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ICPPS 2015

International Conference on Pharmacology and Pharmaceutical Sciences

17-18 November 2015, Vienna, Austria

ICPPS 2015

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Identification of novel inhibitors of *Plasmodium falciparum* Heat Shock Protein 90 (HSP90) for the treatment of malaria

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In 2010, an estimated 3.3 billion people were at risk of malaria. There were about 219 million malaria cases and an estimated 660 000 malaria deaths. Malaria caused by *Plasmodium falciparum* is the most deadly of the five species. Parasite resistance is increasing and thus there is a critical need for identification of drug targets for anti- malarial agents. In this study, Heat shock protein 90 (HSP90) was investigated as drug target for the treatment of malaria. HSP90 has many functions in the parasite cells including; protein folding, gene expression regulation and signal transduction. Compounds were selected from a virtual high-throughput docking study. The top 20 compounds and the worst 5 compounds for Heat Shock Protein 90 (HSP90) were tested to assess their effect in a thermal melt assay. Thermal shift assay was used in this study to identify the effect of these compounds on the protein stability by measuring the variability of melting temperature. 11 compounds out of the top 20 for HSP90 shifted the melting temperature of the protein by greater than 10 degrees. These effects are very significant and confirmed that those compounds are binding to HSP90. Some of these compounds are similar in structure containing different hetero-aromatic rings such as benzidmidazole ring, pyrazole ring and indole ring. These results are promising and more investigation is needed to understand and develop these compounds.

Terbinafine hydrochloride loaded nanovesiclar systems for trans-ungual delivery: *In-vitro* characterization and *Ex-vivo* evaluation

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Abstract

Onychomycosis is a fungal infection that affects the nail apparatus. Treating a nail infection like onychomycosis is challenging, as the human nail plate acts as a formidable barrier against all drug permeation. Available oral and topical treatments have several setbacks. Terbinafine hydrochloride (TBH), belonging to the allylamine class, is a broad spectrum antifungal that is mainly used for treatment of onychomycosis. Colloidal carriers have been investigated as drug delivery systems for the past 30 years in order to achieve specific drug targeting, facilitate the drug transfer through biological membranes, improve bioavailability, control release characteristics, and reduce or prevent side effects. This study aims to formulate TBH in a nanobased spanlastic vesicular carrier that enables and enhances the drug delivery through the nail. The nanovesicles were formulated by ethanol injection method, using either Span 60 or Span 65, together with Tween 80 or Sodium deoxycholate as an edge activator. A full factorial design was implemented to study the effect of different formulation and process variables on the prepared TBH-loaded spanlastic nanovesicles. TBH entrapment efficiency percentages, particle size diameter, percentage drug released after 2 hrs and 8 hrs were selected as dependent variables. Optimization was performed using Design-Expert® software to obtain an optimized formulation with high entrapment efficiency (62.35±8.91%), average particle size of 438.45±70.5 nm, and 29.57±0.93% and 59.53±1.73% TBH released after 2 and 8 hrs, respectively. The optimized formula was further evaluated using differential scanning calorimetry and X-Ray diffraction after lyophilization and was also morphologically examined using transmission electron microscopy. An *ex-vivo* study was conducted to determine the permeation and retainment of the optimized formulation in a human cadaver nail plate and confocal laser scanning microscope was used to show the extent of formulation permeation. In conclusion, the results confirmed that spanlastics exhibit promising results for the trans-ungual delivery of Terbinafine Hydrochloride.

Enantiospecific interactions of selected calcium channel antagonists with human cytochrome P450 CYP3A4 *in vitro*

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Amlodipine, benidipine, felodipine and isradipine, members of a class of dihydropyridine calcium channel antagonists (CCBs), are widely prescribed drugs for the treatment of hypertension and ischemic heart disease. The key of their effect is their inhibition of entry of calcium ions via a subset of channels, thereby leading to impairment of contraction. All these chemical entities have an asymmetric carbon atom in their structures and they are used as racemic mixtures. In the most cases, calcium channel blockers are coadministered with other drugs. Therefore, the interactions between them and other drugs should be well explained. Single enantiomers may act differently in the body as a consequence of stereoselectivity of the interactions with three dimensional structures of proteins acting as enzymes, receptors or ion channels.

These dihydropyridine calcium channel antagonists, which are mainly metabolized by CYP3A4, have been already described as possible inhibitors of human liver microsomal drug-metabolizing enzymes, cytochromes P450 (CYP). The aim of this study was to examine the difference in the inhibitory potency of individual enantiomers of selected CCBs towards catalytic activity of individual cytochromes P450, especially of the CYP3A4 enzyme. The 6β-hydroxylation of testosterone, and midazolam 1´-hydroxylation, specific substrates for CYP 3A4, were used to evaluate the influence of (+)- form and (-)- form of individual drugs on the CYP 3A4 enzyme activity in human liver microsomes *in vitro*.

