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Yi-Ching Lo et al., J Pharma Care Health Sys 2017, 4:2(Suppl)
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Liuwei dihuang, a traditional Chinese medicine, attenuates methylglyoxal-induced activation of oxidative stress and protein degradation in C2C12 skeletal muscle myotubes

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Introduction: Liuwei dihuang (LWDH) is a widely used traditional Chinese medicine for neurosis, diabetics and renal disorders. Methylglyoxal (MG) is the most potent precursor of advanced glycation end products, which has been implicated in diabetic complications, cardiovascular diseases and central nervous system disorders. The present study aimed to investigate the protective effects of LWDH on MG-induced myotoxicity in C2C12 myotubes.

Methods: C2C12 myoblasts were differentiated by differentiation medium to form myotube structure. C2C12 myotubes were then pre-treated with LWDH water extract (LWDH-WE) for 1h before MG treatment. Protein expressions were analyzed by Western blot analysis. Morphological changes were observed by an inverted microscope. Mitochondria membrane potential and reactive oxygen species (ROS) production were measured by flow cytometer using JC-1 staining and H2DCF-DA staining, respectively.

Results: In C2C12 myotubes, LWDH-WE attenuated MG-induced reduction of mitochondrial membrane potential. Moreover, MG-induced NADPH oxidase (Nox) activation and ROS production were inhibited by LWDH-WE treatment. Furthermore, LWDH-WE attenuated MG-induced myotubes atrophy accompanied with down-regulating signaling of protein degradation pathway including Foxo3a, atrogin-1 and MuRF-1 in C2C12 cells.

Conclusion: LWDH might provide protection against MG-induced myotoxicity via attenuating oxidative stress and protein degradation in C2C12 myotubes, suggesting the potential benefits of LWDH on treatment of skeletal muscle atrophy.

Biography

Yi-Ching Lo has her expertise in drug development and in improving the aging health. Her research interest focuses on the development of neuroprotective and muscle enhancing agents, including chemical and natural products.

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Notes:

Tetsuo Narumi et al., J Pharma Care Health Sys 2017, 4:2(Suppl)
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Novel oleanoic acid-derived HIV entry inhibitor: Design, synthesis and bioevaluation

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Naturally occurring pentacyclic triterpenoid derivatives, such as betulinic acid derivatives, have been shown to exhibit various biological activities including anti-HIV activity. IC9564 and RPR103611 are statin derivatives of betulinic acid tethered by 8-aminooctanoic acid linker, which was reported as a novel class of HIV-1 entry inhibitors. Although those betulinic acid derivatives show nanomolar order potency against diverse HIV-1 strains, relatively high cytotoxicity is one of the drawbacks of them. In this study, the structure-activity relationship study of a series of triterpenoid derivatives was conducted to identify the potential triterpenoid-based HIV entry inhibitors with lower cytotoxicity than betulinic acid derivatives. Significant potency gains were made by replacing the betulinic acid moiety with the oleanoic acid, resulting in the discovery of several potent compounds. This study identified a novel lead compound OKS3-019 with significant anti-HIV activity against 89.6 strain of HIV-1 and lower cytotoxicity than those of known betulinic acid derivatives. Design, syntheses, bioevaluation and docking models of the newly identified oleanoic acid derivatives will be discussed.

Biography

Tetsuo Narumi studied Organometallic Chemistry at Waseda University in Shinjuku, Tokyo, where he worked in the research group of Prof. Isao Shimizu. After PhD studies at Waseda University with Prof. Shimizu followed by Kyoto University with Prof. Nobutaka Fujii, he spent a year in US as a JSPS Postdoctoral Fellow with Prof. Jeffrey W Bode at the University of Pennsylvania. In 2009, he began his academic career in Japan, at Tokyo Medical and Dental University with Prof. Hirokazu Tamamura. In 2013, he began his independent career at Shizuoka University in Japan, as an Associate Professor in Bioorganic Chemistry.

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Notes:

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Validation of epigenetic therapeutic target proteins for homogenous assay performance

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Results from sequencing large numbers of normal vs. tumor samples from the cancer genome atlas project (TCGA) revealed a great number of potential new therapeutic targets among epigenetic enzymes and factors. An outcome of this large scale project is that there is significant interest in developing screening strategies for many of the classes of these proteins, including histone deacetylases (HDACs), histone acetyltransferases (HATs), lysine methyltransferases (KMTs), lysine demethylases (KDMs), and bromodomain containing proteins (bromodomains). While the activity of some of these targets have yet to be validated using screening platforms that use peptide substrates (i.e. Perkin Elmer AlphaLISA), Active Motif has produced a protein toolbox of reagents including active enzymes, recombinant substrates, and detection antibodies for homogenous assay platforms such as AlphaLISA. We have validated reagents used in these assays for targets HDAC3, LSD1, p300, SETDB1, and BRD family members to ensure our ability to validate these proteins for performance in screening assays. As assessed by AlphaLISA assays, the IC50 values for various reference compounds for the enzymes under evaluation, are well within published results. The protein toolbox continues to expand to include a range of enzyme substrates, from peptides and recombinant histones bearing post-translational modifications to more complex, and biologically contextual recombinant octamers, oligonucleosomes, and "designer" mononucleosomes, which include site directed post-translational modifications. The expanding portfolio of substrates will enable screens of additional therapeutic targets using these higher order nucleosome substrates which may enable identification of small molecule compounds which exhibit greater specificity or selectively in vivo relative to those currently identified using peptide substrates.

Biography

Masato Yonezawa has nearly a total of ~20 years of experience in epigenetics research in academia and industries. Analyzed epigenetic phenomena by means of molecular biology and biochemistry. He is highly skilled with enzymatic assays and screened several inhibitors of chromatin modifying enzymes and would like to contribute to epigenetic research and therapy by developing useful tools and drugs.

