CU409B1 (patent pending on chemical structure) improved rats osteoblast differentiation. In this study, we tested the hypothesis that a combination of CU409B1 (10, 30 and 100 nM) and Vitamin D_3 (at the lowest plasma concentration, 10 nM) provided synergistic bone anabolic effects.

Primary rat osteoblasts were isolated from Sprague Dawley (SD) (normal and ovariectomized, OVX) rats (sacrificed using an overdose of pentobarbital). Iliac crests collected were immediately immersed in PBS (for 10 min) supplemented with 10X antibiotics, and bone marrow was removed, and the crests were washed thoroughly twice with plain low glucose-DMEM (LG-DMEM). Trabecular bones were harvested, cut into small pieces, and immersed in LG-DMEM (10% fetal bovine serum with 1X antibiotics) for culture in a humidified incubator (37°C and 5% CO_2). When cells reached over 90% confluence, they were treated with either 1,25α dihydroxyvitamin D3 (10 nM, the active form of Vitamin D3), CU409B1 (10, 30 and 100 nM) or a combination of Vitamin D3 (10 nM) plus CU409B1 (10, 30 and 100 nM) for 7 and 14 days before subjecting them to analysis of various biomarkers. mRNA was isolated and subjected to quantitative real-time reverse transcription polymerase reaction (qRT-PCR) to determine the levels of osteogenesis-related mRNAs, including alkaline phosphatase (ALP), bone morphogenetic protein 2 (BMP2), osteopontin (OPN), osteocalcin (OCN) and pro-collagen type I. The extent of osteogenesis was determined by measuring the activity of extracellular ALP and Alizarin Red staining of Ca^{2+} deposits.

At day 7, CU409B1 (10 nM) increased the mRNA levels of ALP in osteoblast-like cells of normal rats ($P < 0.05$ compared to untreated controls). A combination of CU409B1 (10 nM) and Vitamin D_3 (10 nM) significantly enhanced the mRNA levels of ALP, OPN and BMP2 ($P < 0.05$) whereas there was no apparent change in procollagen type I expression. At Day 14, there was no significant change of Ca^{2+} deposition (as determined by Alizarin Red staining) after drug treatment in cells harvested from normal rats. Osteoblast-like cells harvested from OVX rats have higher basal mRNA levels of ALP and OCN. CU409B1 (10 nM), Vitamin D_3 (10 nM) and the combination of CU409B1 plus Vitamin D_3 treatment increased the mRNA levels of OCN at Day 7 and Day 14. However, CU409B1 (30 and 100 nM), with and without Vitamin D_3 (10 nM), reduced the mRNA levels of ALP and OCN in ovariectomized (OVX) rats at Day 7 and Day 14 after treatments.

In conclusion, a synergistic effect of CU409B1 at therapeutic concentration (i.e. 10 nM) on the pro-osteogenic effect of Vitamin D_3 (10 nM) at transcriptional levels was demonstrated in osteoblast-like cells of normal rats. Osteoblast-like cell isolated from ovariectomized rats demonstrated a higher basal mRNA level of ALP and OCN. A trend of a greater increase in OCN mRNA expression in response to drug treatment (CU409B1 with and without Vitamin D_3) was observed.