There are in the literature data indicating the interactions of amlodipine, benidipine and felodipine with the CYP3A4 enzyme suggesting also the possibility of drug interactions *in vivo* [1, 2]. In the study presented here, the enantiospecific manner of the inhibitory effect of dilution series of selected CCBs enantiomers on the CYP3A4 activity is presented.

The K_i values and IC_{50}s have been calculated to evaluate the differences in the inhibition of CYP by individual enantiomers.

Financial support from Czech Science Agency GACR 13-01809S project and IGA UPOL_LF_2015_004 is acknowledged.

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THE INFLUENCE OF OXIDATIVE STRESS ON STATINS BINDING TO ALBUMIN COMPLEXED WITH FATTY ACIDS.

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Albumin is the most important serum binding protein. The main function of albumin is binding and transport of endo- and exogenous compounds such as hormones, bilirubin, metal ions, drugs. Albumin acts as main fatty acid (FA) binding protein in extracellular fluids. Simvastatine (SIM) is highly bound (approximately 95%) to human plasma proteins. SIM is a statin used for the treatment of hypercholesterolemia and for the reduction in the risk of cardiac heart disease mortality and cardiovascular events. Hypercholesterolemia induces oxidative stress and thereby leads to cardiac dysfunction in the heart. Oxidative stress reflects an imbalance between the systemic manifestation of Reactive Oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. ROS result in oxidation of serum albumin, which causes a number of structural changes in the spatial structure. This phenomenon may influence the binding and cause significant drug interactions. During the oxidation, modification of amino acid residues may occur.
The aim of the study was to investigate the influence of oxidative stress on human serum albumin (dHSA, HSA) structure and evaluate disorders in the binding of simvastatine to oxidized human serum albumin (odHSA, oHSA), in the absence and presence of fatty acids, respectively. Changes in albumin structure were examined by comparison of modified (odHSA, oHSA) and nonmodified human serum albumin (dHSA, HSA) absorption spectra, emission spectra, second derivative spectra, red-edge shift (REES) and synchronic spectroscopy. Studies of absorption, emission and second derivative spectra indicated changes in the environment of Trp, Tyr and Phe residues of albumin. Also synchronic fluorescence spectroscopy technique confirmed changes of position of tryptophanyl and tyrosyl residues fluorescent band caused by oxidative stress. Moreover analysis of REES effect allowed to observe structural changes in the region of the hydrophobic pocket containing the tryptophanyl residue.
Effect of oxidative stress on binding of simvastatine (SIM) to defatted and fatted protein was investigated by spectrofluorescence spectroscopy. SIM caused the fluorescence quenching of modified (odHSA, oHSA) and nonmodified (dHSA, HSA) albumin molecule. The values of association constants and a number of binding site on the protein molecule in the high affinity binding site confirmed highly bound of SIM to albumin, influence of oxidative stress on SIM-protein binding and competition between fatty acid and SIM for a binding sites in human protein.

Acknowledgements
This work was supported by the grants of Medical University of Silesia: KNW-1-034/K/5/0.

Antileukemic Activity and Cellular Internalization of Curcumin Thermosensitive Nanodelivery

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Abstract—Curcumin, a yellow bioactive compound, is present in many kinds of herbs, especially in *Curcuma longa* L. (turmeric). It has been reported that curcumin has cytotoxic effects on cancer cells through several mechanisms such as inhibition of cell proliferation, induction of apoptosis and disturbing different signaling pathways, but the obstacle as a cancer therapeutic agent is the low aqueous solubility leading to the low bioavailability of curcumin. To overcome this limitation, thermosensitive block copolymer of poly(ethylene glycol)-b-(*N*-(2-hydroxypropyl) methacrylamide dilactate) (PEG-HPMA-DL) was used to enhance solubility and delivery of curcumin. Taking advantage of the thermosensitivity of PEG-HPMA-DL, the polymeric micelles were fabricated to obtain curcumin thermosensitive nanodelivery (CTN). The characteristics of CTN including loading efficiency and loading capacity using UV-spectrophotometry, size and size distribution using particle size analysis were studied. Moreover, the cytotoxicity against K562 leukemic cells by MTT assay, cellular internalization under a fluorescence microscope, cell cycle arrest by flow cytometry analysis and Wilms' tumor 1 (WT1) protein expression (the leukemic biomarker) by Western blotting were performed to investigate the biological activity. The results demonstrated that CTN substantially enhanced curcumin's solubility. CTN showed high loading efficiency and loading capacity. The average size of CTN was 62 ± 1 nm with the narrow size distribution. CTN revealed good cytotoxicity against K562 leukemic cells with the 50% inhibition concentration (IC_{50}) value of 32.2 ± 2.5 μM. The cytotoxicity was due to the cellular internalization of CTN which was confirmed by the strong fluorescence intensity after 4 h. In addition, CTN arrested in G_2/M phase of the cell cycle progression and also inhibited WT1 protein expression. These findings were indicated that CTN can effectively inhibit the proliferation in leukemogenesis. In conclusion, this present study suggested that CTN is a promising nanocarrier for solubilization and delivery of curcumin and provides the great opportunity for cancer clinical applications.