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Notes:

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Systems toxicology analysis of cardiovascular and respiratory endpoints from ApoE-/- mice showed similar effects when switching to a candidate modified risk tobacco product, THS2.2 or ceasing smoking

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Cigarette smoking is a risk factor for chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD). ApoE-/- mice are prone to developing premature atherosclerosis and emphysema making it an ideal model in which both pathologies can be assessed simultaneously. We evaluated the effects of cigarette smoke (CS) from a standard reference cigarette (3R4F) and aerosol from Tobacco Heating System 2.2 (THS2.2), a candidate modified risk tobacco product (cMRTP). ApoE-/- mice were exposed for up to 8 months to the test aerosol for 3 hours/day, 5 days/week to a target nicotine concentration of 30 µg/l. After 2 months of exposure to CS, cessation and switching groups were further exposed for up to 6 months to fresh air, or THS2.2, respectively. Multiple markers of disease progression were investigated including atherosclerotic plaque formation, pulmonary inflammation, pulmonary function and lung emphysema. Exposure to CS induced time-dependent molecular, physiological and inflammatory pulmonary responses in ApoE-/- mice consistent with emphysematous changes. The area and volume of atherosclerotic plaques measured in the aortic arches were higher in CS-exposed animals compared to both sham and cMRTP-exposed animals at all time-points. Significant changes in the lung transcriptome and proteome of ApoE-/- mice were observed in response to CS-exposure compared to sham-exposed mice. Smoking cessation and switching to THS2.2 resulted in lower activation levels compared to continuous exposure to CS. Both the cessation and switching groups showed similar effects on the histopathological and molecular endpoints, indicating significant reduced effects in comparison to the continued exposure to CS.

Biography

Xia W has earned his Bachelor and PhD degrees from National University of Singapore. He has worked in Takeda and GSK prior to joining PMI and has profound expertise in using preclinical *in vivo* models to evaluate products/compounds and to understand the mechanism of action.

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Chemical assay-guided natural product isolation using solid-supported chemodosimetric fluorescent probe

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Newly isolated compounds from natural sources are important in the lead development of new therapeutics for various diseases. Currently, bioassay-guided isolation is the preferred method for identifying new natural products. However, bioassay guidance alone does not guarantee the complete exploration of natural product compounds. We believe that the systematic exploration of the natural product chemical space will be significantly accelerated by the availability of competent chemical assay systems that can reliably isolate compounds with a specific functional group. We present a new and efficient system for chemical assay-guided natural product isolation. This model system was devised for the identification and isolation of terminal alkyne-containing natural products. This new chemical assay system features a fluorogenic chemodosimeter immobilized onto a solid support. In order to isolate compounds with only the terminal alkyne functionality, copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction was adopted. Our newly designed sensory bead can quantitatively identify terminal alkynes on the basis of the fluorescence signal. With the guidance of our sensory chemical assay system, we were able to detect and isolate a terminal diyne from the methanol extract of *Chrysanthemum morifolium*. We believe that our chemical assay system is applicable in many other fields, such as metabolomics and food science.

Biography

Sanghee Kim has expertise in the synthesis of natural/endogenous products, design and evaluation of their mimetics, and preparation of natural product-like compounds library. Over the last decade, he has been interested in lipids which play essential roles in signal transduction, membrane trafficking and morphogenesis evaluation.

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Identification of small molecular compounds that are useful to suppress mHTT expression, the cause of human Huntington's disease

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Huntington's disease (HD), an inherited neurodegenerative disorder, is caused by aberrant expansion of CAG tri-nucleotide repeats in huntingtin (*HTT*) gene, which results in a production of mutant proteins that are detrimental to neurons. HD clinical symptoms include motor, cognitive and psychiatric disturbances, and patients usually die 10-15 years after the onset of disease. The behavior deficits and neuronal loss can be alleviated by lowering mutant HTT (mHTT) expression in a variety of model systems, suggesting mHTT is causative for genesis and progression of disease and also a target for therapeutic intervention. Despite mHTT is deleterious to neurons, the normal wild-type HTT has a neural protective role. As a result, allele-specific reduction of mHTT is a relatively favorable approach for HD treatment. Supt4h, forming a heterodimer complex with Supt5h, is a transcription elongation factor that aids RNA polymerase II during the process of transcription elongation. Earlier studies we demonstrated that disturbance of Supt4h/5h complex by lowering Supt4h protein levels results in a substantial decrease of transcript production from mHTT allele while leaving wild-type HTT allele affected marginally. We further demonstrated that the motor function deficits of HD transgenic mice are ameliorated by Supt4h genetic knockout and that the life-span of HD mice is prolonged accordingly, suggesting Supt4h is applicable for targeting against mHTT expression and HD. Here, we designed a novel assay platform to screen small molecule compounds that are capable to interfere with the complex formation of Supt4h/5h. Among more than 200 thousands compounds tested, we identified and validated multiple hits with the nature of suppressing mHTT in mouse striatal neural cells or lymphoblastoid cells derived from HD patients. These compounds also showed a rescue effect on rough eye and declined eclosion rate that are caused by mutant HTT in HD-*Drosophila* models, supportive of their potential use in HD.

Biography

Yun-yun Wu is interested in drug development, and glad to have the opportunity to involve from the beginning of the process. She got master degree in learning cell-based experiment from Institution of Biochemistry and molecule biology in National Yang-Ming University. Now she is studying PhD program to find hit and lead compound for ameliorating Huntington's disease progressing.

