Joint Event

4th EUROPEAN BIOPHARMA CONGRESS

&

6th International Conference and Exhibition on

PHARMACOLOGY AND ETHNOPHARMACOLOGY

November 09-11, 2017 Vienna, Austria

Scientific Tracks & Abstracts

Day 1

Sessions

Ethnopharmacology of Medicinal Plants | Ethnoveterinary Medicines | Phytochemical Studies of Plants and Plant Extracts | Pharmacology and Toxicology | Clinical pharmacology | Natural Products Chemistry and Pharmacology | Immunopharmacology | Pharmacogenetics and Pharmacogenomics

Session Chair
Ning-Sun Yang
Agricultural Biotechnology Research Centre, Taiwan

Session Introduction

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Title: **Neuroprotective effect of SNC-1, a biocatalysis processed ayurvedic plant extract as a promising ethnopharmaceutic treatment for Alzheimer's and Parkinson's disease**
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Title: **Immune cell Type or signaling-specific effects of four candidate phytomedicines for cancerous and inflammatory diseases**
Ning-Sun Yang, Agricultural Biotechnology Research Centre, Taiwan

Title: **Isolation and structure elucidation of new cytotoxic polypeptide from bee venom**
Hesham El-Seedi, University of Malaya, Malaysia

Gabriela Hrckova et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
DOI: 10.4172/2167-065X-C1-025

Hypoxic and aerobic culture systems influence activity of selected flavonoids in eukaryotic parasitic organism *in vitro*: An interesting model for pharmacological research

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Larval stage of flatworm, tetrathyridium, has ability of survival and asexual multiplication in a wide range of vertebrate hosts what indicates a high biochemical and physiological potential for adaptation. We recently developed axenic long-term *in vitro* cultivation systems of larvae under both hypoxic and aerobic conditions, representing the unique eukaryotic model for pharmacological and molecular studies. This *in vitro* system allowed us to study the dose- and time-dependent effects of various natural compounds on multiple biochemical and molecular pathways in larvae. We have focussed on flavonolignans (silybin, dehydrosilybin and silychristin) prepared from silymarin, the main component of herb Silybum marrianum and two other flavonoids: bergenin and arbutin. Activity of individual compounds on metabolic activity of larvae, which reflects activity of enzymes of complex I and II in mitochondria, was dependent on oxygen tension in culture. Flavonolignans also modulated activity of other enzymes like GST, SOD, enzymes involved in glucose transport, in lipogenesis, cell death and motility, indicating their complex activity on multiple targets in larvae.

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Guillaume Arcile et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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Neuroprotective effect of SNC-1, a biocatalysis processed ayurvedic plant extract as a promising ethnopharmaceutic treatment for Alzheimer's and Parkinson's disease

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The three ayurvedic medicinal plants *Withania somnifera, Emblica officinalis,* and *Bacopa monnieri*, were extracted by high-pressure static extraction using the Zippertex technology. The extracts were mixed to reach quantifiable amounts of active compounds identified by HPLC-MS analysis. The mixture of extracts was incubated with resting cells of the fungus *Beauveria bassiana* ATCC 7159. The fermentation promoted the fluidization of the starting dense mixture, while HPLC monitoring evidenced the disappearance of glucogallin from *E. officinalis* extract and the concomitant increase in Gallic acid content. While topical exposure of the chick embryo chorioallantoic membrane (CAM) to the non-fermented extract led to an extensive necrosis, the fermented extract was not toxic and reduced the CAM vascularization, supporting its antiangiogenic potency. The innocuity of the fermented extract was demonstrated using the *in vivo* LD_{50} test, the morphological examination of internal organs of treated rats, as well as the evaluation of blood biomarkers of liver damage (aspartate aminotransferase and alanine aminotransferase). The fermented extract SNC-1 was developed as a nutraceutical antiangiogenic treatment of age-related macular degeneration and commercialized in an oral form named Ethnodyne-Visio. Furthermore, study showed that SNC-1 (dried from of Ethnodyne-Visio) was able to significantly protect neurons (cortical as well as dopaminergic neurons – *in vitro* models of Alzheimer's and Parkinson's diseases) from different injuries (β amyloid, mitochondrial toxins, glutamate). Additionally, SNC-1 stimulated neurite outgrowth). Interestingly these effects were still observed at low doses and were still efficient when the extract was applied up to 4h after the toxins application. Extensive efforts are dedicated to the identification of the active compound responsible of these effects. Clinical trials are underway to confirm the benefit of SNC-1 for Alzheimer and Parkinson patients.

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A Review of ethnopharmacological research of some cameroonian medicinal plants

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The use of herbal medicines as complements or alternatives to modern medicines has been on the increase. This review summarizes research carried out on some Cameroonian medicinal plants between 2007 and 2017 by our research team at the Faculty of Science of the University of Douala in collaboration with some national and international scientists. Medicinal plants are resources of traditional medicines and modern medicine derived from plants. This study was designed to outline some biologic activities of various medicinal plants extracts used by the Cameroonians' and central African people. Several medicinal plants growing in Cameroon were identified as having pharmacological properties. These plants species which include *Crassocephalum bauchiense, Dichaentanthera africana, Harungana madagascariensis, Milletia conraui, Nauclea latifolia, Pecedanum zenkeri, Ptelopsis hylodendron, Schefflera barteri, Strychnos icaja, Strychnos malacoclados* with healing properties are listed alongside their traditional use and a summary of the scientific research achieved are given. They were tested for their in vitro or *in vivo* biological activity by standard protocols. These ten plants have shown antimicrobial, antioxidant, antiplasmodial, immunomodulatory, analgesic, antipyretic, anti-Herpes simplex virus and α-glucosidase inhibitory activities. Natural products (diterpenoids, alkaloids and flavone) have been identified from five of them. The results suggest that the plants extracts could be a promising rough material for the development of new and more effective modern drugs. Based on these results, drugs from Crassocephalum were developed but no patent is obtained till today.