A Prospective Randomized, Double-Blind, Double-Dummy, Placebo-Controlled Trial of Solid Lipid Boswellia serrata Particles (SLBSP) Versus Standardized Boswellia Serrata Gum Resin Extract (BSE) For Symptomatic Treatment of Osteoarthritis of Knee.

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Introduction: Osteoarthritis of the knee (OA knee) is a chronic, progressive, skeletal, degenerative disorder often associated with restricted mobility and poor quality of life. NSAIDs are often employed in OA for symptomatic treatment of pain. Boswellic acids (BA) have anti-inflammatory properties and are traditionally used for the treatment of OA knee. However, pharmacokinetic studies have shown low bioavailability of BA including 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA). Phospholipid complexation enhances the bioavailability of BA, which led to the development of Solid Lipid Boswellia serrata Particles (SLBSP). In the present study we investigated the efficacy of SLBSP versus standardized Boswellia serrata gum resin extract (BSE) for symptomatic treatment of OA knee.

Methods: It was a prospective, randomized, double-blind, double-dummy, placebo-controlled, single-center clinical trial in patients with symptomatic OA knee. Subjects were randomized to receive either SLBSP capsules or BSE tablets using a computer generated random sequence. Allocation concealment was achieved with the help of sealed envelopes to eliminate selection bias. Matching placebos resembling SLBCP capsules and BSE tablets were formulated and each patient received an active drug and a placebo (i.e., SLBSP capsule+BSE Placebo or BSE tablet+SLBSP placebo). Each tablet of BSE contained 333 mg of standardized BSE gum comprising 40% Boswellic acids (BA) whereas each SLBSP capsule contained 333mg of the formula equivalent to 100 mg of 40% Boswellic acids Treatment was continued for two months. Patients were allowed to take rescue analgesics (Acelofenac 100mg) as and when required. Improvement in pain and function was assessed with the help of Western Ontario and McMaster Universities OA index (WOMAC), Visual Analog Scale (VAS) and need for rescue analgesics at one month and two months. The outcomes were compared between the two groups using ANOVA.

Results: Twenty patients were enrolled in each arm. Both treatments resulted in marked improvement in pain and function scores compared to baseline. WOMAC score improved by 18.2% and 15.4% at 1 month and two months respectively in the SLBSP arm ($p<0.05$) whereas the corresponding figures for BSE was 18.8% and 23.1% ($p<0.05$) respectively. Similar change was observed in VAS score i.e., 19% and 26% improvement respectively ($p<0.05$) during the same period for BSBSP and 18.2% and 20.4% improvement respectively ($p<0.05$) in the BSE arm. The difference in VAS and WOMAC scores between the two arms was not statistically significant. However, the most significant effect was observed in the need for rescue analgesics which reduced markedly by 67% during the first month and 76% in the second month in SLBSP arm whereas it was 65% and 34% respectively in the BSE arm compared to baseline ($p<0.01$). SLBSP resulted in markedly lower dependence on rescue analgesics compared to BSE at the end of 2 months ($p<0.05$). No adverse effects were observed due to treatment. Compliance to treatment was greater than 80% in all patients in both arms.

Conclusion: Both SLBSP and BSE caused marked improvement in pain and function scores in patients of OA knee but SLBSP was superior to BSE in reducing the need for rescue analgesics.

Clinical Pharmacokinetics Of 3-hour Extended Infusion Of Meropenem In Adult Critically Ill Cancer Patients: Implications For Targeting Susceptible And Intermediate Strains Of Gram Negative Bacteria

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Introduction: Meropenem is an ultra-broad-spectrum injectable carbepenam indicated for for empirical treatment of severe sepsis due to gram negative bacilli (GNB). It is a time-dependent antibiotic, whose antibacterial activity is related to the time for which the free concentration of the drug is maintained above the minimum inhibitory concentration (MIC) during a dosing interval (fT >MIC). The fT >MIC required for optimal bactericidal activity of carbapenems has been reported to be at least 40% (fT >MIC>40) as per in vitro and in vivo data from animal models. This study was designed to determine whether extended infusion regimen of Meropenem (1g over 3hrs administered 8 hourly) achieves plasma concentratioon greater than MIC within 1 hour of starting infusion for intermediate strains of Enterobacteriaceae GNBs (MIC=2µg/mL) and sensitive strains of Non-Lactose fermenting bacteria including Acinetobacter and Pseudomonas (MIC=4µg/mL) and if the fT>MIC>40 is achieved.