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Critical roles of the Histone methyltransferase MLL4 in metabolic syndrome

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The pathophysiologic continuum of non-alcoholic fatty liver disease begins with steatosis. Despite recent advances in our understanding of the gene regulatory program directing steatosis, how it is orchestrated at the chromatin level is unclear. PPARγ2 is a hepatic steatotic transcription factor induced by overnutrition. Here, we report that the histone H3 lysine 4 methyltransferase MLL4/KMT2D directs overnutrition-induced murine steatosis via its coactivator function for PPARγ2. We demonstrate that overnutrition facilitates the recruitment of MLL4 to steatotic target genes of PPARγ2 and their transactivation via H3 lysine 4 methylation because PPARγ2 phosphorylated by overnutrition-activated ABL1 kinase shows enhanced interaction with MLL4. We further show that Pparg2 (encoding PPARγ2) is also a hepatic target gene of ABL1-PPARγ2-MLL4. Consistently, inhibition of ABL1 improves the fatty liver condition of mice with overnutrition by suppressing the pro-steatotic action of MLL4. Our results uncover a murine hepatic steatosis regulatory axis consisting of ABL1-PPARγ2-MLL4, which may serve as a target of anti-steatosis drug development.

Biography

Seunghee Lee is working as a Professor at Seoul National University in South Korea.

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T-type calcium channel blocker, KCP10043F inhibits G_1 cell cycle phase and induces apoptosis in caspase-dependent pathway on lung adenocarcinoma

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KCP10043F (3,4-dihydroquinazoline derivative) is a selective T-type Ca^{2+} channel blockers and other studies showed that selective Ca^{2+} channel bloackers could inhibit the growth of cancer cells such as ovarian, lung, and pancreatic cancer cells. we now investigated that KCP10043F induced G_1 arrest dose-dependently and cyclins and CDKs are regulated at protein expression level. In addition, KCP10043F made synergic effect on cell death with Etoposide, a novel anti-cancer agent. Then we continuously studied that KCP10043F induced apoptosis at high dose, detected by Annexin V-FITC / PI staining assay, and this cell death was dependent on caspase-activation, both capsase-3, 8 and -9. moreover, Bcl-2(anti-apoptotic protein), was down-regulated and Bax(pro-apoptotic protein) was up-regulated by this agent. This pathway was confirmed by using z-VAD-FMK, pan-caspase inhibitor, blocked the cell death induced by KCP10043F. Co-treatment with etoposide in low-dose also activated caspase-8,9. So our results suggested pathway how KCP10043F makes synergic effect on A549 cell line and it has possibility to be a potential anti-cancer agent reducing chemoresistance of lung cancers that show the lowest viability among many cancers.

Biography

Jeong hun Lee majored in Oriental Pharmacy at Kyunghee University, and now studying on master course, belonging to Department of Life and Nanopharmaceutical Science, Kyunghee University. He has studied about cancer biology, especially related with apoptosis and cell cycle arrest in cancer cells and how apoptosis is induced in specific cancer cells, like lung carcinoma, by using derivatives of natural product or chemical compositions.

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Do-Hyung Kim et al., J Pharma Care Health Sys 2017, 4:2(Suppl)
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Development and validation of a high-performance liquid chromatography-tandem-tandem mass spectrometry for quantitative determination of Fimasartan and Amlodipine in human plasma: Its application to a pharmacokinetic study of 60mg Fimasartan and 10mg Amlodipine

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A rapid, specific and fully validated high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-MS/MS) method was developed for the determination of Fimasartan and Amlodipine using BR-A-563 and Clarithromycin as an internal standard, respectively. Liquid-liquid extraction (LLE) was carried out on 0.05 mL of human plasma using ethyl acetate and hexane for Fimasartan and 0.2 mL of human plasma using methyl tert-butyl ether (MTBE) and methyl chloride (MC) for Amlodipine. Detection was performed in positive ion multiple reaction monitoring (MRM) mode by monitoring the transitions: m/z 502.4 → 207.1 for Fimasartan, m/z 526.48 → 207.2 for BR-A-563, m/z 408.9 → 238.0 for Amlodipine and m/z 748.2→158.0 for Clarithromycin, respectively. Chromatographic separation was performed on Kinetex C18 (75 × 2.1 mm, 2.6 µm) using a mobile phase consisting of 0.05% formic acid- methanol (30:70, v/v) at a flow rate of 0.2 mL/min for Fimasartan and Luna C18 (50 × 2.0 mm, 3.0 µm) using a mobile phase consisting of 0.1% acetic acid-methanol (30:70, v/v) at a flow rate of 0.2 mL/min for Amlodipine. The linear calibration curves were 1-500 ng/mL for Fimasartan and 0.2-20 ng/mL for Amlodipine. The total runtime was 2.5 min for Fimasartan and 3 min for Amlodipine, retention time was about 1.6 and 1.0 min for Fimasartan and Amlodipine, respectively. The intra-day and inter-day reproducibility was less than 12% for each analyte. The proposed method shows good separation of analytes, without interference from endogenous substances. Fimasartan and Amlodipine were found to be stable under these conditions and the method was successfully applied to the pharmacokinetic study of complex tablet (Dukarb®, 60 mg Fimasartan and 10 mg Amlodipine).

Biography

Do-Hyung Kim majored in life sciences at Kyung Hee University in South Korea and had experience with many natural plants in Thailand and Indonesia. He is currently a master of medicine analysis at Kyung Hee University. He has a lot of experience in drug analysis and has a lot of experience in bioequivalence experiment, clinical experiment and pharmacokinetic experiment.