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Searching for drugs from Vietnamese ethno-medicine plants

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Vietnam is easternmost country in Southeast Asia where the climate changes considerably from place to place due to differences in latitude. There are two World Natural Heritage Sites (Halong Bay and Phong Nha - Ke Bang National Park), and six biosphere reverses (Can Gio Mangrove Forest, Cat Tien, Cat Ba, Kien Giang, the Red River Delta and Western Nghe An) in Vietnam. We are one of 25 countries which possess a uniquely high level of Biodiversity. Vietnam is hometown to an estimated 12,000 species of high-value plants; approximately 3,780 (~ 36%) medicinal plants; and account for approximately 11% of the 35,000 species of medicinal plants known worldwide. There are 54 ethnic groups in Vietnam with their own characters (language, lifestyle and cultural heritage). That's why many ethno-medicine plants are not yet known and are used only by ethnic groups. Until now, hundreds of Vietnamese medicinal plants were studied such as *Physalis angulata, Cleistanthus indochinensis, Ophiopogon japonicus, Garnoderma lucidum, Camellia bugiamapensis, Momordica charantia, Hedychium coronarium, Annona glabra, Callisia fragrans, Eurycoma longifolia, Cudrania tricuspidata, Tacca* species, *Mallotus* species, *Glochidion* species, *Solanum* species, *Trichosanthes* species, etc. A lot of potential compounds were discovered from Vietnamese medicinal plants such as physalin B, D, F, G; malloapelta B, peaonol, cleistantoxin, desgalactotigonin, berberin, rotundin, rutin, artemisinin, artesunat, etc. The opportunities to discover new drugs from Vietnamese ethno-medicine plants with the collaborators worldwide are so big.

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Harnessing phytochemicals to protect neuronal and glial cells from oxidative stress

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Oxidative stress and amyloid beta toxicity are involved in the pathogenesis of Alzheimer's diseases. We have previously demonstrated that an extract prepared of the plant *Achillea fragrantissima* (*Af)* protected cultured brain astrocytes from oxidative stress-induced cell death and down regulated microglial activation. Using activity guided fractionation, we have purified from Af an active flavonoid named 3,5,4-trihydroxy-6,7,3-trimethoxyflavone (TTF). TTF protected cultured astrocytes from H_2O_2–induced cell death via interference with cell signaling (inhibition of SAPK/JNK, ERK 1/2, and MEK1 phosphorylation) and by reducing the levels of oxidative stress-induced intracellular reactive oxygen species (ROS). The mechanism of the protective effect of TTF against H_2O_2-cytotoxicity could not be attributed to a direct H_2O_2 scavenging but rather to the scavenging of free radicals as was shown in cell free systems. In addition, TTF protected cultured neuronal cells from amyloid beta cytotoxicity via interference with cell signaling events and by reducing the amyloid beta - induced levels of intracellular ROS. Moreover, TTF exhibited anti-inflammatory activities and inhibited the LPS-elicited secretion of the proinflammatory cytokines Interleukin 6 (IL-6) and IL-1beta from microglial cells. Our results suggest that TTF might be a therapeutic candidate for the treatment of Alzheimer's disease as well as other neurodegenerative diseases where oxidative stress, neuroinflammation and amyloid beta toxicity are part of the pathophysiology.

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From popular medicine knowledge to evidence ethnopharmacolgy efficacy can nature give us the tools to fight human neurodegenerative diseases?

Vincenzo Rispoli
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From the night of time, Man has always tried to relieve his suffering by turning his attention to Nature and seeking remedies to cure the ills of his body. In recent decades, phytotherapy has been re-evaluated, not only because of the growing number of patients who use it, but, above, all, since numerous preclinical and clinical researches have been able to demonstrate and confirm the pharmacological bases of plant-based treatment, since acquired in the past from traditional medicine in empirical way. Today phytotherapy has become a medical discipline in all aspects, because it applies the method and the rigor of scientific evidence. Here it is addressed the history, current as well as the future perspective of AD treatment by Ethnomedicine, taking into consideration the probable causes and preventive mechanisms together with the treatment methods. Alzheimer's disease (AD) is an irreversible, slowly progressive neuodegenerative disease of the brain, and it is characterized by memory deficits and progressive cognitive impairment, accompanied by neuropsychiatric changes. It has become the fourth leading cause of death in developed countries. The main symptoms of AD are primarily caused by a cholinergic dysfunction due to degeneration of basal cholinergic forebrain (BCF). in particular, consistent neuronal loss into the *nucleus basalis* of *Meynert* (NBM), which produces a reduced cholinergic input to target areas such as cerebral cortex and hippocampus. Pathogenic cause of AD remains incompletely understood. So, currently acetylcholinesterase inhibitors (AChEIs), which decrease the breakdown of the neurotransmitter, has been the main symptomatic therapy for mild to moderate Alzheimer's patients, approved by FDA. To date, AChEIs are considered the main pharmacological strategy in the palliative approach in the therapy of AD; they undoubtedly, temporarily restore the disrupted cholinergic transmission in brain. Consequently, the search for novel compounds is necessary. In recent times, several chemical and pharmacological studies have searched for new drugs, in an attempt to extract and isolate from plants novel compound or better understand the effects of those already known, in an effort to fight this terrible disease.

Conclusions: AD is a multi-causal and multi-factorial progressive neurodegenerative disease with complicated pathogenesis. Thus it is likely that multiple drugs or drugs with poly-pharmacological activities will be the best therapeutic approaches to address the diverse pathological aspects of the disease.Anti-cholinesterasic activities, anti-Aβ aggregation and anti-Aβ-induced oxidative injury such as anti-NMDA-induced toxicity and anti-inflammatory activity showed by many herbal compounds, encourage their use as potential disease-modifying drugs for neurodegenerative disorders, opening to new insight and future perspectives for a multi-functional phytomedicine. From this point of view, we have to overcome our way to think Ethnomedicine and official medicine opposed each other, or that orthodox medicine is better than traditional medicine, as well as phytotherapeutic remedies as an alternative to synthetic drugs. The two pharmacological approaches are often, and it should always be, complementary; that is, to be able to complement each other. Science and consciousness of physician will depend on the correct integration of. In conclusion, Evidence based pharmacology (EBP) is the conscientious, explicit, judicious and reasonable use of modern, best pharmacotherapeutic evidence in making decisions about the care of individual patients. EBP must integrate clinical experience and patient values with the best available research information.