Methodology: Twenty five eligible patients receiving Meropenem as a 3 hour infusion were enrolled from the ICU of a tertiary cancer hospital in Western India . Blood samples were collected in EDTA tubes from an arterial line at the following time points: predose, 5, 15, 30, 60, 90, 120,180,240, 300,360 and 480 min after the first dose. The same set of 12 samples was repeated on day 3 (7th dose) of the regimen. Plasma Meropenem concentration was determined using a validated reverse phase HPLC assay. Modelling and simulation of the PK data was done using Phoenix® WinNonlin®, Certara, USA.

Results: Of the 25 patients enrolled, 24 pharmacokinetic (PK) sample sets of day 1 and 23 PK sample sets of day 3 were available for the final analysis. One patient was excluded from

analysis on day 1 because the PK samples were severely hemolysed and not suitable for bioanalysis, while one patient expired before the day 3 samples could be collected. We found that extended infusion of Meropenem consistently achieved one hour target (first dose) and fT>MIC>40 of 2 µg/mL on both Day 1 and 3. However it failed to achieve fT>MIC>40 in 8 out of 24 patients for target MIC of 4 µg/mL to cover against sensitive strains of Non-Lactose fermenting bacteria. The pharmacokinetic data on day 1 fitted one compartment model in 20 out of 24 patients. Simulation of individual patient data at various doses showed that a dose of 1.5g administered as 3 hr infusion 8 hourly achieved fT>4ug/mL>40 in all but one patient. This patient couldn't achieve fT>4ug/mL>40 even at a dose of 2g (as per simulation), and therefore could be considered as an outlier.

Conclusion: This study clearly demonstrated that the present practice of 1g TID as 3 hour infusion is adequate to treat susceptible and intermediate strains of Enterobacteriaceae but not for susceptible strains of Acinetobacter and Pseudomonas. Simulation established that a dose of 1.5g would be needed to achieve fT>MIC> 40% for these strains, which should be confirmed in a prospective study.

Theoretical Study on the Geometrical and physico chemical Properties of Paclitaxel Conjugated to Nanoparticle Chitosan Biopolymer Along with ethylene glycol chains

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Abstract

During the recent years, computational chemistry has been very ardent about drug release and delivery. Paclitaxel (PTX) is a well-known anti-cancer agent. The cytotoxicity of paclitaxel can be minimized by linking it to an affinity succinate linkage is used to improve the interaction between an anti-cancer agent, paclitaxel and a chitosan biopolymer. This chitosan sheet could be used as drug carrier for controlled release [1,2].
Low molecular weight chitosan nanoparticles (LMWC) is one of the best carriers. These carriers bind to the drug succinate linker connected and form a stable complex.
It is possible to use these nanoparticles to reduce toxicity and increase its solubility. The loop connecting poly ethylene glycol (PEG) can prolong his time in the blood circulation of the drug. In this report, the Molecular Structure, Dipole Moment (DM) and some physicochemical properties, some geometrical parameters, such as bond length, bond angle and energy structures of paclitaxel, chitosan and paclitaxel conjugated to nanoparticle chitosan were investigated using the Hartree Fock (HF) calculations. The computational method used was HF/6-31g**.
Keywords: HF calculations, Paclitaxel, LMWC-PTX ,geometrical parameter, PEG

Effect of Novel Bisnaphthalimidopropyl derivatives (BNIPs) designed for targeting DNA in a human breast cancer cell system.