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Xanthotoxin, a furanocoumarin compound expresses anti-inflammatory effects through suppression of iNOS, COX-2, TNF-α, and IL-6 via AP-1, NF-κB, and JAK-STAT inactivation in RAW 264.7 cells

Seung-Bin Lee, Woo Seok Lee, Ji-Sun Shin, Dae Sik Jang and Kyung Tae Lee
Kyung Hee University, South Korea

Xanthotoxin has been reported to possess skin-protective and anti-oxidative properties. However, anti-inflammatory property has not been studied to date. Therefore, we investigated the role that xanthotoxin plays on anti-inflammatory activity as well as its underlying molecular mechanisms in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. In LPS-induced macrophages, xanthotoxin was found to inhibit nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6) in a concentration-dependent manner. It also suppressed the LPS-induced inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression at the protein level and iNOS, COX-2, TNF-α, and IL-6 at the mRNA level. Molecular mechanism shows that xanthotoxin attenuated the LPS-induced transcriptional and DNA-binding activity of activator protein-1 (AP-1), and this was associated with a decrease in the phosphorylation of c-Fos instead of c-Jun. It played a suppressive effect on the transcriptional and DNA-binding activity of nuclear transcription factor kappa-B (NF-κB) through the inhibiting of p65 nuclear translocation. In addition, the LPS-induced phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 and p38 mitogen-activated protein kinase (MAPK) was found to be suppressed by xanthotoxin. Taken together, these results indicate that xanthotoxin decreased NO, PGE_2, TNF-α, and IL-6 production through downregulation of NF-κB, AP-1, and JAK/STAT signaling pathways in LPS-induced RAW 264.7 macrophages.

Biography

Seung-Bin Lee is a student at Kyung Hee University in South Korea and has been intensively studied on screening anti-inflammatory effect among various natural product derived compounds. In an idea to alleviate these tendency, he has been investigated the underlying molecular mechanism of several drugs which elicit significant decrease of inflammatory endpoints such as nitric oxide, prostaglandin E_2, and pro-inflammtory cytokines.

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Saravanan Govindaraju et al., J Pharma Care Health Sys 2017, 4:2(Suppl)
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Curcumin-conjugated fluorescent gold cluster synthesis and its biocompatibility

Saravanan Govindaraju and Kyusik Yun
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Fluorescent gold cluster (AuNCs) have gained much attention due to their wide spread property like fluorescence, small size, non-toxic, stable and can be easily conjugated to the biomolecules. Curcumin (CUR), a polyphenol compound derived from *Curcuma longa* plant, is a promising anticancer agent for various tumors. We hypothesized the synthesis of fluorescent gold cluster with the conjugation of curcumin for the evaluation of biophysics, biomechanics and cytotoxicity study in cancer cell. Synthesized nanomaterial gives strong red photoluminescence at 650 nm and FTIR confirms the conjugation of CUR to the AuNCs. HRTEM analysis divulges size range from 4-6 nm of monodispersed particles. CUR-AuNCs didn't show any toxicity to the human fibroblast cell line and more toxic to HeLa cells. Optical fluorescence microscopes exhibited that CUR-AuNCs killed specifically the cancer cells. Bio-AFM image provide the morphological changes of HeLa cells after the treatment of CUR-AuNCs at different time intervals. In future, we extend our research to biophysics and biomechanics study of CUR-AuNCs by Bio-AFM, and the cluster treated for the anticancer effect in xenograft model.

Biography

Saravanan Govindaraju is a PhD student in Prof. Yun's lab, Department of Bionanotechnology, Gachon University, South Korea. He graduated with Master's degree from the same university and Bachelor's degree from Anna University, India. His research experience includes synthesis of nanomaterials, cell cytotoxicity study and bio-AFM imaging.

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An investigator-initiated, open-label, single-center, proof-of-concept-study of Omalizumab in patients with poorly controlled acute Urticaria

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Approximately 20~30% of the general population experiences at least one episode of urticaria in their lifetime. Research has also shown that itch, the dominant symptom in most patients with acute urticaria, severely interferes with sleep and daily activities, and has a detrimental impact on the quality of life. This prospective, interventional, single-arm open–label trial recruited 20 consecutive patients aged 20-75 years with a diagnosis of acute urticaria persistant (wheals between 3 days ~ 6 weeks) and baseline urticaria activity score (UAS) ≥ 4 despite oral/intravenous antihistamines with or without systemic corticosteroid therapy who attended our clinic between January 2015 and October 2015. At day 0, patients received a single dose of 300 mg of Omalizumab subcutaneously. Treatment with Omalizumab resulted in mean UAS decrease from 5.0 ± 0.8 at baseline to 1.6 ± 2.1 at day 7. Compared with baseline, a statistically significant reduction in UAS had been observed since day 1, and continued through week 6 (Figure 1). Ten patients (50%) achieved complete remission (UAS= 0 or UCT=16) at day 7 of Omalizumab therapy. As with the primary efficacy outcome, similar improvements were also observed for ISS, UCT, and DLQI. The mean change from baseline in ISS was -0.15, -0.8, -1.30, -1.35, -1.45 at days 1, 3, 5, 7 and week 6, respectively. Mean DLQI score also decreased by 16.4 points at day 7 after Omalizumab treatment. Similar with UAS, sustained therapeutic improvement in ISS, UCT, and DLQ was also observed till week 6. The mean of the patients' percentage improvements in UCT and DLQI was 75.1% and 55.6 % at day 7, respectively. Our study demonstrated that despite significantly improved symptoms and quality of life in patients with acute urticaria after Omalizumab treatment, a rapid control was observed in a minority.

Biography

Hsien-Yi Chiu is an attending physician of dermatology department and research scientist at the Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University. He has contributed significantly to a number of important studies on immune-related skin disorders, including urticarial and psoriasis.