Keywords: Alzheimer's disease, Neurodegeneration, Multi-target drus, Disease-modifying therapy, Phytotherapy, Preventive Mechanism, Treatment Methods.

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Essential oil of *Citrus lumia* risso: Phytochemical profile, antioxidant properties and activity on the central nervous system

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Background: The use of essential oils (EOs) is known since long time in traditional medicine and aromatherapy for the management of various oxidative stress-related disorders and is further increased recently for their neuroprotective and anti-aging potential as well as for reducing anxiety and stress.

Purpose: To evaluate, for the first time, the chemical composition of *Citrus lumia* Risso EO and its antioxidant, anti-cholinesterase as well as neuroactive properties by cell-free and cell-based assays. *Citrus lumia* Risso is one of the oldest cultivated limettes in Sicily, Mediterranean Europe and North Africa by which distinguished for the fruit shape and mainly for the sweet, no-acidic juice.

Methods: The distribution and morphology of oil glands in the fruit peel were analysed microscopically, by SEM. A phytochemical profile elucidation, by GC-FID and GC-MS analysis, an *in vitro* evaluation of antioxidant and free-radical scavenging properties of the EO, using different *in vitro* methods (Folin-ciocalteu, DPPH, TEAC, FRAP, Fe^{2+}-chelating capacity, ORAC and β-carotene bleaching assays) as well as anti-cholinesterase activity were carried out. The impact on the spontaneous electrical activity of rat neuronal networks by means of microelectrode array (MEA)-based system, was evaluated.

Results: The EO has shown strong antioxidant and free radical scavenging properties, particularly in hydrogen atom transfer based assays (β-carotene bleaching and ORAC assay, IC_{50} 22 μg/mL and 46 μg/mL, respectively), that can be attributed to the high content of monoterpene and monoterpene derivatives, especially d-limonene (48.905%), and linalool (18.245%). Furthermore, has shown an interesting anti-acetylcholinesterase activity (IC50 258.25 μg/mL). Data from MTT analysis indicate that the cytotoxicity of OE, evaluated on L929 mouse fibroblasts, is very low, with an IC50 higher than 500 ug/mL at 48 h. Rat neuronal networks subjected to EO showed a concentration-dependent inhibition of spontaneous electrical activity.

Conclusions: Results indicate that this EO could be an important source of natural antioxidants potentially useful in the detoxification mechanisms of the organism, suggesting an important preventive role in the onset of oxidative stress-related pathologies.

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Immune cell type or signaling-specific effects of four candidate phytomedicines for cancerous and inflammatory diseases

Ning Sun Yang
Agricultural Biotechnology Research Centre, Taiwan

In our recent studies, we showed that many phytochemicals or their derivatives can confer diverse pharmacological activities in preventing tumor metastasis (See References). These phytochemical activities regulate the immune system or non-malignant cells in a tissue microenvironment under various *in vivo* conditions. And these activities cannot be effectively addressed by the conventionally used cell culture systems *in vitro*. Our current strategy is to initiate our study through a combination of omics approaches and specified *in vivo* tumor model systems. Specifically, we first make predictions for candidate specific pharmacological activities according to the "omics screening" profile of differential responsive genes, proteins or involved metabolites. Then we make a list of hypothesis in priority sequence. Finally, we detect/evaluate the candidate mechanistic signaling cells/molecules for suppression of well-defined tumor metastasis activity. With this strategy, we have been successful in evaluating several pharmacological effects of nature plant phytochemicals or their derivatives on immune cell systems or the surrounding nonmalignant cells in defined tumor microenvironment. The omics approaches we used to predict and reveal the specific pharmacological activity of phytochemicals or medicinal herbal extracts/fractions, including genomics, transcriptomics, proteomics, metabolomics and the next generation sequencing (NGS) systems. The systematic analysis of the observed data is to "contemplate" the cellular or physiological responses, according to the various pattern changes detected in different response elements. Technically, in our task on pathway and net-working analyses, the overall or big trend/pattern of the different responsive elements or/and their signaling systems is the key for predicting specific pharmacological mechanisms, instead of the "super" inducer or suppressor single gene activities. For instance, the "expression pattern or trend", rather than the "fold change", of specific microRNA species is a much more important factor for predicting their suppressive effect on target genes. As a result, the understanding and background knowledge of specific targets or signaling networking pathways of specific disease targets are quite important and need to be carefully reviewed "first" before "searching the omics data" in a totally randomized way. With this approach, whether the expression trend of their downstream genes can fit the proposed hypothesis is also considered as a key factor.

Biography

Ning-Sung Yang is a Distinguished Professor and Distinguished Research Fellow of Academia Sinica and the associated universities in Taipei, Taiwan. He has helped the development of gene gun technology and pioneered its application to mammalian transgene experimental systems and gene therapy approaches. After thirty years of a research career in USA, Dr. Yang established the Agricultural Biotechnology Research Center in Academia Sinica, Taipei. He was elected in 2006 as a member of the American Association for the Advancement of Science (AAAS, USA). He has published more than 160 research papers, and obtained 14 USA patents.