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Bisnaphthalimidopropyl (BNIP) derivatives are a family of compounds that were initially synthesised with natural polyamines incorporated into their linker chain and were found to exert anti-cancer activities[1, 2]. According to previous studies[3, 4], variations in the linker sequence seem to improve the aqueous solubility and cytotoxic activity of BNIP derivatives, enhancing their potential application as anticancer drugs. The aim of this project was to synthesise and characterise three novel BNIP derivatives, bisnaphthalimidopropyl-piperidylpropane (BNIPPiProp), bisnaphthalimidopropyl- ethylenedipiperidine dihydrobromide (BNIPPiEth-HBr) and (*trans(trans)*)-4,4'-methylenebis-cyclohexylamine *(trans,trans*-BNIPDaCHM). ^{1}H-NMR, ^{13}C-NMR, MS and melting point determination confirmed the structural identity of derivative. The cytotoxicity of novel BNIP derivatives was assessed against human breast cancer MDA-MB-231 cells by MTT assay[5]. In parallel, DNA binding studies investigated whether the BNIP derivatives can successfully target DNA. Fluorescence-binding experiments and UV binding studies were used to determine their DNA binding properties[6]. Propidium Iodide (PI) flow cytometry was conducted in order to evaluate the cellular DNA content in breast cancer cells and assess the induction of apoptotic cell death by BNIP derivatives. All the derivatives exhibited strong cytotoxic activity against MDA-MB-231 cells, with IC_{50} values ranging between 1.4-2.3 μM after 24 hours treatment. Competitive ethidium bromide displacement experiments revealed that BNIPPiProp, BNIPPiEth-HBr and *trans,trans*-BNIPDaCHM competitively displace EtBr from DNA, with C_{50} values in a range of 1.1-5.6 μM. Corresponding values for K binding constants varied from 3.25 x 10^4 to 12.23 x 10^4, according to UV spectroscopy studies. Furthermore,

cell cycle distribution of MDA-MB-231 cells after cell synchronisation indicated that *trans,trans*-BNIPDaCHM induces a sub-G1 cell cycle arrest which is associated with apoptotic cell death. In particular, an increase of 139.3% and 142.2% in sub-G1 cell population after 24 hours treatment with 1μM trans,trans-BNIPDaCHM and 6μM Camptothecin (a known control for sub-G1 arrest), respectively was observed compared to untreated cells. In conclusion, the above findings signify that novel BNIP derivatives exhibit strong cytotoxic and DNA binding properties *in vitro* and further investigation in mode of cell death, in relation to apoptosis, would be beneficial for their development as potential anticancer agents.

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The toxicity of gold nanoparticles

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Introduction

In recent years the use of gold nanoparticles due to favorable physical characteristics (plasmonic properties), chemical (synthetic, functionalize and easy biofunctionalize) and biological (compatibility in the body), are increasing in various fields of medicine.

Methods

This article examines the toxicity of nanoparticles in the body and is based on the books and papers.

Findings

Several factors contribute to the toxicity of gold nanoparticles as the dosage, individual characteristics, the pharmacokinetics gold nanoparticles synthesis, size, shape, chemical composition and surface properties.

Discussion and Conclusions

Gold nanoparticles with different mechanisms induce toxicity, which are briefly described; ROS, this factor can also be useful in the treatment and toxicity is involved. Inflammatory cytokines also play an important role in the toxicity of gold nanoparticles. DNA on gold nanoparticles also affects the interaction between them but it is not well understood; nanoparticle core covered with Login of the damage to DNA. On the other mechanisms of toxicity and oxidative reactions can affect many different ways, such as membrane, causing cell death and eventually leads to toxicity in the host. Apoptosis, or programmed cell death of the other factors that cause the toxicity of gold nanoparticles through it. Nanoparticles may also interact with proteins and enzymes in performance are affected and cause toxicity.

Antioxidant Enhancing and Cytotoxicity of Curcumin to Normal cells

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***Abstract*—**Curcumin, a well-known natural antioxidant which can be found in many plants especially in turmeric rhizomes, has been investigated in pharmacological researches for recent years. It demonstrates various health benefits ranging from antioxidant, anti-inflammatory and anticancer properties. Curcumin can inhibit free radicals from mediating lipid peroxidation of membranes or oxidative DNA damage which are the important initiator for many chronic diseases. However, there was no data on activity comparison and the biological interactions of curcumin with other natural antioxidants. The objective of this present study was to explore the antioxidant power of cucumin compared to the three potential natural antioxidants; gallic acid, ascorbic acid, and xanthone on free radical scavenging activity. The enhancing effects on antioxidant activity of curcumin in combination with these antioxidants are also explored. Moreover, cytotoxicity of curcumin towards normal red blood cells (RBCs) and normal peripheral blood mononuclear cells (PBMCs) was also investigated. The results showed that the antioxidant activity of cucumin and the other three natural antioxidants increased as the dose dependent manner. The 50% effective concentration (EC_{50}) of curcumin was 30 μM. Curcumin showed significantly higher antioxidant activity than ascorbic acid and xanthone but less than gallic acid. Interestingly, enhancement of antioxidant activity of curcumin was significantly demonstrated as the synergistic effect when combined with gallic acid, whereas the antagonistic effects were shown in its combination with ascorbic acid or xanthone. The toxicity study on RBCs revealed low value of 4.0 ± 0.8 hemolysis which was below the 5% of the critical safe hemolytic ratio according to ISO/TR 7406. Curcumin also showed a good cytocompatibility on PBMCs even at the high concentration. These findings can be concluded that curcumin is a potential antioxidant and has a good safety profile with normal cells. Its antioxidant activity can be synergistically enhanced by combination with gallic acid.