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Phillips B W et al., J Pharma Care Health Sys 2017, 4:2(Suppl)
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Toxicological assessment of the mainstream aerosol of a carbon heated tobacco product in Sprague-Dawley rats: A 90-day sub-chronic inhalation study

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CHTP (carbon heated tobacco product) 1.2 is a potential modified risk tobacco product in which the tobacco plug in a specially designed stick is heated to ≤ 300°C using a carbon heat source. The operating temperature is below the combustion temperature of tobacco, resulting in generation of aerosol with significant reduction of harmful and potentially harmful constituents compared with cigarette smoke. The toxicity of CHTP 1.2 was characterized in a 90-day sub-chronic inhalation study according to the OECD 413 testing guidelines. Sprague-Dawley rats were exposed for 6 hours per day, 5 days per week for at least 13 weeks to filtered air (control), mainstream smoke of reference cigarette 3R4F at 23 µg nicotine/L, or aerosol of CHTP 1.2 at three target concentrations of 15, 23 and 50 µg nicotine/L, respectively. Additional animals from control and CHTP 1.2 high exposed groups were included to observe reversibility of toxicity over a period of 42 days after the exposure. Reduction in respiratory minute volume and frequency typically observed in 3R4F-exposed group was less pronounced in animals breathing CHTP 1.2 aerosol. The number of inflammatory cells and levels of excreted pro-inflammatory cytokines in bronchoalveolar lavage fluid of animals exposed to CHTP 1.2 were lower than in the 3R4F-exposed group. Clinical pathological changes such as higher blood neutrophil counts, elevated liver enzymes and decrease of cholesterol and glucose levels were observed in 3R4F and lesser extent in CHTP 1.2 high groups, compared with control. Microscopic findings in respiratory tract organs including epithelial cell hyperplasia and squamous metaplasia were reduced in CHTP 1.2 as compared with 3R4F-exposed group. In summary, the results indicate that the inhalation of aerosol from CHTP 1.2 caused minor effects in rats mainly attributed to nicotine, and the effects on respiratory tract organs were much lower compared with those from 3R4F reference cigarette.

Biography

Initially trained as a cellular and molecular biologist, Blaine began his scientific career studying the potential of differentiated embryonic stem cells as cellular replacement therapies in a biotechnology start-up company. This led to a journey spanning 3 continents working in the pharmaceutical and the tobacco industries in research and development departments focusing on assay development and drug discovery, as well as inhalation toxicology.

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Prescription audit with special emphasis on drug-drug interactions study in a tertiary care teaching hospital

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Drug-Drug Interactions (DDIs) may be defined as, interaction of two or more drugs in such a manner that the effectiveness or toxicity of one or more drugs is altered. DDI in patients receiving multidrug therapy is a major concern. Although drug-drug interaction constitute only small portion of adverse drug reactions, they are often predictable and therefore avoidable or manageable. The aim of our present study was to assess the incidence and severity of DDIs in patients admitted in a tertiary care teaching hospital. A prospective, observational study was carried out for a period of 6 months (Jan–June 2013). During the study period, a total of 300 prescriptions were analyzed and was found that 242 prescriptions had DDI. The average number of drugs in each prescription was 8. Regarding the severity of clinical results, the interaction was classified as minor, moderate, major from the 242 prescriptions. The 40 major DDIs are reported from 32 prescriptions, leading to increased hospitalisation and health care cost of the patients. DDI was identified by using micromedex, Stockley's drug interaction book and other reputed journals. Many physicians were unaware of various DDIs. Hence, education, computerised prescribing system and drug information along with collaborative drug selection and pharmaceutical care are strongly encouraged for physicians and pharmacists to avoid such incidences.

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Influence of mobile phase modifier and flow-rate on retention time, peak area, HETP and tailing factor in analysis of Levocetirizine hydrochloride by HPLC

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Aim of the present study was to investigate the influence of mobile phase modifier and flow-rate in the chromatographic separation of anti-histaminic drug. Here, simplest full 3-level factorial design with 2-factors (32 design) was applied to obtain best chromatographic separation of anti-histaminic drug Levocetirizine hydrochloride. Two experimental variables selected were (i) % of acetonitrile in mobile phase ammonium acetate buffer (10 mM, pH 4.8) (mobile phase modifier) and (ii) flow rate of mobile phase. Studied chromatographic separation parameters were (i) retention time, (ii) peak area, (iii) HETP and (iv) tailing factor. These experimental variables were simultaneously varied in the region of +1, 1, and -1. Using Response Surface Methodology (RSM), quadratic mathematical models were obtained to predict the chromatographic responses upon varying the experimental variables. From RSM, best level for separation was found to be 1 and -1 for % of acetonitrile in mobile phase and flow rate, respectively. Fittingness of selected set of variables was checked by applying it to the assay of tablet formulation and by performing the validation of method as per ICH guidelines.

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Stability testing of botanicals: An exploratory study

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Background: The role of herbal as drugs, neutraceuticals and dietary supplements is gaining popularity. There have been several examples of poor quality of these products. The formulation and development of botanicals is challenging due to their complex physical and chemical properties. Stability study of herbal is important as instability modifies three important attributes of product i.e. quality, safety and efficacy. Botanicals mentioned under Ayurveda are receiving attention globally. Scientifically validated and technologically standardized botanicals are currently needed for global market. *Emblica officinalis* is mentioned under Ayurveda as a Rasayana drug and is present in many formulations. In recent years, much success has been obtained in documentation, ensuring contaminants limits, safety and standardization. However, the stability testing has not been adequately addressed. The present study was done as per WHO and ICH guidance with the following objectives.