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Isolation and structure elucidation of new cytotoxic polypeptide from bee venom

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Honeybee is an important economic insect, which have vital role in the pollination for crops and wild flowers. In addition to ecological importance, honeybee supplies with various products, including bee venom (BV). This venom has been used in traditional medicine for thousands of years and there is an increasing interest in their applications in modern medicine. BV has diverse biological activities as anticancer, antimicrobial, anti-inflammatory, antiviral and hepatoprotective. Today there is an urgent call to find anticancer agents from the natural products with less ecological damage and minimum health and environmental hazards. Our main aim is to identify and characterize the bioactive peptides from bee venom. These peptides have been poorly characterized, partly because they are generally present in trace quantities. Isolated active peptides from the bee venom have been identified using techniques including High Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS, LC/MS, MS /MS), Amino Acid Analysis (AAA) and 2D-Nuclear Magnetic Resonance Spectroscopy (2D-NMR). Polar fractionation prior to screening of anticancer has been done. The bioassay-guided isolation for bee venom leads to isolation of four peptides melittin, apamin, MCD and secapin. Melittin showed cytotoxic activity on three cancer cell lines: lymphoma cells U-937GTB, myeloma cells RPMI 8226/s, leukaemia cells CCRF-CEM and two drug-resistant sub-lines (PRMI 8226/Dox40 and CEM/VM-1), with IC50 values of 1.3, 1.1, 1.4, 1.7, 2 μM, respectively.

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Notes:

Sessions

Drug Delivery System of Biopharmaceutical Products | Clinical Trials on Biopharmaceutical Products | Drug Therapy | Biologics and Biosimilars | Biopharmaceutics: Drug Discovery and Development | Cellular and Gene Therapy | Biopharmaceutical Companies & Market Analysis

Session Chair
Vladimir P Torchilin
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Session Introduction

Title: **Steroid-harboring nanoparticles provide anti-inflammatory response with less adverse effects**
Krisztian Kvell, University of Pecs, Hungary

Title: **Biosimilars in the UK's NHS: Attitudes, appetites and acceptance**
Theo Christie, Nation Institue for Health Research, UK

Title: **2,2'Dithiodinicotinyl ligands: Key To more reactive thiomers**
Claudia Menzel, University of Innsbruck, Austria

Title: **An ethnopharmacological evaluation of indigenous plants used by the communities of district Kotli, Azad Jammu Kashmir**
Humaira shaheen, Agriculture University Rawalpindi, Pakistan

K Kvell et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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Steroid-harboring nanoparticles provide anti-inflammatory response with less adverse effects

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University of Pécs, Hungary

Statement of the Problem: Steroids are acknowledged anti-inflammatory drugs used in multiple conditions, including autoimmune disease. Steroids provide strong suppression of inflammation however, their long-term utilization triggers numerous adverse effects including obesity, diabetes, osteoporosis, edema retention etc. As a result, only inflammatory flares are treated with steroid compounds, for short term.

Methodology & Theoretical Orientation: Our collaborative research team has produced nanoparticles of specific size harboring steroid compounds. In theory, due to their specific size, steroid-harboring nanoparticles trigger phagocytosis in monocytes and macrophages, but leave other (non-phagocytic) cells unaltered.

Findings: Our human *in vitro* data indicate that steroid particles show potent anti-inflammatory effect on monocytes / macrophages, equivalent to that of steroid solution. However, their adverse effects are reduced using non-phagocytic cells. Liver cells, for example, show increased viability with steroid particles as opposed to steroid solution.

Conclusion & Significance: Our working hypothesis was that steroid-particles of a specific size range can preferentially target monocytes/macrophages, the major mediators of inflammation. Other (non-phagocytic) cell types shall largely be unaltered by steroid particles, as opposed to steroid solution. This is confirmed by our data. Our technology allows for the production of regular steroid compounds with significantly reduced side effects, with the promise of long-term use in human.

Biography

K Kvell, MD PhD has primary expertise in Immunology and Biotechnology. He works in the field of Immune Senescence and its relation to steroid compounds. Currently he is working at the Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, at the University of Pecs, Hungary. He is currently involved in interdisciplinary research utilizing steroid compounds and nanoparticles. This field encouraged the collaborative research team to develop novel drug delivery strategies of steroid treatment, to allow for targeted steroid treatment with potent anti-inflammatory effect, yet with reduced level side effects.

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Biosimilars in the UK's NHS: Attitudes, appetites and acceptance

Theo Christie
NIHR Clinical Research Network, UK

Is the NHS ready for the biosimilars boom? to most, biosimilars are a no-brainer. But as with anything new, there are early adopters and sceptics. The NIHR CRN is an independent, government-funded organization which supports delivery of the majority of clinical research in England - and with that comes unique insight. The NIHR CRN can report that attitudes, appetites and acceptance in relation to biosimilars in the UK are changing. Despite being approximately ten years ahead of the US our approach and acceptance of biosimilar drugs, in 2015 it came to light, some commercial trial sponsors were overlooking the UK as a destination for biosimilar trials - claiming that the appetite for delivering these types of trials was low. The NIHR Clinical Research Network was drafted to sharpen the UK's competitive edge. In this presentation we will reveal why life science companies were overlooking the UK to deliver their trials, and how these challenges are being overcome using the Clinical Research Network structure which is unique to the NHS in England. Companies can now continue to place their biosimilar trial in UK with confidence and get ahead of the game when it comes to study set-up, feasibility, and patient recruitment. This presentation will present a range of perspectives (via video clips) which illustrate how the UK's appetite, capacity and capabilities to deliver biosimilar clinical trials have developed in parallel with the expansion of the biosimilars market. You will hear from those involved in conducting biosimilar trials - the clinicians, investigators and nurses at the coal face of research delivery in the NHS. We've also captured the NHS Trust R&D viewpoint, along with some thoughts from the NHS pharmacy team. The British Biosimilar Association offers up some interesting ideas, but probably the most memorable perspective is that of the patient.

Biography

Divya Chadha Manek is the Head of Business Development (Commercial) for the NIHR Clinical Research Network (CRN). Her role is to maintain strategic relationships with global and UK life sciences companies and the Clinical Research Network. She also leads on ensuring that the Clinical Research Network is abreast of new study delivery innovations to ensure that the organization is evolving to service life sciences industry requirements. With a degree in Clinical Psychology and a Master's in Clinical Research, she has worked with the Clinical Research Network for the past nine years. Her first role with the CRN was delivering commercial contract clinical research within a National Health Service (NHS) hospital. Prior to her current role, she worked as the Industry Manager within the Mental Health team, performance managing and maintaining oversight of the national mental health portfolio of studies. She has experience of clinical research from a site level and from a national perspective in the UK. She is currently studying PhD in Dementia Care.