Antileukemic Activity and Cellular Internalization of Curcumin Thermosensitive Nanodelivery

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***Abstract*—**Curcumin, a yellow bioactive compound, is present in many kinds of herbs, especially in *Curcuma longa* L. (turmeric). It has been reported that curcumin has cytotoxic effects on cancer cells through several mechanisms such as inhibition of cell proliferation, induction of apoptosis and disturbing different signaling pathways, but the obstacle as a cancer therapeutic agent is the low aqueous solubility leading to the low bioavailability of curcumin. To overcome this limitation, thermosensitive block copolymer of poly(ethylene glycol)-b-(*N*-(2-hydroxypropyl) methacrylamide dilactate) (PEG-HPMA-DL) was used to enhance solubility and delivery of curcumin. Taking advantage of the thermosensitivity of PEG-HPMA-DL, the polymeric micelles were fabricated to obtain curcumin thermosensitive nanodelivery (CTN). The characteristics of CTN including loading efficiency and loading capacity using UV-spectrophotometry, size and size distribution using particle size analysis were studied. Moreover, the cytotoxicity against K562 leukemic cells by MTT assay, cellular internalization under a fluorescence microscope, cell cycle arrest by flow cytometry analysis and Wilms' tumor 1 (WT1) protein expression (the leukemic biomarker) by Western blotting were performed to investigate the biological activity. The results demonstrated that CTN substantially enhanced curcumin's solubility. CTN showed high loading efficiency and loading capacity. The average size of CTN was 62 ± 1 nm with the narrow size distribution. CTN revealed good cytotoxicity against K562 leukemic cells with the 50% inhibition concentration (IC_{50}) value of 32.2 ± 2.5 μM. The cytotoxicity was due to the cellular internalization of CTN which was confirmed by the strong fluorescence intensity after 4 h. In addition, CTN arrested in G_2/M phase of the cell cycle progression and also inhibited WT1 protein expression. These findings were indicated that CTN can effectively inhibit the proliferation in leukemogenesis. In conclusion, this present study suggested that CTN is a promising nanocarrier for solubilization and delivery of curcumin and provides the great opportunity for cancer clinical applications.

Metformin IR tablets: partial *in vitro* dissolution profiles differences do not preclude in *vivo* bioequivalence

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Abstract

Objectives: To investigate whether Metformin Profarma 850 mg tablets (test product) are bioequivalent to Glucophage® 850 mg tablets (Merck Santè laboratories - reference product) despite partial *in vitro* dissolution profiles differences observed.

Methods: A randomized, open-label, single-dose, two-period, one-week wash out, crossover study was performed in 20 healthy male and female volunteers at "Mother Theresa" University Hospital Centre, Tirana, Albania, after obtaining the approval by National Ethics Committee. A single 850mg dose of metformin was administrated with 200 ml of water after overnight fasting and blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 14 h after dosing. Plasma concentrations were measured by using a validated HPLC method with UV-DAD capable to detect metformin in the range of 50 - 2000 ng/ml. Non-compartmental pharmacokinetic parameters such as C_{max}, $AUC_{0\text{-}14\ h}$, $AUC_{0\text{-}\infty}$, and T_{max} were determined using PKSolver Version 2. The formulations were considered bioequivalent if the logarithmic mean ratios of ln-transformed Cmax, AUC0-14 and AUC0-∞ values were within the equivalence range of 80%-125%. Analysis of variance (ANOVA) was carried out using logarithmically transformed AUC0-t, AUC0-∞ and Cmax, and untransformed Tmax.

Results: Administration of single Metformin Profarma 850 mg and Glucophage® 850 mg tablets resulted in comparable systemic exposures to metformin, as determined by C_{max}, $AUC_{0\text{-}14}$ and $AUC_{0\text{-}inf}$. ANOVA analysis of the ln-transformed C_{max}, $AUC_{0\text{-}14}$ and $AUC_{0\text{-}inf}$ values indicated that none of the effects examined (formulation, period, sequence and carry over) was statistically significant. The geometric mean ratios of C_{max}, $AUC_{0\text{-}14}$ and $AUC_{0\text{-}inf}$ were 103.3%, 99.3% and 98.8%, respectively, and 90% confidence intervals of C_{max}, $AUC_{0\text{-}14}$ and $AUC_{0\text{-}inf}$ were contained within the bioequivalence acceptance limits of 80% to 125%.

Conclusions: Metformin Profarma 850 mg and Glucophage® 850 mg tablets were shown to be bioequivalent despite the *in vitro* dissolution profiles indicate a faster dissolution rate for Metformin Profarma 850 mg, at least in one dissolution medium.