Objectives: 1. To develop the analytical method for Gallic acid estimation using HPTLC and validation as per ICH guidelines. 2. To elucidate the physical, chemical, pharmaceutical and biological attributes of the Amla extract with respect to real and accelerated storage conditions. 3. To establish shelf life of spray dried amla extract with respect to storage conditions and retest periods.

Methodology: Mobile phase optimization: Mobile phase consisting of toluene, ethyl acetate, formic acid in the ratio of (4.0: 5.5: 0.5, v/v/v/v) was optimized and good resolution with R_f value of 0.36±0.02 for Gallic acid was obtained when densitometry scanning was performed at 277 nm.

Method Validation: The optimized method was validated as per ICH guidelines.

Results: Pharmaceutical properties were measured i.e., particle size and flow, extract showed poor free flowing properties and very moisture sensitive. It showed significant change in physical moisture content 4%-7% at real with respect to 4%-11% at accelerated forms. Significant change in form was also observed at real time (clumps) and accelerated (cake) at end of six months. In conclusion, extract when stored at real time showed significant change in physical (moisture content, form) and chemical (peak areas at R_f 0.47) and pharmaceutical (flow and compressibility) properties on 6 months storage. In accelerated conditions, these changes were seen at 1-3 months of storage. Biological stability of extract was studied using DPPH assay.

Conclusion: No significant change in activity was found at 6 months storage at room and accelerated storage. This suggests that extract retest period should be within 6 months and proper storage conditions needs to be optimized with respect to container and temperature.

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Mechanistic study of phlorofucofuroeckol A for the induction of apoptosis in human colorectal cancer cells

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Phlorofucofuroeckol A (PFF-A) as one of the phlorotannins found in the brown algae has been reported to exert anti-cancer property. However, the molecular mechanism for anti-cancer effect of PFF-A has not been known. Activating transcription factor 3 (ATF3) has been reported to be associated with apoptosis in colorectal cancer. The present study was performed to investigate the molecular mechanism by which PFF-A stimulates ATF3 expression and apoptosis in human colorectal cancer cells. PFF-A decreased the cell viability through an apoptosis in human colorectal cancer cells. PFF-A increased ATF3 expression through regulating transcriptional activity. The responsible cis-element for ATF3 transcriptional activation by PFF-A was CREB located between -147 to -85 of ATF3 promoter. Inhibitions of p38, JNK, GSK3β and IκK-α blocked PFF-A-mediated ATF3 expression. ATF3 knockdown by ATF3 siRNA attenuated the cleavage of PARP by PFF-A, while ATF3 overexpression increased PFF-A-mediated cleaved PARP. These results suggest that PFF-A may exert anti-cancer property through inducing apoptosis through ATF3-mediated pathway in human colorectal cancer cells.

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Grape seed extract nanosuspension: A green chemotherapy against colon cancer cells

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Colorectal cancer is the third leading cause of cancer death in both men and women. Medicinal plants are mainly used for traditional Indian medicine and act as dietary agents for the treatment of various diseases including cancer. The poor water soluble drugs/herbal drugs have always been a challenging problem faced by pharmaceutical scientists to make pharmaceutical formulations. The objective of the study was to develop grape seed nanosuspension (GSNS) and investigate its *in vitro* anti-cancer activity against colon cancer cell line (HCT- 116). Grape seed nanosuspension was prepared by nano-precipitation method. Characterization of GSNS was performed using Zetal potential analysis and particle size termination by SEM analysis. GSNS was subjected to solubility study as well as stability study. Further, GSNS was subjected to *in vitro* anti-cancer activity against colon cancer cell line. The study results indicated that the prepared GSNS by nano-precipitation include several advantages, such as suitable for poor water soluble drugs/herbal drugs and suitable for large scale production. Particle size of GSNS showed range from ~ 140 to 201 nm. Zeta potential value of formulated nanosuspension was obtained as 3.42 mv. Solubility study indicated that formulated GSNS enhanced the solubility of the grape seed extract. Stability study showed GSNS was stable in room temperature and cold temperature. GSNS exhibited significant anticancer activity against HCT- 116 cell line at dose dependent manner. The study concluded that nano-precipitation method could be suitable to improve the solubility of grape seed extract and GSNS exhibited effective anti-cancer activity against colon cancer cell line.

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Erlotinib nanosuspension: *In vitro* anti-cancer study using colon cancer cell line

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Erlotinib is used for the treatment of lung and pancreatic cancer. The clinical usage of erlotinib is limited due to its poor solubility and dissolution rate. The objective of the research work is to formulate erlotinib nanosuspension with improved solubility and dissolution rate and investigation of its anticancer activity against colon cancer cell line (HCT 116). Erlotinib nanosuspension (ENS) was prepared by high pressure homogenization method. Characterization of ENS was performed using zeta potential analysis and particle size termination by SEM analysis. ENS was subjected to solubility study as well as stability study. Further, ENS was subjected to *in vitro* anti-cancer activity against colon cancer cell line. The study results indicated that the prepared ENS by high pressure homogenization method include several advantages, such as suitable for poorly water soluble drugs/ herbal drugs and suitable for large scale production. Particle size of ENS showed range from ~5 to 26 nm. Zeta potential value of formulated nanosuspension was obtained as 3.16 mv. Solubility study indicated that formulated ENS enhanced the solubility of the erlotinib. Stability study showed ENS was stable in room temperature and cold temperature. ENS exhibited significant anti-cancer activity against HCT-116 Cell line at dose dependent manner. The study concluded that high pressure homogenization method could be suitable to improve the solubility of erlotinib and ENS exhibited effective anti-cancer activity against colon cancer cell line.