Theo Christie is a Business Development Manager (Commercial) for the NIHR Clinical Research Network (CRN). Theo facilitates key discussions between industry and the Clinical Research Network and is a point of contact for the life sciences companies engaging with the Clinical Research Network. Theo is able to provide advice to companies on how they are able to tap into the Clinical Research Network study support services to ensure clinical studies are set up efficiently and recruit to time and target. With a degree in Clinical Sciences, Theo has been with the Clinical Research Network for over four years. He previously worked within the Research Delivery Directorate of the CRN, collaborating with the life sciences industry and national specialty groups across 10 therapeutic areas, providing operational support through feasibility, set up and patient recruitment.

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2,2'Dithiodinicotinyl ligands: Key to more reactive thiomers

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The goal of this study was to establish a new type of preactivated thiomers showing comparatively higher reactivity with mucus and improved mucoadhesive properties. The dimeric form of 2-mercaptonicotinic acid (MNA-MNA) was directly attached to the polymeric backbone of chitosan (CHI) to achieve a higher reactivity. Amide bond formation mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) was used as a coupling reagent. The remaining free amino groups were reacted with succinic anhydride (Succ) to gain a homogeneously anionically charged polymer (CHI-Succ-MNA-MNA). Within our study, various coupling rates of up to 170 µmol MNA-MNA per gram polymer were obtained. The coupling of the dimeric ligand resulted in a preactivated thiomer with a more reactive disulfide substructure due to the additional nitrogen atom in conjugation over the aromatic moieties. Furthermore, the obtained polymer is completely preactivated and therefore protected against unwanted oxidation reactions. Our kinetic studies of disulfide exchange reactions showed a 3.8-fold higher reactivity of CHI-Succ-MNA-MNA compared to a state-of-the-art preactivated thiomer. Rheological measurements showed that CHI-Succ-MNA-MNA with a coupling rate of 170 µmol (CHI-Succ-MNA-MNA 170) lead to a 5.7-fold higher mucus viscosity than the non-thiolated control polymer (CHI-Succ). This indicates a rheological synergism due to mucoadhesive properties. These results were confirmed by an additional mucoadhesion experiment, which showed a significantly prolonged retention time of CHI-Succ-MNA-MNA on the small intestinal mucosa compared to CHI-Succ ($P<0.02$). According to the presented results, the double preactivation seems to be a promising strategy to obtain entirely preactivated polymers with improved mucoadhesive properties.

Biography

Claudia Menzel is a PhD student from the Institute of Pharmacy at University of Innsbruck. Her work focuses on the improvement of drug bioavailability and the development of drug delivery systems for the non-invasive administration of poorly absorbed drugs. She has published three papers and one review article in reputed journals.

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Humaira shaheen, Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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An ethnopharmacological evaluation of indigenous plants used by the communities of district Kotli, Azad Jammu Kashmir

Humaira shaheen
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Aim of the Study and Ethnopharmacological Relevance: District Kotli is a mountainous area ranging from scrub to alpine forests which is sporadically known with reference to ethnopharmacological research. Such studies are mandatory for discovering new crud drugs based on the folk knowledge. This study was aimed to collect knowledge of medicinal plants and folk herbal remedies from the local inhabitants.

Materials and Methods: In all, 100 local informants including 55 were males and 45 were interviewed using semi-structured questionnaire in addition to group discussions and field observations. Different ethnobotanical indices such as Spearmann test, relative frequency of citation (RFC), Relative Importance (RI), Informants Consensus Factor (FIC) and Medicinal Importance (MI) were calculated from the recorded data. Besides, to check the novelty of information, the recorded data was compared with the literature from the recent past.

Results: In all, 80 medicinal plants were used in treating 58 diseases/ailments by the indigenous communities. Comparing the knowledge held by men and women, men had much higher knowledge on medicinal plants ($Z = -2.8$; $p < 0.05$) and their uses ($Z = -0.252$; $p < 0.005$): they reported 14.05 (±10.18) species and 6.12 (±4.13) uses, while women 8.55 (±6.06) species and 5.83 (±3.65) uses. Abdominal pain was the most prevalent problem treated with nine species (7.38%), followed by acute injuries & pain (7 spp., 5.74%) and diabetes (5 spp., 4.10%). The Informants' Consensus Factor (FIC) analysis indicated that among the 19 disease categories used, mouth, ear and eye problems (0.91), skin and related symptoms (0.91), circulatory problems (0.90), allergies (0.90), hair related problems (0.90) and diabetes (0.90) had the highest FIC values. Aerial parts (21spp.) and leaves (20 spp.) were highly utilized for making recipes. The oral application of powder was the leading mode of application (21 spp., 26.25%). Zanthoxylum alatum possessed the highest relative importance (93.75), followed by Adhatoda zeylanica (91.67).

Conclusions: The high informant consensus suggests that current use and knowledge of medicinal plants are still strong and local inhabitants have a high dependency on medicinal plants in meeting their primary health care. This knowledge can be exploited in validation of this knowledge for the drug development and pharmacological activities in addition to the conservation and management of these valuable plant resources of this territory.

Keywords: Ethnopharmacological research, Local inhabitants, Frequency of Citation, Relative Importance, Informants Consensus Factor, Medicinal Importance.