Key words: Metformin, Dissolution profile, Pharmacokinetics, Bioequivalence

Mixed Hydrotropic Zaleplon Oral Tablets: Formulation and Neuropharmacological Effect on Plasma GABA Level

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Abstract: Zaleplon (ZP) is a non-benzodiazepine poorly soluble hypnotic drug indicated for the short-term treatment of insomnia having a bioavailability of about 30%. The aim of the present study is to enhance the solubility and consequently the bioavailability of ZP using hydrotropic agents (HA). Phase solubility diagrams of ZP in presence of different molar concentrations of HA (Sodium benzoate, Urea, Ascorbic acid, Resorcinol, Nicotinamide, and Piperazine) were constructed.
ZP/Sodium benzoate and Resorcinol microparticles were prepared adopting melt, solvent evaporation and melt-evaporation techniques followed by XRD. Directly compressed mixed hydrotropic ZP tablets of Sodium benzoate and Resorcinol in different weight ratios were prepared and evaluated compared to the commercially available tablets (Sleep aid® 5 mg). The effect of shelf and accelerated stability storage (40°C ± 2°C/75%RH ± 5%RH) on the optimum tablet formula (F5) for six months were studied. The enhancement of ZP solubility follows the order of: Resorcinol > Sodium benzoate > Ascorbic acid > Piperazine >Urea > Nicotinamide with about 350 and 2000 fold increase using 1M of Sodium benzoate and Resorcinol respectively. ZP/HA microparticles exhibit the order of: Solvent evaporation > melt-solvent evaporation > melt > physical mixture which was further confirmed by the complete conversion of ZP into amorphous form. Mixed hydrotropic tablet formula (F5) composed of ZP/(Resorcinol: Sodium benzoate 4:1 w/w) microparticles prepared by solvent evaporation exhibits in-vitro dissolution of 31.7±0.11% after five minutes (Q5min) compared to 10.0±0.10% for Sleep aid® (5 mg) respectively. F5 showed significantly higher GABA concentration of 122.5±5.5mg/mL in plasma compared to 118±1.00 and 27.8±1.5 mg/mL in case of Sleep aid® (5mg) and control taking only saline respectively suggesting a higher neuropharmacological effect of ZP following hydrotropic solubilization.

Keywords: Zaleplon, hydrotropic solubilization, plasma GABA level, mixed hydrotropy.

Effects of the *Caulerpa* Species Ethanol Extracts on Biofilm Formation of *Staphylococcus aureus* Bacteria

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Microbial biofilms are classified as serious problems for public health. These biofilms are generally occurred by attaching of the microorganisms to the various surfaces and producing extracellular polysaccharides. There have been a great interest in discovering new antimicrobials as a result of the increased resistance of microbial organisms to currently used antimicrobial agents (Donlan et al., 2001). It is known that the macro algae species secreted various secondary metabolites in order to cope with its predators (Mollo et al., 2008). Beside the defensive functions of these secondary metabolites, they can also be used as an important source in other industrial fields such as pharmaceutical, cosmetics and so on. In this respect, the purpose of the present study is to determine the antibiofilm effect of ethanol extracts of *C. racemosa* var. [*lamourouxii*] f. *requienii* and *C. taxifolia* var. *distichophylla* on *Staphylococcus aureus* bacteria.

The *Caulerpa* species were collected manually from Kemer/Üç Adalar (36°27'35.44"K, 30°33'2.92"D) and transferred to the laboratory immediately. The samples were washed with firstly the tap, then the distilled water to remove salt and epiphytes. After freeze-drying the sample at -52 ° C, the ethanol extracts were prepared for further use. The antibiofilm effect of the extracts against three *Staphylococcus aureus* strains that formed biofilm (*S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 33862) was tested via crystal violet assay (Meritt et al., 2005).

Eventually, it was determined that both extracts have antibiofilm effects on studied microorganisms and the biofilm inhibition activity increased with the increase in extract concentration. The maximum inhibitory activity with the ratio of 81.29 % was observed for *C. racemosa* extract (0.8 mg/ml concentration) on *S. aureus* ATCC 29213 species. In contrast, 0.8 mg/ml *C. taxifolia* extract inhibited the biofilm formation of *S. aureus* ATCC 29213 species with the ratio of only 69.58 %. Similar results were observed for other species of *S. aureus*. The difference between the amounts of secondary metabolites in *Caulerpa* species may be the reason of these results. There are a lot of papers in the scientific literature which specified that the main secondary metabolite of *Caulerpa* species is caulerpenyne (Jung et al., 2002). Hence, the reason of the increased antibiofilm activity of *C. racemosa* may have been caused from the higher amount of caulerpenyne in that species than *C. taxifolia.* Srivastava et al. (2010) and Nagaraj and Osborne (2014) also reported the antibacterial activity of *C. racemosa* extract against the *S. aureus* bacteria. Briefly, the ethanol extracts of *C. racemosa* can be a promising candidate for the developing new and effective antimicrobial agents, especially for Gram positive bacteria.