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A retrospective study on the pharmacoeconomic impact of pharmacists' interventions in a tertiary hospital

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Background: Clinical pharmacy, a fast-emerging trend in healthcare, is concerned with promoting rational medication use by providing a variation of patient care that seeks to promote health, wellness and disease prevention. In the Philippines, the role of the clinical pharmacist is not fully recognized with only a handful of institutions that implement the use of this particular resource.

Objectives: This study aims to provide insight on the important role of pharmacists in medication safety by assessing the frequently occurring medication errors intercepted and identified by the pharmacists in a selected tertiary hospital and gauging the monetary equivalence of pharmacists' interventions.

Methods: The Medication Safety Through Interventions (MSTI) forms of the hospital were used to gather data on commonly occurring medication errors and the specific interventions done by pharmacists within the hospital. Qualified interventions were selected for the study for the estimation of cost savings and cost avoidance, using the formula developed by Nesbit *et al.* (1990).

Results: 1,068 pharmacy interventions have been reported from January to February 2016. These interventions were documented as errors of commission (23.03%), errors of omission (13.30%), and drug therapy monitoring (63.67%). 201 of the reported interventions have demonstrated a reduction in patients' overall drug therapy cost. Direct cost savings and cost avoidance of the interventions made by pharmacists amounted to 4,571,661.62 PHP (96,245.51 USD) and 75,004.37 PHP (1,579.04 USD), respectively. In total, cost saving and cost avoidance amounted to 4,646,665.98 PHP (97,824.55 USD).

Conclusion: The results indicate that medication errors have a direct impact on a patient's overall healthcare cost and that pharmacy interventions have had an overall positive pharmacoeconomic impact in the hospital setting.

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Astashine capsules: World's most powerful antioxidant on earth

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Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection. Based on these facts a Super Antioxidant ASTASHINE Capsules has been developed by R&D Centre, Lactonova Nutripharm (P) Ltd, Hyderabad. The present paper Reviews the Role of Astashine capsules in maintaining optimum health and wellbeing.

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Ciprofloxacin for the treatment of non-resolving pneumonia in a tertiary care pediatric hospital

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Purpose: Data regarding the use of ciprofloxacin in children with non-resolving pneumonia are scarce. The present study aims to evaluate the effect of ciprofloxacin therapy in pediatric patients with non-resolving pneumonia.

Methods: Over the past year, all pediatric patients with non-resolving pneumonia who received ciprofloxacin treatment in the pulmonary unit of Al-Rantisy specialized pediatric hospital in Gaza, Palestine, were included in this retrospective study. Ciprofloxacin was given for all patients in a dose of 20 mg/kg/day divided into two doses. Patient demographic data, clinical symptoms recorded, sputum culture findings and ciprofloxacin therapeutic outcome were gathered. Data was analyzed using computer software SPSS version 11.

Results: The study included 57 patients with non-resolving pneumonia, 36 males and 21 females with mean age of 3.4 years, ranged from 2 month to 8 years. Fever (73.7%) and cough (89.5%) were the most common symptoms. Positive culture was obtained in 42 (73.6%) patients while 15 (26.4%) showed negative results. The most common organism isolated in the positive cultures was *Pseudomonas aeruginosa* 26 (62.0%). Among the study sample, 23 (40.4%) patients received ciprofloxacin as empirical therapy and 34 (59.6%) received this drug depending on culture sensitivity results. There was a significant decrease in body temperature levels ($P<0.001$) at day 1, 2 and 3 of ciprofloxacin treatment. Overall, ciprofloxacin was effective in the treatment of 53 (93.0%) patients of the present study. Only 4 (7%) cases showed resistant to this therapy. The mean length of hospital stay was 7.5 days. No side effects were reported during the course of this study.

Conclusion: Data of the present study suggest that ciprofloxacin is effective and safe, including as initial monotherapy, for the treatment of pediatric patients with non-resolving pneumonia.

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Investigation into hepatoprotective and antioxidant potentials of *Epilobium hirsutum* on iron dextran induced hepatotoxicity in Sprague Dawley rat

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Present study deals with investigation of hepatoprotective and antioxidant potentials of *E. hirsutum* in iron overloaded rats. The hepatotoxicity was induced by administering six IP injections of iron dextran (12.5 mg/100 g) uniformly distributed over a period of 30 days. Different fractions of *E. hirsutum* were given orally whereas Deferoxamine (DFO) was given subcutaneously for 30 days. The various biochemical parameters were estimated on 15th and 30th days of treatment whereas antioxidant parameters were estimated on 30th day of treatment. The methanolic fraction of methanolic extract (MFME) and methanolic fraction of aqueous extract (MFAE) of *E. hirsutum* significantly ($P<0.01$) decreases Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH) whereas significantly ($P<0.01$) increases Malondialdehyde (MDA) as compared to the disease control (DC) rats. There were significant ($P<0.01$) hepatoprotective effects shown by MFME and MFAE of *E. hirsutum*. Hence present study concluded that MFME and MFAE of *E. hirsutum* have hepatoprotective and antioxidant effect. The possible mechanism of action as hepatoprotective may be due to its antioxidant potential by scavenging free radicals through iron chelation.