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Joint Event

4th EUROPEAN BIOPHARMA CONGRESS

&

6th International Conference and Exhibition on

PHARMACOLOGY AND ETHNOPHARMACOLOGY

November 09-11, 2017 Vienna, Austria

Scientific Tracks & Abstracts

Day 2

Sessions

Latest Trends in Ethnopharmacology | Ethnopharmacology of Alkaloids | Ethnoveterinary Medicines | Pharmacognosy and Phytochemistry | Applied pharmacology | Cardiovascular Pharmacology | Ethnobotany | Ethnopharmacy | Biologics and Biosimilars

Session Chair
Anupam Bishayee
Larkin University, USA

Session Introduction

Title: **Antineoplastic activity of cannabidiol containing products**

Spiro Konstantinov, Medical University of Sofia, Bulgaria

Title: **Curcumin based possibilities for targeting protein kinase B and nuclear factor kapa B In cutaneous T-cell lymphoma**

Maya Zaharieva, Bulgarian Academy of Sciences Sofia, Bulgaria

Title: **Synergistic effects of *chuanxiong-chishao* herb-pair on promoting angiogenesis at network pharmacological and pharmacodynamic levels**

Wei Hong Cong, Beijing University of Chinese Medicine, China

Spiro Konstantinov et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
DOI: 10.4172/2167-065X-C1-025

Antineoplastic activity of cannabidiol containing products

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Malignancies are the second cause of death in humans worldwide. Despite many newly introduced and approved targeted drugs, the problem of multidrug resistance and serious side effects remain unsolved and open the search for effective natural products with fewer side effects. Curcumin is a practically non-toxic compound of plant origin with own antineoplastic and NF-kB inhibitory activities. Curcumin is one of the most popular drugs derived from Ayurveda traditions. We investigated the cytotoxic activity of curcumin loaded electrospun mats in human hepatic and colon carcinoma cell lines (HEP-G2 and HT-29). Disks with 5 mm diameter of the electrospun mats caused nearly 80% inhibition in HT-29 colorectal cells. The efficacy against the HEP-G2 hepatocellular carcinoma cells was weaker. The mats showed antibacterial features and were found to be biocompatible with human tissues of different origin. The tested polymeric mats increased the accumulation of curcumin inside of the malignant cells as estimated by fluorescent microscopy. One of the polymeric mats represents fully water soluble form of curcumin that may have distinct pharmacokinetic advantages. Taken together our experimental findings indicate that the non-toxic yellow pigment curcumin after inclusion into electrospun polymeric mats can be used for topical treatment of liver lesions (e.g. colorectal cancer metastases) and these results are promising in terms of further clinical application.

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Maya M Zaharieva et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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Curcumin based possibilities for targeting protein kinase B and nuclear factor kapa B In cutaneous T-cell lymphoma

Maya M Zaharieva[1], Antonios G X Trochopoulos[2], Ludmila Dimitrova[1], Alexander D Kroumov[1], Stanislav Philipov[3], Yana Ilieva[2], Theodora Atanassova[2], Iva Tsvetkova[1], Vanya Pencheva[2], Krassimira Yoncheva[2], Martin R Berger[4], Hristo M Najdenski[1] and **Spiro M Konstantinov[2]**

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Cutaneous T-cell lymphoma (CTCL) is an orphan disease which primarily affects the skin by clonal accumulation of neoplastic T-lymphocytes and is characterized by a 5-year overall survival of 32% of the patients, if the skin is involved and only 7% for extracutaneous involvement. CTCL therapy is challenging, often empiric because of the limited insight into the genetic basis and single drug therapy is usually not applicable. Curcumin is one well-known ethnopharmacological non-toxic drug with limited bioavailability. The objective of our study was to investigate new treatment modalities for targeting CTCL by combining nanoencapsulated curcumin with alkylphosphocholines thus affecting malignant cell proliferation, skin inflammation and related infections. The nanoparticle size and zeta-potential of Nano-systems containing curcumin were determined by photon correlation spectroscopy and electrophoretic laser Doppler velocimetry. Curcumin concentration was measured by HPLC. MTT- (ISO 10993-5) and CFU-assays were performed on CTCL cell lines for evaluation of cell viability and clonogenicity. Cell death ELISA, microscopy, Hoechst staining and Western blotting were used to monitor hallmarks of apoptosis. Antimicrobial activity was evaluated by ISO 20776-1:2006 (E). Chou & Thalalai software and response surface analysis of combination effects were used to design the experiments and to estimate drug-drug interactions. The nano-sized curcumin delivery systems were prepared using two copolymeric carriers with diameter less than 200 nm and negative surface charge. Encapsulated curcumin penetrated through the cell membranes of CTCL cells faster than curcumin solubilized in ethanol. Combination effects were evaluated as additive to slight synergistic. We have observed in treated samples induction of apoptosis and modulation of PKB/Akt and related signal proteins. Erufosine has exhibited bacteriostatic activity against Gram-positive bacteria in concentrations ranging from 32 up to 100 μM. Combination of erufosine with nano-sized curcumin has led to bactericidal effect. Taken together, our experimental findings clearly indicated that properly designed combinations of curcumin with alkylphosphocholines may show higher antineoplastic potential than single compounds and could be beneficial for the treatment of CTCL as orphan disease.

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Synergistic effects of *chuanxiong-chishao* herb-pair on promoting angiogenesis at network pharmacological and pharmacodynamic levels

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Objective: The objective of the study is to investigate the synergistic effects of Chuanxiong-Chishao herb-pair (CCHP) on promoting angiogenesis *in silico* and *in vivo.*

Methods: The mechanisms of action of a herb-pair, Chuanxiong-Chishao, were investigated using the network pharmacological and pharmacodynamic strategies involving computational drug target prediction and network analysis, and experimental validation. A set of network pharmacology methods were created to study the herbs in the context of targets and diseases networks, including prediction of target profiles and pharmacological actions of main active compounds in Chuanxiong and Chishao. Furthermore, the therapeutic effects and putative molecular mechanisms of Chuanxiong-Chishao actions were experimentally validated in a chemical-induced vascular insufficiency model of transgenic zebrafish *in vivo*. The mRNA expression of the predicted targets was further analyzed by real-time polymerase chain reaction (RT-PCR).