Key words: Biofilm, *C. racemosa* var. [*lamourouxii*] f. *requienii*, *C. taxifolia* var. *distichophylla, Staphylococos aureus*

Acknowledgements: *Ministry of Science, Industry and Technology supported this study as 0651.TGSD.2014 number project.*

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Encapsulation of venlafaxine-Dowex® resinate: A once daily multiple unit formulation

Dr. Salwa Salaheldin

INTRODUCTION

Major depressive disorder affects high proportion of the world's population presenting cost load in health care. Extended release venlafaxine is more convenient and could reduce discontinuation syndrome. The once daily dosing also reduces the potential for adverse events such as nausea due to reduced C_{max} . Venlafaxine is an effective first-line agent in the treatment of depression. A once daily formulation was designed to enhance patient compliance. Complexing with a resin was suggested to improve loading of the water soluble drug. The formulated systems were thoroughly evaluated in vitro to prove superiority to previous trials and were compared to the commercial extended release product in experimental animals.

MATERIALS AND METHODS

Venlafaxine-resinates were prepared using Dowex®50WX4-400 and Dowex®50WX8-100 at drug to resin weight ratio of 1: 1. The prepared resinates were evaluated for their drug content, particle shape and surface properties and in vitro release profile in gradient pH. The release kinetics and mechanism were evaluated. Venlafaxine-Dowex® resinates were encapsulated using O/W solvent evaporation technique. Poly-ε-caprolactone, Poly(D, L-lactide-co-glycolide) ester, Poly(D, L-lactide) ester and Eudragit®RS100 were used as coating polymers alone and in combination. Drug-resinate microcapsules were evaluated for morphology, entrapment efficiency and in-vitro release profile.The selected formula was tested in rabbits using a randomized, single-dose, 2-way crossover study against Effexor-XR tablets under fasting condition.

RESULTS AND DISCUSSION

The equilibrium time was 30 min for Dowex®50WX4-400 and 90 min for Dowex®50WX8-100. The percentage drug loaded was 93.96 and 83.56% for both resins, respectively. Both drug-Dowex® resintes were efficient in sustaining venlafaxine release in comparison to the free drug (up to 8h.). Dowex®50WX4-400 based venlafaxine-resinate was selected for further encapsulation to optimize the release profile for once daily dosing and to lower the burst effect.The selected formula (coated with a mixture of Eudragit RS and PLGA in a ratio of 50/50) was chosen by applying a group of mathematical equations according to targeted values. It recorded the minimum burst effect, the maximum MDT (Mean dissolution time) and a $Q_{24}h$ (percentage drug released after 24 hours) between 95 and 100%. The 90% confidence intervals for the test/reference mean ratio of the log-transformed data of AUC_{0-24} and $AUC_{0-\infty}$ are within (0.8–1.25), which satisfies the bioequivalence criteria.

CONCLUSIONS

The optimized formula could be a promising extended release form of the water soluble, short half lived venlafaxine. Being a multiple unit formulation, it lowers the probability of dose dumping and reduces the inter-subject variability in absorption

Anti-inflammatory activity of lectin purified from *Morus nigra* against lipopolysaccharide (LPS) induced renal stress in rats.

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The study was designed to investigate the possible protective role of lectin of *Morus nigra* in lipopolysaccharide renal inflammatory, by using biochemical approaches. The effects of lectin of *Morus nigra* on LPS induced oxidative and renal stress were evaluated by serum creatinine, urea and uric acid levels, kidney tissue lipid peroxidation, GSH levels, SOD, GSH-Px , GST and catalase activities. Administration of LPS induced significant increase in serum: creatinine, urea and uric acid concentration showing renal inflammatory. LPS also induced oxidative stress, as indicate by decreased kidney tissue of GSH level, SOD, GSH-Px, GST and catalase activities along with increase the level of lipid peroxidation. Furthermore, treatment with LPS caused a marked elevation of kidney weight and decreased body weight. lectin of *Morus nigra* treatment markedly reduced elevated serum: creatinine, urea and uric acid levels and conteracted the deterious effects of LPS on oxidative stress markers and attenuated histological changes caused by LPS in kidney. Our results indicate that lectin of *Morus nigra* could have a beneficial role against LPS induced nephrotoxicity and oxidative stress in rat.

Keywords: Antioxidant enzymes, LPS, lectin, *Morus nigra*, renal inflammatory.