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Identification of potential inhibitors for Ebola virus: An *in silico* approach

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Ebola virus (EBOV) is a fatal virus that causes severe hemorrhagic fever in human and animals. However, there is currently no FDA-approved drug for treating Ebola virus infection. Identification of potential inhibitors for Ebola virus has gained much attention of medicinal chemists in the last few years. Although few lead compounds were identified, the drug discovery for Ebola virus is significantly more challenging. In this study, *in silico* approaches were applied to explore potential inhibitors for EBOV infection. Initally, four protein targets for EBOV were identified through their important roles in viral pathogenesis and disease, namely VP24 (PDB id: 4MOQ), VP30 (PDB id: 5DVW), VP35 (PDB id: 3FKE), và VP40 (PDB id: 1H2C), respectively. The ligands were taken from some drugs which are in clinical testings from other anti-viruses potential compounds. Through blind dockings and focused dockings, the potential inhibitors and binding sites were discovered for different protein targets of EBOV. The docking results of the trial drugs are consistent with the experiment data. In the group of other potential compounds, there were some ligands which had abilities to well-bind with Ebola proteins such as Silybin (-9.5 kJ.mol-1), Harringtonine (-8.0 kJ.mol-1), Homoharringtonine (-8.3 kJ.mol-1), Chat 5 (-8.2 kJ.mol-1). Among these ligands, after screening through Lipinski 5 rules, Silybin was the only suitable one which could be used as lead compounds for EBOV drug discovery. This study provided helpful information to considerably assist in drug discovery of antiviral agents for Ebola virus.

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Indispensible biological processes offer exceptional cellular and molecular windows for pharmacological interventions in protozoan parasites

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Responses to stressful environmental conditions, endoplasmic reticulum stress, reactive species (e.g. free radicals, reactive oxygen and nitrogen species) and apicomplexan parasite sequesteration are crucial biological processes that deserve extensive review at a formative time for the development of our knowledge concerning the state-of-the-art data on their cellular and molecular mechanisms of action and the ways in which they can be manipulated in and by protozoan parasites. We have attempted to provide a comprehensive overview to reveal intervention points that could be exploited to discover novel therapies, vaccine strategies and prophylactic intervention points for broad-spectrum host-oriented inherent measures and eukaryotic parasite counter-measures, and to understand the parasitic disease progression and the infection consequence.

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Solubility enhancement of Furosemide and its fabrication into dosage form

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Bioavailability is defined as the rate and extent of the drug concentration in the systemic circulation after oral administration. The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. Furosemide is a class IV drug. Present experimental work was aimed to prepare optimized, stable solid self emulsifying drug delivery system containing Furosemide. The combination of the various solubilizers and hydrophilic surfactants like Poloxamer 188, Polysorbate 80 and medium chain triglycrides were used in the present study. PEG-40 Hydrogenated castor oil was used as solvent cum cosurfactant on the basis of solubility of Furosemide. The formulations were so designed that they form nano dispersion in contact with water or GI fluids which increases the permeability through GI membrane. All the prototype formulation tested for *in vitro* dissolution formed nano emulsion in 15 minutes. Trend of drug dissolution of prototype A and B remain constant or increase marginally as the time increases, dissolution rate of drug remains constant or increases marginally until 60 minutes in case of prototypes A and B. This indicates that upon contact with dissolution media, formulations A1 to A3 and B1 to B3 form emulsions which have poor thermodynamic stability and eventually drug particle size in dispersion increases. This was not observed in the case of the prototype C3 formulation where the drug dissolution enhances with time indicating good thermodynamic stability of nanoemulsion produced on contact with aqueous fluids. Thus prototype C3 is optimized formulation and this optimized batch was evaluated for average weight of tablet, hardness, friability, disintegration time, dissolution and stability study was carried out.

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Synthesis, characterization and pharmacological evaluation of hybrid urea/thiourea derivative as a potential antidiabetic activity

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The prevalence of diabetes is rising all over the world due to population growth, aging, urbanization and an increase in obesity and physical inactivity. Type 2 *Diabetes mellitus* (T2DM) presents a major challenge to healthcare system around the world. Urea and thiourea derivatives possess many promising biological activities. Here, urea/thiourea derivatives have been synthesized and screened for the antidiabetic activity. This study involves the synthesis of a series of hybrid urea/thiourea derivatives (5a-5h) containing chalcone moiety. The synthesized compounds were characterized by FT-IR, 1H NMR, mass spectroscopy and evaluated for their both *in vitro* and *in vivo* antidiabetic activity. The *in vitro* antidiabetic activity was done by α-glucosidase inhibitory activity of synthesized compounds. Acute toxicity study of the synthesized compounds was conducted by OECD guidelines, from which dose levels were calculated. The *in vivo* antidiabetic activity was performed on streptozotocin induced diabetic Swiss albino rats. The blood glucose level, different enzymatic studies (SGPT, SGOT, ALT) and lipid profile (HDL, LDL, Cholesterol) of the studied animal were estimated. The results indicated that the hybrid urea/thiourea derivatives displayed promising antidiabetic activity. Among the series, compound 5a showed potent α-glucosidase inhibitory activity when compared to the standard drug Acarbose. In *in vivo* study, the compound 5a was found more effective when compared to the standard drug Metformin. It may be concluded that hybrid urea/thiourea derivatives will be a new class of antidiabetic compound in future.

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Evaluation of film-forming potential and drug release profile of a *Lepidium sativum* Linn. gum

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Films were prepared using mucilage of gum of *Lepidium sativum* with different proportions of plasticizers. The films were casted on glass plates and dried under controlled evaporation. Films prepared with 0.15, 0.2 part of PEG 400; 0.15 part of glycerin and propylene glycol showed satisfactory drying after 24 h. They were evaluated for following parameters water uptake, tensile strength, folding endurance, and water vapor transmission rate. Microspheres were coated by using gum and Diclofenac sodium as model drug. *In vitro* drug release parameter was checked by dissolution apparatus using solution pH 7.2. From the physical parameters and drug release profile it is found that gum is having capacity to retain drug up to 12 hrs so it can successfully be employed as once a day oral controlled release drug delivery system.

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