Results: The computational prediction results found that the compounds in Chuanxiong have antithrombotic, antihypertensive, antiarrhythmic, and antiatherosclerotic activities, for hypoxic-ischemic encephalopathy, ischemic stroke, myocardial infarction and heart failure.In addition, compounds in Chishao were found to participate in anti-inflammatory effect and analgesics. Particularly, estrogen receptor α (ESRα) and hypoxia-inducible factor 1-α (HIF-1α) were the most important potential protein targets in the predicted results. In vivo experimental validation showed that post-treatment of tetramethylpyrazine hydrochloride (TMP•HCl) and paeoniflorin (PF) promoted the regeneration of new blood vessels in zebrafish involving up-regulating ESRα mRNA expression. Co-treatment of TMP•HCl and PF could enhance the vessel sprouting in chemical-induced vascular insufficiency zebrafish at the optimal compatibility proportion of PF 10 μmol/L with TMP•HCl 1 μmol/L.

Conclusions: The network pharmacological strategies combining drug target prediction and network analysis identified some putative targets of CCHP. Moreover, the transgenic zebrafish experiments demonstrated that the Chuanxiong-Chishao combination synergistically promoted angiogenic activity, probably involving ESRα signaling pathway.

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Notes:

Sessions

Clinical Trials on Biopharmaceutical Products | Drug Delivery Systems | Therapeutic Biological Products | Drug Delivery System of Biopharmaceutical Products | Drug Delivery System | Biotechnological Products | Drug Disposition & Pharmacokinetics | Pharmacodynamics

Session Chair
Hiroshi Ohrui
Yokohama University of Pharmacy, Japan

Session Introduction

Title: **New compounds from Withania Somnifera with neuroprotective activities. Isolation, structure elucidation bioassays and scale-up production**
Géraldine Le Goff, National Center for Scientific Research CNRS, Institute for Chemistry of Natural Products ICSN, France

Title: **High throughput optimization and mass spectrometric analysis of covalently labeled proteins and antibody drug conjugates**
Chawita Netirojjanakul, Amgen, USA

Title: **Oral booster vaccine against hepatitis B in the form of low-dosed lyophilised plant tissue bearing S-HBsAg VLPs**
Marcin Pyrski, Institute of Plant Genetics Polish Academy of Sciences, Poland

Title: **Identification of bioactive peptides from enzymatic hydrolysis of royal jelly**
Hesham El-Seedi, University of Malaya, Malaysia

Title: **Achieving Quality by Design for parenterals filling with a time-pressure setup**
Yannick Elias, Janssen (Cilag AG), Switzerland

Title: **Antitumor prperties of curcumin loaded polymeric electrospun mats**
Spiro M Konstantinov, Medical University of Sofia, Bulgaria

Géraldine Le Goff et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
DOI: 10.4172/2167-065X-C1-025

New compounds from *Withania Somnifera* with neuroprotective activities. Isolation, structure elucidation bioassays and scale-up production

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The prevalence of neurodegenerative diseases are increasing worldwide due to extensions in lifespan with more than 2.1 billion people aged 60 and more in 2030. The most representative diseases are Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). Available treatments are limited in number and efficacy and extensive efforts are dedicated to alternative herbal therapy. Among various promising plants, *Withania somnifera* roots and leaves extracts demonstrated large spectrum activities on neural dysfunction (common name Ashwagandha). Based on our previous encouraging results, our ongoing efforts are dedicated to identify new compounds from Ashwagandha and demonstrate their mechanism of action and their relevance in neuroprotection. Besides the known major constituents, withanolides, withanone and withaferin, now steroidal components were identified and their biological activity investigated.

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Chawita Netirojjanakul et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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High throughput optimization and mass spectrometric analysis of covalently labeled proteins and antibody drug conjugates

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The use of automated high throughput screening in large molecule discovery research still lags behind that of small molecule discovery. Recently we developed a high-throughput large molecule discovery platform to automate hundreds of bioconjugation reaction setup in one setting. In addition, given LC-MS is a widespread analytical bottleneck, we also established a high-throughput mass spectrometry (HT-MS) platform to accurately detect and rapidly quantitate protein conjugates. We showed that our HT-MS platform can be used to quantitate the extent of covalent inhibitor adducts to a cysteine-containing protein construct (~19 kDa) and of biotinylated adducts to mAbs and Fc domains (~150 and ~50 kDa, respectively). Sample acquisition time was ~20 seconds per sample, 10-50x shorter time than traditional LC-MS methods. Site-specific bioconjugation of human Fc domains with cysteine engineered at different positions were conducted under a matrix of reaction conditions varying equivalents of reductants, oxidants, and alkylating agents using the high-throughput large molecule discovery platform. Using HT-MS, 4 x 384 well plates were analyzed in ~8 hours, as opposed to ~11 days using traditional LC-MS. This approach facilitated rapid determination of DAR values for the reduced and intact huFc domains and selection of optimized conditions for different cysteine-engineered Fc constructs which will be used in preparation of Fc-peptide conjugates as therapeutic leads.

Biography

Chawita Netirojjanakul received her BSc in chemistry from MIT, conducting research in the laboratory of Prof. John Essigmann (MIT) and Prof. Steve Ley (Cambridge). After graduation, she pursued her interest in science policy and commercialization studying MPhil in Technology Policy at University of Cambridge, UK, as a Gates Scholar. She received HHMI International Student Research Fellowship to conduct PhD research under the supervision of Prof. Matthew Francis in the Chemistry Department at UC Berkeley with a focus on "development and applications of well-defined antibody and antibody fragment bioconjugates." She is a Scientist in Therapeutic Discovery Department at Amgen.

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Oral booster vaccine against hepatitis B in the form of low-dosed lyophilised plant tissue bearing S-HBsAg VLPs

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The continued HBV high prevalence coupled with deficiencies in vaccination programmes stimulate research on a new type of vaccines. Potential orally administered plant-based vaccine is highly attractive regarding efficacy, cost-effectiveness and availability of mass hepatitis B prevention. Freeze-dried oral formulations facilitate elimination of complex purification steps, size reduction and better stability during storage, as well as ensure controlled administration regime in minimised medical facilities. Micropropagation of lettuce expressing S-HBsAg was optimised to provide repeatable uniform feedstock for plant-derived oral vaccine manufacturing. Lyophilisation protocol facilitating successful processing of lettuce leaf tissue containing S-HBsAg assembled into VLPs (Virus-Like Particles) was developed. Several drying profiles and excipients as well as effects of freezing rate and post-process residual moisture were analysed. The profile of 20°C for 20 h for primary and 22°C for 2 h for secondary drying as well as sucrose proved the most efficient stabilisation of S-HBsAg during freeze-drying. The process was highly reproducible (86-97%), and provided a product with VLP content up to 200 µg/g DW. Atmosphere of nitrogen proved to preserve S-HBsAg VLPs for minimum one year at temperatures up to 37°C. Low-dosed (5-200 ng) preparation used as oral booster vaccine elicited anti-HBs response in animals at level of commercial injection vaccine (around 1000 mIU/ml). As a result, a plant-derived semi-product with good long-term stability and immunogenicity of S-HBsAg was obtained for the definite formulation of oral booster vaccine against HBV.

Biography

Marcin Pyrski is a PhD student in Bioengineering Team led by Dr. Tomasz Pniewski in the Institute of Plant Genetics, PAS. He completed Engineering and Master's degrees in Biotechnology at the University of Life Sciences in Poznań. He had attended one year training in plant micropropagation in Floralab Company. Actually he is working with plant-based HBV antigens on their expression, functionality and immunogenicity.

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Notes:

Hesham El-Seedi et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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Identification of bioactive peptides from enzymatic hydrolysis of royal jelly

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Royal jelly (RJ) has been widely used in traditional consumables and in skin creams and ointments for health promotion. RJ is rich in bioactive constituents such as jelleines, 10-hydroxy-2-decenoic acid, royalisin as well as the major royal jelly proteins (MRJPs), all of which have shown antimicrobial effect *in vitro*. However, the characterization of RJ is far from complete, and the development of new characterization techniques is allowing the discovery of new compounds. Many new bioactive peptides have been identified using enzymatic hydrolysis as a tool. Enzymatic hydrolysis of RJ has verified the presence of peptides with anti-oxidant and anti-hypertensive activity. In the current work, using bioassay guided fractionation of RJ enzymatic digests; a total of 42 peptides were identified. The peptides, all belonging to the Apis mellifera genome, were identified using a combination of mass spectrometry and bioinformatics tools. Bioassay guided isolation led to the isolation and structure elucidation of three peptides with promising antimicrobial activity. These finding support the use of RJ as food preservative and its potential application as antibiotic.

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Achieving Quality by Design for parenterals filling with a time-pressure setup

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Particulate matter in parenteral drug products is recognized by Health Authorities as a critical quality attribute. A strong focus of fill and finish process development is put on the appropriate selection and operation of the filling system to reduce intrinsic particle formation, often related to shear induced drug product degradation. Rotary Piston Pumps (RPP) have been a standard selection in Janssen for many years, as they offer a simple, compact and robust design and show a very high dosing accuracy. However, as the drug product acts as a pump lubricant, the product is exposed to very high shear rates, which in turn can cause enhanced intrinsic particle formation. Thus, alternative filling technologies, such as peristaltic pumps (PP) or a time-pressure (TP) system, are required for shear-sensitive products. A TP system consists essentially of a pressurized tank and a pinch valve. Dosing accuracy is achieved by harmonizing the vessel over pressure and the valve opening duration. To improve the overall product quality during filling of shear-sensitive products, a Quality by Design and Right the First Time approach is chosen to ensure proper equipment operation. For this, understanding the critical process parameters, risks associated with those parameters and their impact on critical product quality attributed is crucial. Thus, a Design of Experiment study was performed to identify and characterize the impact of six distinct process and equipment parameters on different solutions to obtain a Design Space for optimal TP filling operation.

Biography

Yannick Elias completed his PhD from Swiss Federal Institute of Technology Zurich (ETH Zürich) in Process Engineering, focusing on continuous heterogonous reactions and has published several papers in reputed journals. Currently, he works for Janssen Pharmaceutical Development and Manufacturing Sciences (PDMS) as a Process Engineer, focusing on development for parenterals fill and finish operations. He worked with Janssen for more than one and a half years and is currently leading the strategic expansion of a large molecule drug product development laboratory.

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Notes:

Spiro M Konstantinov et al., Clin Pharmacol Biopharm 2017, 6:4(Suppl)
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Antitumor prperties of curcumin loaded polymeric electrospun mats

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Malignancies are the second cause for death in humans worldwide. Despite many newly introduced and approved targeted drugs the problem of multidrug resistance and serious side effects remains unsolved and opens the search for effective natural products with fewer side effects. Curcumin is a practically non-toxic compound of plant origin with own antineoplastic and NF-kB inhibitory activities. Curcumin is one of the most popular drugs derived from Ayurveda traditions.We investigated the cytotoxic activity of curcumin loaded electrospun mats in human hepatic and colon carcinoma cell lines (HEP-G2 and HT-29). Disks with 5 mm diameter of the electrospun mats caused nearly 80% inhibition in HT-29 colorectal cells. The efficacy against the HEP-G2 hepatocellular carcinoma cells was weaker. The matsshowed antibacterial features and were found to be biocompatible with human tissues of different origin. The tested polymeric mats increased the accumulation of curcumin inside of the malignant cells as estimated by fluorescent microscopy. One of the polymeric mats represents fully water soluble form of curcumin that may have distinct pharmacokinetic advantages.Taken together our experimental findings indicate that the non-toxic yellow pigment curcumin after inclusion into electrospun polymeric mats can be used for topical treatment of liver lesions (e.g. colorectal cancer metastases) and these results are promising in terms of further clinical application.

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